Cardiac Commitment on Compliant Polymeric Substrata

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Statement of Purpose: Engineered myocardium may help the heart regain function after damage. To maintain consistent and synchronous beating, the engineered tissue must be able to deform the growth substrata. Elastomeric materials promote contraction while rigid materials can inhibit beating. Therefore, we chose four materials with differing elasticity poly(D,L-lactic-co-glycolic acid) (PLGA) (85:15), poly(D,L-lactide-co-caprolactone) (PLCL) (50:50), thermoplastic polyurethane (PU), and poly(glycerol sebacate) (PGS) [1], and created porous scaffolds to examine the effects of elasticity on cardiac commitment. We used two cardiomyocyte cell models. DMSO treated P19 embryonal carcinoma cells were the initial test model of choice [2-3], followed by clinically relevant human mesenchymal stem cells (hMSCs).

Cells were seeded onto 3D porous scaffolds and examined for several cardiac markers by immunocytochemistry and qRT-PCR (α-actinin, myosin heavy chain, tropomyosin, sarcomeric actin, troponin T). The contractile function of the cells was determined by monitoring voltage of the pulsations. Morphology of the cells in contact with the scaffolds was observed with scanning electron microscopy (SEM). Results indicate that the PGS elastomer and PU thermoplastic elastomer allow for contractility of the cardiomyocyte-like cells and may be appropriate substrates for fully functional heart patches. The hMSC-derived cardiomyocyte-like cells displayed numerous cardiac markers and may be an acceptable cell type for engineering myocardium.

Methods: The porous scaffolds were prepared by a solvent casting/salt leaching method [4], and tested for mechanical compliance and biodegradation rate. Embryonal carcinoma (EC) P19 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and hMSCs were maintained in MSC Basal Medium and SingleQuot Kit (Lonza).

P19s and hMSCs were induced to differentiate into cardiomyocytes by cultivating into embryoid bodies using the hanging drop method [3,5]. After 2-3 days (depending on cell culture model), the embryoid bodies were transferred to the bottom of the petri dish, and further cultivated for 5 days. They were then seeded on scaffolds and grown for an additional 11-14 days.

Protein expression and mRNA were analyzed for α-actinin, myosin heavy chain, tropomyosin, sarcomeric actin, troponin T, and nuclei were conducted, and expression observed using a *Zeiss Observer Z1*. SEM was conducted using a *Philips XL30 ESEM*. Contractile ability was assessed by voltage readings.

Results: SEM was used to examine the morphology of the porous scaffolds (Fig. 1). The images confirm that the scaffolds are highly porous, with large pores (approximately 150-300 μm). The mechanical compliances of the individual materials are: 0.07 MPa (PGS), 63.05 MPa (PLCL), 40.23 MPa (PLGA), and 0.7754 MPa (PU).

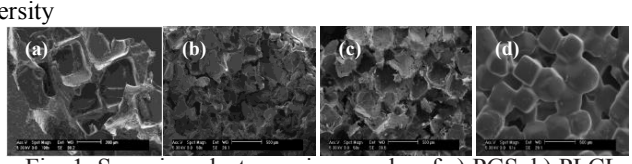

Fig. 1. Scanning electron micrographs of a) PGS, b) PLCL, c) PLGA, and d) PU before cell seeding.

The contractile ability of the cells on the scaffolds was assessed. PGS, the only material with a comparable elastic modulus to myocardium, supports the greatest amount of cell contraction. The hMSC-derived cardiomyocyte-like cells displayed an aligned structure in the control condition, while the P19-derived cardiomyocyte-like cells did not (Fig. 2). Additionally, myofibrillar organization in the hMSC-derived cells could be detected by the α-actinin expression. On scaffolds, the protein expression is the most significant on PGS and PU for both cell types, signifying successful cardiomyocyte growth and development. Protein expression is limited on PLCL and PLGA, indicating limited differentiation. The mRNA expression levels of cardiac markers (ex. β-myosin heavy chain) are significantly higher than undifferentiated markers (ex. Oct-4) on the polymers that supported significant contractile ability.

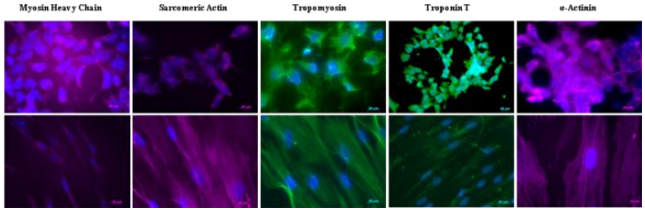

Fig. 2. Expression of cardiac markers in the differentiated P19 cells (top) and the differentiated hMSCs (bottom)

Conclusions: This study reports the differentiation and function of P19 and hMSC-derived cardiomyocyte-like cells on four different porous polymeric substrates of differing elasticity. PGS, an elastomer with an appropriate elastic modulus for a heart patch, supported the greatest amount of cell contraction, followed by PU. Contractility on the other substrates may not be sustainable. Protein expression for cardiac markers was greatest in the cells on PGS and PU. Genes for cardiac markers were up-regulated in both cell types on PGS and PU, but were down-regulated in cells on PLCL and PLGA. Therefore, PGS or PU scaffolds appear to be suitable scaffolds for engineering hMSC-based myocardium.

References:

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