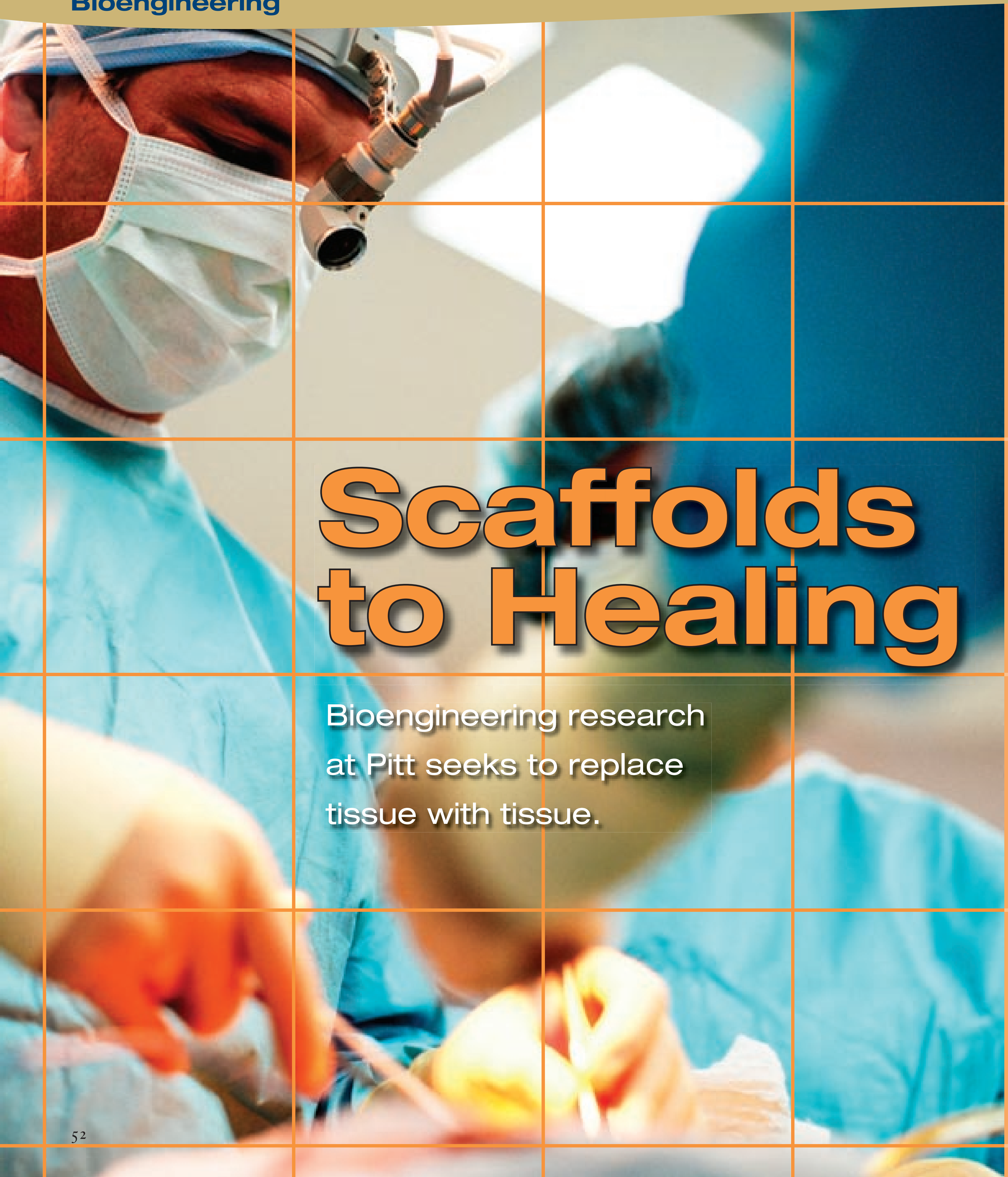
Bioengineering



Scaffolds to Healing

Bioengineering research at Pitt seeks to replace tissue with tissue.

In Colorado, a 55-year-old woman races down a double-black diamond slope, spraying clouds of snow in her wake as she slices through the powder. Suddenly, she hears a "pop!" and instinctively reaches for her right knee, collapsing to the ground in pain.

