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Effective Stiffness of Suspended Gels

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EFFECTIVE STIFFNESS OF SUSPENDED GELS

A Major Qualifying Project Report:
submitted to the Faculty
of the
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in partial fulfillment of the requirements for the
Degree of Bachelor of Science
by

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Date: April 24, 2008

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Abstract

Pathological wound contraction and chronic wound healing can lead to scleroderma, hypertrophic scarring and fibrosis. In chronic wound healing, fibroblast differentiation is uncontrolled, resulting in the over-production of myofibroblasts. The stiffness of the surrounding environment may contribute to the excessive differentiation of fibroblasts into myofibroblasts. In order to better understand this process, wound healing is commonly studied in vitro using collagen gels. In these models, stiffness is often determined using destructive methods, such as uniaxial testing, which render the gels useless for future testing. The goal of this project was to develop a non-destructive device which maintains the integrity of the gel during testing. A novel device which implements transverse loading methods was developed to displace the center of fibroblast-populated collagen gels to assess changes in stiffness. The device measures force and displacement with 0.1mN and 0.0254mm resolutions, respectively. These measurements can be used to determine the stiffness of collagen gels and the changes that result from remodeling. The device was validated by testing thin silicone membranes (PDMS) to compare with published values of stiffness measured by transverse loading methods. Additionally, testing was completed on both acellular and fibroblast-populated collagen gels to demonstrate repeatability and illustrate that the device was compatible with relevant wound healing models. The device was also integrated with existing laboratory research focusing on the effect of varying boundary conditions on collagen gel remodeling. The device achieved the project objective of non-destructively measuring stiffness while aiding in the advancement of novel wound healing models.

1   Introduction

Wound healing is a complex biological process of regenerating dermal and epidermal tissue. This process is divided into three phases – inflammation, proliferation, and remodeling. These three phases work together to repair damage by inducing cell migration to the wound site, forming of granulation tissue, and eventually producing collagen and fibronectin to form a new extracellular matrix (ECM). During the remodeling phase of wound healing, it is necessary for the resident fibroblasts in the connective tissue to differentiate into contractile myofibroblasts to aid in the synthesis and remodeling of the ECM at the wound site (Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002). The differentiation of fibroblasts to myofibroblasts is important because wound contraction, a phenomenon in which the size of a wound is mechanically reduced, is driven by myofibroblasts.

The different mechanisms through which fibroblasts differentiate into myofibroblasts have yet to be understood by researchers. It is widely accepted that myofibroblast differentiation is modulated by both mechanical and biochemical stimuli and it has been shown that the increases of overall matrix stiffness within the ECM can play a significant role in regulating cell phenotype (Frederick Grinnell, 2000). Therefore, it is important to understand the link between stiffness and the differentiation of fibroblasts *in vivo* by measuring and controlling the stiffness of the surrounding matrix.

There are different 3D matrices which have been used in previous studies to examine the effects of stiffness on myofibroblast differentiation (Freyman, Yannas, Yokoo, & Gibson, 2002). One example is an unrestrained gel which has a zero force boundary condition. In this type of gel, cells are embedded in the gel, the boundary is not restricted, and the matrix cannot resist the traction forces generated by fibroblasts. This can lead to the compaction of the matrix and the inability of cells to differentiate into myofibroblasts (Tomasek et al., 2002). Alternatively, if the gel is rigidly anchored or restrained around its boundary, this creates a zero displacement boundary condition and the matrix is able to resist the cell-generated force and develop mechanical stress within the matrix. In the presence of a stiff matrix, along with biochemical stimuli such as TGF-β, cells in a restrained gel are able to differentiate into myofibroblasts (F. Grinnell, 2003).

Current methods in studying matrix stiffness cannot fully account for all of the stiffness experienced by the cells. While unrestrained and restrained models provide two extreme environments which result in two very different phenotypes of cells, they cannot account for intermediate stiffness values that often occur *in vivo*. The Boundary Controlled Stiffness Device (BCSD), a novel device developed by Dr. Kristen Billiar of Worcester Polytechnic Institute, was created to make it possible to study the effects of such intermediate boundary stiffness conditions. This device employs the use of compliant springs, attached to the edges of the gel to vary boundary stiffness. Using the BCSD, changes in cell phenotype, which result from a change in boundary stiffness, can be observed. In addition to observing cell phenotype, it is also important for the matrix stiffness of the gel to be quantified. This measurement is necessary as the stiffness experienced by the cells in the gel is a combined result of both boundary stiffness and gel stiffness.

The primary goal of this project is to develop a novel device to determine the composite stiffness of a gel as experienced by its resident cells. This novel device will be able to measure the composite stiffness, a combination of the boundary stiffness and the matrix stiffness, of the gel. This project will evaluate current methods used for testing stiffness to determine the best method for testing. The device will output force and displacement readings which will be used to calculate the composite stiffness of the gel through finite element analysis. The deliverable for this project will be a novel testing device which will work in conjunction with the BCSD to provide intermediate composite stiffness readings of gels.

2 Background

2.1 Importance of Studying Stiffness

Extracellular remodeling is an important stage in tissue healing as well as in fibrocontractive diseases. One stage of extracellular remodeling is wound contraction, the time during which the size of a wound is mechanically reduced until a defect is closed. When wound contraction is carried out incorrectly and there is too much contraction, chronic wound healing is observed. In chronic wound healing the wound does not maintain structural integrity and there is not an orderly progression of wound contraction. The driving force behind wound contraction is the myofibroblast – a transformed fibroblast that expresses α-smooth muscle actin (α-SMA). Chronic wound healing results in the over-production of myofibroblasts, which can lead to excessive scar tissue production (Stadelmann, Digenis, & Tobin, 1998).

The mechanism through which fibroblasts differentiate into myofibroblasts has yet to be completely understood. One theory suggests that fibroblast differentiation into myofibroblasts could be caused by mechanical stresses on the cells (Gabbiani, 2003). In normal connective tissue, resident cells are protected from such stresses by their accompanying ECM (this is commonly known as "stress-shielding", a concept very familiar to engineers). However, in damaged or remodeling tissue, the ECM is compromised and the stresses that were once absorbed by the matrix are transferred to the cells. This indicates that there is a correlation between matrix stiffness and fibroblast differentiation. This concept has been verified by Pelham et al. who proved that the stiffness of the matrix has a direct effect on cell phenotype and differentiation (Pelham, 1997). Matrix stiffness can be controlled *in vitro* by altering the external environment through the use of different models.

2.2   Two- and Three-Dimensional Models

Two and three-dimensional models have been used to study wound healing. Two-dimensional models allow for simple analysis while three-dimensional models more accurately depict conditions *in vivo*. When studying fibroblast interactions with 2D substrata, the fibroblasts are fixed in place and are asymmetrically distributed over the surface (F. Grinnell, 2003). Flexible substrata have been used to determine traction forces generated by cells in order to characterize the mechanical interactions (Beningo & Wang, 2002); but this approach cannot mimic the reciprocal and adaptive interactions that occur between fibroblasts and surrounding matrices in terms of mechanical responses and signaling in a dynamic environment (F. Grinnell, 2003). Since fibroblasts are forced to adjust to flat and rigid 2D surfaces they are not able to exert the mechanical force and signal transduction required to induce expression of proteins which are necessary for ECM remodeling (F. Grinnell, 2003).

In contrast to 2D matrices, 3D matrices allow fibroblasts to interact in an environment where cells can migrate and cause changes to the mechanical properties of the matrix. In a 3D matrix, cells are housed in a more complex physical environment and have a more geometrical pattern of distribution than on 2D surfaces. As fibroblasts are able to contract under this environment, mechanical loading begins to develop. This mechanical loading causes an increase in the expression of α-SMA, which is essential for differentiation into myofibroblasts (Cukierman, Pankov, & Yamada, 2002). Furthermore, as the matrix is remodeled the mechanical signals provided by the matrix direct cell phenotype and in part regulate fibroblast differentiation. Due to their ability to better replicate *in vivo* conditions, 3D matrices provide a more accurate model and are more commonly used in wound healing research (Weadock, Miller, Bellincampi, Zawadsky, & Dunn, 1995).

2.3   Determining Gel Stiffness

Currently, many methods of determining mechanical properties of biomaterials exist. Simple methods such as uniaxial mechanical testing are some common ways to characterize biomaterials, butcan lead to complications. Due to the compliant nature of these soft materials, gripping can become difficult and slipping can occur. Additionally, misalignment and increased edge effects can also lead to inaccurate results (Scott el al., 2004). Furthermore, to prepare for uniaxial testing, a sample of the soft materials must be cut out and inserted at the grips of the machine. This can be destructive to the materials, therefore, no further testing can be performed. During uniaxial testing, the fibers of these materials may also realign along the test axis and alter the mechanical properties of the materials. Since these soft materials have very low moduli, the test system should be highly sensitive to low force measurements and small displacements. The test system should also be non-destructive to the materials and be able to securely hold the materials being tested.

2.4   Alternative Methods of Determining Gel Stiffness

In previously conducted studies, several different methods have been used to determine the stiffness of collagen and other soft gels. These methods can be divided into two categories, those that measured stiffness in plane and those that measured stiffness by transverse loading. In this study, two cantilever beams, separated by a thin layer of copper foil, were used to measure stiffness (Figure 1). In terms of transverse loading, the most common testing method was transverse deflection, although methods involving inflation and positive or negative pressure were also used.

