Highly Aligned Elastin Incorporated Collagen Fibers for Vascular Tissue Engineering

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Introduction: Coronary artery disease caused by atherosclerosis is a significant health problem worldwide. In the United States alone, over 370,000 people die from the disease each year [1]. The current gold standard for the treatment of coronary artery disease is the transplantation of autologous blood vessels. However, lack of sufficient donor tissue and donor-site morbidity are major limitations [2]. While synthetic grafts are viable alternatives, occlusion and intimal hyperplasia limit their use only to large-diameter blood vessels (> 6mm). Development of a fully functional tissue-engineered vascular graft that mimics the composition, topography and mechanical properties of native blood vessel is a viable strategy for the treatment of diseased arteries. In this study, we employed an electrochemical processing methodology to synthesize elastin incorporated electrochemically aligned collagen (ELAC) fibers. We hypothesized that the elastin incorporated ELAC fibers will provide compositional and topographical cues to stimulate the orientation and contractile phenotype of rat aorta smooth muscle cells (rSMCs).

Materials and Methods: Dialyzed type I collagen (3.1mg/ml, Advance Biomatrix, CA) was mixed with insoluble elastin (IE; Elastin Product Company, MO) at ratio of 60:40 w/w and loaded between two stainless steel electrodes. An electric field of 3V was applied for 30 min to form elastin incorporated ELAC fibers (Col-IE). Collagen only fibers were used as control. Monotonic tensile tests were performed to assess the effect of elastin incorporation on the mechanical properties of ELAC. For the cell studies, rSMCs were seeded on the fibers at a density of 10,000 cells/well (24-well plate, Corning) and cultured for 21 days in culture medium composed of high glucose DMEM supplemented with 10% FBS. Expression of markers specific to the contractile phenotype of SMCs (α-SMA and calponin) were assessed by real-time PCR (days 3 and 14) and immunofluorescence (days 7 and 21).

Results and Discussion: We have previously confirmed the incorporation of IE within ELAC fibers via autofluorescence imaging [3]. Tensile test results showed that elastin incorporation decreased ($p = 0.0076$) the yield stress of ELAC fibers by about 2-fold from 0.43 MPa to 0.22 MPa. Young's modulus was also observed to decrease upon elastin incorporation from 4.53 MPa to 3.04 MPa; however, this difference was not statistically significant ($p = 0.36$). Yield strain was comparable between groups at around 12%. Real-time PCR results showed an increase in α-SMA and calponin expression over time on both collagen only and Col-IE fibers (Fig. 1E). More importantly, α-SMA expression increased significantly on Col-IE fibers from day 3 to day 14 ($p = 0.0138$). Further, α-SMA expression was significantly higher ($p = 0.0457$) on Col-IE fibers compared to collagen only fibers at day 14. Immunofluorescence results showed a significant increase in calponin expression from day 7 to day 21 on both fibers (Fig. 1A-D).

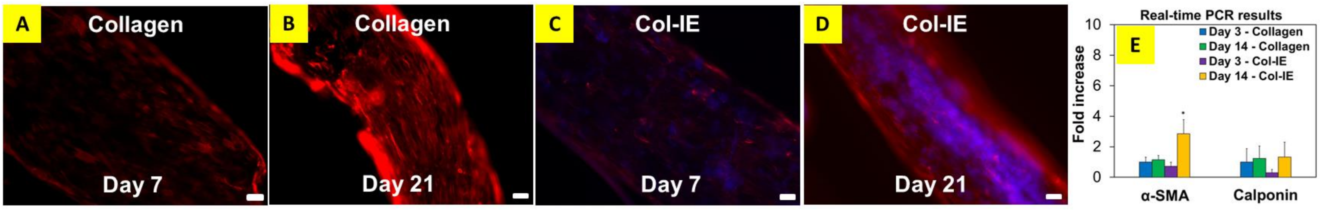

Figure 1. (A-D) Immunofluorescence showed an increase in calponin expression over time on both fibers; (E) Real-time PCR results showed significantly higher expression of α-SMA on col-IE group at day 14. Scale bar: 100µm.

Conclusions: In summary, incorporation of elastin into highly aligned dense collagen fibers significantly enhanced α-SMA expression suggesting that the cells on the Col-IE fibers express a more contractile phenotype. Future studies will focus on assessing the mechanical strength and matrix organization of cell-synthesized remodeled matrix at the end of the culture period. Further, the concentration of elastin incorporated within the ELAC fibers will be optimized to improve the mechanical properties of the fibers. ELAC fibers incorporated with the most optimal elastin concentration will be used for the development of a three-dimensional tubular tissue-engineered graft for vascular applications. Overall, the results from the current study demonstrate that elastin incorporated ELAC fibers have considerable potential to be developed for vascular tissue engineering applications.

References: [1] CDC, NCHS., CDC Wonder Online Database, 2015; [2] Song, Y., Clinical hemorheology and microcirculation, p358, 2011. [3] Nguyen, T. TERMIS, Abstract #184, 2014.