2016 Medicine by Design Symposium Report

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Abstract

More than 250 stem cell researchers from across the University of Toronto (U of T) and its affiliated hospitals, as well as leading Canadian and international regenerative medicine experts, gathered on Nov. 28, 2016, at Toronto's MaRS Discovery District for the inaugural Medicine by Design symposium. Celebrating the first year of Medicine by Design, a U of T initiative to accelerate breakthroughs in regenerative medicine and translate them into new therapies, the symposium covered a wide diversity of topics ranging from basic stem cell biology insights to translational advances in regenerative medicine. Speakers included principal investigators leading eight of Medicine by Design's collaborative team projects; members of the initiative's Scientific Advisory Board; and Nadia Rosenthal of The Jackson Laboratory, who delivered the keynote address. Twenty graduate students and postdoctoral fellows presented their research during the poster session.

Back to basics: A better understanding of the immune system, stress and molecular diversity will shape future regenerative therapies

One of the prevailing themes of the symposium was the need to better understand the basic biology of stem cells and tissue regeneration, including the role of the immune system, stress responses and transcriptional diversity between different cell types. Emerging insights in these areas already have the potential to accelerate regenerative therapies, both by means of transplantation of replacement tissue and via endogenous repair, which is driven by recruitment and differentiation of native stem cells.

Nadia Rosenthal (The Jackson Laboratory) reminded the audience there is a wide range of regenerative capacities across the phylogenetic tree of life. Furthermore, the ability to regenerate tissues and organs changes during development, generally diminishing with age. To understand regeneration, she said, it is crucial to study development, as well as to look beyond the animal models typically used in research. Rosenthal's work on axolotl, a salamander that can regrow entire limbs, established an important difference in the way the immune system is recruited to the site of injury in axolotl and mice. In axolotl, there is an immediate and simultaneous recruitment at the wound site of the whole spectrum of immune cells (Godwin et al., 2013). In contrast, mammalian wound healing occurs in a step-wise fashion where different immune cells arrive at the wound in succession over several days to drive scar formation. The axolotl immune response is orchestrated by regenerative so-called M2 macrophages, whose ablation, she said, stops regeneration and leads to scarring as in mammals. These and other findings paint a picture in which macrophages play a key role in tipping the balance of post-

injury tissue modulation via scarring, which blocks regrowth, to regeneration, possibly by reactivating a developmental program. Further work identified IGF-1 as the key signalling factor, which is secreted by the macrophages and promotes scar resolution and regeneration of skeletal and heart muscle in mammals (Forbes and Rosenthal, 2014). These studies underscore the importance of understanding how different organisms have adapted different regenerative abilities and can give us mechanistic insight into how to switch wound repair from scarring to regeneration.

One way to do this would be to use biomaterials as a way of stimulating a regenerative immune program locally. Medicine by Design team project leader Michael Sefton of U of T's Institute of Biomaterials & Biomedical Engineering (IBBME) reported that poly-methacrylic acid-co-methyl methacrylate (MAA) beads, which are known to stimulate blood vessel growth in the model of diabetic wound healing and in the absence of exogenous cells and growth factors, do so through activation of so-called M2 macrophages and Shh signalling although the underlying molecular mechanisms remain unclear (Fitzpatrick et al., 2012; Wells et al., 2017). IGF-1 also plays a key role in this system and IGF-1 inhibitors block the MAA-induced vascularization. Sefton's intriguing proposal to design therapeutic polymers could even elicit regenerative signalling in the absence of macrophages. Using the structure of a particular receptor, he said, it may be possible to create a biomaterial that activates the receptor to trigger downstream signalling events and activate tissue regeneration.

Just as these insights open the door to modulating immune signalling as a way of boosting regeneration, recent findings about endogenous stem cell signaling pathways provide unexpected avenues for harnessing their therapeutic potential. Medicine by Design project leader John Dick (Princess Margaret Cancer Centre, University Health Network) reported a link between the stress response pathway in the hematopoietic stem cells (HSCs) and their ability to engraft into the host bone marrow (van Galen et al., 2014). Unlike committed progenitors, which can endure higher levels of stress, the HSCs are primed for apoptosis induced by the ER-mediated unfolded protein response, presumably to ensure that any damage in the life-long HSC pool is rapidly purged. Overexpression of ERDJ4, a co-chaperone in the ER stress response that suppresses apoptosis, in HSCs increased engraftment success in nude mice, suggesting that similar strategies may improve future transplants in patients.

