Project Proposal

Miniature Biaxial Testing Device

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Project for Dr. Wei Sun
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**Executive Summary**

The goal of the project is to design a biaxial testing device that is compact enough to fit under a 2-photon microscope so that the microstructure of the tissue can be captured as it is stretched. The analysis of the orientations of the elastin and collagen fibers are of particular interest with regard to the tissue’s microstructure. The elastin provides the tissue with its ductility and the collagen contributes to the strength of the tissue; changes in these fibers could directly affect the tissue’s mechanical properties. These mechanical properties are important for determining the material properties which help to create constitutive models of the specific tissue type that is tested.

This proposal will discuss the design and development of our device beginning with an analysis of previous biaxial devices and the how our device will fit the specific needs of our client as it varies from current biaxial devices in the market. We will continue the proposal with project objectives and detailed methods describing how the device is to be built. SolidWorks will be used for evaluation of preliminary designs. Upon agreement between the client and our design team, the project will progress with machining and assembly of the device. Finally, we will discuss how our device will be implemented and the implications this will have. The logistics of this project with a budget is given in the pages that follow.

**1 Introduction**

**1.1 Background**

This project is for Dr. Wei Sun, an Assistant Professor in the Mechanical Engineering department at the University of Connecticut. His research focuses on tissue mechanics with both experimental work and computational models to further the understanding of behavior of soft tissues. Soft tissues present in the body include skin, heart tissues such as the myocardium, heart valve leaflets, or membranes. Changes in
tissue microstructure as humans age often contributes to tissue degradation and mechanical failure which plays a large part in human disease or death. Heart valve replacement devices could be assessed before implantation by creating 3-dimensional computational models that replicate the performance of these devices in the body. In this way, the ability of the devices to function properly could be tested without endangering the patients’ lives. Three-dimensional models could also be used to visualize the smooth muscle contractions of the myocardium. There have been limitations in models previously synthesized to evaluate this mechanism. Three-dimensional models are also of use to surgeons who need to know the precise area of the tissue to cut to execute their task. The mechanical properties of various tissues are also essential to the surgeons’ knowledge in that the surgeon needs to know how much force is required to make an incision and how the tissue will deform in response to any manipulation.

1.2 Purpose of Project

The purpose of the project is to design a miniature biaxial testing device for soft connective tissues. The device must be small in order to fit underneath a two-photon microscope. A method of analyzing the orientation of the microfibers viewed through the two-photon microscope will be devised once the biaxial machine is assembled. The project also involves the design of a type of cookie-cutter mold to cut the tissue into a desirable shape for mounting in the biaxial testing machine.

Small tissue samples have been tested using the device currently located in the Bronwell building; however, the tissue samples are prone to tearing when they are stretched due to the difficulty of attaching hooks to the specimen. A different means of gripping the tissue is needed because it is hard to attach the same number of hooks used for average-sized specimens onto small tissue samples.

Soft tissues exhibit non-linear stress-strain behavior, high elasticity and mechanical anisotropy. Soft tissues are also generally characterized as incompressible. Due to their complexity, it has been a challenge to construct accurate models for their behavior. Data
from biaxial testing experiments can be used to identify material parameters for 3-D constitutive models. These models can be used to visualize the tissue deformation *in vivo* and can be used for predicting the functionality of bio-prosthetics as an example. Understanding a tissue’s mechanical properties by creating accurate models will be critical for the design of implantable devices. Further applications of this device, or the knowledge collected from it, may be for tissue engineering. Here a scaffold to support cell growth will depend on the strength and mechanical cues of its environment.

### 1.3 Previous work done by others

Dr. Wei Sun and Dr. Michael Sacks built a large scale biaxial machine that is currently located in the Tissue Mechanics Laboratory on the University of Connecticut Campus. This state of the art machine is one of the most accurate devices in the world in acquiring soft tissue mechanical properties.

Five undergraduate WPI students designed a miniature biaxial testing device for compliant tissue in 2005 under Dr. Kristen Billiar of Worchester Polytechnic Institute as their advisor. The design team made a four-axis planar biaxial testing device that fit into a small space. The device used screw-driven slides and two rotational force transducers. A motion controller board and filter system were purchased from National Instruments. A stepper motor was also chosen for the design. The sample was mounted in a saline bath with fishhooks and sutures.

Additionally, there are commercially available biaxial devices used in several research laboratories for similar purposes that provide researchers the capability of mechanical testing on any desired soft tissue samples. This data could be applied to a variety of studies in the soft tissue mechanics field.
1.3.1 Products

The ElectroForce® Systems Group of BOSE Corporation produces materials testing devices including biaxial testing devices for research. The company manufactures a wide array of testing devices for the characterization of many different types of materials. Among their products are the ElectroForce test bench instruments. These devices feature a 200N motor and a reaction bracket. The linear motor works without producing friction which allows the product to display a high resolution. The test bench instruments can be multi-axis. A saline bath can also be added to the device as an environmental control.

