FINAL PROGRESS REPORT
MECHANICALLY-INDUCED REMODELING OF TISSUE ENGINEERED BLOOD VESSELS
APPLICATION ID: R21-HL088156

Specific Aims.
The ***goals of this proposal*** are **(*i*)** to develop and test an experimental device to that can precisely and independently control multidirectional mechanical loading and is capable of performing intermittent pressure-diameter (P-d) and axial force-length (f-ℓ) tests and multiphoton laser scanning microscopy (LSM) to observe changes in the biomechanical response and microstructural organization, respectively, at multiple time-points in culture, **(*ii*)** to develop a microstructurally-motivate computational model to describe TEBV remodeling to mechanical stimuli, and **(*iii*)** to employ these experimental and computational models to characterize remodeling of combined collagen-fibrin gel-derived TEBVs exposed to unidirectional and multidirectional loading. Our ***central hypothesis*** is that axial extension will induce remodeling in collagen-fibrin TEBVs at rates that exceed that of pressure- or flow-induced remodeling and combining these multidirectional mechanical stimuli will increase remodeling rates beyond the additive response of these unidirectional stimuli alone. The specific aims were to:
Aim 1: Characterize remodeling of collagen-fibrin gel-derived TEBVs exposed to gradual increases in (a) axial extension, (b) pulsatile pressure, and (c) luminal flow. .
Aim 2: Characterize remodeling of collagen-fibrin gel-derived TEBVs exposed to simultaneous increases in axial extension, pulsatile pressure, and luminal flow.

Studies and Results.
We have completed much of the work specified in the original aims of this grant. This award has supported the work disseminated in four publications, summarized below.

1.Theory and experiments for mechanically-induced remodeling of tissue engineered blood vessels. Gleason RL and W Wan (2008) *Advances in Science and Technology.* 57: 226-234. In this paper we describe experimental and computational frameworks to characterize the use of mechanical stimuli to improve the mechanical properties of TEBVs. We model the TEBV as a constrained mixture and track the production, degradation, mechanical state, and organization of each structural constituent. Specifically, we assume that individual load bearing constituents can co-exist within each neighborhood and, although they are constrained to deform together, each constituent within this neighborhood may have different natural (i.e., stress-free) configurations. Motivated by this theoretical framework, we have designed a bioreactor and biomechanical testing device for TEBVs. This device is designed to provide precise and independent control of mean and cyclic luminal flow rate, transmural pressure, and axial load over weeks and months in culture and perform intermittent biaxial biomechanical tests. This device also fits under a two-photon laser scanning microscope for 3-dimenstional imaging of the content and organization of cells and matrix constituents. These data directly support our theoretical model.

Relation to the grant aims: This paper presents the overall approach of the combined theoretical-experimental paradigm describe in Aims 1 and Aim 2 and presents data demonstrating the utility and effectiveness of this combinded approach.

A Novel Biaxial Computer Controlled Bioreactor and Biomechanical Testing Device for Vascular Tissue Engineering. Zaucha M, J Raykin, W Wan, R Gauvin, FA Auger, L Germain, RL Gleason (2009) *Tissue Engineering*.15(11): 3331-3340. PMID: 19385725. It is becoming evident that tissue-engineered constructs adapt to altered mechanical loading, and that specific combinations of multidirectional loads appear to have a synergistic effect on the remodeling. However, most studies of mechanical stimulation of engineered vascular tissue engineering employ only uniaxial stimulation. Here we present a novel computer-controlled bioreactor and biomechanical testing device designed to precisely and simultaneously control mean and cyclic values of transmural pressure (at rates up to 1Hz and ranges of 40 mmHg), luminal flow rate, and axial length (or load) applied to gel-derived, scaffold-derived, and self assembly- derived tissue-engineered blood vessels during culture, while monitoring vessel geometry with a resolution of 6.6 mm. Intermittent monitoring of the extracellular matrix and cells is accomplished on live tissues using multi-photon confocal microscopy under unloaded and loaded conditions at multiple time-points in culture (on the same vessel) to quantify changes in cell and extracellular matrix content and organization. This same device is capable of performing intermittent cylindrical biaxial biomechanical testing at multiple time-points in culture (on the same vessel) to quantify changes in the mechanical behavior during culture. Here we demonstrate the capabilities of this new device on self-assembly-derived and collagen-gel-derived tissue-engineered blood vessels.

Relation to the grant aims: This paper presents the culmination of the first milestone of Aim 1 and preliminary long-term culture work proposed in Aim 2. We found that mechanical stimulation of gel-derived tissue engineered blood vessels presented challenges that we difficult to overcome; namely, the vessels could withstand only very low pressures <5 mmHg. Application of any meaningful loads would result in vessel damage and plastic deformation.

