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Developing a Bioreactor for Biaxial Mechanical Testing and Conditioning of Vascular Tissue

Steve Marcous
University of South Carolina - Columbia

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Steve Marcous

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Accepted by:

Tarek Shazly, Director of Thesis

Abdel Bayoumi, Reader

Lacy Ford, Vice Provost and Dean of Graduate Studies

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ABSTRACT

Cardiovascular diseases are known to be one of the major causes of death around the world. One of the major causes of cardiovascular disease is arterial malfunction. Arteries malfunction when they experience chronic perturbations in their local environment above normal levels. Hence, we developed a bioreactor system in order to study the effect of chronic changes in the arterial global parameters (axial force, luminal pressure, and flow rate) on the behavior of local parameters (circumferential stress, axial stress, and flow induced shear stress). The bioreactor system was designed to (i) perform biaxial mechanical testing, (ii) incorporate, for the first time, the outer pressure as a fourth global parameter, (iii) independently control flow rate, transmural pressure (inner pressure minus outer pressure), and axial force, (iv) continuously monitor the change in arterial geometry, and (v) automatically reach/maintain constant level of user defined target values for mean axial and circumferential stress. The bioreactor system was capable of performing inflation Extension tests at axial stretch ratios of 1.2, 1.4, and 1.6 for a porcine renal artery at a pressure range from 10 to 180 mmHg. The bioreactor system was also capable of achieving target values of circumferential stress (5, 10, 15, and 20 kPa) with a tolerance of 10% at a constant level of axial stress (100 kPa) and vice versa; 60, 100, 140, and 180 kPa axial stress at constant circumferential stress of 5 kPa. A 95% Confidence Level was used to remove outliers form the collected data points.

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LIST OF SYMBOLS

V	Wall volume of the cultured construct.
r_o	Deformed outer radius of the cultured construct.
R_o	Undeformed outer radius of the cultured construct.
r_i	Deformed inner radius of the cultured construct.
R_i	Undeformed inner radius of the cultured construct.
h	Deformed thickness of the wall of the cultured construct.
l	Deformed length of the cultured construct.
L	Undeformed length of the cultured construct.
τ	Wall shear stress in the lumen of the cultured construct.
μ	Viscosity of the cell culture media.
Q	Mean flow rate of the cell culture media.
σ_z	Mean Axial stress.
λ_z	Axial stretch ratio.
σ_θ	Mean circumferential stress.
λ_θ	Circumferential stretch ratio.
F	Axial force.
P_t	Transmural pressure (inner pressure – outer pressure).
P_o	Outer pressure.
P_i	Inner pressure.

List of Abbreviations

ECs ..... Endothelial Cells

SMCs ..... Smooth Muscle Cells

LA1 ..... Linear Actuator 1

LA2 ..... Linear Actuator 2

DMEM ..... Dulbecco's Modified of Eagle's Medium

BV1 ..... Ball Valve1

BV2 ..... Ball Valve2

CCD ..... Charge Coupled Device

SV1 ..... First Solenoid Valve

SV2 ..... Second Solenoid Valve

SNP ..... Sodium Nitroprusside

mV ..... millivolt

CHAPTER 1

INTRODUCTION

The purpose of this chapter is to provide the necessary background that will introduce the motivation as well as the significance of this work. The chapter starts with a discussion of the significance of cardiovascular disease that provides the core motivation for cardiovascular research. The next section describes the function and structure of arteries. Section 1.3 talks about adaptive versus maladaptive remodeling. Next, a comparison of available biaxial mechanical testing devices in the field will be illustrated. Finally, the objectives of this work will be stated.

1.1 Cardiovascular disease

Cardiovascular diseases are one of the major causes of death around the world. Cardiovascular diseases can develop due to a host of reasons, among which are diet, exercise, smoking habits, irregularities in the electrochemical signaling of the heart, and malfunction of the arteries that supply blood to the heart muscle.

1.2 Function and Structure of arteries

The main function of an artery is to transport oxygenated blood as well as nutrients into the whole organ system in the human body including the heart itself. Blood is pumped from the heart into the aorta and travels through elastic arteries that dampen

the pulsatile nature of the pumped blood. After that, the blood travels through more muscular arteries, then through smaller arteries, passing by arterioles where pressure is reduced tremendously. After the arterioles, the blood spreads into a large network of smaller blood vessels called capillaries, where gas and nutrients exchange occurs. Deoxygenated blood leaving the capillaries goes through venules, large veins, inferior and superior vena cava, and finally back to the heart. (Humphrey 2002).

Regardless of the type of an artery, whether elastic or muscular, an artery consists of three layers: Intima, Media, and Adventitia. Figure 1.1 shows these three layers. The Intima is the innermost layer. The Intima is a uni-layer of Endothelial Cells (ECs) that allows for nutrients/waste exchange. The Intima is separated from the Media by a dense elastic layer called internal elastic lamina. The Media is the second innermost layer. The Medial layer plays an important role in giving an artery its structural support and in regulating arterial vaso-activities. The Medial layer is very thick and is composed mainly of Smooth Muscle Cells (SMCs), elastin, and collagen. The elasticity of an artery is determined by the ratio of elastin to collagen. The more elastin the Medial layer has, the more elastic an artery becomes. On the other hand, the more collagen the Medial layer has, the more muscular an artery becomes. The Media is separated from the Adventitia by a dense elastic layer called the external elastic lamina. The Adventitia is the outermost layer of an artery. The Adventitia is the load bearing layer and it is composed mainly of collagen, elastin, and connective tissues. The percentage of collagen to elastin is much higher in the Adventitia than in the Media (D'Souza, Histology of Blood Vessel).

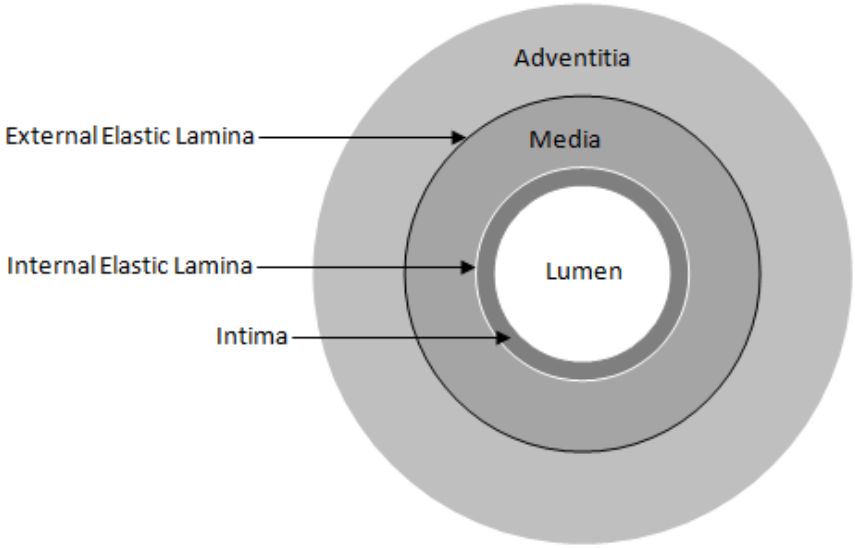


Figure 1.1: A schematic representation of the layers of an artery.

Cardiac arteries can malfunction for a number of reasons such as chronic alcoholism, atherosclerosis, inflammation, and coronary artery disease (Drexler et al., 1992; Gheorghiade & Bonow, 1998; Kriszbacher, Koppán, & Bódis, 2005; Libby, 2002). In addition, one of the major causes of arterial malfunction is when an artery experiences chronic perturbations in the local arterial environment above the normal levels. Chronic perturbations include, but they are not limited to, hypertension, an increase in blood flow rate, and accumulation of plaques.

1.3 Adaptive versus maladaptive remodeling

All perturbations (e.g. acute and/or chronic) are first sensed by the ECs that line up the inner surface of the Intima. ECs then send signals to the Media where SMCs exist. In case of acute perturbations, SMCs either relax or recoil in response to the signals received from the ECs in order to maintain a homeostatic arterial environment. In case of chronic perturbations, the chronic change in transmural pressure (pressure inside the

artery minus pressure outside the artery), blood flow rate, and/or axial force leads to maladaptive remodeling. Maladaptive remodeling occurs by breaking down and building up (with a different percentage) arterial constituents (e.g. collagen, elastin, and fibroblasts) in a trial to reach a homeostatic condition (Gerdes & Capasso, 1995). Maladaptive remodeling, therefore, changes the percentage of arterial constituents such that an artery becomes less/more compliant and hence, unable to function efficiently compared to a healthy artery.

In normal conditions, cells are known to adopt a certain level of residual stress/strain to achieve an equal distributed amount of axial stress along the arterial length and of circumferential stress across the thickness of the arterial wall. Axial residual stress/strain can be noticed by the shortening of an artery when that artery is excised. Circumferential residual stress/strain can be seen when an artery is cut into rings and a ring is cut radially. When a ring is cut radially, it springs open and loses its circular shape, which is indicative of the existence of a residual stress/strain (Rachev & Greenwald, 2003). When changes occur in any of the mechanical cues such as transmural pressure, flow rate, and/or axial force (whether acutely or chronically), the arterial wall experiences different level of stresses. As a result of the change in the stress sensed by the arterial wall, an artery remodels itself to alleviate the change in stress level and to return to a homeostatic condition. Thus, controlling mechanical cues is a key in investigating the arterial remodeling process in general.

1.4 Comparison of available biaxial mechanical testing devices

In order to study the effect of changing the mechanical cues (transmural pressure, flow rate, and/or axial force) ex vivo, researchers developed many different types of bioreactors. Kim et al. (Kim et al., 2011) developed an inflation extension bioreactor to study the effect of increasing transmural pressure at a constant axial stretch ratio (deformed length divided by undeformed length) on the compliance of their porcine thoracic aorta. However, an inflation extension bioreactor is only valid to study the effect of acute changes in the mechanical cues on the cultured construct. In order to investigate how an artery adapts to chronic changes in the mechanical cues, researchers developed another kind of bioreactor that allows for long term culturing (7-14 days). Gleason et al. (Gleason, 2004) described a bioreactor that is capable of long term culturing of small caliper (50-5000 micron in diameter) arteries. In addition to the capabilities of the inflation extension bioreactor of Kim et al., the bioreactor of Gleason et al. allowed for simultaneous biaxial mechanical testing as well as functional testing to quantitatively describe how an artery remodels when subjected to chronic changes in the arterial mechanical cues over the culturing period. Zaucha et al. (M. Zaucha et al., 2009) guided by the design of the bioreactor of Gleason et al. developed another type of bioreactor that is capable of performing a multiphoton microscopy on the cultured construct to monitor the change in the conformation and composition of collagen, elastin, and SMCs during the culture period when subjected to various mechanical cues.

Although many advances have been made to develop various types of bioreactors to study arterial remodeling, no one has developed a bioreactor that cannot only be used to perform simultaneous biaxial mechanical testing and functional testing over long term

culturing, but also to utilize outer pressure in achieving/maintaining mean circumferential stress.

1.5 Objectives

Towards fulfilling the needs described at the end of the previous section, the objective of this work is to (i) perform biaxial mechanical testing, (ii) incorporate, for the first time, the outer pressure as a fourth global parameter, (iii) independently control flow rate, transmural pressure (inner pressure minus outer pressure), and axial force, (iv) continuously monitor the change in arterial geometry (outer diameter) of the cultured construct, and (v) automatically maintain constant level of desired mean axial and circumferential stress by automatically adjusting axial force and transmural pressure, respectively based on the change in the geometry of the cultured construct.

CHAPTER 2

MATERIALS AND METHODS

The design of the bioreactor will be described in terms of five key features: the culture chamber, control of axial stretch/load, control of outer pressure, imaging system, and computer interface system, data acquisition, and analysis. Figure 2.1 shows a conceptual schematic of the bioreactor. The function of the components of the bioreactor in satisfying the key features/objectives of this research will be explained subsequently.

2.1 The culture chamber

The function of the culture chamber is to provide a suitable incubation of the cultured construct throughout the experimental period. The culture chamber has to allow for axial stretching of the cultured construct, facilitate continuous monitoring of the geometry (outer diameter) of the cultured construct, and be pressurized/depressurized as necessary. In order to meet these features, we designed the culture chamber described below. Figure 2.2 can be used as a guide to visualize the components of the bioreactor system.

The culture chamber is made of clear polycarbonate rectangular sheets that are glued together to form the walls of the chamber. The inner dimensions of the culture chamber are 10.20 x 5.10 x 5.00 cm (length, width, height). The thickness of the walls of the culture chamber is 1.24 cm. The walls of the culture chamber are glued on a polycarbonate base. The dimensions of the polycarbonate base are 15.28 x 10.18 x 1.24

cm (length, width, height). The walls of the culture chamber are centered on the polycarbonate base such that there is an extra 1.30 cm distance remaining all around the polycarbonate base. The reason for the remaining 1.30 cm distance is to allow for the assembled chamber (walls and base) to be fixed to an Aluminum base using four Allen head bolts (not shown); one bolt on each corner of the polycarbonate base. The chamber is sealed with (i) a 0.30 cm thick silicone gasket that is oriented throughout the perimeter of the top surface of the chamber walls and (ii) a polycarbonate cover via 12 bolts. The dimensions of the polycarbonate cover are 12.68 x 7.58 x 1.24 cm (length, width, height). The chamber has three sets of double threaded ports and a single set of a single threaded port. The function of each set of the threaded ports will be explained in the sections where these sets of threaded ports are utilized.

The first set of double threaded ports is used to exchange the static cell culture media (adventitial cell culture media) surrounding the outer surface of the cultured construct under a laminar flow hood to minimize contamination. The first set of double threaded ports is to mount cell culture media exchange connectors; inlet port and outlet port. The inlet port is on the polycarbonate cover and the outlet port is on the front wall of the chamber. The inlet port is 1.84 cm from the right surface of the polycarbonate cover and 1.84 cm from the front surface of the polycarbonate cover. In order to keep the adventitial cell culture media at 37 degree Celsius, there exists a silicone heat plate (Statesville Process Instruments, USA) that sets into an extruded cut in the bottom surface of the polycarbonate base. A thermostat (not shown) that is fixed onto the Aluminum base controls the temperature of the silicone heat plate. Underneath the

silicone heat plate is a Styrofoam sheet (not shown) that insulates the bottom surface of the heat plate, so that heat does not dissipate into the Aluminum base.

