Technique may help stem cells generate solid organs

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Stem cells can thrive in segments of well-vascularized tissue temporarily removed from laboratory animals, say researchers at the Stanford University School of Medicine. Once the cells have nestled into the tissue's nooks and crannies, the so-called "bioscaffold" can then be seamlessly reconnected to the animal's circulatory system.

The new technique neatly sidesteps a fundamental stumbling block in tissue engineering: the inability to generate solid organs from stem cells in the absence of a reliable supply of blood to the interior of the developing structure.

"Efforts to use tissue engineering to generate whole organs have largely failed," said Geoffrey Gurtner, MD, associate professor of surgery, "primarily due to the lack of available blood vessels. Now we've essentially hijacked an existing structure to overcome this problem." The key, the researchers discovered, is to keep the tissue adequately supplied with oxygen and nutrients while outside of the body.

In the near future, the researchers believe that the stem cells in the tissue could be induced to become an internal, living factory of healthy, specialized cells churning out proteins missing in people with conditions such as hemophilia or diabetes. In the long run, they hope to encourage the cells to become entire transplantable organs such as livers or pancreases.

Gurtner, who is also a member of Stanford's Cancer Center, is the senior

author of the study, which is featured in the March issue of the *FASEB Journal*.

The technique devised by Gurtner and his colleagues does more than provide the versatile stem cells with a readily accessible blood supply and a pre-formed cellular framework within which to begin differentiating. It also eliminates the chance of rejection or complications caused by the use of artificial or donor scaffolding materials by utilizing the animal's own tissue.

The researchers capitalized on a portion of the circulatory system shared by animals and humans called microcirculatory beds. To understand what they are, spread the fingers of each of your hands apart and then touch your fingertips together. One wrist represents the inflow of blood, and the other, the outflow. The fingers are the tiny capillaries that supply oxygen and nutrients to the surrounding tissue wrapping itself invisibly around your hands.

In many cases these beds create a flap of expendable tissue that can be easily removed. (With your fingertips still touching, bring your elbows together. Now imagine lopping off your hands midway down the forearm. Your fingers and wrists now represent a free microcirculatory bed.)

Gurtner and his colleagues removed microcirculatory beds about the size of a half-dollar coin from the groin of laboratory rats and attached the ends of the two main blood vessels to a modified piece of equipment called a bioreactor designed to keep livers and kidneys healthy outside the body. The modified bioreactor pumps an oxygenated soup of nutrients into one vessel and recovers it from the other; Gurtner referred to it as a "kind of life support, or cardiopulmonary bypass, machine for tissue."

The scientists showed that, once the appropriate blood pressure and nutrient balance was achieved, the bioreactor could keep the tissue healthy enough for reimplantation into a second, genetically identical animal for up to 24 hours. In many cases, the tissue became nearly indistinguishable from surrounding skin within 28 days of transplant, although the success rate of the procedure decreased as time spent on the bioreactor increased. In contrast, control tissue not connected to the bioreactor after removal died within six hours of transplantation.

The team then used the bioreactor to pump multipotent stem cells from a variety of sources, including bone marrow and fat tissue, through the tissue. Unlike embryonic stem cells, which can become any type of cell in the body, multipotent cells are more restricted in their potential. The researchers found that the cells could migrate out of the vascular spaces and into the surrounding tissue. Once there, they set up shop and began to form colonies. Unlike stem cells injected directly into the tissue, the stem cells that had been seeded into the tissue continued to thrive even eight weeks after reimplantation.

"This is an incredible opportunity to bulk-deliver cells that don't just die," said Gurtner. "Conceivably, we could use this technique at least to supply the synthetic function of an organ by stimulating the cells to form insulin-producing pancreas cells or albumin-producing liver cells."

Members of Gurtner's team are now trying to use the technique to deliver Factor VIII and Factor IX — crucial blood-clotting components that are missing in people with hemophilia. The researchers concede, however, that much remains to be done before the technique could be used to generate whole organs. Indeed, Gurtner readily agrees that other methods might be developed that could be more effective. But for now, they've overcome a major hurdle in tissue engineering.

"Eventually science will find a way to fabricate an organ in all its

complexity," said Gurtner. "But in the short term we need to find more options for patients who are dying while waiting for transplants."

Source: Stanford University Medical Center