Fresh blood for damaged tissues via alginate hydrogels

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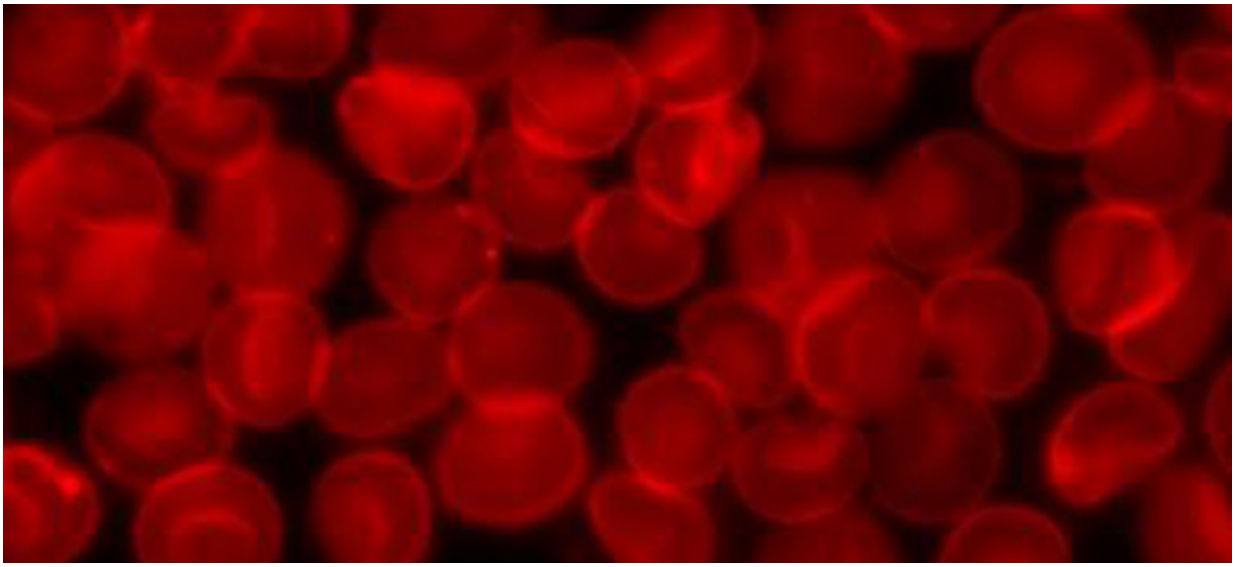

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Ischemia is a serious medical condition in which the flow of blood and delivery of oxygen to tissues is restricted, thus resulting in pain, weakness, and more seriously, tissue and organ damage. Ischemia in muscle tissue, most commonly as a result of atherosclerosis, leads to life-threatening diseases like coronary artery disease and stroke, but also to chronic peripheral artery disease (PAD). PAD symptoms can vary from discomfort and difficulty walking to debilitating pain and even limb amputations from irreversible muscle damage. Treatment methods

include blood-thinning medications and angioplasty or, in more severe cases, bypass surgery. However, a promising alternative to surgical intervention involves stimulating angiogenesis, or growth of new blood vessels, in order to increase blood flow at the ischemic site via delivery of angiogenic growth factors like Vascular Endothelial Growth Factor (VEGF) and Insulin-like Growth Factor-1 (IGF).

Injection of growth factors systemically, or directly into the blood vessels, is a straightforward approach to deliver recombinant growth factors into the body. This method, however, is not very safe and reliable as higher doses of factors are required for an effect, there is no specificity to targeting to the ischemic site, and proteins are not protected from degradation. To overcome these limitations, researchers are increasingly turning to biomaterial-based delivery of growth factors. One widely-used biopolymer found in brown seaweed is alginate. In its hydrogel, or water-swollen form, it is biocompatible, stable, cost-effective, non-toxic and easily crosslinks, or gelates in the presence of calcium. Doctors use alginates extensively for wound dressing and healing. Scientists have utilized them to build 2D and 3D matrices for cellular culture, and when imbued with growth factors and stem cells released as the gel dissolves, alginate hydrogels promote growth and regeneration in a variety of tissues.