Across the country, on a parched field in southwestern Pennsylvania, a 21-year-old star running back takes the handoff from the quarterback and sprints up field. After a few quick steps through a small hole in the defense, the player cuts sharply to avoid a tackle. His cleats cling to the turf, bending his knee awkwardly as his body crumbles on the ground. Lying in the dirt, he reaches for his knee, hoping his career isn't over.

Knee injuries like these are all too common today, where "weekend warriors" and elite athletes alike push their bodies until something gives. And often it is the anterior cruciate ligament (ACL), the knee's main stabilizing force. In the United States, approximately 200,000 people tear their ACLs each year, although many of their injuries are not through contact sports or strenuous exercise.

"It's an epidemic," famed bioengineer Savio L-Y. Woo, director of the University of Pittsburgh Musculoskeletal Research Center (MSRC) in the School of Engineering, says without a hint of exaggeration.

Depending on the severity of the injury, reconstructive surgery may be unavoidable. This can involve a surgeon replacing the damaged ligament with healthy tissue, usually harvested from the patellar tendon or hamstring, or from donor tissue. With the help of metal or plastic screws and pins, the healthy band of tendon is threaded through the knee joint and attached to the thighbone (femur) and shinbone (tibia).

But is there possibly a gentler, more natural way for this ligament to heal? If a doctor could wrap flexible, strong biodegradable scaffolding—packed with collagen and anti-immune properties—to the damaged tissues, the structure would signal the surrounding cells to climb aboard and regrow tissue naturally. The scaffolding would leave behind strong and healthy ligaments—perhaps close to their preinjured state.

At MSRC, possibilities like this excite Woo and his colleagues.

"We aren't interested in helping only the elite athletes," says Woo, the William Kepler Whiteford Professor of Bioengineering. "We're interested in helping everybody."

Funded by a $2.1-million National Institutes of Health grant, Woo and his colleagues are testing the regenerative powers of natural scaffolding built from submucosa, a layer of tissue harvested from a pig's small intestines.

The scaffolding, which looks like a white piece of mesh cloth, was developed by then-Purdue University senior scientist Stephen Badylak. Badylak has since joined the University of Pittsburgh as a research professor in the Department of Surgery and director of the Center for Pre-Clinical Tissue Engineering at the McGowan Institute for Regenerative Medicine.

During initial tests, the researchers grafted the scaffolding in rabbits with injured medial collateral ligaments (MCLs)—the ACL's lesser-known but more-often-injured counterpart.

The results?

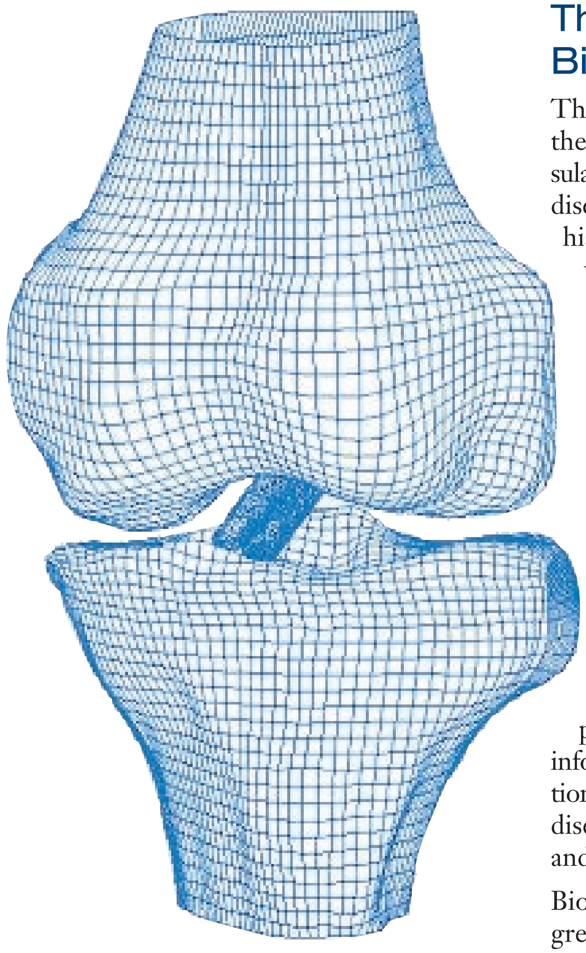
The tissue in rabbits that received the scaffolding was able to withstand more stress than the tissue in the "nonscaffolded" rabbits. The Pitt researchers are now looking at the mechanics involved in how cells align themselves to further improve the healing process.

