Robert Taylor

Regenerating

Hope

Research in vascular tissue engineering offers new possibilities for better transplant outcomes from cardiology to oral surgery.

By Debra Levy Martinelli

We have all seen it: A new building in the early phases of construction, all concrete slabs filling in an intricate matrix of steel rebar protruding this way and that. The rebar scaffolding anchors the structure, providing the strength, support and stability that prevent the concrete from caving in on itself.

Once the walls and roof go up, the windows and doors are installed, and the brick, siding, trim and paint are added, the rebar recedes from view and memory. But we know that without it the completed structure before us could not exist.

Now picture a similar scaffold, this one made of tissue extracted from discarded umbilical cord vessels (no fetal or embryonic stem cells are used), where the rebar is such structural proteins as collagen and elastin, and the concrete is lipids, carbohydrates and non-structural proteins. In this model, though, the "concrete" is stripped away, leaving only the rebar, from which new tissue can be reconfigured and implanted to regenerate into healthy tissue to replace the tissue that is damaged or diseased.

Step into the research laboratory of Peter McFetridge, University of Oklahoma assistant professor of chemical, biological and materials engineering and a researcher at the OU Bioengineering Center, and you can do more than imagine this mind-bending breakthrough. You can see why McFetridge's novel approach to biomedical tissue engineering is gaining the attention and respect of scientists and doctors around the world searching for better ways to treat heart disease, stroke and other vascular conditions.

"Our main objective is to develop viable alternatives to existing transplant materials that, when implanted, result in improved repair or regeneration of diseased tissues," he explains. The resulting material can be used as a direct implant or as a re-seeded "living" construct. In the latter process, human cells, isolated from the patient, are seeded onto the scaffold and grown under controlled chemical and mechanical environments within specifically designed bioreactors.

When McFetridge began working in the field of vascular tissue engineering, he wondered why his fellow researchers were ignoring the umbilical cord as a source material for bypass vessels. "It seemed so obvious. The cord is long, relatively straight and uniform. It sounded perfect," recalls the New Zealand native, who holds a bachelor's degree in applied biology and a doctorate in chemical engineering, both from the University of Bath in the United Kingdom.

The challenge became how to process the cord. "You can't just implant a piece of tissue. It's very sensitive, and the body's immune system would reject the implant and start to degrade it," he explains. "In the past, scientists have 'fixed' the material in formaldehyde, creating a wall that immune cells can't break through to attack it. It works but is not significantly better than current techniques, and is a tedious and time-consuming way to process the tissue.

"On the other hand, you could implant the whole cord, but it wouldn't regenerate. The idea is to get the cord relatively thin to allow nutrients and oxygen to support cell growth, while removing all of the non-structural material. This reduces the potential for an immune reaction but preserves the ability for the tissue to regenerate."

continued

The Human Umbilical Vein (HUV) at top is mounted on the mandrel prior to freezing and machining. The lower image is the HUV immediately after machining and thawing showing a uniform surface structure, cut precisley to a set wall thickness of 0.75 mm.

After much trial and error, McFetridge hit upon a simple mechanical solution: Put a tube through either the vein or the artery of the cord then freeze it and put it in a lathe, then machine off all the extraneous matter. The end material, after machining and chemical treatments—the scaffold or matrix—has no surviving cells to trigger an immune reaction. This makes it an ideal structure for growing new cells that regenerate the new blood vessel.

The benefits of the umbilical cord material are numerous. Chief among them: Because it is human tissue, there is less immune response and no risk of interspecies viral transfer; and because it is one long continuous piece without smaller ancillary vessels that would have to be tied off, there is no leakage.

Once the vessel has been transformed into the scaffold, it is placed in a bioreactor—a glass tube-like container with a pipe going into one end and another going out the other. By applying internal and external pressures, the bioreactor mimics mechanical forces that occur naturally in the body, like blood flowing through a vessel.

"Cells on a flat surface in tissue culture (*in vitro*) grow wonderfully, but there are no mechanical forces telling them they need to regenerate in a way that builds strength. They're just sitting there without the normal cues that direct their growth, behaving the way a muscle atrophies with lack of use. They produce collagen, for example, but not in a way that provides strength," says McFetridge. "But if you put them in a bioreactor and apply some sort of force field, they lay down the collagen in a way that applies strength. The pressure also allows for the delivery of oxygen and nutrients.

