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Inosculation and perfusion of pre-vascularized tissue patches containing aligned human microvessels after myocardial infarction

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ABSTRACT

A major goal of tissue engineering is the creation of pre-vascularized tissues that have a high density of organized microvessels that can be rapidly perfused following implantation. This is especially critical for highly metabolic tissues like myocardium, where a thick myocardial engineered tissue would require rapid perfusion within the first several days to survive transplantation. In the present work, tissue patches containing human microvessels that were either randomly oriented or aligned were placed acutely on rat hearts post-infarction and for each case it was determined whether rapid inosculation could occur and perfusion of the patch could be maintained for 6 days in an infarct environment. Patches containing self-assembled microvessels were formed by co-entrapment of human blood outgrowth endothelial cells and human pericytes in fibrin gel. Cell-induced gel contraction was mechanically-constrained resulting in samples with high densities of microvessels that were either randomly oriented (with 420 ± 140 lumens/mm^2) or uniaxially aligned (with 940 ± 240 lumens/mm^2) at the time of implantation. These patches were sutured onto the epicardial surface of the hearts of athymic rats following permanent ligation of the left anterior descending artery. In both aligned and randomly oriented microvessel patches, inosculation occurred and perfusion of the transplanted human microvessels was maintained, proving the *in vivo* vascularization potential of these engineered tissues. No difference was found in the number of human microvessels that were perfused in the randomly oriented (111 ± 75 perfused lumens/mm^2) and aligned (173 ± 97 perfused lumens/mm^2) patches. Our results demonstrate that tissue patches containing a high density of either aligned or randomly oriented human pre-formed microvessels achieve rapid perfusion in the myocardial infarct environment - a necessary first-step toward the creation of a thick, perfusable heart patch.

1. Introduction

The creation of engineered tissues containing microvascular networks that can be rapidly perfused within a few days following implantation remains a major goal of tissue engineering [1,2]. Applications that involve highly metabolic tissues, such as myocardium and liver, are especially dependent on the presence of a rapidly perfusable microvascular network to prevent the formation of a necrotic core beyond the diffusion limit in the implanted tissue [3–5]. Recruitment of vasculature via angiogenesis is a common method for vascularizing tissue engineered constructs *in vivo*, but this vascularization strategy is too slow to permit the survival of thick, highly metabolic tissues [2]. Implantation of tissues with preformed microvascular networks could enable rapid perfusion of thick engineered tissues by inosculation of the implanted microvessels with adjacent host blood vessels.

Many examples of creating “engineered microvessels” exist [6–11], and many groups are striving to create functional microvessel patches in macroscopic, implantable materials [6,12–18]. The most impressive of these studies demonstrate that

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implantation of pre-cultured human microvessels in immune-compromised mice can result in inosculation with the host vasculature and perfusion of the human microvessels [13,14,17]. However, these studies were carried out in the healthy subcutaneous environment, and no study has implanted pre-cultured human microvessels in the especially challenging myocardial infarct environment. With the ultimate goal of treating myocardial infarctions and heart disease with a perfusable, beating cardiac patch containing both microvessels and cardiomyocytes, the only fair way to assess the vascularization potential of a precursor patch containing only microvessels would be to implant it at the site of a myocardial infarction. This way, the insights gained from such a study could be leveraged in the future development of a patch that also contained cardiomyocytes.

Many studies in rodents have administered tissue-engineered heart patches onto myocardial infarcts containing various cell types [19–21], but implantation of human microvessels has not yet been reported. The Okano Group has shown that thin microvascular cardiac patches made from neonatal rat cardiomyocytes and rat endothelial cells can be perfused by the host and result in improved cardiac function after 4 weeks [22]. In our lab's prior studies we have implanted heart patches made from either neonatal rat heart isolates or human induced pluripotent stem cell derived cardiomyocytes co-entrapped with human pericytes. These heart patches were made in fibrin gel and cultured for 1–2 weeks to align the cells and fibrin. In both studies, the patches had no microvessels at the time of implantation, yet we observed perfused host-derived capillaries invading the patch as early as 1 week [19,20]. These results suggest the possibility that a similar patch that contained pre-formed human microvessels could rapidly inosculate with the invading host vessels and be perfused within 1 week.

The microvessel density and organization also needs to be addressed. In tissues that require efficient perfusion, the capillaries are generally highly organized in microvascular beds, with many capillaries spanning across the same arteriole and venule. In myocardium, the capillaries are not only very dense, but they are also aligned [23–25]. Therefore, emulating these two features in the engineered microvessels of a heart patch is highly desirable. Previous studies of pre-vascularized engineered tissues do not control microvessel alignment, nor do they achieve lumen densities within the same order of magnitude as native adult myocardium (2000 lumens/mm^2) [23,24]. To date, reports of pre-implant cross-sectional lumen density range from fewer than 200 lumens/mm [2] up to 650 lumens/mm^2, the latter of which was reported by our lab [6,26–29].