Woo understands the MCL intimately. In the early 1980s he led a team of researchers that changed the way doctors treat patients with MCL injuries. The researchers discovered that surgery, followed by wrapping the leg in a cast for six weeks—the common practice at the time—had "no benefit."

The MCL will heal itself, Woo and his colleagues learned, and "controlled motion" during rehabilitation helped the knee to heal. Today, orthopaedists usually treat this injury by splinting the knee to limit its excessive and uncontrolled motion.

For his work, the International Olympic Committee awarded Woo an Olympic Gold Medal and a prize for Sports Science, making him the highest honoree in the field, at the 1998 Winter Games in Nagano, Japan.

Woo, a member of the National Academy of Engineering as well as the Institute of Medicine, is part of what is quickly becoming an all-star team of bioengineers at the University of Pittsburgh. He and his colleagues' research on ligament healing represents one example of the University's commitment to strengthening a world-class



This computer-generated model of a knee joint shows the anterior cruciate ligament (ACL) in the center. The knee's main stabilizing force, the ACL may require replacement surgery if it is damaged or torn. Pitt researchers are investigating new treatment options using strong, flexible, biodegradable scaffolding that would support ACL tissue regrowth.

bioengineering research effort that excels in bringing technologies from the bench to the bedside—a department that *U.S. News and World Report*'s "America's Best Colleges" ranked in 2003 in the Top 10 among public universities, and in the Top 20 among all universities, in the category Undergraduate Engineering Specialties: Biomedical/Biomedical Engineering.

The Growth of Bioengineering

The roots of bioengineering are, for the most part, untraceable, as it encapsulates many intersecting and interwoven disciplines. Yet its footprints mark the history of humankind: from wine to wooden teeth, from angioplasty to artificial hearts and lungs.

According to NIH's Bioengineering Consortium (www.becon.nih.gov/becon.htm), "Bioengineering integrates physical, chemical, or mathematical sciences and engineering principles for the study of biology, medicine, behavior, or health. It advances fundamental concepts, creates knowledge from the molecular to the organ systems levels, and develops innovative biologics, materials, processes, implants, devices, and informatics approaches for the prevention, diagnosis, and treatment of disease, for patient rehabilitation, and for improving health."

Bioengineering education and research grew in strength and national prominence during the last few decades of the 20th century, particularly in the 1990s, thanks, in large part, to The Whitaker Foundation, established in 1975 to fund "interdisciplinary medical research and education."

In 1991, the foundation began offering grants to help universities create biomedical engineering departments and degree programs. The University of Pittsburgh, which already offered master's and PhD degrees in the field, received a $5 million grant from the foundation to create its undergraduate program. In 1996, the University enrolled its first freshman class. (Today, the bioengineering program has the fastest-growing enrollment within Pitt's School of Engineering.)

Bioengineering is not a new concept at Pitt. Engineers, basic scientists, and clinicians have long been joining forces to move ideas out of the lab and into daily life. In 1987, the school created the Center for Biotechnology and Bioengineering to strengthen the bond between science and health care.

In 2001, Pitt and UPMC created the McGowan Institute to position the University and the city as leaders in the burgeoning industry of regenerative medicine. The McGowan Institute, on Pittsburgh's South Side, is an offspring of the McGowan Center for Artificial Organ Development, which was established in 1992 with an initial research focus in cardiopulmonary organ replacement.

How does Pitt stack up against the competition?

As of September 2003, the University ranked first in the nation in NIH bioengineering research grants, with 13 awards since 2001—far more than any other school.

Real Patients, Real Problems

"We're not interested in designing pie-in-the-sky products that have no relevance," says Harvey S. Borovetz, Robert L. Hardesty Professor of Surgery, professor of chemical and petroleum engineering, and chair of Pitt's Department of Bioengineering. "We're talking about real patients with real problems and physicians who want to help…from cellular therapy to medical imaging to artificial hearts to orthopaedic surgery. Every one of these is a critical area where bioengineers can make a real contribution, and I think we do."

These "real problems," says Borovetz, are what fuels research at Pitt.

"You always start with the patient and the caregiver. The patient has problems, and the caregiver understands how to best help the patient.

Using a specific type of collagen and stem cells, Pitt research teams are constructing blood vessels that they hope will be the biological and functional equivalents of patients' own.

