Mechano-dependent biosynthetic response of micro-integrated cells in elastomeric scaffolds.

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Introduction

The rapidly growing field of tissue engineering combines the principles of biology and engineering in an effort to create biological substitutes that recapitulate the requisite mechanical and structural properties of healthy native tissues to restore, maintain, or improve tissue function [1]. One such area of interest within the field lies in tissue engineered heart valves. Approximately 75,000 patients per year in the US receive prosthetic heart valves, and it is estimated that this number increases to 250,000 worldwide [2]. Although the current solutions have proven to prolong the lives of those living with valvular disease, there still remains to be an ideal solution to valve replacement. For example, pediatric patients who receive prosthetic heart valves must have them replaced periodically because current prosthetic options do not have a capacity for tissue growth and remodeling [3]. Additionally, anyone receiving a prosthetic heart valve puts themselves at risk for numerous blood related problems (prosthetic valve thrombosis (PVT), thromboembolism, anticoagulant-related hemorrhage, etc.) [4]. Continued research and development in the field of tissue engineering will serve to increase the available solutions to valve failure and further decrease the complications and risk associated with prosthetic heart valves.

The proposed study investigates the biomechanical events that take place during in-vitro tissue development within poly (ester urethane) urea (PEUU) scaffolds and its comparison to the behavior of native tissues. Engineered tissue that mimics the critical load bearing response of native tissues must develop an organized and functional extracellular matrix by responding to external signals [3]. This project will examine the biosynthetic effects of cyclic mechanical strain placed on a PEUU scaffold densely integrated with rat vascular smooth muscle cells with the overall goal of developing an engineered tissue that mimics the load bearing capabilities of native tissue.

Methods

This project consists of multiple phases which include cell expansion, construct production, dynamic conditioning (Fig. 2), and quantification of the construct biosynthetic activity. Vascular smooth muscle cells isolated from rat aorta will be expanded onto tissue culture plates under Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 2% anti/anti and 1% HEPES solution [5]. Scaffold preparation involves concurrently electrospinning of the PEUU scaffold and electrospraying of the vascular smooth muscle cells. The process involves depositing a solubilized polymer across a voltage gradient onto a collection surface resulting in a continuous mat of tissue that can mechanically behave in a similar manner to native soft collagenous tissue (Fig. 1) [6]. After the specimen is produced, it will be placed in the tension bioreactor that will mechanically condition the specimen in a controlled manner. The specimen will be assessed in groups as follows: day 0 control, day 7 static,

day 7 15%, 30%, and 50% strain. Soluble collagen, proteoglycan, and DNA content will be quantified compared to day 0 controls. Additionally, traditional histology and immunohistochemistry will be performed to generalize the cellular distribution between the ECM and new scaffold and qualitatively analyze cell phenotype respectively.

Expected Results

Based on preliminary work (Fig. 3), it is expected that ECM production will be strain level dependent, and this dependency will have a non-linear relationship. Large strain will cause a statistically significant increase in the production of extracellular matrix, and this anticipated result is consistent with those trends seen in native tissue [3]. It is anticipated that the cell-scaffold deformation relationship will evolve with ECM growth towards what has been observed in native tissues (Fig. 4).

Works Cited

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[3] Stella, John A., Nicholas J. Amoroso, William R. Wagner, and Michael S. Sacks. Effects of mechanical stimulation on matrix synthesis of microintegrated cells in fibrous elastomeric scaffolds. Society for Biomaterials Annual Meeting, San Antonio, TX, April 2009.

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[5] Stankus JJ, Guan J, Fujimoto K, Wagner WR, Microintegrating smooth muscle cells into a biodegradable, elastomeric fiber matrix. Biomaterials 2006;27:735-44.

[6] Stella, John A., Jun Liao, Yi Hong, W. D. Merryman, William R. Wagner, and Michael S. Sacks. "Tissue-to-cellular level deformation coupling in cell micro-integrated elastomeric scaffolds." Elsevier (2008): 3228-236.

