

# Background

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## **Tissue Engineering in the Pittsburgh Region**

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Broadly defined, tissue engineering is the development and manipulation of laboratory-grown molecules, cells, tissues or organs to replace or support the function of defective or injured body parts.

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Although cells have been cultured or grown outside the body for many years, the possibility of growing complex, three-dimensional tissues – literally replicating the design and function of human tissue – is a recent development. The intricacies of this process require input from many types of scientists, including the problem-solving expertise of engineers, hence the name tissue engineering.

Tissue engineering crosses numerous medical and technical specialties. Cell biologists, molecular biologists, biomaterial engineers, computer-assisted designers, microscopic imaging specialists, robotics engineers and developers of equipment, such as bioreactors, where tissues are grown and nurtured, all are involved in the process of tissue engineering.

Tissue engineers in the United States and abroad have set out to grow virtually every type of human tissue – liver, bone, muscle, cartilage, blood vessels, heart muscles, nerves, pancreatic islets and more. Commercially produced skin is already available for use in treating patients with diabetic ulcers and burns.

Many current medical therapies may be improved upon by tissue engineering with significant financial savings. In standard organ transplantation, for example, a mismatch of tissue types necessitates lifelong immunosuppression, with its attendant problems of graft rejection, drug therapy costs and the potential for the development of certain types of cancer.

Furthermore, there is always the potential for rejection of the tissue, but as the field of tissue engineering progresses, it inevitably will provide many improvements because the costs of tissue harvest and postoperative patient costs will be reduced significantly.

By actually designing replacements to mimic the native tissue being reconstructed, the adequacy of tissue function will be optimized, leading to improved patient care at less expense.

As a world leader in organ transplantation, it is little wonder that Pittsburgh became a world-class center of excellence in tissue engineering.

### **McGowan Institute for Regenerative Medicine**

To realize the vast potential of tissue engineering and other techniques aimed at repairing damaged or diseased tissues and organs, the University of Pittsburgh School of Medicine and the University of Pittsburgh Medical Center (UPMC) Health System established the McGowan Institute for Regenerative Medicine (MIRM). As an entity, the MIRM serves as a single base of operations for the university's leading scientists and clinical faculty working to develop tissue engineering, cellular therapies, biosurgery and artificial and biohybrid organ devices. Established in 1992, MIRM has grown to 228 faculty and more than 800 students and staff, and it has invested in excess of \$70 million a year in research and clinical delivery.

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The mission of the institute is to devise innovative clinical protocols, as well as to pursue rapid commercial transfer of its technologies related to regenerative medicine.

The faculty and programs of the McGowan Center for Artificial Organ Development have been incorporated into the MIRM, and other university faculty has joined its forces as well. These include researchers working in tissue engineering, adult-derived stem cell research, wound healing and biomaterials research, among other branches.

In 2006, two McGowan Institute researchers were named to the *Scientific American 50*, a tribute from the magazine that recognizes research, business and policy leaders who have played a critical role in driving key science and technology trends over the last year. William Wagner and Michael Sacks were recognized for their development of novel biodegradable scaffolds, matrices that resemble the scale and mechanical behavior of the native extracellular matrix.

The MIRM is considered to be the most ambitious tissue engineering program in the nation, coupling biology, engineering, organ transplantation and biomedical research in all facets of its work. One of the attractive features of the MIRM is that it enables cutting-edge basic and clinical research to be performed across disciplines, allowing organ and tissue engineering and cellular and regenerative therapies to be developed and swiftly evaluated in the clinical setting.

