Comparison of Elastomeric Polymers for Bladder Regeneration

Srikanth Sivaraman[a], Nicholas Amoroso[b], William Wagner[b], Shilpa Sant[c], Jiro nagatomi[a]
[a] Department of Bioengineering, Clemson University, Clemson, SC, [b] McGowan Institute for Regenerative Medicine,[c] School of Pharmacy, University of Pittsburgh, Pittsburgh, PA

Statement of Purpose: The urinary bladder is one of the few organs that has been successfully engineered and implanted in humans for a long-term clinical trial[1]. Although the recent success of bladder tissue engineering demonstrated the feasibility of this technology, most polyester scaffolds such as PGA used in the previous studies[1] possess inadequate mechanical properties for organs that exhibit large deformation. The present study explored the use of biodegradable elastomers, poly-glycerol sebacate-polycaprolactone (PGS-PCl), poly (ether-urethane) urea (PEUU), and poly (carbonate-urethane) urea (PCUU) for urinary bladder tissue engineering since these materials have previously been shown to exhibit high extensibility and biocompatibility[2, 3]. 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[4]. Therefore, the present study compared mechanical properties and bladder smooth muscle cell (BSMC) response to various biodegradable elastomers.

Methods:

Fabrication of scaffolds: All elastomer scaffolds were prepared using conventional electrospinning methods with conditions described in previous reports[2, 3]. The PCUU sample was stored in cell culture media to maintain hydration until use.

Cell Culture: BSMC were isolated from adult female Sprague- Dawley rats following established methods[5]. 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.

Cell-Biomaterial Interactions: BSMC were seeded on 0.5 x 0.5 cm elastomer samples at $2x10^5$ cells per sample. BSMC morphology on the elastomers samples was confirmed by rhodamine –phalloidin staining after a 7 day time period using an Olympus spinning disc confocal microscope.

Mechanical Characterization: PGS-PCL, PEUU and wet PCUU specimens were prepared according to the ASTM standards D3039/D3039D and subjected to uniaxial tensile loads under hydrated conditions (PBS at 37ºC) at a rate of 18mm/min until rupture[2] using MTS synergie 100. The mechanical behaviors of these materials were analyzed by plotting the calculated values of tension (N/mm) against stretch ratio λ (ratio of deformed to reference lengths) and comparing the maximum tensions and stretch ratios at failure.

Results: Images of rhodamine-phalloidin stained BSMC showed well spread morphology and uniform cell distribution throughout the PGS-PCL, PEUU & PCUU scaffold surfaces indicating the suitability of the elastomers for BSMC adhesion and growth. The uniaxial testing of the PGS-PCL scaffolds in the present study revealed that although the maximum stretch was 1.75 (175% elongation), the maximum tension at failure was on average 0.079 N/mm. This is significantly lower than the maximum tension for the normal human bladder 0.54-1.1 N/mm, which was estimated from study data in the literature[6]. The PEUU, on the other hand, exhibited much greater maximum tension and stretch ratio, 2.5 N/mm and 2.2, respectively. Although these values exceed the levels of normal human tissues, the relatively high stiffness at lower tensile force makes this material not suitable for the bladder application. The uniaxial testing of the PCUU scaffolds, in contrast, revealed that the maximum tension (0.48N/mm) and stretch (1.75) were similar to those of the native bladder determined from literature[6].

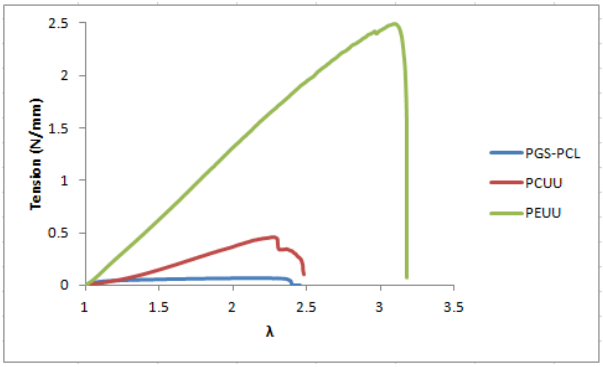

Figure 1: Representative mechanical behavior of elastomeric scaffolds

Conclusions: The results demonstrated that all the elastomeric scaffolds tested in the present study promoted BSMC spreading and growth. The Mechanical characterization indicated that the PCUU may be more suitable for the bladder tissue engineering application than PEUU and PGS-PCL. Further studies, however, are necessary to quantify the mechanical behavior of PCUU scaffolds in the presence of cells via in vitro and in vivo experiments.

References:

1. Atala, A. et al. (2006) *Lancet* 1241-1246
2. Sant, S.et al. (2010) *J Tissue Eng Regen Med* 283-291
3. Hong Y.et al. (2010) *Biomaterials* 4249-4258
4. Isenberg BC. (2009) *Biophys J* 1313–1322
5. Roby T et al. (2008) *Ann Biomed Eng* 1744-1751
6. Dahms SE. et al. (1998) *J Urol* 411-416

Acknowledgements: Funding source NIH RR021949