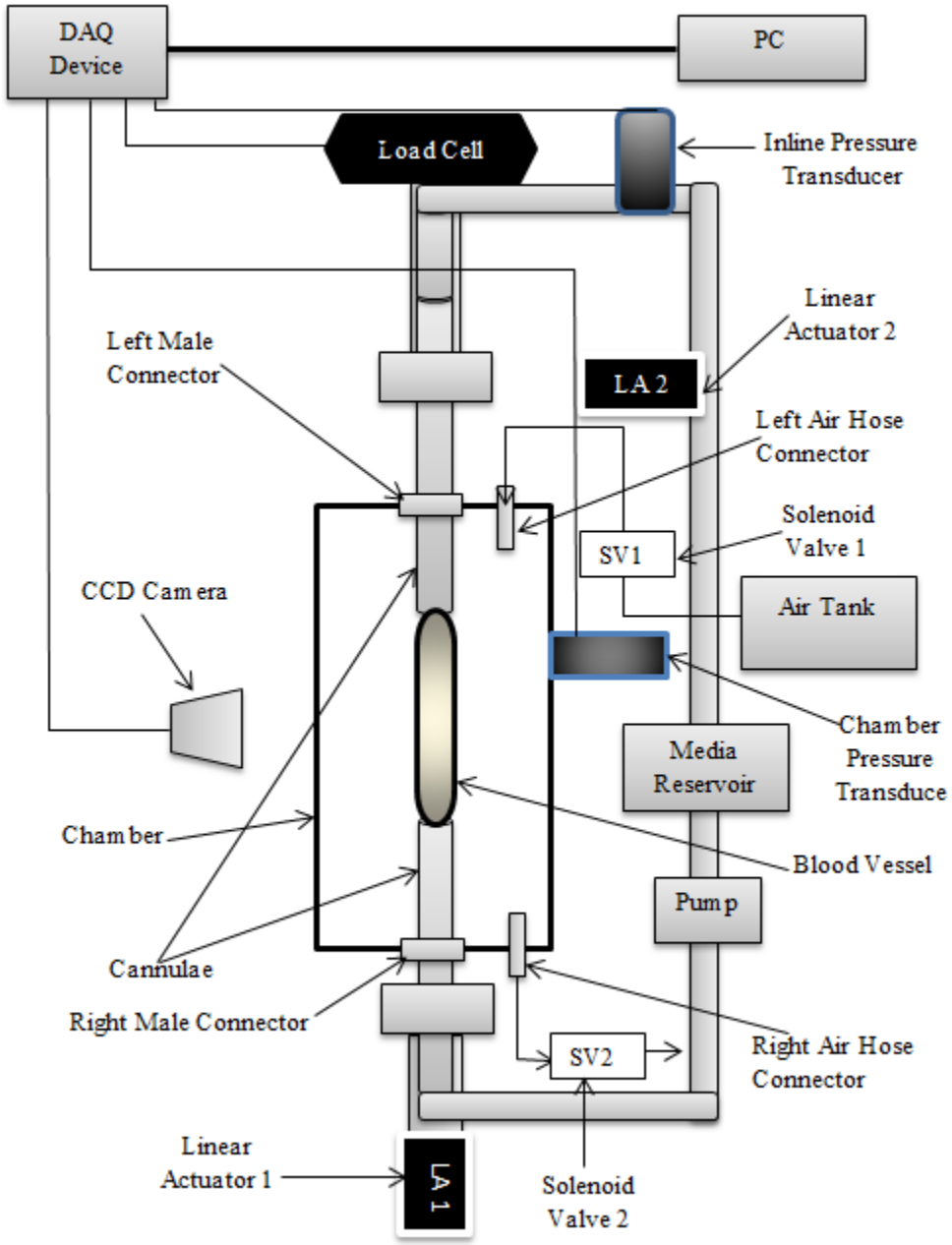


Figure 2.1: A top labeled view of the conceptual schematic diagram of the bioreactor system. The left hand side of the chamber is the front wall of the bioreactor.

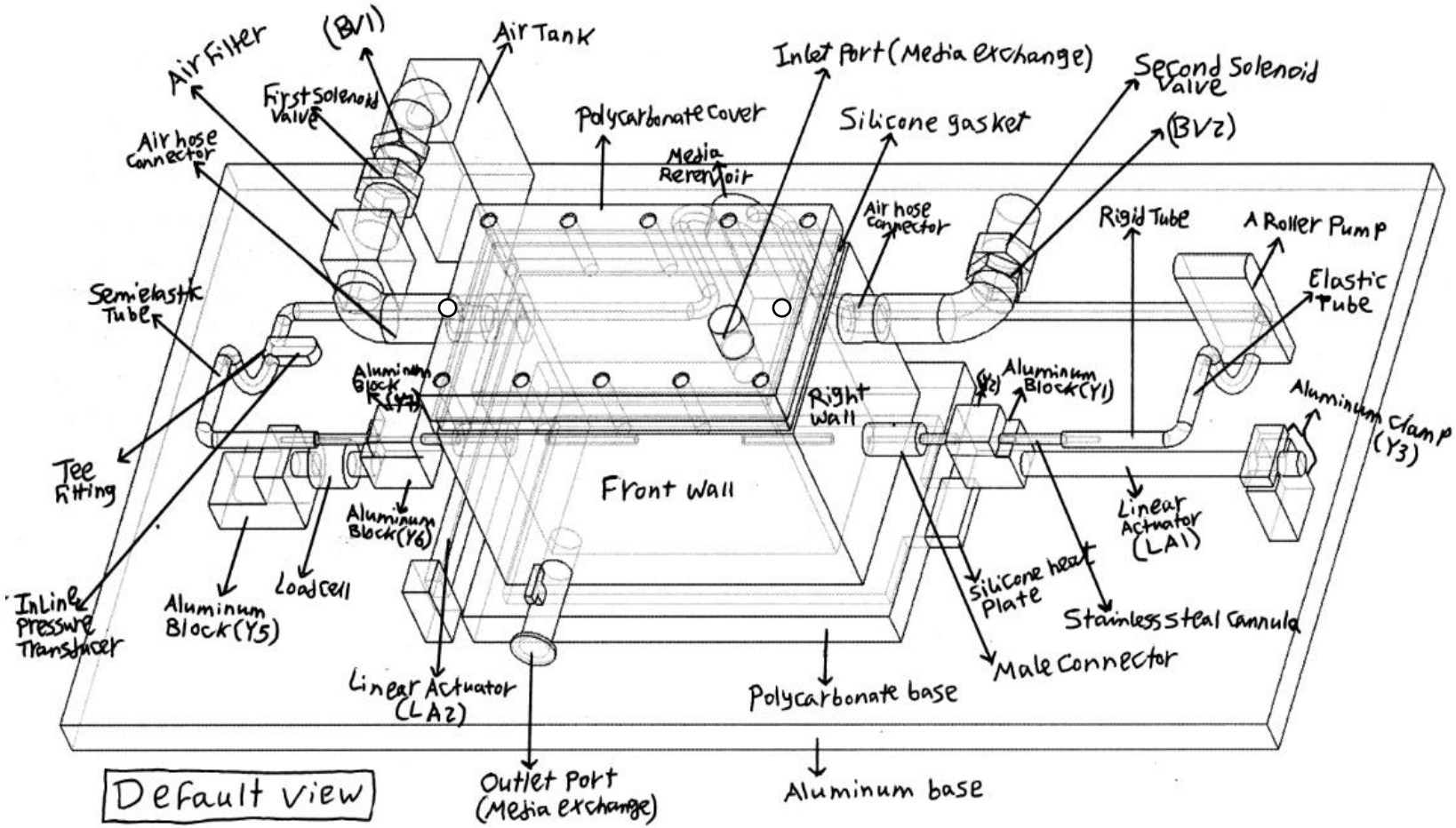


Figure 2.2: A labeled schematic representation for the components of the bioreactor

The single threaded port is to mount a outer pressure transducer that is shown in Figure 2.1 (Omega Engineering, USA). The outer pressure transducer monitors the pressure inside the sealed culture chamber. The outer pressure transducer is mounted on the back wall of the culture chamber 4.30 cm from the top surface of the polycarbonate base and 2.5 cm from the edge of the left wall of the culture chamber.

2.2 Control of Axial Stretch/Load

Axial stretch and load of the cultured construct ex vivo are accomplished by sliding cannulae (Swagelok, model SS-T2-S-028-20, Columbia, SC) through two male connectors (Swagelok, model SS-2-UT-1-2BT, Columbia, SC). The two male connectors are attached to the culture chamber using the second set of double threaded ports. The two male connectors face one another on opposite sides of the right and left walls of the culture chamber. The male connectors are mounted vertically 1.80 cm above the top surface of the polycarbonate base and horizontally in the middle of the right and left walls of the culture chamber. The male connectors allow for two bright annealed steel cannulae (0.31 cm outer diameter 'OD') to slide horizontally back and forth through a silicon O-ring in order to stretch and relax the cultured construct. The ends of the cannulae where the cultured construct is to be sutured are both tapered and grooved to allow for easy and snug mounting of the cultured construct on the cannulae. The snug mounting helps prevent any leakage.

On the right-hand side of the chamber there exists a linear actuator 1 (LA1) (Physic Instrumente, model M-227.50, Auburn, MA) that controls the axial length of the cultured construct. LA1 has a stroke (travel distance) of 5 cm and is controlled by

Mercury C-863 DC motor controller (Physic Instrumente, model M-227.50, Auburn, MA). The tip of the shaft of LA1 is bolted to a custom made Aluminum block (Y1) that is 2.86 x 2.56 x 2.86 cm (length, width, height). The distance between the bottom surface of Y1 and the top surface of the Aluminum base is 0.83 cm, which allows for free movement of the shaft of the linear actuator. Another custom made Aluminum block (Y2) is mounted on top of Y1. The dimensions of Y2 are 1.92 x 1.92 x 1.30 cm (length, width, height). A centered hole with a 0.31 cm diameter is cut through Y1 and Y2, so that Y2 can clamp on the right-hand side cannula via 4 screws (not shown) that are bolted to Y1. This configuration of Y1-Y2 allows the movement of the right-hand side cannula back and forth via the linear actuator without interfering with the flow of cell culture media inside the cannula.

In order to fix LA1 onto the Aluminum base, I custom made a third Aluminum clamp (Y3). The dimensions of the bottom block of Y3 are 1.95 x 6.37 x 2.16 cm (length, width, height) and those of the top block of Y3 are 1.95 x 6.37 x 1.97 (length, width, height). Y3 has a centered hole of 1.90 cm in diameter that fits the outer diameter cylindrical body of LA1. I chose the height of the bottom block of Y3 to be 2.16 cm, so that the right-hand side cannula is aligned with the hole of right-hand side male connector. Because LA1 does not have automatic limit switches to stop the movement of its shaft when the shaft reaches its min/max limit, I bolted a custom made Aluminum bar (not shown) 5 cm away from the right-hand side male connector to mechanically stop the shaft without damaging the motor of LA1. Hence, the travel distance is only 5 cm, which is the stroke capacity of LA1.

On the left-hand side of the chamber, there exists a load cell (Delta Metrics Inc. model XLU68s-500G, Worthington, OH) that measures the axial force being applied on the cultured construct when the shaft of LA1 moves horizontally back and forth. The load cell has an intensity of 500 gram of force. The load cell is mounted horizontally into two custom-made Aluminum blocks. The first custom-made Aluminum block (Y5) is attached to the Aluminum base via two Allen head bolts (not shown). The dimensions of Y5 are 5.00 x 1.32 x 2.00 cm (length, width, height). Y5 has a threaded hole into which the distal side of the load cell is screwed. The threaded hole is 1.53 cm away from the bottom of (Y5) resulting in a 0.26 cm clearance between the bottom surface of the load cell and the top surface of the Aluminum base. The 0.26 cm clearance allows the load cell to measure the axial force without any frictional losses that would have been experienced if the load cell were to touch the top surface of the Aluminum base. The second custom made Aluminum block (Y6) has a threaded hole into which the proximal side of the load cell is screwed. The dimensions of Y6 are 2.60 x 1.33 x 2.90 (length, width, height). Thus, there is a distance of 0.20 cm between the bottom surface of Y6 and the top surface of the Aluminum base to allow for frictionless movement. On top of Y6 there is another custom made Aluminum block (Y7) that has dimensions of 1.90 x 1.33 x 1.28 cm (length, width, height). A centered hole with a 0.31 cm diameter is cut through Y6 and Y7, so that Y7 can clamp on the left-hand side cannula and be bolted to Y6 (bolts are not shown). The Y6-Y7 configuration allows for the load cell to measure the axial force sensed by the cultured construct without any friction that would result, if Y6 touched the top surface of the Aluminum base. I chose the dimensions of Y5, Y6, and Y7 such that the left-hand side cannula is aligned with the hole of the left-hand side male connector.

2.3 Control of Luminal Flow/Pressure

In order to achieve/maintain a physiological level of shear stress, a roller-pump (New Era Pump Systems Inc. model NE9000, Farmingdale, NY) that is shown in figure 2.2 is used to withdraw cell culture media from a media reservoir that is pressurized with 95% oxygen and 5% carbon dioxide air to maintain proper humidity and pH level. The cell culture media is Dulbecco's Modified of Eagle's Medium (DMEM) with 4.5 g/L glucose, L-glutamine and sodium pyruvate. The roller-pump infuses the DMEM into 0.46 cm OD elastic tubing, through 0.31 cm OD rigid tubing to dampen the pulsatile flow, then to the right-hand side cannula, through the cultured construct (figure 2.1), into the left-hand side cannula, through 0.66 cm OD semi-elastic tubing, into a tee fitting, through 0.46 cm OD elastic tubing, and finally back to the media reservoir. I placed the media reservoir into a heat bath (not shown) to maintain the temperature of the cell culture media at 37 degree Celsius.

Figure 2.2 also shows an inline pressure transducer (Omega Engineering Inc., model PX309-015GV, Sunbury, OH) that is attached to the tee fitting (Lowe's, Columbia, SC) and both the tee fitting and the pressure transducer are attached to the Aluminum base via a custom made polycarbonate clamp (not shown). The inline pressure transducer measurements were calibrated as shown in Figure 2.3 to read the luminal pressure of the cultured construct as follows: first, I borrowed and attached a catheter-based pressure transducer (Millar Inc., model MPR-500, Houston, TX) to the tee side of a push-to-connect tube fitting (McMaster-Carr, model 7880T263, Atlanta, GA). Second, I attached two separate 0.31 cm OD elastic tubes to the remaining two sides of the push-to-connect tube fitting. Third, I connected the other ends of the 0.31 cm OD elastic tubes

into the right-hand side and the left-hand side cannulae of culture chamber. Fourth, I measured the luminal pressure using both the inline and the catheter-based pressure transducers simultaneously. Finally, I developed a calibration curve, so that the readings of the inline pressure transducer (that is located past the left hand side cannula) can be adjusted to read the pressure in the lumen of the cultured construct. The reason why a catheter-based pressure transducer was not used in lieu of the inline pressure transducer was because of the inability to connect the catheter based pressure transducer anywhere close to the cultured construct such that the catheter based pressure transducer does not interfere with the flow of the cell culture media.