Recent advances in microfluidics and genomic technologies have enabled molecular profiling of individual cells in unprecedented detail (Macosko et al., 2015; Tanay and Regev, 2017). These studies continue to reveal enormous molecular diversity among cells previously thought to be of the same type, calling for a redefinition of cellular subtypes and lineages. Using single cell transcriptomics, Dick and colleagues uncovered, for example, that the common myeloid progenitor (CMP) population, previously thought of as an essential oligopotent intermediate state in adult human hematopoiesis, consists in fact of three unipotent cell types (Notta et al., 2016). This finding upended the classical view of human hematopoiesis and led to a new and dynamic model in which oligopotent progenitors gradually lose their ability to differentiate into multiple cell types during development to become unipotent progenitors in the adult. This new

paradigm of how blood is formed will undoubtedly lead to a better understanding of the origins of blood disorders.

Nowhere is tissue complexity more bewildering than in the human brain, thought to consist of 100 billion neurons that connect via 100 trillion synapses. The growing power and dwindling costs of single-cell technologies, however, now make it possible to survey this diversity to reveal not only transcriptomic and proteomic profiles but epigenetic states as well. Medicine by Design team project leader Gary Bader (Donnelly Centre for Cellular and Biomolecular Research, U of T) outlined his plans to use these approaches to map cell-to-cell communication networks during brain development. His goal is to identify pathways guiding endogenous repair, which may in the future provide a way to heal damage cause by injury or disease. A recent study from Freda Miller and David Kaplan's groups at the Hospital for Sick Children demonstrated the value of this approach. Using microfluidics-based single-cell transcriptomics of precursors and neurons in the developing mouse cortex, they were able to reveal a previously unknown diversity among precursor types and identify new ligands that promote neuronal differentiation (Yuzwa et al., 2016). Siraj Zahr, a graduate student in the Miller lab who won third place in the symposium poster competition, revealed another layer of complexity in the way neural precursor cells (NPCs) are specified by showing that NPCs are transcriptionally primed to generate diverse types of neurons. He also revealed translational repression as the underlying molecular mechanism ensuring that the neuronal type-specific transcripts are translated at an appropriate time during development.

Further advances in microfluidics could bring into the spotlight rare cells types, such as circulating tumour cells, which originate from primary tumours and can vary widely in their metastatic potential. To this end, Peter Aldridge, a graduate student in Shana Kelley's lab at the IBBME, is developing an innovative cell sorter that uses a ferromagnetic alloy to separate cells that bind to magnetic nanoparticles coated with a cell surface marker. Using a combination of cell surface markers, this approach could enable a characterization of functional diversity of circulating tumour cells and improve disease diagnostics. Aldridge won second prize in the symposium poster competition.

Despite the amazing power of new technologies, classical approaches continue to reveal surprising new aspects of stem cell biology with profound effects on understanding — and possibly treating — disease. Michael Rudnicki (Ottawa Hospital Research Institute and chair of Medicine by Design's Scientific Advisory Board) reported on the link between dystrophin localization in muscle stem cells (satellite cells) and the onset of Duchenne muscular dystrophy (DMD). He showed that asymmetric localization of dystrophin in satellite cells is necessary for the assembly of the polarity PAR complex and subsequent segregation of myogenic factors into muscle progenitors (Dumont et al., 2015). These findings suggest that DMD, previously thought to be the consequence of fragile muscle fibres, might also stem from the satellite cells' inability to differentiate, leading to a diminished progenitor pool. Intriguingly, Rudnicki also showed that dystrophin is required for asymmetric localization of a protein kinase pathway that leads to epigenetic de-repression of the progenitor muscle fate driver Myf5, raising a possibility that DMD might be targeted by epigenetic agents.

