

Taking tissue engineering to **HEART**



More than a decade after Japanese scientists implanted the first bioengineered blood vessel into a child with a congenital heart defect, the experimental treatment has finally made its way into clinical testing in the US. **Elie Dolgin** asks what took so long and what lessons have been learned along the way.

Last month, a team of around a dozen doctors and nurses in Connecticut performed a 12-hour operation to insert a cigar-shaped plastic tube, seeded with bone marrow cells, around the heart of a toddler born with only a single functioning ventricle. The delicate surgery, performed at the Yale-New Haven Hospital, represents the first time that surgeons have implanted a tissue-engineered blood vessel into someone on US soil. It could radically alter the future treatment of this type of congenital heart defect—which affects around 3,000 babies born each year in the US—and could have implications for more common heart procedures down the road.

For the trial investigators, the surgery has been more than a decade in the making. “See the black binders,” says Christopher Breuer, gesturing to a bookshelf in his small, sunlit office. Breuer, a pediatric surgeon at the Yale University School of Medicine who has spearheaded the experimental surgery, stands up and pulls out one of more than a dozen thick binders lining the wall. “All but three of these constitute a single copy of the first application” to the US Food and Drug Administration (FDA) to approve the clinical protocol, submitted in August 2009. “Things moved along very slowly,” he admits.

That’s especially true when you consider that the same surgery had already been performed on 25 people, starting ten years earlier, by Toshiharu Shinoka and his colleagues at the Tokyo Women’s Medical University Hospital in Japan.

To demonstrate the contrast, Breuer opens one of the binders to produce the original Japanese application—a slim, six-page document, seven if you include the signatures on the last page. At the time, authorities in Japan required only that Shinoka’s local institutional review board approve his experimental treatment. But times have changed, and such an approach would never pass muster at the FDA.

So, slowly and methodically, Breuer and Shinoka, who came to Yale in 2007, have been making their case to the US authorities. “We’ve really bent over backwards to be as careful as possible,” Breuer says, “especially because we’re starting with children.”

The two surgeons have worked arduously to document the safety of the tissue-engineered conduits. They’ve detailed the growth potential of the tissue-engineered blood vessels in young sheep¹, used mouse models to demonstrate the role the immune system has in vascular remodeling², and shown how cells from the

adjacent vessel wall around the implant site in mice populate the scaffold to give way to the new blood vessel³.

“It’s a ton of work up front to collect all this stuff,” says Breuer. But it should pay off. By working with scientists at the FDA to fully understand the surgery at a molecular level, Breuer thinks his landmark study will be more robust, be safer and yield more useful information than any tissue-engineered cardiovascular trial previously performed anywhere else in the world.

“So, am I frustrated? Maybe I wish we could have gone a bit faster. But I think the wait was worth it,” Breuer says. “God forbid we have a bad outcome; at least I can look myself in the mirror and say I did everything I could.”

Onlookers familiar with bringing tissue-engineered products to market applaud the undertaking. “It’s addressing an unmet need,” says Ali Khademhosseini, a bioengineer at Harvard Medical School in Boston. “It’s not something that can be addressed with a purely synthetic product that doesn’t degrade away.”

“These are the kinds of things we need—not just in the [tissue engineering] industry, but in the health system,” adds Tim Bertram,

executive vice president and chief scientific officer of Tengion, a biotechnology company headquartered near Philadelphia that is developing tissue-engineered bladders to treat bladder cancer.

Engineering enterprise

Breuer and Shinoka met as postdocs at Children's Hospital Boston in the mid-1990s. The two budding physician-scientists were working in different labs at the time but shared a common interest in the idea of constructing heart valves by tissue engineering. Rather than replacing diseased or defective valves with mechanical, animal- or cadaver-derived substitutes—a surgery performed on around 100,000 people each year in the US alone—they wanted to create a living valve that could respond to growth and physiological forces in the same way as native tissue.