While transverse loading is a generally more simplistic and common approach to mechanically testing soft gels, methods of in plane measurements have also been conducted. An example of this is the use of vibration to determine the viscoelastic properties of the gel as seen in the study by Parsons and Coger (2002). In this study two cantilever beams were separated by a thin layer of copper foil as seen in Figure 1. One of these beams was used to propagate the vibration while the other beam acted as a sensor for the signal. By vibrating the gel at a known

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rate and then detecting what the actual level of vibration that was seen at another point in the gel, the storage modulus and loss modulus could be determined. These factors show how much of the vibration was allowed to travel through the gel and from these, the stiffness of the gel can be determined. This method is susceptible to error due the nature of the setup. Additionally, complex computation is required as well as the manufacturing of small parts that need to be placed with a high level of precision (such as the distance between the beams).
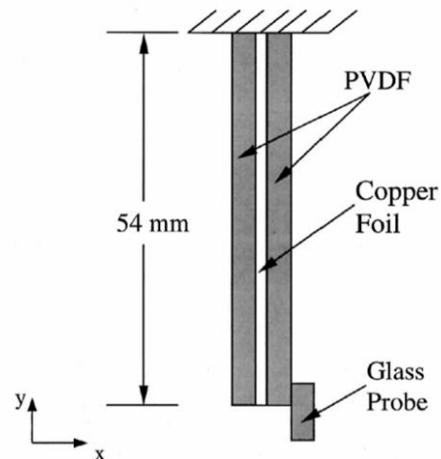
Figure 1: Diagram showing the cantilever beams of the vibration test. The copper foil separates the two by 33 μm (Parsons & Coger, 2002).

The most documented method of measuring the stiffness of collagen or similarly soft gels is by transverse deflection. This method involves applying a load to the gel that is perpendicular to the gel. This method involves a load being applied to the gel that is perpendicular to the gel. This force is measured and the amount of deflection of the material caused by the force application is also measured. Force and deflection can be measured through various means which include using a beam attached to a force transducer, an actuator to control movement and determine displacement, a water jet to indent the surface, and ultrasound waves to measure deflection or objects of known weight to cause the indentation. In studies using transverse deflection, the gels were generally strained to between 5-7% in order to stay well under the 10% strain limit as given by Bueckle's limit, which states that after 10% stiffness values become disproportionately high (Scandiucci de Freitas et al., 2006).

In one study, Ju et al. developed a method for determining the stiffness of thin biomaterials (2002). This method involved using a circularly fixed material that is transversely

loaded in the center to cause a point indentation. The displacement was measured with a resolution of 1 nm and the force with a resolution of 10 μN. After a series of complex calculations were carried out, this author's determined the stiffness of a non-bending ultra thin material when loaded with a central point force.

Other studies have also been conducted using a similar approach. Scandiucci de Freitas et al. indented a gel with a flat tip steel rod attached to a force transducer capable of 0.001 N resolution (2006). The deflection of the material was measured and controlled with a resolution of 0.001 mm. The results of this these tests yielded stiffness values by creating a linear fit to a stress-strain curve. This approach was similar to the study conducted by Ju et al., however, it was more simplistic in nature and was conducted on materials that are more like the collagen matrices that will be tested in this study.

A slightly different method of indentation was conducted in an experiment using a water jet for indentation and ultrasound waves to measure the deflection values (Lu, Zheng, & Huang, 2007). The test setup can be seen in Figure 2. The force used was determined based on the pressure from the water jet. Additionally, there was a pressure sensing load cell placed under the tissue to capture force data. The strain was held to 5% to keep inside of the linear elastic range and then the stiffness was determined by the slope of the stress-strain curve. This method used photo analysis to look at the deformation profile.
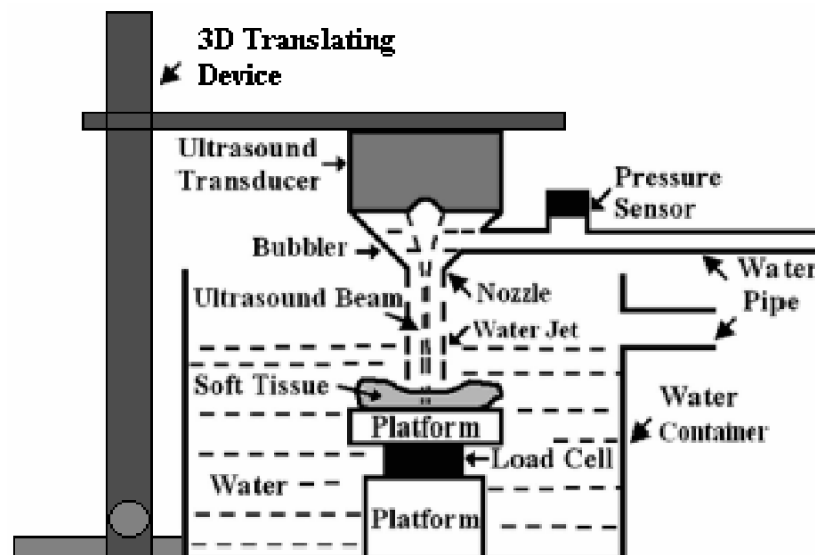
Figure 2: Diagram of the water jet test setup (Lu et al., 2007)

Overall, transverse loading methods are more prominent than in-plane loading methods as they require fewer complicated equations to determine a closed form solution and testing is generally more simplistic. Existing transverse loading methods each have their individual strengths; however, many are incompatible with collagen gels and the gel clamping system. In previously conducted experiments, many complex equations which are beyond the scope of this project or sophisticated testing setups which are not available and beyond the budget of this project were used. Furthermore, approaches which feature a water jet or pressure change also require the gel to be fixed either in place or on a platform, a configuration which cannot be achieved with our current testing requirements. Based on previous research, a new device which determines the stiffness of the gels in a circular clamp using transverse loading must be developed.

3   Project Approach

Before moving forward with the design process, it is necessary to outline assumptions being made and detail specific aims of the project.

3.1   Assumptions

Several assumptions have been made for this project, most of them regarding finite element analysis. These assumptions can be seen below:

- The behavior of the gel can be described by a closed-form solution

- Bending effects are negligible

- Finite contact effects are accounted for in the model

- The gel may be modeled as a circular gel clamped with a fixed outer edge

- The gel is of uniform thickness

- Contact between the indenter tip and the gel is frictionless

- Poisson's ratio is equal to 0.5

3.2   Specific Aims

The ultimate goal of this project is to create a novel device to be used to determine the stiffness of circularly clamped collagen gels. The specific aims for this project are presented below:

- Develop a conceptual design in which the stiffness of the test matrix can be determined through physical contact with the matrix.

- Design a novel device to collect and output quantitative measurements of force applied and displacement of the collagen gel.

- Develop a modeling equation to predict gel stiffness

- Create a mathematical model to relate force and displacement in order to further quantify composite stiffness.

4 Design

4.1 Client Statement

The purpose of this project is to develop and validate a device to measure the stiffness of circularly clamped collagen gels. This new device will be used to determine the composite stiffness experienced by the cells growing within the test matrix. The device will output quantitative measurements of both force and displacement which will be used to calculate the composite stiffness. The device must be accurate, easy to use, safe for the user and material, compatible with data acquisition software, and appropriate for the laboratory setting.

4.2 Objectives, Functions, Constraints

In order to develop a final design, it is necessary to determine the best way to measure the stiffness of suspended gel. In determining this, it is necessary to outline objectives, functions, and constraints. The latter were identified and weighted to determine their importance in deciding the appropriate final design.

4.2.1 Objectives

The design team identified over 10 objectives for this design. Based on the inputs of the two clients, Dr. Kristen Billiar and Dr. Marsha Rolle, of Worcester Polytechnic Institute, three pairwise comparison charts (two from two separate clients and one from the design team) three main objectives were determined and a table of objectives and sub-objectives were created for the final design. The weighted overall design objectives are shown in Table 1.

Table 1: Weighted design objectives based on pairwise comparison charts

Objectives	Sub-Objectives
Accurate - 50%	- *Results must be reproducible* - *The device must be easily calibrated* - *The device must provide measurements with high resolution*
Easy to Use - 31%	- *Must be easy to transport* - *Must be easy to operate* - *Must be easy to assemble* - *Must be time efficient*
Easy to Maintain - 19%	- *Must be made of parts that are easy to remove or insert* - *Designed with cost and maintenance in mind* - *Easy to clean* - *Robust enough to withstand cleaning chemicals and range of temperatures*

Based on Table 1, it is clear that the final prototype must produce accurate results in terms of reproducibility, correct calibration, and high resolution. This is especially important where accurate and reproducible data is needed for research. The device must be easy to calibrate and it must provide reproducible measurements with high resolution. In contrast, maintenance of the device in terms of cost and materials are only minor factors in this design. The ease of transporting, operating, and assembling the results is more important than the cost of the device, but not as important as its accuracy and the reproducibility. In this case, the importance of having accurate results clearly outweighs the cost of the device and the cost of maintenance.

4.2.2 Functions

Based on the client statement, this novel device must be able to collect the quantitative measurement of force and displacement upon contact with the gel. Since the goal of the project is to analyze gel stiffness, one must be able to measure the force the device is inflicting on the gel. Additionally, it must be able to detect the displacement of the gel.

