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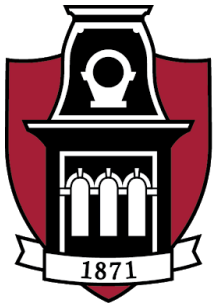

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Development of a Model for Accelerated Fatigue Testing In Venous Valves

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Table of Contents

Abstract

Malfunctioning venous cause issues ranging from cosmetic to life threatening situations for millions of people in the U.S. (1). Venous valve bioprosthetics often fail due to a loss in leaflet tissue flexibility following chemical fixation of donor tissue (2). A viable solution for testing venous valves prior to and post chemical fixation is in high demand for the development of a more durable prosthetic replacement. As a result, this research aims to create a fatigue apparatus that provides a means to model the durability of venous valves. The design criteria for this project included modeling physiological conditions in an accelerated time frame for fatigue. A big consideration was minimizing the allotted time for testing as much as possible. The completed apparatus can hold 5 vein segments at the same time in a clear acrylic chamber. A system of syringes with two linear actuators is used to propel the plungers to circulate saline solution through the valves. A preliminary test was run using this apparatus for 10 hours with 4 valves. Uniaxial mechanical testing was completed, however the condition of the leaflets upon harvest was very poor due to holes and they varied in size. Stress strain curves were plotted for 5 leaflet samples and the results of the peak tangent moduli made it evident the valves would need to be placed in the apparatus for longer than 10 hours. The preliminary testing made it evident that valves would need to be removed from sample data if they are found defective prior to fatiguing. The built apparatus meets all the design criteria and can model 1 year of valve life in 2.6 days. Further work is needed in the form of biaxial testing with comparisons of mechanics made among fresh, fatigued, and chemically fixated valves.

Article I. Introduction

Section 1.1 Motivation

The focus of this research is venous valves, which are located in the veins of the lower extremities. When these valves malfunction, blood cells become lodged between the valve leaflets and walls of the vein, leading to poor blood circulation throughout the body. Venous valve issues can range from cosmetic to life threatening situations for millions of people in the U.S. (1). Chronic Venous Insufficiency (CVI) refers to the hypertension in the veins caused by weaknesses existing in the vein wall and valve leaflets that leads to pain, swelling, ulcers, and edema, as shown in Figure 1 (1). When left untreated, CVI can lead to Deep Vein Thrombosis (DVT), which is the formation of blood clots in the lower extremities (1,2). These clots become problematic as they are circulated to the rest of the body and can result in a pulmonary embolism (1). Despite the widespread prevalence, venous disease continues to be an understudied area within cardiovascular research (1).

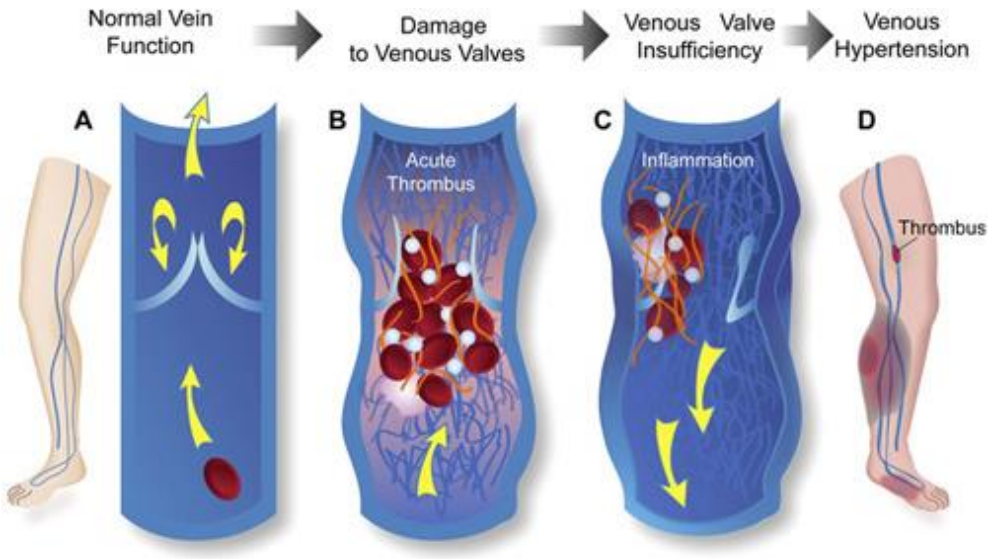

Figure 1. *Depiction of normal and diseased venous valves. Poorly functioning valves can cause thrombus formation, which if dislodged can travel to other organs.(3)*