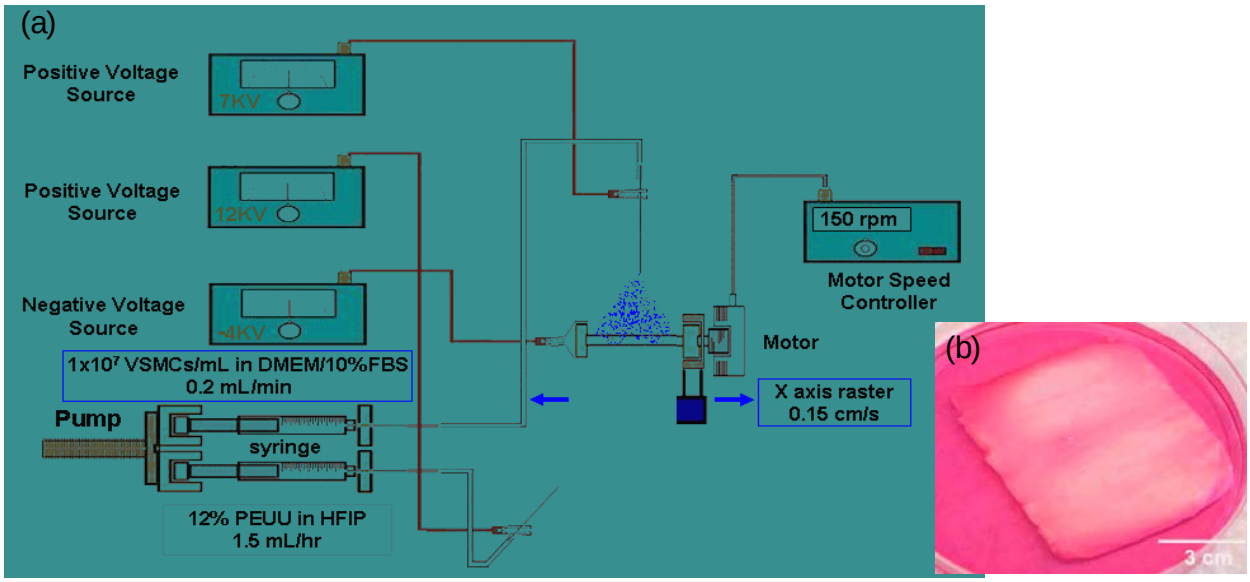

Figure 1: (a) Setup for the electrospinning PEUU polymer and electrospraying of cells onto a rotating shaft, which results in (b) a continuous tissue like mat [3]

Figure 2: Cyclic tension bioreactor [3]

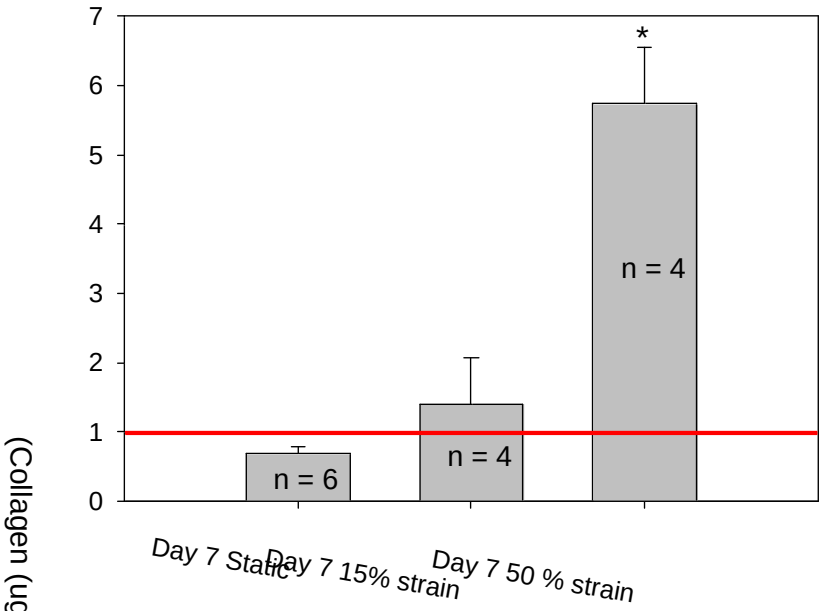

Figure 3: Collagen quantification compared to day 0 controls [3].

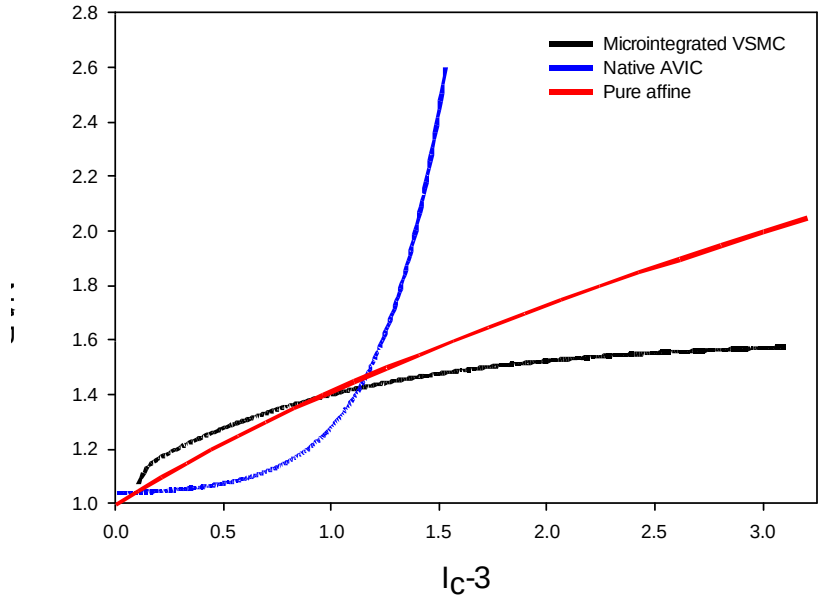

Figure 4: Comparison of measured cell-scaffold deformation behavior of native and engineered tissues. For comparison purposes, a pure affine response is plotted highlighting the unique deformation response of cells within fibrous scaffold architectures.