GROWING THE DISTANCE

How regenerative medicine is changing the future of personalized therapy.

WRITTEN BY JEANNE BERKOWITZ, PhD

IMAGINE HAVING SKIN SO FRAGILE that even pressure from the seams in a piece of clothing could cause painful blistering and injury. For children with epidermolysis bullosa, a rare genetic disease that can be caused by a single point mutation in a structural protein of the skin, this scenario is a painful daily reality. An ongoing clinical research program in Colorado holds promise to provide relief to these patients, and highlights the intense activity currently ongoing in Colorado in the field of regenerative medicine.

Ganna (Anya) Bilousova, PhD, an assistant professor in the Department of Dermatology at the Charles C. Gates Center for Regenerative Medicine at the University of Colorado Anschutz Medical Campus, heads a research program that aims to harness the regenerative power of induced Pluripotent Stem (iPS) cells for renewal and replacement of aging tissues and organs. Together with Dennis Roop, PhD, a professor and director of the Gates Center, and with the assistance of colleague Igor Kogut, PhD, an instructor in the Department of Dermatology, Bilousova's laboratory is also developing applications to ameliorate the debilitating effects of inherited genetic diseases like epidermolysis bullosa.

IPS cells represent a relatively recent advance in the field of stem cell research. By artificially inducing expression of a handful of specific genes in adult somatic cells, these cells can be reprogrammed to revert to an immature, highly undifferentiated, genetically rejuvenated state with properties similar to those of embryonic stem cells (ESCs). Like ESCs, iPS cells can be propagated and expanded indefinitely in vitro and, in theory, be induced to differentiate into any cell type in the body with the goal of growing replacement tissues or organs to treat diseases or the degenerative effects of aging.

"We can take cells from a patient as old as 80 years of age, induce those cells to become iPS cells, and they seem to revert to a rejuvenated phenotype," Bilousova says. "In theory, we can potentially use these cells to grow entire, new, young organs, with improved functionality, that are specific to the patient the iPS cells came from."

Colorado State University Clinical Sciences professor David Frisbie with a horse at the Equine Orthopaedic Research Center.

AlloSource employee working in the tissue processing area.

It's not difficult to see the potential of iPS cells for use in regenerative medicine. Unlike ESCs, iPS cells are derived from adult somatic cells, and therefore do not involve the use of in vitro fertilized embryos or ESC lines—sources associated with significant societal, ethical, legal, and scientific hurdles. They are also specific to the patient being treated, presumably avoiding or reducing the possibility of immune rejection after transplantation.

Bilousova's research program is tackling some of the technical hurdles of translating the basic science into the clinic. Her team is interested in identifying and developing optimal tissue sources for somatic cells that will be induced to become iPS cells, and optimizing differentiation to produce the desired cell types. Perhaps most fundamentally, Bilousova, together with Korgut and Roop, is further improving so-called non-integrating approaches to reprogramming somatic cells using mRNA, in order to avoid the risks of introducing new, permanent genetic mutations that could, in themselves, cause disease.

"Our work is based on the fact that, fundamentally, proteins are the molecules that do the jobs within the cells, so that is what we need to express in order to reprogram the cells," Bilousova says.

The ultimate goal is to adapt iPS cells for therapeutic applications. Kogut's research program is working to identify strategies for manipulating iPS cell genomes to safely correct faulty genes in order to treat epidermolysis bullosa as proof of concept for other genetic disorders.

"We want to make genetic corrections," Kogut says, "but that has to be achieved at the genome level, without introducing any additional nucleotide changes, and without leaving behind any kind of selection marker."

Kogut is therefore working with two different cutting-edge genome editing techniques: TALEN (Transcription Activator-Like Effector Nucleases) and CRISPR/Cas9 (Clustered, Regularly Interspaced, Short Palindromic Repeats) to repair the disease-causing mutation, after which the cells will be differentiated and grown into a skin substitute. Ultimately, the goal is to develop these advances for commercialization, leveraging the considerable resources and expertise of the Gates Center.

> The partially demineralized allograft bone – the foundation for the AlloStem tissue [developed by AlloSource] – provides a natural scaffold for new bone formation. The naturally occurring growth factors present in allograft bone have been shown to promote new bone growth.

