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Previews

# Mending the Failing Heart with a Vascularized Cardiac Patch

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**Functional, stem-cell-containing cardiac grafts will require vascularized myocardial constructs to support their survival and integration into the host vasculature. Recently in *Tissue Engineering, Part A*, Lesman et al. (2009) reported the successful integration of vascular cells and hESC-derived cardiomyoctyes into stable grafts in rat recipients.**

Heart failure remains the leading cause of death in the world. As one of the least regenerative organs in the body, the heart wall is primarily composed of cardiomyocytes (CMs) and fibroblasts that are tightly packed with supporting vasculature and collagen-rich extracellular matrix. Following myocardial infarction, an enormous number of dead CMs are replaced by thick, stiff fibrous scars, resulting in the heart's reduced contractile function and, ultimately, heart failure. Clearly, understanding how such a complex organ develops from various different progenitors in the body will help us to unlock the full potential of stem cell biology for cardiovascular therapy (reviewed in Hansson et al., 2009). Cardiac tissue engineering endeavors to generate functional tissue constructs that can reestablish the structure and function of the injured myocardium. Human embryonic stem cells (hESCs) can generate bona fide CMs, as well as the supporting vascular cells. In an earlier study (Caspi et al., 2007), the Gepstein laboratory showed that, when CMs and endothelial cells (ECs) derived from hESCs are cultured with mouse embryonic fibroblasts (MEFs) within a three-dimensional scaffold, they recapitulate early embryonic heart development. Now, in their current work, the same group demonstrates that tissue-engineered vascularized cardiac muscle, generated ex vivo, can form stable grafts in vivo (Lesman et al., 2009).

Implantation of thick, complex constructs has always been a challenge in tissue engineering. Once implanted, the engineered cardiac grafts must endure a hostile ischemic microenvironment with a minimal diffused oxygen supply and high metabolic demand. Consequently, significant cell death follows transplantation, even in the absence of an injury model, mainly due to insufficient graft vascularization. The degree of graft vascularization is crucial not only for the survival and integration of the engineered cardiac grafts, but also for their proper contractile function. Prevascularization of cardiac grafts can be done either in vivo or in vitro. Recently, the Cohen laboratory designed a strategy for the in vivo prevascularization of cardiac grafts by heterotypic transplantation onto the omentum (Dvir et al., 2009). A porous alginate scaffold was seeded with neonatal CMs in a mixture of Matrigel with prosurvival and angiogenic factors; once tissue organization was achieved, the engineered cardiac graft was transplanted onto the omentum, a blood-vessel-enriched membrane, for further maturation and vascularization. A sustained release of angiogenic factors was able to attract ECs and other perivascular cells, resulting in a highly vascularized engineered cardiac graft. This graft was then explanted and transplanted onto an infarcted heart, demonstrating structural and electrical integration into the host myocardium.

An alternative strategy involves prevascularizing the engineered construct in vitro by providing vascular cells and angiogenic factors. Previously, the Langer laboratory demonstrated an approach for the in vitro prevascularization of skeletal tissue constructs (Levenberg et al., 2005). In vitro prevascularization was found to require the triculture of myoblasts, ECs, and fibroblasts to generate stable vascularization within the engineered skeletal muscle constructs. In their recent report, the Gepstein laboratory describes a well-designed and well-controlled study to engineer a vascularized cardiac muscle in vitro. Engineered cardiac tissue constructs were generated by culturing hESC-derived CMs, human umbilical vein endothelial cells, and MEFs in synthetic biodegradable scaffolds coated with Matrigel (Figure 1). After 2 weeks in culture, the engineered constructs were fully vascularized and exhibited synchronized beating. Upon grafting onto rat hearts, these triculture cardiac constructs exhibited a superior and more complex vascularization than transplanted scaffolds containing only hESC-derived CMs. Additionally, more $CD31^+$ and $\alpha$-$SMA^+$ cells were found in the triculture constructs, suggesting their integration with the host vasculature. These exciting findings support the hypothesis that perivascular cell precursors, such as embryonic fibroblasts, can serve as active partners with ECs in forming stable and functional blood vessels (Hirschi et al., 1998). The research suggested three vital roles played by embryonic fibroblasts in the formation of stable blood vessels. First, this population was spread diffusely throughout the cardiac graft, suggesting their role in laying down extracellular matrix to direct vascular morphogenesis. Second, MEF-derived cells were found mostly near the vessel wall and were positive for $\alpha$-SMA, suggesting that they differentiated into vascular smooth muscle cells to provide physical integrity to the vessels. Third, the triculture systems were found to highly express vascular endothelial growth factor, platelet-derived growth factor BB, and angiopoietin-1—all angiogenic factors necessary for the survival of

ECs. In a larger context, these findings highlight the importance of combining different cell types to capitalize on their synergistic roles in engineering functional tissue constructs.

