The Georgia International Convention Center was host to the annual meeting of the Genetics Policy Institute (GPI) and the Regenerative Medicine Foundation (RMF). The event is described as “the most expansive interdisciplinary, networking meeting of stem cell science and regenerative medicine stakeholders, uniting the diverse community. With the overarching purpose of fostering biomedical research, funding and investments targeting cures, the Summit is the single conference charting the future of this burgeoning field.”

Lattice Biologics Ltd.’s resident Ph.D.s, Dr. Christopher Bradley and Dr. Milla Zakharova attended the event to represent our technologies and products, research the presentations, and collaborate with other regenerative medicine pioneers.

**Christopher Bradley, Ph.D.**

**Product Development Director**

**BACKGROUND**

**Further Insights into the Complexity of the Extracellular Matrix**

Naturally, I’m interested in learning more about the extracellular matrix (ECM), specifically the kind secreted by stem cells, and its potential for promoting regeneration in tissues and its application to regenerative medicine. I was inspired by an article I read in *Genetic Engineering & Biotechnology News* titled, “Such Stuff As Biofab Organs Are Made On.” ([Read article here.](#)) The article talked about whole organ decellularization as a way of fabricating new functional organs populated with a patient’s own cells in order to meet the demand for organ transplants.

In particular, the article focused on the challenges in characterizing the ECM that is left behind after decellularization. The author makes the point that “Extracellular matrix components are a key determinant of the cell-cell and cell-matrix interactions that are established once the scaffold is repopulated with cells, and these conditions are, therefore, a fundamental determinant of the functionality of the future organ.” So the importance of the ECM, a complex reservoir of growth factors, adhesion factors, and signaling molecules, is clear.

However, understanding the organization of the ECM is still a challenge, given the “paucity of tools that can reliably quantitate [the ECM’s] composition.” This lack of understanding has stymied efforts to refine the process of decellularization and still preserve the function of the precious ECM, since “a comparison of decellularization protocols… revealed that elastin, laminin, and fibronectin retention [differs under varying chemical conditions].”
I can appreciate that experimental conditions can change the ECM composition that is left behind, but, due to my personal research on fibronectin I also understand that each of these identified proteins hold their own additional complexity. “Fibronectin” is so exquisitely nuanced and encompasses such a huge family of slightly varied proteins, that it must be considered a generalized catch-all term. As language currently fails to capture the breadth of differences between fibronectin family members, our understanding of the intricacies of the mixture that is the ECM is inherently limited.

The following presentation restored my belief in the painstaking minutiae of scientific research because it dared to dive into the dirty details of another ECM component protein, laminin.

PLURIPOTENCY AND DIFFERENTIATION
(Moderator: Gary D. Smith, PhD, HCLD, University of Michigan)

On December 10th, I attended the “Pluripotency and Differentiation” session which featured three notable speakers who deciphered the cryptic nature of the ECM and how stem cells respond to their fascinating micro-environment. I have highlighted two of the presentations from this group here.

Father, Son and the Holy Trimer
(Karl Tryggvason, MD, PhD, Karolinska Institutet, Duke-NUS)

Karl Tryggvason, MD, PhD honed in on the generally underappreciated differences in types of laminin, present in the ECM of tissues. The laminins occur in multiple isoforms (variations on the same protein). Twelve different genes code for the alpha, beta, and gamma chains of the laminins, and at least 16 different combinations of these subunits (assembled into a three chain “trimer”) have been identified in mammals.

In vitro (or “in the dish”) cell culture is the traditional method of growing tissue cells in the laboratory; however, these cells encounter a very different environment that only partially mimics the conditions that cells experience in the body. This results in laboratory-grown cells that de-differentiate (or lose their original identity), which renders them potentially useless for transplantation.

The need to define the conditions required to duplicate the “stem cell niche” in an in vitro system led Dr. Tryggvason and co-workers to:

1. characterize the specific laminin types present in various tissue microenvironments, and
2. test their effects on laboratory-grown cultures for their ability to stabilize cellular phenotypes.

The application of purified, recombinant (produced by genetic engineering) laminins appeared to restore biorelevant cell-matrix interactions and preserve the behavior of stem cells and tissue-specific cells. This led to a systematic, chemically-defined approach to expanding cells in vitro which holds great promise for custom cell therapies to restore organ function and treat liver, heart, and other degenerative diseases.

It also gave rise to a family business, BioLamina -- the biotech company managed by Dr. Tryggvason’s son, that specializes in the production of recombinant laminins and promises to “revolutionize cell culture.”

References:
Controlling Stem Cell Fate Using Multivalency and Optogenetics

(Ravi Kane, PhD, Georgia Institute of Technology)

Another fascinating talk I caught at the Pluripotency and Differentiation session was given by Ravi S. Kane. Any attempt to pin down this guy’s expertise on the Internet is futile, as he seems to have his hand in all manner of research, from neural cell physiology to nanomaterials to bacterial pathogenesis. I guess this is because -- at the heart of it -- he is a tissue engineer who is working on the cutting edge of materials science that borrows heavily from biology.

