Chemistry & Biology *13*, 233–234, March 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.chembiol.2006.03.003

Innovations

On the Trail of Prometheus

The Greek gods punished Prometheus for giving their fire to humans. Each day, an eagle would tear out his liver; every night it would grow back again. Although bodily regeneration has existed in myth for millennia, only certain tissues will, in fact, regenerate. Over the last two decades, many companies have tried to transform regenerative ability into reality.

The first wave of tissue-engineering companies, founded in the 1990's, were impaled on an ugly reality: making a complex functional organ was not possible with the scientific knowledge at hand. "The two big ones that went down, Advanced Tissue Sciences and Organogenesis, really gave the field a wakeup call," says Dr. Stephen Badylak, director of the McGowan Institute for Regenerative Medicine at the University of Pittsburgh. The first companies did not have products that could pass the FDA or show significant advantages over existing technology and practices for their cost.

Between 2000 and 2002, the overall activity in the field dropped 90%, according to a survey conducted by Dr. Michael Lysaght, director of the Brown University Center for Biomedical Engineering. Yet over the last few years, tissue engineering, now dubbed "regenerative medicine," has begun cropping up again at investor conferences. "Almost every university has research in this area," says Dr. Anthony Atala, director of the Institute of Regenerative Medicine at Wake Forest University and founder of Tengion (http://www.tengion.com/). In its new incarnation, tissue engineering covers all aspects of the repair or replacement of tissues and organs by incorporating the use of cells, genes, or other biological building blocks along with bioengineered materials and technologies. Atala notes that advances in stem cell research have brought new vigor to the field.

Small companies are sprouting up again, and giants, like Smith & Nephew, Johnson & Johnson, Medtronic, and Genyzme, have a variety of tissue-engineering programs. Organogenesis was revived and is now churning out artificial skin. But for most companies, commercialization beyond the research stage still lags. Few products are making it to market, and they tend to be relatively simple components: tissues, such as artificial skin, or matrices for seeding human cells, such as decellularized bone and cartilage. One promising approach is to make hybrid devices, like those made by Rhode Island-based RenaMed Biologics, Inc. (http://www.nephrostherapeutics.com/). The company's product being developed in collaboration with Genzyme is an external device meant to supplement dialysis for people with acute renal failure. Patients' blood is filtered through a hollow fiber cartridge containing renal epithelial cells, which perform certain metabolic functions in lieu of a natural kidney.

"Tissue engineering covers all aspects of the repair or replacement of tissues and organs by incorporating the use of cells, genes, or other biological building blocks along with bioengineered materials and technologies."

Blood Vessels on a Shoestring

Cytograft Tissue Engineering, founded in 2000 (http://www.cytograft.com/) in Novato, California, is constructing autologous blood vessels for peripheral and coronary bypass surgery. Last year at the American Heart Association conference, Cytograft presented the first human use of a small diameter engineered blood vessel. "The real home run in vascular surgery is high-pressure arterial bypass grafts for coronary, for lower limb bypass, or for AV [arteriovenous—directly connecting artery to vein] shunts," says Todd McAllister, company cofounder.

So far, the company has inserted their engineered blood vessels in an AV shunt application in two patients with end stage renal disease. The company is now enrolling patients in clinical trials at three different centers worldwide and anticipates submitting an Investigational New Drug application in June.

Cytograft constructs its veins by growing sheets of cells and rolling them over a temporary support mandrel. Once the roll has fused into a uniform tissue, the tube is lined with a second cell type to prevent blood from clotting. Both cell types are harvested from each patient. "We can achieve requisite mechanical strength without the inclusion of synthetic materials," McAllister says. Building a personalized blood vessel currently takes six months, but McAllister expects to gain efficiencies through process optimization. The company is now working to produce an off-the-shelf product with a tube built from a universal donor and then lined with autologous endothelial cells. The ten-person company has raised $3.5 million from NIH and $4.5 million in private equity.

The Skin Game

The granddaddy of tissue engineering is Integra (http://www.integrals.com/), a pliable artificial scaffold developed thirty years ago in the laboratory of Ioannis Yannas, professor of polymer science and engineering at MIT, and Dr. John F. Burke, then director of the Burn Center at Massachusetts General Hospital and Shriners Burn Institute. Integra enables badly burned patients to regenerate the dermis layer of skin without scarring. The dermis does not regrow spontaneously in adults. The process takes 18 days and requires two operations. Once Integra

is applied and the dermis grows, it must then be covered with an epidermal graft. In the fresh wound, the scaffold disrupts the scarring process by providing binding sites that are specific for scar-producing cells. As the new tissue forms, the scaffold degrades. Integra was approved by the FDA in 1996.

