JUST WHAT IS
REGENERATIVE MEDICINE,
ANYWAY?

UNPACKING ONE OF THE HOTTEST PHRASES IN MEDICAL SCIENCE TO UNDERSTAND HOW RESEARCHERS AT SUNNYBROOK ARE ENGINEERING TISSUE AND DELIVERING THERAPY TO REPAIR AND RESTORE THE HUMAN BODY

BY JIM OLDFIELD

ILLUSTRATION: JIM FRAZIER

IN 2006, the National Institutes of Health (NIH), the largest funding body for medical science in the U.S., issued a state-of-the-field report on regenerative medicine. The report's cover boasted an image of the Greek deity Prometheus, chained to a rock and under siege by an eagle. According to myth, Zeus sent an eagle to eat Prometheus's liver as punishment, but Prometheus was able to regenerate the organ, and survived despite daily attacks by the eagle.

The NIH report claims that Prometheus is a fitting symbol for regenerative medicine, a field that, broadly defined, means restoring health by growing organs and engineering tissue. And, the field has seen spectacular success. In 2006, Dr. Anthony Atala of Wake Forest University announced that his team had implanted lab-grown bladders in seven patients, and that all seven had been doing well—some for up to six years.

But after 30-plus years of research, such breakthroughs are rare, and the field is more complex. Where scientists once thought the right type of cells, properly placed next to the right scaffold, would on their own become a tissue or organ, they now know that's often not enough. Replicating the intricate signals exchanged among cells and how those cells interact with their environment is increasingly important. For Dr. Graham Wright, whose team has spent years using imaging to visualize experimental regenerative techniques, one word comes to mind on hearing regenerative medicine: "caution."

"Regenerative medicine is one of those fields that can be oversold easily," says Wright, director of the Schulich heart research program at Sunnybrook Research Institute (SRI) and a professor at the University of Toronto. "It's enticing to say, 'I'm going to grow a heart and put it in.' But I think the field has recognized that this is a complex challenge, and that we have to understand the processes associated with tissue *response* and *repair* to advance this goal."

One approach, Wright says, is to focus on damage caused by disease, and then work to limit and repair it. Doing so, he says, first requires understanding how the body—especially the vascular and immune systems—interacts with tissues. One problem in this regard has been ensuring the survival of cells and tissue meant to repair damage once injected or implanted. A considerable challenge in small animals, the issue is even more complex in larger systems, like humans, where cells take longer to reach their destination and require significant vasculature (blood supply) and nutrients to function. "It's not simply a matter of growing and implanting tissue," says Wright. "We also have to think about the system from a molecular and cellular point of view, and work with the system to repair damage."

To that end, researchers at SRI are working on clinically relevant problems in tissue engineering and therapy delivery. They have made advances that could shape patient care within a few years for those with diabetes, degenerative disc disease, heart disease and cancer. At the same time, they are extending the foundational knowledge of regenerative medicine that may yet allow scientists to fulfil the field's Promethean promise.

Healing Wounds, Healing People

One advance that may prove a boon for diabetics is Vasculotide, a compound developed at SRI that can be applied as a cream to help wounds heal better and faster. Many people with diabetes have poor circulation, and this leaves them prone to injuries that don't heal. Up to 15% of these people will develop ghastly, painful sores, usually on the feet or lower legs. There are few effective treatments, and as many as one in five patients with these wounds will require limb amputation.

Vasculotide speeds up wound healing in diabetic mice by 30% to 40%. Co-invented by Dr. Dan Dumont, director of molecular and cellular biology at SRI and a scientist in SRI's Advanced Regenerative Tissue Engineering Centre (ARTEC), and his research associate Dr. Paul Van Slyke, the fully synthetic compound mimics the properties of the protein growth factor Angiopoietin (Ang) 1. This is desirable, explains Van Slyke, because Ang 1 is a "master regulator" of blood-vessel growth, development and stability—useful, therefore, in closing wounds. While Ang 1 is difficult to purify on a large scale, unstable and potentially unsafe, Vasculotide has none of these problems. Moreover, it can be made cheaply.

