Project Statement & Specifications

Miniature Biaxial Testing Device

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Project for Dr. Wei Sun,
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**Statement of Need**

Our client is in need of a miniature biaxial testing device for small, thin tissue samples. A biaxial testing device applies tensile force to a small tissue specimen in two-dimensions. Currently, biaxial testing devices accommodate large tissue samples (~100mm²). The small tissue samples are difficult to secure in the current biaxial machine setup and tear easily.

In addition to the physical device, the client desires a type of cookie-cutter mold to cut the tissue into a cross-like shape which is desirable for homogenous stress-strain behavior. Additionally, a LabView program to capture the mechanical data and tissue deformation efficiently is also desired. The client is in need of a compact and transportable biaxial testing device that fits under a two-photon microscope. The two-photon microscope would allow the client to visualize the tissue microstructure as it deforms. The dimensions of the biaxial device currently used are too large for the device to be positioned under and transported to the two-photon microscope.

**Introduction and Overview**

Accurate modeling of soft tissue is an important component of biological research and biomedical engineering. Several applications in tissue engineering and prosthetic design rely on tissue mechanical and behavioral characteristics as a necessary foundation for their creation and efficacy.

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 quantify the
tissue deformation \textit{in vivo} and can be used for predicting the functionality of bio-
prosthetics as an example. Understanding a tissue’s mechanical properties with
accurate models will be critical for the design of implantable devices. Another
application of this device, or the knowledge collected from it, may be within tissue
engineering. Here, a scaffold to support cell growth will depend on the strength and
mechanical cues of its environment.

Current biaxial testing in the Tissue Mechanics Lab uses four markers that are
placed in the center of the sample at the corners of a small square region to track tissue
movement and deformation. The stress and strain measurements in this region are
considered homogeneous due to its small size. A thin tissue sample is then mounted in
a phosphate buffered saline bath and stretched along two perpendicular axes that are
aligned with the preferred fiber orientation and the cross-fiber direction. Pairs of hooks
are anchored into the tissue sample and sutures that loop around rotators are tied to the
hooks. Load cells, a transducer, and a motor are used to move the tissue and capture
an image. A CCD camera is placed above the bath to capture the deformation of the
markers. Before data is collected, the tissue sample undergoes several precondition
cycles. A number of stress protocols, e.g. seven, are used to comprehensively
document the tissue’s stress-strain relationship.

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.

To further analyze samples as they undergo biaxial testing, coupling the device
with a two-photon microscope will yield more information by presenting real-time tissue
microstructure as the test is performed. Two-photon microscopy is a fluorescence non-
linear imaging technique that allows for deep tissue imaging. Two-photon microscopy
can penetrate tissue specimens up to 1 mm in depth. Biological tissues strongly scatter
light. Nonlinear signal generating systems make it possible to capture high-resolution, deep penetrating images. A laser emits pulses of near-infrared light, which passes through three lenses before reaching the specimen. The two-photon excited fluorescence can be collected using epi- and/or trans-collection mode with photomultiplier tubes (PMTs). The ability to couple the biaxial device with microscopy allows us to track collagen and elastin movement. This is an advantage over the current use of the CCD camera, which can only track the overall tissue movement with the four markers.

**Realistic Constraints**

Specimens to be tested using the miniature biaxial device will be on the range of 5mm$^2$ whose small size will present some challenges. Currently, sutures are hooked into a larger sample, but this method will not work in a miniature design. In order to grip the specimen four clamps will be used attached to each side of a cross shaped tissue sample. To obtain accurate results, the tissue must not slip from the clamps. If slipping occurs, the load created by the device will not transfer in entirety to the sample. Additionally, a separate device will be needed to cut the sample into the required cross shape, which is repeatable and accurate and does no significant damage to the tissue.

In order to couple this device to two-photon microscopy an additional step to specimen preparation will be required. This may require fluorescent staining of the sample before testing and visualizing with the two-photon microscope. A method for tracking and measuring the tissue movement with the microscope will need to be addressed.

Another constraint is the transportability of the device and its set-up. Set-up at the microscope may require additional time because the device and sample have to be transported to this site. For example, maintaining the specimen in a heated PBS or
saline bath may be required to prevent tissue damage. The biaxial device is powered by motors, meaning that when transported a power supply must be available. This may require either battery power, or close proximity to an outlet. Lastly, because the device will be testing dissected tissue samples, proper health and safety regulations must be met and taken into design consideration.

**Other Information**

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. More accurate knowledge of tissue structure can lead to the creation of more well designed implantable devices for disease treatment. The miniature biaxial testing device will be used by Dr. Sun and his assistants in his laboratory. It will also be portable in order to test soft tissues and simultaneously image a specimen with a two-photon microscope. All of the work and instruments will be done on the Storrs University campus.

**Preliminary Questions**

- What type of tissues will be tested with the machine? If there will be multiple types, will the device need to be adjusted for each?
- Will the sample be set up in the biaxial machine before transporting to the two-photon microscope test area?
- What is the greatest load that will need to be generated by the machine for the smaller samples?
In a standard biaxial test machine, the saline bath is monitored and kept at 37°C by means of a convection current bath. The source of the heated water for this bath is a large pump alongside the biaxial machine. For the device to be portable, this convection bath cannot be included. The question arises whether a 37°C constant temperature is necessary to have accurate results, or can the bath be allowed to cool as the procedure progresses?

In terms of portability, how will the data be collected? Will it be stored on an external hard drive-like device and then transferred to a computer back at the lab, or will data analysis occur immediately at the test station?