"We can synthesize organs in adults," says Yannas. "They are not perfect, but they are clinically useful." In addition to adult skin (albeit without hair or sweat glands), Yannas' lab has grown peripheral nerves to certain distances and conjunctiva, the organ underneath the eyelid that induces tearing. "We do not believe that the best way to reach the clinic is to try to synthesize an organ in vitro," Yannas says. "In my opinion, there is no limit to how you can grow organs in adults using scaffolds with very high biological activity. You do not have to add stem cells. You do not need to add wound growth factors. That's because the wound already has all these things in it. What the wound doesn't have is an active scaffold because it goes about destroying its own matrix in order to form a scar."

Other autologous skin grafts include Epicel, developed by Drs. Howard Green of Harvard and Eugene Bell of MIT and licensed by Genzyme Tissue Repair (http://www.genzyme.com/), Organogenesis' (http://www.organogenesis.com/) graft Apligraf, which has living cells in it and was approved by the FDA for venous ulcers, and Ortec International's (http://www.ortecinternational.com/) OrCel, a collagen-based skin dressing designed on similar principles as Integra.

Grafting in the Burn Ward

Dr. David Barillo (COL USAISR-Ft. Sam Houston) is an Army surgeon who treats soldiers as well as civilian burn victims at the Army Burn Center in San Antonio, Texas.

"We get a lot of business from Iraq," Barillo says. Because of improved medical care and logistics, the Army can save more soldiers, but the ones they save are more severely injured. "We try to get as much of the burn off as quickly as possible," Barillo says. The faster the burn is excised and covered, the better. But patients with large burns over 50% or 60% of their bodies may not have skin to spare for grafts; thus, Barillo and his fellow surgeons use temporary dressings, "a lot of Integra," as well as cadaver skin. Barillo emphasized the need for off-the-shelf skin that can be applied when needed, especially critical for patients who have large burns on 80% to 90% of their bodies. Saving their lives uses up all their available skin; there is none to spare for reconstruction.

According to Barillo, the more difficult patients are the ones who suffer from deep injuries. The doctors can only replace skin, not muscle. "There is a tremendous interest: can we regenerate muscle, can we regenerate functional tissue, can we regenerate nerves to hook everything up?" Barillo notes that the center is planning some collaborative research with the Wake Forest Institute of Regenerative Medicine on tissue engineering for reconstructive surgery.

Making It to the Transplant

Organ transplantation techniques have improved immensely over the past fifty years, to the extent that the number of available organs doesn't meet demand. The United Network for Organ Sharing website lists over 91,000 people waiting for various organs. Even when a transplant is successful, patients depend on immunosuppressant drugs that have numerous side effects that impact quality of life.

Growing organs or tissues from a patient's own cells could potentially solve problems both of availability and compatibility. But creating a fully functional organ proves to be an extremely difficult technical challenge. Growing an organ in vivo holds the promise of harnessing the body's healing capacity. Many researchers working in the field are taking this path by seeding matrixes with a patient's own cells and implanting them, hoping the body can provide better growing conditions than can be generated in a lab.

"Everything South of the Kidneys"

In his 16 years working as a urologic surgeon, Dr. Anthony Atala has built up a number of patents regarding the urogenital system. At the Wake Forest Institute of Regenerative Medicine, which he has directed since 2003, Atala and other researchers grow pancreas tissue, blood vessels, livers, and heart tissue.

At Children's Hospital in Boston, Atala and colleagues developed autologous organs such as bladders, kidneys, and penis tissue. In 1999, Atala's team published results on growing functional bladders in animals. The proof of concept was followed by a limited human trial. Atala's bladder patents from his work at Children's changed hands a few times and in 2003, were licensed by Tengion. The 45-person company received approximately $39 million in venture capital funding as well as financial inducements from the Winston-Salem city council and business community to locate there. Tengion recently signed a collaborative "multimillion dollar and multiyear" deal with Wake Forest University, funding research there in exchange for the right to commercialize the results.

A bladder is a reservoir made of smooth muscle and connected to the brain and spinal cord by nerves that sense when it is full. "It is actually quite a complex organ," Atala says. Currently, if a bladder replacement is needed, the solution is substituting a piece of bowel tissue. Because bladder and bowel tissue serve different metabolic purposes, the procedure results in complications.

Tengion's "neobladder" is a three-dimensional bioresorbable scaffold layered with epithelial and smooth muscle cells biopsied from the patient. In 6–8 weeks, the neobladder grows enough to be implanted, after which the body takes over the regenerative process and fills in the appropriate tissue layers and cell types. Wrapping the organ in the omentum, a sheath-like blood-vessel-rich tissue in the abdomen, encourages vascularization. Whether the appropriate neural connections can be established is unclear, but the initial patient population targeted has a dysfunctional nervous system.

Tengion anticipates its first human trial of a bioengineered bladder to take place at the end of 2006. "We have had patients on wait lists for years," says Atala. "If we can guarantee them an organ in six months, it is better than no organ at all."

Wendy Wolfson (wendywolfson@nasw.org) is a science and technology writer based in Oakland, CA.