Section 1.2 Background

Surgical treatments are highly invasive and not optimal for treatment of venous valve diseases (3,4). Instead, venous valve bioprosthetics are used to treat the specific issues associated with the valves. Bioprosthetics are made from animal donor tissue and require chemical fixation to ensure biocompatibility of the tissue with the human body (5). However, current fixation techniques increase the stiffness of tissue, which results in a decrease in flexibility (6). Leaflet tissue flexibility is essential for venous valves to maintain their biomechanical characteristics for optimal function (2). The loss of function results in failure of implanted valves. As chemically treated bioprosthetic tissue has been widely used for several decades in valve replacement (3,4), a viable solution for testing venous valves prior to and post chemical fixation is in high demand.

A fatigue test provides an effective, objective way to predict the durability of a valve and analyze changes in core tissue characteristics. Examining the performance of venous valves prior to any fixation treatment and after fixation can guide future research in the development of a more durable prosthetic replacement. Although methods of fatigue testing have been extensively researched for mitral valves located in the heart, the application to venous valves is unclear and requires a different approach due to structural differences. Since no other method can truly predict the longevity of a venous valve or its suitability for prosthetic development, this research is significant to the cardiovascular field.

Section 1.3 Objectives

Therefore, the purpose of this study is to create a fatigue test that provides an effective, objective way to predict the durability of a venous valve and analyze changes in primary tissue characteristics. The main objective of the project is to design an apparatus that could open and close a valve in an accelerated time frame to guarantee valve fatigue. Additionally, the fatigue

test must adhere to physiological constraints of venous valves to better predict their longevity as a source of prosthetic tissue and later analyze the mechanical characteristics of the valve leaflets. To accomplish these objectives, the research efforts outlined below have been completed.

Article II. Materials and Methods

Section 1.1 Design Criteria

The design considerations for the development of the fatiguing apparatus have been focused on achieving a way to fatigue venous valves at an accelerated rate. This can be achieved by creating alternating pressures that open and close the valves within normal physiology. The flow rate through the vein segments should be maintained in order to match the physiological conditions of flow in human veins of similar size. Additionally, the design included increasing the number of valves fatigued simultaneously in the setup to minimize the allotted time for testing within the apparatus.

Section 1.2 Design

For the creation of the fatigue apparatus, there were several designs and assemblies generated that all had certain strengths and weaknesses. Ultimately, the chosen design was one of the simpler models that contained a housing unit, or chamber, to hold the tissue, attachments to circulate the fluid through the vein segments, and a motor system to propel this movement. Five donor veins containing a valve could be placed in the housing unit to be fatigued at the same time, making the fatigue test more efficient by cutting time and costs. This design was the most feasible under the constraints and allowed ease of access to attach and detach the venous tissue.

To monitor the tissue during the actual testing, acrylic was used for the chamber, as it is transparent and easy to handle. All parts and assemblies were designed in SolidWorks. For the housing unit, the acrylic sheets were laser cut to fabricate the specific parts created in

SolidWorks, and then assemble them together to form the chamber. To ensure no liquid leakage from the housing unit, the laser cut portions were adhered together using Weld-On 4 Acrylic Adhesive.

Once the chamber was built, the next step involved constructing the attachments to the chamber to move the fluid through the valve. 2-inch sections of PVC rod were inserted into the openings of the tank and on the opposite side attached a cut syringe that modeled the movement of a piston pump. Lastly, the setup needed to be motorized, for which two actuators were used and programmed using code on an Arduino Uno. A working circuit was created for the use with the two actuators and code. Finally, I combined all the pieces of the setup and attached them to a wooden frame, which finished the process of building the assembly (Figure 2).