TOP TO BOTTOM:
DR. JUAN CARLOS ZÚÑIGA-PFLÜCKER; DR. GRAHAM WRIGHT;
DRS. CARI WHYNE AND ALBERT YEE

PHOTO OF DR. JUAN CARLOS ZÚÑIGA-PFLÜCKER: CURTIS LANTINGA

"REGENERATIVE MEDICINE is one of those fields that can be oversold easily," says Wright.

Critically, Vasculotide also improves the quality of the wound closure. In Dumont's lab, Van Slyke picks up an image series of purple, pink and white cross-sections showing three wounds—each treated differently—and their underlying tissue. The first two reveal tissue layers beneath typical diabetic wounds. "You can see [the tissue] is spongy, fatty and lacking support," says Van Slyke, pointing out several large white clumps just below the skin. The third image shows a wound treated with Vasculotide. Several layers of purple and pink fibres, or "granulation" tissue, are packed between a small layer of fat and the wound surface. "This is all connective tissue and blood vessels feeding the wound," says Van Slyke. "Its thickness would provide resistance to the wound reopening. And that's a major issue in diabetic wound healing—patients get up to walk around, and the wound just blows back open."

Vasculotide is so promising that the NIH selected the compound for its Type 1 Diabetes Preclinical Testing Program, a precursor to its "rapid access" program, which fast-tracks therapies from the bench to the clinic. The program will fund four San Diego-based trials of Vasculotide in increasingly large animals over the next year. Dumont, who also holds the Canada Research Chair in Angiogenic and Lymphangiogenic Signalling, and is a professor at U of T, has formed a spin-off company to commercialize his technology.

While Vasculotide has implications for diabetes, Dumont and Van Slyke have evidence, which they continue to collect, that it may be useful in other conditions where improving blood supply can improve treatment, including age-related macular degeneration (loss of vision), stroke and heart disease. It may also help solve the key question facing regenerative medicine researchers: how do you provide blood supply and nourishment for cells, tissue and organs grown in the lab?

A Matter of Environment

In 2002, Dr. Juan Carlos Zúñiga-Pflücker, a scientist at SRI, found a way to grow T cells from stem cells in a Petri dish. T cells are virus- and infection-fighting white blood cells, which many scientists believe will one day be able to be harnessed to restore

YEE, WHYNE AND WOODHOUSE are working to develop a treatment that would slow disc degeneration at a much earlier stage, before expensive, invasive and variably effective surgery is the only option.

immunity in patients with severe immune deficiencies. The breakthrough transformed the field of immunology by allowing immunologists to observe and manipulate T cell development in unprecedented detail.

Since then, Zúñiga-Pflücker has been searching for a means to maintain these lab-grown T cells over time. One of his ideas was to build an artificial thymus, the main organ in which T cells grow in the body. This led to talks with Dumont. "If we ever have this 3-D [thymic] structure to implant, it would have to be vascularized [provided with a blood supply]," says Zúñiga-Pflücker, who holds the Canada Research Chair in Developmental Immunology and is the director of ARTEC. "So what Dan's lab is doing will be applicable to a lot of regenerative medicine approaches where achieving vascularization is important for any tissue you're growing."

From those talks, the two developed a method, which they're refining, to improve the generation of the blood stem cells Zúñiga-Pflücker's lab uses to grow T cells.

At the same time, Zúñiga-Pflücker and his postdoctoral fellow Dr. Mahmood Mohtashami discovered a way to support T cells that doesn't require an entire organ-like structure. "It turns out we didn't need to be as sophisticated as we thought, so we scaled down the idea of a fully 3-D thymus to something simpler," says Zúñiga-Pflücker.

Their method combines a select mix of molecules in a 2-D environment, and increases T cell numbers with cells harvested from the same animal that could receive them as therapy, opening a door to more "personalized" T cell regeneration.

