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Vascular Perfusion of Implanted Human Engineered Cardiac Tissue

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Abstract

Regeneration of muscle tissue in the heart after a myocardial infarction requires delivering human cardiomyocytes that will survive and integrate with the host myocardium. Of primary importance is the development of a vascular bed to nourish the implanted cardiomyocytes, whether delivered via injection or in engineered tissues. Co-culture of hESC-derived cardiomyocytes, human endothelial cells, and human stromal cells provides a prevascular network in scaffold-free engineered tissue patches. As a result, the density of lumen structures in the graft increases by histological analysis, but perfusion of these vessels must be assessed. In this study, we develop a method for perfusing the host heart and engineered human cardiac tissue graft that is compatible with confocal microscopy for obtaining 2D images and 3D reconstructions of the graft vasculature. We demonstrate that, although vascular density is substantial in the grafts, flow remains sluggish. Further improvements in arterial remodeling or vascular engineering are required for physiological levels of blood flow.

Keywords

cardiac tissue engineering; vascular perfusion; stem cell biology; myocardial infarction

I. INTRODUCTION

Regeneration of the infarcted heart using engineered tissue relies heavily upon successful vascularization of the tissue after implantation. Spontaneous vascularization of engineered human embryonic stem cell (hESC)-derived cardiac tissue by the host is far below the capillary density ($3000/\text{mm}^2$) of native adult human myocardium [1,2].

One approach to promoting rapid vascularization upon implantation is the use of co-culture where endothelial cells and stromal cells are added to cardiomyocytes during tissue formation. In doing so, in vitro endothelial cell networks form and can create vessel-like structures with densities up to $175/\text{mm}^2$ [3]. Upon implantation of these cardiac patches, human endothelial cells form lumens ($50/\text{mm}^2$) that contain host red blood cells [3]. However, to better understand how blood flow reaches the tissue, we must assess perfusion.

Intravenous lectin injection has been used to assess vessel perfusion (primarily from two-dimensional images) of cancer tumors [4], the infarcted heart after angiogenic therapy [5], and engineered tissue on uninjured hearts [1]. Thus, while this technique appears robust in a number of contexts, perfusion of the engineered tissue is minimal and limited to the periphery of the implant, suggesting a possible non-myocardial source of vessels (e.g. vascularization from chest adhesions) [1].

To assess perfusion of engineered cardiac tissue through either graft-derived or host-derived coronary vessels, we developed new procedures using retrograde perfusion of fluorescent Texas Red-tomato lectin via the aorta. Two-dimensional images showed perfusion of the graft. Imaging of thick sections with confocal z-stacks enabled visualization of three-dimensional vessel geometry. These methods are therefore tailored to assess graft perfusion from the host heart and offer a greater understanding of the morphology of vasculature and dynamics of blood flow in this system.

II. METHODS

Derivation of hESC-cardiomyocytes and formation of pre-vascularized scaffold-free engineered cardiac tissue patches containing cardiomyocytes, human umbilical vein endothelial cells, and human mesencymal stem cells are as previously described [3]. The patches were implanted onto the epicardial surface of uninjured athymic rat hearts as previously described and analyzed after one week [3]. All in vivo protocols were approved by the University of Washington Institutional Animal Care and Use Committee.

Perfusion was examined in the host tissue and implanted patch using injection of Cell Tracker CM-DiI (120 $\mu\text{g/mL}$; Molecular Probes) or Texas Red-conjugated *Lycopersicon esculentum* (tomato lectin; 1 mg/mL; Vector Labs). While tail vein injection was sufficient to label host heart vasculature, in situ retrograde perfusion through the aorta was required to visualize perfusion of graft and ensure a coronary source of the perfusion. To do this, the animal was anesthetized with inhaled isoflurane, the chest was opened, and the heart was exposed. A 25g butterfly needle was inserted into the ascending aorta (prior to the aortic branches) and a suture tied around it to secure it in place. The needle was connected with saline-filled tubing to a syringe containing the dye which was mounted on a syringe pump for delivery of the dye at constant, slow flow. (No pressure measurement was made.) The heart blanched with brief saline perfusion and turned bright pink/purple with perfusion of either DiI (4.2 mL, 500 μg) or Texas Red-lectin (0.7 mL, 700 μg). With DiI infusion, the right atrium was punctured to allow for drainage prior to dye infusion. With Texas Red-lectin, the right atrium was punctured 1 min after dye infusion. The dye was followed by perfusion of 10 mL saline and 5 mL 4% paraformaldehyde (PF). The heart was excised, rinsed in saline, and fixed in 4% PF for 1 hr at 4°C, followed by 30% sucrose dehydration overnight, embedding in OCT compound and sectioning of thin (6 μm) and thick (200 μm) sections mounted on glass slides. Tissue sections were imaged dry or with Vectashield with DAPI (Vector Labs) to counterstain nuclei and maintain tissue integrity for confocal microscopy.

III. RESULTS AND DISCUSSION

Initial experiments to visualize perfused vasculature used tail vein injections of DiI. Although air dried sections showed sharply delineated vessels, addition of Vectashield blurred the DiI signal. Because the grafts showed no perfusion with intravenous delivery of DiI, we next used retrograde perfusion of the aortic canula. This offered some evidence that large vessels were indeed perfused in the engineered tissue grafts (arrowheads), albeit not to the extent of the host heart (Fig. 1).

Improved imaging was achieved using Texas Red-lectin that specifically binds endothelial cells and did not blur with Vectashield and DAPI nuclear counterstain (Fig. 2).

Further, frozen sections were cut up to 200 μm thick to create 3D reconstructed images using confocal z-stacks to visualize large and small vessels (Fig. 3). These results suggest that tissue perfusion can be quantified for vascular volume, branching, and tortuosity within the limitations of the 200 μm thick sections. Future use of GFP-positive graft cells will provide clear delineation between graft and host, enabling comparison of perfused vasculature within the engineered cardiac tissue graft and host heart.

IV. CONCLUSION

Developing methods for assessing vascular perfusion in engrafted engineered cardiac tissue originating from the host heart is essential for understanding survival and integration of implanted cardiomyocytes. Our studies demonstrate that the graft vessels are not perfused as well as those of the host heart, likely reflecting the absence of a branching hierarchy at one week post-engraftment. In the future, perfusion-based imaging will influence the design of novel systems to pre-vascularize tissues and induce host vascular ingrowth.

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Fig. 1.
Retrograde perfusion of DiI in graft and host.

Fig. 2.
Host perfusion with Texas Red-lectin and Vectashield with DAPI (left, red channel; right, red and blue channels).

Fig. 3.
Three-dimensional reconstruction of confocal z-stack of 200 μm -thick host tissue with Texas Red-lectin.