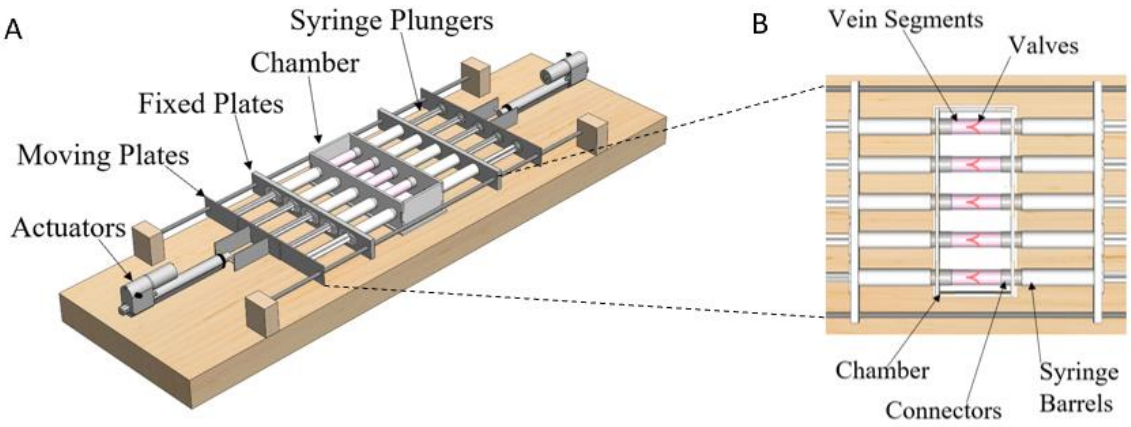

Figure 2. A). *SolidWorks rendering of the fatigue apparatus. Two linear actuators are attached to syringes that circulate fluid to open and close the valves placed inside the acrylic chamber. B). The vein segments are attached to PVC connectors that are joined to the syringe barrels.*

Section 1.3 Preliminary Uniaxial Testing

Preliminary mechanical testing was conducted to observe if the apparatus is capable of movement for prolonged periods of time and if any additional considerations need to be made for future mechanical tests. Due to time and resource constraints, the fatigue apparatus ran for 10 hours with 4 bovine vein segments. The jugular vein segments were obtained from a local abattoir from steer. After harvesting, the veins were stored in 0.9%w/v sterile saline and transported back to the ENRC. The veins were dissected and cut into segments of about 2 inches with a venous valve in each segment. The veins were not selected for branching and the quality of the valves was not inspected. The veins were placed in the fatigue apparatus with 0.9%w/v sterile saline in the chamber and fatigued for 10 hours (Figure 3).

Figure 3. *Four valves were placed in the fatigue apparatus for ten hours for the preliminary mechanics testing. The vein segments are attached to the PVC connectors using zip ties.*

After fatiguing, valve leaflets were extracted from each vein segment using dissecting equipment. Upon dissection, multiple valve leaflets were missing, torn, or too small to be harvested (Figure 4). Only 4 valve leaflets moved on to uniaxial testing. Square portions were cut out of the leaflets, with the largest leaflet providing 2 square samples of 3-4 mm in length/width (a total of 5 samples for testing). Although cardboard and staples were considered for mounting the leaflets, the only feasible method due to the small size of the tissue proved to be

using alligator clamps. These were attached following extension in the radial direction of the fiber orientation in the venous valves.

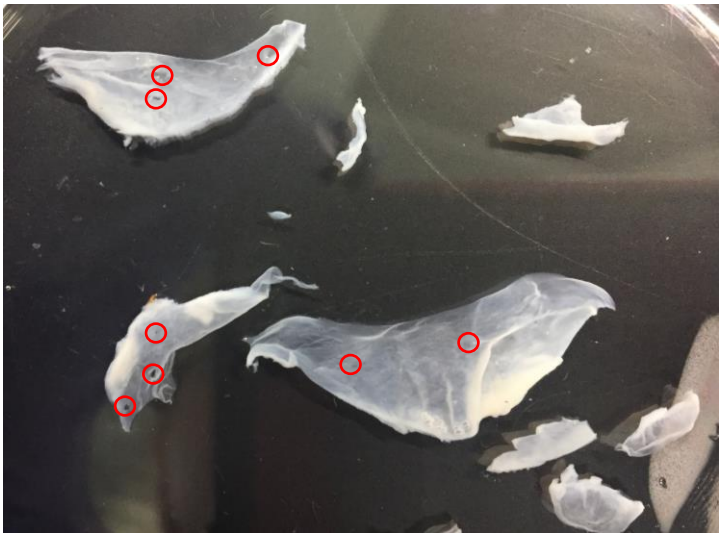

Figure 4. *Harvested venous valves after the ten hour fatigue. The valves were variable in size and multiple specimens had holes or were too small to mount for the remainder of the experimentation.*

The Instron 2710-004 in the teaching lab in White Engineering was used for the uniaxial testing (Figure 5). The load readings were offset to zero once the valves were pulled taut. Ten preconditioning cycles were done at an extension rate of 10%/s up to a maximum value of tensile extension of 30%; the length of each valve was also recorded. After the preconditioning was complete, a constant strain rate of 10%/s was applied until 60% extension in the radial direction was achieved. Example values for the specimens are provided below in Table 1. Parameters for this testing were obtained from literature studies done on fresh jugular venous valves (7). Stress strain data was calculated from the load and extension data gathered.