As bioengineers, we're able to work with the caregiver to try and provide the devices and therapies and procedures that he or she needs to help the patient."

Along with a "team of dozens"—including UPMC leading heart surgeons Robert Kormos, professor of surgery, and Sanjiv Gandhi, assistant professor of surgery, and pediatric cardiologists Bradley Keller, professor of surgery, and Steven Webber, associate professor of surgery, all in Pitt's School of Medicine—Borovetz and his colleagues are tackling the problem of congenital heart failure in newborns. According to a 2003 report by the American Heart Association, eight of every 1,000 infants (or 40,000 annually) are born with heart defects.

Borovetz and his colleagues have developed a prototype of a neonatal blood pump that they hope to begin testing in animals soon. The pump, about the size of a quarter, would be surgically attached to the infant's left ventricle to temporarily keep the body fed with blood until transplantation or cardiac recovery is possible. The prototype is based on research begun at the McGowan Institute in the 1990s.

"In the short term, these mechanical blood pumps are still being used," explains Borovetz. "But as the biology becomes better understood and we develop organs—tissue replacements, if you will—then ultimately that will be the best solution. For decades, researchers have been using metals and plastics, man-made materials. The ultimate goal is to replace tissue with tissue and have that be able to ultimately improve the quality and duration of life for these patients."

While Borovetz works to build a "bridge" to transplantation or recovery, colleagues William Wagner, the McGowan Institute deputy director and associate professor of surgery, chemical and petroleum engineering, and bioengineering, and David Vorp, associate professor of surgery and bioengineering, are working on ways to mend a broken heart.

> "We're talking about real patients with real problems and physicians who want to help…from cellular therapy to medical imaging to artificial hearts to orthopaedic surgery. Every one of these is a critical area where bioengineers can make a real contribution, and I think we do."
>
> —Harvey S. Borovetz

Resting on Wagner's desk at the McGowan Institute is a thin, soft, white tube, about one-and-a-quarter-inch long. The tiny, inconspicuous hose is a biodegradable scaffolding, or "cellular bandage," that researchers hope will be used to repair a damaged heart.

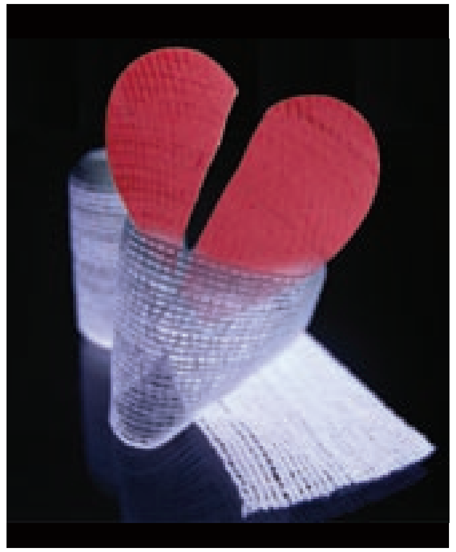
The scaffolding, in preclinical trials, is a specialized polymer constructed of a porous and pliable biodegradable polyurethane—unlike the synthetic, indestructible materials used in reconstructive cardiac surgeries today.

When surgically implanted into the wall of the heart, the scaffolding allows cells to "crawl" through its pores and repair and regenerate the damaged tissue. Initial tests in rats look promising, says Wagner. The scaffolding slowly disappeared and left behind healthy tissue.

For Vorp and one of his research teams, it's tissue-engineered blood vessels that have their collective hearts racing. They are constructing blood vessels from a specific type of collagen and stem cells that they hope will be the "biological and functional equivalent to the patient's own blood vessels, such as those used for coronary artery bypass surgery," according to a University new release.

This could be particularly great news for patients with congestive heart disease (CHD). Of the estimated 550,000 new CHD cases each year, the American Heart Association estimates that nearly 50 percent of those diagnosed will die within five years.

The NIH's National Heart, Lung, and Blood Institute recently awarded the McGowan Institute a five-year,



$4.78-million bioengineering research grant for the Wagner and Vorp teams to continue with their research.

In the University of Pittsburgh Engineered Tissue Mechanics Laboratory that he heads, Michael Sacks, CNG Faculty Fellow and associate professor of bioengineering, also has set his sights on another matter of the heart: improving replacement heart valves, small one-way doors that allow blood to pass from the atria to the ventricular chambers and that can deteriorate from degenerative diseases and infection. Sacks and his team are investigating the mechanics and structure of bioprosthetic heart valves harvested from animals to improve tissue-engineered valve designs—in a nutshell, to make them safer and stronger.