His talk left me a bit agog, as I am woefully unable to explain the complexity of its content with the same level of familiarity as he. The title of the talk, “Controlling Stem Cell Fate Using Multivalency and Optogenetics” includes the term, optogenetics, which is likely new to most people.

Perhaps a quick trip to Wikipedia would help.

Optogenetics (from Greek optikós, meaning “seen, visible”) is a biological technique which involves the use of light to control cells in living tissue, typically neurons, that have been genetically modified to express light-sensitive ion channels.

It is a neuromodulation method employed in neuroscience that uses a combination of techniques from optics and genetics to control and monitor the activities of individual neurons in living tissue—even within freely-moving animals—and to precisely measure the effects of those manipulations in real-time.

[1] The key reagents used in optogenetics are light-sensitive proteins.

To “shed some light” on the matter, I would describe optogenetics as a classification for a molecular biology technique that uses light as a tool to invoke controlled intra- and intercellular changes by means of light-sensitive proteins.

References:
3http://www.well.ox.ac.uk/may-10-cell-cell-communication

In the case of Dr. Kane’s work, he was exploring how proteins sensitive to light present on the cell surface of plant tissues (photobodies) can be engineered to trigger gene expression in animal cells. When the gene for the photobodies is spliced together with a known signaling factor and expressed in mammalian cells, a burst of blue light can cause reversible clustering of the photobodies which sends a message to the nucleus to turn on protein production from a gene in the β-catenin/Wnt pathway. The Wnt pathway is known to act as a switch that pushes stem cells to differentiate into bone tissue.

As such, the applications for this technology extend well beyond this particular demonstration.
Further exploration into the phenomena of receptor clustering involved the idea of controlled multivalency, that is, spatially organizing the presentation of certain receptor-activating proteins onto polymers (hydrogels) modeled after components in the ECM. These elaborately contructed biomaterials can change the differentiation fate of stem cells by biophysical means, i.e., contact with receptors on the cell surface.

If these kinds of light-induced tissue engineering technologies are being developed today, how long is it before a wave of a “magic wand” (or Star Trek tricorder, if you prefer) can initiate healing at the cellular level?

- Christopher Bradley, Ph.D.
Lattice Biologics Inc.

Milla Zakharova, Ph.D.
Senior Research Scientist

BACKGROUND
A Tale of Two Trends

The World Stem Cell Summit (WSCS) is the largest global networking meeting of stem cell science and regenerative medicine stakeholders. More than 1,200 attendees and 200 speakers from 40+ countries participated in this year’s summit. It brings together policy, science, industry, advocates and clinicians in a dynamic and powerful forum with a mission to transform the treatment of human diseases and injuries through advancing and accelerating stem cell technologies.

From a scientific perspective, I observed two main trends dominated WSCS2015 discussions:

1. Switching from autologous to allogeneic stem cells for the Regenerative Medicine Industry
2. Shifting from cell replacement therapies to tissue and organ engineering using multiple cell sources and 3D printing technologies

Read on for more about each of these trends...

AUTOLOGOUS VS. ALLOGENEIC CELL THERAPIES

Many speakers repeatedly raised this topic throughout the Summit, including Joshua Hare (University of Miami, FL), Julie Allickson (Wake Forest Institute for Regenerative Medicine), Jan Nolta (UC Davis, CA), and Mahendra Rao (Q Therapeutics & NYSCF, CA).

Comparison of autologous and allogeneic stem cell therapy.

Autologous Stem Cell Therapies:
How it works: Autologous cell therapies utilize patients’ own cells. The process starts when a tissue biopsy is obtained from a patient, followed by cell isolation, purification, and expansion in culture to enrich the target cell population. At this stage, cells can be modified to improve their therapeutic properties. After the expansion, cells are collected and delivered back to the patient.

What the FDA says: From the FDA’s perspective, this approach is considered to be the “gold standard” of cellular therapies for several reasons:

- Autologous cell protocols are safer for patients.
- They require fewer tests.
- They cause fewer tracking issues.

What Scientists and Biotech says: From scientific and manufacturing standpoints, the autologous therapy approach has many flaws:

1. The patients, themselves, are often diseased or older, thereby likely compromising the regenerative potential of the isolated cells.
2. Patients must undergo two different invasive procedures (first to collect the tissue specimen and then to deliver the cells).
3. Manufacturing autologous cells can be a logistic nightmare due to low yield, lack of batch-to-batch consistency, failure-dependency, and a high overall cost of production.

Allogeneic Stem Cells Therapies:
Allogeneic therapy is clearly a disruptive concept in biology.