4.2.3   Constraints

In designing this device, several constraints must be taken into account during the design process. These constraints are:

- The device must not cause harm to the user.
- The device must not inflict damage to the tested gel so that it becomes unusable.
- The device must use Data Acquisition software to capture data.
- The device must be built and validated by March 2008.

Since the transverse loading device is used to measure stiffness of gels, the final device must be compatible with circularly clamped collagen gels. The device must also not cause any harm to the user and must not inflict damage to the tested gel that it becomes unusable. It is important that the tested gel can be reused for testing to create reproducible results. The device must use National Instrument's Data Acquisition software to capture the data collected by the device as this is one of the few means available to the design team. Finally, the device must be built and validated by testing with cell-seeded gels by March 2008.

4.2.4   Specifications

Once the objectives and the functions of the device were identified, the specifications of the device were further evaluated to determine the parameters that the device must meet in performing the functions. Table 2 illustrates the device specification and the parameters at which they must be met:

Table 2: Device Specifications

Specifications	Description
Force Measurement	The device must be able to measure a minimum force of 2N at a resolution of 2mN
Displacement Measurement	The device must be able to record the displacement at a resolution of 0.1mm

For the measurement of force, the preliminary study with collagen gel showed that the gel can withstand a force of roughly about 2N. Studies have also shown that in order to accurately record the force of the tested gel, the device must have a resolution at 100x smaller than the force (Scott, Begley, Komaragiri, & Mackin, 2004). Similarly, the displacement reading must be accurate at a resolution of 0.1mm. Based on the preliminary studies with different materials, it was determined that the gel would be tested to a displacement of 3 mm in order to collect the greatest amount of data without causing damage to the gels.

4.3   Feasibility Studies

4.3.1   Jell-O Stiffness Testing

Mock gels were poured using Jell-O as a substitute for collagen gels. The purpose of this testing was to perform mock testing on a low cost material (Jell-O) to determine stiffness and establish methods which would be successful for testing collagen gels. This testing was carried out on October 3, 2007. Jell-O was poured in rectangular foam anchors and paperclips were hung from the Jell-O to determine stiffness. The protocol for this testing can be seen in Appendix I.

4.3.2   Collagen Gel Stiffness Testing

Testing was done on collagen gels to preliminarily determine the stiffness of collagen gels to compare with literature. Based on the testing done prior to this with paperclips, it was determined that paperclips were too heavy so ¾” kidney wire was use as a substitute in this testing. Additionally, the amount of force applied and amount of displacement were recorded. Testing was completed on October 4, 2007. The protocol for this testing can be seen in Appendix II.

4.3.3   Latex Mock Testing

Mock testing was carried out with a testing setup similar to the one which will be used for actual testing. In this testing, a 50g force transducer was used in conjunction with an actuator and both were operated using LabVIEW. Force and displacement readings were recorded during the test and used to determine stiffness values of the mock material. The mock material used for this test was latex. Testing was completed on November 28, 2007. The protocol for this testing can be seen Appendix III.

4.3.4   Circular Jell-O Mock Testing

These tests built upon the testing which was completed on November 28, 2007 and was also carried out with a setup similar to the one which will be used for actual testing. In this testing a 50g force transducer was used in conjunction with an actuator and both were operated using LabVIEW. The mock material used for this test was Jell-O. Two different concentrations of Jell-O were used and the Jell-O was poured into foam anchors of three different diameters. Force and displacement readings were recorded during the test and used to determine stiffness values of the mock material. Testing was completed on December 3, 2007. The protocol for this testing can be seen in Appendix I.

4.4   Alternative Designs

Upon identifying the objectives, functions, and the constraints of the design, several stiffness measurement prototype designs alternatives were created. These design were drawn from past research as well as new ideas which came up during brainstorming sessions. To evaluate the design alternatives, a design selection matrix was used with weighted design objectives and constraints to ensure the final design prototype were fully compatible and meet the needs of the client. The morphological chart in table 3 shows the possible means to carry out each function.

FUNCTIONS	MEANS						
Measure Force	Point Compression	Transverse Loading	Balloon Inflation	Clamp Model	Free Weight	Magnet	Vacuum
Measure Displacement	Linear Actuator	Displacement Transducer	Optic Laser	LED with Sensor	Straightedge		

The design team came up with several design alternatives with the possible means stated in the morphological chart to carry out each function. The first design alternative is known as the Point Contraction Model (Figure 3). This model works by slightly indenting two points onto the surface of the gel. These points are then moved toward each other causing the gel and the springs to deflect. This deflection would be measured and modeled with FEM to determine the stiffness.
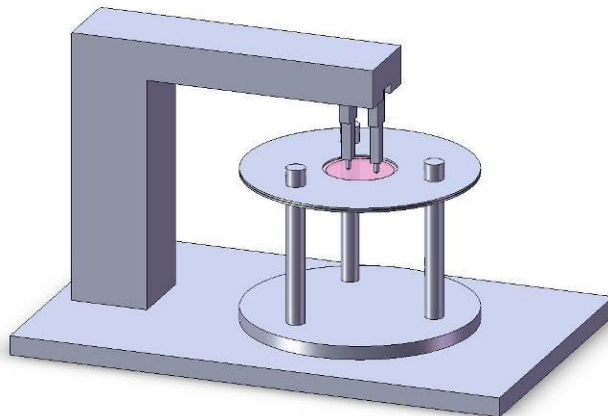
Figure 3: The point compression design alternative

The next design alternative is known as the Clamp model (Figure 4). This model uses a clamp that compresses at the center of the gel and then slowly begins to retract. It is important that the clamp be able to fit between two of the springs and that it clamps on the center of the gel.
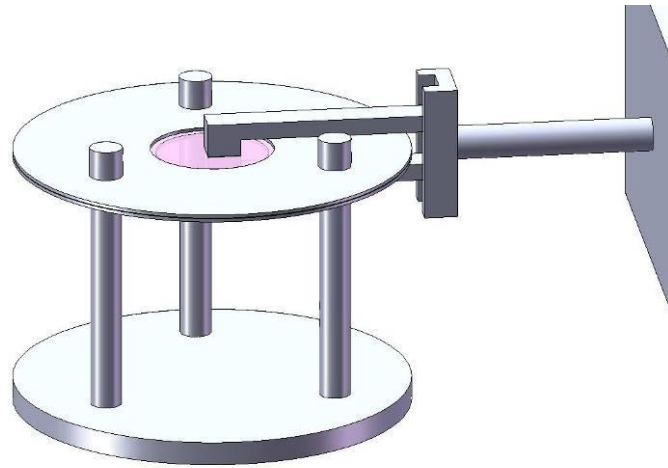
Figure 4: The clamp model design alternative

The next design alternative is known as the Balloon Inflation model (Figure 5). This model uses a balloon to cause the deflection of the gel. Then by determining the pressure inside of the balloon, the amount of deflection and the surface in contact with the gel, the stiffness could be determined.
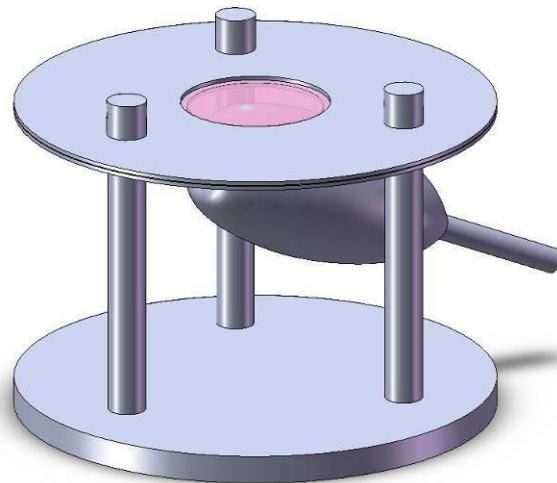
Figure 5: The Balloon Inflation design alternative

The Transverse Loading design is the next design alternative (Figure 6). This model uses an indenter tip attached to a force transducer to cause deflection in the gel. The deflection is measured by the position of the transducer and the force is registered by the transducer. This information can then be used to determine stiffness.

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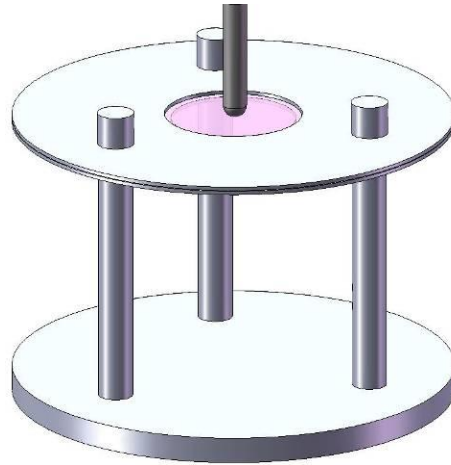
Figure 6: Transverse Loading design alternative

The next model for the design alternatives is the Free Weight Model (Figure 7). This design uses a weight of a know value that is placed on the center of the gel. The deflection of the gel is then measured by image analysis or a similar method and the stiffness can then be calculated. This would then be repeated for weights of different magnitude.
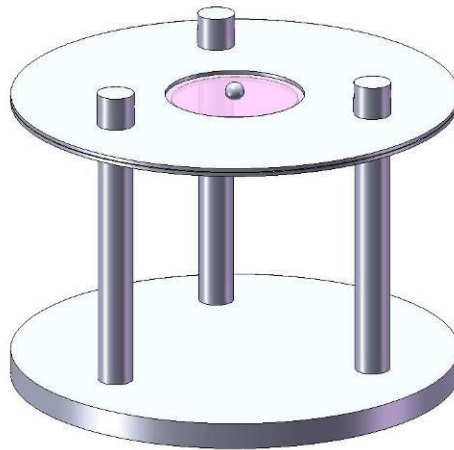
Figure 7: The Free Weight design alternative