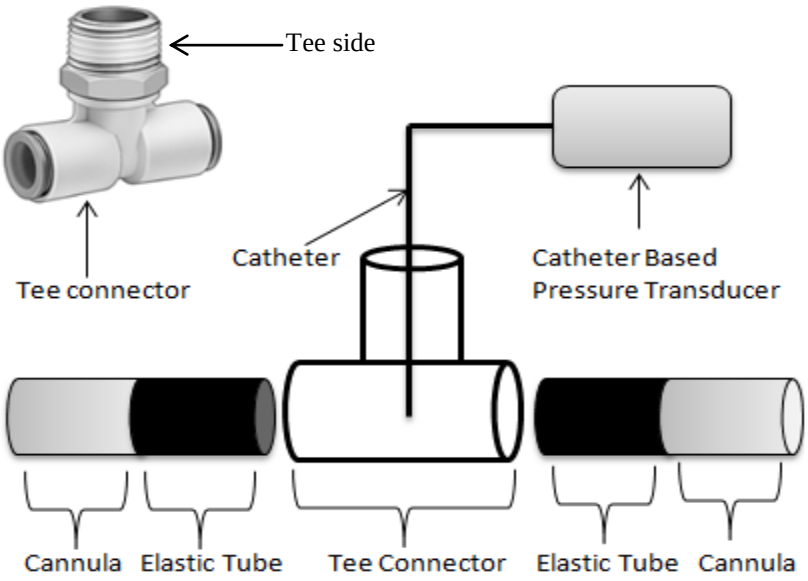


Figure 2.3: A schematic representation that shows the mechanism by which the inline pressure transducer (not shown) is calibrated to read the luminal pressure of the cultured construct. The tee connector is placed where the cultured construct is to be mounted.

I used another linear actuator (LA2) (Firgelli Technologies Inc., part # L12-100-210-6-p, Swanton, VT) to independently control the luminal pressure apart from the flow rate. The shaft of LA2 pushes on the 0.46 cm OD elastic tube distal to the inline pressure transducer creating a back pressure. Hence, the luminal pressure can be manipulated.

2.4 Control of Outer pressure

Decoupling stress from strain is achieved by controlling the outer pressure utilizing the third set of double threaded ports. The third set of double threaded ports is to mount two air hose connectors as shown in Figure 2.2. These two air hose connectors are facing one another on opposite sides of the right and left walls of the culture chamber. The air hose connectors are mounted 4.22 cm from the top surface of the polycarbonate base and 2.31 cm from the surface of the 10.20 x 5.00 cm back wall of the chamber. The left-hand side air hose connector is to supply air to the chamber to pressurize it. The right-hand side air hose connector is to release air from the chamber to depressurize it.

In order to achieve that objective of decoupling stress from strain, I used a 1 gallon air tank (VIAIR corp., part # 91010, Irvine, CA) that is filled with air (95% oxygen and 5% carbon dioxide) to pressurize the chamber. I connected a pressure gauge (not shown) to the air tank to read the pressure inside the air tank. The factory recommended maximum air tank capacity is 150 psi. There are two solenoid valves (Norgren Herion USA Inc., model 3803, Warrendale, PA) that are used to control the process of pressurizing/depressurizing the chamber. When the first Solenoid Valve (SV1) is energized, air leaves the air tank to pass through a slightly open Ball Valve1 (BV1) (Lowe's, Columbia, SC) as shown in Figure 2.2, into 1.00 cm OD semi-elastic tube, through the first solenoid valve, into another 1.00 cm OD semi-elastic tube, through an air filter (Norgren Inc., model F74G-3AD-AD1, Louisville, KY), and into the chamber through the left-hand side air hose connector. When the second Solenoid Valve (SV2) is

energized, air leaves the chamber through the right-hand side air hose connector, into 1.00 cm OD semi-elastic tube, through another slightly open Ball Valve2 (BV2), into the second solenoid valve, and to the laminar flow hood.

2.5 Imaging System

In order to calculate axial and circumferential stress/strain on the wall of the cultured construct, the outer diameter and the thickness of the wall of the cultured construct are to be determined. A Charge Coupled Device (CCD) camera (Basler Inc., model scA640-70fm, Exton, PA) is used to monitor the change in the outer diameter of the cultured construct using a FUJINON lens (not shown) (Fujifilm Corp., model HF50HA-1B, Edison, NJ). The thickness of the wall of the cultured construct is calculated based on the assumption of incompressibility from the current length and the initial volume using the formulas below:

$$\mathbf{V = \pi (r_o^2 - r_i^2) l} \qquad \mathbf{r_i = sqrt\left(r_o^2 - \frac{V}{\pi l}\right)} \qquad \mathbf{h = r_o - r_i}$$ (Gleason et al., 2004)

where V is the wall volume, r_o is the deformed outer radius (outer diameter divided by 2), r_i is the deformed inner radius, l is the deformed length, and h is the deformed wall thickness.

The CCD camera that is shown in Figure 2.1 points towards the front wall of the culture chamber. A cylindrical tube of known diameter was used to calibrate the number of pixels into an outer diameter (distance between the top and the bottom edges of the tube) in millimeter. The camera code in LabVIEW (National Instruments Inc., V11.0,

Austin, TX) utilizes the pixel-distance conversion to accurately monitor the change in outer diameter of the cultured construct.

2.6 Computer Interface System, Data Acquisition and Analysis

I used LabVIEW to build a code that controls the bioreactor. All LabVIEW codes are presented in the Appendix A. The code can be broken down as follows: first, I used a Data Acquisition device (DAQ) (National Instruments Inc., model NI USB-9219, Austin, TX) that has four differential channels. I used only three channels; a channel to acquire a signal from the load cell, a channel to acquire a signal from the inline pressure transducer, and a channel to acquire a signal from the outer pressure transducer. The acquisition mode of the module is "continuous samples" and the number of samples to read is 5 samples at a rate of 2 Hz. Second, a For loop that is nested in a While loop generates 10 datum points from the three sensors (load cell and the 2 pressure transducers) and from the camera (outer diameter) simultaneously in millivolt (mV) and in millimeter (mm), respectively at each iteration of the While loop. These datum points are converted to force in Newton and pressure in mmHg accordingly. Third, an outlier test with a 95% confidence level is applied to the 10 datum points to remove any outliers from the acquired datum points. Fourth, the remaining datum points after performing the outlier test are averaged into a single value for each sensor. Finally, the averaged values are saved and are used to calculate necessary parameters and perform designed analyses.

For the linear actuator (LA1)-load cell subsystem, the averaged actual value of the force from the load cell is compared with a user defined force value within a user defined tolerance. If, for example, the averaged force value is greater than the user defined force

value, the linear actuator extends its shaft to reduce the axial force sensed by the cultured construct and vice versa.

For the linear actuator (LA2)-inline pressure transducer subsystem, the averaged actual value of the luminal pressure from the inline pressure transducer is compared with a user defined luminal pressure value within a user defined tolerance. If, for example, the averaged actual luminal pressure value is greater than the user defined luminal pressure value, LA2 retracts and releases the pressure on the semi-elastic tube to reduce the actual luminal pressure value and vice versa.

For the outer pressure transducer- air tank subsystem, the averaged actual value of the outer pressure from the outer pressure transducer is compared with a user defined target outer pressure value within a user defined tolerance. If, for example, the averaged actual outer pressure value is greater than the user defined outer pressure value, SV2 will open to release air from the chamber to reduce the outer pressure and vice versa.

The flow rate is determined by a user defined wall shear stress value and the deformed inner radius assuming constant viscosity at each iteration of the While loop using the formula below.

$$\tau = \frac{4\mu Q}{\pi r_i^3} \qquad \text{(Rachev \& Greenwald, 2003)}$$

where τ is the wall shear stress, Q is the mean flow rate, μ is the viscosity of the media, and r_i is the deformed inner radius.

Axial and circumferential stress/strain are calculated based on the averaged actual values of inner pressure, outer pressure, axial force, and deformed length as well as

inner/outer radius, and thickness measurements of the cultured construct using the following formulas:

$$\sigma_\theta = \frac{P_t r_i}{h} - P_o \qquad \sigma_z = \frac{F}{\pi(r_o^2 - r_i^2)}$$

$$\lambda_\theta = \frac{(r_o + r_i)/2}{(R_o + R_i)/2} \qquad \lambda_z = \frac{l}{L} \qquad \text{(Gleason et al., 2004)}$$

where σ_θ is the circumferential stress, P_t is the transmural pressure (luminal pressure minus chamber outer pressure "P_o"), r_i (deformed inner diameter/ 2) is the deformed inner radius, h ($r_o - r_i$) is the wall thickness of the cultured construct, r_o (deformed outer diameter/2) is the deformed outer radius σ_z is the axial stress, F is the axial force, and r_o is the outer radius (outer diameter/ 2), λ_θ is the circumferential strain, λ_z (deformed length "l" / undeformed length "L") is the axial strain, and R_o and R_i are the undeformed outer and inner radius, respectively.

On the other hand, the user can set values for the circumferential and axial stress and LabVIEW code would achieve/maintain these values of circumferential and axial stress via changing the transmural pressure and the axial force, respectively. The transmural pressure will be changed by manipulating the luminal pressure, the outer pressure, or both. The axial force will be changed by controlling the position of the linear actuator 1 (LA1).

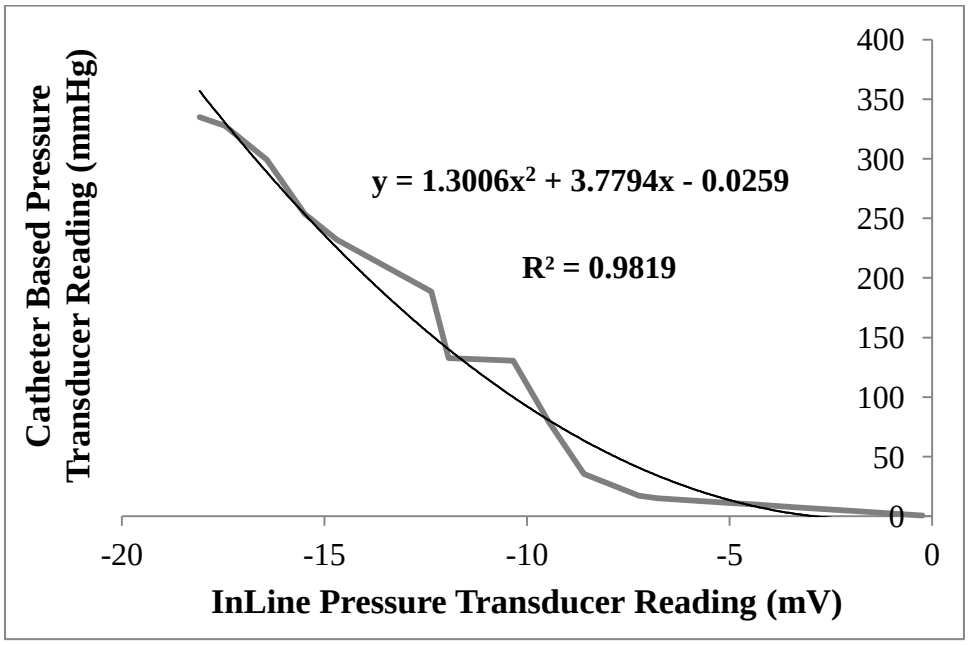
CHAPTER 3

RESULTS

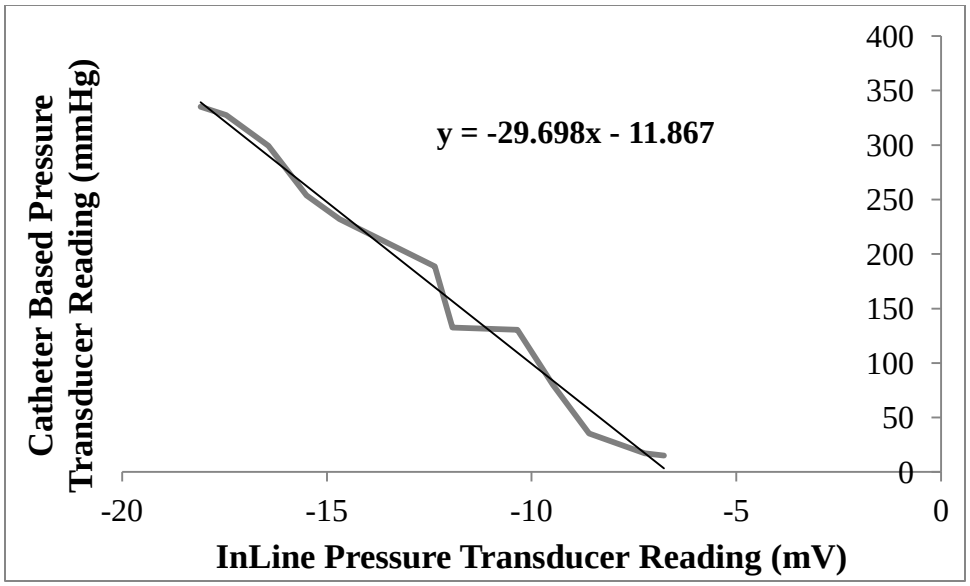
The results of this work are demonstrated below. Each section describes a specific aspect of the results

3.1 Inline pressure transducer calibration

Figure 3.1(a) shows the simultaneous measurement of luminal pressure using both the inline pressure transducer and the catheter-based pressure transducer. Only the linear part of the graph was used to come up with a linear equation that converts the millivolt (mV) signal into a luminal pressure in mmHg. Figure 3.1(b) shows the linear portion of the curve.



(a)



(b)

Figure 3.1: (a) Shows the full calibration of the inline pressure transducer to read the luminal pressure inside the cultured construct. (b) Shows only the linear portion of the calibration curve and the equation of the line that was used to convert mV signal into a luminal pressure.

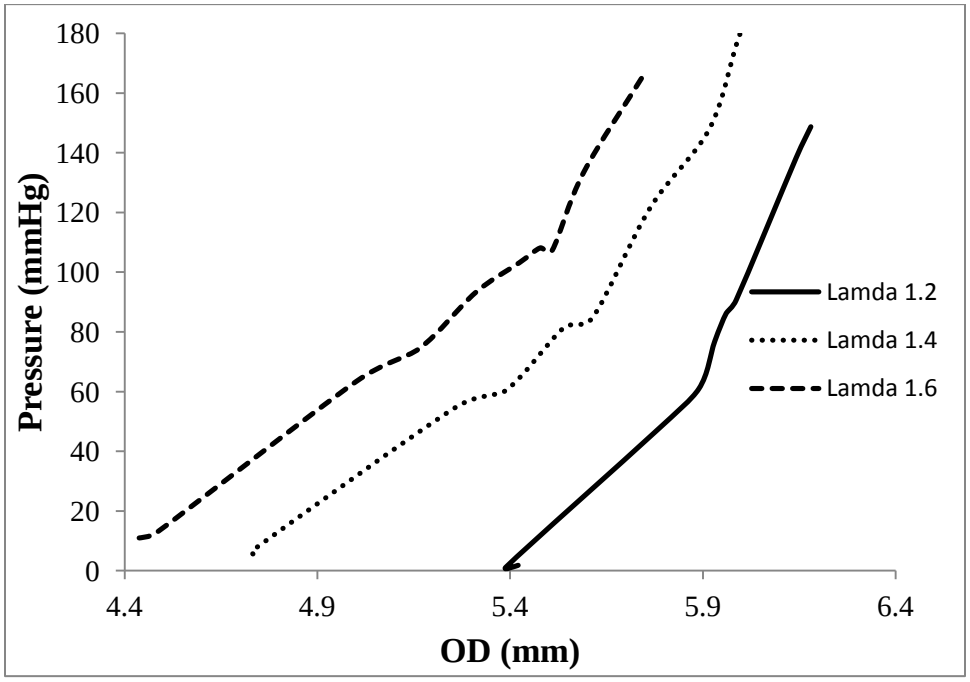
3.2 Biaxial mechanical testing

An inflation extension test was made on a porcine renal artery to prove the capability of the bioreactor to perform bi-axial mechanical testing. Figure 3.2 (a) shows passive (the artery was infused with and submerged in water) pressure-diameter curves of the porcine renal artery at three different axial stretch ratios; Lamda 1.2, Lamda 1.4, and Lamda 1.6. When the porcine renal artery was stretched 120% of its original length, its outer diameter was 5.39 mm at zero luminal pressure. As the luminal pressure increased, the outer diameter increased as well. Higher stretch ratios of 140% and 160% led to a smaller outer diameter of the porcine renal artery of 4.73 and 4.44 mm.

