Increased Mature Elastin Synthesis in Arterial Constructs Based on Elastomeric Scaffolds

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Statement of Purpose: Elastic fibers provide elasticity and compliance, which is critical to develop arterial constructs with mechanical responsiveness similar to native arteries.[1] Most engineered vessels lack elastic fibers in the medial layer and those present are poorly organized. Therefore, mature elastin synthesis is a key challenge in arterial tissue engineering. The objective of this study is to increase mature elastin synthesis in small-diameter arterial constructs by using porous tubular scaffolds seeded with vascular smooth muscle cells (SMCs) *in vitro*.

Methods: Porous tubular scaffolds were fabricated from a biodegradable elastomer, poly(glycerol sebacate) (PGS)[2] using the salt fusion method with three different sized salts. Adult primary baboon SMCs (passage 4-6) were seeded in the lumen of tubular scaffolds and cultured in a pulsatile flow bioreactor for 3 weeks. Morphological and morphometrical properties of scaffolds were examined from scanning electron microscopy and microcomputed tomography, respectively. Cell infiltration of each construct was examined using H&E staining after 1-day culture and measured by a distance from the lumen to attached cells. Extracellular matrix (ECM) contents and mechanical properties were measured from biochemical assays and uniaxial tensile testing.

Results: In Fig.1A, all scaffolds had homogeneous wall thicknesses, but different pore structures in their lumens. As shown in Fig. 1B, scaffolds with 25-32 μm pores had narrower pore size distribution and significantly higher surface area (data not shown) than those with large pores. Fig.2. shows that constructs with 25-32 μm pores increased SMC organization at their lumens and decreased cell infiltration. These results provide the evidence that this small pore increased SMC attachment and made more SMCs packed densely at the lumen of scaffolds. Fig.3. also shows that constructs with 25-32 μm pores produced significantly higher insoluble elastin and collagen than those with large pores. The highest contents of insoluble elastin and collagen in constructs with 25-32 μm pores corresponded to 23 and 10 % of native arteries. These results indicate that this small pore increased mature elastin synthesis, which is closely related to increased SMC organization.

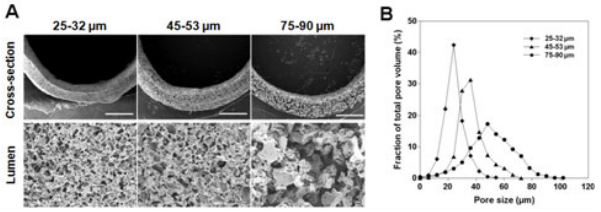

Fig. 1. (A) Morphology of each scaffold. Scanning electron micrographs of PGS scaffolds with three different sizes of porogens. Scale bar: 1 mm (top row), 20 μm (bottom row). (B) Pore size distribution of PGS scaffolds with three different pore sizes.

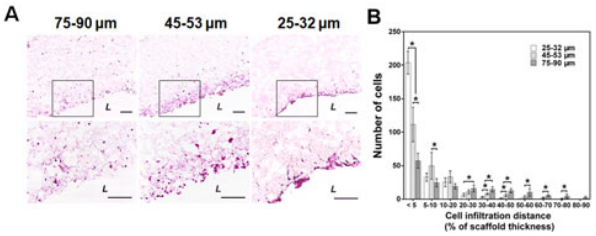

Fig. 2. Cell infiltration from the lumen of PGS constructs. (A) H&E staining of each construct (top row) and partial magnification (bottom row) of the box shown in top images. L: lumen. Magnification: 10× (top row), 30× (bottom row). Scale bar: 100 μm. (B) Cell infiltration distance from the lumen in each construct. * represents $P<0.05$ ($n = 3$)

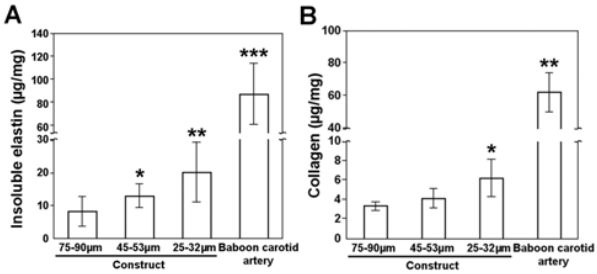

Fig. 3. Biochemical analysis of PGS constructs and native arteries. (A) Insoluble elastin contents. *$P<0.05$ (45-53 vs. 75-90 μm), **$P<0.05$ (25-32 vs. 45-53 and 75-90 μm), and ***$P<0.05$ (Baboon carotid artery vs. all PGS constructs) ($n = 3$). (B) Total collagen contents. *$P<0.05$ (25-32 vs. 45-53 and 75-90 μm), **$P<0.05$ (baboon carotid artery vs. all PGS constructs) ($n = 3$).

Conclusions: A relatively small pore size of approximately 30 μm in the constructs increased SMC organization and mature elastin synthesis than larger pore sizes, indicating that the smaller pores increased cell self-organization and decreased cell infiltration.

References:

1. Patel A. Cardiovasc Res. 2006:71:40-49.
2. Wang Y. Nat Biotechnol. 2002:20:602-606.

Acknowledgments

This study was supported by a grant from the National Institutes of Health (R01 HL089658).