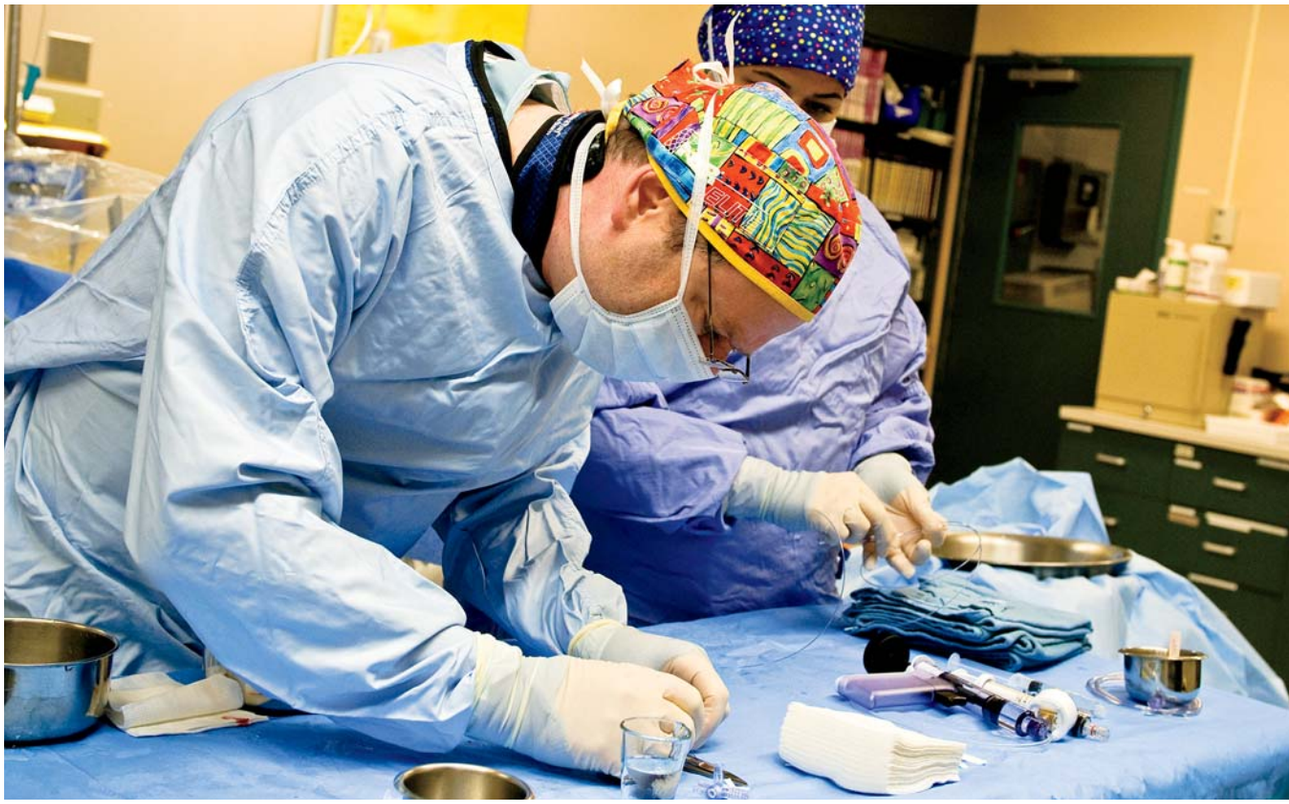
The trouble is, this approach produces only a small number of T cells. Hence, says Zúñiga-Pflücker, it's still possible that some type of 3-D structure that reproduces the thymic environment might work better. To that end, he continues to collaborate with Dumont and with Dr. Kimberly Woodhouse, associate director of ARTEC, who specializes in chemically engineered scaffolds that can support vasculature and 3-D tissue growth.

Woodhouse is dean of the faculty of applied science at Queen's University and a professor at U of T, who maintains an appointment as scientist at SRI. She works with ARTEC scientists Drs. Cari Whyne and Albert Yee on regenerative approaches to degenerative disc disease.

