Controlling the 3D architecture of hydrogel scaffolds for tissue engineering

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Statement of Purpose: The design of the scaffold has increasingly become an important factor in tissue engineering. The ability to create scaffolds with a wide range of geometries that can be tailored to suit a particular cell type or tissue is key to the success of tissue engineering. Photopolymerized hydrogels are attractive for tissue engineering because of the high water content, tissue-like elastic properties and spatial control over the polymerization.[1,2] Herein, we describe a simple method to create patterned structures in porous hydrogels. Our approach combines a sphere-templating technique to control the pore microstructure[3] with a novel photo-lithography process to control the gel macrostructure.[4] Poly(2-hydroxyethyl methacrylate) (pHEMA) and poly(ethylene glycol) (PEG) hydrogel scaffolds were fabricated. Cardiomyocytes were seeded onto these scaffolds to assess their potential as scaffolds for cardiac muscle tissue engineering.

Methods: A microsphere template was prepared with sieved uncrosslinked poly(methyl methacrylate) microspheres placed between two glass slides separated by 750 μm spacers, sonicated for 10min, and heated to 140°C for 19hrs to fuse the beads. For patterned gels, a photomask (i.e., a pattern printed onto transparency film) was used. The monomer solution was purged with nitrogen for ~2min, poured over the microsphere template and irradiated with 365 nm collimated light for 35s. The monomer solution contained 80% of either purified HEMA and 2 mol% crosslinker/mol HEMA (poly(ε-caprolactone)-b-tetraethylene glycol-b- poly(ε-caprolactone) dimethacrylate) or PEG dimethacrylate (1000 MW) in 20% ethylene glycol/dH_2O with 1.5% (w/v) 2,2-dimethoxy-2-phenyl-acetophenone. Post polymerization, the sphere template was dissolved with 90% acetone:10% water. The gels were imaged using scanning electron microscopy (FEI Sirion 30, 1kV beam). Cell adhesive proteins (collagen) or oligopeptides (RGD) were immobilized on the scaffolds. Rat neonatal ventricular cardiomyocytes isolated from 1-2 day old pups were seeded onto the scaffolds at 1-2 million cells/ scaffold and cultured for up to 7 days. Cell-scaffolds were assessed via histology or live cell imaging.

Results/Discussion: Porous hydrogels based on p(HEMA) and PEG were fabricated via a light-initiated chain polymerization reaction around a microsphere template to produce highly uniform and monodisperse pore structures. The addition of the monomer solution to the sphere-template caused a change in appearance from opaque to translucent enabling the transmittance of initiating light and subsequent polymerization around the microsphere template. Two pore sizes were produced: 60±5 or 150±15 μm. Cardiomyocytes adhered to pHEMA and PEG scaffolds when treated with cell adhesive proteins or oligomers as evidence by positive staining for live cells (a membrane integrity fluorescent marker) and the cardiomyocyte specific marker, α-sarcomeric actin. For example, Fig 1 illustrates neonatal cardiomyocytes seeded onto porous PEG hydrogels modified with RGD after 5 days of culture. Cardiomyocytes adhered to the scaffold and evidence of spreading was observed. Qualitative assessment of these scaffolds indicated that there was a higher cell density in the larger pore scaffold. This observation is likely due to the enhanced seeding efficiency with larger pores.

A photo-patterning technique based on manipulations in the polymerization kinetics was recently developed for patterning thick hydrogels.[4] Here, we demonstrate that this patterning technique may be used to create patterns in porous hydrogels. A photomask was prepared as described previously[4] with patterns consisting of 200 to 500 μm circles spaced 1 mm apart. The resulting patterned and porous p(HEMA) structures for the 200 μm diameter photomask are shown in Fig 2. Through this patterning process, aspect ratios of ~2 were obtained in porous gels that were ~750 μm thick. An attractive feature of this novel photo-patterning technique is that it may be applied to a wide array of chemistries where sufficient differences in polymerization kinetics occur. We are currently adapting this technique to photo-polymerized PEG hydrogels. Additional studies are underway to create patterned macrostructures (vertical channels for enhance nutrient transport (Fig 2) and horizontal channels to promote cell alignment) to support long-term survival and growth of cardiomyocytes.

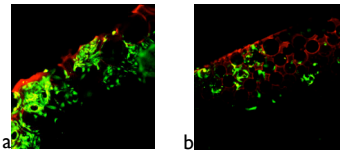

Fig1. Confocal microscopy images of a cross-section of porous PEG hydrogel seeded with cardiomyocytes and cultured for 5 days. Pore sizes are (a) 150 μm and (b) 60 μm. The polymer stains red while the live cells stain green.

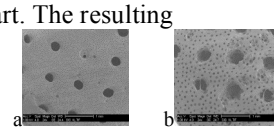

Fig 2. Patterned and porous pHEMA scaffolds with pore diameters of (a) 60 and (b) 150 μm. Bar=1 mm.

In conclusion, photopolymerized pHEMA and PEG gels can easily be fabricated with precise control over the macroscopic architecture with respect to pore size and 3D patterning (to date for pHEMA) to support cardiomyocyte attachment. This study presents for the first time the use of photolithography techniques to generate a patterned and porous hydrogel structure. Photolithography offers facile construction of highly complex scaffold architectures.

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