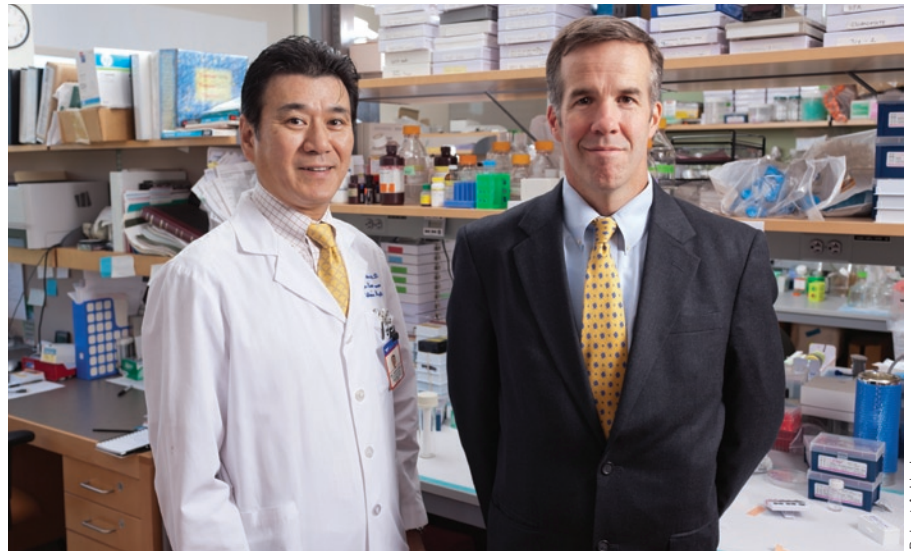
In their first paper together, published in 1995, Breuer and Shinoka showed in a lamb model that they could successfully engineer part of a working heart valve using a synthetic biodegradable scaffold seeded with the animal's own fibroblasts and endothelial cells⁴. The researchers eventually tested their tissue-engineered heart valves in a few dozen young sheep, demonstrating the feasibility of the approach. But then each man went his own way—Shinoka back to his native Japan to practice medicine, Breuer to complete a clinical fellowship at Brown University in Providence, Rhode Island, and then to the US Air Force as a condition of his medical training.

The two men lost touch for several years. But then, around a decade ago, Shinoka wrote to Breuer to tell him about an operation he had just completed. He had successfully treated a four-year-old girl with a malfunctioning ventricle using a tissue-engineered graft similar to the construct that the two had implanted in sheep years earlier.

In May 1999, Shinoka had taken a small snippet of vein tissue from the young girl's leg, cultured the tissue for eight weeks, seeded the millions of cells onto a biodegradable scaffold for ten days and then transplanted the tube-shaped graft. Seven months after the surgery, the patient was doing well, with no signs of graft blockage or other complications, Shinoka told Breuer (and reported in 2001 in the *New England Journal of Medicine*⁵).

"I found out about it and almost jumped out of my skin," recalls Breuer, who was stationed in Turkey and Texas at the time, attending to soldiers injured in Afghanistan and their families. "It was so cool to go from an idea to actually making it a reality."

After repeating the same procedure in two other children, Shinoka switched to a



Taking heart: Yale's Shinoka (left) and Breuer engineered blood vessels for congenital heart disease.

protocol that didn't require culturing cells for months. He aspirated bone marrow from the hips of his patients on the morning of their surgeries, centrifuged the samples to obtain only the mononuclear cell layer—blood cell precursors previously shown to promote vessel formation⁶—and then seeded millions of these uncultured white blood cells right onto biodegradable tubular scaffolds. After just a few hours of incubation, the grafts were ready for implantation.

Between 2001 and 2004, Shinoka used this approach to treat 25 people, ranging from 1 to 24 years of age, all of whom suffered from what are known as 'single-ventricle anomalies'. Many different cardiac defects can cause this problem—such as a defective heart valve or a misconnected atrium—but they all share the common feature that only one of the two ventricles is working properly. As a result, oxygen-rich blood returning from the lungs and unoxygenated blood from the veins gets mixed in the single ventricle and then pumped back out into the aorta and pulmonary artery indiscriminately, often leading to a condition known as 'blue baby syndrome'.

Without surgical intervention, 70% of babies born with univentricular hearts die in the first year of life, so most children undergo a series of three or more procedures to stop the mixing and move more blood to the pulmonary system. The surgeries typically culminate at around age 2 or 3, with what's known as a 'Fontan operation'. This procedure basically reconfigures the vascular plumbing so that deoxygenated blood bypasses the heart and travels straight—but passively—to the lungs.

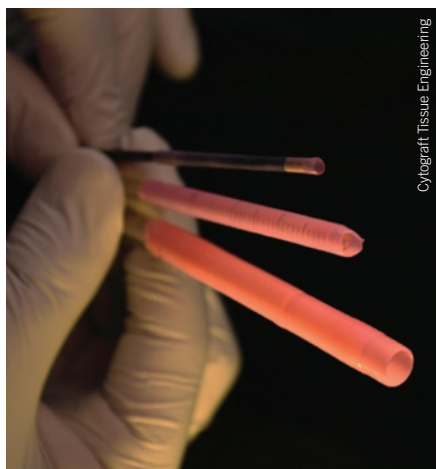
In the modern-day Fontan operation, which has undergone several modifications since it was first successfully completed more than 40 years

ago, surgeons connect the inferior vena cava and the right pulmonary artery using a tube made of a material called polytetrafluoroethylene, sold commercially as Gore-Tex. This bypassing of the heart on the way to the lungs, in effect, creates a single-loop circulatory system; the sole functioning heart ventricle in affected individuals is assigned to pump oxygenated blood returning from the lungs to the body. Although not curative, the procedure substantially prolongs life and improves people's standard of living.