The next design alternative is similar to the previous model in that it uses a round weight on the surface of the gel, but this model incorporates a magnet underneath the gel which makes it possible to vary the force applied to the gel. The electromagnet under the gel can be used to

attract the metal weight and with different currents can provide different amounts of force. This design is known as the Magnet Model (Figure 8).
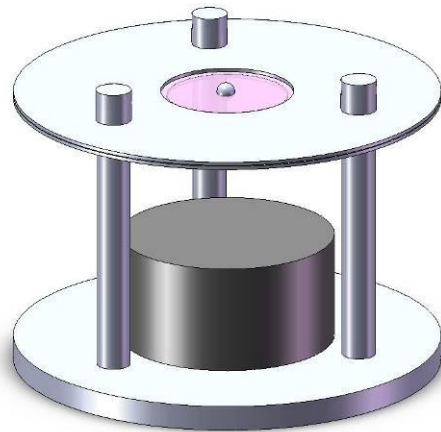
Figure 8: The Magnet Model design alternative

The final design alternative is known as the Vacuum model (Figure 9) because it uses a vacuum chamber underneath the gel. This vacuum will provide an area of less pressure underneath the gel, causing it to deflect into the chamber. The amount of deflection along with the pressure inside of the chamber will be used in order to determine the stiffness.
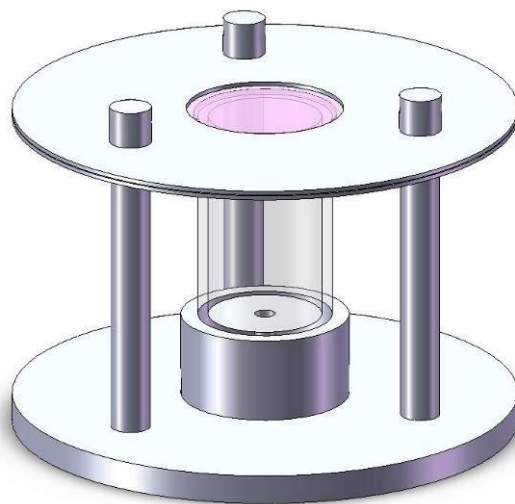
Figure 9: The vacuum design alternative

Each alternative design has its own advantages and disadvantages. In order to determine which means of applying force and measuring displacement are most suitable for our device, each device was evaluated in relation to weighted objectives. Additionally, each design alternative was evaluated with the design constraints previously outlined. A decision matrix was

created to compare each weighed objective with each design alternative to ensure the final design best met the objectives of the project. The decision matrix is shown in Table 4.

Table 4: Force and displacement measurement selection matrix with objectives and constraints

Design	Weight (%)	Side Compression	Point Compression	**Transverse Loading**	Balloon Inflation	Clamp Model	Free Weight	Magnet Model	Vacuum Design
Design Constraints and Objectives									
C: Must not cause harm to user		Y	Y	**Y**	Y	Y	Y	Y	Y
C: Must not inflict damage to tested gel that it becomes unusable		Y	Y	**Y**	Y	Y	Y	Y	Y
C: Must use Data Acquisition software to capture data		Y	Y	**Y**	Y	Y	N	Y	Y
O: Accurate	50	0.8 x 50%	0.7 x 50%	**1.0 x 50%**	0.6 x 50%	0.6 x 50%	0.9 x 50%	0.7 x 50%	0.6 x 50%
O: Easy to Use	31	0.5 x 31%	0.6 x 31%	**0.8 x 31%**	0.7 x 31%	0.4 x 31%	0.9 x 31 %	0.7 x 31 %	0.7 x 31 %
O: Easy to maintain	19	0.6 x 19%	0.6 x 19%	**0.8 x 19%**	0.7 x 19%	0.6 x 19%	0.8 x 19%	0.8 x 19%	0.8 x 19%
Totals	100	66.9	65.0	**90.0**	71.4	53.8	88.1	71.9	66.9

Among all the alternative designs, transverse loading has the highest score in terms of accuracy, ease of use, and ease of maintenance. The transverse loading method also meets all the constraints of this project. As a result, transverse loading was selected as the final design.

4.5   Design Decisions

4.5.1   Force transducer selection

After deciding on the design to use for the device, a force transducer needed to be selected. Force transducers from 10 different companies were researched and the best one was

selected according to five different criteria: cost, resolution, force range, overload, and type. The force transducer selection matrix is shown in Table 5.

Table 5: Force transducer selection with criteria

Criteria	Weight	Singer	Cooper	WPI	Omega (LCMKD)	Transducer Techniques
Cost	10%	x	3	7	5	10
Resolution	50%	50	50	35	35	35
Force Range	25%	25	15	25	15	15
Overload	10%	10	10	x	5	5
Type	5%	5	5	3	2	2
Total	**100%**	**90**	**83**	**70**	**62**	**67**

Criteria	Weight	SMD	Omega (LCUB)	A.L. Design	Sol Tech	Futek
Cost	10%	9	3	5	5	2
Resolution	50%	50	30	50	25	50
Force Range	25%	15	10	20	20	20
Overload	10%	5	3	6	3	10
Type	5%	1	3	2	3	4
Total	**100%**	**80**	**49**	**83**	**56**	**86**

Cost – 10 points for under $100, less points as the price increases

Resolution – 50 points for .1% or better, 35 points for .25% and 25 points for .5%

Range – 25 points for 5 grams, 20 points for 10 gram, 15 points anything higher

Overload – 10 points for better than 500%, 5 points for 150%, 1 point for 100%

Type – 5 points for in plane, 3 points for cantilever, 1 for hard to use design

4.5.2   Indenter Tip Selection

Once the Transverse Loading design was selected, a proper tip to apply the force to the gel needed to be selected. Many differing types of indenters used for other types of hardness testing were considered. These different types included spherical tip, diamond tip, cone tip, blunt tip and several other variations. It was decided that a spherical tip would be used as previous studies have shown the spherical tip to provide a better strain profile (Scott et al., 2004). Additionally, a spherical tip is the most ideal for soft materials such as collage as it will eliminate the potential of the tip piercing effect of other shaped tips. Once the project team decided on a spherical indenter, quotes were obtained from various companies and one certified indenter was even found on eBay. This indenter was the lowest cost option to purchase at a price of $65. It was then recommended that a spherical indenter tip be made out of standard materials such as a rod and ball bearing. This will help save cost while providing a similar outcome as the more expensive options.

4.5.3   Frame Material Selection

In order to save time in the construction of the of the device's frame, materials from the website, 8020.net will be purchased. The site describes its products as the "Industrial Erector Set". The site provides custom cut aluminum pieces that fit together with simple nuts and bolts. This will save the design team time in the machine shop to custom make the frame out of aluminum stock. Compared to the price of the stock aluminum and other pieces needed for custom machining, the 80 20 opinion is not much more expensive and will allow the group to assemble the frame quickly and efficiently with a more professional look than probably could be achieved by our project group which have limited machining experience. The difference in cost is minimal because the amount of stock material that is needed to machine the frame would result in a lot of excess material.
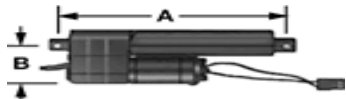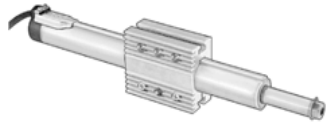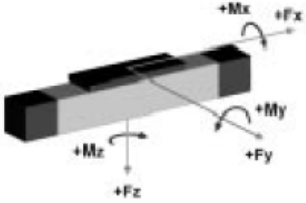
4.5.4 Actuator Selection

In order to select an actuator, the project team researched many different companies which make actuators. The most significant specification for the actuator, as outlined in project specifications, is that the actuator must be accurate within 0.1mm. Furthermore, the stroke length of the actuator must be at least five inches to accommodate for the height of the BCSD. Additionally, the actuator must be able to support at least 50g, the weight of the force transducer and indenter tip. Seen below is the actuator selection matrix (Table 6). The first column outlines the specifications the actuator must meet. Followed by this, each column outlines a different actuator. The cells highlighted in blue are cells which meet the design specifications, whereas the cells highlighted in red are cells which do not meet the design specifications. Columns which are highlighted in gray and have a price highlighted in green are actuators which meet all design specifications. Based on this table, it was determined that actuator which best satisfied the requirements of this project was the Ultramotion actuator as it was the lowest cost and met all specifications.