1.3.2 Patent Search Results

Ghosh and Tushar K. patented a biaxial testing device which uses clamping systems to grip textile and membrane material samples in 1999. The clamping systems are oriented along an X-axis and a Y-axis which are orthogonal to each other. The device was designed to analyze the stress-strain behavior of textile fabrics since these materials exhibit nonlinear and complex stress-strain relationships. The device has spaced-apart segmented clamping systems interconnected by pantographs so that the clamps can contract and extend with respect to each other. The design aims to allow strain to occur naturally near the anchored edges and minimize the boundary effects of the clamped edges.

2 Project Description

2.1 Objective

The aim of this project is to construct a miniature sized biaxial testing device for measuring the mechanical properties of soft tissues. The requirements for this device are that it is a portable version of the current technology. It will be easily transported to the two-photon microscope and allow for easy set-up at this site. The reason for this is that as tissue samples are loaded and deformed their properties can be measured with two imaging modalities; microscopy and a charge coupled device (CCD) camera. Two-photon microscopy will collect real time images of the tissue microstructure during testing. The
CCD camera will track the movement and position of the specimen. A mirror will be used in order to capture data from both techniques. A proposed set-up allowing for this is shown below in Figure 1.

![Figure 1. General schematic for dual imaging set-up capability](image)

A variation in our device will be the implementation of clamps to secure small tissue specimens. To optimize this technique the sample will be cut into a basic cross-shape, where clamps can attach to each free straight edge. Another objective to achieve this is the creation of a die which can be used to cut samples in this contour. Figure 2 below demonstrates the desired shape from which any shaped specimen can be cut with a simple die in a cookie-cutter type process.

![Figure 2. Cross-shape of specimen which can be cut with a die and easily clamped to the miniature biaxial device](image)
For compatibility with two-photon microscopy our tissue samples will require extra preparation and fluorochrome marker staining to image collagen and elastin fibers. Continuous data from both the microscope and CCD camera may be collected onto a USB drive and transported for data analysis.

2.2 Methods

2.2.1 Device Design via SolidWorks modeling

The miniature biaxial machine will first be designed using SolidWorks software. This design will be modeled off of currently available biaxial machines with altered dimensions, and parts to fit our needs. Dimensions of this device require capability of operation in conjunction with the two-photon microscope, portable and easy to transport. It will include a four motor design to pull the tissue specimen from four attachment points. These attachments will be via clamps onto tissue prepared in a cross shape. Reference to the previous figure 2 visualizes this set-up. The motors will be connected to transduce a desired force onto the specimen. Additionally, both the load and rate at which the load is applied will be controlled with NI software. This procedure allows the device to produce a given force at given time intervals for a specified period in order to examine the tissue behavior under such conditions. Two load cells for the x and y directions will be included for strain measurement. The system will require a convection heated saline bath to keep the tissue at body temperature during all testing procedures. This entire schematic will require easy set-up at the two photon microscope. A simple base with four holes for clamping into the microscope bench will be used to secure the device for experiments. The parts mentioned here and additional specifications will be taken into account and modeled via SolidWorks. Designs via this software, analyses and predicted efficacy of multiple designs can be tested on. Simulations can be performed to test the machine before building. Upon agreement between the group and client on an appropriate SolidWorks design the machine will continue forward with assembly.

2.2.2 Device Control and analysis via NI LabVIEW

The motors used in our device will require NI control with LabVIEW. This way, the exact load placed onto the specimen, by a pulling force generated from the motors, will be
controlled by the user with a LabVIEW interface capability. The load, rate and duration of the cycles can all be decided by the user. Dave Kaputa in the Biomedical Engineering Department will be responsible for this function due to the time and complexity required for accurate motor control.

If time permits, the group will also use LabVIEW as a data analysis means. The transportable nature of the miniature device will be a completely new feature for the client’s laboratory. Allowing the biaxial testing to be performed while collecting information on the tissue microstructure provides a whole new type of data. This data will be the images of the collagen and elastin fibers in the sample over the period of a mechanical test. A LabVIEW program may be created to transform these images into useful data requested by the client. For example, monitoring how the position or orientation of these fibers changes over the time of a test. This type of information can then be paralleled to the strain rate, load, and deformation properties of the material. It will be possible to correlate a particular fiber direction to a specified load or rate with proper analysis and sufficient testing. LabVIEW will prove a very efficient tool for our purposes. Using just this one software will provide the ability to control experimental variables, acquire the data from instruments and finally analyze the data that is collected.

