Tissue-engineered pro-angiogenic fibroblast scaffold improves myocardial perfusion and function and limits ventricular remodeling after infarction

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Objective: Microvascular malperfusion after myocardial infarction leads to infarct expansion, adverse remodeling, and functional impairment. Native reparative mechanisms exist but are inadequate to vascularize ischemic myocardium. We hypothesized that a 3-dimensional human fibroblast culture (3DFC) functions as a sustained source of angiogenic cytokines, thereby augmenting native angiogenesis and limiting adverse effects of myocardial ischemia.

Methods: Lewis rats underwent ligation of the left anterior descending coronary artery to induce heart failure; experimental animals received a 3DFC scaffold to the ischemic region. Border-zone tissue was analyzed for the presence of human fibroblast surface protein, vascular endothelial growth factor, and hepatocyte growth factor. Cardiac function was assessed with echocardiography and pressure–volume conductance. Hearts underwent immunohistochemical analysis of angiogenesis by co-localization of platelet endothelial cell adhesion molecule and alpha smooth muscle actin and by digital analysis of ventricular geometry. Microvascular angiography was performed with fluorescein-labeled lectin to assess perfusion.

Results: Immunoblotting confirmed the presence of human fibroblast surface protein in rats receiving 3DFC, indicating survival of transplanted cells. Increased expression of vascular endothelial growth factor and hepatocyte growth factor in experimental rats confirmed elution by the 3DFC. Microvasculature expressing platelet endothelial cell adhesion molecule/alpha smooth muscle actin was increased in infarct and border-zone regions of rats receiving 3DFC. Microvascular perfusion was also improved in infarct and border-zone regions in these rats. Rats receiving 3DFC had increased wall thickness, smaller infarct area, and smaller infarct fraction. Echocardiography and pressure–volume measurements showed that cardiac function was preserved in these rats.

Conclusions: Application of a bioengineered 3DFC augments native angiogenesis through delivery of angiogenic cytokines to ischemic myocardium. This yields improved microvascular perfusion, limits infarct progression and adverse remodeling, and improves ventricular function. (J Thorac Cardiovasc Surg 2010;140:667-76)

Ischemic cardiovascular disease is an increasingly prevalent global health concern. Traditionally, patients with ischemic cardiomyopathy are treated with either percutaneous coronary intervention or bypass surgery. However, a significant proportion of patients do not have anatomically correctable coronary disease. Additionally, conventional revascularization methods do not adequately address the microvascular destruction that accompanies a significant ischemic myocardial injury.[1-4] These factors have driven investigators to develop novel revascularization techniques that specifically target the microvasculature in ischemic myocardium.[5-7]

Induction of microvascular angiogenesis by angiogenic growth factors has been studied as one such approach. Vascular endothelial growth factors (VEGFs) are potent inducers of vascular growth and have been shown to produce transmural angiogenesis and improve myocardial perfusion.[8-10] Hepatocyte growth factor (HGF) likewise enhances myocardial angiogenesis, limits apoptosis, and improves postinfarct cardiac function.[11-13]

Despite these promising results, optimal delivery of angiogenic growth factors remains problematic. Systemic or intracoronary delivery, while preferable owing to ease of use and clinical applicability, does not achieve adequate concentrations in the myocardium to maximize efficacy.

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Abbreviations and Acronyms

α-SMA	= alpha smooth muscle actin
3DFC	= 3-dimensional human dermal fibroblast culture
dP/dt max	= maximum rate of pressure rise
EDV	= end-diastolic volume
ESV	= end-systolic volume
HFSP	= human fibroblast surface protein
HGF	= hepatocyte growth factor
IU	= intensity units
LAD	= left anterior descending coronary artery
LV	= left ventricular
LVID	= left ventricular internal diameter
LVIDd	= left ventricular end-diastolic diameter
LVIDs	= left ventricular end-systolic diameter
PECAM	= platelet endothelial cell adhesion molecule
SCID	= severe combined immunodeficient
VEGF	= vascular endothelial growth factor

Therefore, local delivery is desired to maximize effect.[14] Although recombinant proteins have a good safety profile, delivery via either direct myocardial injection or percutaneous catheter-based injection is confined to a single administration, temporally limiting the effects. Alternatively, viral gene transfer may achieve greater efficacy but is hampered by immune factors that may cause toxicity and diminished transgene expression.[15] The ideal delivery vehicle would achieve sustained growth factor expression in the zone of interest with limited toxicity.