How it works: Allogeneic cell-based products utilize cells from unrelated donors. Cells for allogeneic therapies are typically obtained from young, healthy donors to ensure their regenerative qualities.
Benefits: Allogeneic based products are amenable to a scale-up manufacturing approach and available as “off-the-shelf” or “on-demand” products. Allogeneic cells can be expanded in large quantities, fully tested, stored in cell banks, and released and shipped upon request. This manufacturing strategy is very similar to the production of other biologics, including vaccines, antibodies or recombinant proteins.

Challenges:

- Lack of clear regulatory guidelines from FDA.
- Complicated testing before product release.
- Potential host immune response.

The latter issue could be overcome in the case of therapeutic products based on mesenchymal stem cell (MSCs), as MSCs are well known for their immunomodulatory capabilities. Their immunoprivileged properties are believed to permit their allogeneic or even xenogeneic transplantation into immunocompetent recipients without the use of immunosuppressants.1 (See study here.)

Conclusion:
My impression is that there is a high level of demand for the FDA to update its regulation guidelines and facilitate larger randomized clinical trials. Despite the issues to be considered as the field evolves, allogeneic cell therapies have a strong potential and I look forward to hearing more at future conferences about progress in this area.

FROM TISSUE TO ORGANS: 3D BIOPRINTING, SCIENCE AND INDUSTRY PERSPECTIVES

These exciting sessions covered progress and challenges in tissue and organ bioengineering. Priorities for advancing the field focused on four parallel pathways:

1. Layer-by-layer building of organs and tissues
2. Recellularization strategies
3. Cellular Repair or Regeneration
4. Xenotransplantation

The first session focused on scientific advances, including:

- Laryngeal transplantation (David Lott, Mayo Clinic)
- Building a heart (Doris Taylor, Texas Heart Institute)
- Case studies for bioengineering organs, such as the bladder (Shay Soker, Wake Forest)

The second session covered current clinical and industry approaches aimed to provide some relief to the challenge of organ shortage:

- 3D printing organs layer-by-layer
- Developing off the shelf extracellular matrix tissue products

Enjoy these brief summaries of some of the talks.

Shannon L.M. Dahl, PhD, Humacyte

Shannon Dahl (Co-Founder, VP, Technology and Pipeline Development, Humacyte) presented recent developments in creating acellular biovessels. Her company, Humacyte, developed a proprietary technology platform to isolate and grow investigational human acellular vessels that have the potential to provide stable “off-the-shelf” human tissue replacements for vascular surgeries.

Humacyte’s investigational products are acellular extracellular matrices, which are formed in vitro from banked vascular smooth muscle cells and then decellularized. No cells from the patient are required for this production process. The resulting end product is designed to have significant shelf life for on-site storage at hospitals and -- if approved -- may have potential to be readily available for patients in need of vascular access or replacement. Humacyte received Fast Track designation to use their products for vascular access in hemodialysis patients from the U.S. Food and Drug Administration in 2014 and has ongoing Phase II trials in the U.S. and EU for patients with the end-stage renal disease.

Keith Murphy, Organovo

Keith Murphy discussed how his company, Organovo (San Diego), designs and creates multi-cellular, dynamic and functional human tissues for use in drug discovery and medical research. Organovo claims that their proprietary 3D bioprinting technology enables creation of tissues that mimic key aspects of native tissues, ensuring that each layer contains relevant cell type(s) and has dimensions that approximate those of native tissue. The final products are not yet cGMP-compliant and are currently tested only in academic research or industrial R&D settings.

References:

Doris A. Taylor, PhD, FAHA, FACC, Texas Heart Institute at St. Luke’s Episcopal Hospital

Doris Taylor (Director of Regenerative Medicine Research at Texas Heart Institute).

She is a pioneer in cardiovascular cell therapy research who is credited with a number of important scientific breakthroughs related to cell therapy.

Doris Taylor's research group is most famous for creating the first decellularized heart and attempting to bring it back to life through engineered recellularization. Her presentation was focused on trials and errors of this process. At that point, researchers had been successful in recellularizing small organs (mouse heart) and organs of limited complexity (bladder).

Advancing this process to more complex organs of human size presents a set of tremendous challenges:

- What cells to use, and how many?
- Should they be mature cells, embryonic stem cells, or iPSC (induced pluripotent) cells?
- What is the optimum cell source?
- What does it take to grow sufficient number of cells to populate a human heart?

**Conclusion:**

Despite some skepticism among the research community regarding success of organ engineering projects in the near future, the effort may be worthwhile. The ultimate objective is creating brand new organs for transplantation and overcoming donor shortage; however, besides this goal, there is a lot to learn from the first generation engineered organs.

- Milla Zakharova, Ph.D.
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Images:

Heart image: http://www.vtnews.vt.edu/articles/2014/05/052014-vtc-heartcenter.html