Once the prevascularized cardiac constructs can anastomose with the host vasculature, the next challenge is to promote CM maturation and synchronization with the electrical syncytium of the existing myocardium, rather than maintaining their own spontaneous contractile activity. Indeed, upon engraftment, the prevascularized cardiac constructs were able to induce CM maturation into elongated and multinucleated myotubes, which contained gap junctions and were organized anisotropically (Lesman et al., 2009). Interestingly, the degree of CM maturation varied among rats, raising the question of whether the degree of graft vascularization affects CM maturation.

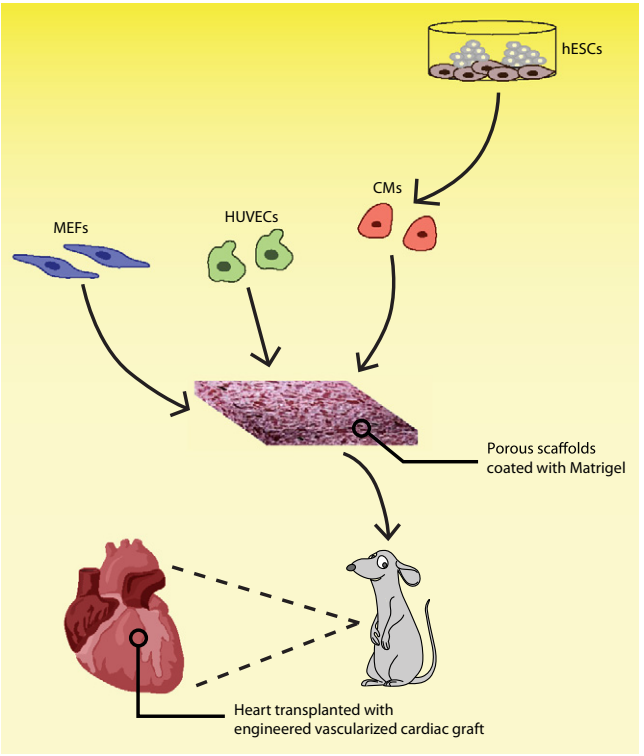


**Figure 1. Engineering Cardiac Tissue Constructs**
hESC-derived cardiomyocytes (CMs), human umbilical vein endothelial cells (HUVECs), and mouse embryonic fibroblasts (MEFs) were cultured in synthetic biodegradable scaffolds coated with Matrigel and transplanted into a rat heart.

Early and late markers of CM differentiation and maturation were highly upregulated in the triculture system but not observed in scaffolds containing only hESC-derived CMs (Lesman et al., 2009). These findings add to the growing body of evidence linking vascularization and tissue differentiation (Jain, 2003), which is crucial when bioengineering a complex and highly vascularized organ. Yet, a critical issue remains: the functional status of the engineered cardiac grafts. Because the hallmark of a functional adult heart is its ability to propagate intricate electrical impulses and to beat synchronously, a relatively immature CM must be further developed, for example by electromechanical stimulation (Vunjak-Novakovic et al., 2009), and tested in vitro prior to transplantation (Song et al., 2009). It would be interesting to investigate how the degrees of CM maturation, vascularization, and tissue perfusion affect the electrophysiological function of the engrafted heart.

As the emerging field of cardiovascular engineering moves closer to clinical application, this elegant work from the Gepstein laboratory raises several new questions for investigation. Recent exciting findings on induced pluripotent stem cells (iPSCs) open the possibility of creating patient-specific therapies for cardiac regeneration. However, engineering an autologous cardiac graft from iPSCs without genetic transformation remains to be addressed. Another critical challenge is to engineer a fully defined construct that can be transplanted into a patient without any animal byproducts. As a proof of concept, most cardiac grafts to date have used Matrigel or other animal-derived matrices to facilitate cell adhesion and vascularization. The challenge remains to develop a xeno-free matrix that mimics the in vivo microenvironments, directs CM differentiation, induces vascularization, and supports tissue organization. Combining the best of both worlds—stem cell biology and tissue engineering—the next era of cardiac regeneration could be closer than ever.

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