Table 6: Linear Actuator Selection Matrix

Color	Meaning
Blue Cell	Meets design specification
Red Cell	Does not meet design specification
Green Cell	Price of actuator which meets design specifications
Gray Column	Actuator meets all design specifications

Company	Firgelli Automations	Cleveland Motion Controls	McMaster-Carr	McMaster-Carr
Model	Mini Style (FA-MS-15-12)	Platform MS 65	6509K83	Unguided Linear Motor (2280K17)
Stroke Length (5" minimum)	6"	5.9"	6"	5.51"
Load Capacity (50g minimum)	15lbf	Up to 65lbf	25lbf	9.85lbf
Input Voltage	12VDC, 2A at full load	N/A	12VDC	12VDC
Maximum Speed	0.0125 in/min	0.875 in/min	120 in/min	2.75 in/min
Resolution (0.1mm minimum)	0.1"	7.87E-7"	Has start/stop pin	0.004"
URL	http://www.firgelliauto.com/product_info.php?cPath=110&products_id=128	http://www.cmccontrols.com/linear_actuator_ms65.asp	http://www.mcmaster.com/	http://www.mcmaster.com/
Price	$63.99	$1500.00 (quoted)	$203.80	$1,603.00
Notes				Also need to buy servo controller ($575.62) and power supply ($320) these are included in price listed above

Company	Trossen Robotics	Ultramotion	Precision Technology	Digital Linear Actuators	Haydon Switch and Instrument
Model	LACT6P	The Digit	Wiesel POWERLine	35DMB-K	8700 Series
Stroke Length (5" minimum)	6"	8"	197"	0.875"	2.5"
Load Capacity (50g minimum)	110lbf	400lbf	6.3E5lbf	6.5lbf	10.1lbf
Input Voltage	12VDC	24VDC	12VDC	5VDC or 12VDC	
Max Speed	0.0013in/min	0.0042in/min	1.64in/min	425 steps/s	
Resolution (0.1mm minimum)	Controller only three pre-sets which it extends to	0.00004"	3.93E-4"	0.0001"	3.93E-5"
URL	http://www.trossenrobotics.com/store/p/5184-6-STROKE-110-LB-12-VDC-LINEAR-ACTUATOR.aspx	www.ultramotion.com/products/digit.php	http://www.pt-usa.net/products/pdf/Precision%20Technology%20POWERLine%20WM60-370%20Specification%20Sheet.pdf		http://www.hsi-inc.com/hybrid_linear_actuators.php
Price	$143.63	$550.00	$1500.00 + cost of controller	Did not get quote because insufficient stroke length	Did not get quote because insufficient stroke length
Notes	Also needs a controller ($57.44) included in price listed above			This is the actuator the other MQP group is using, has insufficient stroke length	

4.6   Optimization

The design chosen as the best for our testing purposes was the Transverse Loading model. In order to get the most out of this design, it has been determined that it must conform to the following specifications:

- Frame must be constructed of metal (aluminum or other) to provide a solid base for the sensitive measuring equipment.

- Frame will be equipped with anti-vibration feet to reduce noise.

- The indenter tip will be round and of a known diameter.

- The frame will be larger enough to allow easy loading of the clamping system which will be fixed into the frame.

- The clamping system will be fixed in such a manner that the center of the gel will be positioned in-line with the indentation tip.

4.7   Preliminary Data

4.7.1   Jell-O Stiffness Testing Data

Data gathered from the preliminary Jell-O stiffness tests was more qualitative than quantitative in nature. The purpose of this testing was to determine the feasibility of applying the testing methods to actual gels. From this testing it was determined that paperclips were too heavy to be used as an incremental measurement of weight. The paperclips weighed on average 1.3g so an alternative of ¾" kidney wire which was much lighter was selected for the next round of testing when collagen gels were used.

4.7.2   Collagen Gel Stiffness Testing Data

The collagen gel stiffness testing allowed project team to estimate the stiffness of collagen gels which would be used in final testing once the device was constructed. This testing used collagen gels which had been allowed to compact for three days in an incubator. After each

weight was added a picture was taken which was analyzed using ImageJ Analysis to determine how much each weight displaced the gel. It was assumed that there were no bending effects in this testing. Of the six tests were run, three were eliminated from analysis based on observations made during testing. The cross sectional area that the force was applied to was equal to the width of the gel multiplied by the thickness (0.079cm^2). The data from this testing can be found in Appendix VI. Of the stiffness values calculated for the collagen gels, 10.6kPa was used for future testing as this data had the best R^2 value and also the least amount of standard deviation for the stiffness reading.

4.7.3   Latex Mock Testing Data

Similar to the Jell-O stiffness tests, the purpose of the latex mock testing was also to determine the feasibility of the proposed testing setup before using collagen gels. The latex used in this testing was much stiffer than predicted for the collagen gels with which the project team will be working. As a result of this, the latex did not fail with 50g of force applied. However, since a 50g force transducer was being used, when the load reached 50g the test was terminated. In these tests, the project team was able to come up with a mock indenter tip, calibrate the actuator, and make a pre-existing LabVIEW program compatible with the project's requirements.

4.7.4   Circular Jell-O Mock Testing

Below is a summary of the information found in the preliminary study conducted on two different concentrations of Jell-O. The first samples are identified as the Blue samples which were made with 2 packets of Jell-O to one cup of water. The second set of samples, identified as the Red samples, was made with 3 packets of Jell-O to one cup of water. Samples 1 – 3 were the "large size" with 70 mm outer diameter and 55 mm inner diameter. Samples 4 – 6 were the "medium size" with a 55 mm outer diameter and 33 mm inner diameter. Samples 7 and 8 were the "small size" with a 28 mm outer diameter and 20 mm inner diameter. All of the graphs and

data analysis were completed in Microsoft Excel 2007. A sample graph can be seen below in Figure 10. The stiffness of this sample was determined to be 57.05gf/cm.
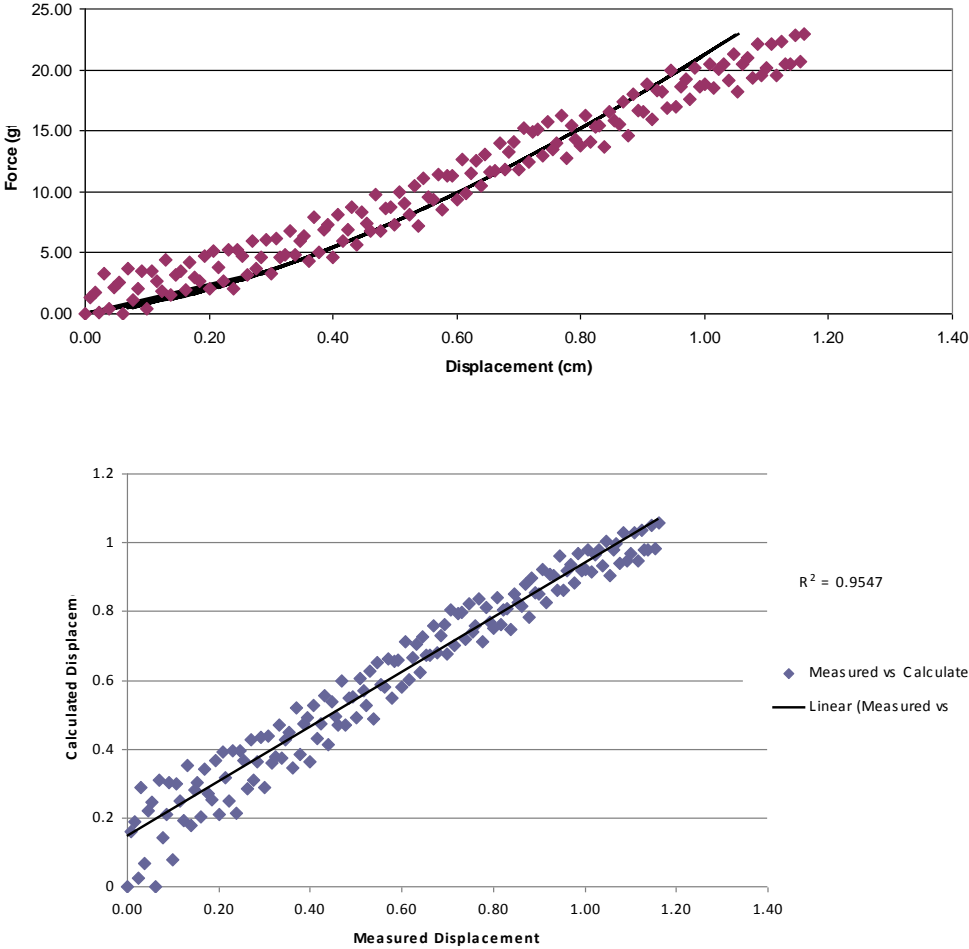
Figure 10: Top: Force vs displacement for sample R7; Bottom: Measured vs Calculated displacement for sample R7

Table 7 below shows the sample identification number paired with the stiffness reading for the test. The mean and standard deviation was calculated for each size of the samples. Additionally, the mean and standard deviation was calculated for all of the Blue samples and all of the Red samples. A t-test was performed on the data assuming unequal variances and it was determined the stiffness of the two sets of samples was statistically different with a 90% confidence.

Table 7: Circular Jell-O Testing Stiffness Measurements

Sample ID	Stiffness		Sample ID	Stiffness
B1	12.6		R1	29.6
B2	18.8		R2	16.7
B3	19.1		R3	25.7
Mean	16.8		Mean	24.0
Stdev	3.7		Stdev	6.63
B4	15.9		R4	30.1
B5	29.9		R5	31.2
B6	28.3		R6	29.3
Mean	24.7		Mean	30.2
Stdev	7.7		Stdev	0.96
B7	34.9		R7	57.1
B8	37.9		R8	48.7
Mean	36.4		Mean	52.9
Stdev	2.15		Stdev	5.90
All Blue Samples			**All Red Samples**	
MEAN	24.7		MEAN	33.5
STDEV	9.33		STDEV	13.0

Table 8: t-test for Circular Jell-O Testing Comparing Red and Blue Samples

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	24.6838	33.54361
Variance	87.04718	168.5091
Observations	8	8
Hypothesized Mean Difference	0	
df	13	
t Stat	-1.56757	
P(T<=t) one-tail	0.070496	
t Critical one-tail	1.350171	
P(T<=t) two-tail	0.140993	
t Critical two-tail	1.770933	

5   Validation

Several experiments were done to validate the device. The first set of experiments conducted was indentation testing on circularly clamped PDMS samples. The purpose of this testing was to match results found in the article by Scott et al. (2004) to determine the stiffness of PDMS. The next set of tests usedcircularly clamped acellular collagen gels to show that the device is compatible with testing collagen gels. Following this, testing was done on cellular gels with varying concentrations of cells and collagen to demonstrate that the device can accurately detect differences in stiffness resulting from changes in these variables.