2.2.3 Sample Preparation

Prior to tissue handling, frozen cardiac tissue samples (human, bovine, porcine, etc.) will be thawed and acclimated to a 9% saline solution using a timed-exposure procedure for 50 minutes. Desired samples will then be cleaned and dissected to a square specimen with side length of approximately 15 mm. The tissue will then be cut into cruciform shape using a stainless steel die-cut to produce clamp lips a length of 5 mm each. Graphite markers will be placed to form a 1mm² test area in the center of the specimen as seen in figure 3 below. The four markers will be tracked by the CCD video camera for further analysis.
To visualize the specimen with the two-photon microscope a staining process will be done on the specimen. A typical dye such as the combination of Masson trichrome & Verhoeff stains would allow for visualization of the specimen microstructure. With these particular stains, the elastin would appear black and collagen would be a blue or a green color. If desired, other stains could potentially be used as well. This preparation is necessary to properly visualize the sample during testing. Once cut, marked with graphite and staining tissue samples are ready for loading into the device and beginning experimentation.

**2.2.4 Biaxial Machine**

Accurate physical testing of a tissue sample on the biaxial machine is the most critical component of this project. There are several steps in the overall testing procedure before data analysis can occur.

*Sample Mounting*

The clamps to be used are 7 mm stainless steel clamps with #180 grit waterproof sandpaper lining as tissue contact grips to prevent slippage (Langdon et al.). The clamps will be attached to the motor, and placed in the saline bath at 37°C. The motors are designated X1 and X2 representing two major physiological directions on the tested tissue sample. If the specimen is an excised portion of a vessel, X1 represents circumferential and X2 represents longitudinal directions. For leaflets or other non-cylindrical based cardiac tissue, X1 remains circumferential, but X2 is the radial direction.
The tissue must be placed such that it is perfectly centered in the apparatus. The graphite markers must be able to be seen through the hole in the mirror, and the CCD camera must be able to view the entire specimen reflection on the mirror. The specimen must also be equidistant from motor bases to ensure accurate load and true equibiaxial properties.

**Preconditioning**

At this point, tissue preconditioning will occur, a step critical to reducing tissue hysteresis and creating a constant reference between trials. To begin preconditioning, the sample will be equiaxially stretched until the tissue in the bath appears to have high stiffness and resistance to further stretching. Based on past trials, this point will occur between 150 – 250 gram load for a specimen this size. This load may vary due to individual tissue fiber configuration and possible diseased states of the patient. Finding this fatigue point is key in determining the approximate max test load for the subsequent trials. Preconditioning will continue for no more than 40 cycles starting at a low load and incrementing to the desired maximum load. Every 5 to 10 cycles in the preconditioning phase, a new reference marker is taken to calculate stress-strain levels.

**Sample Testing**

After preconditioning, the sample is ready for testing. Due to portability of the device, testing can occur at the 2-photon microscope or at any standalone location. Advantages to testing at the microscope station is the ability to view collagen and elastin responses to applied loads in real time and in great detail; however testing procedures are the same regardless of locale.

The sample is tested for 10 cycles at 11 different protocols, summarized in the table below. The protocols describe the ratios that the two axes pull at with respect to the selected maximum load. The data from the biaxial machine is then analyzed using LabVIEW, and the data from the 2-photon microscope is sent to NI Vision Assistant and LabVIEW software.
Table 1. Test protocols for the biaxial machine.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Ratio (X1 : X2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconditioning</td>
<td>1.00 : 1.00</td>
</tr>
<tr>
<td>1</td>
<td>1.00 : 1.00</td>
</tr>
<tr>
<td>2</td>
<td>0.75 : 1.00</td>
</tr>
<tr>
<td>3</td>
<td>0.50 : 1.00</td>
</tr>
<tr>
<td>4</td>
<td>0.30 : 1.00</td>
</tr>
<tr>
<td>5</td>
<td>0.10 : 1.00</td>
</tr>
<tr>
<td>6</td>
<td>1.00 : 1.00</td>
</tr>
<tr>
<td>7</td>
<td>1.00 : 0.75</td>
</tr>
<tr>
<td>8</td>
<td>1.00 : 0.50</td>
</tr>
<tr>
<td>9</td>
<td>1.00 : 0.30</td>
</tr>
<tr>
<td>10</td>
<td>1.00 : 0.10</td>
</tr>
<tr>
<td>11</td>
<td>1.00 : 1.00</td>
</tr>
</tbody>
</table>

2.2.5 Post-data analysis

A custom built LabVIEW program will be the primary source of post data analysis and calculations. The program will be designed to calculate deformations from given the images captured by the CCD camera. The program will compare the contrast and intensity of the markers for a maximum 8 bit value of 0, and the tissue, with a maximum 8 bit value of 225. A frame rate proven to be successful is that of 15 Hz, so this will be taken into consideration (Sacks, M.S and Chuong C.J.). If during testing the sample experiences low elasticity and small deformation, a higher sample rate may be necessary to accurately capture data.

