Impact of mechanical conditioning and elastogenic factors on biomimetic cell-mediated elastic matrix regeneration in 3-D tissue constructs

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Statement of Purpose: Elastin, a major component of elastic matrix structures within several soft connective tissues provides them mechanical resilience and maintains healthy cell phenotype. Specifically, within physically injured blood vessels, or those afflicted with inflammatory disease (e.g., aortic aneurysms or AAs), enhanced production/activity of matrix metalloprotease (MMPs) enzymes is triggered, to rapidly disrupt the elastic matrix. The elastin peptides thus generated further induce inflammatory reactions that can lead to greater loss of elasticity, and ultimately, loss of tissue integrity and function. Since diseased vascular cells are poorly capable of in situ regenerative elastic matrix repair, an alternative is to tissue-engineer elastic vessel replacements in vitro using healthy, autologous vascular smooth muscle cells (SMCs) seeded on biomimetic scaffolds. However, this has been challenged severely by their poor elastogenicity, and lack of knowledge of materials and methods to stimulate elastic matrix production and assembly. Previously, our lab determined the elastogenic benefits of hyaluronan oligomers (HA-o) and TGF-β1 to 2-D-cultures of healthy rat aortic SMCs (RASMCs) [1]. However, to better mimic the spatio-temporal characteristics of the ECM environment within intact tissues, we presently study the dose-specific effects of these factors on RASMCs seeded within 3-D compacted collagen gel constructs.

Methods: The inherent nature of collagen to shrink and form a compacted gel was utilized to fabricate 3D constructs. The constructs consisted of rat aortic SMCs (RASMCs; passage 3-5, 10^6 cells/ml) and acid-solubilized Type-I collagen (BD Biosciences), cultured within rectangular (36x18x18mm) wells created in silicone rubber molds. The wells were provided with porous polyurethane end holders that constrained the gels longitudinally, but enabled transverse contraction. The RASMCs were cultured for up to 4 weeks in DMEM/F12 medium containing 10% v/v FBS and each of several dose combinations of exogenous HA-o (0.2ug/ml and 2ug/ml) and TGF-β (0.1ng/ml, 1ng/ml and 10ng/ml) (n=6/dose). The constructs were imaged every 3 days to monitor the rate and extent of contraction. At 4 weeks, the constructs were harvested and analyzed for (a) contraction (n = 6), (b) cell proliferation using a DNA assay (n = 3), (c) tropoelastin in pooled medium fraction and alkali-soluble and –insoluble matrix elastin using a Fastin dye binding assay (n = 3), and (d) elastic matrix distribution and ultrastructure using IF and histology.

Results: (a) Minimum % contraction, calculated based on the central widths of constructs, was obtained for constructs cultured with 2μg HA and 0.1ng TGF-β, while maximum contraction was seen with 0.2μg HA and 1ng TGF-β. Differences between the other cases were insignificant, (b) cell proliferation was maximal (4.24± 0.49-fold at 4 weeks) in constructs cultured with 2μg HA and 0.1ng TGF-β, and minimal (1.24± 0.495-fold) in cases cultured with 10ng TGF-β, (c) tropoelastin production on a per cell basis was the least by constructs cultured with 0.1ng TGF-β and though they exhibited the maximal increases in matrix elastin (3.4± 0.68-fold vs. non-additive controls) and matrix yield (matrix elastin/total elastin; 3.6± 0.21-fold vs. controls). Constructs cultured with 10ng TGF- β showed greatest increases in tropoelastin production and alkali-insoluble matrix elastin (1.29± 0.21-fold and 2.38± 0.12-fold vs. control), but exhibited the lowest yields of matrix elastin (0.88± 0.49-fold vs. control),(d) Verhoeff-van Gieson elastic stain and immunofluorescence performed on paraffin embedded constructs showed that those cultured with 0.1ng and 1ng TGF-β to contain a more fibrillar matrix, and those cultured with 10ng TGF-β to contain more disorganized elastin clumps.

Conclusions: A 30-day study was performed to determine the synergistic elastogenic effects of HA and TGF-β added exogenously in various doses to the 3-D collagen constructs. While HA is known to influence the assembly of tropoelastin into an extracellular elastin network, TGF-β is known to primarily influence trpoelastin production and improve matrix yield[1]. Significant amounts of matrix and tropoelastin production was observed in all cases. Although higher concentrations of TGF-β increased tropoelastin production, total matrix elastin produced was seen to be significantly lower than other cases. However, amount of alkali-insoluble elastin produced in these cases was higher than at lower concentrations of TGF-β. Further tests to study MMP activity and mechanical properties like Young's Modulus are required to identify constructs capable of performing sufficiently *In vivo*. Future studies also include studying the effects of the above factors in constructs under cyclic stretch, on elastic matrix production and mechanical properties. One of the final goals of this project is to develop a suitable, long-term substitute for elastic arteries. Depending on the application, a dose combination can be chosen. For example, vascular grafts that match tensile properties of native elastic arteries would need higher matrix elastin produced at a faster rate, therefore requiring a faster cell proliferation. However, if these constructs are used as regenerative tools for restoring normal phenotype from an aneurysm, then a lower cell density would be ideal. Once a protocol for optimum elastic matrix production is obtained, it can be used to restore elastic properties of other soft tissues.

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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