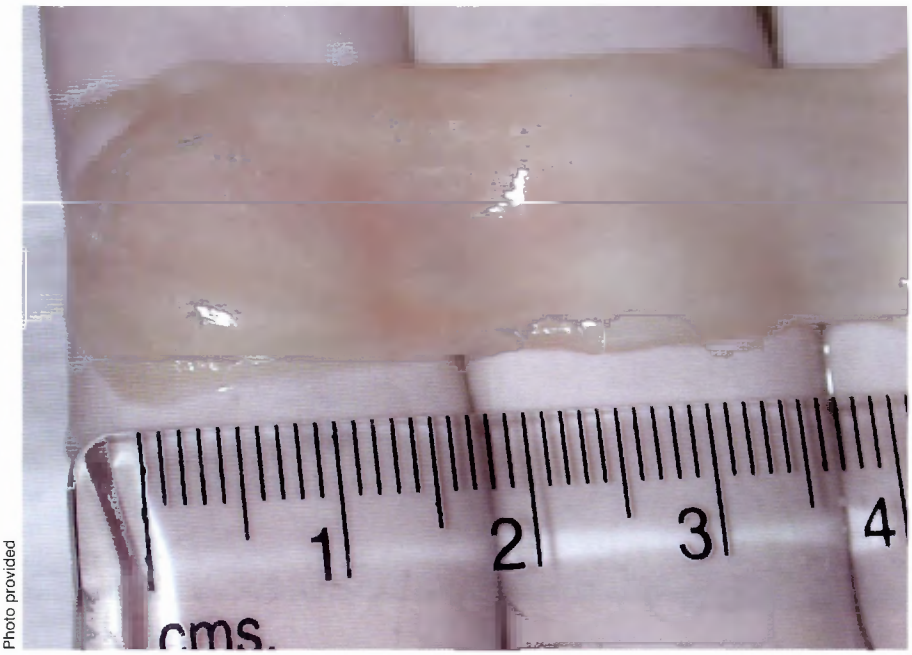

Photo provided

The machined human umbilical vein is opened out into a flat sheet for the periodontal graft therapy. If FDA requirements are met, this treatment could be available in the next 10 years.

"We're only just scratching the surface in understanding how physical forces affect our material," adds McFetridge, who has been awarded a five-year, $1.8 million grant from the National Institutes of Health to support his work.

The grant is a coup for both the OU College of Engineering and OU Bioengineering Center. "We are thrilled to see Dr. McFetridge's research in tissue engineering recognized with an NIH grant," says Ed O'Rear, director of the Bioengineering Center and Francis W. Winn Professor of chemical, biological and materials engineering. "He looks at important problems in interesting and

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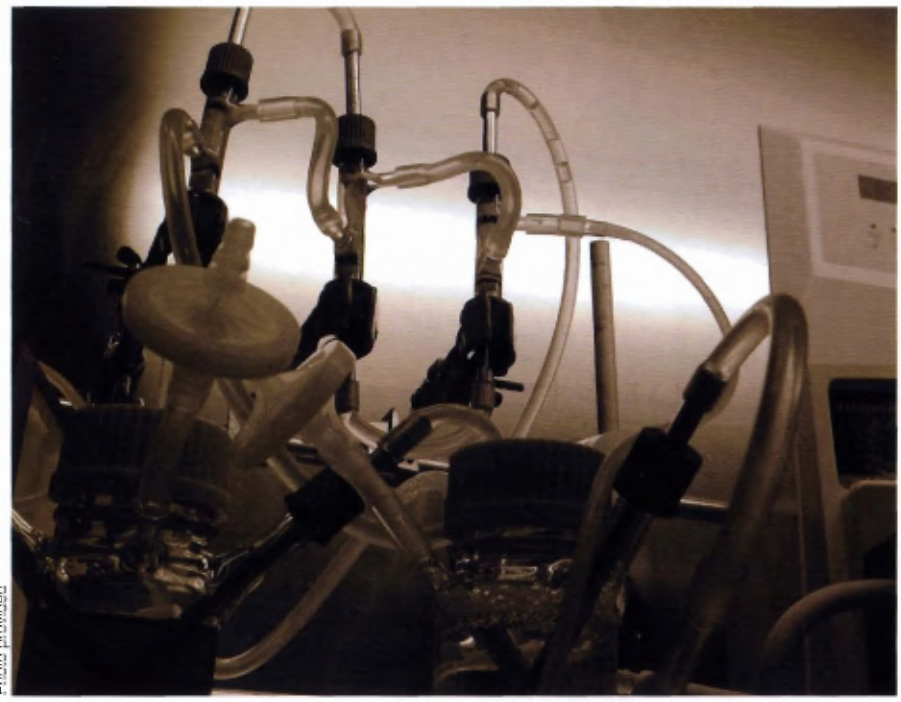

Photo provided

The dual-circuit perfusion bioreactors use peristaltic pumps that deliver nutrients and gases to the growing blood vessels. These systems are computer controlled and monitored so that the pumps, pressure and flow conditions are regulated to maximize the growing vessels' development.