Still, conduits made of Gore-Tex and other synthetic materials are not a panacea. They often narrow, leading to a condition known as stenosis, and they can trigger clot formation, necessitating that kids take blood-thinning drugs such as warfarin and aspirin for the rest of their lives. What's more, these artificial materials lack growth potential, so doctors often wait until children weigh at least 15 kilograms to carry out the operation; in rare instances, children can also outgrow their grafts and require larger replacements.

"Now, if you can put a tube in that would be made of their own cells and grow with their inferior vena cava, then potentially you would take care of that issue [of limited growth], which would be a big deal," says Harold Burkhart, a congenital heart surgeon at the Mayo Clinic in Rochester, Minnesota. Carl Backer, head of cardiovascular-thoracic surgery at Children's Memorial Hospital in Chicago, adds: "in theory, a tissue-engineered graft would not require anticoagulation," referring to the blood-thinning medications.

Patient advocates similarly see a benefit in an engineered blood vessel that could be implanted in infancy. "I think having repairs done as soon as possible is better for the child,"



Totally tubular: Cytograft's blood vessels.

says Susan North, president of the Children's Heart Society in Edmonton, Canada, whose daughter Madison, now 17, underwent a Fontan operation as a three-year-old. In the year-plus period that doctors waited to perform the operation, Madison was in and out of the hospital every month for severe respiratory infections. Worse, Madison now suffers from chronic headaches, circulation problems and cognitive problems, including attention deficits and language-processing difficulties. North worries that the low oxygen levels in her daughter's blood before the Fontan may have caused irreversible brain damage. With a tissue-engineered product going in earlier, children "would have less time with low oxygen in their blood and hopefully fare better," she says.

The heart of the matter

Four of the 25 patients in Shinoka's earlier study experienced graft stenosis⁷. All of these individuals were successfully treated by balloon angioplasty and are now doing fine, but the frequency of stenosis left Shinoka wondering why some people experienced this complication and others did not. "Our results from Japan aren't perfect," he says. "We still have many questions."

Unfortunately, the Japanese authorities changed the rules before Shinoka was able to get answers. In 2005, the government issued more stringent regulations for clinical trials, meaning his local institutional review board oversight wasn't good enough any more. Rather than try to regain authorization in Japan, however, Shinoka decided to rejoin his former labmate at Yale and work with the FDA to try the treatment in the US.

"We went to the FDA, and we shared all the data from the Japanese study, everything that we had," recalls Breuer, who has been part of Yale's Vascular Biology and Therapeutics Program

since 2003. "But we fell short on some level, and it all had to do with quality control and quality assurance."

Breuer and Shinoka eventually got their protocol up to scratch. Before, during and after last month's surgery, their team ran 25 safety and monitoring tests on the naked polymer scaffold, six tests on the patient's white blood cells before placing them on the scaffold, five after the cells were seeded to ensure they were ready to go into the patient and eight more on the pieces of the graft not implanted. They also have a 300-page data collection form for tracking each child's development for at least three years after the surgery. "I'll have every ounce of information on these kids," Breuer says.

"It's exciting that they've gotten this far," says Robert Langer, a biomedical engineer at the Massachusetts Institute of Technology in Cambridge who Breuer worked with as a postdoc in the 1990s. "It's a long, slow process—but it's an important one."

The FDA declined to comment for this story, as the trial is ongoing. But people familiar with developing tissue-engineered products in the US say these demanding requirements are par for the course. "When you come to regenerative medicine, you're talking about a lot of new ground that has to be covered," says Anthony Atala, director of the Institute for Regenerative Medicine at Wake Forest University in Winston-Salem, North Carolina, who, in 1999, transplanted the first of several tissue-engineered bladders into young people with bladder disease in the US, and, in 2004, followed up with tissue-engineered urethras.

But not everyone is inclined to jump through all of the hoops necessary to get the FDA's go-ahead for experimental trials. "The US regulatory framework for cell-based therapeutics right now is extraordinarily conservative," says Todd McAllister, cofounder and chief executive of Cytograft Tissue Engineering, a Novato, California company developing tissue-engineered blood vessels for people on dialysis for end-stage kidney disease.

