Electrochemically Aligned Collagen-Elastin Fibers for Vascular Tissue Engineering

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Statement of purpose: Coronary artery disease due to occlusion causes high mortality worldwide. In the United States, approximately 7 million people died from coronary disease in 2008 costing nearly 20% of national health care expense[1]. The main cause for this disease is atherosclerosis. Transplantation of autologous vessels is the common treatment for arterial reconstruction. However, up to 30% of patients do not have suitable donor site[2] and morbidity is also a major concern. Nondegradable polymeric vascular grafts such as ePTFE have been widely used but are limited to large blood vessels (>5mm) due to mechanical mismatch with medium and small blood vessels (≤5mm). Therefore, development of functional tissue engineered grafts that mimic the composition, structure and mechanics of native tissue is a viable alternative strategy to replace diseased arteries. In this study, we synthesized highly aligned elastin incorporated collagen fibers that compositionally mimic native blood vessels. Elastin constitutes ~50% of elastic arteries. Rat aortic smooth muscle cells (rSMCs) were seeded onto the fibers (w or w/o elastin) and morphology, proliferation and phenotype of the cells was assessed.

Methods: Dialyzed type I collagen (3 mg/ml, Advanced Biomatrix, CA) was mixed with insoluble elastin (Elastin Products Company, MO) at a mass ratio of 1:1. The mixture was loaded between two wire electrodes and an electric field of 20V was applied for 5 min to form elastin incorporated electrochemically aligned collagen fibers. Collagen only fibers were also synthesized in a similar manner and used as controls. The resultant fibers were crosslinked with 10mM EDC & 5mM NHS (Sigma-Aldrich, USA) for 4hrs and washed with 0.1M Na_2HPO_4. To confirm the incorporation of elastin into collagen fibers, autofluorescence imaging with a DAPI filter set was performed. For the cell studies, the fibers were sterilized overnight in 70% ethanol, washed with PBS and placed in ultralow attachment plates (Corning). Rat aortic smooth muscle cells (rSMCs) were seeded on the fibers at a density of 20,000 cells/cm^2 (based on the area of the well) and cultured for up to 14 days. Culture medium composed of DMEM supplemented with 10% FBS, 1% pen/strep and L-glutamine. Cells proliferation was analyzed at days 1, 4, and 7 using alamarBlue assay. Cell morphology was assessed using confocal microscopy of phalloidin stained cell cytoskeleton at days 7 and 14. Calponin expression for rSMCs was analyzed at day 7 to evaluate cell phenotype.

Results: Autofluorescence imaging confirmed the incorporation of elastin into electrochemically aligned collagen fibers (Fig. 1). Confocal microscopy at day 7 revealed that rSMCs exhibited a more elongated fibroblastic morphology on collagen only fibers (Fig. 2A). On the other hand, cells on collagen-elastin fibers appeared more cuboidal in morphology (Fig. 2B). By day 14, a highly confluent layer of cells was observed on collagen-elastin fibers (Fig. 2C). Results from the alamar blue assay indicate that cell proliferation on collagen only and collagen-elastin fibers was comparable. Furthermore, calponin expression was observed on both groups (Fig. 3) suggesting that rSMCs developed a more contractile phenotype after 7 days in culture.

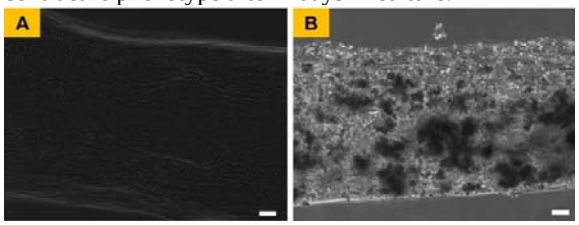

Fig. 1. Autofluorescence images for (A) Collagen only fiber and (B) Collagen-Elastin fiber. Scale bar: 50 μm.

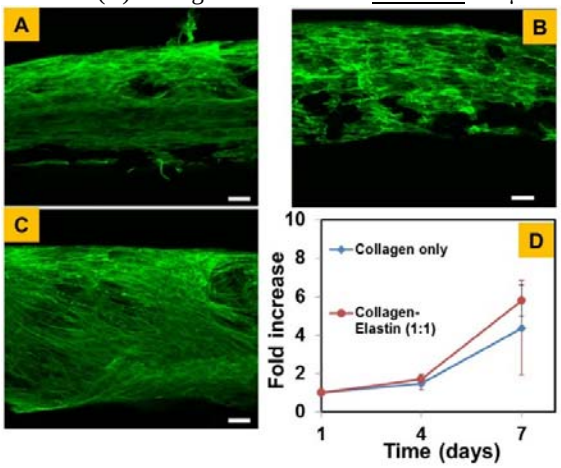

Fig. 2. Confocal images for rSMCs at day 7 (A&B) and day 14 (C) for (A) Collagen fiber and (B&C) Collagen-Elastin fiber. (D) Cell proliferation on collagen only and collagen-elastin fibers. Scale bar: 50 μm.

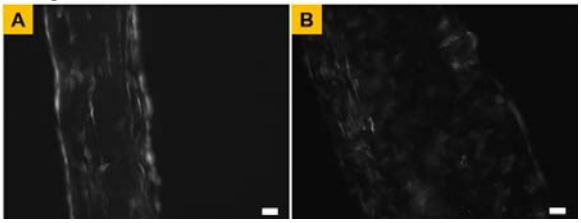

Fig. 3. Calponin expression for rSMCs cultured on (A) Collagen only fibers and (B) Collagen-Elastin fibers at day 7. Scale bar: 50 μm.

Conclusions: In summary, we have demonstrated that elastin can be incorporated uniformly within electrochemically aligned collagen fibers. Further, incorporation of elastin induces morphological changes in rSMCs. Future studies will focus on mechanical characterization of these collagen-elastin fibers as we believe that mimicking the native composition of elastin will impart mechanical properties comparable to those of the small diameter blood vessels. Further, long term cultures will be performed to assess the effect of elastin incorporation on cell phenotype and gene expression. Overall, results from this preliminary study demonstrate that elastin incorporated electrochemically aligned collagen fibers have the potential to be developed for vascular tissue engineering applications.

References: [1] Catto, V., et. al. 2014. ISRN Vascular Medicine; [2] Song, Y. 2011. Clinical hemorheology and microcirculation. p358.