BUSINESS NEWS

Cover Story

November 15, 2008

Limb Regeneration: Replacing What Was Lost

Researchers around the world attempt to awaken a process in humans that would allow them to regrow lost cells, digits and, eventually, limbs.

By Stephanie Z. Pavlou

If a salamander loses its arm, it immediately begins to grow a replacement. Over the course of a few weeks, muscle, bone and skin cells develop in place of the missing limb. The salamander regenerates a new arm just like the one that was lost.

Imagine the possibilities if this were applied to humans.

Researchers and clinicians around the world have pressed their imaginations to find clinical applications for this idea. *O&P Business News* spoke to several of the top minds in cell regeneration today to determine how far they have progressed.

Mimicking animals

The idea that humans might be able to replicate animals is not new, and biologists have spent years trying to apply that process to humans, both in tissues and entire limbs.

As an undergrad at Tulane University, Robert A. Warriner, III, MD, researched limb regeneration in mammals, specifically focusing on the North American opossum, which has embryonic hind limbs. Warriner and his classmates found that, the younger the tissue, the better the odds of regeneration capability.

Now chief medical officer for Diversified Clinical Services, an outsource wound management provider headquartered in Jacksonville, Fla., Warriner understands that humans still are quite far from the ability to regenerate the most basic cells without a great deal of scientific intervention.

“The unfortunate reality is that, although there is a great deal of research on tissue regeneration today … we are far away from generating a replacement extremity, though we know a great deal more than we knew back in the early 1970s,” he said.

This knowledge, however, did not lead to significant breakthroughs for many years. Until the concentration on regenerating cells met the field of tissue engineering, said David Baer, PhD, director of research at the U.S. Army Institute of Surgical Research at Fort Sam Houston in San Antonio. This field has grown quietly in the background, working with technology like extracellular matrices, biologically active proteins and stem cells to improve healing outcomes.

“These two worlds are starting to come together, as tissue engineering has produced single tissue constructs that can regenerate some individual tissues,” he said.

Now those involved in the field are looking to apply this idea in a more complex structure, such as a limb.

Regenerating cells

Stephen Badylak, DVM, PhD, MD, professor in the department of surgery and director of tissue engineering at the McGowan Institute for Regenerative Medicine at the University of Pittsburgh, sees regenerative medicine as encompassing both tissue engineering and limb and digit reconstruction.

First, the marriage of tissue engineering and regenerative medicine came about because of the inability to treat certain health conditions, like amputation and scarred heart tissue in heart attack patients, Badylak told *O&P Business News*. Researchers in the medical community began working on reversing these issues.

Several of the medical specialties designed their own products with this thought in mind. Organogenesis Inc. developed Apligraf, which was the first biologically engineered skin patch for wound therapy to receive FDA approval, in 1998 according to the company’s Web site. Advanced BioHealing Inc. developed Dermagraft, a skin substitute derived from human fibroblast, extracellular matrix and a bioabsorbable scaffold. DePuy Orthopaedics created a biological scaffolding material for the treatment of rotator cuff injuries. Over time, other manufacturers followed suit, and the field grew into what it is today.

Another matter to consider is humans’ innate ability to generate cells. In the womb, life begins with only a few cells; but within 9 months, a tiny person emerges, comprised of countless bone, skin and muscle cells from head to toe. Moreover, a fetus can regenerate complex tissues for several months in early gestation, with no trace of a wound or scar.

In addition, young children — with evidence of those as old as 2 years — can regenerate fingertips that do not include joints after amputation.

“That process is more robust the earlier it happens in childhood,” Baer said. “The failure rate starts to go up as the child ages. With adults, you typically do not see it.”

The key to this ability, he explained, is trapped within the human body. Unlocking this ability would open the door to scarless wound healing.

Stephen Badylak, DVM, PhD, MD

Image reprinted with permission of McGowan Institute of Regenerative Medicine.

Robert A. Warriner, III, MD

Image reprinted with the permission of Robert A. Warriner, Diversified Clinical Services.

William Zamboni, MD

Image reprinted with the permission of The University of Nevada School of Medicine.

Unlocking the process

“We do not know exactly why we can’t regenerate tissues, whether that is scarless wound healing or regenerating lost tissues. We do not know exactly what it is that [allows us to do this] early on in development or [why some] animals can do that through their whole lives,” Baer said. “Basic research needs to go on to figure that out.”

The eventual goal is to regrow complex structures like limbs, he said. For the time being, researchers have taken small steps in this direction.

Badylak provided one example: an Achilles tendon rupture. Options for the surgeon include pulling together tendon and muscle tissue, which can be difficult and often causes various gait issues, or harvesting donor tissue from another site on the patient’s body to fill in the gap, which has a limited success rate.

