Ascending and Descending ECM Hydrogels for Modeling Aortic Aneurysms

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Statement of Purpose: Biomechanical weakening of the vessel wall, medial degeneration, and vasa vasorum remodeling are all hallmarks of aneurysmal diseases via mechanisms that are not fully understood. Understanding what factors disrupt the multi-layer biology of large blood vessels during the progression of diseases such as aneurysm can aid in the unmet clinical need to slow or halt disease progression after diagnosis. In particular, an adventitial microvascular network known as the vasa vasorum provides the primary blood supply to the outer aortic wall and is a key component of inter-layer vascular health. The vasa vasorum ("vessels of the vessels") play an essential role in maintaining aortic homeostasis by delivering oxygen and nutrients to the outer layers of the aortic media. In the ascending aorta, the vasa vasorum originate from the coronary and brachiocephalic arteries whereas in the descending aorta, the vasa vasorum stem from the intercostal arteries. The different origins of the vasa vasorum correspond to the anatomically specific functions of the aortic regions, which can further pertain to the differing origins of vascular wall cells and putative differences in the composition of extracellular matrix (ECM). Biologic scaffolds produced from ECM are useful biomaterials to understand biological processes and address wound healing, stem cell differentiation, and angiogenesis for both *in vitro* and *in vivo* disease models. In the present study, we investigated putative differences in composition, structure, and bioactivity between ascending and descending aorta-derived ECM to better understand intra- and inter-layer cell-matrix interactions relevant to vasa vasorum function in the aorta.

Methods: Porcine aortic adventitia (pAdv) and media (pMed) ECM were prepared by a modified version of the decellularization process by Reing et al[1]. Decellularization of ECM was evaluated by quantifying residual dsDNA within ECM and performing gel electrophoresis to determine DNA fragment length. ECMs were stained using H&E to visualize nuclear material. Hydrogels were prepared by pepsin digestion (4 mg/mL) of ECM (20 mg/mL) for 24 hours at room temperature, followed by neutralization and addition of Type I collagen (2.5 mg/mL). Turbidimetric measurements were obtained to determine gelation kinetics of hydrogels at 37°C as described previously[2]. Glycosaminoglycan content was measured from ECMs using papain digestion and determined from a standard curve of chondroitin-6-sulfate. The influence of pAdv and pMed (1 mg/mL) on immortalized human aortic pericyte contractility was determined using a collagen compaction assay. The ultrastructure of ECM hydrogels was visualized using scanning electron microscopy (SEM).

Results: Qualitative assessment of DNA using gel electrophoresis revealed low fragments of DNA.

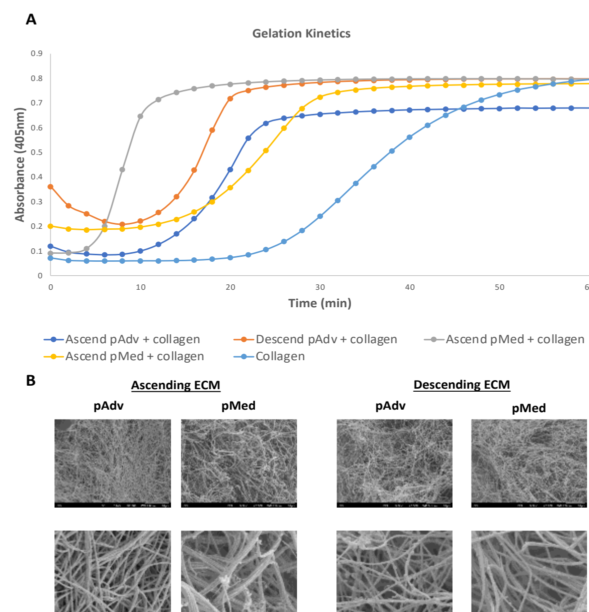

Figure 1. (A) Turbidimetric gelation kinetics of ECM hydrogels compared with collagen gel. **(B)** SEM images of ECM hydrogels showing fiber architecture at 2,500x (top) and 20,000x (bottom) magnification

Quantitative assessment of DNA content for ascending and descending pAdv and pMed showed <125 ng DNA/mg dry tissue weight. Little to no nuclei were noted in H&E stained decellularized tissues. Optical density of hydrogels from ascending and descending aorta-derived ECM revealed a logarithmic curve during the gelation period at 37°C. Peak gelation of ECM hydrogels was achieved within 30 minutes while collagen alone gelled within 1 hour **(Fig. 1A)**. Ascending pAdv and pMed ECM gelled faster when compared with descending aorta-derived ECMs. Compaction of pericyte-embedded collagen gels was higher in the presence of either ascending or descending pAdv or pMed when compared with gels lacking aortic ECM (cell only controls). SEM imaging of ECM hydrogels revealed acellular fibrous architecture for ascending and descending ECMs **(Fig. 1B)**. Interestingly, pAdv hydrogels exhibited single fiber microarchitecture, whereas pMed hydrogels were comprised of collagen fiber bundles.

Conclusions: Ascending and descending aortic ECM hydrogels retain bioactivity to influence human perivascular cells and can potentially be used as disease models for investigating aortic aneurysms. A comprehensive understanding of the influence of layer-specific ECM on cells in different aortic regions could help uncover novel disease mechanisms and serve as less invasive treatments for aortic aneurysms.

References: 1. Reing, JE. Biomaterials, 2010. 31(33): p. 8626-8633. 2. Freytes, DO. Biomaterials, 2008.29.11 (2008): 1630-1637