Disease Modelling in 3D

In the body, stem cells and progenitors exist in a complex and tightly regulated environment, or niche, where intercellular cross-talk regulates tissue maintenance and repair. In recent years, *in vitro*-grown three-dimensional organoids have come to the forefront as a model for understanding cell-cell communication at the molecular level and how it contributes to disease, as well as a platform for drug discovery. Conceptually rooted in developmental biology, organoids are derived from pluripotent stem cells through step-wise activation of specific pathways that drive development of a given organ.

Taking cues from molecular pathways that drive gut development in the embryo, Medicine by Design team project leader Jeffrey Wrana (Lunenfeld-Tanenbaum Research Institute, Sinai Health System) established a protocol for differentiating human intestinal organoids *in vitro*. Composed of epithelial cells, myofibroblasts and mesenchymal cells, the intestinal organoids provide a window into the cross-talk between Tgf-ß, Wnt and Hippo pathways, which are known to be important in gut development (Varelas and Wrana, 2012). The team's recent finding that Hippo and Wnt pathways intersect to regulate intestinal crypt formation during mouse development (Gregorieff et al., 2015) led to the discovery that in organoids, Hippo activation in the surrounding mesenchyme represses organoid growth. The finding may help researchers overcome the slow rate of organoid growth, which typically takes several months and limits their use, by inhibiting Hippo in the mesenchyme. Wrana also reported on using organoid differentiation protocol as a platform for small molecule and CRISPR screens, which enabled them to identify new epigenetic regulators and tumour suppressor genes as key drivers of differentiation. In the future, such screens may also be helpful in identifying metabolic products, or factors secreted by the microbiome, that affect gut regeneration.

Similarly, the knowledge of molecular pathways that govern lung development in embryo enabled Hans-Willem Snoeck (Columbia University) to drive *in vitro* differentiation of human embryonic stem cells into lung organoids (Huang et al., 2014). Marker gene expression revealed that these organoids are composed of at least six different cell types organized into a typical three-dimensional structure, with proximal airways cells and distal branching alveolae. Importantly, the organoids recapitulate milestones of human lung development *in utero*, which cannot be modelled in the mouse due to striking differences in developmental timing (Snoeck, 2015). By introducing into the ESCs familial mutations that cause idiopathic pulmonary fibrosis (IPF), which causes incurable scarring of the lung, Snoeck's team was able to generate organoids with the typical disease phenotype, demonstrating their potential in modelling disease and drug discovery. He was also able to establish a model for a respiratory infection caused by the respiratory syncytial virus (RSV), which can be fatal in newborns, with no vaccine available.

In addition to organoids, tumours can be cultured in a complex three-dimensional environment to better understand how they progress. Tumour-secreted factors can change their environment in a way that facilitates growth and invasion and these processes cannot be modelled in a standard monolayer culture. Gordana Vunjak-Novakovic (Columbia University) presented her work on establishing a 3D culture system to study interactions between the Ewing's sarcoma

type I, an aggressive bone cancer, and the healthy mesenchyme of the bone. After co-culturing bioengineered 3D bone scaffolds (Bhumiratana et al., 2016) with sarcoma cells, her team was able to collect and analyze tumour-secreted factors. Gene expression profiling of the tumour secretome revealed high levels of Polycomb histone methyltranferase EZH2 mRNA, suggesting epigenetic regulation as a mechanism by which tumours modify their environment (Villasante et al., 2016). Furthermore, the EZHZ mRNA was also found in patient plasma, but not in the monolayer culture, emphasizing the value of studying cancer *in vitro* in a 3D system.

Cells in Translation: Advancing Towards the Clinic

Regenerative medicine has a potential to overcome the shortage of organ donors with new *in vitro*-grown replacement tissues. For a stem cell-derived tissue to be used as a transplant graft, it is critical to ensure that it is composed of the right kind of cells in terms of lineage, purity and maturation. As discussed below, cell therapy has shown promise in driving regeneration of damaged cardiac, liver and retinal tissues in animal models without triggering tumour growth.