"While we can all debate the risk-benefit analysis that drives the FDA approval process, the fact remains that groups like Chris and Toshi's spend years in preclinical work, followed by a years-long negotiation with the FDA to get a US clinical trial approved," says McAllister. "Without the [academic] support that a Yale researcher has or the revenue support that a larger company has, this type of time lag to first-in-man studies simply cannot be justified to the investors that drive a small business venture. The FDA's paradigm is clearly designed to push initial clinical trials outside the US."

In 2004, Cytograft traveled to Argentina to treat its first kidney dialysis patients with blood vessels

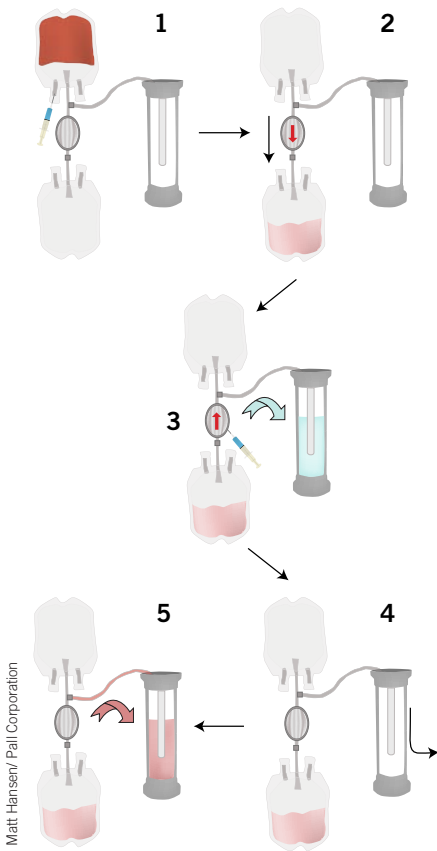
grown in a laboratory dish from snippets of their own skin. Like many people on dialysis, these trial volunteers needed an artificial passageway between the arteries and veins in their forearms to provide proper pressure and access for the repeated needle punctures where the blood is drawn and returned during their regular dialysis treatments. In the treatment, Cytograft used cells taken from the backs of patients' hands and grew these into sheets of fibroblast cells; these sheets were later rolled around steel rods into tubes and strengthened in culture.

McAllister and his colleagues eventually used this approach to treat ten people in Argentina and Poland between 2004 and 2007. They found that three grafts failed within the first three months—a failure rate lower than generally seen with synthetic shunts—but, in the rest of the volunteers who stayed in the trial, the tissue-engineered shunts kept their mechanical stability and remained effective 6 to 20 months after implantation⁸. The company has since switched to an off-the-shelf approach so that the product can be available whenever patients need them. Last year, they treated three people in Poland with bioengineered tubes made from donor cells. More than eight months after implantation, all three grafts were holding up well, McAllister reported at the American Heart Association's Emerging Science online conference in June. This month, the company expects to launch a phase 3 trial in Colombia involving 40 patients who will get the tissue-engineered blood vessels and 20 patients getting traditional synthetic shunts.

Meanwhile, in the Raleigh-Durham area of North Carolina, a company called Humacyte is also growing long tubes from banked cells, although the company uses smooth muscle cells, rather than fibroblasts, and seeds them onto a plastic scaffold instead of rolling up sheets of cells. Using mechanical forces, these cells are stimulated to secrete extracellular matrix proteins that form a robust tube. Over the span of around two months in the lab, the scaffold degrades away. The researchers then add detergents to wash away the cells, leaving only a collagenous, water-tight cylinder that can be stored for up to a year in refrigeration.

"What's left, our product, are the proteins produced by the cells," explains Shannon Dahl, Humacyte's cofounder and senior director of scientific operations. "We're left with a tissue—it looks like a tissue—but it doesn't have cells, and without cells the risk of immunogenicity is very low."

Reporting earlier this year, Dahl and her colleagues showed that the grafts functioned well for up to a year in nine baboons, with no evidence of scarring, calcification or dilation of the vessel wall⁹. "That gives us a high index of



Matt Hansen/Pall Corporation

Filter kilter: Pall's closed seeding system. Bone marrow is loaded in (1). The mononuclear cells are captured on the filter (2). The captured cells are transferred to the chamber (3). A vacuum pulls the seeded cells onto the scaffold (4). After the chamber is filled with effluent, the scaffold is incubated for two hours (5).

confidence that when we move into humans, which we hope to do soon, we won't have any problems," notes fellow cofounder and Yale bioengineer Laura Niklason, who says the company plans to start clinical trials early next year in Eastern Europe.

