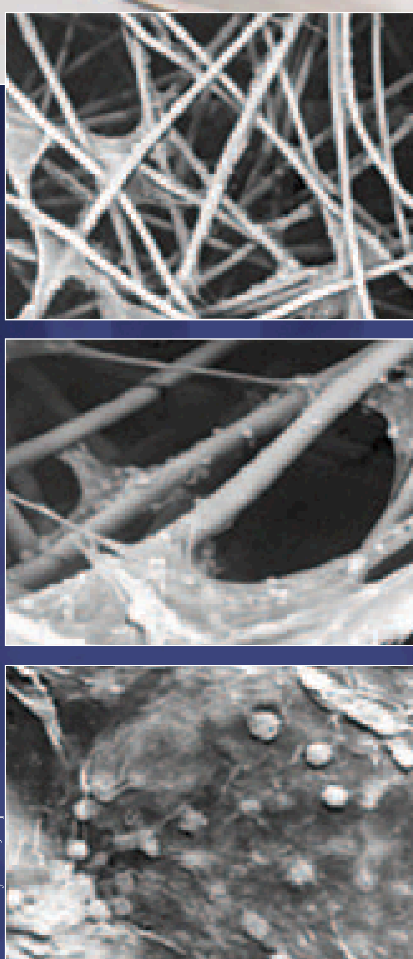
Photo courtesy of *Tomorrow's World*



Photos courtesy of Dr. Joseph Vacanti

A mouse, its naked body pink and wrinkled, peers out of a petri dish and sniffs the air, oblivious to the large elliptical protrusion stretching taut the skin of its back. And oblivious, too, to the impact it would have on the lives of the scientists who would become known, for better or worse, as "the guys who did the mouse with a human ear."

At left: A microscopic view of cells proliferating on one of the Vacantis' biodegradable polymers and, over time, forming new tissue. The top image is at one week, the middle at three weeks and the bottom at six weeks.

Vacanti Brothers: Pioneers in Tissue Engineering

By Elizabeth Gehrman

That image — which prompted double-takes worldwide as newspaper readers awoke to it one day in October 1995 — was the general public's introduction to the nascent field of tissue engineering, a science begun in the 1970s with laboratory-grown human skin, but developed exponentially in the past 15 years, largely due to the work of four physician-brothers and Creighton alumni. They are Joseph (Jay) Vacanti, BS'70, John Homans Professor of Surgery at Harvard Medical School at the Massachusetts General Hospital; Charles (Chuck) Vacanti, BS'72, professor and chair of anesthesia at UMass Medical Center, Worcester; Martin (Marty) Vacanti, BS'74, MD'82, associate professor and director of hospital laboratories at UMass/Memorial Owned Affiliate Hospitals; and Francis (Frank) Vacanti, BS'74, administrator of Massachusetts General Hospital (MGH) operating rooms and an associate anesthetist at MGH.

"It was very significant," said Chuck, the brother who showed the mouse to the BBC film crew that got the media ball rolling. "That picture was really the, um ..."

"Turning point? Fulcrum? Watershed?" Marty interjected. The two, who are the closest in age of the four men and were "always in trouble together, since we could walk," frequently finish each other's sentences and speak the same words simultaneously.

"Well," Chuck said, "I think it started a chain reaction of events that has snowballed beyond anything I had ever even imagined."

The image's release increased attention to the field, attracting the research funding, enthusiastic young scientists and enhanced international cooperation that have brought tissue engineering tantalizingly close to everyday clinical application. It spurred the development of the Tissue Engineering Society, with hundreds of members in 17 countries, and helped to catapult the science from obscurity to what *Time* magazine's "Visions of the 21st Century" issue called the number one "job of the future" in May 2000. It hastened a human trial that resulted in the successful implantation of a tissue-engineered thumb onto the stump of a machinist who had severed his finger at work. And it resulted in the transplantation of Marty Vacanti, the last of the brothers to leave Omaha, to the East Coast to join in the family collaboration as a leader of the team at Chuck's lab.


Photo courtesy of *The Boston Globe*

The Vacanti brothers — front, Joseph (left) and Charles; back, Francis (left) and Martin. The Creighton alumni are leaders in the field of tissue engineering.

The celebrity came overnight — but the success, of course, did not.