Heart disease is a leading cause of death in the developed world and so it is not surprising that some of the greatest focus in the field is on efforts to repair heart muscle damage caused by myocardial infarction. Some of the pioneering work came from the Medicine by Design team project leader Gordon Keller (McEwen Centre for Regenerative Medicine, University Health Network) who presented his team's success in driving *in vitro* differentiation of multiple heart lineages from pluripotent stem cells. What made this possible, Keller said, was the fundamental understanding of how the heart develops in an embryo. This allowed them to induce different cell fates, including ventricular and atrial cardiomyocytes, sino-atrial node (SAN) pacemaker cells, as well as epicardial and endocardial cells, through a sequential activation of developmental pathways, including BMP and WNT (Kattman et al., 2011; Witty et al., 2014). He stressed the importance of manipulating signalling pathways early — within a few days of commitment to mesoderm — to drive a particular fate, and that this can be optimized through monitoring marker gene expression. Keller has been working with CCRM, Medicine by Design's commercialization partner, to scale up the production of cardiomyocyte from a pluripotent stem cell source in stir-tank bioreactors for cost-effective manufacturing, a key requirement for any future application. Among the remaining challenges are confirming that the cells derived in the scale-up process have the same properties and efficacy as those initially derived in smaller batches, as well as separating the desired therapeutic cell type from residual PSCs or other cells that result from the process.

In vitro differentiated cardiomyocytes have shown the ability to resolve fibrosis caused by myocardial infarction with subsequent tissue regeneration. After transplanting hESC-derived cardiomyocytes into infarcted areas in a number of animal models, including rodents, guinea pigs, pigs and primates, Medicine by Design team project leader Michael Laflamme (Toronto General Research Institute, University Health Network) reported that the engraftments became electromechanically coupled with the host cardiac tissue and improved its contractile function without causing tumours (Chong et al., 2014; Laflamme et al., 2007). However, both primates and pigs, which are considered the best preclinical model because their organs are the same size as human organs, developed arrhythmias about two weeks after the transplant, which later

resolved. While these studies suggest that re-muscularization of the human heart may also be possible, potential arrhythmias remain a concern and this will be a key area of focus in future studies.

Advances in cardiac regeneration also pave the way for tackling other diseases. Among the most challenging is liver disease, which affects one in ten Canadians with death rates that have increased by a third in the past decade. So far, however, generating functional liver cells *in vitro* has been challenging. This may be due to missing environmental signals, which could be addressed by differentiating 3D liver organoids. This is the goal of Keller's interdisciplinary Medicine by Design team, which will use the principles learned from cardiac cell differentiation to create functional liver *in vitro*. To this end, Mina Ogawa, a research associate in the Keller lab and winner of the Best Poster Award, was able to generate functional biliary organoids from hPSCs by modulating Notch signalling, which the team previously showed to be important for the cholangiocyte fate. Furthermore, Ogawa was able to co-culture hPSC-derived hepatocytes and cholangiocytes and recapitulate initial stages of biliary tree development (Ogawa et al., 2015; Ogawa et al., 2013).

Historically, cell transplants using cadaveric hepatocytes showed some success in treating inborn metabolic liver diseases, sometimes even obviating the need for organ transplant (Fox et al., 1998; Strom et al., 1997). However, cadaveric hepatocytes are of limited use given their poor quality and their inability to be expanded in culture. This led Markus Grompe (Oregon Health and Science University) to seize on the hepatocytes' ability to divide *in vivo* and use animals as living bioreactors for expansion of human hepatocytes. His team developed mice with humanized livers, using the FAH deficiency-based mouse model of tyrosinemia, which allowed mouse hepatocytes to be replaced with human counterparts (Azuma et al., 2007; Paulk et al., 2012). Furthermore, human hepatocytes could be vastly expanded through serial transplantation without losing functionality or causing tumour growth. Grompe said that "hepatocyte farming" in pigs could further boost the yield of human hepatocytes to achieve the amounts required for patient transplants. He also presented a viral rAAV-based system for *in vivo* expansion of genetically modified hepatocytes (Nygaard et al., 2016). While the *in vivo* expanded human hepatocytes are a powerful tool for disease research and drug discovery, Grompe warned about the risk of contamination with animal cells, an issue that would have to be overcome before these grafts can be used on patients.