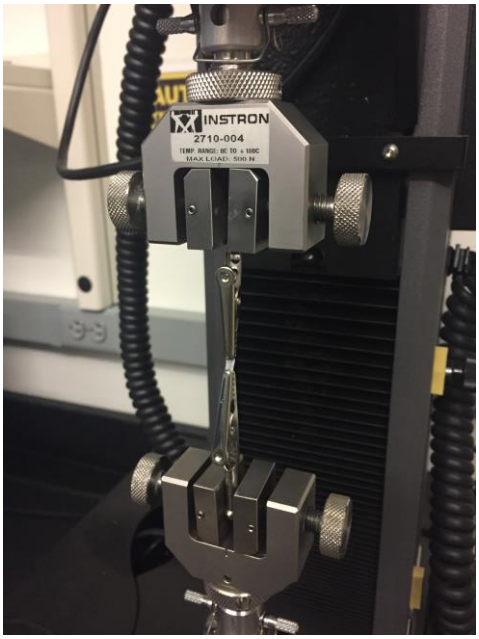

Figure 5. *Uniaxial testing was carried out on five samples of leaflet tissue. Alligator clips were utilized as the mounting method for the leaflet squares.*

Table 1. *Example parameter values for uniaxial testing of the venous leaflet sections.*

Number of Preconditioning Cycles	Length of Leaflet Section	Preconditioning Extension Rate (10%/s)	Preconditioning Maximum Tensile Extension (30%)	Strain Rate (10%/s)	Maximum Extension (60%)
10	4 mm	0.4 mm/s	1.2 mm	0.4 mm/s	6.4 mm

Article III. Results

Section 3.1 Fatigue Apparatus

An in vitro model was designed to fatigue venous valves, creating alternating pressures to open and close the valves at an accelerated rate of 4 cycles/s. Flow rate through the valves is 14 mL/s, which matches the physiological conditions of flow in human veins of similar size of 12-15 mL/s (3). To minimize the allotted time for testing, the setup was maximized to fatigue five treated donor valves at the same time. The completed apparatus is shown in Figure 6.

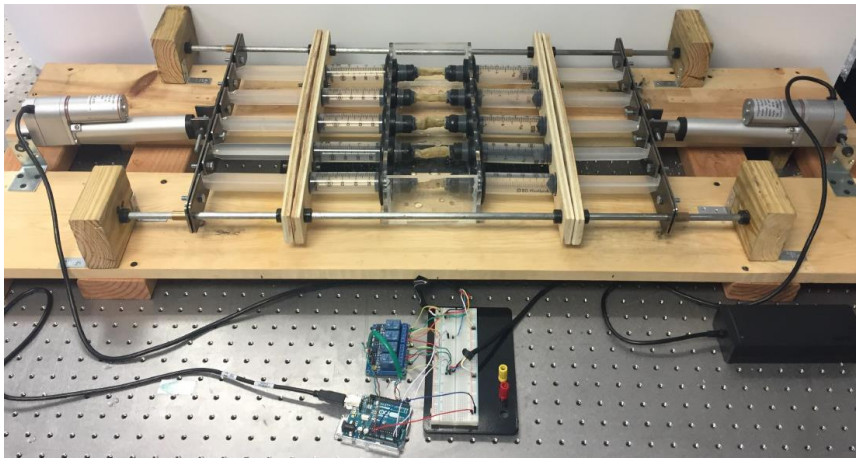

Figure 6. *The model contains an acrylic chamber to hold the vein segments, attachments to circulate saline fluid through the vein segments, and actuators to propel this movement. The linear actuators are synchronized and programmed using a microcontroller board and customized software.*

