Efficacy of induction of aneurysmal cell-mediated elastin regeneration for stabilizing aortic aneurysms

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Statement of Purpose: Elastin critically maintains native vessel structure and regulates cell-signaling pathways involved in morphogenesis, injury response, and inflammation. Thus, the failure to repair or replace degraded elastin matrices can undermine vessel structure and function. Unfortunately, elastin regeneration in situ or within tissue engineered constructs is challenged by very poor elastin synthesis by adult vascular cells, and inadequacy of known cellular cues to (a) up-regulate synthesis of elastin precursors, (b) recruit and assemble them efficiently into mimics of native elastin structures, and (c) mature and stabilize elastin. We have shown that hyaluronan (HA) tetramers (HA4 mer; MW: 756 Da; 0.2 μg/ml) and TGF-β (1 ng/ml) synergistically upregulate elastin precursor (5-fold vs. non-additive) and matrix synthesis (8-fold) by healthy, adult rat vascular smooth muscle cells (SMCs), and further enhance elastin matrix yield (45 vs. 5.3%), crosslinking, stability, and fiber formation[1]. While these results greatly benefit tissue engineering elastin rich constructs using healthy SMCs, it is unknown if aneurysmal vascular SMCs will likewise respond to the cues, and if so, if dose-re-optimization is necessary. This information will guide ongoing attempts to achieve in situ elastin regeneration/ repair within rat models of aortic aneurysms (AAs).

Methods: To induce AA formation, adult rat infrarenal abdominal aortae were treated peri-adventitially with 0.5M of $CaCl_2$ for 15 minutes. This model has been conclusively shown to induce proteolytic elastin degradation and aortal expansion similar to human AAs[2]. Rats were euthanized, and aortae size-analyzed at 28 days prior to excision. Primary cells were isolated from the aortal media layer using an explant technique, and cultured in basal DMEM: F12 containing 10% v/v FBS. The isolated cells were cultured for 3 weeks and their matrix production under basal conditions and when induced with TGF-β (1 ng/ml) alone, HA 4mer (0.2 μg/ml) alone, or both cues together were compared to additive-free healthy control SMCs of identical passage (n = 3/culture). At 21d, cell layers were assayed for DNA (flurometric assay of Labarca and Paigen), tropoelastin precursors, and alkali-soluble and –insoluble matrix elastin (Fastin dye-binding assay) and collagen (Hydroxyproline assay). Desmosine crosslinks (ng/ng of matrix elastin) were quantified using ELISA. Matrix ultrastructure was assessed using TEM and matrix calcification by van Kossa Staining. Western blot and zymography quantified MMP production of active elastolytic MMPs 2 and 9. Production of the elastin crosslinking enzyme LOX was quantified by western blot and its activity with an Amplex Red assay kit (Molecular Probes). All averaged biochemical outcomes were normalized to the DNA content of the respective cultures and further normalized to outcomes for non-additive control cultures to gauge relative fold-change.

Results: Aneurysm development was confirmed by a 45% local increase in aortal diameter at 28 days post-injury. The cells derived from injured aortae appeared to represent a mixed population with a significant number amongst them exhibiting decreased volume/spreading. Almost all the cells however expressed SMC α-actin. Also, additive-free aneurysmal SMCs generated greater amounts of MMPs 2- and 9 than healthy SMCs (1.67 ± 0.16 and 2.08 ± 0.35-fold vs. healthy SMCs; $p < 0.05$) and contained a greater number of calcific deposits, suggesting an activated phenotype. **A**dditive-free aneurysmal SMCs proliferated more slowly than healthy SMCs (2.5 ± 0.32 vs. 7.6 ± 3.3-fold number increase in 21 days for control; $p < 0.05$), produced far less tropo and matrix elastin than did healthy control cells **(see Table 1)**, and crosslinked a smaller fraction of generated tropoelastin precursors into matrix elastin (9.6 ± 2.1 vs. 20.1 ± 2.5% for healthy SMCs; $p = 0.02$). At the provided dose (1 ng/ml) TGF-β did not influence basal levels of proliferation or elastin production by aneurysmal SMCs. HA 4mers and TGF-β together however suppressed cell proliferation (0.66 ± 0.15-fold **vs. additive-free aneurysmal SMCs**), enhanced synthesis of elastin matrix (1.66 ± 0.32-fold) and total elastin (1.49 ± 0.06-fold), and total collagen (1.78 ± 0.18-fold) while elastin matrix yield was not impacted (1.11 ± 0.21-fold). Encouragingly, elastogenic cues reduced calcific deposits, and production of active MMPs-2 and 9 down to healthy control levels. TEM showed that elastin fiber formation was significantly enhanced in aneurysmal cultures that received both cues together, relative to additive free cultures, and healthy SMC cultures.

Aneurysmal Cells	Fold-change relative to additive-free healthy SMC cultures			
	No additives	HA 4mer	TGF-β	HA 4mer + TGF-β
Proliferation Ratio	0.33 ± 0.04	0.26 ± 0.03*	0.32 ± 0.03	0.22 ± 0.03*
Total Matrix Elastin	0.05 ± 0.01	0.07 ± 0.00*	0.06 ± 0.01	0.09 ± 0.01*
Total Elastin	0.11 ± 0.01	0.13 ± 0.00*	0.11 ± 0.01	0.16 ± 0.01*
Matrix/ Total Elastin Ratio	0.48 ± 0.10	0.54 ± 0.04	0.55 ± 0.08	0.54 ± 0.10
Total Collagen	0.94 ± 0.08	1.33 ± 0.02*	1.26 ± 0.05*	1.68 ± 0.17*
Active MMP-2	1.67 ± 0.16	1.49 ± 0.16	1.43 ± 0.18	1.13 ± 0.3*
Active MMP-9	2.08 ± 0.35	1.91 ± 0.17	1.34 ± 0.60	0.90 ± 0.32*

Table 5. Matrix production by adult rat aneurysmal RASMCs relative to **additive-free healthy RASMCs**. Cells were cultured for 21 d. Ratios shown represent mean ± SD of n = 3 cultures/case. * is significance of differences vs. **additive-free aneurysmal** deemed for $p<0.05$ (green cells)

Conclusions: Our outcomes provide evidence that aneurysmal SMCs enhance tropo/matrix elastin synthesis, and exhibit suppressed proliferation in response to elastogenic cues, and (c) can be restored by elastogenic cues to a healthier phenotype (i.e., inhibited proliferation, calcification, & MMP production). However, our results indicate that doses of elastogenic cues must be re-optimized independently for aneurysmal SMCs for elastin regeneration within AAs.

References: [1]Joddar B et al. Biomaterials. 2006; 27(33): 5698-707; [2] Isenberg JC et al., Circulation. 2007; 115(13):1729-37; [**Support:** NIH grant EB006078-01A1]