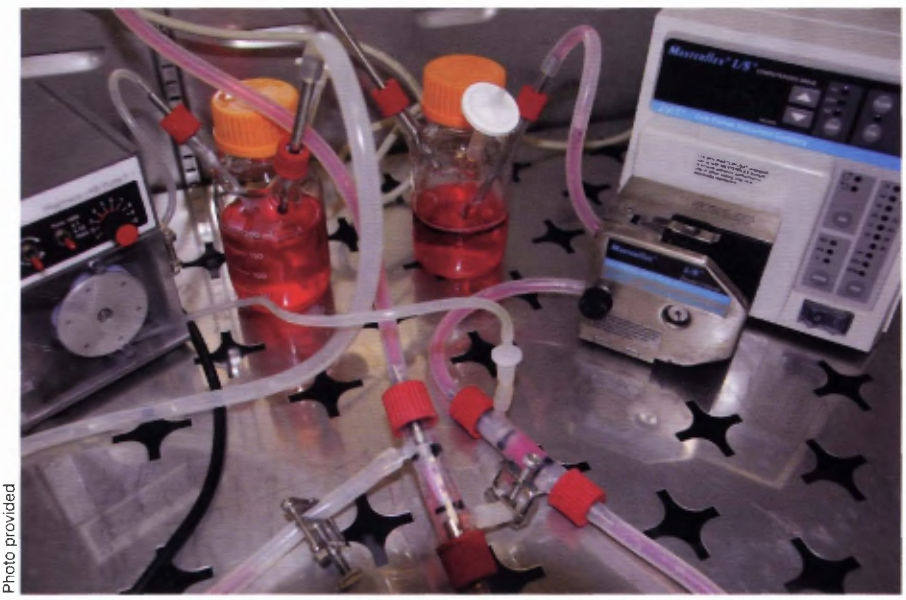

Photo provided

These bioreactors contain the regenerating blood vessel, with human smooth muscle cells growing and proliferating on the human umbilical vein, or HUV. Over time these cells will migrate into the HUV to develop fully functional blood vessels .

unusual ways. He does great science."

In McFetridge's lab, as soon as it comes off the lathe, the umbilical cord vessel is thawed and placed directly into the bioreactor.

"If a bioengineered material is ultimately going to be submitted for FDA approval for clinical application, it must be handled as little as possible, so that's the last time we actually have to touch the matrix. We strip out the cells, sterilize and pH balance it inside the bioreactor. Then we seed the cells through it," he explains. "We're developing newer designs that are entirely disposable. You process the material, seed the cells, culture the vessels and ship the end product in a transportation case."

One of the most promising applications is as a graft material for coronary bypass surgeries. When a blood vessel is constructed through tissue engineering, conditions must be created that allow platelets to mimic the natural, healthy condition that will not lead to blood clots.

"A patient arriving in an emergency room needing a heart bypass needs it then, not in a few months. So the faster this matrix can be regenerated, the better off the patient will be. If regeneration takes more than a week or two, using this material isn't a viable option. Our goal is to reduce the time frame so it is not only viable, but optimal."

A similar procedure, the peripheral femoral bypass graft, is likely to be ready for use in the operating room before the heart bypass graft is perfected. The elderly

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McFetridge and Maritza Rodriguez, a Ph.D student, assess properties of the HUV scaffold to determine any mechanical variation that may have occured as a result of the freezing, machining, and decellularization of the blood vessels. These techniques ensure that each vessel maintains its natural biomechanical properties, critical to the success of a bypass graft.

are most at risk to develop the condition requiring the bypass—blood clots in the femoral artery resulting from prolonged immobilization or injury. McFetridge says human implantation of his femoral graft material could come as soon as two years from now.

His work is not limited to vascular applications. McFetridge is collaborating with John Dmytryk, associate dean for research in the OU College of Dentistry, to develop soft-tissue implants for periodontal wound repair.

"Periodontal disease is a major cause of tooth loss in adults. An inflammatory response to bacteria, periodontal disease affects the majority of the population to some degree. The most common form is gingivitis, but if untreated it can progress to loss of not only the gums but also the bones and connective tissue supporting the teeth," Dmytryk explains.

Depending on FDA requirements for the material, it could be available in less than a decade.

He says that recent attempts to regenerate these tissues through surgical implantation can work but are unpredictable. The material being developed by McFetridge, however, has the potential to be uniquely biocompatible for implantation. Depending on FDA requirements for the material, Dmytryk says it could be available in less than a decade.

Despite the enormous potential of tissue engineering, McFetridge stresses that there is much to be done. "This kind of research is expensive, time-consuming and very challenging technically," he says. "At the end of the day, it's a great idea and works reasonably well in the lab. The proof of the pudding is when we look how the scaffold behaves when it's implanted."

That proof, he maintains, may take some time. But for McFetridge, the journey is every bit as exciting as the result.

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