Hydrogel Scaffolds for Bladder Tissue Regeneration

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Purpose: For patients with dysfunctional or injured bladders, few options exist for restoring normal, functional tissue. A tissue engineering option, using autologous cells from bladder urothelium (UCs) and smooth muscle (SMCs) offers a potential solution. However, only a small number of scaffolding materials are being extensively explored by urologic researchers, and only a fraction of these are synthetically-derived. Unlike biologically-derived matrices, polymeric scaffolds offer reliable synthesis and characterization. Our laboratory makes extensive use of a series of well-defined methacrylate triblock copolymers for studies in polymer adhesion. Under aqueous conditions, the terminal hydrophobic endblocks of these polymers segregate to form non-covalent crosslinked domains, yielding a network polymer with appropriate physical properties for tissue engineering applications. This study explored the feasibility of this elastomeric material as a bioscaffold, and initial cellular response after seeding.

Methods: Triblock copolymers with poly(methyl methacrylate) (PMMA, degree of polymerization = 370) endblocks and a poly(methacrylic acid) (PMAA, dp = 1450) midblock were synthesized by anionic means. Porous hydrogel scaffolds were obtained by a porogen leach method. Briefly, the PMMA-PMAA-PMMA copolymer was dissolved at 10% (w/v) in DMSO, a good solvent for both blocks, and mixed with sodium chloride (NaCl) salt crystals of defined size. The mixture was filled into circular washers and immersed in 18.2 MΩ•cm distilled water to form gels. Gels were immersed in multiple exchanges of water to remove all salt crystals, then re-equilibrated in PBS. Porous hydrogels were characterized by Confocal Laser Scanning Microscopy (CLSM) (Zeiss ConfoCor3/510 Meta) in the Biological Imaging Facility at Northwestern University. In order to get a good contrast to image the pores, a hydrophobic fluorophore, Hostasol 3G, was added to the DMSO solution prior to solvent exchange. For cell studies, 3E5 human bladder SMCs were seeded onto each scaffold for 90 min, followed by 3E5 human UCs. Three replicate samples were incubated in culture medium for 3 days and evaluated by a live/dead assay (Invitrogen) and by DAPI staining of formalin-fixed samples.

Results/Discussion: The PMMA-PMAA-PMAA copolymer solution in DMSO was easily formed into scaffold discs. Exposure to DI water induced immediate polymer gelation, followed by gradual dissolution of the NaCl crystals. The porous polymer scaffolds expanded to ~ 3x their original size after NaCl removal and then back to ~2x original size after equilibration in PBS. CLSM imaging of Hostasol-dyed scaffolds yielded the 3-D construction shown in Figure 1a. Uniform, open pore structure was observed throughout the entire gel thickness. We were also able to create bilayer scaffold systems, using one layer of sieved < 40µm salt, covered by a second layer of 40-120 µm salt (Figure 1 b,c). This anisotropic structure would be beneficial in cell seeding, to match cell size requirements to pore size. (e.g. seeding UCs in small pores and SMCs in large pores) In addition, an outer layer of large pores would help offset tangential stresses in the scaffold under pressure.

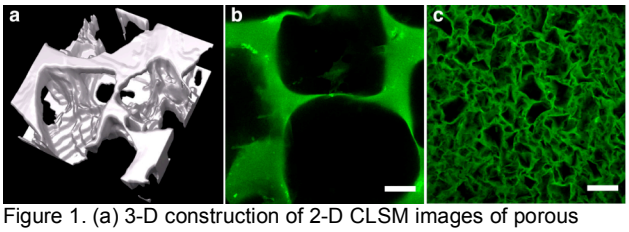

Figure 1. (a) 3-D construction of 2-D CLSM images of porous hydrogel, compiled from 13 slices at 50µm intervals; (b,c) Large (b) and small (c) pores from a bilayer scaffold structure. Scale bars are 200 µm.

SMCs/UCs were seeded onto the polymer scaffolds, and found to attach and spread over time. Live/dead imaging showed a majority of cells were live and attached to the scaffold (Figure 2). Imaging throughout the scaffold depth demonstrated cell penetration into the porous structure, and biocompatibility *in vitro* for short-term cultures.

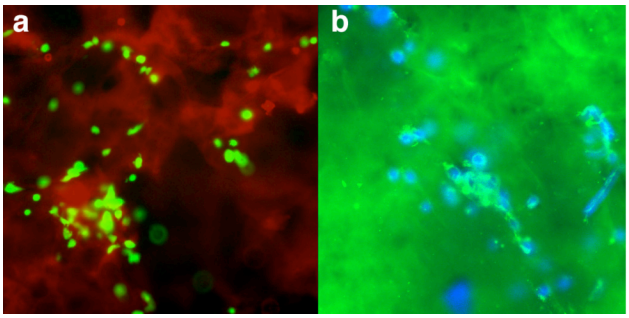

Figure 2. (a) Live/dead assay of cells on the polymer gel. The majority of cells were alive (green). Red stain for dead cells was retained by the polymer, but few dead cells were seen. (b) DAPI-stained cells (blue nuclei) on a Hostasol-loaded scaffold (green).

Conclusions: PMMA-PMAA-PMMA block copolymers were found to easily form stable hydrogels in aqueous media, and could be formed into porous scaffolds, capable of supporting cellular attachment and growth. A bilayer system was developed, to match the cellular and physical requirements of the bladder. SMCs and UCs were able to attach to these scaffolds and spread. We are continuing our investigations of these polymers to explore cell growth, morphology, and phenotype as functions of seeding density and pore size distribution. These offer a unique example of a biocompatible elastomer with a potential urologic application.