5.1   PDMS Testing

The PDMS testing was set up to mirror testing done by Scott et al. (2004). Samples were tested with an h/a (thickness to span radius) ratio of 0.005 which ensures they are in the membrane region rather than the plate region because bending effects become negligible and accurate non-linear analytical solutions exist (Scott et al., 2004). Below a figure can be seen illustrating a cross section of the testing setup with labeled variables (Figure 11).
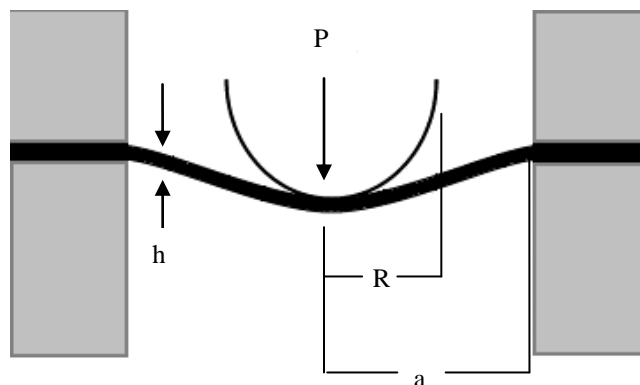
Figure 11: Schematic diagram of indentation testing

For this series of testing, two samples of PDMS were tested, each forfive time, using the device. The first sample was 0.18 mm thick with a radius of 32mm, giving an h/a ratio of .0056,

which is in the membrane region. The second sample was 0.3 mm thick with a radius of 35mm, giving an h/a ratio of 0.00857. Both samples were tested with a 1.5 mm radius spherical indenting tip giving an R/a ratio of .047 for the first sample and .043 for the second sample.

Samples were tested using the device and force (P), displacement (δ), span radius (a), and film thickness(h) were used to determine stiffness according to the following equation from Scott et al.:

$$\delta = f(v)a\left(\frac{P}{Eah}\right)^{1/3},$$

(4)

Where *f(v)* is equal to $1.049 - .0146v - 0.158v^2$ and *v* is equal to 1/3. Equation 4 is a modified equation of the classical Schwerin solution for point loads and has been shown to be appropriate for use with circularly clamped gels (Scott et al., 2004). This approximation has shown to be accurate for small R/a ratios (Scott et al., 2004). In Scott et a.l, an R/a ratio of 0.03 was used and for the testing done with the device a similarly small R/a (indenter tip radius to gel span radius) ratio of 0.04 was used. In Figure 12, a plot can be seen showing the R/a ratio of 0.03 with the point load solution as well as the data.
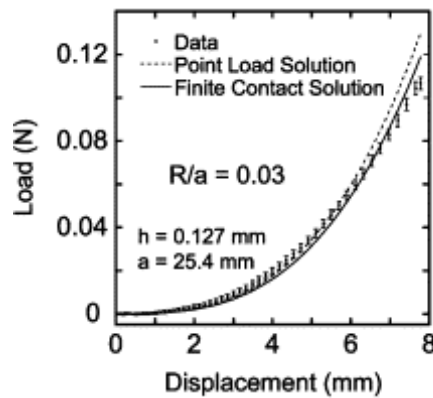
Figure 12: Load vs Displacement for Point Load Solution (Scott et al., 2004)

The plot of load versus displacement of the data was compared to Figure 12. A plot can be seen below which contains test data from one of the trials of PDMS testing, The R/a ratio for the test data was 0.04 and the test data can be seen below in blue.
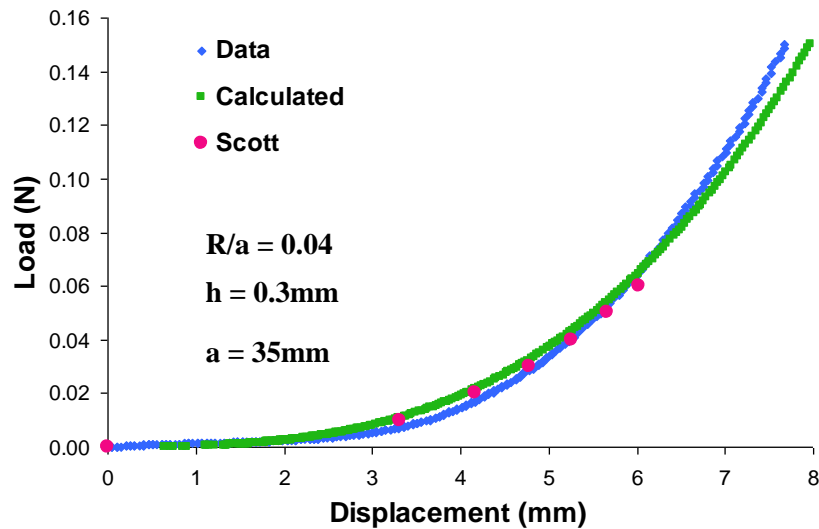
Figure 13: Load vs Displacement Comparing Test Data to Scott et al

Also on this plot are the load and displacement pairings from the Scott article which can be seen in pink. Additionally, displacement points which were calculated using Equation 4 and the stiffness value determined for the sample. To solve for the stiffness of the sample, calculated displacements were compared to measured displacements and the difference was minimized by altering the stiffness value using the Solver function in Microsoft Excel.

Below a summary table can be seen of the Stiffness values calculated for each trial for samples 1 and 2. It should be noted that the percent deviation for each sample is less than five percent. This demonstrates that the d is able to take measurements with a relatively high precision. Additionally, it is also important to note the calculated stiffness for sample 2 is equal to 1.15MPa which is equal to the stiffness for the sample used by Scott et al in Figure 12.

Table 9: Summary of PDMS Data

Sample	Trial	Stiffness (Pa)
Sample 1	Trial 1	2.01E+06
	Trial 2	2.00E+06
	Trial 3	2.04E+06
	Trial 4	1.98E+06
	Trial 5	1.97E+06
	Mean	2.00E+06
	Standard Deviation	2.49E+04
	Coefficient of Variation	1.25%
Sample 2	Trial 1	1.15E+06
	Trial 2	1.23E+06
	Trial 3	1.16E+06
	Trial 4	1.07E+06
	Trial 5	1.15E+06
	Mean	1.15E+06
	Standard Deviation	5.74E+04
	Coefficient of Variation	4.98%

The protocol for this testing can be found in Appendix VII and raw data can be found in Appendix VIII.

5.2   Circularly Clamped Acellular Collagen Gel Testing

Acellular circularly clamped collagen gels were tested with the device. The gels were be poured in 60mm Petri dishes with a foam anchor that is 7.5mm thick to provide an inner diameter of 45mm. Force, displacement, span radius, and film thickness will be used to determine stiffness of the gels using Equation 4 to derive E.

Table 10 below summarizes the stiffness data for each trial. No data is provided for sample 1 as the sample was pierced on the first trial. The standard deviation was very high for this set of tests.

Table 10: Circularly Clamped Collagen Data

Sample	Trial	Stiffness (Pa)
2	1	5.51E+05
3	1	1.71E+06
	2	3.67E+05
	3	1.06E+06
4	1	1.61E+06
	2	1.55E+06
	3	1.00E+06
5	1	1.64E+06
	2	1.54E+06
	3	2.51E+06
Mean		1.35E+06
Standard Deviation		6.24E+05
Percent Deviation		46.08%

However, when the data is analyzed by Trial for each sample rather than all grouped together the standard deviation decreases significantly. For the first trial the percent deviation is only three percent while for trials two and three this increases to 58% and 60%. From this it was concluded that each sample can only be tested once.

Table 11: Circularly Clamped Collagen Data, Trial 1

Sample	Stiffness (Pa)
3	1.71E+06
4	1.61E+06
5	1.64E+06
Mean	1.66E+06
Standard Deviation	5.01E+04
Percent Deviation	3.03%

An FEM was made through which for a given force, the derived displacement from the model should match the displacement reading from testing. The purpose of this testing is to show that the FEM model works for acellular gels. The protocol for this testing can be found in 0 and the raw data for this testing can be found in Appendix X.

5.3   Circularly Clamped Collagen Gels with Cells Testing

Circularly clamped collagen gels seeded with cells were tested with the device. Force, displacement, span radius, and film thickness were used to determine stiffness of the gels using Equation 4 to derive E. The same FEM model from the circularly clamped acellular collagen gel testing was used to analyze the results. The E for the collagen gels seeded with cells was expected to be higher than the E for the acellular collagen gels. The purpose of this testing is to set up a control for testing other variables.