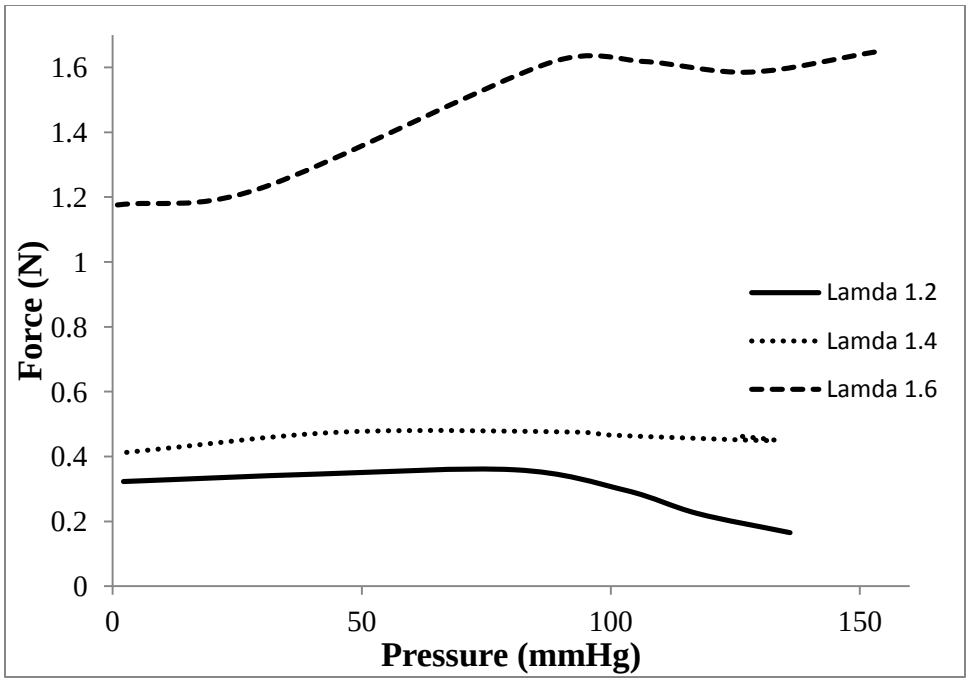
Figure 3.2 (b) shows concurrent passive force-pressure curves for the same porcine renal artery at the same axial stretch ratios mentioned above. At 1.2 axial stretch ratio, the force sensed by the porcine renal artery stayed the same until the luminal pressure reached 80

mmHg. Past a luminal pressure value of 80 mmHg, the porcine renal artery started to relax. The relaxation of the porcine renal artery can be shown by the reduction of the force sensed by the porcine renal artery past the 80 mmHg luminal pressure.

At 1.4 axial stretch ratio, the force sensed by the porcine renal artery stayed the same regardless of the change in the luminal pressure. At 1.6 axial stretch ratio, the force sensed by the porcine renal artery continued to go up.



(a)



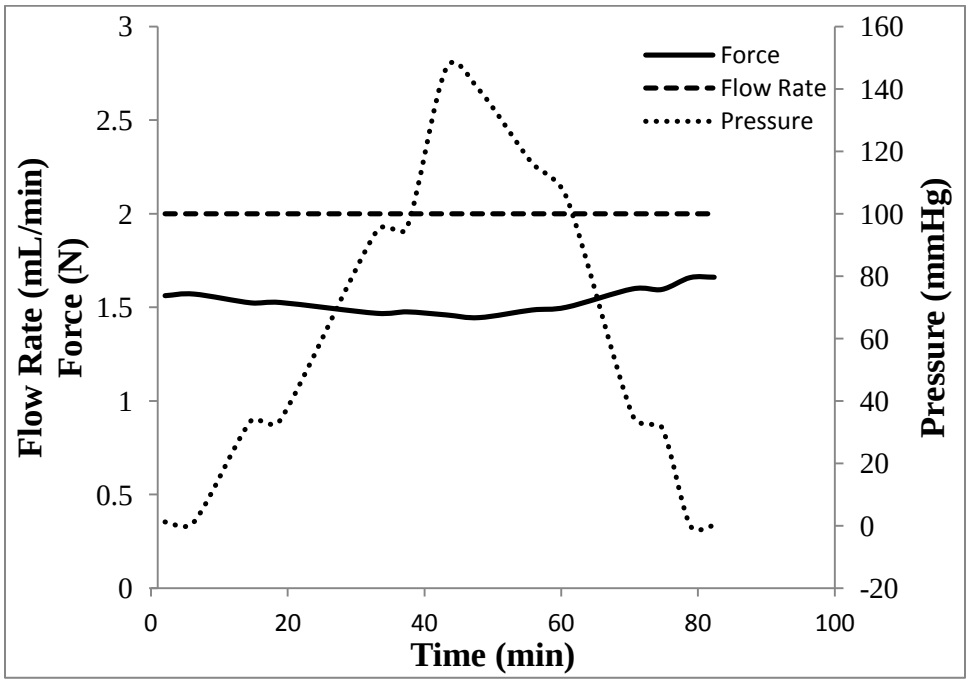
(b)

Figure 3.2: Shows the results of an inflation extension test of an artery, where (a) shows the pressure diameter relation at three constant axial stretch ratios; Lamda 1.2, Lamda 1.4, and Lamda 1.4, and (b) shows the force pressure relation at the same axial stretch ratios.

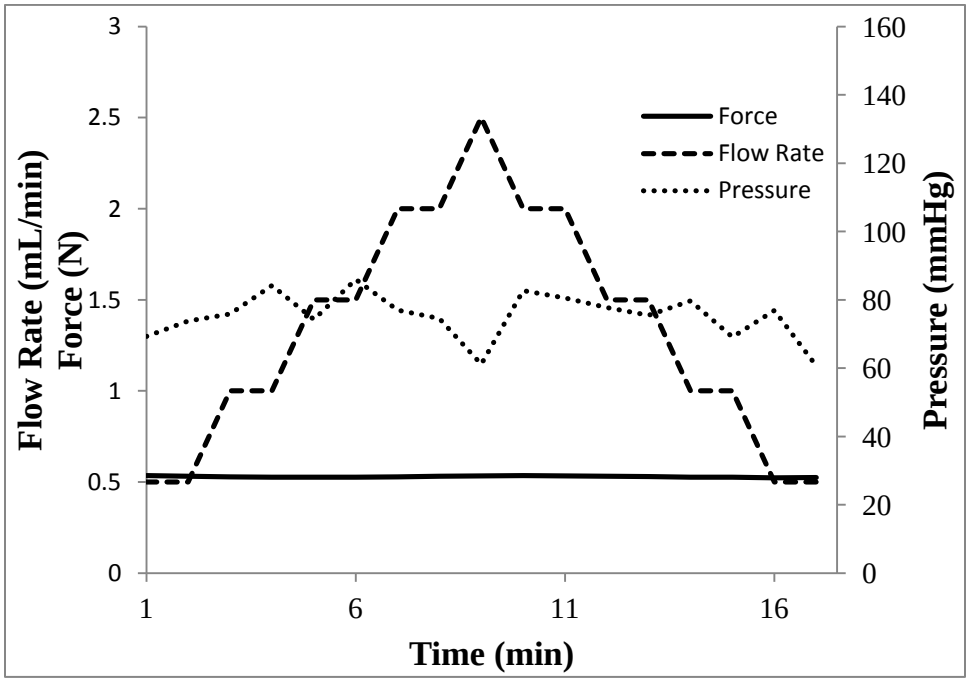
3.3 Independent control of transmural pressure, flow rate, and axial force

Three experiments were run to demonstrate the capability of the bioreactor system to independently control flow rate, axial force, and transmural pressure. Figure 3.3 (a) shows a stepwise increase of 20 mmHg in transmural pressure from 0 to 140 mmHg flowed by a stepwise decrease of 20 mmHg from 140 to 0 mmHg at constant flow rate of 2 mL/min and axial force of about 1.59 N. Figure 3.3 (b) shows a stepwise increase of 0.50 mL/min in flow rate from 0.50 to 2.50 mL/min followed by a stepwise decrease of 0.50 mL/min from 2.50 to 0.50 mL/min at constant pressure of about 78 mmHg and axial force of about 0.59 N.

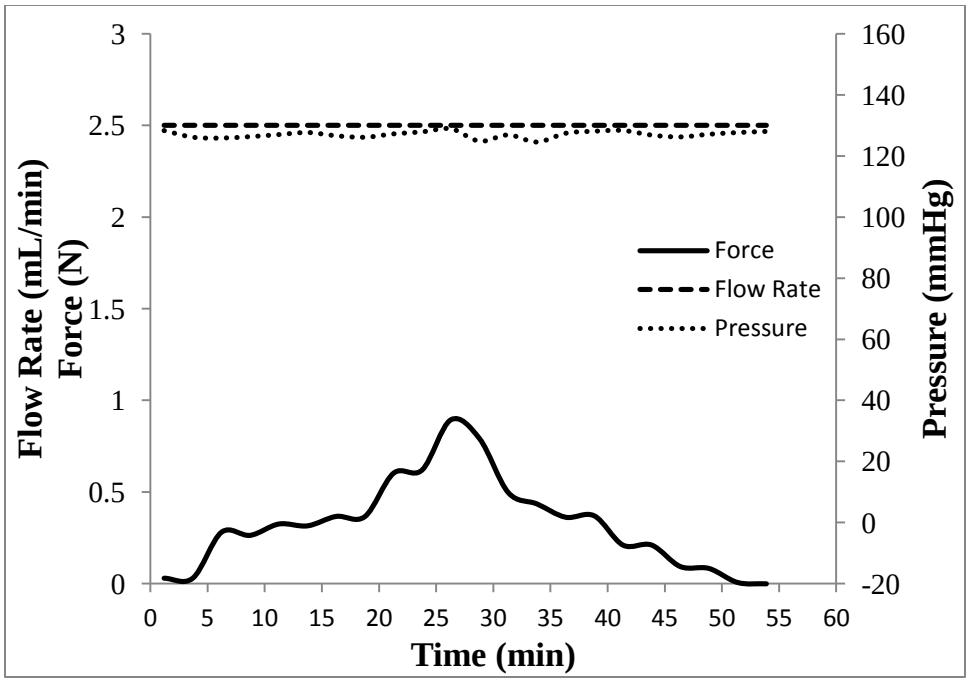
Figure 3.3 (c) shows a stepwise increase of 0.30 N in axial force from 0 to 0.90 N followed by a stepwise decrease of 0.3 mL/min from 0.9 to 0 N at constant pressure of about 127 mmHg and flow rate of about 2.50 mL/min.



(a)



(b)



(c)

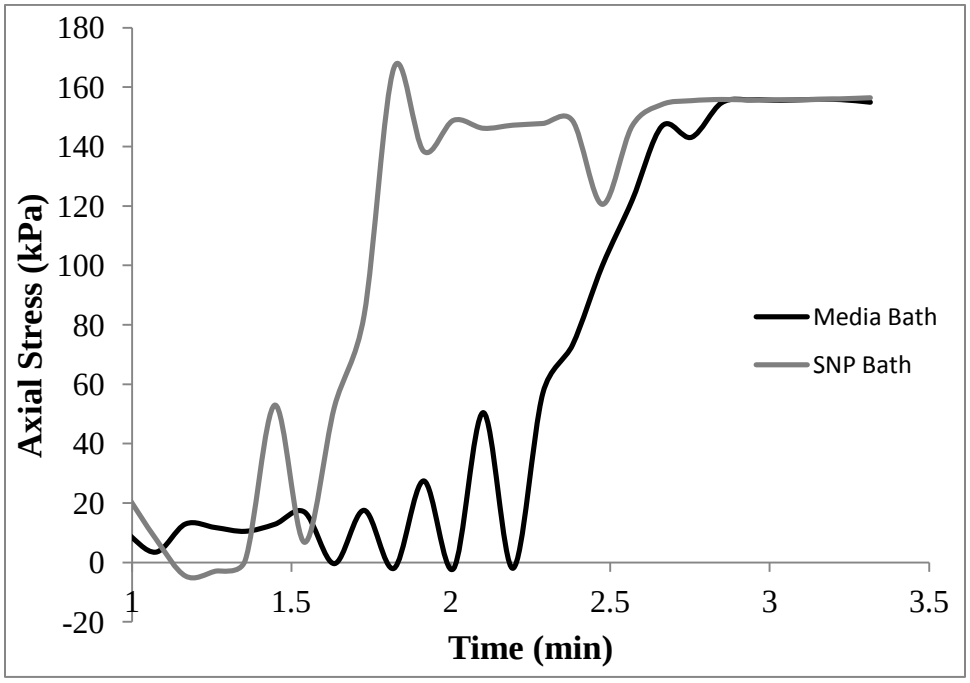
Figure 3.3: Shows the capability of the bioreactor system to independently control transmural pressure (a), flow rate (b), and axial force (c) while maintaining the other two variables constant.

3.4 Target test of axial and circumferential stress

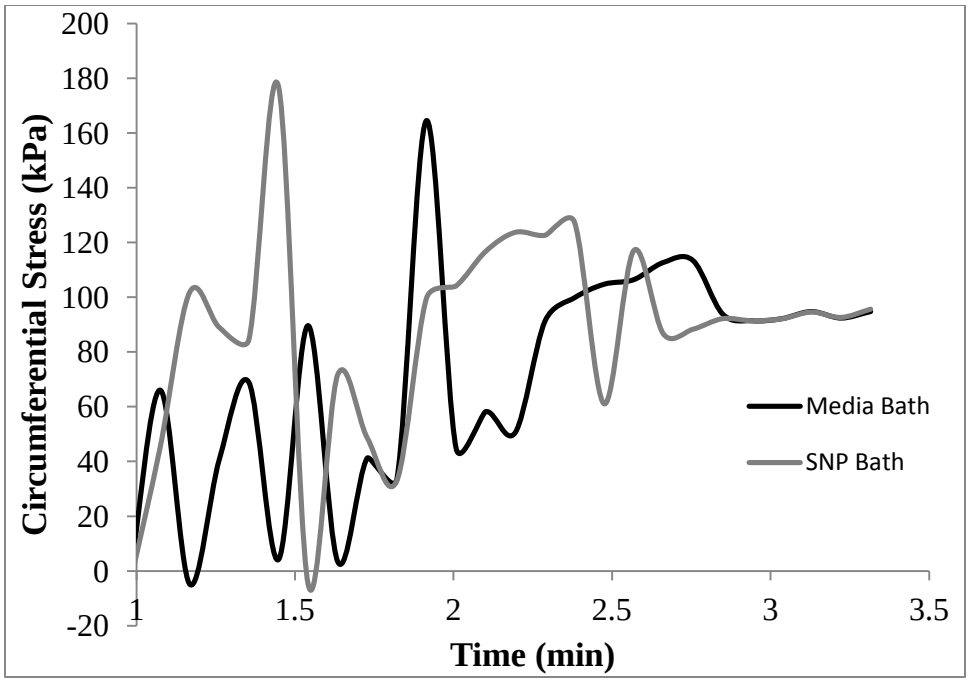
In order to demonstrate the capability of the bioreactor system to reach/maintain user defined values for axial stress and circumferential stress, two experiments were run concurrently. Figure 3.4 shows an active testing (meaning that the cultured construct is kept alive) of a porcine renal artery. In the first experiment, the porcine renal artery was infused as well as submerged with DMEM cell culture media (Media bath). However, in the second experiment, the porcine renal artery was infused with DMEM cell culture media, but was submerged into Sodium Nitroprusside (SNP) (SNP bath). SNP is a vasodilator that is known to inhibit SMCs activities such that the artery becomes more compliant. SNP was used to show that the bioreactor system is capable of

reaching/maintaining target values of axial stress and circumferential stress regardless of the vaso-activity of the cultured construct.