Bringing Products to Market

"The ultimate goal is to bring new technologies to patients," says Alan Russell, the McGowan Institute director and Pitt professor of surgery and chemical and petroleum engineering. "That involves going through markets. The goal is science that leads to technology transfer [that reaches] patients."

Russell understands what it takes to bring a product to market. His own research, using enzyme polymers to detect and decontaminate specific chemical agents, particularly for defense purposes, led to the creation of Agentase, a South Side business that makes such products as towelettes that decontaminate.

Funded by a grant from the U.S. Department of Defense, Russell's laboratory is turning its focus away from developing materials like clothing and sponges, and toward developing coatings. Ultimately, the researchers would like to create a paint that would change colors in the presence of a nerve agent and simultaneously decontaminate the affected area.

One promising technology that is marching to market is an implantable artificial lung developed and patented by Brack Hattler, Pitt professor of surgery and executive director of the McGowan Institute's Artificial Lung Laboratory. The Hattler Respiratory Support Catheter (or intravenous membrane oxygenator), set to begin clinical

A Pitt research team is investigating the mechanics and structure of bioprosthetic heart valves in order to make them safer and stronger.

trials in Europe in 2004, will be only the second implantable artificial lung tested on humans, and the first new one in about a decade.

The 18-inch device is inserted through a vein in the leg and placed into the superior and inferior vena caves, or veins that taxi blood back to the heart. Hollow fiber membranes in the catheter's body pass oxygen into the blood while removing the waste, carbon dioxide, like a normal functioning lung. An internal helium balloon inflates and deflates, at 300 beats per minute, to keep the blood moving and the oxygen flowing.

Hattler's device provides seven to 10 days of relief to the lungs, thus allowing them to heal. It might be used to help people with chronic obstructive pulmonary diseases, like chronic bronchitis and emphysema, which affect 110,000 people each year; or possibly even to treat soldiers infected by chemical or biological agents.

The Department of Defense awarded Hattler and his team—which includes close collaborator William Federspiel, director of the Artificial Lung Laboratory and associate professor of chemical and petroleum engineering, bioengineering, and surgery—a two-year, $2 million grant to investigate with Army scientists possible military uses for the Hattler Catheter.

Another bioengineer and new McGowan Institute recruit, Jörg Gerlach, a professor in the University's Departments of Surgery and Bioengineering, hopes that his potentially pioneering technology can someday be a common bedside treatment for patients with chronic liver disease. Approximately 26,000 people die each year in the United States awaiting transplantation.

Gerlach and his lab partners use bioreactors, similar in function to a dialysis machine, which temporarily keeps kidneys working properly. Bioreactors are locker-sized machines used to regenerate cells that extend the life of a diseased liver until a healthy liver is found for transplantation. By using harvested human cells from donor livers "found to be unsuitable for transplantation," Gerlach and his team are able to grow functioning liver tissue in the bioreactors.

In phase I clinical testing conducted in Germany, all of the eight patients treated were successfully "bridged to transplantation." And a similar study involving nine patients was almost equally successful.

In Pitt's Center for Biotechnology and Bioengineering, just two buildings away from the McGowan Institute, Jerry Schultz and his partners are working on a biosensor for diabetes with the potential to break into what he says is a "billion-dollar industry."

According to the NIH Web site, biosensors are "devices that detect specific molecules or biological processes and convert this information into a signal" that can be read or measured (i.e., blood pressure monitors or home-pregnancy tests). The researchers' sensor monitors glucose levels in people with diabetes, a disease that afflicts 6.2 percent of the U.S. population, according to the American Diabetes Association.

The biosensor that Schultz—a member of the National Academy of Engineering and Distinguished Service Professor of Engineering and professor of bioengineering and medicine—and his partners are working on is a porous tube about the size of a piece of thread. It's filled with carbohydrate beads that are covered with a red dye, which blocks out light. Inside the beads are proteins, called concanavalin A, which have been "tagged" with a chemical that glows a fluorescent green when exposed to a bright light.

Once the sensor is implanted into the skin's dermis layer, probably near a person's wrist, glucose in the surrounding tissue flows through the tube and pulls the proteins out of the beads and binds to them. By shining a light onto the skin above the sensor, the glowing proteins tell the person (or caregiver) how much glucose is in the blood. The brighter the light, the more glucose present.