A number of projects are underway at the MIRM, including:

- promising research on the possibility of developing a vaccine for the treatment of cervical cancer
- continued development of a modular extracorporeal liver support concept which integrates dialysis and detoxification into hybrid liver devices that brings portable life-saving technology to the aid of liver patients
- an axial-flow left-ventricular assist system for patients with end-stage heart disease
- a respiratory assist device for patients with acute lung failure
- a biohybrid artificial lung intended for long-term use
- a novel blood additive that improves blood flow to oxygen-deprived tissues
- a bioengineered blood vessel and a myocardial patch of muscle cells intended to repair heart tissue damaged by heart attack
- the development of an extracellular matrix, a medical product derived from pig bladders that has been demonstrated to regenerate tissue, including fingers, limbs, skin and heart muscle

The new McGowan Institute for Regenerative Medicine and the former McGowan Center for Artificial Organ Development are named after the late William G. McGowan, who as chief executive officer at MCI Communications, underwent a successful heart transplant at the University of Pittsburgh Medical Center in 1987.

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In 2004, the McGowan Institute opened a new, state-of-the-art histology laboratory in its Bridgeside Point facility. The lab development was funded by a grant from the Health Resources and Services Administration, and it provides the ability to examine the morphology of tissues as a result of tissue engineering or regenerative medicine interventions. Viewed as an essential step in bringing potential new therapies to patients, the lab offers standard histology services including frozen and fixed tissue preparation, and immunohistochemistry. Bridgeside Point is the hub for the Institute's tissue engineering research.

In 2003, the McGowan Institute announced a new Pre-Doctoral Tissue Engineering Program and worked on tissue-engineered solutions for heart disease.

### **Artificial Heart**

Practically overnight, the availability of heart transplant surgery gave many patients new hope for survival, but in addition to being a gravely serious surgery with no guarantees of success, there were few donor hearts available to help the thousands of people who enter the transplant waiting list each year. Heart failure accounts for more than 250,000 deaths in the United States every year, and at any time, approximately 3,500 patients are on the national heart transplant waiting list; many die waiting for a donor organ.

Researchers at UPMC, one of the partners in the MIRM, have given hope to these patients by developing artificial pumping devices that give the failing heart a bit of help. Such heart assist devices have proven their efficacy, keeping many people alive long enough to receive a donor heart. Even better, as each new generation of devices has been developed, they have become smaller and easier to manage, affording patients a far more normal lifestyle.

A funny thing happened on the way to transplant surgery; some of these patients bucked what once was conventional wisdom. Their heart tissues, relieved of the full burden of pumping the body's blood by the assist device, began to heal.

HeartMate II, a new heart assist device controlled by an electronic system, has given patients unparalleled improvement in independence and quality of life. HeartMate's researchers at UPMC comprise one of only four centers in the United States studying this ventricular heart assist device (or VAD, so called because it helps the heart's main chambers pump blood) in patients who are candidates for heart transplantation.

HeartMate II is a miniature rotary heart pump intended for patients with end-stage heart failure. A key feature of the VAD design is a sophisticated control system developed by researchers at MIRM. It senses when to increase or decrease the rate of blood flow based on the patient's level of activity, allowing someone to climb stairs, for instance. Accommodating varying degrees of physical activity was not possible with other experimental devices that require manual adjustments in flow.

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The controller was the brainchild of James Antaki, a member of the McGowan Institute who is a faculty member at both Carnegie Mellon University and the University of Pittsburgh. Weighing 12 ounces and approximately 1.5 inches in diameter and 2.5 inches long, the HeartMate II is about the size of a D-cell battery. That's significantly smaller than current VADs, which weigh closer to 3 pounds. Because of its size and quiet, simple-to-use design, the new device could be used for smaller patients.

In addition to work on the HeartMate II as a bridge to transplant, McGowan Institute researchers also have demonstrated that in certain instances the VAD can be used temporarily to allow for recovery of native heart function. Using new diagnostic tests and a VAD weaning trial, the UPMC investigators have shown how some patients with heart failure can be successfully weaned. Clinicians and scientists are working to identify people who can be removed from the transplant list. In an age where donor organs are limited, weaning a patient from the transplant list actually saves two lives – that of the person weaned off the VAD system and that of the person obtaining the organ that would have gone to the recovered individual.

