Elastic Matrix Regeneration in Collagen-Rich Tissues Require Elastogenic Induction of Cells

Lavanya Venkataraman, BS[1] and Anand Ramamurthi, PhD[1, 2, 3]
[1]Department of Bioengineering, Clemson, SC; [2]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, and [3]Department of Biomedical Engineering, Cleveland Clinic, Cleveland OH

Statement of Purpose: An intact elastic matrix is a prerequisite for normal functioning of a cyclically distending elastic artery. Elastin, the primary elastic protein of such a matrix, determines vessel recoil and compliance and regulates healthy vascular smooth muscle cell (SMC) behavior within. Synthetic vascular grafts (eg. Dacron®) used to bypass diseased blood vessels can reinstate vessel elasticity and compliance, but do not support healthy vascular cell phenotype leading to homeostatic abberations. Lately, completely tissue engineered vessel replacements using autologous SMCs seeded on biodegradable scaffolds, natural or synthetic, have shown promise as alternatives, but fail to incorporate the elastin content and matrix architecture of native vessels, due to (a)poor elastogenicity of adult vascular SMCs, and their (b) poor ability to recruit elastin precursor, crosslink them, and organize them into directionally-oriented fibers.

Previously, our lab established the elastogenic benefits of exogenous hyaluronan oligomers (HA-o; ~756 Da) and TGF-β1 in 2-D-cultures of healthy rat aortic SMCs (RASMCs) [1]. Since the presence of collagenous matrix is centric to replicating vascular tissue architecture and mechanics and since vascular cells, generally, regardless of the choice of scaffolds, robustly synthesize collagen, it is imperative to examine the impact of a pre-existing collagenous microenvironment on ability of the cells to synthesize fibrous elastic matrix on their own and also their response to provided elastogenic factors. To investigate these aspects, the first phase of our project involved testing the dose-specific effects of HA-o and TGF-β on RASMCs embedded within statically-loaded 3-D collagen constructs. After establishing an optimal elastogenic factor dose-combination, in order to enhance crosslinking, alignment and overall maturity of the regenerated elastic fibers, phase 2 of our project investigated synergy between dynamic pulsatile conditioning and factor delivery to enhancing elastic matrix deposition within compacted collagen tubes.

Methods: 3D collagen constructs consisted of RASMCs (P3-5, 10^6cells/ml) and acid-solubilized Type-I collagen (BD Biosciences). Statically loaded constructs were cultured within rectangular silicone rubber wells with polyurethane end holders that constrained the gels longitudinally, but enabled transverse contraction, for 21 days. Dose combinations of HA-o (0.2μg/ml or 2μg/ml) with TGF-β (0.1ng/ml, 1ng/ml or 10ng/ml) (n=6/dose) were supplemented to the culture medium. In phase 2, RASMC-embedded collagen tubes were compacted around silastic tubing (5 mm inner diameter, 4cm length) and was perfused with saline to exert pulsatile circumferential distension on the tube wall at frequencies of 0.5 Hz or 2 Hz, at a constant strain rate of 2.5%, in presence of exogenous factors (dose optimized in phase 1) contained in a hydrating reservoir of unperfused DMEM-F12 medium. After 21 days, the constructs were harvested and analyzed for (a) contraction ratio (n = 6), (b) cell proliferation using a DNA assay (n = 5), (c) tropoelastin in pooled medium fraction and alkali-soluble and –insoluble matrix elastin using a Fastin dye binding assay (n = 5), and (d) elastic matrix distribution and ultrastructure using IF and histology, (e) Matrix metalloprotease (MMP) and Lysyl oxidase content using Western blotting (n=3), MMP activity using zymography, and (n=3) (f) RT-PCR for mRNA expressions of LOX and tropoelstin (n=6).

Results: In phase 1, **c**ell proliferation ratio was greatest (4.2±0.8) in presence of 0.1 ng/ml of TGF- and 2 μg/ml of HA-o. Overall tropoelastin synthesis/cell was enhanced significantly (1.54±0.2 fold vs control) within constructs with the highest dose of TGF-β1 (10 ng/ml) and HA-o (2 μg/ml). Elastic matrix deposition/cell was significantly higher in all the experimental cases compared to control; greatest increases (5.44±0.76 and 5.26±1.09 fold) noted in lower doses of 0.1 ng/ml TGF-β1 and 0.2 μg/ml or 2 μg/ml of HA-o respectively. LOX protein synthesis was most increased in constructs with 10 ng/ml of TGF-β1 and 0.2 μg/ml of HA-o (3.46±0.48 fold). MMP-2 protein amounts were significantly enhanced, and MMP-9 decreased within all factor-treated constructs, relative to controls; constructs cultured with the lowest doses of the factors seeing the greatest increase. Von Kossa staining revealed no calcific deposits. VVG staining and fluoroscopy revealed aligned fibers more towards the edges of the construct, than at the center. Based on above results, 0.1ng/ml of TGF-β1 and 2μg/ml HA-o combination was concluded to be the optimal and was delivered in phase 2 experiments. Robust, intact tubular constructs were harvested at 21 days of culture. Ultrastructure analysis revealed circumferentially aligned elastin fibers throughout the constructs with factors. Further tests are ongoing to establish quantitative comparison between test cases and control.

Conclusions: A study was performed to investigate benefits of HA-o, TGF-β1 and pulsatile conditioning in inducing synthesis and assembly of a mature vascular elastic matrix by adult RASMCs within non-elastogenic collagen constructs. Based on several contributing indicators towards elastogenesis, optimum dose combination was concluded to be 0.1 ng/ml TGF-β1 and 2 μg/ml of HA-o. This dose of elastogenic factors together with pulsatile conditioning of SMC-embedded collagen tubes improved elastin synthesis and elastic fiber deposition and circumferential orientation, and greater crosslinking of elastic matrix, as desired.

References: Kothapalli, C.RTaylor, P.M., Smolenski, R.T., Yacoub, M.H., and Ramamurthi, A et al. Tissue Eng 15, 501, 2009.

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