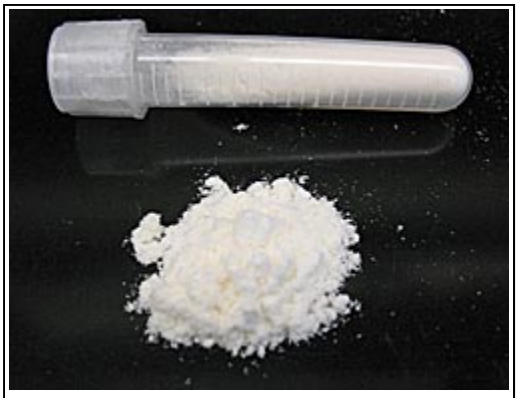
On the other hand, the surgeon could combine a scaffold material, like collagen, with stem cells taken from the patient’s own blood or bone marrow. This mixture then would be exposed to stress in a bioreactor — or inside the patient’s own body — to turn them into tendon tissue. Because this tissue was created from the patient’s own cells, the surgeon can use this material to repair the tendon and avoid any rejection issues that might arise.

Warriner agrees that this is where the field is headed.

“It is a great concept to think that at some point — by chemical stimulators or mediators, or by the addition of specific stem cell types — we will be able to create in man the spontaneous regeneration that happens in salamanders and newts all the time,” he said. “That capacity is certainly inherent in mammals. It is a question of unlocking the mechanism, and we are not sure how to do that yet.”

Reversing the trauma

Another population of patients who would benefit from this technology is the severely wounded. For many with traumatic injuries or burns, scarring tightens the skin and leaves the patient with a limited range of motion.



Extracellular matrix, shown in powder form, lays the foundation for the regrowth of tissue.

Image reprinted with permission of The University of Pittsburgh Medical Center.

Baer currently works to implement some of this technology to help wounded soldiers, specifically with extracellular matrix materials. Millions of extracellular matrices, taken from places like demineralized bone and commercially prepared skin, are implanted each year.

For Baer's soldiers who suffered severe burns, he simply wants to generate length in the residual fingers that would enable the patients to create a pinch motion.

"We are not trying to grow a whole new finger," he said. "If they can pinch enough to pick up a pen or a toothbrush, it increases their quality of life and their independence, as opposed to having hands with no fingers at all."

Soldiers' injuries stand apart from other traumatic cases because often they are from penetrating injuries. These follow a different set of rules for wound healing. Often these wounds need an extracellular matrix even to begin the healing process. These injuries seldom come without the chance of infection as well, so Baer and his team are exploring ways to ways to deliver antimicrobials and antibiotics to prevent infection and promote healing.

In addition to regrowing skin, the Army Institute of Surgical Research has been working on regenerating muscle by implanting extracellular matrix derived from intestinal submucosa in areas of the body where large amounts of muscle have been lost. Here, the clinical team hopes to improve function in those with significant functional deficits.

Later in the process, Baer wants to use the extracellular matrix material to replace ears and other appendages that can be lost when patients are badly burned.

Explaining the Terms

Extracellular matrix
The extracellular matrix is a framework within tissues in which all composite materials — such as cells and blood vessels — reside. Extracellular matrices hold the ability to recreate the skin's structure.

Growth factor
Growth factors are molecules that regulate and promote growth of cells and tissues. They also initiate cell repair.

Stem cell
Stem cells are precursor cells, which have the ability to both replicate other cells or become other types of cell.

Source: *Dorland's Illustrated Medical Dictionary*

Gauging success

Determining the success rate of such technology rests on several factors. First, for any of these solutions that require growing cells outside of the body in a bioreactor, the time necessary for the procedur, the regulatory requirements and the cost increase significantly. For example, a procedure using Carticel, biologically engineered cartilage made by Genzyme:

"In this approach, a surgeon harvests some of the patient's own cells from the knee and cleans up the defects. They send it off to the company. The company grows the cells for about 2 months in a bioreactor to make a new piece of cartilage, sends it back to the surgeon, a second surgical procedure is done and then you hope that the new cartilage takes," Badylak said. "The total cost to the patient is about $30,000-$35,000."

That is a great deal of money for a procedure whose success rate is questionable, he continued. For the medical community to consider this a success, is it enough that the Carticel stays in place, or should it also decrease arthritis in the knee for the foreseeable future?

"That is an application where I think almost everyone agrees that this rather sophisticated approach has a chance to be successful, but the jury is still out on the degree of success," Badylak said.

Reconstructing tissue

Tissue reconstruction and tissue regeneration are inextricably linked. Prior to being able to regenerate tissue from cells, science must be able to rebuild those tissues that are damaged or lost. Reconstruction also centers around limiting the impact of aging on tissue and cellular function, and rests heavily on the outcomes of stem cell and gene-based therapy, Warriner said.

Limb regeneration may be in the early stages, but successful limb reattachment surgeries take place each day in the United States.