Figure 3.4 (a) shows that the bioreactor system has reached and maintained a target value of axial stress of 160 kPa with a tolerance of 5%, when the porcine renal artery was submerged in DMEM cell culture media and in SNP. Figure 3.4 (b) shows that the bioreactor system has reached and maintained a target value of circumferential stress of 100 kPa with a tolerance of 5%, when the porcine renal artery was submerged in DMEM cell culture media and in SNP. Important to mention is that the outer pressure feature of the bioreactor system was not utilized in achieving the pre-mentioned target values of the axial stress and the circumferential stress.



(a)



(b)

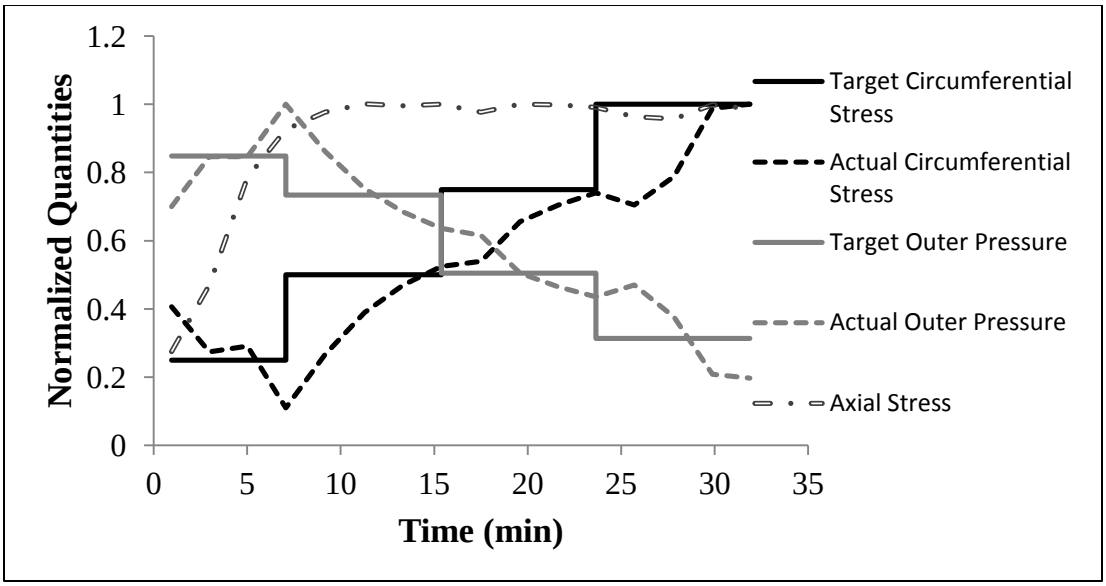
Figure 3.4: Shows the capability of the bioreactor system to reach target values of axial stress (a) and circumferential stress (b) regardless of the vaso-activity of the porcine renal artery, in terms of the fluid (DMEM cell culture or SNP) in which the porcine renal artery is submerged in.

3.5 Outer pressure Incorporation

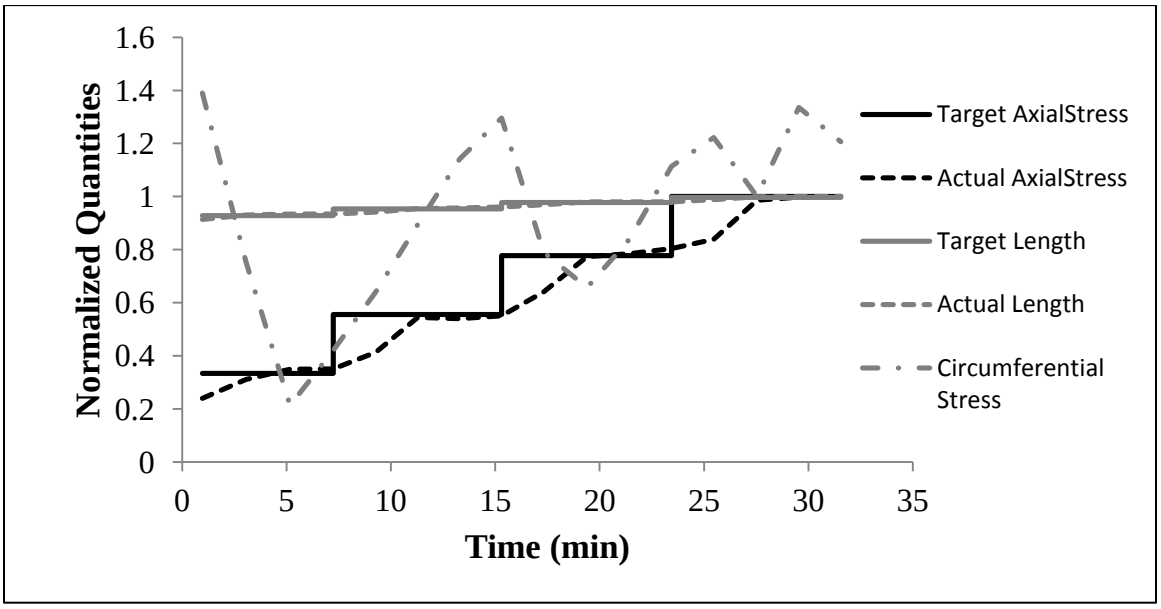
The outer pressure is a newly introduced global parameter that has a great effect on the circumferential stress calculation. The outer pressure also has a slight effect on axial stress level. Assuming incompressibility, when the outer pressure exerts a force on the cultured construct upon increasing the luminal pressure, the cultured construct does not shorten as much. This experiment of incorporating the outer pressure was done on an elastic tube. Figure 3.5 shows normalized values of actual circumferential stress, outer pressure, axial stress, and length within a 5% tolerance of the corresponding target values.

Figure 3.5 (a) shows how the bioreactor system responded to reach target values of the circumferential stress by changing the outer pressure, given that the luminal pressure was held constant at 232 mmHg. According to the circumferential stress equation, when the luminal pressure was held constant at 232 mmHg, the outer pressure decreased to maintain target values of circumferential stress of 5, 10, 15, and 20 kPa. Figure 3.5 (a) also shows that the target circumferential stress values were reached at a constant axial stress of 100 kPa (shown by the "- . - . -" line). The broken lines indicate the normalized actual change in outer pressure and in circumferential stress to meet the normalized target values of the outer pressure and the circumferential stress, which are indicated by the solid lines.

Figure 3.5 (b) shows how the bioreactor system changed the length of the elastic tube to reach target values of axial stress at a constant circumferential stress (shown by "- . - . -" line). Axial stress was targeted to reach 60, 100, 140, and 180 kPa. The linear actuator 1 (LA1) stretched the elastic tube accordingly from its original length of 4.53 cm to achieve the prescribed axial stress values. Again, the broken lines in figure 3.5 (b) indicate the actual normalized change in axial stress and length of the elastic tube. The solid lines, however, indicate the normalized target change in the axial stress and the length of the elastic tube.



(a)



(b)

Figure 3.5: shows how the bioreactor system changed global parameter (outer pressure and length) to achieve/maintain target values of local parameters; (a) circumferential stress and (b) axial stress. All values were normalized.

CHAPTER 4

DISCUSSION

The motivation of this work is the need to better control global parameters (flow rate, axial force, luminal pressure, outer pressure) both independently and in conjunction with one another. Hence, the goal of this work is to develop a bioreactor system that can be used for mechanical characterization and conditioning of vascular tissues as well as for assessing the remodeling process of arteries under prescribed and sustained target values of axial and circumferential stress/strain independently. The developed bioreactor system would allow studying arterial remodeling based on the independent control of either a single global parameter that is held unchanged while manipulating the rest of the global parameters or a selected group of these global parameters.

One of the most important features of the developed bioreactor system is the addition of the outer pressure component into the global parameters of flow rate, axial force, and luminal pressure. The significance of the outer pressure stems from the ability to change circumferential stress values without the need to change the luminal pressure. This feature of changing the circumferential stress based on the change in outer pressure would reveal how an artery would remodel when experiencing a change on its outer surface rather than its lumen.

4.1 Changes in arterial geometry

The biaxial mechanical testing of the porcine renal artery indicated that at higher axial stretch ratios, the vessel becomes stiffer. The stiffness of the cultured artery can be recognized by the left shift in the pressure-diameter curves, which means that at higher axial stretch ratios, the increase in luminal pressure causes less increase in the arterial outer diameter. If higher (critical) pressure values were realized, there would be no change in the outer diameter of the cultured artery. The reason why the cultured artery behaves in such a way is because below the critical pressure value of the porcine renal artery the elastin that exists predominantly in the medial layer carries the load. Elastin, as mentioned in the introduction, is what gives an artery its elastic behavior. However, when the luminal pressure of the porcine renal artery reaches its critical value, collagen is the protein that takes over bearing the load. Collagen is what makes an artery more muscular and stiffer. When collagen starts to bear the increasing load in luminal pressure, the geometry of the porcine renal artery does not change any more.

In the force-pressure curves, the relaxing behavior of the porcine renal artery shown by a decrease in the axial force at 1.2 axial stretch ratio indicates that the 1.2 axial stretch ratio is below the physiological axial stretch ratio of the porcine renal artery. However, the increase in the force sensed by the porcine renal artery when the luminal pressure was increased at 1.6 axial stretch ratio indicates that the 1.6 axial stretch ratio is above the physiological axial stretch ratio of the porcine renal artery.

When the porcine renal artery was excised, the residual strain in the porcine renal artery allowed the porcine renal artery to shorten. After that, when the porcine renal artery was sutured onto the cannulae of the bioreactor system and was stretched to a

length below its original length, the porcine renal artery started to pull on the cannula that is attached to the load cell. As a result of this pulling, a force is sensed by the load cell. However, when the luminal pressure started to increase, the porcine renal artery reduced the amount of pulling off the load cell by relaxing and increasing its length accordingly to maintain homeostasis. The result of the increase in the porcine renal artery can be shown by the reduction in the axial force as the luminal pressure increase.

On the other hand, when the axial stretch ratio went beyond the physiological axial stretch ratio of the porcine renal artery, the porcine renal artery tried to maintain homeostasis by pulling on the cannula attached to the load cell. Hence, the axial force continued to peak as the luminal pressure continued to increase. However, there was no change in axial force as the luminal pressure increased, when the axial stretch ratio was set to a value that matched the physiological axial stretch ratio of the porcine renal artery. These results agrees well with Gleason et al., kim et al., and Zaucha et al.(Gleason et al., 2004; Jang-ho Kim et al., 2009; M. T. Zaucha, Gauvin, Auger, Germain, & Gleason, 2011; M. Zaucha et al., 2009)

4.2 Independent change in transmural pressure, flow rate, and axial force

One of the features of the developed bioreactor system is its capability of independently manipulate the global parameters such as axial force, flow rate, and transmural pressure (inner pressure minus outer pressure). The importance of controlling these global parameters independently stems from the flexibility of the bioreactor system to study the effect of each one of the global parameters on the change in the local

parameters such as axial stress/strain, circumferential stress/strain, and flow induced shear stress.

DMEM cell culture media is less viscous than blood (1 cP of DMEM compared to 3.4 – 4 cP of blood). Hence, in order to achieve the physiological level of flow induced shear stress, the flow rate has to be increased to 2.7 – 3 mL/min (Gleason et al., 2004). The experiment of the independent control of flow rate, transmural pressure, and axial force was done at 1 axial stretch ratio. Although the axial force would tend to go down as the pressure is increased when the axial stretch ratio of a cultured construct is below its physiological level, the change in the axial force was minimal when the transmural pressure increased and decreased in a stepwise scenario at constant flow rate. Also, there was a slight fluctuation in the transmural pressure, when the flow rate was increased and decreased in a stepwise scenario. However, the average of the transmural pressure stayed the same. These results agrees with what Gleason et al. have done before (Gleason et al., 2004)

4.3 Realization of target values of axial and circumferential stress

The developed bioreactor system distinguishes itself in that it is designed not only to perform biaxial mechanical testing through inflation extension tests, but also it is capable of achieving as well as maintaining user defined target values of axial stress and circumferential stress independent of the vaso-activity status of the cultured construct. When the porcine renal artery was submerged into SNP, it lost its vaso-activity and became more compliant (being able to be stretched and inflated more than normal porcine renal arteries). The increased compliance in the SNP treated porcine renal artery,

can be seen by (i) the quick realization of the bioreactor system to the target axial stress and circumferential stress values in comparison to the same porcine renal artery that was DMEM cell culture media treated, and (ii) the less number of perturbations (peaks and valleys) in reaching those target values of axial stress and circumferential stress. This quick realization of the target axial and circumferential stress values was manifested by the left shifting of the curves that indicated a quicker increase towards target values.

4.4 Effect of outer pressure on circumferential stress

The addition of outer pressure as one of the global parameters is a novel idea that provides a new spectrum into mechanical testing of arteries. For the first time a target value of circumferential stress can be reached while the luminal pressure was held constant. This was done by manipulating the outer pressure to reach the targeted values of circumferential stress. At a constant luminal pressure, as the outer pressure increases, the circumferential stress decreases according to the formula of calculating circumferential stress.

On the other hand, various target axial stress values were realized by simply increasing/decreasing the length of the cultured construct. It is important to mention here that the results of the target circumferential stress and axial stress in which the outer pressure was incorporated were performed on an elastic tube rather than on an artery. The elastic tube was chosen to prove the capability of the bioreactor system, because of its consistent wall thickness and constant volume. However, this part of utilizing the newly introduced feature of the outer pressure could have been done on any artery given enough

time and is expected to deem the same results of reaching and maintaining the set target values of axial stress and circumferential stress.

In summary, we were able to develop a bioreactor system that is capable of performing biaxial mechanical testing and of providing representative pressure diameter curves and force pressure curves. The developed bioreactor system is also capable of controlling flow rate, axial force, and transmural pressure independently. In addition, the bioreactor system allows for continuous monitoring of the geometry of the cultured construct. Based on the assumption of incompressibility, the wall volume of the cultured construct remains unchanged. Hence, monitoring the change in outer diameter and length of the cultured construct allows for calculating the corresponding inner diameter.