Some of the most advanced translational efforts are those aiming to repair vision loss. This is, in part, due to the fact that human embryonic stem cells can be easily differentiated to retinal pigment epithelial cells (RPE). Importantly, the cell type and location are known for those cells that are lost in blindness associated with age-related macular degeneration and retinitis pigmentosa — RPE and photoreceptors — making the retina a good testing ground for new transplant procedures. Several ongoing clinical trials are testing the ability of stem cell-derived retinal pigmented epithelial cell grafts to treat tissue degeneration in the eye (Kimbrel and Lanza, 2015). These grafts, however, do not replace damaged photoreceptors, which are the primary cells that perceive light and which have been more challenging to generate and engraft in animal models. Studies in mice carried out by the Medicine by Design team project leader

Molly Shoichet (Department of Chemical Engineering & Applied Chemistry) and Derek van der Kooy at the Donnelly Centre suggest that adult -retinal stem cell-derived rod photoreceptor integration into the host retina can be enhanced by hyaluronan and methylcellulose (HAMC) — an injectable hydrogel that also boosts cell survival *in vitro* (Ballios et al., 2015). While rod photoreceptor grafts were able to restore some pupil constriction in blind mice in response to light, Arturo Ortin Martinez (Valerie Wallace's lab) called for caution in interpreting cell integration data based on GFP fluorescence alone, after demonstrating the occurrence of GFP transfer between donor and host cells in the retina (Ortin-Martinez et al., 2017). In the future, efficient vision repair will also depend on the ability to generate pure populations of other retinal cell types, especially cone photoreceptors that are responsible for central and colour vision.

In addition to transplantation, another way to ameliorate degenerative disease is to stimulate endogenous repair. To this end, Medicine by Design team project leader David McMillen (University of Toronto Mississauga) reported on a strategy to counteract inflammatory bowel disease (IBD), caused by a deficiency of NOD2 protein that stimulates repair of the gut lining. McMillen's team is applying expertise in synthetic biology to engineer a bacterium that can act as a sensor to detect low NOD2 levels and secrete more protein when needed to trigger tissue repair in IBD patients.

Although mammalian synthetic biology is still in early stages, it has a potential to overcome a number of challenges in regenerative medicine, from immune rejection to scalable manufacturing of desired and quality controlled cell types (Lipsitz et al., 2017). Jeffrey Harding from Andras Nagy's group at the Lunenfeld-Tanenbaum Research Institute presented their efforts in designing cell grafts that harbour sensor systems linking expression of immunomodulatory genes to the immune response at the graft site. The goal is to be able to modulate the host's immune response locally and avoid systemic immune suppression, which carry serious side-effects, following allogeneic transplants. And Laura Prochazka from Peter Zandstra's group in the IBBME presented an ambitious plan for a stable integration of multi-input genetic circuits into human iPSCs for an enhanced and monitored generation of iPSC-derived progenitor T cells for immune cell therapies.

Ashton Trotman-Grant, a PhD student in the laboratory of Juan Carlos Zúñiga-Pflücker (Sunnybrook Health Sciences Centre) presented an alternative approach to boosting progenitor T cell production by developing an *in vitro* cell-free system based on Delta-like-4 multimers, which are immobilized on beads to trigger Notch signalling in the HSCs. The strategy was successful in driving T cell development in mice and Trotman-Grant won the Blueline Therapeutic Translation Award, in recognition of the poster with the greatest commercial translation potential.

Conclusion

The breadth of presentations by Medicine by Design team project leaders confirmed Canada's position as a leading global force in regenerative medicine research. However, as Nicole Forgione (CCRM) emphasized in her talk, based on investment in research and innovation data, Canada falls behind other regenerative medicine hubs in discovery commercialization. One way

to boost research translation, Forgione said, is to streamline access to venture capital, one of CCRM's main goals. Joined by other stakeholders in academia, industry and government, Medicine by Design is well placed to bridge the gap to commercialization by recognizing and supporting research with the greatest translational potential.

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