Another function of the program will be to analyze images acquired by the microscope for collagen and elastin levels. This will allow for both a quantitative and qualitative correlation of tissue behavior and composition with mechanical properties.

**Strain and Stress Calculations**

For cardiac tissue, the samples are assumed to be orthotropic, hyperplastic, incompressible and pseudoelastic. In accordance with Sacks and Sun (2003), the in-plane second Kirchhoff stresses are derived from the two dimensional strain energy function \( W \):

\[
S_{ij} = \frac{\partial W}{\partial \varepsilon_{ij}}
\]  

(1)
Where $S$ is the stress, and $E$ is the Green’s strain. Typically with respect to soft biological tissues, a Fung based model is used to fit the data such that

$$\rho_0 W = \frac{c}{2} \left[ \exp \left( a_1 E_{11}^2 + a_2 E_{22}^2 + 2a_4 E_{11} E_{22} \right) - 1 \right]$$  \hspace{1cm} (2)$$

Where $\rho_0$ is the tissue initial density, and $a$ and $c$ are material constants. LabVIEW will take the deformations, calculate the deformation gradient, calculate the Green’s Strain, and apply them to calculating stress knowing the strain energy functions. It will then create stress-strain curves allowing for further analysis and interpretation.

3 Budget

Several of the supplies needed to build the miniature biaxial machine are already in the possession of Dr. Wei Sun and will therefore not be included in the budget. These items include:

- CCD monochromatic camera
- 2 NI compatible motor
- 2 2-N Load cells
- NI controller board
- Two-photon microscope (located in the Pharmacy building)

Therefore, the bulk of the purchases made will be raw materials to build the bath, the stands, and the physical biaxial device. The following budget is derived from the specifications and includes primitive research on pricing. Until the device is fully built on SolidWorks, the exact parts to be ordered are still to be determined.

<table>
<thead>
<tr>
<th>Item</th>
<th>Material Type</th>
<th>Price per Unit</th>
<th>Amount to be Ordered</th>
<th>To be used for</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>316 Stainless Steel</td>
<td>$9.60</td>
<td>12” x 12” x 0.018”</td>
<td>Die Cut Razor</td>
<td>onlinemetals.com</td>
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<tr>
<td>2</td>
<td>PVC Sheet</td>
<td>$144.54</td>
<td>24” x 48” x 0.625”</td>
<td>Saline Bath</td>
<td>professionalplastics.com</td>
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<td>PVC Rod</td>
<td>$20.10</td>
<td>24” x 2.250”</td>
<td>Load cell base</td>
<td>professionalplastics.com</td>
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<td>quote inquired</td>
<td>24” x 0.500”</td>
<td>Rods for clamps</td>
<td>vincentmetals.com</td>
</tr>
<tr>
<td>5</td>
<td>316LVM Stainless Steel</td>
<td>quote inquired</td>
<td>12” x “12” x 0.300”</td>
<td>Clamps</td>
<td>vincentmetals.com</td>
</tr>
<tr>
<td>6</td>
<td>#180 grit waterproof sandpaper</td>
<td>$0.79</td>
<td>9” x 11”</td>
<td>Clamp interior</td>
<td>paint-and-supplies.hardwerestore.com</td>
</tr>
<tr>
<td>7</td>
<td>Biochemika</td>
<td>$40.70</td>
<td>1 kit</td>
<td>fluorescent markers</td>
<td>sigmaaldrich.com</td>
</tr>
</tbody>
</table>

**Table 2. Preliminary Budget**

TOTAL (without items 5 and 6) $215.73
4 Conclusion

The proposed biaxial machine will be the first of its kind to analyze tissue components in real time under high magnification. It also allows for the analysis of tissue samples that are very small in size, making testing of leaflets and other small physiological components easier. This machine is designed for the use of the Tissue Mechanics Laboratory at the University of Connecticut, but because of its practical nature, it could be used in all laboratories with access to a two-photon microscope.

Mechanical properties calculated by the biaxial machine could contribute to the overall field of diagnosis and repair of cardiac care. Cardiac diseases claim more than 17 million lives from all over the world each year, indicating that diagnosis and treatment is a definitive focus in biomedical research (World Health Organization Statistics). By knowing mechanical properties, not only can the failure strength of vessel walls be accurately calculated for a patient, making a personalized diagnosis for several cardiac diseases, including aortic aneurysms. Additionally the ability to see the collagen and elastin alignment in conjunction with the stress and strain levels produced allow for better bioprosthetics to be created. The aims of this project intend to address these problems and provide the means to improve current solutions within soft tissue mechanics.