Fabrication of highly cellularized tissue constructs mimicking anisotropy and mechanical properties of the cardiac muscle

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Statement of Purpose: Congestive heart failure (CHF) is a high mortality cardiovascular disease affecting more than 5 million Americans and increasing at a rate of 550,000 new cases each year. CHF is characterized by a dilated, relatively thin-walled ventricle, and inability to pump a sufficient amount of blood to meet the metabolic requirements of the body. Tissue engineering approach has been explored to treat CHF aiming to attenuate myocardial remodeling, improve cardiac function, and/or regenerate new myocardium. It involves engineering cellularized 3-D cardiac patch that can be subsequently placed on the infarcted epicardium. Current cardiac patch tissue engineering has significant limitations: 1) the mechanical properties of the cardiac patches often mismatch those of the myocardium and thus mechanical signals from the myocardium do not transfer well to the cells inside the cardiac patch; 2) the patches do not possess structural properties similar to those of the myocardium; and 3) the patches lack a large cell population. To overcome these limitations, we fabricated highly cellularized tissue constructs that mimic structural and mechanical properties of the myocardium, by employing electrospinning and electrospraying techniques. The low modulus polycarbonate based polyurethane was used as scaffolding polymer, and mesenchymal stem cells (MSCs) capable of differentiation into cardiomyocytes were employed as cell type.

Methods: Polyurethane (PU) elastomer was synthesized by using a pentablock copolymer PTMC-PEO-PPO-PEO-PTMC as soft segment, 1,4-diisocyanatobutane as hard segment and 1,4-diaminobutane as chain extender. The pentablock copolymer was synthesized by ring-open polymerization of trimethylene carbonate (TMC) initiated by PEO-PPO-PEO. The synthesized PU had tensile strength of 8.1 MPa, breaking strain of 362% and modulus of 5.6 MPa. Tissue constructs were fabricated by simultaneously electrospinning PU nanofibers and electrospraying human MSCs (modified from [1]), both were deposited on a rotating drum (1500 rpm). Three different cell densities (8, 15 and 30 million/mL) were utilized. This fabrication process generally lasts for 40 min to yield a tissue construct with thickness ~200 um. The tissue constructs were cultured either statically or dynamically in a spinner flask for 14 days. Cell growth was characterized by MTT and DNA assays. Extracellumar matrix GAG content was also measured. Cell morphology was characterized by live cell staining using CMFDA and observed under confocal microscope.

Results: To examine if MSCs can tolerate high voltage, cells electrosprayed under 20 KV were evaluated in terms of their growth, morphology and differentiation capability. The electrosprayed MSCs were found to have the same growth rate and morphology as those un-electrosprayed cells. Moreover, they showed their ability of osteogeneic and chondrogenic differentiation. These data demonstrated that MSCs can tolerate electrical treatment under appropriate voltage. The fabricated tissue constructs had aligned structure with degree of alignment greater than 72.5%. The tissue constructs exhibited anisotropic mechanical properties as confirmed by biaxial mechanical testing, with alignment direction possessing higher mechanical strength and modulus than its perpendicular direction. Tissue constructs were flexible and relatively strong with breaking strains ranging from 115% -165%, tensile strengths ranging from 0.9-1.4 MPa and moduli ranging from 0.3-0.8 MPa (alignment direction), depending on the cell density. The breaking strains and tensile strength were higher than those of the myocardium while moduli were similar or slightly higher than those of the myocardium. These mechanical properties are hypothesized to effectively decrease wall stress of the infarcted myocardium. MSCs were found to be alive within the tissue constructs and distributed at different thickness of the tissue constructs (Figure 1). MTT, DNA and GAG assays demonstrated that MSCs can grow within the tissue constructs when culturing dynamically in a spinner flask during a 14-day culture period. For statically cultured tissue constructs, the cell number significantly decreased ($p<0.05$), attributing to poor nutrient transport within the tissue constructs.

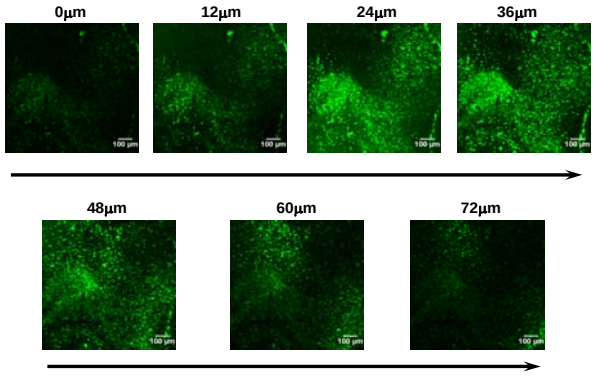

Figure 1. Z-series CLSM images of CMFDA stained MSCs in tissue constructs.

Conclusions: We present here a technique to rapidly fabricate stem cell integrated, aligned fibrous scaffolds mimicking anisotropy and mechanical properties of the myocardium.

References:

1. Stankus JJ. Biomaterials 2006, 27: 735–744.