Although a bioengineered sustained delivery mechanism is appealing, perhaps the ideal method of achieving prolonged local expression of angiogenic growth factors is recruitment or transplantation of fibroblasts. As a key mediator of wound healing and angiogenesis, fibroblasts are a robust source of VEGF and HGF, as well as other known and unknown factors. Additionally, a cellular source is more likely than a bioengineered delivery system to secrete the precise concentrations of growth factors required for an optimal angiogenic response. Because multiple investigators have shown that direct myocardial injection of cells results in poor cell survival,[16-19] and studies show that cell-seeded biocompatible sheets result in improved cell engraftment,[20,21] the current study uses a tissue-engineered, 3-dimensional human dermal fibroblast culture (3DFC) scaffold as a sustained local source of angiogenic growth factors. A similar 3DFC (Dermagraft; Advanced BioHealing, Inc, La Jolla, Calif) is approved by the Food and Drug Administration in the treatment of diabetic foot ulcers and is a safe, effective method of improving ulcer closure.[22] Our 3DFC, composed of structural extracellular matrix proteins and human dermal fibroblasts, currently marketed as Anginera (Theregen, Inc, San Francisco, Calif), is being studied as an angiogenic therapy for myocardial ischemia. Two previous studies have shown that the 3DFC supports myocardial angiogenesis and attenuates reduction in cardiac function in a severe combined immunodeficient (SCID) mouse model of ischemic cardiomyopathy.[23,24]

The purpose of this study is to examine in depth the angiogenic mechanisms of the 3DFC. It is hypothesized that the 3DFC serves as a sustained local source of VEGF and HGF to support angiogenesis after ischemic myocardial injury. It is further hypothesized that enhanced microvascular perfusion results in diminished adverse ventricular remodeling and cardiac functional loss in a rat model of ischemic cardiomyopathy.

METHODS

The 3DFC (Figure 1), composed of fibroblasts cultured on pieces of polyglycolic acid mesh, was stored at –80°C and then rapidly thawed, rinsed, and used immediately according to the previously published protocol.[25]

In Vitro Growth Factor Expression

A portion of 3DFC was cultured in endothelial basal medium-2 (Lonza Walkersville, Inc, Walkersville, Md), free of growth factors and supplements. A time course of growth factor expression by the 3DFC was generated. Media samples were collected at 0.5, 1, 1.5, 2, 3, 4, 10, and 24 hours after culture and analyzed via enzyme-linked immunosorbent assay for human VEGF-A (R&D Systems, Minneapolis, Minn) and human HGF (R&D Systems). Media-only samples served as a control.

Animal Care and Biosafety

Male Lewis rats weighing 250 to 300 g were obtained from Charles River (Boston, Mass). Food and water were provided ad libitum. This study was performed in accordance with the standard humane care guidelines of the ''Guide for the Care and Use of Laboratory Animals'' and the Institutional Animal Use and Care Committee of the University of Pennsylvania.

Ischemic Cardiomyopathy Model

Per a previously published protocol,[26] rats were anesthetized with ketamine (75 mg/kg) and xylazine (7.5 mg/kg), intubated with a 16-gauge catheter, and mechanically ventilated (Hallowell EMC, Pittsfield, Mass) with a tidal volume (mL) = $6.2 \times M^{1.01}$ (M = animal mass, kg) and respiratory rate (min^{-1}) = $53.5 \times M^{-0.26}$. A thoracotomy was performed in the left fourth intercostal space. A 7-0 polypropylene suture was placed around the mid–left anterior descending coronary artery (LAD) and ligated to produce a large anterolateral myocardial infarction of 30% of the left ventricle. The extent of infarction is highly reproducible in our hands and cardiomyopathy has been well documented.[11,27-29] In experimental animals, a 3DFC scaffold (0.75×0.75 cm^2) was sutured to the ischemic area. Control animals received a similar array of sutures with no scaffold. Notably, the two previous SCID mouse 3DFC studies showed no significant angiogenic response in animals treated with an acellular scaffold, suggesting that the angiogenic response results entirely from the cellular component of the 3DFC.[23,24] A fibroblast implantation control group was not used because the poor survivability of therapeutic cells when directly injected into ischemic myocardium would render this an inadequate control population.[16-19] The thoracotomy was closed and animals were implanted with identification

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