Biodegradable Spherical Porous Polyurethane Scaffold for Urinary Bladder Tissue Engineering

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Statement of Purpose: Tissue engineering replacement for the urinary bladder has shown promising results as an alternative to the use of gastrointestinal segments [1]. We designed a process to manufacture biodegradable sphere-templated porous synthetic scaffolds to be used as a matrix for urinary bladder tissue engineering. Our goal is to produce a biodegradable matrix with adequate mechanical properties, geometry, and chemical composition that will allow human bladder urothelial and smooth muscle cells (SMC) adhesion, proliferation and new tissue formation. The 38µm pore size used has been found to be optimal for angiogenesis and biointegration [2].

Methods: We synthesized a biodegradable poly(ester urethane) (PU) using poly(caprolactone) triol MW 900 (PCLT), 2-isocyanatoethyl-2,6-diisocyanato methyl caproate, and 1,1,1 tris(hydroxymethyl) propane at different ratios. We designed and manufactured a two-piece Teflon mold to build the spherical scaffold, adapting the sphere templating technique [2] to create uniform porosity. The mold is designed to produce a sphere with an internal diameter of 25mm and 1.5mm thick, but the mold can be scaled up for larger size or different thickness. Polymer composition is infiltrated into the mold under vacuum pressure, polymerization is done at 80ºC. Porous polymer scaffold tensile strength was evaluated using uniaxial tensile testing (Instron 3340). SEM microscopy was used to evaluate uniformity of pore structure and pore size. Scaffold porosity was quantified analyzing polymer histological cross sections imaged with optical microscope and using ImageJ (NIH) software. *In vitro* cytotoxicity was evaluated by the metabolic assay WST-1 (Roche Applied Science). Preliminary cell seeding on porous scaffolds with immortalized bladder urothelial and smooth muscle cells (SMC) was evaluated using histology and optical microscopy.

Results: Our manufacturing process allows fabrication of spherical porous scaffolds with the intended dimensions as shown in Fig. 1.Tensile strength for porous PU scaffolds range from 2.70 MPa to 37.0 MPa depending on fractional PCLT content. Pore size was found to be uniform (38µm) in cross-sections taken from different areas of the scaffold and analyzed by SEM. Porosity estimation by histological imaging is 65%. Metabolic assays performed at day 2 and day 7 of cell-seeded scaffolds found minimal toxic effects from the compositions. Human urothelial cells recovered metabolic activity up to 70% of control cell cultures, whereas smooth muscle cells metabolic activity reached almost 120% of control by day 7 (Fig.2). Preliminary cell seeding experiments show infiltration of cells after day 7 up to approximately 100µm into the scaffold. Cell morphology shows urothelial cells and SMC adhered to pore walls and creating interconnections.

Conclusions: We are able to fabricate porous spherical scaffolds with our method. The mold can be used to fabricate scaffold with difrerent pore size and polymer compostions. The design can be adapted to create spheres with different outer diameters and scaffold wall thickness. Mechanical properties of our polymer compositions show satisfactory strength values for our application. Uniform porosity was found across the spherical scaffold walls, with pore size of approximately 38µm and inter-pore interconnections of 10µm. There is a significant difference on the metabolic response per cell type, altough we were able to observe recovery in both populations after 7 days of culture. Bladder smooth cells adapt faster to the scaffold environment. Cell culture experiments for longer periods of time are planned to furhter evaluate cellular response.

References:

[1] Atala A. et al., The Lancet. 2006;367:1241-46
[2] Ratner BD, Marshall AJ. *US Pat Appl* 20080075752

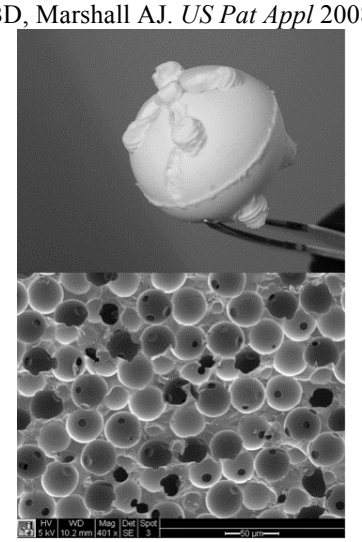

Fig. 1 Top, spherical porous PU scaffold, 28 mm outer diameter. Bottom, SEM image of scaffold porosity.

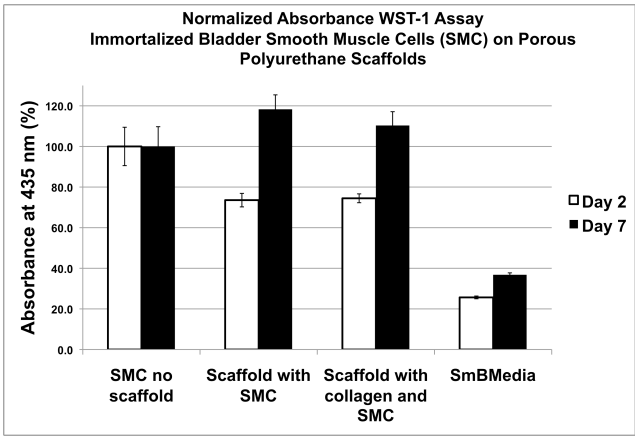

Fig. 2 WST-1 metabolic assay results from urinary bladder SMCs seeded on PU porous scaffolds.