Incorporation bFGF in an Injectable Pericardial Matrix Gel: Effect on Acute Neovascularization Post-MI

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Statement of Purpose: Heart failure following a myocardial infarction (MI) continues to be the leading cause of cardiovascular disease-related deaths. Recent work has focused on the development of clinically relevant biomaterials for use in tissue engineering approaches to cardiac repair, including the design of scaffolds from natural materials such as collagen, fibrin, matrigel, and different materials derived from the extracellular matrix (ECM) of decellularized tissues including bladder, small intestinal submucosa and the myocardium [1]. Injected alone into the infarct in small animals, these materials promote vessel growth; some have also been shown to preserve cardiac function post-MI. Additionally, pro-angiogenic growth factors have been investigated for use in the repair of ischemic injury in the heart [2].

We have recently developed a potentially autologous scaffold derived from the extracellular matrix of decellularized pericardial tissue [3]. Previous work with a variety of ECM components has shown that when growth factors are immobilized on a scaffold, angiogenic potential is enhanced [4,5]. This can be done via chemical crosslinking or by loading the growth factor into materials that contain glycosaminoglycans (GAGs) that bind growth factors natively. We have previously shown the pericardial matrix to be biochemically complex, retaining sulfated GAG content. We therefore hypothesize that the incorporation of basic fibroblast growth factor (bFGF) with the injectable porcine pericardial matrix (PPM) will have an increased effect on acute neovascularization in the infarct region when compared to collagen combined with the growth factor

Methods: Porcine pericardia were collected from juvenile Yorkshire pigs and decellularized with 1% sodium dodecyl sulfate (SDS). Samples were lyophilized, milled, and pepsin-digested in 0.1 M HCl as modified from [6] . Solubilized ECM was brought to pH 7.4 and a concentration of 6 mg/mL before injection. Before dilution, bFGF was added to the matrix such that the final 75 µL injection contained 10 µg of growth factor. All solutions were sterile-filtered before use and all manipulation was performed in a biosafety cabinet.

Female Sprague Dawley rats were given an MI via 25 min occlusion-reperfusion. One week later, all rats received an injection of 75 µL of one of the following groups: PPM (6 mg/mL) with bFGF, collagen (2.5 mg/mL) with bFGF, bFGF in saline, collagen (2.5 mg/mL), PPM (6 mg/mL), or saline. On day 5 post-injection, all animals were euthanized. Before euthanasia, two animals from each group were perfused with dextran-conjugated fluorescent microbeads retrograde via the abdominal aorta in order to establish anastomosis of the vessels formed. All hearts were excised, fresh frozen in OCT, sectioned and stained with hematoxylin and eosin. Infarct screening was done to exclude hearts with small infarcts. For the remaining hearts, 5 sections throughout the center of the infarct were co-stained with anti-smooth muscle α-actin and fluorescently tagged isolectin to visualize vascular cells. Infarct regions were outlined and the arteriole density within the region was quantified. Any vessel that stained with the anti-SMA and had an average lumen diameter greater than 10 µm was included.

Results: The Blyscan assay indicated the PPM retained 26.5 ± 3.4 µg of GAG per mg dry ECM. Collagen contained no sulfated GAGs. Quantification of arteriole density showed that only the PPM+bFGF group had a significantly increased arteriole density within the infarct region ($p < 0.05$) when compared to the controls (Figure 1A, B). PPM+bFGF had a density of 33.4 ± 11.8 arterioles/mm^2 while the collagen+bFGF, bFGF alone, PPM, collagen, and saline groups which had arteriole densities of 15.7 ± 6.6, 14.6 ± 5.1, 11.9 ± 6.1, 11.1 ± 6.6, and 10.4 ± 2.8, respectively (n = 5-7). Binning the data into diameter ranges, it was observed that the majority of vessels (70-75%) have average diameters of 10-25 µm; PPM+bFGF produced a significantly greater number of arterioles in this range compared to the other groups. Additionally, anastomosis was confirmed by the presence of microbeads within the arterioles formed in the infarct region, as seen in Figure 1C.

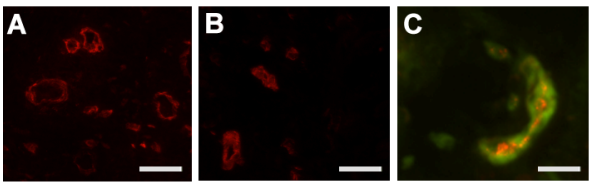

Figure 1: Smooth muscle cells (red) in the infarct region of PPM+bFGF (A) and Collagen+bFGF (B) injected animals, scale bar = 50 µm; endothelial cells (green) with fluorescent microbeads (red) in a PPM+bFGF-injected animal, scale bar = 25 µm (C).

Conclusions: As previously shown, decellularized and processed porcine pericardial matrix retains GAG content and therefore the potential ability to bind growth factors. The increased acute vascularization response seen in the PPM+bFGF group indicates that this may be allowing the growth factor to have a prolonged effect when compared to injection in saline or collagen. The greater proportion of small vessels is understandable because quantification was done only 5 days post-injection. Thus, this approach to growth factor incorporation in a biochemically complex ECM material could allow for growth factor immobilization via the mechanisms observed natively, circumventing the need for additional chemistry to link the small molecules to an ECM protein such as collagen.

References:

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