One recent study found that seven percent of patients implanted with heart assist devices were weaned successfully from them without the need for a heart transplant. The VAD support appears to allow long-term restoration of cardiac function. These results are prompting physicians across the nation to appreciate that even patients who are gravely ill can experience dramatic recovery through ventricular support. The VAD allows the heart to relax and shrink down, while doctors restore circulation and try to repair the damage that heart failure has done to the other organs.

### **National Tissue Engineering Center**

In 2002, the McGowan Institute established the National Tissue Engineering Center (NTEC) in Pittsburgh to serve the Department of Defense as a single base of operations for leading civilian and military scientists and clinicians working to advance the science of tissue engineering, cellular therapies, biosurgery and artificial and biohybrid organ devices. The goal of the NTEC is to translate these new technologies to clinical practice, save lives and reduce soldier downtime. In order to meet this goal, its work centers on the following general areas:

- designing surgical adhesives, sealants and hemostatics that will revolutionize wound closure
- understanding the molecular determinants of scarless wound healing
- developing bone regenerative therapies for craniofacial wounds
- verifying that a bioresorbable scaffold bulk-seeded with stem cells and cultured in-vitro will yield
- a vascular graft suitable for implantation.
- evaluating the use of amnion-derived pluripotential cells for early, rapid wound closure of partial-thickness and full-thickness burns

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It is clear that optimal progress in developing new regenerative medicine methods only can be realized by multi-disciplinary teams that range across a wide spectrum of scientists, including but not limited to engineers, clinicians, cell biologists, materials engineers, specialists and practicing clinicians. The National Tissue Engineering Center brings this wide expertise together, tapping the southwestern Pennsylvania resources of Carnegie Mellon University's Bone Tissue Engineering Center, Duquesne University, MIRM and UPMC.

The pioneering research of Dr. Stephen Badylak led to the discovery of a bioengineered tissue scaffold that promotes wound healing. The bioengineered material is playing a crucial role in treating conditions ranging from incontinence to burns. His discovery has evolved into a significant advance in tissue engineering, laying the groundwork for a host of new medical treatments.

The material, which is derived from the small intestines of pigs, is increasingly used by surgeons to restore damaged tissues and support the body's own healing process. Physicians rely on the material, called small intestinal submucosa (SIS), for everything from reconstructing ligaments to treating incontinence. Today, SIS is most commonly used to help the body close hard-to-heal wounds such as second-degree burns, chronic pressure ulcers, diabetic skin ulcers and deep skin lacerations.

The SIS tissue, for example, which relies on an extracellular matrix, can be configured into sheets, gels, powders and multilaminar forms for orthopedic use and hernia repair. In its early stages, scientists engineered SIS primarily from a mechanical perspective. Researchers were looking for a material shaped like a tube, the size of blood vessels, and strong enough to be sutured, while also sustaining the contraction and expansion of a pulsating artery. Scientists have since realized that engineering SIS from a biochemical standpoint is paramount. For successful healing to occur, the graft tissue must foster a molecular environment that can speed up the body's own healing process.

Similarly, Promethean LifeSciences has developed another bioengineered tissue, GammaGraft, which is allograft skin from cadavers that has been processed and treated to reduce the risk of bacterial and viral infection. The structural integrity of the skin is preserved so that after application GammaGraft mimics the patient's own skin to protect a wound from the outside environment while facilitating healing

Originally Promethean thought that burn doctors would be primary users of the product, but because GammaGraft can be stored at room temperature for up to two years, it is used by a wide range of other physicians who have never had access to allograft skin because of costly procurement and storage conditions. It is packaged and stored in a pouch, and because it is about the size of a large bandage, GammaGraft is ready for application when the pouch is torn open.

Pennsylvania State University owns the company's intellectual property, for which Promethean has an exclusive license. The company, which is also working to commercialize other intellectual

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property, moved from Hershey to Pittsburgh in 1997 when plastic surgeon and company founder Dr. Ernest Manders joined UPMC. Demand for GammaGraft has grown by 100-fold since it went on the market in 1998, and most recently it has been widely used in Iraq and Afghanistan, as well as for victims of hurricane Katrina and the Pacific tsunami. It is also stocked by the State Department in American embassies.