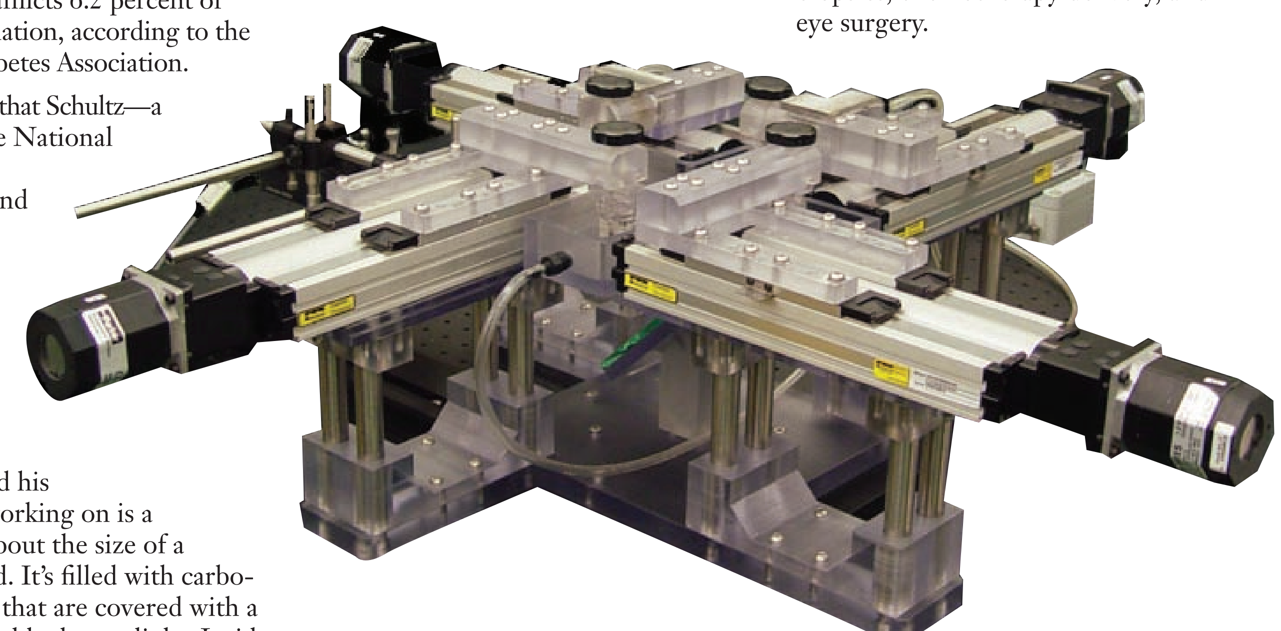
If the sensor proves workable, it could eliminate the current method of pricking a finger with a needle. To date, it has been tested successfully on lab animals.

Another device with dreams of leaving the bench and ultimately improving the lives of patients is the Sonic Flashlight, an instrument akin to the old X-ray glasses advertised in comic books that allowed the wearer to see through skin. Developed by a team of researchers headed by George Stetten, assistant professor of bioengineering at Pitt, the Sonic Flashlight has grabbed national headlines since

> "We aren't interested in helping only the elite athletes. We're interested in helping everybody."
> — *Savio L-Y. Woo*

information about it was released in December 2001.

Based on what Stetten and his collaborators refer to as real-time tomographic reflection, the portable handheld device sends high-frequency sound waves through the body. When the waves bounce back, they pass through a thin, half-silvered mirror attached to the side of the instrument that reflects images of what lies under the skin. Blood vessels, tissue, and tendons can appear directly in a physician's line of vision, an advantage over current ultrasound technology, which requires doctors to turn and look at a monitor. The turning away can cause doctors to lose the natural and precise hand-eye coordination needed for delicate procedures like amniocentesis, needle biopsies, chemotherapy delivery, and eye surgery.



This newly developed, high-speed biaxial tester is used in Michael Sacks' laboratory to measure the mechanical properties under stress of natural biological tissues and tissue-derived soft biomaterials.

Stetten has received NIH funding to test the Sonic Flashlight and to study the perceptual and motor skills involved in using the device. His collaborators include Roberta Klatzky, professor of psychology at Carnegie Mellon University, and Carnegie Mellon Robotics Institute graduate student Damion Shelton.

