SB³C2022
Summer Biomechanics, Bioengineering and Biotransport Conference
June 20-23, Eastern Shore, MD, USA

DESIGN AND VALIDATION OF A BIOREACTOR FOR DYNAMIC BIAXIAL MECHANICAL STIMULATION OF ENGINEERED TISSUE VASCULAR GRAFTS

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INTRODUCTION

Cardiovascular disease remains as the leading cause of death worldwide, with mortality strongly correlated with coronary artery disease and commonly observed as stenosis or occlusion of blood vessels leading to myocardial infarction. Engineered tissue vascular grafts (ETVGs) promise an attractive alternative option to autologous grafts typically employed in coronary artery bypass grafting surgery.[1] In theory, ETVGs hold many advantages such as relieving supply limitations, the ability to grow and repair in vivo, and lacking the need of either using the internal mammary artery or harvesting the saphenous vein. However, more research is necessary before this technology can reach clinical application. The main hurdles lie in achieving similar mechanical properties of native vessels as compliance mismatch is a strong factor correlating with grafting success. It is known that mechanical conditioning of engineered tissues during incubation improves their properties and physiological function; specifically, biaxial loading of ETVGs has been shown to improve vascular mechanics.[2] However, there is a lack in systematic understanding of the parameters driving ETVG growth and remodeling during in vitro incubation and the resulting patency of the ETVG once deployed in vivo. Therefore, more research is necessary in determining the ideal protocols of mechanical stimulation to produce viable ETVGs. Unfortunately, access to bioreactors capable of providing this stimulation is a limiting factor in the field due to complexity and cost.[3] A low-cost, automated, robust, and reproducible method for experimenting with in vitro culture of ETVGs is needed to progress the field through systematic collection of data for model generation. Here, we propose and validate a custom designed dynamic biaxial bioreactor to meet these criteria.

METHODS

Axial Stimulation. The bioreactor is designed to accept four electrospun PCL scaffolds of 2mm diameter, 3cm length, and 200μm thickness suitable for deployment as an aortic interposition graft in rats. A novel scaffold mounting system and associated fixturing procedures were developed to grip the scaffolds. The scaffolds are attached to stainless steel cannulas at both ends, and these cannulas are gripped by collets that hold the scaffold and stretch it between two spaced brackets made of stainless steel. The system ensures consistent mounting results while minimizing leakage and scaffold damage at mounting points. Axial stretch on the scaffolds is achieved through the use of a linear actuator acting on one of the brackets through a linear bearing system with minimal resistance. The use of a single linear actuator simplifies the bioreactor design, yet it retains the capability of providing uniform axial displacement to four scaffolds simultaneously.

Circumferential Stimulation. A closed bioreactor flow system was developed to provide cell media circulation through the scaffolds as well as control over the pressure and velocity of the fluid, and it serves as an analogic fluid level sensor on the culture chambers. The system utilizes a single multi-channel pump and a four-channel pinch valve placed downstream to regulate the pressure in the loop. Circumferential strain is controlled by coordinating the controls of the pump and pinch valve via software to achieve predetermined pressure levels in the scaffolds. To prevent cross-talk and possible cross-contamination, each scaffold has an independent flow loop with an isolated bioreactor chamber and cell media reservoir.

Bioreactor Design and Construction. The bioreactor was designed in Solidworks. The main body with 4 separate ETVG chambers was developed with 3D printing constraints in mind, specifically for FDM manufacturing with ABS thermoplastic. The remaining pieces of the bioreactor system are primarily off-the-shelf parts and electronics. While some of the brackets are custom, they were designed for reproducibility and ease of manufacture. Overall, the design is not technologically demanding, and the construction can be easily and quickly performed with basic tools. Sterility during seeding and incubation is another integral design feature. A custom room-

temperature-vulcanizing silicone gasket was developed, which was formed and cured in a 3D printed mold; this method is simple to recreate and could be easily scaled. The gasket provides an air-tight seal for the lid of the incubator to avoid contamination in culture. The linear bearing system and the inlets and outlets of the flow system were also designed to prevent exposure of the inner chamber of the bioreactor to the incubator environment.

Software Control. A custom user interface was developed to control the bioreactor system in LabView. The user can adjust all inputs to control the pump velocity, axial stretch magnitudes and frequencies, max pressure, time to hold pressure, number and duration of cycles, etc. The fine control over these parameters allows for future experimentation with different mechanical stimulation procedures on the scaffolds. Additionally, a single Texas Instruments data acquisition board controls all the electronic components of the bioreactor. The entire assembly can easily be transported in a single tray and placed on an incubator shelf with the possibility being able to run 2 bioreactors simultaneously in a standard 160L incubator.