### **University of Pittsburgh Department of Bioengineering**

The University of Pittsburgh's bioengineering department has an active, interdisciplinary graduate program in conjunction with faculty from the School of Medicine, the School of Health and Rehabilitation Sciences and the clinical staffs at the UPMC hospitals.

This program is directed toward engineering and life science education and research, with particular emphasis on the Ph.D. Its scope is broadly defined to incorporate the application of engineering principles, methods and technology in two broad areas:

- scientific inquiries into fundamental biological phenomena
- development of instrumentation, arterials, devices and systems relative to application in the biological sciences and medicine.

Active, externally funded areas of research include, but are not limited to:

- computer processing of biologically derived signals
- development of prostheses, artificial organs and implantable sensors
- development of medically related instrumentation
- mathematical modeling of physiological systems
- tissue engineering
- biomaterials
- orthopedic biomechanics and sports medicine
- vascular mechanics
- other related fields.

Thus, the bioengineering faculty is applying various forms of engineering principles, mathematics computation, technology and methodology to a broad variety of medical and life sciences problems.

### **Bone Tissue Engineering Center at Carnegie Mellon University**

The need for bone substitutes is particularly important. They often are required to help repair or replace damaged or diseased tissues in cases that include congenital and degenerative diseases, cancer and cosmetic surgery.

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There are approximately 500,000 surgical procedures performed every year in the U.S. that require bone substitutes. Currently available bone substitutes, including autografts, allografts and synthetic materials, are the most implanted materials second only to transfused blood products.

However, these substitutes are far from ideal and have many associated problems. Autografting is expensive and can have significant donor site morbidity, and synthetic materials wear and do not behave like true bone. The goal of the Bone Tissue Engineering Center is to provide an alternative solution by creating large-scale, tissue-engineered bone.

A major technology for creating tissue-engineered bone is an advanced computer-aided-design/computer-aided-manufacturing (CAD/CAM) bioreactor system capable of growing large-scale, customized bone substitutes. A CAD model of the desired bone substitute first would be derived from CAT scans or MRI data of the patient. The synthetic bone then would be fabricated outside the body in an advanced CAM bioreactor by depositing layers of biodegradable scaffolding material, while simultaneously embedding donor cells and growth factors within the layers.

Synthetic vasculature also would be embedded within the scaffold as it is being built up, until the new bone was mature enough to be removed from the bioreactor and implanted into the patient. Such a system would also have applicability to other tissues and whole organs.

Current research involves not only laying the foundation for several of the components required for realizing such an advanced system, but also gaining knowledge and developing components that will have clinical relevance in the nearer term. Projects include scaffold materials, solid free-form fabrication scaffolds, synthetic vessels and growth factors.

The Bone Tissue Engineering Center brings clinicians, scientists and engineers together from Carnegie Mellon's School of Engineering, Mellon College of Sciences and Robotics Institute, UPMC, the University of Pittsburgh, Children's Hospital of Pittsburgh and Duquesne University.

### **Carnegie Mellon University Develops Novel Ink-Jet Printer**

Researchers at CMU's Robotics Institute have created and used an innovative ink-jet system to print "bio-ink" patterns that direct muscle-derived stem cells from adult mice to differentiate into both muscle and bone cells. The technology could revolutionize the design of replacement body tissues and one day benefit millions of people whose tissues are damaged from a variety of conditions, including fatal genetic diseases like Duchenne Muscular Dystrophy (DMD), wear and tear associated with aging joints, accidental trauma and joint deterioration due to autoimmune disorders.

Researchers previously have been limited to directing stem cells to differentiate toward multiple lineages in separate culture vessels, but this is not how the body works; it is one vessel in which multiple tissues are patterned and formed.

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The ink-jet printing technology allows precise engineering of multiple unique microenvironments by patterning bio-inks that could promote differentiation towards multiple lineages simultaneously. Controlling what types of cells differentiate from stem cells and gaining spatial control of stem cell differentiation are important capabilities, if researchers are to engineer replacement tissues that might be used to treat disease, trauma or genetic abnormalities.