The developed bioreactor system is also introduced with a fully integrated LabVIEW code that allows the user to do the following (i) calculate the axial stress, circumferential stress, and flow induced shear stress, when the cultured construct is axially stretched, pressurized, and infused with a certain flow rate, respectively and (ii) set target values for axial stress, circumferential stress, and/or flow induced shear stress, and the bioreactor system will correspond by changing the length of the cultured construct, the luminal or outer pressure, and/or the flow rate, respectively. Thus, this developed bioreactor system promises to provide new insights into vascular mechanical testing and conditioning.

CHAPTER 5

SYSTEM LIMITATIONS AND FUTURE WORK

Although we were successful in developing a bioreactor system that met the stated objectives, there are still some limitations in the developed bioreactor system to be considered for further solutions.

5.1 Limitations

Among the limitations that the developed bioreactor system has are: first, the ability to accurately monitor online the inner diameter rather than calculating it based on measurements of undeformed inner diameter, outer diameter, and length. These measurements of the undeformed inner and outer diameters are usually taken at either side of the excised vessel. These measurements, therefore, neglect unintentionally the true values of the undeformed inner and outer diameter at the region where the deformed outer diameter is monitored with the CCD camera.

Second, the left-hand side cannula that is attached to the load cell distally and to the cultured construct proximally experiences some frictional forces when it travels across the o-ring of the left-hand side male connector. Third, the resolution of the linear actuator 2 (LA2) used is in the range of 1 -2 mm. Hence, LA2 is not precise enough to pinch on the elastic tube distal to the inline pressure transducer repeatedly with the same accuracy. The imprecision of LA2 leads to a greater fluctuation in the luminal pressure,

which consequently leads to a fluctuation in the corresponding calculation of the circumferential stress.

Fourth, although the developed bioreactor system is too bulky to fit into an incubator, the system has a silicone heat plate with an adjustable thermostat that keeps the adventitial media at 37^{o} Celsius. The developed bioreactor system can, however, fit underneath a laminar flow hood to maintain sterility.

Fifth, the inner length of the culture chamber 10.42 cm, which means that there is a limit on how much a cultured construct could be axially stretched. However, all of the used porcine renal arteries could be axially stretched more than 250% of their original length. Thus, it is not an issue for such short cultured constructs, but might be an issue for longer cultured constructs, if they were to be axially stretched beyond the length limit of the culture chamber.

5.2 Overcoming limitations

In order to alleviate the limitations of the developed bioreactor system mentioned in section 5.1, the following steps should be taken. To address the first limitation regarding monitoring online the inner diameter, a system of laser beam along with a laser detector is advised to be used. The laser system would shine a column of laser that possesses a long wavelength (greater than 35 nm) for a fraction of a second through arteries of different thicknesses. Long wavelengths are recommended, so that the laser beam would not be fully absorbed by the cultured construct. A laser detector that is situated on the same line (180 degrees apart from the laser beam) of the laser beam would detect the intensity of the received laser beam after the laser beam passes through the

cultured construct and converts the received intensity into a mV signal. Arteries of different thicknesses are to be used in order to build a calibration curve of laser intensity versus mV signals. A DAQ is then used to relay the mV signal into the computer. A calibration curve will then be developed to convert mV signals into vascular thickness. Knowing the outer diameter of the cultured construct and its thickness would allow to calculate the inner diameter [outer diameter – (2*thickness)].

In order to overcome the second limitation of the opposing friction force that develops when the left-hand side cannula moves, the cannula is first allowed to move with an equal distance back and forth axially through the o-ring of the left-hand side male connector. Second, two force values are to be recorded for the back and forth move of equal distance of the left hand side cannula. Third, the two recorded force values are averaged. Finally, the average of the two force values represents the frictional force that is to be subtracted from the registered force in order to get the force sensed by the cultured construct without any frictional losses. I got this idea from Dr. Alexander Rachev.

Regarding the third limitation of the fluctuating luminal pressure caused by the imprecision of LA2, we would recommend buying a more precise with higher resolution incremental steps. Regarding the fourth limitation of the bulkiness of the bioreactor system, no recommendations are available, because it is very hard to reduce the system size to fit a regular size incubator. The fifth limitation, however, is easily overcome simply by machining a longer culture chamber, if necessary.

5.3 Future projected work

Although a lot has been done into bringing the described bioreactor system into a workable model, there still much work to perform, if I have the chance to continue working on this project. Among the projected tasks that I would perform are the following: (i) I would perform active mechanical testing on renal, carotid as well as other easily accessible arteries in order to study their behavior under various mechanical loading scenarios while the arteries are kept alive, (ii) I would experiment the effect of changing luminal pressure while maintaining outer pressure constant and vice versa on the change in circumferential stress at a prescribed axial stress target value, and (iii) I would experiment the effect of changing axial stress while keeping the circumferential stress at a prescribed target value.

The outer pressure is considered to be a plus in the design of this bioreactor system, because it can be used to decouple stress from strain. Decoupling stress from strain would allow to answering a novel question regarding whether cells sense and respond to stress or strain. Finally, I would perform long term culturing (more than 7 days) to study remodeling and the effect of each single global parameter (flow rate, inner pressure, outer pressure, and axial force) on vascular adaptation.

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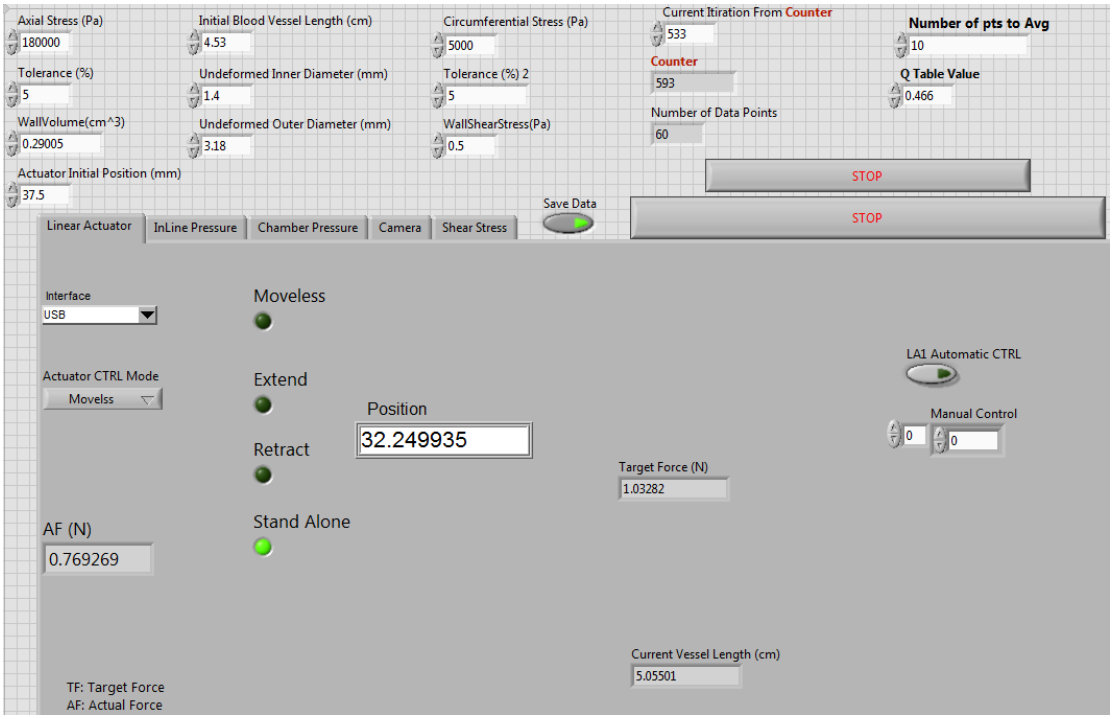
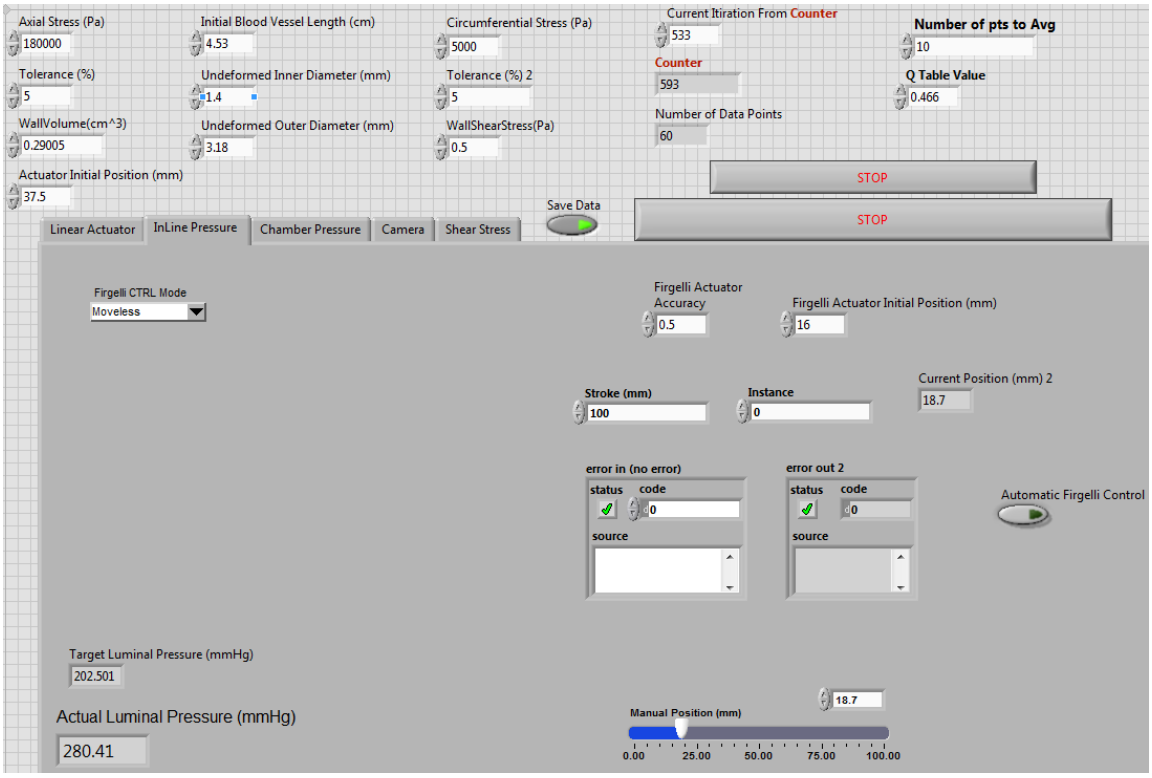
APPENDIX A – LABVIEW CODE SCREEN SHOTS