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According to his younger siblings, Jay

Vacanti is accustomed to getting what he wants. "Jay was the top of his class in grade school, high school, college and medical school," said Marty. "He was also the most outgoing, most well-liked, best speaker, you know, all of that." As a young pediatric surgeon at Mass General in the late 1970s, Jay came across something he couldn't obtain — or at least not often enough to save the lives of all of his young patients: transplant organs. Unlike those who had gone before him, however, Jay saw this not as a defeat, but as a challenge. If he couldn't get the organs, he thought, he would simply make them; or, more accurately, he would provide the conditions that would allow organs to make themselves.

"I said, 'Let's take cells from a tissue, dissociate it down, put them in culture, and reconfigure them, and they'll help rebuild the tissue,'" Jay recalled.

He knew the procedure was possible from a cell-biology standpoint. Given the proper chemical signals, along with a healthy dose of oxygen, nutrients and some incubation time in a bioreactor, just a few cells will divide until they become chunks of tissue. Unfortunately, chunks of tissue alone are of little use.

What was needed was a structure that would allow these bits of tissue to become a working organ. Creating these structures, however, turned out to be a difficult task. For fabrication expertise, Jay turned to MIT chemical engineer Robert Langer, with whom he had worked in the Harvard Medical School lab of pioneering cancer researcher Judah Folkman. They decided to tackle the liver first, both because of the complexity of the organ and because, Jay said, "there is no surgical procedure in care that's bigger or harder than liver transplantation in children." For months, he and Langer smeared living cells onto various kinds of plastic polymers, scaffolding onto which the cells could build a three-dimensional organ. But there was a problem. The cells would divide on the outside of the scaffolding, but not on the inside — where the thickness of the organ limited the cells' access to needed oxygen and nutrition.

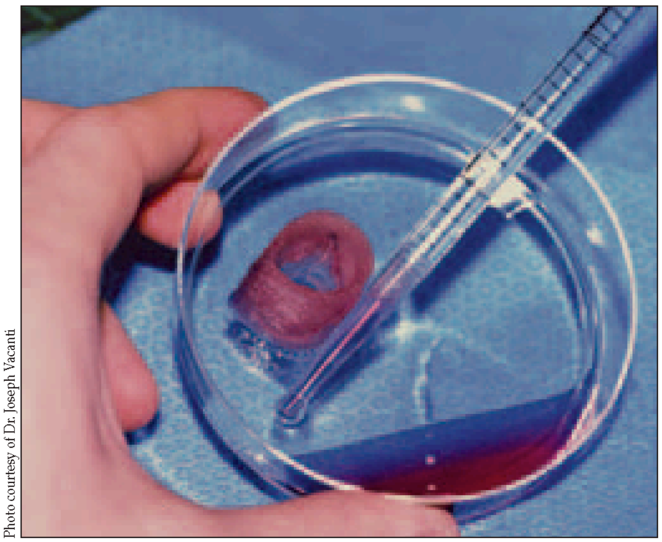

Photo courtesy of Dr. Joseph Vacanti

A heart valve polymer is seeded with cells.

Then, during a 1986 family vacation to Cape Cod, Jay had a eureka moment. The branching structure of seaweed was similar to that of the liver's blood vessels; he and Langer needed to mimic that structure inside permeable, water-soluble molds that would harmlessly dissolve in the body. "The solution was enough cells of the correct type," Jay said, "and a temporary scaffold that was 99 percent porous, so that exchange could occur across the necessary thickness. When you implanted that into an animal, it would signal blood vessels to grow into it. The polymer disappeared and you had a permanent tissue with its own blood supply."

Today, "the whole world uses that configuration successfully," said Jay, who is working to improve his scaffolding systems using state-of-the-art computer technology. At his last count, 23 kinds of tissue were in various stages of development.

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By 1987 Jay had invited Chuck, who was working as an anesthesiologist at Mass General, to his lab to design experiments using lung tissue. "I don't have any interest in lungs," was Chuck's reply. "Can I work on bones?" Jay quickly agreed.

"We used degradable suture material," Chuck said, "knotted and frayed at one end so it looked like a feather duster. I put some cells on it and implanted these things onto the backs of animals to give them a blood supply, and they turned into cartilage. It was my first experiment, and I didn't think anything of it, but Jay and Bob Langer were amazed. It was the first time a specific, free-standing tissue had ever been generated."

Chuck began sending Marty, then doing advanced training in pathology at Creighton's Saint Joseph Hospital, tissue specimens to evaluate their histology. "I was just astounded at how close they were to the real thing," Marty said. But when Chuck and his team drafted a paper and submitted it to a major peer-reviewed journal, he said, "they wrote back and said, 'Well, this is nice. But we see no practical use for it.'"

