Muscle Cells Align in 3D when Seeded in Channels Patterned into Porous Hydrogels

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Statement of Purpose: Myocardial infarction causes permanent damage to heart muscle, but current therapies only alleviate symptoms and do not heal the tissue. This research focuses on tissue engineering as a strategy to create living, organized muscle tissues integrated into 3D scaffolds. We aim to design mechanically robust scaffolds with tailored architectures whereby 3D channels are created to guide cell alignment and porous substrates are employed to promote nutrient transport. The goals of this study were two-fold. First, we investigated the mechanical properties of several porous hydrogel substrates. We evaluated their compatibility with the mechanical requirements of the native heart tissue where the scaffold must easily and repeatedly stretch 10%[1]. Secondly, we investigated a range of open, horizontal 3D channels within a hydrogel to promote high-density culture of muscle cells and promote their alignment in 3D. While previous studies have indicated good cell alignment and formation of gap junctions with thin, 2D patterned lanes[2,3], we present here the ability to promote alignment in high-density cultures within thicker and wider 3D patterned channels.

Methods: For tensile testing of porous hydrogels, monomer solutions were made of 65 or 80% (v/v) poly (ethylene glycol) diacrylate (PEGDA) (MW 500), 20% (w/w) PEGDA (MW 3000), or 50, 65 or 80% (v/v) 2-hydroxyethyl methacrylate (HEMA) containing photoinitaitor and photopolymerized (365 nm, 4 mW/cm^2, 10 min) around poly (methyl methacrylate) (PMMA) templates made with spheres of diameters of 57 ± 5 μm or 163 ± 10 μm in a dog bone shaped mold. The spheres were dissolved in acetone. After equilibrating in water, samples were stretched at 15% / min, and the modulus, maximum stress and strain were calculated.

For channel studies, a thiol-ene chemistry[4] was used to create hard, photopolymerized 3D inverse molds, with dimensions ranging from 40 x 40μm to 300 x 300μm. PMMA sphere templates were fabricated around the mold, and porous scaffolds were made as described above with a 20% (w/w) PEGDA (MW 3000), .1% (w/w) Irgacure 2959 and 5 mM acryloyl-PEG-RGD precursors. Scaffold width and depth were analyzed by confocal microscopy after incorporation of acrylated rhodamine. The scaffolds were rinsed in PBS overnight, and either skeletal myoblasts (C2C12, ATCC) or primary neonatal rat cardiomyocytes were seeded at a density of 40,000 cells / cm^2 into the channels. Cultured cell-scaffold constructs were analyzed at 24 hr intervals. Cell viability was analyzed with calcein AM (live) and ethidium homodimer-1 (dead). Cytoskeletal structure, alignment and gap junction formation were observed through immunohistochemistry with phalloidin, Troponin I, and connexin-43.

Results: Tensile testing indicated no statistical change in the maximum strain of samples with either 57 ± 5 μm or 163 ± 10 μm pores from the maximum strain values of non-porous hydrogels for a given hydrogel formulation. For example, 65% pHEMA resulted in the largest maximum strain at 0.42 ±.03, while 65% PEGDA (MW500) resulted in the smallest maximum strain at 0.12 ±.01. Incorporation of pores did affect the ultimate tensile stress (UTS), however. In pHEMA samples of all densities, the maximum stress decreased significantly with small pores and decreased even more with larger pores. In the PEG samples, pores caused a significant decrease in the UTS, but there was no difference as a function of pore size.

When channels were integrated into the hydrogels, alignment of skeletal myoblasts (used as screening cells) were observed exhibiting aligned intracellular F-actin filaments when cultured in channels with widths of 40, 100, or 200μm. Cell alignment was observed at least four cell layers deep, but appeared to be dependent on the location. The bottom layers exhibited similar isotropy as 2D patterned scaffolds, but more superficial layers within the channels exhibited superior alignment. Cells remained viable over the course of the study of several days. We recently combined porous scaffold development with channeled architectures (Fig. 1c) to enhance nutrient transport via large pores.

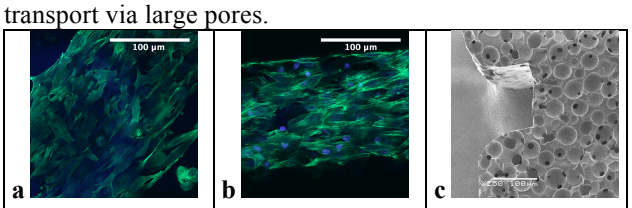

Figure 1. F-actin (green) and DAPI (blue) in skeletal myoblasts cultured for 48 hours on a) a 2D, 100 μm wide, .5 mM RGD-patterned PEG hydrogel surface and b) a 3D 100x100 μm channel with 0.5 mM RGD. c) SEM image of pHEMA hydrogel with simultaneous pores (40 μm) and channels (130μm wide, 130μm tall).

Conclusions: Our findings indicate that hydrogels can be tailored with robust mechanical properties even with the addition of pores reaching maximum strains well above those required for the heart. In addition, 3D architectures in the form of macro-channels are capable of guiding cell alignment in 3D in channels that are 200 μm wide and multiple cell layers deep. Studies are underway to investigate cardiomyocyte alignment and the formation of gap junction within these 3D structures. With our ability to create patterned and porous scaffolds, we hypothesize that these scaffolds will support high-density cell culture of viable cardiomyocytes, while providing cues for alignment making them attractive platforms for cardiac muscle regeneration.

References: [1]Zimmermann WH. Circ Res. 2002; 90:223-230. [2]McDevitt TC. J Biomed Mater Res A. 2002; 60: 472-479. [3]Rohr S. Circ Res. 1991; 68:114-130. [4]Lu H. Dent Mater. 2005; 21:1129-1136.