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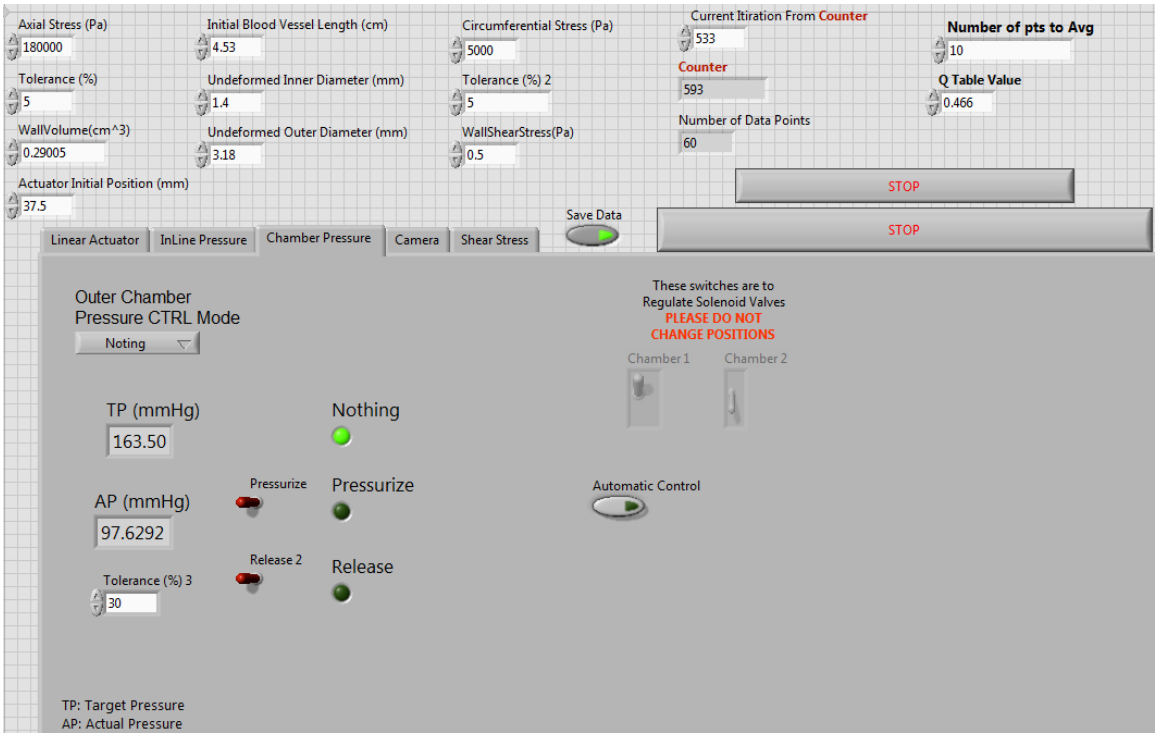
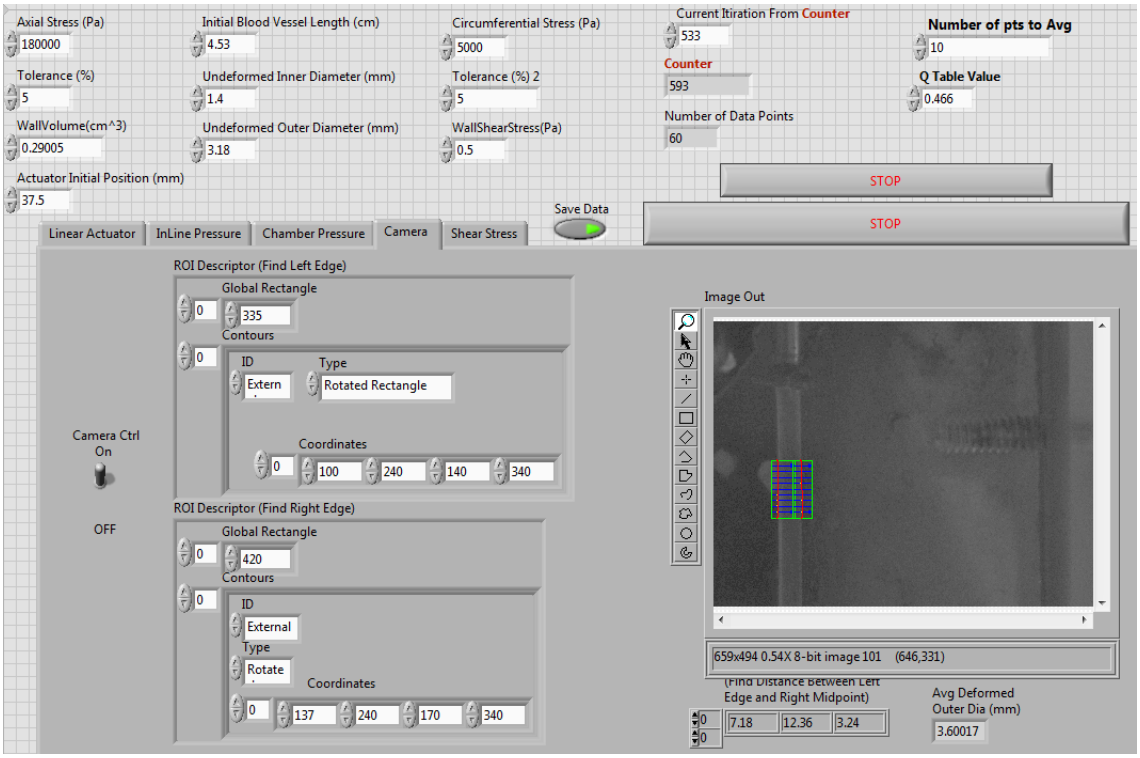
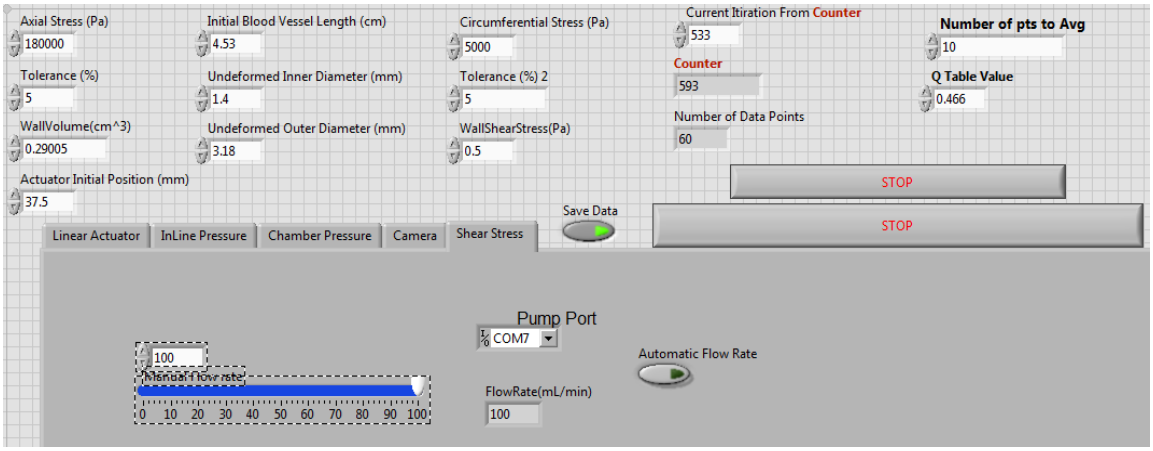
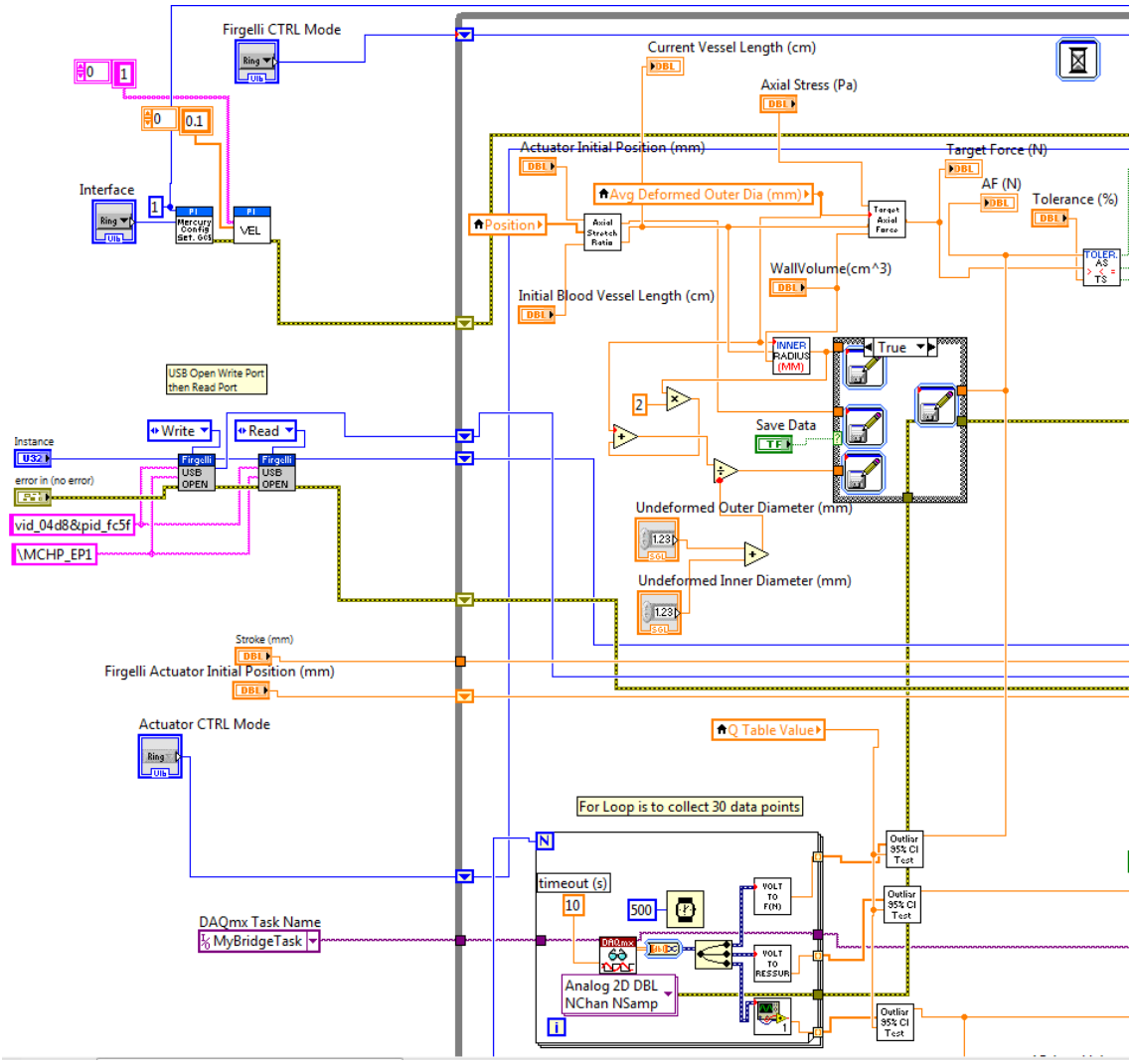
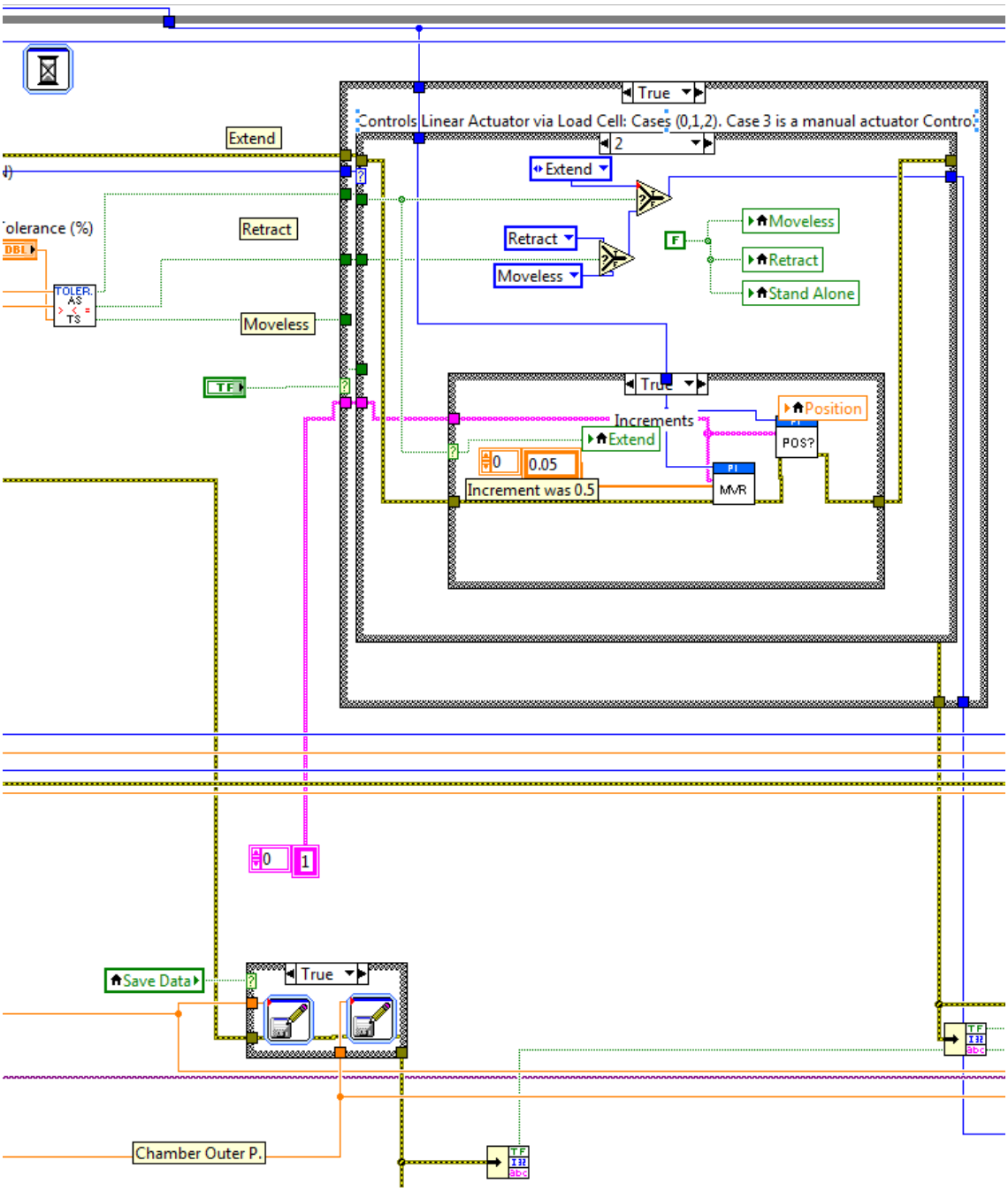
Initial Blood Vessel Length (cm)
4.53
Circumferential Stress (Pa)
5000
Current Itiration From Counter
533
Number of pts to Avg
10
Tolerance (%)
5
Undeformed Inner Diameter (mm)
1.4
Tolerance (%) 2
5
Counter
593
Q Table Value
0.466
WallVolume(cm^3)
0.29005
Undeformed Outer Diameter (mm)
3.18
WallShearStress(Pa)
0.5
Number of Data Points
60
Actuator Initial Position (mm)
37.5
STOP
Save Data
STOP
Linear Actuator
InLine Pressure
Chamber Pressure
Camera
Shear Stress
Firgelli CTRL Mode
Moveless
Firgelli Actuator Accuracy
0.5
Firgelli Actuator Initial Position (mm)
16
Stroke (mm)
100
Instance
0
Current Position (mm) 2
18.7
error in (no error)
status
code
0
source
error out 2
status
code
0
source
Automatic Firgelli Control
Target Luminal Pressure (mmHg)
202.501
Actual Luminal Pressure (mmHg)
280.41
18.7
Manual Position (mm)
0.00
25.00
50.00
75.00
100.00

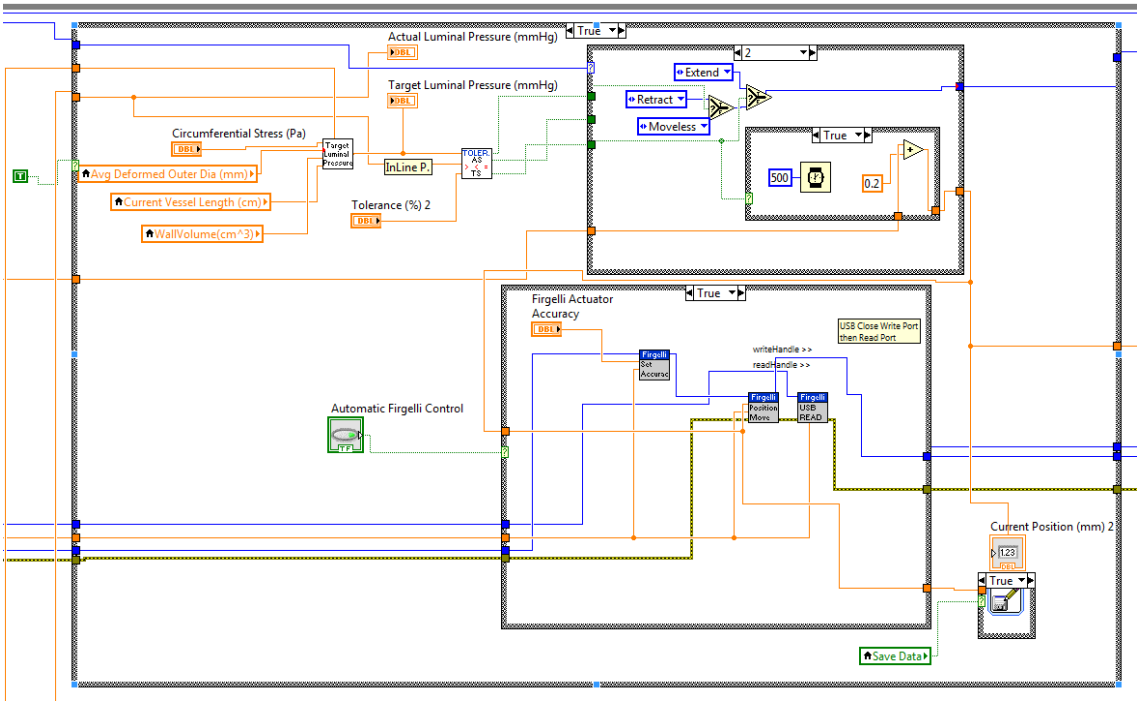

Axial Stress (Pa)
180000
Initial Blood Vessel Length (cm)
4.53
Circumferential Stress (Pa)
5000
Current Itiration From Counter
533
Number of pts to Avg
10
Tolerance (%)
5
Undeformed Inner Diameter (mm)
1.4
Tolerance (%) 2
5
Counter
593
Q Table Value
0.466
WallVolume(cm^3)
0.29005
Undeformed Outer Diameter (mm)
3.18
WallShearStress(Pa)
0.5
Number of Data Points
60
Actuator Initial Position (mm)
37.5
STOP
Save Data
STOP
Linear Actuator
InLine Pressure
Chamber Pressure
Camera
Shear Stress
Outer Chamber
Pressure CTRL Mode
Noting
These switches are to
Regulate Solenoid Valves
PLEASE DO NOT
CHANGE POSITIONS
Chamber 1
Chamber 2
TP (mmHg)
163.50
Nothing
Pressurize
Pressurize
AP (mmHg)
97.6292
Automatic Control
Release 2
Release
Tolerance (%) 3
30
TP: Target Pressure
AP: Actual Pressure