While some disc degeneration with aging is normal, as much as 85% of the population will have chronic (lasting three months or more) back pain. For those who require invasive treatment, options are limited. "Right now, a lot of surgical therapies are directed toward the end stages of the condition," says Yee, a researcher at SRI, who is also a surgeon at Sunnybrook Health Sciences Centre and an associate professor at U of T. "If you have a worn-out disc and you're symptomatic with a concordant constellation of symptoms, then we either fuse the disc or give an artificial disc replacement—but both have varied results."

Yee, Whyne and Woodhouse are working to develop a treatment that would slow disc degeneration at a much earlier stage, before expensive, invasive and variably effective surgery is the only option. They have had some success with a liquid hydrogel that solidifies once injected, providing a structural support that is flexible but can withstand repetitive spinal load-bearing. They will

DRS. DAN DUMONT AND JENNIFER ALAMI



DR. BRADLEY STRAUSS

publish those results this year, and are moving on to the next stage: how the implant interacts with the environment of the spine at the molecular and cellular level. "We want to reinstate the mechanical environment, but we may need to include additional cells or growth factors to help the cells get back on track, otherwise they'll just go off down the wrong path again," says Whyne, who is director of the Holland musculoskeletal research program at SRI and an associate professor at U of T.

Imaging for Cell-Based Therapies

A key aspect of translating this musculoskeletal and other regenerative medicine research to patients is imaging—particularly magnetic resonance (MR). Imaging enables disease diagnosis, but it is also increasingly essential to monitor the delivery of regenerative therapies and the body's response to those therapies. Wright is partnering with other scientists in SRI's Centre for Molecular and Cellular Response and Repair (CMCRR) and the Imaging Research Centre for Cardiac Intervention to develop novel imaging techniques for cell-based regenerative therapies.

One such therapy is for chronic total occlusions (CTOs), which are coronary or peripheral arterial blockages lasting more than six weeks. Peripheral CTOs can result in leg pain with walking and, in severe cases, amputation; coronary CTOs produce chest pain and lower life expectancy.

Pioneered by Dr. Bradley Strauss, a scientist in molecular and cellular biology at SRI, cardiologist at Sunnybrook and professor of medicine at U of T, the treatment uses an enzyme called collagenase to help restore blood flow in the blocked areas, or lesions. Strauss found that injecting collagenase softens the blockages enough to enable minimally invasive percutaneous (through the skin) intervention, where a surgeon draws a guide-wire over the blockage before doing angioplasty—a preferable alternative to bypass surgery and drugs, the current standards.

By tagging capsules and molecules with iron or gadolinium, each of which alters the MR signal to create a local positive contrast around the agent, Wright and Dr. Charles Cunningham, an imaging scientist at SRI and assistant professor at U of T, are working to monitor the delivery of Strauss's therapy and its effect on microvasculature. "Collagenase is an exciting new development, but one of the questions around it is how far you can get the collagenase into the lesion; it likely relies on a microvascular network to penetrate beyond the lesion's surface," says Wright.

They've validated the techniques in preclinical models, and expect it will provide the quantitative feedback to take the therapy, which Strauss is now testing in a clinical trial at Sunnybrook, to a new level of efficacy.

Wright's overriding goal is to improve the ability of researchers and clinicians to track, in a way that can be measured over time, the physiological changes with disease and repair. Traditionally, imaging has provided mostly anatomical information, but long-term studies of regenerative interventions require measurements of blood volume and flow, local oxygen consumption, inflammation, and their effects on tissue. These measurements will be essential in tracking disease evolution and patients' response to emerging treatments.

Wright looks forward to locating his lab alongside the labs of Dumont, Zúñiga-Pflücker, Strauss, Cunningham and about 100 other SRI staff in the CMCRR's new home, now being built on the seventh floor of the hospital's M wing. "We've got basic biology, imaging physics, molecular targeting and clinical expertise. Having that group together will be valuable in moving this whole area ahead."

As with Strauss's work, says Wright, success in regenerative medicine will be incremental. "But I think we'll see quicker translation to the clinic with this approach," he adds. "These will perhaps be smaller steps than some people originally pictured, but through those small steps we will get closer to the long-term goal of providing solutions for people."

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