Circularly clamped collagen gels seeded with cells were tested with the device. Force, displacement, span radius, and film thickness were used to determine stiffness of the gels using Equation 4 to derive E. The same FEM model of the circularly clamped acellular collagen gel tests was used to analyze the results. The E for the collagen gels seeded with cells was expected to be higher than the E for the acellular collagen gels. The purpose of this testing was to set up a control for testing other variables.

Proposed variables for testing include varying the concentration of collagen in the gels and varying the concentration of cells in the gels. The varying concentrations of collagen in the gels include 0.5, 1.75, 3.0 (mg/mL). The purpose of testing other variables was to demonstrate that the device is able to detect differences in stiffness which result from altering gel properties. It is believed that the stiffness of collagen gels increases as the concentration of collagen gel within the matrix increases (Delvove et al, 1991). The testing protocol can be found in Appendix XI. An overview of the LabVIEW program which was used to carry out this testing can be found in Appendix XII.

5.4   FEM Analysis

Finite element analysis was conducted using both ANSYS 11.0 and ABAQUS 6.6. Both pieces of software are industry standard for finite element analysis. ANSYS was selected primarily for its simple user interface and quick calculation times and advanced features including interactive time stepping, animation creation, and advanced CAD integration with other drafting programs.

The first FEM test that was conducted was of PDMS. This test used a PDMS sample with a radius of 32mm and a thickness of 0.18 mm (dimensions measured from PDMS sample testing).The modeled PDMS was given an elastic modulus of 1.72 MPa and a Poisson's ratio of 1/3. Additionally, the PDMS was subjected to forces ranging from 0.01 N to 0.1 N (approximately 1 to 10 grams). Unexpectedly, these material parameters do not yield to a calculatable solution through ANSYS. This may be attributed to inherent problems with ANSYS and extremely thin substances.

Alternatively, when a sample was given a higher thickness (in this case, 3mm), ANSYS was able to calculate a reasonable deformation (as seen below in Figure 14). Because of this inherent inability for ANSYS to account for extremely thin samples ABAQUS, another Finite Element Modeling program was used. This program was also used by Jeffrey John of the Worcester Polytechnic Institute Tissue Mechanics and Mechanobiology lab to create a model that matched with the theoretical data from Scott et al.
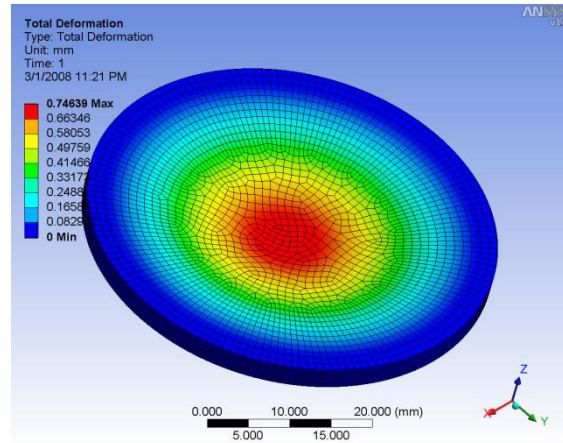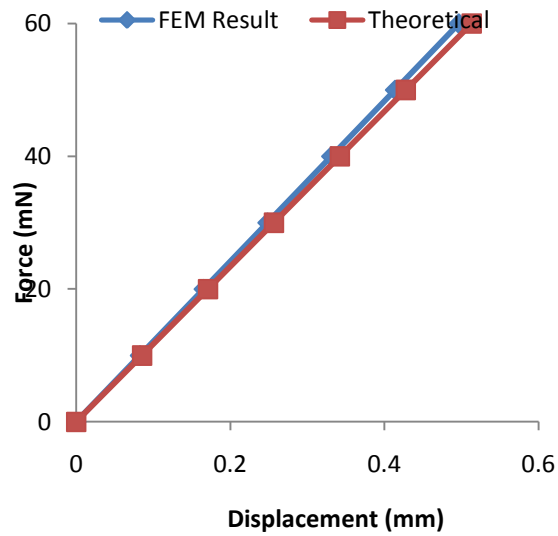
Figure 14: Comparison of Scott et al. to PDMS FEM (left) and
deformation of PDMS model subjected to up to 100 mN of Force (right)

Using ABAQUS, a second deformation analysis was performed using a simulated 3.0 mg/ml collagen gel subjected to varying forces from 0 to 80 mN. When compared to the actual results from the 3.0 mg/ml trials, there is a strong correlation between the modeled displacement and the measured displacement.
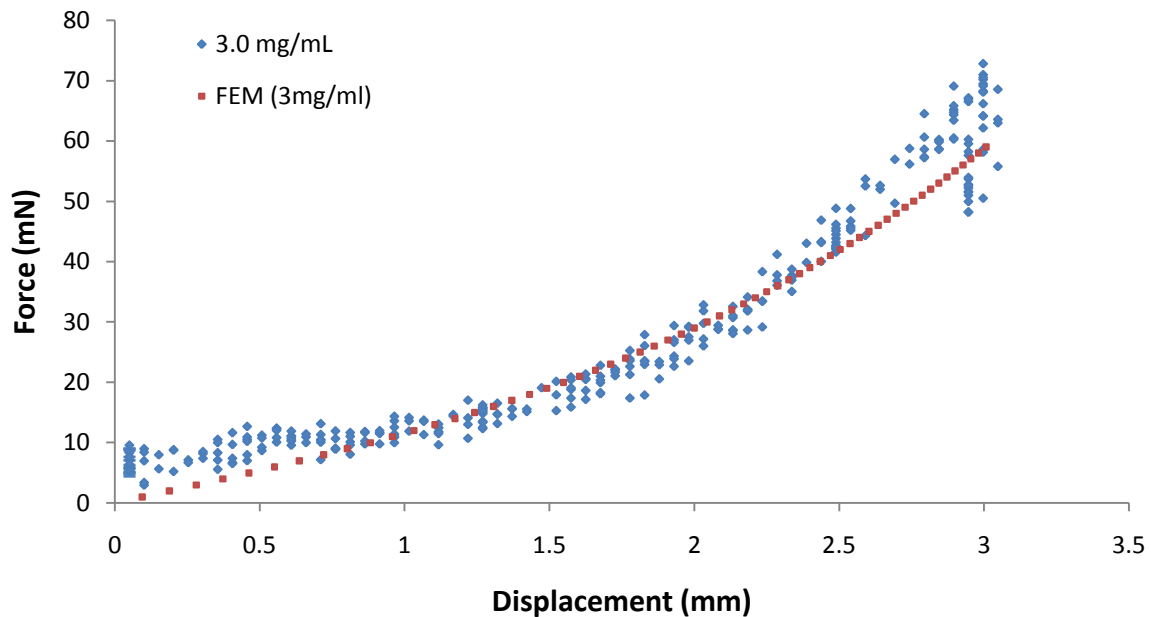
Figure 15: Modeled vs. Measured Force-Displacement Curves for collagen at 3.0 mg/ml

To reference all of the points generated from FEM, this analysis is reproduced in Appendix XV.

In addition to deformation analysis, strain analysis was performed on the finite element models in order to assess the non-destructiveness of the indentation method. In order to accomplish this, the ANSYS model was changed so that it would account for strains instead of deformation. A picture of the strain analysis can be seen below in Figure 16.

Model Color	Strain (%)
	17.6
	15.8
	13.9
	12.0
	10.1
	8.19
	6.29
	4.39
	2.49
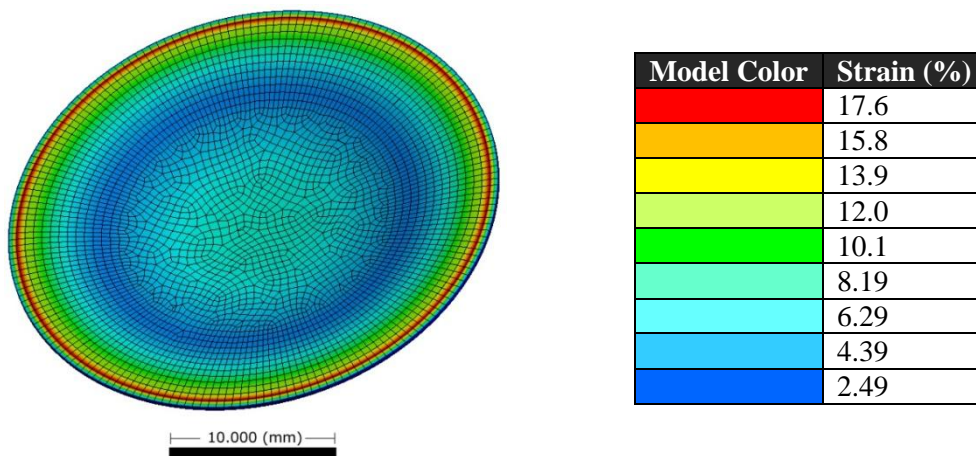
Figure 16: Strain profile on collagen FEA Model subjected to 90 mN of Force

Additionally, a table can be seen below summarizing the strains encountered at different distances from the center of the gel (Table 12).

Table 12: Strain away from center of FEA model

Radial Distance From Center (mm)	Strain (%)
0	4.4
2	4.2
4	3.8
8	4.39
10	2.50
12	3.19
14	11.1
15	18.0

As can be seen in the table, very low strains are present in the majority of the gel. It is only at the outer edges of the gel near the clamping where high strains are present. These results suggest, as specified by our device objectives, that our indentation method is mostly nondestructive, despite the large displacement in relation to the thickness of the sample itself.

