Microchanneled and Microporous Fibrin Scaffolds for Cardiac Tissue Engineering

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Statement of Purpose: Cellular cardiomyoplasty to replace non-functional tissue following cardiac infarction appears clinically viable. Current strategies utilizing direct injection of cell suspensions are limited by low cell retention, poor cell localization, and high cell death. Synthetic biomaterials developed to enhance cell delivery can lead to problems with immune rejection, degradation, and mechanical mismatch, preventing functional integration of constructs with host myocardium. The goal of this project is to develop a functional cardiac tissue construct with enhanced host integration capabilities as a novel and improved strategy to replace damaged myocardium. We have developed a novel templated fibrin scaffold seeded with a tri-cell mixture of cardiomyocytes, endothelial cells, and stromal support cells, in order to promote functional integration of the scaffold with host myocardium. The novel scaffold architecture includes 1) microchannels spanning the length of the scaffold, allowing alignment of cells to mimic native cardiac tissue structure, and 2) micropores to enhance construct survival by improving nutrient delivery, waste removal efficiency, and encouraging vascular invasion (figure 1). Fibrin is an ideal scaffold material because it can be autologous, it induces angiogenesis, improves cell attachment and growth, and degrades into natural byproducts. Linnes *et al*. have developed a method for templating a microporous fibrin scaffold with mechanical properties much closer to cardiac tissue than fibrin gels or glues. By combining this fibrin templating technique with our novel microchanneled architecture and tri-cell seeding, we have designed a cardiac tissue scaffold uniquely suited to enhancing functional integration.

Methods: Optical fibers (Paradigm Optics) with a 60 µm inner polycarbonate (PC) core and a 30 µm poly(methyl methacrylate) (PMMA) outer shell are bundled and sintered to form a solid PMMA matrix with PC cores. The PMMA matrix is selectively dissolved away and replaced with PMMA microbeads (30 µm diameter), which are then sintered to form a neck diameter 50% of the bead diameter. A dense (200 mg/mL) fibrinogen solution is centrifuged into this template, and a concentrated thrombin solution is used to polymerize the fibrinogen in place. Both polymers are dissolved away, resulting in a microporous fibrin scaffold with 60 µm channels spaced 60 µm apart. Scaffolds are centrifuge-seeded down the length of the channels with a tri-cell mixture of cardiomyocytes, endothelial cells, and stromal support cells (1:1:0.5) at a density of 1.0×10^6 cells/scaffold. Seeded constructs are incubated at 37 °C in static or rotational culture. Constructs are evaluated based on mechanical stiffness, and via histological and SEM analysis.

Results: Fibrin scaffolds had a stiffness of approximately 16.0 ± 3.0 kPa. Centrifuge seeding with the tri-cell mixture increased scaffold stiffness to 38.3 ± 8.9 kPa. Stiffness decreased over time in culture (25.2 ± 3.1 kPa, Day 6). Patches of beating cells were observed inside channels within two days in culture. After three days in culture, histological analysis showed uniform seeding down the length of the channels and in cross-section, as well as cardiomyocyte and lumen formation by endothelial cells within channels.

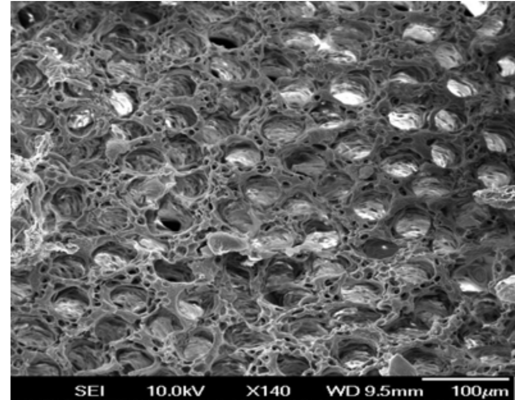

Figure 1. SEM image of microporous and microchanneled fibrin scaffold.

Conclusions: The microchanneled and microporous fibrin scaffolds were shown to have mechanical stiffness (16.0 ± 3.0 kPa) closer to native myocardium than fibrin gels (0.5 to < 7 kPa). Upon the addition of the tri-cell mixture, the construct stiffness increased to values near that of neonatal myocardial tissue (~40 kPa). Matching the stiffness of the constructs to that of myocardial tissue is vitally important in order to prevent mechanical mismatch upon implantation, which would ultimately prevent functional integration with the host myocardium. In addition, the construct stiffness decreased over time in culture, which may indicate the cells have begun to degrade the scaffold and produce their own replacement extracellular matrix (ECM), an ideal property of a biodegradable scaffold. Histological analysis indicates a nearly uniform seeding of the scaffolds both in cross-section and down the length of the channels. Staining for a cardiomyocyte-specific marker showed the presence of seeded cardiomyocytes within the 60 µm channels. Additionally, staining for an endothelial-specific marker showed immature lumen formation within the channels. This indicates the microchanneled architecture will likely enhance anastomosis with the host vasculature upon implantation, a process that is vital to support cardiomyocyte survival and contractile function. These results indicate micro-templated fibrin scaffolds are a unique and viable platform for cardiac tissue engineering. Future studies will focus on optimizing the cell seeding and culture conditions in order to obtain a more developed and organized cellular structure, which will improve the overall functionality of the tissue construct.

References: (Linnes M. et al, Biomaterials. 2007;28(35):5298-5306.)