A team at the Wyss Institute for Biologically Inspired Engineering led by David Mooney Ph.D., a Founding Core Faculty of the Wyss and the Robert P. Pinkas Family Professor of Bioengineering at Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS), engineered an alginate hydrogel infused with VEGF and IGF in order to promote vascularization in the ischemic hind limbs of aged mice and young rabbits and increase perfusion, or blood flow in the damaged tissue. This work was done in collaboration with the University of Michigan under Paul Grossman, where the rabbit studies were performed. The findings are reported in the *Journal of Vascular*

Research.

"Alginate hydrogels allow for gentle and protective encapsulation of therapeutic proteins and other biologically active molecules and cells," said Mooney. "With minimally invasive injections we can deliver a combination of these active molecules while controlling degradation rates, and our tests in different ischemia animal models are very promising," he explained.

To validate their gel-based delivery method, the Wyss team addressed a number of key questions. The first question concerns age. Previous animal work has been carried out in young mice that are not the age equivalent of older PAD patients and individuals enrolled in therapeutic angiogenesis clinical trials. A 17-month old mouse is roughly the equivalent of a 70-year old human; therefore 20-month old mice were selected for the aged cohort of this study. Another critical question is that of scalability. Mice are 3000 times smaller than humans and the protein dose must be appropriately scaled according to body mass. The team utilized young rabbits to assess the treatment and dose efficacy for a larger animal than the mouse. And to more accurately model the effect of their method on chronic artery disease, the researchers tested delayed treatment with growth factors.

The Wyss team began by ligating the major hind limb arteries of mice in order to generate ischemia models, and then injected the hydrogel containing VEGF and IGF directly into the ischemic site of the hind limb muscles. "We found that blood flow at the ischemic site in aged mice increased by 2-fold compared to the gel control without factors, or gel-less injection of VEGF and IGF. Additionally, these animals also showed more muscle strength and a higher number of small blood vessels at the injury site" said Alexander Stafford, a staff scientist at the Wyss and a leading author of this study. Younger mice also greatly benefited from the treatment as the blood flow in their muscles reached

levels comparable with the pre-ischemic state. The scientists also tested the stability of the new blood vessels over time and found that perfusion continues even at 3 months after treatment.

The findings in the young rabbit cohort mirrored those in mice as gel-delivery of IGF and VEGF promoted perfusion and growth of smaller blood vessels stemming from arteries adjacent to the ischemic site. Local delivery of gel-based angiogenic factors also enabled restoration of blood flow using a smaller and safer amount of growth factors as rabbits are 100-times larger than mice, but only required a 6.5-fold increase of the mouse dose. The Wyss researchers also compared perfusion recovery between rabbits treated immediately or 30 days post-ischemia, which models chronic ischemia. They found that the delayed treatment works just as well to increase perfusion to levels comparable with the immediately treated cohort. A larger number of capillaries, or small blood vessels, per area were also observed compared to the controls. This study establishes the efficacy and safety of localized alginate-based delivery of angiogenic recombinant growth factor proteins to promote vascularization and treat arterial blockage in older and larger animals.

Mooney's team plans to use these findings as groundwork for future clinical trials aimed at restoring perfusion to ischemic human tissues, such as the muscle damage caused by PAD. Additionally, the team plans to test the use of alginate hydrogel-based delivery in regrowth of damaged peripheral nerves.

"As we focus on developing new technologies that can bring positive impact on the world, we are very excited to see this demonstration that biomaterial-mediated growth factor delivery can restore perfusion of blood vessels in both mice and rabbits, as it brings us one step closer to treating disease in human patients," said Donald Ingber, M.D., Ph.D., Founding Director of the Wyss Institute, the Judah Folkman Professor

of Vascular Biology at Harvard Medical School (HMS) and the Vascular Biology Program at Boston Children's Hospital, as well as Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS).

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