Sean McCormack saw a use for it. Born without bone or cartilage protecting his heart, the Norwood, Mass., 12-year-old was told by doctors he would have to wait until he stopped growing to have an artificial plate implanted; meantime, any unlucky bump in the chest could kill him. But in 1994, the Food and Drug Administration allowed a procedure so innovative there were no regulations governing it. Working with Jay and Chuck, McCormack's doctors harvested cells from the youngster's malformed sternum, brushed them onto a scaffold awash in growth factors, and let them do their thing in a bioreactor for a few weeks. The resulting implant — which McCormack's body easily accepted, since it was made from his own cells — grew along with him. Today, McCormack is a champion BMX racer whose chest looks perfectly normal and is as strong as any other young man's.

Further indication of the new technology's promise came with the human ear — the structure reconstructive surgeons consider their biggest headache — allowed to grow on the back of a mouse. And in 1998, Chuck seeded a phalanx-shaped piece of surgical coral with cells from Raul Murcia, who had crushed his thumb in a cargo elevator. The initial operation was a success, and Murcia again has a complete thumb.

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When Chuck opened his new lab, at UMass Worcester, he asked Marty to come try his hand at tissue-engineering a section of spinal cord. "My initial response," said Marty, "was, 'Well, you're absolutely crazy.' Everybody knows central nervous system tissue doesn't regenerate."

But Chuck presented his hypothesis from a logical standpoint: When you cut your finger, the skin repairs itself; something must be driving that and similar healing processes throughout the body. There have even been reports of limited spontaneous healing in brain and spinal cord injuries. Marty became convinced the concept was sound — and, like his older brothers, he has always been inclined to disregard conventional wisdom. He packed his bags.

"Everyone said it was impossible," he said. "That meant absolutely nothing to me. I just didn't care."

They thought the key to spinal cord repair lay in a newly described cell type: adult stem cells. These cells, they concluded, might have some of the advantages of fetal stem cells — such as a greater ability to survive an oxygen-depleted environment — without the ethical and political drawbacks.

"Around this time," said Chuck, "adult stem cells seemed to be mysteriously popping up in cell culture, but no one could identify where they were coming from. And we decided they weren't manna from heaven; they had to be coming from somewhere."

Marty set out to find the cells' precursors, examining slide after slide for more than a year. Finally, one day he noticed a few dots of an almost infinitesimal size and quantity — a spherical shape about a tenth the diameter of a normal cell.

"These had been seen for years," said Chuck, "but they were so small that people had always been taught they were cell debris. Marty looked at them and said these are too round, too uniform in size, to be debris. He made the intellectual leap."

Marty placed some of the "junk" — later dubbed "sporelike cells" — into a bioreactor, and a few days later they had multiplied. After awhile, he observed these cells growing larger as well, appearing to go through the stages of development of the specific organ type from which they were derived. Marty became convinced beyond doubt that these were essentially baby adult stem cells — not quite differentiated, but "committed" to becoming a certain kind of tissue.

> "I think someday you're going to have people coming in to have their own body parts grow back. And I think tissue engineering is part of the learning curve to get there."
>
> — Frank Vacanti

"We believe," said Marty, emphasizing that not everyone agrees with the hypothesis but clearly as excited as a kid on Christmas morning, "that in every tissue in the body, Mother Nature has provided a repair or recycling cell. And this repair cell lies dormant in the tissue until you have an injury. We've tested them, and these cells can survive the kinds of hostile chemical environments that result from injury, such as acidosis, as well as freezing, overheating, oxygen-depletion, et cetera."

To see how his sporelike cells would perform in a practical situation, Marty inserted seeded polymer scaffolds into three- to four-millimeter spaces cut into the spinal cords of nine adult female rats. The results could conservatively be called astonishing. The cells differentiated into mature neurons and other cell types, formed some of the fatty protective layer called myelin and exhibited gross synaptic activity. After three months, Marty said, the animals "actually recovered the use of both legs in terms of movement and even some ability to use them to help ambulate." Or, put plainly, paraplegic rats had begun to walk again.

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Frank, the youngest of the doctors Vacanti — they have three sisters and a fifth brother who lives in Omaha — and the last to become interested in tissue engineering, is also looking into cord repair, but from a slightly different perspective. His current research, which he is pursuing in Jay's lab at Mass General, focuses, like Jay's, on optimizing the environment to help cells find the straightest path to their proper connections. His theory is that parallel-track tunnels bored into the spinal cord scaffolding will give the cells "a higher probability of lining up in a functional succession."

