Commentary: If you want blood...

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Intricate vascular network of the heart.

Central Message

Rapid advancements in regenerative biology and tissue engineering will likely provide novel therapeutic approaches to neovascularization.

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Perfusion is fundamental to the function of any organ in the human body, such that one of the most common etiologies of organ dysfunction is malperfusion or ischemia. As development proceeds in the embryo, every developing organ, regardless of the germ layer from which it originates, receives mesodermal angioblasts that form vessels *de novo* via a process called vasculogenesis.¹ As the vascular system continues to grow, these vessels bring oxygenated blood and nutrients into the organ and carry deoxygenated blood with waste products out of the organ. Perfusion is particularly relevant in an organ like the heart, which has a very high metabolic demand, extracting nearly 75% of the oxygen delivered to it.

The development of the highly complex and intricate coronary vasculature (Figure 1) is directed by temporally and spatially regulated molecular cues that are still incompletely understood.^{2,3} What is well known, however, is that impaired blood flow in the coronary vessels due to coronary artery disease has the potential to cause devastating damage to heart function. Every 40 seconds, someone in the United States has a myocardial infarction, and cardiovascular disease is the number one cause of death, carrying an annual health care cost of about \$351.2 billion.⁴ Myocardial ischemia can induce a variable, but routinely inadequate, degree of collateral vessel formation from pre-existing vessels, a process called neoangiogenesis, the extent of which appears to be patient- and disease-context specific.

Collateral vessels are less mature and poorly organized as opposed to native vessels. Heart muscle cells, or cardiomyocytes, in contrast, have severely limited capacity to regenerate in humans such that cell death usually results in the formation of a functionless fibrotic scar. Thus, current interventions in myocardial ischemia are limited to restoring perfusion via the native vessel by angioplasty or by establishing alternate routes of perfusion with coronary bypass. Given that in all of these approaches blood flow still relies to a variable degree on the native (hence diseased)

vasculature, disease progression and recurrence of ischemia is the norm. Biological therapy, borne out of painstaking translational research, holds promise as the next novel approach to tackle this vexing clinical problem.

Early molecular attempts to augment innate collateralization resulted in the formation of leaky immature vessels that did not reliably improve end-organ perfusion.⁵ Subsequent bench research shed light on vessel maturation factors, which allow for the stabilization of nascent blood vessels and the establishment of vascular hierarchy, eventually leading to integration into the native vasculature and improved perfusion. Preclinical trials have demonstrated the ability of these vessel maturation factors to improve recovery from ischemic insults.^{6,7} These positive results, however, have not translated into clinical benefit due, in part, to issues with reliable and sustained delivery of the molecules without systemic adverse effects. Delivery via drug-eluting stents to direct injection into the myocardium have been attempted in large animal models with variable results. Regardless, the search to favorably manipulate molecular control of angiogenesis and blood vessel maturation has intrinsic scientific merit and could have particular benefit for viable ischemic myocardium.

Late in the disease process, however, when a large section of functional myocardium is replaced by fibrotic scar, even robust revascularization cannot re-establish contractile function in this area. Trans-differentiating fibroblasts to cardiomyocytes to replenish the functional cellular

FIGURE 1. Coronary vasculature visualized by postmortem antegrade injection of polymerizing contrast agent.

unit⁸ has met with preclinical success but is yet to mature into clinical reality. Currently, the only viable clinical option for end-stage ischemic cardiomyopathy is transplant. The serious paucity of donor organs results in 20 deaths awaiting transplantation every day in the United States.⁹ Advances in tissue engineering could facilitate “growing” an entire organ, or a functional subunit thereof, in a dish.¹⁰ Science has allowed us to generate induced pluripotent stem cells derived from easily accessible adult cells, such as from the skin or blood. Thus, patient-specific organs or organoids that circumvent issues of rejection can now be generated. These tissues could be genetically engineered to simultaneously address other pathology. For example, a patient-specific heart that has the capability to secrete regulated amounts of insulin and anti-inflammatory molecules would be highly desirable for the patient with diabetic cardiomyopathy. However, as we scale up the size of these organoids, the issue of vascularization and perfusion to maintain their function in vivo becomes a real challenge.

In this issue of the *Journal*, Zhang and Radisic¹¹ eloquently elevate this new frontier by highlighting advances in the bioengineering of vasculature at the organ level. They discuss the application of 3-dimensional printing techniques, fabrication of newer-generation hydrogels, and the use of phenotypic plasticity of induced pluripotent stem cells to overcome the truly Martian hurdle of perfusing tissues generated in vitro. This is exciting progress and brings the vision of bioengineered solutions to complex clinical problems out of the realm of fantasy.

However, a lot remains to be clarified. What is the appropriate “dosage of per-capita” vasculature for a given sheet of cardiomyocytes? How does one ensure viability of the tissue for the time period required for it to integrate and be perfused from the native vasculature? Alternatively, would a sheet of cardiomyocytes come with its own major blood vessel that would be anastomosed in the patient to result in immediate perfusion of the organoid? Or should we be targeting a bioengineered blood vessels in isolation to be used as a conduit? What is our ability to generate smart products that are potentially capable of responding to environmental stimuli and self-repair?¹²

The very fact that we can even pose these questions is a testament to the tremendous progress in this field. We can all agree that the tenacity of human inquisitiveness and the power of rigorous scientific research have paved the way for steadfast advancement of humankind in the past. The progress detailed by Zhang and colleagues¹¹ should reinforce our faith in this process and encourage us to redouble our efforts to accelerate the translation of these exciting bench discoveries to the clinic.

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