The custom-built ink-jet printer can deposit and immobilize growth factors in virtually any design, pattern or concentration, laying down patterns on native extracellular matrix-coated slides, such as fibrin. These slides then are placed in culture dishes and topped with muscle-derived stem cells (MDSCs). Based on pattern, dose or factor printed by the ink-jet, the MDSCs can be directed to differentiate down various cell-fate differentiation pathways (e.g. bone- or muscle-like).

The long-term promise of this new technology could be the tailoring of tissue-engineered regenerative therapies. In preparation for preclinical studies, the Pittsburgh researchers are combining the versatile ink-jet system with advanced real-time live cell image analysis developed at the Robotics Institute and Molecular Biosensor and Imaging Center to further understand how stem cells differentiate into bone, muscle and other cell types.

### **The Pittsburgh Tissue Engineering Initiative**

The mission of the Pittsburgh Tissue Engineering Initiative (PTEI) is to facilitate the recognition of Pittsburgh as an international center of excellence in tissue engineering research and education. The PTEI also fosters economic development by promoting the growth of a regional biotechnology industry rooted in tissue engineering.

The PTEI accomplishes its goals through:

- funding of research with clear potential commercial impact
- playing a leadership role in defining community strategies for regional development
- sponsorship of educational programs for a spectrum of students and professionals
- facilitating access to innovative technology transfer systems
- promoting collaboration among Pittsburgh's leading health and academic centers and within the larger field of tissue engineering
- global dissemination of tissue engineering-related information

To date, through partnerships with the NIH, the U.S. Departments of Defense, Education and Energy, the PTEI has awarded more than \$13 million to teams of researchers that it has formed from both academia and industry to address complex problems in regenerative medicine.

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In 2002, the PTEI debuted a new tissue engineering educational program and planetarium show entitled “Tissue Engineering for Life.” The unique educational program focused on tissue engineering and its applications. Curriculum materials for use in classrooms, a K-12 outreach program and Web-based tools were features of the program.

“Tissue Engineering for Life” was funded by a \$1.62 million Science Education Partnership Award from the National Institutes of Health – the largest award ever given at the time for information science education. Regional partners involved with the project included:

- Carnegie Mellon University’s Center for Light Microscope Imaging and Biotechnology
- Carnegie Mellon’s STUDIO for Creative Inquiry
- Carnegie Science Center
- Catalyst Connection
- Pittsburgh Supercomputing Center
- University of Pittsburgh’s Bioengineering Department
- UPMC Health System

In January 2003, Catalyst Connection, with assistance from the PTEI, obtained a \$300,000 award for the development of a life-science curriculum. The curriculum uses the biotechnology program at the Community College of Allegheny County; the undergraduate research programs and biology programming at Duquesne University; the Technology Studies magnet school in the Pittsburgh School District and the professional development training for middle and high school teachers provided through the PTEI.

During this time frame, the Pennsylvania Department of Community and Economic Development awarded Catalyst Connection an expansion grant of \$100,000 to sponsor a one-week tissue engineering summer camp for middle school students, along with the launch of the life science curriculum at two additional locations. Managed by the PTEI, the grant also guaranteed the continuation of a student internship program, which since 1997 has had more than 268 undergraduate participants.

Strategic industry partnerships fostered by PTEI aim to position and showcase Pittsburgh as a leader in the fields of regenerative medicine and tissue engineering. Partnerships include affiliation with The Society for Biomaterials, the Wound Healing Society, the Society for Regenerative Medicine, the Tissue Engineering Society International and the Tissue Engineering Research Centers in Japan and North America. Early last year PTEI joined a new collaboration between regenerative medicine experts and the military that has launched several clinical trials for treatment of combat wounds.