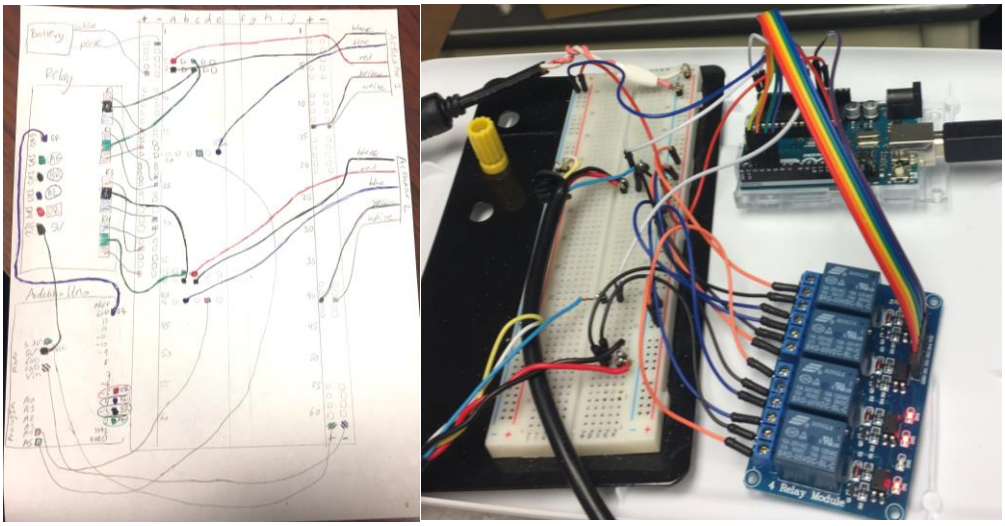

Figure 7*. The sketch and the built circuit that allows for movement of the two linear actuators.*

Through the completion of the design and development of the venous valve fatigue testing setup, movement of tissue was achieved in an accelerated time frame. The system moves the valve leaflets at specified and adjustable conditions set by the code. While the frequency of venous valve closures in the body is dependent on multiple factors, it has been estimated that

venous valves open and close approximately 900k times each year (4) – much less than cardiac valves, averaging at 37M times per year. With this assumption, the rate of 4 cycles/s allows for this setup to achieve 1 year of valve life in 2.6 days and 5 years of usage in 13 days. This model can be used as a first step towards evaluating bioprosthetic venous valves for tissue dynamics and long-term endurance.

Section 3.2 Preliminary Uniaxial Testing

Using the setup displayed in Figure 8, valves were fatigued for 10 hours. The uniaxial testing provided inconsistent results due to small sample size and issues with valve quality. Stress and strain curves for Valve sample 1 are provided in Figure 9. Stress was calculated by dividing load by the cross-sectional area of the sample. As thickness data of the sample could not be collected due to lack of equipment for the small values, the literature value of 0.05 mm was used to substitute thickness measurements.

Strain was calculated by dividing extension by the length of the sample. It was observed valve sample 3 had a hole, which is hypothesized to be responsible for the valve failure during preconditioning; as a result, no data was collected from sample 3. For samples 1, 2, 4, and 5 peak tangential moduli were recorded for the linear regions of the stress and strain curves. The modulus is calculated by dividing the change in stress by the change in strain. The literature values for comparison are shown in Table 2, and the results of the uniaxial testing are shown in Table 3.