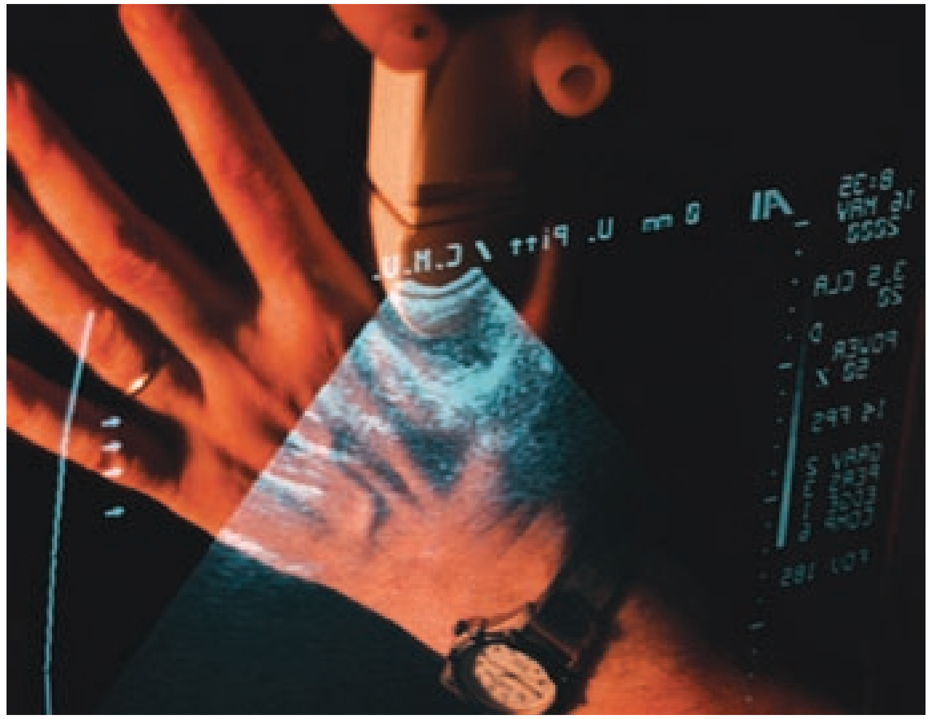
A new prototype of the device has been constructed by Wilson Chang, a student in the Pitt/Carnegie Mellon Medical Science Training Program. It may soon be used—pending NIH approval—in initial tests on patients at UPMC for the placement of catheters in the veins of the arm. Stetten also has received a grant from the National Science Foundation to develop an extension of the Sonic Flashlight for nonmedical uses based on holography, three-dimensional laser photography, in collaboration with Andreas Nowatzyk, associate professor in Carnegie Mellon Robotics Institute.

Linking Research to Practice

"The biggest single advantage that our bioengineering activities have is the closeness and tie-in they have to the clinical departments and medical centers. It's unique in the world," says Russell. "For 25 years, our clinical departments in the medical school have been appointing engineering professors to their faculty, and our students and faculty are tightly interwoven with the delivery of health care and the designing of medical devices. That's different from most places."

One of the most obvious examples of researchers and clinicians working side by side can be seen in Pitt's Musculoskeletal Research Center.

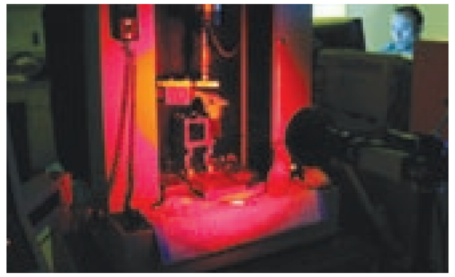
Along with the tissue engineering work to heal damaged knee ligaments, Woo and his colleagues—including Steven D. Abramowitch, a research assistant professor in the department of bioengineering, who works with Leaf Huang, professor of pharmacology and bioengineering, and Cy Frank of the University of Calgary—are testing antisense gene therapy to target a specific collagen that appears to hinder ligament healing in damaged MCLs. By decreasing the expression of Type V collagen by healing cells, ligaments heal with collagen fibrils of a larger diameter, which leads to improved quality. In addition, Woo and his colleagues at the MSRC have found that the small-intestinal submucosa scaffolding discussed previously also decreases the Type V collagen in healing MCLs. It is envisioned that these two functional tissue engineering approaches can be combined as a more effective treatment strategy for ligament injury.