Validation of Mechanical Stimulation. A computer vision program was implemented for tracking the circumferential and axial strain response of the scaffolds. Grayscale-based edge tracking of the scaffold reports changes in the diameter. For the axial component, three dots are drawn on the scaffolds and the distances between them are used to compute axial stretch. Additionally, a pressure sensor was added to the flow system to measure luminal pressure inside the ETVG. Test scaffolds were produced by casting silicone around a brass rod of 2mm diameter to match the desired scaffold dimensions. After curing, the thin silicone tubes were pulled off the rod and cut to length for use in validation of the axial and circumferential strain feedback in the bioreactor flow loop. Impermeable silicone model scaffolds were a useful tool for prototyping the pressure system and the actuation for axial stretch by providing repeatable strain values from the various inputs.

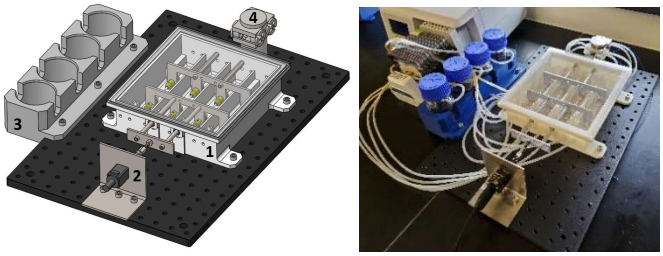

Figure 1: Final 3d-CAD assembly (left) and constructed bioreactor (right). Legend: 1) Bioreactor Chambers, 2) Linear Actuator, 3) Reservoir Fixture, 4) Pinch Valve.

RESULTS

The custom-designed ETVG bioreactor (Figure 1), was demonstrated to be effective in producing axial and circumferential stretch on small-diameter tubular scaffolds. The control programs and hardware effectively delivered the desired inputs to the bioreactor, and outputs could be measured with sufficient accuracy. The computer vision measurement system was capable of recording deformations less than 0.1mm in the scaffolds. We were able to prescribe any type of combination of axial and circumferential stretches. Circumferential stretch is achieved with a three-phase cycle (Figure 2) that involves a sequential activation of pump driving flow with pinch valve closing downstream to build luminal pressure. We found a linear correlation between time and pressure/diameters achieved, which could then be held for any duration until valve opens and initial diameter is recovered. Axial strain implementation was also validated; we found that displacement of the linear actuator applied strain directly and uniformly to the scaffolds as expected. We were able to reproducibly impart axial strains of ~10% and circumferential strains of ~4%, which are typical values of physiological strains observed in vivo due to pulsatility.[2] These results were met while maintaining a low cost for the bioreactor. In total, the system costs about $450 in hardware and $5,800 in electrical components, most of which are standard laboratory equipment that may already be available.

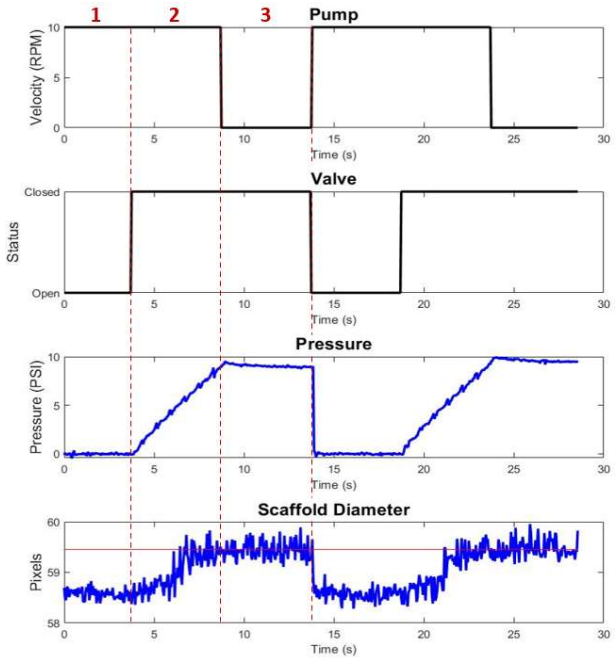

Figure 2: Cyclic circumferential strain data from bioreactor operation. Inputs are shown in black and outputs in blue. Vertical red dashed lines show different phases of the pressure cycle.

DISCUSSION

3D printing holds many advantages as a manufacturing process in this application.[4] First, it allows the bioreactor to remain low-cost relative to commercial alternatives. Additionally, 3D printing allows for rapid prototyping of the bioreactor, making it simple to perform future design iterations if desired. The technology is also easily accessible, which contributes to the reproducibility of the design. Increased availability of bioreactor technology for dynamic mechanical stimulation of engineered tissues during their incubation will enable systematic experimentation of ETVG development and determination of ideal mechanical stimulation protocols. Additionally, the bioreactor is easily scaled; a sample size of n=8 can be achieved with two bioreactors in one standard-size incubator. Due to its low cost, reliable results, and ease of manufacture and assembly, the proposed dynamic biaxial bioreactor holds potential for broader impact in the field of small-diameter vascular graft tissue engineering.

ACKNOWLEDGEMENTS

This research was funded by the NSF and VCU through the Mechanobiology REU program. The work was guided and supported by the members of the ETM[3] laboratory.

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