2. A phenomenological model for mechanically-mediated growth, remodeling, damage, and plasticity of gel-derived tissue engineered blood vessels. Raykin J, AI Rachev, RL Gleason (2009) *Journal of Biomechanical Engineering*. 131(11):101016 (12 pgs). PMID: 19831486. Mechanical stimulation has been shown to dramatically improve mechanical and functional properties of gel-derived tissue engineered blood vessels (TEBVs). Adjusting factors such as cell source, type of extracellular matrix, cross-linking, magnitude, frequency, and time course of mechanical stimuli (among many other factors) make interpretation of experimental results challenging. Interpretation of data from such multifactor experiments requires modeling. We present a modeling framework and simulations for mechanically mediated growth, remodeling, plasticity, and damage of gel-derived TEBVs that merge ideas from classical plasticity, volumetric growth, and continuum damage mechanics. Our results are compared with published data and suggest that this model framework can predict the evolution of geometry and material behavior under common experimental loading scenarios.

Relation to the original aims: This paper is directly related to the modeling components of Aim 1 and Aim 2. In addition to the original growth and remodeling of engineered arteries, our experimental data suggested that mechanical damage and plastic deformation were important factors in the evolution of geometry and mechanical behavior of gel-derived tissue engineered blood vessels; these phenomena are captured in this model.

3. Biaxial biomechanical properties of media and adventitial equivalents and bilayer blood vessel substitutes created by self assembly. Zaucha M, R Gauvin, FA Auger, L Germain, RL Gleason (2010) *Journal of the Royal Society Interface.* ePub ahead of print. PMID: 20554564. Along with insights into the potential for graft success, knowledge of biomechanical properties of small diameter tissue-engineered blood vessel (TEBV) will enable designers to tailor the vessels' mechanical response to closer resemble that of native tissue. Composed of two layers that closely mimic the native media and adventitia, a tissue-engineered vascular adventitia (TEVA) is wrapped around a tissue-engineered vascular media (TEVM) to produce a self-assembled tissue-engineered media/adventia (TEVMA). The current study was undertaken to characterize the biaxial biomechanical properties of TEVM, TEVA and TEVMA under physiological pressures as well as characterize the stress-free reference configuration. It was shown that the TEVA had the greatest compliance over the physiological loading range while the TEVM had the lowest compliance. As expected, compliance of the SATEBV fell in between with an average compliance of 2.73 MPa21. Data were used to identify material parameters for a microstructurally motivated constitutive model. Identified material parameters for the TEVA and TEVM provided a good fit to experimental data with an average coefficient of determination of 0.918 and 0.868, respectively. These material parameters were used to develop a two-layer predictive model for the response of a TEVMA which fit well with experimental data.

Relation to the original aims: Due to the challenges described above associated with mechanical damage and failure of gel derived tissue engineered blood vessels, we decided to test the hypotheses of the grant proposal on a different class of tissue engineered arteries. Thus, this paper presents the mechanical characterization of self-assembly derived tissue engineered arteries.

This support has initiated a new line of research in our lab that employs this combined theoretical-experimental paradigm to quantify the role of mechanical stimulation on tissue self-assembly and allow to tuning of mechanical properties of self-assembly derived tissue engineered blood vessels. In addition, although gel-derived tissue engineered arteries were not able to withstand meaningful pressure, they can be exposed to physiological shear stress. Thus, we are current developing a gel-derived vessel constructed with human cells for the study of mechanically mediated development of cardiovascular disease associated with HIV-1 infection. The NIH supports our research in this area.

Publications.

Zaucha M, R Gauvin, FA Auger, L Germain, RL Gleason (2010) Biaxial biomechanical properties of media and adventitial equivalents and bilayer blood vessel substitutes created by self assembly. *Journal of the Royal Society Interface*. ePub ahead of print. PMID: 20554564.

Raykin J, AI Rachev, RL Gleason (2009) A phenomenological model for mechanically-mediated growth, remodeling, damage, and plasticity of gel-derived tissue engineered blood vessels. *Journal of Biomechanical Engineering*. 131(11):101016 (12 pgs). PMID: 19831486.

Zaucha M, J Raykin, W Wan, R Gauvin, FA Auger, L Germain, RL Gleason (2009) A Novel Biaxial Computer Controlled Bioreactor and Biomechanical Testing Device for Vascular Tissue Engineering, *Tissue Engineering*. 15(11): 3331-3340. PMID: 19385725.

Gleason RL and W Wan (2008) Theory and experiments for mechanically-induced remodeling of tissue engineered blood vessels. *Advances in Science and Technology*. 57: 226-234.

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