George Stetten's Sonic Flashlight uses an ultrasound scanner with a small display and half-silvered mirror to create an in-position image of what lies beneath a patient's skin.

At the MSRC, Woo and his colleagues—including Richard Debski, assistant professor of bioengineering; Zong-Ming Li, assistant professor of orthopaedic surgery, bioengineering, and occupational therapy; and Patrick McMahon, assistant professor of orthopaedic surgery—also are using robotics and computer modeling to improve ligament healing. Researchers mount the femur and tibia from a cadaver knee to a robot and apply forces that simulate real-leg movement. After measuring the results, they cut the ACL and repeat the procedure. By comparing the two sets of data for specific load conditions and movements, the researchers can determine the function of the ACL and ACL graft, and thus can better inform the surgeons and therapists on how to improve techniques and postoperative rehabilitation procedures.

These results are particularly important for orthopaedic surgeon Freddie Fu, a world-renowned specialist in sports medicine. Having performed thousands of knee operations, Fu understands the intricacies of the ACL. According to an article in *Dartmouth Medicine*, Fu "pioneered the repair of torn ACLs, showed that artificial ligaments deteriorate over time, and developed the first dynamic model of the shoulder using a robotic arm and cadaver muscles."



At the MSRC of the University of Pittsburgh, robotic testing systems and computer modeling are used to generate information that enables orthopaedic surgeons and therapists to improve surgical techniques and postoperative rehabilitation.

The David Silver Professor of Orthopaedic Surgery and chair of the Department of Orthopaedic Surgery at Pitt, Fu is the director of the UPMC Center for Sports Medicine and head team physician for the University's athletics department.

In recent years, Fu has worked closely with Johnny Huard, the Henry J. Mankin Associate Professor of Orthopaedic Surgery and Molecular Genetics and Biochemistry in the School of Medicine and associate professor of bioengineering, to study the use of growth factors that improve muscle healing after an injury. Their research has shed light on the role specific growth factors play in the healing process and how gene therapy and tissue engineering might facilitate healing.

Huard also is leading a team of bioengineers to rid the world of Duchenne Muscular Dystrophy (DMD), a fatal genetic disease that almost exclusively afflicts young males. A defective gene in the X chromosome stops muscle cells from producing dystrophin, a key protein in muscle fiber. Without it, muscle cells break down and die.

But recent findings by Huard and his research team offer tremendous hope. They have taken muscle stem cells from healthy newborn mice and injected them into the muscles of mice with a genetic disease similar to DMD.

"Not only did the donor cells continue to grow and make dystrophin in the recipient," Huard told *Science Daily News*, "but they also apparently failed to provoke an immune response, which would protect them from rejection."

While Huard's research focuses on DMD, Stephen Badylak's work with "naturally occurring" scaffoldings (that Woo and his team at MSRC used to repair torn MCLs in rabbits, as mentioned previously) has unearthed a healing bounty for an assortment of ailments. Recently, the FDA-approved material has been used to repair and reconstruct damaged and diseased tissues and organs in 160,000 patients worldwide. The list of applications is still growing and the possibilities seem limitless: hernias, chronic sores and skin wounds, the lining of the brain, blood vessels, rotator cuffs, Achilles tendons, hip capsules, and lower urinary tracts.

Infrared lamps, an infrared-sensitive camera, and reflective markers enable Pitt researchers at the MSRC to record the in-vivo kinematics of daily activities for study.

Team Science

"A lot of challenges that face scientists these days need what NIH calls 'team science,' " says Russell. "In the past, scientists weren't driven to play on teams; they were driven and rewarded to play as individuals, like in a tennis match. And this [movement to team science] may be like a transition from playing tennis to playing volleyball. You have to work together as a team in order to win, in order to answer those complex scientific questions that still remain.…For bioengineering to be successful, it needs to interface with clinicians and with basic scientists."

As evidenced in all of these projects—which represent only a thin slice of the potentially groundbreaking bioengineering research ongoing at Pitt—researchers seem to relish the opportunity to partner with colleagues from other disciplines, perhaps because they share a vision of improving the quality of human life.

"The bottom line is, if [research] doesn't leave the University, it may never help people," says Borovetz. "The goal has to be to develop technologies and procedures that get to the patients who need them." ■