Bladder Smooth Muscle Cell Responses to Contact Guidance and Biaxial Mechanical Stretch

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Statement of Purpose: A major limiting factor in tissue engineering the urinary bladder wall is the lack of proper tissue organization following bladder augmentation surgery. In animal studies where the bladder wall was replaced, smooth muscle cells were observed to aggregate into small, irregular muscle bundles and did not achieve the large-scale tissue structures observed in the native bladder [1]. Recent findings of a clinical study by Atala et al. [2] showed significant success utilizing autologous urothelial and smooth muscle cells on a composite matrix implanted into patients with myelomeningocele. While the patients' bladder capacities and compliance were increased, bladder functionality could not be determined [2]. Proper tissue structure is essential to ensure bladder functionality. The present study was thus undertaken to develop approaches that promote increased tissue organization and function prior to implantation.

Methods: PDMS microgrooves were fabricated using soft photolithography. Bladder smooth muscle cells (BSMC) isolated from rat bladders were seeded 100,000 cells/cm^2 onto the patterned PDMS. Scanning electron microscopy (SEM) was performed, and F-actin staining (Molecular Probes) was used to visualize cell alignment. A custom-made biaxial stretch bioreactor was developed, capable of stretching up to 20% in one or both directions with varying waveforms. Smooth and microgrooved PDMS was affixed to either a uniaxial or biaxial stretch bioreactor for dynamic tissue culture at 0.5 Hz. for up to 10 days. Following stretching, a Sircol Collagen Assay and Fastin Elastin Assay (UK Biocolor) were used to determine de novo ECM production.

Results/Discussion: Microgrooved PDMS was able to be fabricated with uniform grooves of varying dimensions. These grooves provided contact guidance for the bladder SMCs and promoted alignment. Narrower grooves led to qualitatively higher alignment of bladder SMCs. On flat surfaces, BSMC aligned perpendicular to the direction of uniaxial stretch (Fig 2A). Under equi-biaxial stretch and static culture, F-actin staining of BSMC showed little to no alignment (Fig 2 B,C). After 10 days in culture, unaxial stretch down-regulates elastin production and up-regulates collagen production (Fig. 2). In preliminary studies, samples under equi-biaxial stretch performed in our biaxial stretch bioreactor had increased elastin and collagen production compared to uniaxial stretching (Fig. 2). These results may be caused by a switch from a contractile to a synthetic cell phenotype under biaxial mechanical stretch. The phenotype of the cells will be confirmed in future studies.

Conclusions: The use of PDMS microgrooves provided necessary contact guidance for the bladder SMC organization, and biaxial stretch promoted more ECM production than uniaxial stretch. This study suggests that contact guidance and mechanical stretch can be used to manipulate BSMC alignment and ECM production, which may lead toward engineering more organized, functional bladder tissue replacements.

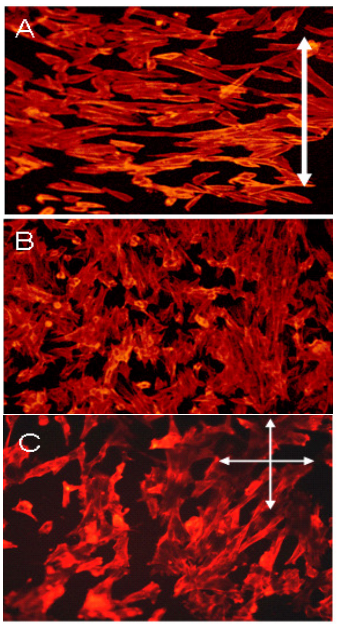

Figure 1. F-actin staining of BSMC under uniaxial stretch for 48 hours (A), in static culture (B), and under equi-biaxial stretch for 48 hours (C). The white arrows in (A) and (C) show the direction of strain.

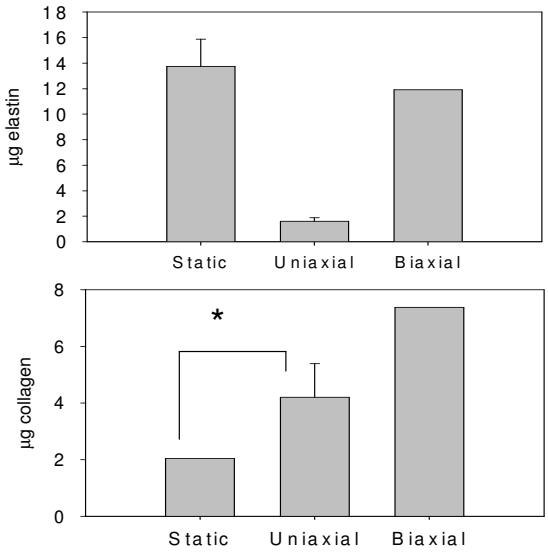

Figure 2. Elastin (A) and collagen production (B) at 10 days. Data are presented as mean +/- sem n=2 for elastin, n=3 for collagen, and n=1 for biaxial stretch. *p<0.05

References:

1. Brown, AL, Biomaterials, 2002. 23(10): p. 2179-90.
2. Atala, A., Lancet, 2006. 367(9518): p. 1241-6.

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