A necessity for a heart patch that would aim to restore mechanical function would be rapid connection to blood flow to maintain viability of the large number and high density of transplanted cardiomyocytes in a thick tissue-like patch. Ideally, this would be achieved by implanting a cardiomyocyte patch containing a pre-formed, perfusable microvascular network that could rapidly anastomose with the host and maintain perfusion. As a step toward that goal, the present study sought to determine the rapid vascularization potential of a remodeled fibrin patch containing either an aligned or randomly oriented microvascular network, but no cardiomyocytes, implanted onto a myocardial infarction for 6 days.

In this study we investigated the rapid *in vivo* vascularization potential of tissue patches pre-vascularized with either aligned or non-aligned human microvessels and implanted over myocardial infarcts. Patches containing human microvessels, were made by entrapping human blood outgrowth endothelial cells (BOECs) [30] and human pericytes (PCs) in fibrin gel, and allowing self-assembly of a microvascular network of tubules. Patches with aligned human BOEC/PC microvessels ("aligned microvessel patches") were anchored at both ends by porous plastic spacers, and aligned via cell-induced gel compaction, as previously described [12]. Briefly, as the samples compact laterally and remain constrained in the longitudinal direction by the spacers, the fibrils, cells, and the formed microvessels become aligned in the longitudinal direction. To investigate the effects of a patch lacking microvessels, patches made in a similar manner with only aligned PCs ("aligned PC patches") were also investigated. Patches with non-aligned microvessels ("isotropic microvessel patches") were made by maintaining gel adhesion to the bottom surface of the culture plate and preventing lateral compaction and longitudinal alignment.

The patches were sutured onto the infarcted region of the heart of nude rats immediately following LAD ligation, as we previously described for heart patches containing cardiomyocytes [19,20], and were implanted for 6 days. Perfusion of the patches was evaluated with species-specific endothelial-binding fluorescent labels injected into the left ventricle to circulate throughout the bloodstream prior to sacrifice. Immunohistochemistry on histological sections from explanted hearts was used to quantify the total number of human vessels in the patches. Aligned microvessel patches were predicted to have a greater number of perfused vessels after 6 days and isotropic microvessel patches were predicted to have some perfused vessels, but less than the aligned microvessel patches. Aligned PC patches were expected to recruit some host-derived microvessels by 6 days, but the total number of perfused microvessels (human + rat) was expected to be much lower than in the pre-vascularized patches.

While we did not expect these patches to have an effect on cardiac function or infarct size, as they were lacking cardiomyocytes, ejection fraction and fractional shortening were measured before implantation and at sacrifice to ensure any changes were recorded. The infarct was characterized by measuring the percent of the left ventricular wall occupied by scar as well as the left ventricular wall thickness.

2. Methods

2.1. Culture of human blood outgrowth endothelial cells and human pericytes

Human BOECs were isolated from adult peripheral blood by the lab of Dr. Robert Hebbel at the University of Minnesota – Twin Cities [30]. Briefly, BOECs were screened for VE-cadherin, flk-1, vWF, CD36, and CD14 (negative). Passage 5 BOECs were thawed and plated on 0.05 mg/ml collagen I – coated flasks in BOEC medium (EGM-2 bulletkit medium (Lonza) supplemented with 10% FBS, 1% penicillin/streptomycin (Gibco)). Medium was changed every other day and BOECs were passaged after 4 days, then plated and cultured for 4 more days prior to harvest.

Human brain vascular PCs (ScienCell, fetal, characterized by immunofluorescence with antibody specific to α-smooth muscle actin) were transduced to express GFP and obtained from the lab of Dr. George Davis at the University of Missouri. Passage 6 PCs were thawed and plated on 1 mg/ml gelatin-coated flasks in PC medium (13% FBS, 1% penicillin/streptomycin (Gibco), 10 ng/ml gentamicin (Gibco) in low-glucose DMEM (Lonza)). Medium was changed every 2–3 days and PCs were harvested after 10 days.

2.2. Creation of aligned microvessel patches

Rectangular molds (18.4 mm × 5 mm) were created by melting ridges into the bottom of a 6 well tissue culture plate. Porous polyethylene spacers (5 mm × 5 mm) were placed on top of a dollop of sterile vacuum grease at both ends of the rectangular mold leaving a central rectangular well (8.4 mm × 5 mm). Droplets