His research may not be as far along as that of his brothers, but his dreams for the future of medicine — in this family of people who dream big — may stretch even further.

"I think someday," he said, "you're going to have people coming in to have their own body parts grow back. And I think tissue engineering is part of the learning curve to get there."

Frank realizes this assertion may raise

a few eyebrows. "Of course, I'm only speculating," he quickly added. "You don't like to speculate about science. You like to say this is the data, these are some of the conclusions you can draw from the data. To say anything more looks bad."

But wait: The most surprising thing about Frank's theory is that once he begins to explain it, it doesn't sound so wacky after all.

"My thought process is that we can mimic nature," he said. "In the salamander, there's a canal that runs the length of the spinal cord, right in the center. When you take out a slice of that canal, the cells in the center multiply and move into the gap. Eventually they redifferentiate and it becomes a functional spinal cord that looks perfectly normal. Humans have essentially the same anatomy — but when we get injured, our cells form scar tissue. What I want to find out is why ours forms scar, when the salamander's grows back."

In experiments with spine-injured animals, Frank has gotten some movement, he said, "but not enough to make you want to rush out and have the operation if you're a patient with spinal injury." His next step will be to try to identify and reproduce the signals and environment that allow regeneration in the salamander, "so that we get not only tissue, but tissue that interfaces properly at either end and becomes functional."

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Jesuits Encouraged Independent Thinking

In the early 1980s, when Jay Vacanti told his surgical chiefs at Harvard how he proposed to save some of the thousands of people who die every year while awaiting a transplant — by making organs out of lab-grown tissue — the concept was, he said, "ten standard deviations beyond anything that anybody would suggest. It was completely way off anybody's radar screen." But rather than try to stop him, his bosses were supportive. "They said, 'Do what you think is right.' So I did."

It's a response the Vacanti brothers' off-the-wall ideas have engendered throughout their lives. "When I was a freshman at Creighton Prep," said Chuck, "we were all lined up at our desks to do our first chemistry experiment. And halfway through I told the teacher I wasn't going to complete it because it couldn't be done, the experiment was flawed. He said, 'What do you mean? I've been doing this experiment for 20 years.' I said, 'It doesn't matter. The way you've described it, it's impossible. It's pointless for me to do it.'" The teacher asked to see Chuck after class, where he sat and listened as his student explained the problem. "When I finished he said, 'You're absolutely right.' And he changed his experiment. He didn't punish me, he didn't say I was an idiot. We were taught to think independently."

Photos courtesy of Joanne Vacanti

Creighton dental professor Charles Vacanti, BS'47, DDS'52, who died in 1994, is the center of his children's attention in this 1956 photo. From left are Mary, Jay, Chuck, Marty, Frank and Joni. Sister Cathy would be born later that year.

The brothers come from a long line of independent thinkers. Their maternal grandfather left Sicily as a young man and took a Union Pacific train across the United States. When asked why he chose to disembark in Omaha, he replied, "Because no one else would." Their father, Charles Vacanti, BS'47, DDS'52, who died in 1994, held the first chair of endodontics at Creighton as well as several dental patents. According to Marty, "His example made us want to do, I think, something that no one else had done." And all four mention their mother, who put herself through Creighton's premedicine studies working in a bomber plant during World War II, as an enormously positive influence.

"I always told them," said Joanne Vacanti, BS'57, who had eight children in 13 years, "if you want to do something different, you don't have to go by what anybody else says. Try it yourself. You don't have to accept what other people say."

Their genetic predisposition to nonconformism was nurtured outside the home as well. "It sounds corny," said Chuck, "but part of it is the Jesuit training." All four went to Creighton Prep and received their undergraduate degrees at Creighton University, where Marty also completed his M.D.

"Educationally," Jay agreed, "the most important global influence on my career was my Jesuit education. The Jesuit habit of mind is expansive, trying to really get at the nature of things philosophically, reason things out, understand logic." Jay also credits Harvard, where his mentors encouraged him to "continually improve while pushing the envelope."

But perhaps the biggest influence the Vacanti boys had was one another.

