Application of PCUU Scaffolds in Urinary Bladder Tissue Engineering

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Statement of Purpose: Although the recent success of bladder tissue engineering demonstrated the feasibility of this technology, most polyester scaffolds such as PGA used in previous studies[1] possess inadequate mechanical properties for organs that exhibit large deformation. The present study explored the use of biodegradable elastomer poly (carbonate-urethane) urea (PCUU) for urinary bladder tissue engineering since this material has previously been shown to exhibit high extensibility and biocompatibility[2]. However, the specific mechanical demands of the bladder are not limited to the high extensibility, but also high compliance at low forces and high strength under physiological bladder pressures. Moreover, recent studies have demonstrated that cell behaviors are influenced by the stiffness of the substrates that the cells attach to[3]. Therefore, the present study examined the mechanical properties and bladder smooth muscle cell (BSMC) response to the electrospun PCUU scaffold.

Methods:

Fabrication of scaffolds: All the PCUU scaffolds were prepared using conventional electrospinning methods with conditions described in previous reports [2]. The PCUU sample was stored at 4ºC in sterile DMEM cell culture media to maintain hydration until use.

Cell Culture: BSMC were isolated from adult female Sprague- Dawley rats following established methods[4]. The smooth muscle phenotype of these cells was confirmed by immuno-staining for α-smooth muscle actin and smooth muscle myosin heavy chain. The cells below seven passages that consistently expressed smooth muscle phenotypic markers were used in all experiments. BSMC were seeded at 0.12 X 10^6 cells/cm^2 & 0.48 X 10^6 cells/cm^2 on the scaffolds and incubated at standard cell culture conditions (37 °C, humidified, 5% CO_2 / 95% O_2) and cultured in RPMI + 10% FBS for 7 days

Mechanical Characterization: The PCUU scaffolds were cut into rectangular specimens (~ 30mm x3 mm) according to the ASTM standards D3039/D3039D and subjected to uniaxial tensile loads under hydrated conditions (submersed in PBS at 37ºC) at a rate of 18mm/min until rupture[5] using MTS synergie 100. The mechanical behaviors of these materials were analyzed by plotting the calculated values of tension (force normalized by the width in N/mm) against stretch ratio λ (ratio of deformed to reference lengths under a tare load) and comparing the maximum tensions and stretch ratios at failure.

Pressure-volume studies: Small defects (~20% of the surface area of rat bladder specimens) were created on the dome of rat bladder specimens and PCUU patches were sutured on using nylon 9-0 sutures to cover the puncture. The bladders with the PCUU patches were then tested using a custom made ex vivo pressure device. Specimens submersed in saline maintained at 37ºC were infused with saline at a flow rate of 0.1 ml/min until failure and changes in pressure were recorded

Results: Images of immuno stained BSMC exhibited well spread morphology and uniform cell distribution throughout the PCUU scaffold surface indicating the suitability of the elastomer for BSMC adhesion and growth. The uniaxial testing of the PCUU scaffolds after one week of BSMC culture revealed that the maximum tension (0.5N/mm) and stretch (3.0) were similar to those of the native bladder determined from literature[5]. BSMC seeding at higher concentrations (0.48 X10^6 cells/cm^2) led to increased stiffness and strength indicating increased secretion of ECM. The initial results of the pressure-volume experiments provided evidence that the PCUU sutured bladders could withstand pressure up to 60 cm H_2O before leakage.

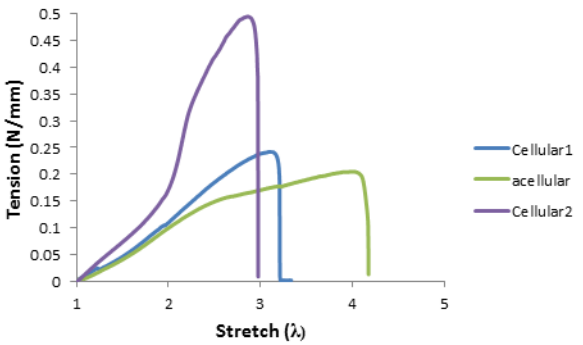

Figure 1: Uniaxial tensile testing of acellular and cellular PCUU; **Cellular 1**- 0.12 X 10^6 BSMC/cm^2; **Cellular 2**- 0.48 X 10^6 BSMC/cm^2

Conclusions: The Mechanical characterization indicated that the cell seeded PCUU scaffolds could mimic the peak physiological stress of native bladders[5]. The initial results of the pressure-volume experiments showed that the PCUU sutured bladders exhibited compliance similar to that of the intact bladder, though with a reduced capacity. Further studies are necessary to quantify the mechanical behavior and contractility of PCUU scaffolds seeded with BSMC.

References:

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