With more than 85 faculty members at the Anschutz Campus and from other institutions all over Colorado, state-of-the-art core facilities, and an advanced cGMP biomanufacturing facility, the Gates Center represents a central hub for translational research in the field of regenerative medicine in Colorado.

The center is not alone in its efforts to harness the powers of this field. Academic and commercial activities across the state indicate that stem cell research and regenerative medicine will be a major focus for bioscience in Colorado in the decades to come.

In Fort Collins, Colorado State University (CSU) recently received an anonymous $20 million donation to fulfill its $65 million fundraising goal to begin construction on the new CSU Institute for Biologic Translational Therapies.

The center was originally launched in late 2014, with a $42.5 million gift – the largest cash gift in CSU history – from Denver-area philanthropists John and Leslie Malone.

The Malones became interested in stem cell research and regenerative medicine through their experiences with the Gail Holmes Orthopaedic Research Center at CSU, which helped them treat lameness in one of their world-class dressage horses through arthroscopic surgery and the use of stem cell injections. After endowing a chair in Equine Sports Medicine at CSU, they decided to broaden their focus. They recognized that research and advances in veterinary regenerative therapies, including stem cells, gene therapies, replacement tissues and organs, and novel proteins, would have extraordinary value, not only for animals and their owners, but also for treating human disease, degenerative disorders and aging. The new institute will serve as a nucleus for that kind of translational approach, in which discoveries in basic and pre-clinical research can develop in parallel for both veterinary and clinical applications.

The commercial potential of veterinary stem cell research at the Holmes Orthopaedic Research Center has been demonstrated by the spin-out of a commercial entity, ART (Advanced Regenerative Therapies, Fort Collins). ART was co-founded in 2007, by Dr. David Frisbie, PhD, and Dr. John Kisiday, PhD, both of whom are associate professors at the center. The company's services focus on providing autologous bone marrow stem cell collection, expansion and banking to treat equine and canine musculoskeletal injuries and disorders.

Another Colorado company, Regenexx, located in Broomfield, Colo., also offers autologous bone marrow stem cell therapies, but its focus is on orthopedic procedures in humans. Its principal therapeutic offering in the US is the so-called "same-day" stem cell transplant, in which bone marrow is aspirated from a patient's hip bone, processed to rapidly isolate the mesenchymal stem cells, and reintroduced into the patient's knee, shoulder, back, and other joints to alleviate pain from tendon, ligament and bone injuries, arthritis and other degenerative conditions. Regenexx tracks all of its patients in a treatment registry in order to collect and analyze treatment outcomes and complications. According to the company, its proprietary isolation and injection protocols are associated with improved outcomes for patients compared to other commercially available stem cell transplant technologies.

Regenexx also conducts extensive research in stem cell therapies, including a procedure using cultured mesenchymal stem cells in place of freshly isolated ones. While that procedure is not currently available as an approved therapy in the US, Regenexx has conducted clinical studies for the procedure and continues to investigate its potential for further development.

"The healthy and expanding ecosystem of academic and commercial activity in this field suggests that our state will be well positioned to address this growth.

The non-profit organization AlloSource, located in Centennial, Colo., is another key player in regenerative medicine in Colorado. Founded in 1994, AlloSource develops, processes and distributes donated human tissue for use as allografts in medical procedures such as knee replacement, joint repair, bone grafting, spinal fusions and skin grafts for wound and burn care. Its tissue bank includes more than 200 kinds of bone, cartilage, skin, soft-tissue and custom-machined allografts for use in an array of life-saving and life-enhancing medical procedures.

Its most recent innovation, AlloStem, is partially demineralized allograft bone combined with adipose-derived mesenchymal stem cells. The partially demineralized allograft bone – the foundation for the AlloStem tissue – provides a natural scaffold for new bone formation. The naturally occurring growth factors present in allograft bone have been shown to promote new bone growth. The adult mesenchymal stem cells present in AlloStem naturally adhere to the bone substrate and may contribute to the formation of new bone.

With the overall aging of the population, both in Colorado and nationally, the demand for therapies for repair, restoration, rejuvenation and replacement of diseased or degenerating tissues and organs is growing. The healthy and expanding ecosystem of academic and commercial activity in this field suggests that our state will be well positioned to address this growth.