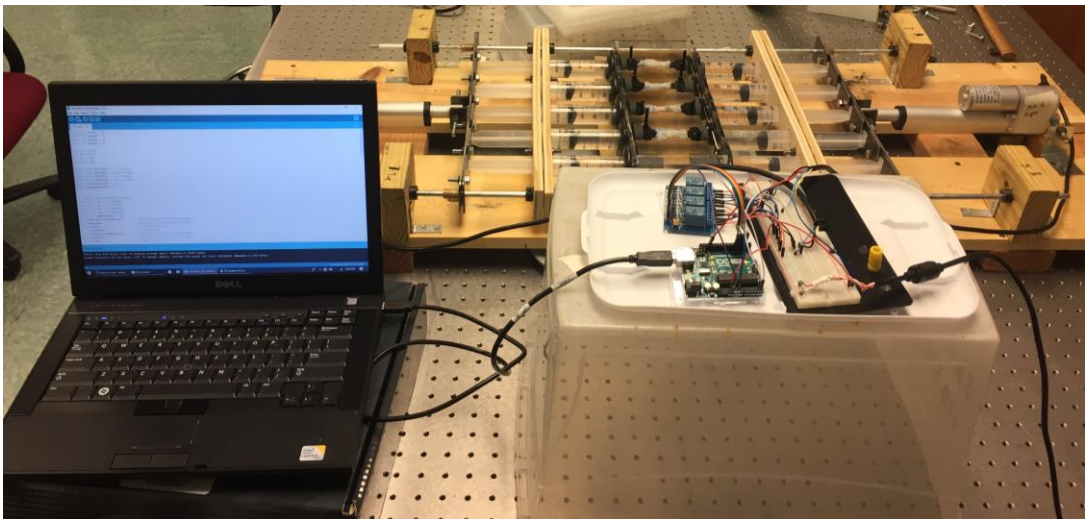

***Figure 8**. Complete setup for the fatigue apparatus during the preliminary testing.*

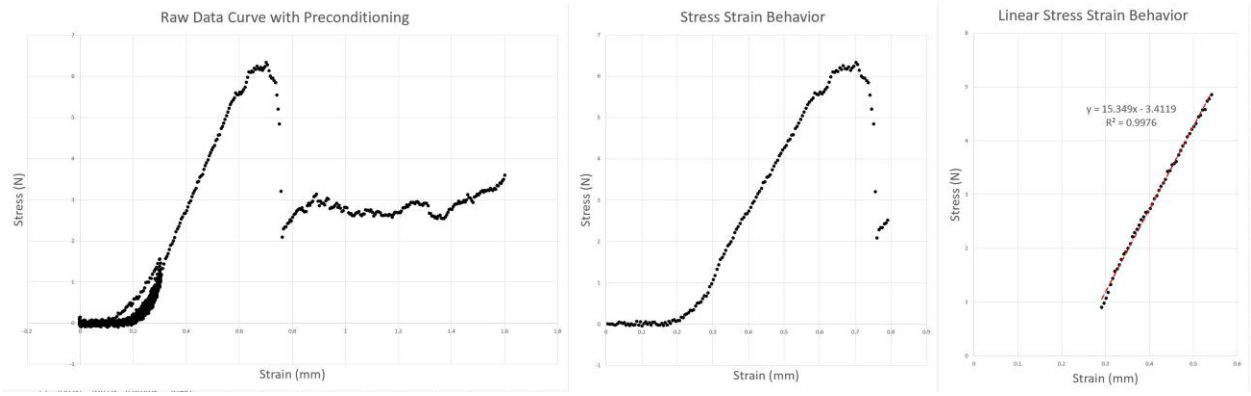

***Figure 9**. Stress and strain curves for sample 1 venous leaflet.*

***Table 2.** Literature values for fresh venous valve leaflet mechanics. Highlighted in red are the values that can be compared to the current experimental results (7).*

Table 1 Peak tangent moduli (in the 45–60% strain range) and peak stresses (at 60% strain) measured for venous valve leaflet tissues excised from bovine jugular veins

	Proximal ($n = 11$)		Middle ($n = 8$)		Distal ($n = 11$)	
	Cir	Rad	Cir	Rad	Cir	Rad
Peak tangent modulus (MPa)	27 ± 9	9 ± 6	24 ± 20	10 ± 16	30 ± 6	15 ± 9
Peak stress at 60% strain (MPa)	6 ± 2	2.5 ± 1	6 ± 6	2.4 ± 4	6 ± 2	4 ± 3

Mean ± standard deviation

Table 3. *Peak Tangent Modulus for 5 valve samples after uniaxial testing in the radial direction.*

Sample #	Peak Tangent Modulus	Issues
1	15.35 MPa	none
2	7.47 MPa	elasticity reached twice
3	-	hole caused failure
4	15.30 MPa	elasticity reached twice
5	21.67 MPa	multiple elasticity regions

Article IV. Discussion

Section 4.1 Project Accomplishments

The success of the research is the level of functionality in the fatigue apparatus. The actuator movement is synchronized, and it reliably moves saline through each vein segment. The valves in the vein segments open and close as predicted, which can be visible when they are mounted. The apparatus can withstand long periods of movement.

Section 4.2 Project Limitations and Possible Solutions

With the initial round of mechanical testing, no confident levels of fatigue were observed. Due to time constraints, 10 hours was chosen for testing; however, this may not have been enough time to show fatigue in venous valves. A future direction of improving planning for the mechanics experiments would be to prolong the fatigue of the valves.