According to Niklason, dialysis shunts are "the lowest-risk place to start with something like this, and it's frankly one of the areas where current technologies work the most poorly." But the company also hopes to adapt the approach to develop coronary and peripheral artery bypass grafts to treat blocked blood vessels in the heart and limbs. Indeed, they've already shown preclinical success of both applications in a dog model⁹. "There's a clear medical need for a bypass conduit that you don't have to harvest from the patient," Niklason says.

Whereas Cytograft and Humacyte have conducted trials abroad, Pervasis Therapeutics, a Cambridge, Massachusetts company, has tested their tissue-engineered vascular implant in close to 50 people in the US¹⁰. It's not quite a replacement vessel, but their 'Vascugel'

product—a spongy Band-Aid-like scaffold seeded with aortic cells that wraps around an injured blood vessel—is designed to surround the surgery site and prevent scar tissue formation, inflammation and thrombosis.

"We're somewhat between the cell therapy and that fully tissue-engineered organ," says Elle Nugent, vice president of research and development at the company. "There aren't too many products like ours."

On the lamb

"Declamping," announces Shinoka, with a soft-spoken urgency. He releases the pressure on the lamb's inferior vena cava, and blood starts to flow again through the major blood vessel. The radiant red color and semirigidity of the newly implanted, tissue-engineered section—a two-centimeter-long tube shaped like a short piece of rigatoni pasta—sets it apart from the elastic, milky white vein attached on either end.

To mark the tissue-engineered graft for later imaging studies, Shinoka sews a metal staple into the blood vessel. He squirts a fibrin sealant over the site of the implant to prevent oozing around the stitches. Then, he leaves the job of closing up the 21-kilogram, seven-week-old lamb's chest cavity to postdoc Yuji Naito and surgery resident Mark Maxfield. After a morning of open-heart lamb surgery, Shinoka is off to treat his human patients. The lamb, meanwhile, is transferred to a pen with other livestock. By dinnertime, he is standing and eating.

This lamb transplant, conducted in late July, was part of an ongoing study to improve the methods for seeding the polymer scaffold. Currently, the researchers must spin down the bone marrow aspirate in a centrifuge and manually apply the cells to the scaffold. Indeed, that's how it worked in last month's human surgery. But, together with collaborators at the Pall Corporation, a Port Washington, New York manufacturer of filtration and separation products, they hope to standardize this step with an entirely closed system (see 'Filter kilter').

According to Frank Pascale, Pall's senior vice president of new business development, this process would limit the opportunity for contamination and speed the time between when the bone marrow is aspirated and when the graft is ready for implantation. Plus, the filter-seeding method eliminates the need for the types of sophisticated clean rooms found only in some elite hospitals, such as Yale's. "When we use the filter system, we can do everything on the [operating room] table," says Narutoshi Hibino, a former Yale postdoc now at the Children's National Medical Center who performed many of the surgeries with Shinoka in Japan. "We don't need a special facility"

Paolo Macchiari, a surgeon at Stockholm's

Karolinska Institute who in June of this year in Sweden conducted the world's first tissue-engineered windpipe transplant involving a cell-seeded artificial scaffold, applauds these efforts at improving the technology. "There are many tools that are part of tissue engineering that are suboptimal right now," he says. "All these devices need to be improved."

But Breuer and Shinoka's number-one focus is their six-person clinical trial. With funding from Yale, New York's Doris Duke Charitable Foundation and Gunze Limited—the "Fruit of the Loom of Japan," as Breuer calls it, owing to their extensive clothing line, but also the country's largest producer of biodegradable medical goods—they expect to complete the single-institutional study in early 2013.

Thanks to their orphan device status from the FDA, the researchers only need to secure financial backing for this phase 1 safety trial. After that, they hope to land a humanitarian use exemption, which would allow them to charge for the experimental device, thereby making further trials cost-neutral at least. They then plan to launch a large, randomized study evaluating the tissue-engineered blood vessel—ideally, seeded with their new closed system—against the synthetic alternative. "What we really need to do is the Pepsi challenge," says Breuer. "You need to compare Gore-Tex to tissue-engineered vascular grafts head-on—look at safety, look at efficacy, look at growth and the implications for that."

Ultimately, Breuer hopes to combine engineered blood vessels with the envisioned product that kicked off his academic partnership with Shinoka close to two decades ago: an engineered heart valve. "If we had to make one thing that, at the end of the day, would make the biggest impact, it would be that." Valves present a particularly pressing challenge. "That's where the most redo surgeries occur," he says, "so if we can do that successfully, we would really help a lot of children."

Elie Dolgin is a news editor with Nature Medicine in New York.

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