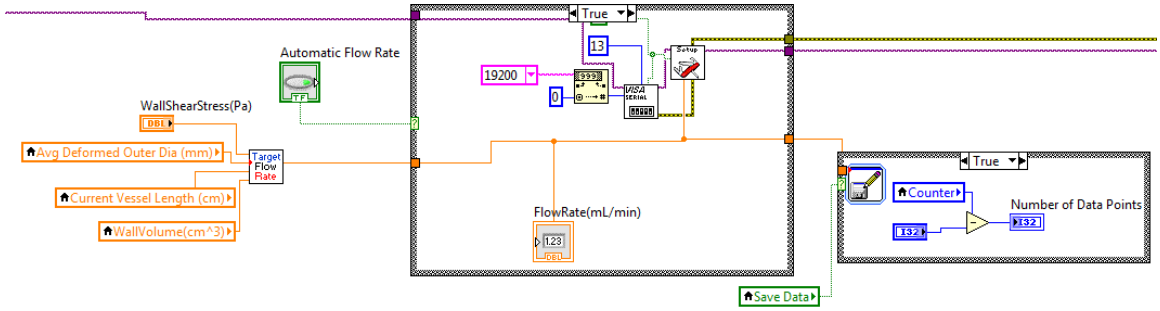

Axial Stress (Pa)
180000
Initial Blood Vessel Length (cm)
4.53
Circumferential Stress (Pa)
5000
Current Itiration From Counter
533
Counter
593
Number of pts to Avg
10
Q Table Value
0.466
Tolerance (%)
5
Undeformed Inner Diameter (mm)
1.4
Tolerance (%) 2
5
Number of Data Points
60
WallVolume(cm^3)
0.29005
Undeformed Outer Diameter (mm)
3.18
WallShearStress(Pa)
0.5
Actuator Initial Position (mm)
37.5
STOP
Save Data
STOP
Linear Actuator
InLine Pressure
Chamber Pressure
Camera
Shear Stress
ROI Descriptor (Find Left Edge)
Global Rectangle
Contours
ID
Type
Extern
Rotated Rectangle
Coordinates
100
240
140
340
Camera Ctrl
On
OFF
ROI Descriptor (Find Right Edge)
Global Rectangle
420
Contours
ID
External
Type
Rotate
Coordinates
137
240
170
340
Image Out
659x494 0.54X 8-bit image 101 (646,331)
(Find Distance Between Left Edge and Right Midpoint)
7.18
12.36
3.24
Avg Deformed Outer Dia (mm)
3.60017

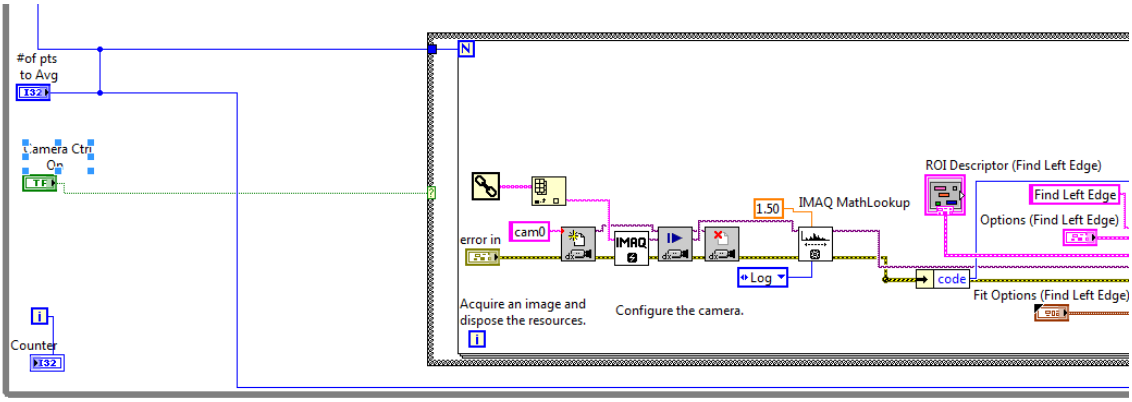

Axial Stress (Pa)
180000
Initial Blood Vessel Length (cm)
4.53
Circumferential Stress (Pa)
5000
Current Itiration From Counter
533
Counter
593
Number of pts to Avg
10
Q Table Value
0.466
Tolerance (%)
5
Undeformed Inner Diameter (mm)
1.4
Tolerance (%) 2
5
Number of Data Points
60
WallVolume(cm^3)
0.29005
Undeformed Outer Diameter (mm)
3.18
WallShearStress(Pa)
0.5
Actuator Initial Position (mm)
37.5
STOP
Save Data
STOP
Linear Actuator
InLine Pressure
Chamber Pressure
Camera
Shear Stress
Pump Port
COM7
Automatic Flow Rate
100
Manual Flow rate
0 10 20 30 40 50 60 70 80 90 100
FlowRate(mL/min)
100

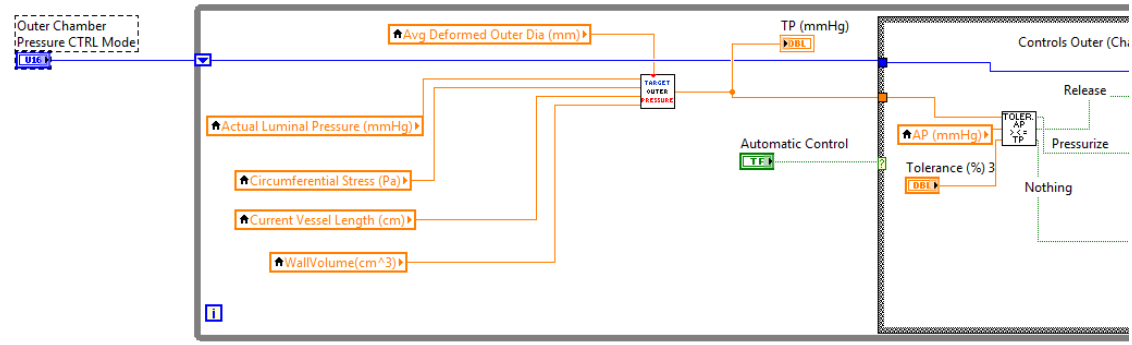

Firgelli CTRL Mode
Current Vessel Length (cm)
Axial Stress (Pa)
Actuator Initial Position (mm)
Target Force (N)
AF (N)
Tolerance (%)
Interface
Avg Deformed Outer Dia (mm)
Position
Axial Stretch Ratio
Target Axial Force
WallVolume(cm^3)
Initial Blood Vessel Length (cm)
INNER RADIUS (MM)
True
USB Open Write Port then Read Port
Write
Read
Instance
error in (no error)
vid_04d8&pid_fc5f
\MCHP_EP1
Firgelli USB OPEN
Save Data
Undeformed Outer Diameter (mm)
Undeformed Inner Diameter (mm)
Stroke (mm)
Firgelli Actuator Initial Position (mm)
Actuator CTRL Mode
Q Table Value
For Loop is to collect 30 data points
timeout (s)
DAQmx Task Name
MyBridgeTask
Analog 2D DBL NChan NSamp
VOLT TO F(N)
VOLT TO PRESSUR
Outlier 95% CI Test

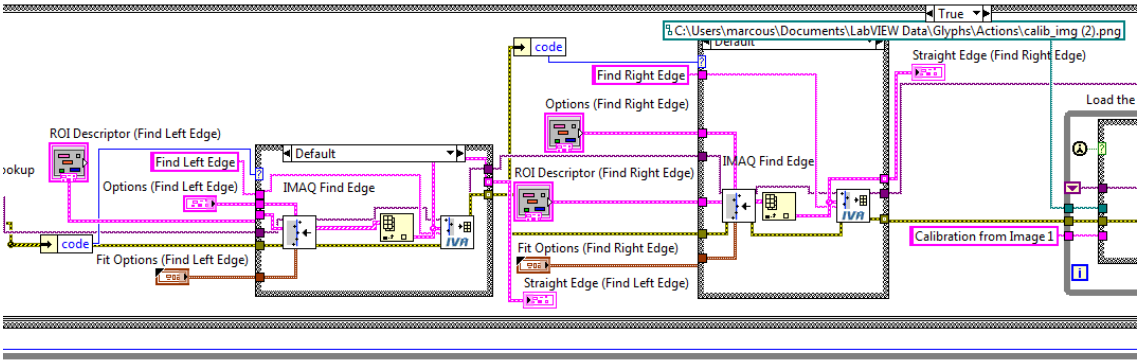

True
Controls Linear Actuator via Load Cell: Cases (0,1,2). Case 3 is a manual actuator Contro
2
Extend
Retract
Moveless
olerance (%)
DBL
TOLER.
AS
TS
Extend
Retract
Moveless
F
Moveless
Retract
Stand Alone
TF
True
Position
Increments
Extend
POS?
0
0.05
Increment was 0.5
MVR
0
1
Save Data
True
Chamber Outer P.

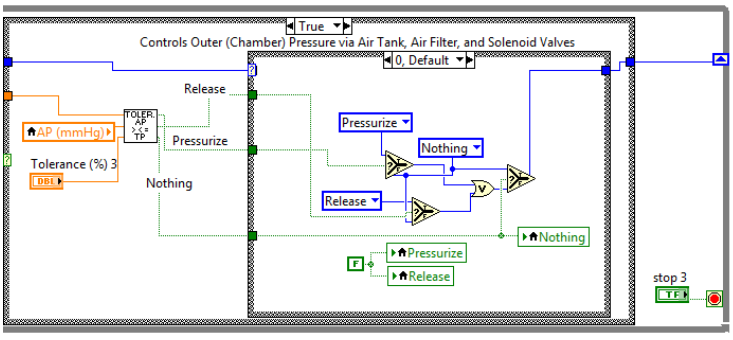

Actual Luminal Pressure (mmHg)
Target Luminal Pressure (mmHg)
Circumferential Stress (Pa)
Avg Deformed Outer Dia (mm)
Current Vessel Length (cm)
WallVolume(cm^3)
Tolerance (%) 2
InLine P..
Extend
Retract
Moveless
500
0.2
Firgelli Actuator Accuracy
USB Close Write Port then Read Port
writeHandle >>
readHandle >>
Automatic Firgelli Control
Current Position (mm) 2
Save Data

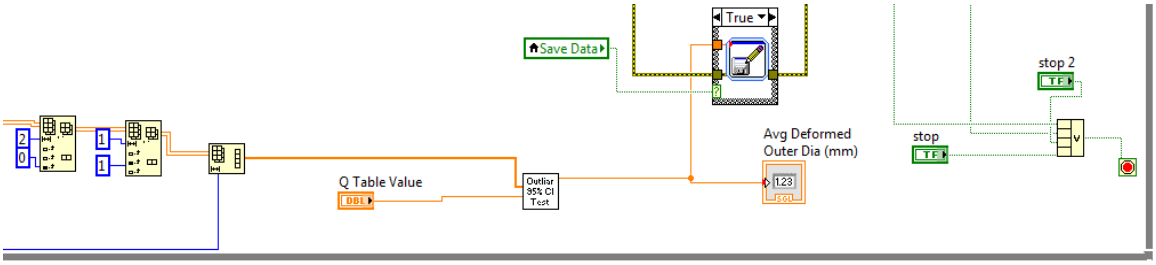

Automatic Flow Rate
WallShearStress(Pa)
Avg Deformed Outer Dia (mm)
Current Vessel Length (cm)
WallVolume(cm^3)
Target Flow Rate
19200
13
0
FlowRate(mL/min)
Counter
Number of Data Points
Save Data

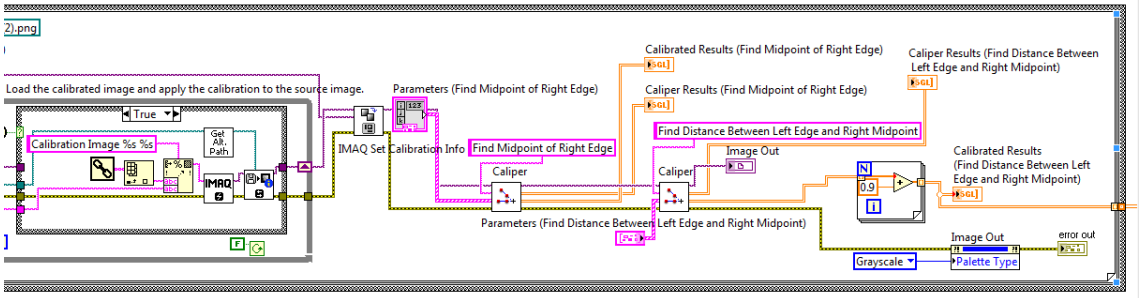

#of pts
to Avg
Camera Ctrl
On
Counter
error in
cam0
IMAQ
1.50
IMAQ MathLookup
Log
ROI Descriptor (Find Left Edge)
Find Left Edge
Options (Find Left Edge)
code
Fit Options (Find Left Edge)
Acquire an image and
dispose the resources.
Configure the camera.


Outer Chamber
Pressure CTRL Mode
Avg Deformed Outer Dia (mm)
TP (mmHg)
Actual Luminal Pressure (mmHg)
Circumferential Stress (Pa)
Current Vessel Length (cm)
WallVolume(cm^3)
Automatic Control
Controls Outer (Ch
Release
AP (mmHg)
Pressurize
Tolerance (%) 3
Nothing


True
C:\Users\marcous\Documents\LabVIEW Data\Glyphs\Actions\calib_img (2).png
ROI Descriptor (Find Left Edge)
Find Left Edge
Options (Find Left Edge)
Default
IMAQ Find Edge
code
Fit Options (Find Left Edge)
Find Right Edge
Options (Find Right Edge)
ROI Descriptor (Find Right Edge)
Fit Options (Find Right Edge)
Straight Edge (Find Left Edge)
Straight Edge (Find Right Edge)
Calibration from Image 1


True
Controls Outer (Chamber) Pressure via Air Tank, Air Filter, and Solenoid Valves
0, Default
Release
AP (mmHg)
Pressurize
Tolerance (%) 3
Nothing
Nothing
Pressurize
Release
stop 3


Save Data
True
stop 2
TF
Avg Deformed
Outer Dia (mm)
stop
TF
Q Table Value
DBL
Outlier
95% CI
Test
1.23


2).png
Load the calibrated image and apply the calibration to the source image.
True
Calibration Image %s %s
Get
All
Path
IMAQ
Parameters (Find Midpoint of Right Edge)
IMAQ Set Calibration Info
Find Midpoint of Right Edge
Caliper
Calibrated Results (Find Midpoint of Right Edge)
Caliper Results (Find Midpoint of Right Edge)
Caliper
Image Out
Find Distance Between Left Edge and Right Midpoint
Parameters (Find Distance Between Left Edge and Right Midpoint)
Caliper Results (Find Distance Between
Left Edge and Right Midpoint)
Calibrated Results
(Find Distance Between Left
Edge and Right Midpoint)
0.9
Image Out
error out
Grayscale
Palette Type