For the mechanical testing additional important considerations need to be made for the selection of the venous valves. Although the sample size should be randomized, the preliminary testing made it evident that some valves would need to be removed from the sample data. Such examples of valves that would be excluded can include leaflets with visible defects such as holes, limit effects of branching, and select for valve size to assist with ease of dissection and mounting

protocols. Venous valves can be bicuspid or tricuspid, and as a result more literature research should be conducted to investigate if this will have an affect on fatigue. The sample size should also be increased to provide enough for statistical analysis. The mechanical experiments conducted in the future will be improved based on the results of the preliminary data. As shown in Figure 10, some preliminary samples expressed multiple regions of elasticity. This is likely due to improper mounting with the large alligator clips that could be off-centered. More care should be taken in future work to ensure correct mounting.

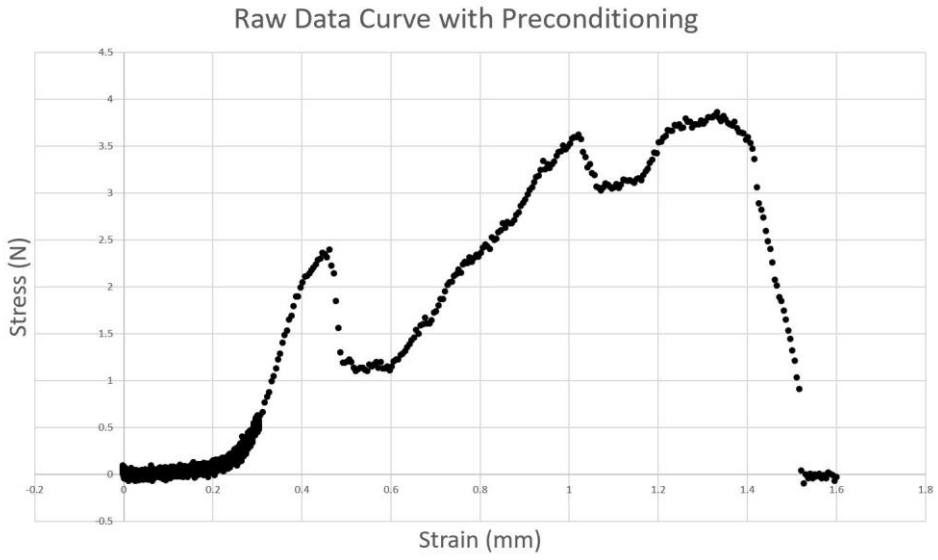

***Figure 10**. Sample venous valve stress and strain curve displaying several elastic regions.*

Article V. Conclusions

Through the completion of the design and development of a venous valve fatiguing apparatus, a mechanical idea was successfully combined with software to achieve movement of the tissue and mimic the physiological environment in an accelerated time frame. The system moves the valve leaflets at specified and easily adjustable conditions set by my program code. The program can match the physiological conditions of flow in human veins, and the valve leaflets will open and close due to the movement of fluid in the apparatus at the specified rate.

The design criteria were met, including being able to hold five vein segments at once. Main challenge in system was leakage, which has been addressed in the new design with additional sealants. With this assumption of venous valves opening and closing approximately 900k times each year[4], the rate of 4 cycles/s allows for this setup to achieve 1 year of valve life in 2.6 days and 5 years of usage in 13 days.

Article VI. Future Work

The fatigue apparatus can be used to quantify the changes in tissue characteristics and performance following chemical fixation. This setup can be utilized in future research to determine the effect of fatigue of fresh bovine venous valves compared to that of chemically-fixed valves. The mechanical characteristics of the valves can be compared, which can be used to better understand the extent of the outcomes of fixation of venous valve fatigue. Biaxial mechanical testing will be conducted on the fatigued valves and differences will be quantified among fresh, fatigued, and chemically fixated valves. Changes in geometric orifice area and rate of opening and closing of the valves using a video capturing technique can also be quantified.

Assessing the longevity of chemically-fixed fatigued valves when compared to fresh and fresh fatigued valves using this fatigue apparatus can provide new biomechanical information on the mechanical causes of poor durability of bioprosthetic venous valves. Fatigue testing can be used to better examine the outcomes of fixation on venous valve fatigue and can guide future research in the development of a more durable prosthetic replacement devices.

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