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The Soldier Treatment and Regenerative Consortium will focus on research that could lead to novel treatments for burns and wounds and perhaps even regenerate digits and limbs. In addition to PTEI, the McGowan Institute for Regenerative Medicine, the U.S. Army Institute of Surgical Research at Fort Sam Houston, Texas, Walter Reed Army Medical Center and other organizations are part of the consortium.

The consortium's studies, which will be conducted at Fort Sam Houston, including attempts to grow fingers and toes for soldiers who have lost digits, closing wounds by reconstituting skin and muscle, and using tissue engineering to regenerate tissue after massive tissue loss. Researchers also will test a powder form of a material that induces regenerative capacity and might prompt tissue, such as an amputated fingertip, to regrow.

Pittsburgh also has been home to the Engineering Tissue Growth International Conference & Exposition (ETG), the world's largest gathering of tissue engineering thought leaders, which has attracted scientists from academia and industry, as well as representatives of government, business and economic development organizations.

The PTEI organizes the annual conferences, which combine a comprehensive scientific program with an exhibition of products, services and technologies. The objective of the conference is to stimulate the exchange of information and ideas, foster collaboration for the advancement of the field and accelerate the pace of scientific discovery so that tissue-engineered products can be more quickly developed to help patients worldwide.

The ETG historically has attracted more than 800 thought leaders from North America, South America, Europe, Asia and the Pacific Rim. Attendees chose from more than 70 scientific sessions in seven focused tracks, and they attended keynote presentations by some of the world's most preeminent scientists, in addition to several innovative panel presentations and roundtable discussions. The conference has since been renamed Termis North America.

In 2005, the PTEI sponsored the Regenerate International Conference and Exposition, an international gathering, organized by PTEI and the Wake Forest University Institute for Regenerative Medicine, which explored scientific and topical issues in the fields of tissue engineering and regenerative medicine. The conference combined a premier scientific program with an industry-focused forum and trade show, bringing together basic life science researchers, leaders, students and entrepreneurs, along with representatives of government funding agencies.

The objectives of the conference are to advance the science of tissue engineering and regenerative medicine and foster interactions that may result in new technologies coming to fruition and available to patients more rapidly.

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## **The Industry Cell**

Approximately 380 life sciences firms call southwestern Pennsylvania home, including. Among these organizations, Cook Myosite, Stemnion and Promethean Life Sciences (referred to earlier in this article), along with Cohera Medical, Celsense and ND Life are examples of companies specializing in tissue engineering.

Through the University of Pittsburgh's office of technology management, Pittsburgh-based Cook MyoSite, has licensed technologies related to stem cells derived from adult muscle tissue. These muscle-derived stem cells have the ability to repair diseased or damaged muscle, bone and cartilage and to deliver therapeutic genes.

Stemnion is a Pittsburgh-based tissue engineering startup that aims to discover, develop and commercialize proprietary methods for stimulating organ regeneration in patients with potentially life threatening disorders. Using stem-cell based cellular therapies, Stemnion assists in the development of new treatments for liver, metabolic, endocrine and other diseases. Its cellular products also may be used for evaluating pre-clinical drug candidates and for use in drug metabolism studies, enzyme induction experiments and toxicology testing. Stemnion is one of five young biotech companies residing in the Pittsburgh Life Sciences Greenhouse incubator space.

Cohera Medical, Inc. is aimed at fulfilling the market demand for a strong, safe tissue adhesive to improve the wound closure process by positioning tissues for optimal healing, while minimizing fluid accumulation. The company's first product under development, TissuGlu™, is an easy-to-use, resorbable adhesive that is as strong after one hour as a naturally healed wound is at one week.

Celsense defines its mission as providing world-class services in the area of adult stem cell collection, processing, storage and related products for the community. The company assists in the development of knowledge and technologies that will enhance the effective utilization of adult stem cells and tissue to treat disease through national and international research affiliations.

At present, stem cells are mostly used in diseases, such as blood disorders, cancers, immune deficiencies and metabolic disorders. Cord blood stem cells are used where bone marrow stem cells are routinely used. To date, more than 94 disorders have been treated with stem cells.

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