Design and Characterization of a Decellularized Pericardial Matrix Gel for Cardiac Tissue Engineering

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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. Thus, there is a need for the development of clinically relevant biomaterials for use in tissue engineering approaches to cardiac repair. Recent work has included the design of scaffolds from natural materials such as collagen, alginate, matrigel, and different materials derived from the extracellular matrix (ECM) of decellularized tissues including bladder, small intestinal submucosa, and myocardium [1-3]. The objective of this project was to determine if the pericardium could be used as a platform for developing a potentially autologous decellularized matrix gel for the treatment of MI.

Decellularized porcine pericardium is a widely used, FDA-approved biomaterial. Pericardial tissue is of particular interest clinically because it is not essential to life; it can be surgically resected without adverse consequences. In fact, the pericardium is routinely left unsutured after a procedure, to avoid the risk of cardiac tamponade. Human tissue-derived ECM has the unique advantage of offering an autologous therapy, circumventing the regulatory, ethical, immunogenic, and disease transfer concerns that hinder the clinical translation of xenogeneic materials. In this work, matrix gels made from both porcine and human pericardia are characterized and proof-of-concept is established for their use as scaffolds for cardiac repair.

Methods: Porcine pericardia were collected from juvenile Yorkshire pigs. Human pericardia were surgically resected from consenting patients undergoing a previously scheduled cardiothoracic surgery. Porcine and human pericardia were decellularized with 1% sodium dodecyl sulfate (SDS); decellularization was verified with histological analysis. Samples were lyophilized, milled, and then pepsin-digested in 0.1 M HCl as modified from [4] . Solubilized ECM was neutralized for experimentation and characterization.

SDS-polyacrylamide gel electrophoresis (PAGE) with an Imperial Protein stain was used to compare porcine pericardia ECM, human pericardia ECM, and collagen. Mass spectroscopy (MS) was used to identify peptide fragments and a Blyscan assay was performed to quantify glycosaminoglycan (GAG) content. To determine *in vivo* feasibility, solubilized ECM was injected into the left ventricular (LV) free wall of male Sprague-Dawley rats. After 45 minutes (n=2) and 14-15 days (n=4) hearts were excised, frozen, sectioned, and co-stained for smooth muscle, endothelial, and stem cells in the injection region using anti-smooth muscle α-actin, fluorescently tagged isolectin, and anti-c-kit, respectively

Results: Histological analysis of the decellularized tissue indicated an absence of nuclei. Gel electrophoresis showed both ECM samples to have bands matching those in collagen as well as additional bands at smaller molecular weights, indicating a diversity of proteins and peptides. MS detected the presence of additional ECM fragments, including those from elastin, fibrillin, fibrinogen, and keratin. GAG content was found to be 125.9 ± 6.6 and 136.5 ± 1.1 μg per mg of ECM for porcine and human samples, respectively. Results of the *in vivo* studies showed vascularization within the injection region after two weeks [Figure 1A,B], with an average of 51 ± 42 and 78 ± 13 arterioles per mm^2 for porcine and human samples, respectively. c-Kit+ cells were found in injection regions for both matrix gels [Figure 1C,D].

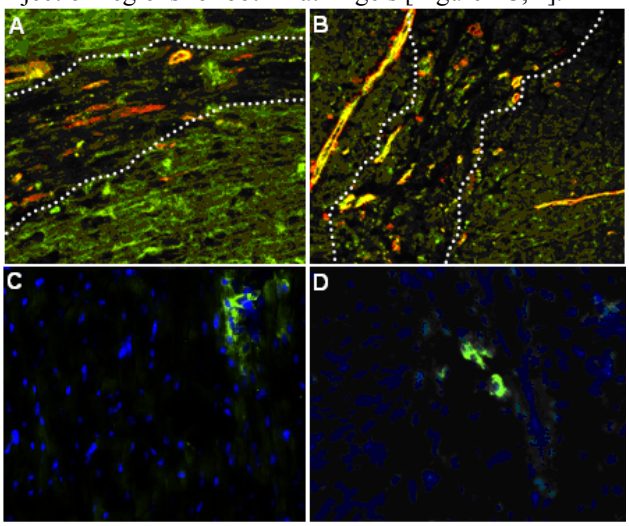

Figure 1: Smooth muscle cells (red) and endothelial cells (green) in human (A) and porcine (B) matrix injection regions. Nuclei (blue) labeled for c-Kit (green) in porcine (C) and human (D) matrix injection regions.

Conclusions: In this proof-of-concept study, it was determined that a solubilized form of decellularized human and porcine pericardia could be injected and induced to gel *in vivo*. With clinical translation as the ultimate goal, it is important to note that as injectable, *in situ* gelling biomaterials, these pericardial matrix gels can be administered via minimally invasive methods. The pericardial matrix gels retained a high degree of compositional complexity and were capable of causing neovascularization *in vivo,* demonstrating feasibility as an injectable treatment. Additionally, c-Kit+ cells were identified within the injection region, indicating the potential for stem cell migration into the matrix gel. It was interesting to note that while the porcine tissue was collected from young, healthy animals and the human tissue was donated by older, diseased individuals the vessel ingrowth was comparable. Finally, an exciting result of this work is the identification of pericardial tissue as a novel source for an autologous scaffold for treating MI.

References:

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