Image reprinted with permission of Robert A. Warriner, Diversified Clinical Services.

Additionally, reconstruction likely will prove its value sooner than regeneration; in functional recovery of severely traumatized limbs, for example.

“Today we are somewhat limited by simply putting the anatomy back together as best we can,” he said. “Frequently those limbs are left with permanent nerve damage, so we do not restore full function even though we get a limb that looks normal anatomically.”

Successful limb reconstruction, however, already works with a combination of existing technologies: extracellular matrices, growth factors and stem cells.

At Diversified Clinical Services, Warriner and his team use these methods — along with hyperbaric oxygen therapy — to salvage tissue in patients with acute injury. Hyperbaric oxygen therapy involves placing patients in a hyperbaric chamber, where they breathe 100% oxygen. At the increased pressure, the oxygen floods the patients’ blood and tissues, especially those that have been damaged.

“If we can raise the oxygen level that can get to that tissue until the blood vessel can be repaired, it will keep [the patient] alive, and we can reduce the residual damage and defect that would happen in that limb,” he said.

Another interesting effect of exposing patients to high-pressure oxygen is the stimulation of tissue growth. Hyperbaric oxygen releases stem cells from bone marrow, and whisks them through the bloodstream toward the areas of tissue injury, Warriner explained. For this reason, providing patients with access to hyperbaric oxygen treatment plays a key role in their recovery.

These therapies also aid in limb reattachment procedures, an area of specialty for William Zamboni, MD, plastic surgeon, professor and chairman of the department of surgery at the University of Nevada School of Medicine. Zamboni, who started the Limb Reattachment Program in Las Vegas at University Medical Center in 1994, has focused his career on improving outcomes for limb reattachment after amputation.

“There used to be a 6-hour window, but with some of the current treatments, it is not unusual to have [a limb unattached] for 12 hours and still [see good outcomes], as long as you treat afterwards with hyperbaric oxygen,” he said.

Altering O&P

Zamboni also has used his combination of therapies to aid amputees. In one example, he had a patient whose arm was ripped off below the shoulder and at the mid forearm. He quickly reattached the patient's elbow, which gave him a functional limb with which to use a prosthesis.

Using hyperbaric oxygen therapy and surgical reconstruction, surgeons were able to salvage this 8-year-old girl's leg.

Image reprinted with permission of Robert A. Warriner, Diversified Clinical Services.

For limb reattachment to become a more widely used option, both health care professionals and patients must be made aware that digits, ears, noses and limbs all can be reattached today if they are salvaged, Warriner said.

Proper care for detached limbs include recovering, cleaning and protecting them, and keeping the limbs from becoming too wet or too dry. Also, avoid packing the detached limb in ice because that may damage the tissues.

"The surgeon has no opportunity to reattach the limb if he does not have the limb in hand," Warriner said. "Perhaps people don't realize how good plastic surgeons today are at reattaching limbs. Because the limb often does not [come in with] the patient, the patient has less of an opportunity for full recovery."

Baer believes that the future of O&P will deal more with the integration of prosthetic devices and interfaces.

"What is coming down the line is interfacing with the patients' residual nerves so that limbs can be powered and moved by patients using their nervous systems, as opposed to pulleys and wires or springs," he said.

By maintaining maximum length in the residual limbs and integrating prostheses with the patients, the prostheses can become extensions of the patients as opposed to objects they wear.

Taking the next steps

Research in limb reattachment, replacement and regeneration has the potential to alter the course of O&P forever. Despite the advances in prosthetic technology, prostheses still cannot replace patients' own tissue. What if the medical community was able to offer these other options to patients?

"Instead of just trying to regrow a limb or a digit in a bioreactor, we are actually going to try to go into the body and try to change the default mechanism by which a body responds to injury," Badylak said. "It is a much more aggressive, challenging type of approach, but … we have some initial findings that suggest that we can get part of the way there."

For Baer, nirvana would be a world where amputation was almost synonymous with blood donation: "It may take a little while, but you get back the tissue that you have lost or you get back your limb."

In the meantime, he wants to avoid preventable amputations, or those that take place later, when an injury becomes infected or nerve loss becomes too great. With help from engineered cells, nerves and blood vessels — and immediate access to hyperbaric oxygen treatment — injured limbs can maintain function, increased chance of survival and the hope of minimal scarring.

"Those are interim steps to saying that the only thing we want is to regrow a limb and we are going to wait 20 years before we have an impact," he said. "We are looking [to make a difference] in the nearer term than that."

For more information:

- www.diversifiedclinicalservices.com
- www.medicine.nevada.edu
- www.mirm.pitt.edu
- www.usaisr.amedd.army.mil

Stephanie Z. Pavlou is a staff writer for *O&P Business News*.

http://www.oandpbiznews.com/200811b/cover_story.asp