"If you think about it," said Frank, who got his M.D. at 23, "how many other families are there where four siblings went to medical school? It leads to a very dynamic interaction." Personality-wise, he said, "We all have bits of each other and we're all different. Jay's very outgoing in a socially proper way. Chuck's more of a person who can go into a room of total strangers and in five minutes everybody's laughing. I'm the kind of person who goes into a room with total strangers and I go off in a corner because I don't want people

"Functional" being the key word. It turns out that tissue engineering, begun as a way to solve the organ shortage, has several important advantages over transplantation beyond its initial mandate. Perhaps most important, since the cells are autologous — taken from the patient himself — there are no rejection-related complications or lifelong immunosuppressants. The degradable scaffolding materials have been used for decades with few adverse effects beyond the very rare polymer allergy, detectable in advance of the procedure. There is no possibility of the transmission of infectious diseases such as HIV. And, even given ideal conditions, a transplanted kidney might live only 15 to 20 years — if the patient happens to reside in a country that offers transplantation; in Japan and many other nations, there are enormous cultural barriers to such procedures.

Cultural barriers — also known as ethical concerns — are one of the main reasons Jay chose to explore tissue engineering in the first place. He cites the story of a U.S. woman who announced her intention to conceive for the sole purpose of aborting the fetus and harvesting its stem cells for use in treating her father, who had Parkinson's disease. "When well-intentioned people are willing to do something like that," Jay said, "you know the slippery slope is real."

But even beyond the moral and religious objections, there may be valid scientific reasons for tissue engineering to ultimately prevail over the currently much-hyped fetal stem cell therapy. A case in point: the highly publicized recent setback in which fetal stem cells injected into the brains of Parkinson's patients not only didn't help, but actually made some patients' symptoms worse. There are a couple of possible reasons for this.

First, fetal stem cells are made to differentiate using external stimuli — chemical signals that force them to grow in the desired direction. But "generally when you remove those stimuli," said Chuck, "they lose the signal. Quite often they break down and revert to what they were, or they simply die." Second, it may be that fetal cells are simply rejected in the long run. "Eventually the body figures out that these cells are foreign," said Marty. "The rejection is just slower than it is with other tissues, which allows for some initial improvement."

Another rarely mentioned problem with fetal cells, said Marty, is that "if they do generate a tissue, more often than not, they're also generating multiple other tissues; they become a hodgepodge of various tissue types. There is the potential to get fully differentiated, functional tissue, but it's very difficult."

The main objection thus far to tissue engineering has been that adult stem cells are hard to obtain. But that doesn't seem to be slowing down research in nearly every major teaching institution in the world. There appears, at this relatively early stage, to be virtually no application for which tissue engineering could not work.

Lab-grown cartilage is already being used to help children born with a congenital urinary tract abnormality. At Mass General, research fellows have successfully replicated sections of colon, with obvious implications for the tens of thousands of people diagnosed yearly with inflammatory bowel disease and colon cancer. Chuck and Marty's team has implanted an entirely tissue-engineered trachea into a sheep. Heart valves, partial livers and complete bladders have flourished; pancreatic progress is coming close to a cure for Type I diabetes, and Jay has gotten a tissue-engineered sheep's heart to actually beat in the lab. Even the holy grail of modern medicine conjures an imaginable scenario: "If you look at cancer as improperly differentiated cells," said Frank, "maybe the solution isn't to poison them and kill them, but to change them — redifferentiate them into their proper state."

In public, the Vacanti brothers are careful to qualify their words and remain scientifically aloof when discussing the latest finds and possibilities. "I'm not happy to say it's had an impact until I actually see it translated into patient care," Jay said soberly. In private, it's another story: "How cool is this?" he asked, jumping out of his chair to find a paper describing tissue-engineered teeth. "It's fabulous!"

About the author: Gehrman is a free-lance writer in Boston.

The Vacanti brothers help their mother, Joanne, celebrate her 75th birthday party at Creighton in 1998. From left are Marty, Frank, Joanne, Chuck and Jay.

laughing at me. And Marty is initially quiet but if you push the right buttons he can turn into Chuck."

The four have collaborated since they were small children, combining their individual strengths to build everything from windmills to engines to animated homecoming floats — though their experiments weren't always as successful as they are today.

"Things always flopped," said Frank. "But if you're afraid of failure, you won't try anything. And if you have enough failure, eventually it works. One time we found some sticks that Chuck said were bamboo, and we found some cloth that Chuck said was muslin, and we decided to build a glider. We got our little sister Cathy to try it by jumping off the roof." He laughs. "She went straight down. But not as quickly as she would have done without the thing."