6   Analysis and Discussion

Circularly clamped collagen gels populated with 0.5million fibroblasts/mL were tested with the device. Gels were poured at three different concentrations: 0.5mg/mL, 1.75mg/mL and 3.0mg/mL. During testing, force and displacement were recorded. Two separate sets of testing were completed; one was completed March 31, 2008 – April 1, 2008 and the other on April 4, 2008. On March 31, 2008 testing was conducted on a batch of gels at 3.0mg/mL. These gels had been allowed to remodel for four days as technical difficulties were encountered on March 30 which prevented testing on that day. The rest of the gels which were not tested on March 31 were tested on April 1 and they were the 0.5mg/mL and 1.75mg/mL concentrations. The gels tested on April 4 were allowed to remodel for 3 days. Due to difficulties encountered while removing the 0.5 mg/mL gels, the testing was not conducted on these samples and therefore they are not included in the data analysis sections.

6.1 Reproducibility

For both sets of testing, force and displacement was recorded. One of the important requirements of the testing was that reproducible results were achieved between trials for samples at the same concentration of collagen. Below are two plots, one for the 3.0mg/mL concentration and one for the 1.75mg/mL concentration, which visually show reproducible results (Figure 17and Figure 18).
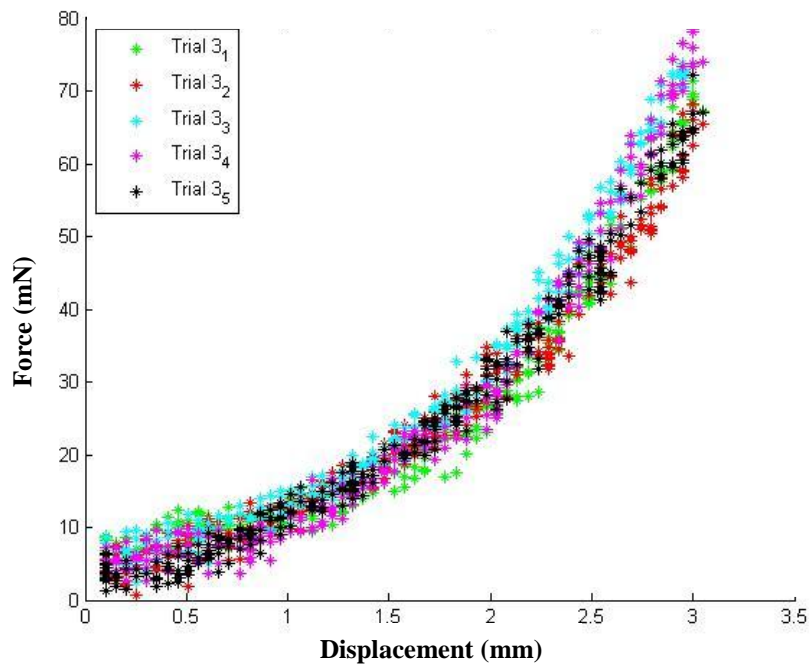
Figure 17: Collagen gel of 3mg/ml were tested showing reproducible results between trials
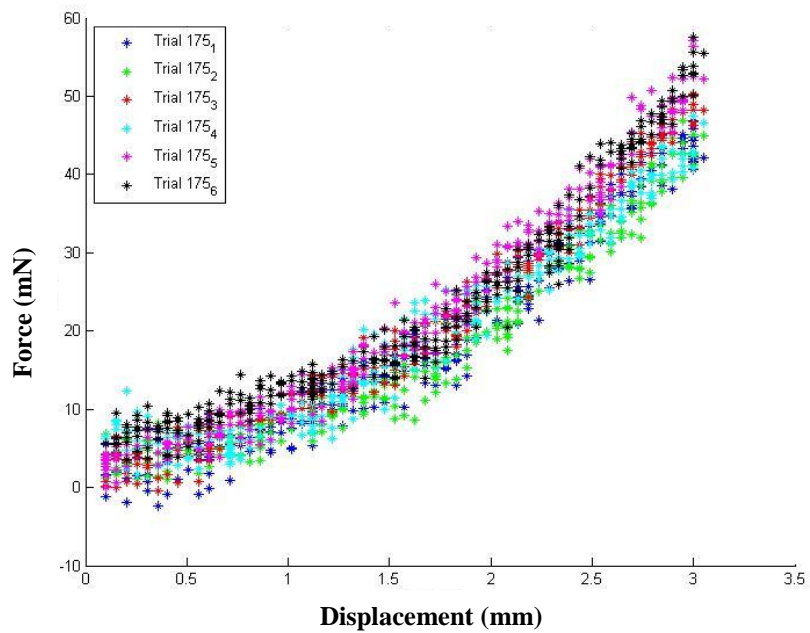
Figure 18: Collagen gel of 1.75mg/ml were tested showing reproducible results between trials

Additionally, to determine how numerically similar the trials were, a comparison of force at different displacements was completed. The force at six different displacements (0.5, 1.0, 1.5,

2.0, 2.5, and 3.0mm) were determined. To get these force values, the average of the force readings for each of the above displacements was taken. The average of these values was then taken to determine the mean force value at each displacement. The standard deviation and coefficient of variation was also computed. It is important to note that the tare load at which displacement began to be recorded was not the same for trials on 3/31 and 4/1 as it was on 4/4. For the testing on 4/4, displacement was recording starting a force reading of 1g. In contrast, the displacement recording began at higher forces for 3/31 and 4/1 which is why a lower displacement, 2.5mm, was reached. A table can be seen below which summarizes the mean, standard deviation, and coefficient of variation for both sets of testing at the two different concentrations:

Table 13: Force-displacement measurements for testing of collagen gels on 3/31/2008, 4/1/2008, and 4/4/200

3.0mg/mL							
Displacement (mm)		**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
4/4/2008	**Mean (mN)**	5.66	12.39	20.65	31.36	48.80	62.75
	Stdev (mN)	1.37	1.06	1.88	3.02	3.84	2.07
	Coeff Var	24%	9%	9%	10%	8%	3%
3/31/2008	**Mean (mN)**	13.78	32.19	56.22	88.38	123.21	
	Stdev (mN)	2.90	3.02	5.84	6.05	4.31	
	Coeff Var	21%	9%	10%	7%	3%	

1.75mg/mL							
Displacement		**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
4/4/2008	**Mean (mN)**	4.99	10.96	16.17	26.39	38.25	46.31
	Stdev (mN)	2.51	2.17	3.07	3.17	3.46	4.54
	Coeff Var	50%	20%	19%	12%	9%	10%
4/1/2008	**Mean (mN)**	3.83	4.11	9.52	18.17	32.25	
	Stdev (mN)	0.74	0.55	1.83	2.44	2.20	
	Coeff Var	19%	13%	19%	13%	7%	

For testing completed on the 3.0mg/mL gels the force values at the given displacements are higher for those tested on 3/31/2008. It is believed that the reason for this discrepancy is because the gels tested on 3/31/2008 were allowed to remodel for an extra day, leading to higher stiffness values. For testing completed on the 1.75mg/mL gels the stiffness values are much closer between the two trials and are higher for the set tested on 4/4/2008. This is the opposite of

the trend seen with the other concentration of gels. A possible explanation for this difference is that since there was less collagen in these samples there was not a significant increase in the stiffness and in fact the gels began to degrade due to the remodeling period of five days. The Force-displacement pairings were also plotted for both of the testing days and can be seen below in Figure 19. These plots visually reinforce the low standard deviation and coefficient of variation seen between samples in the same trials.
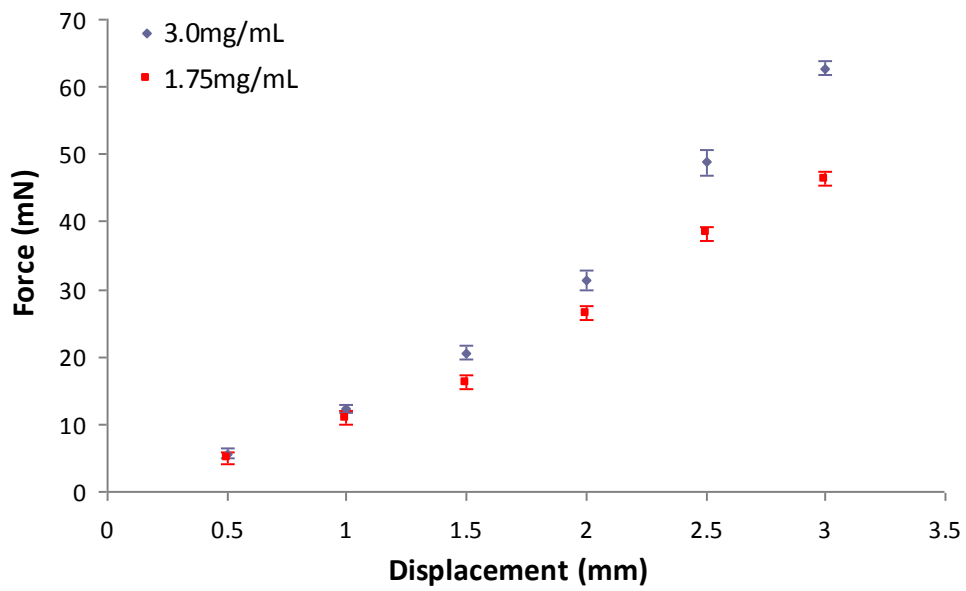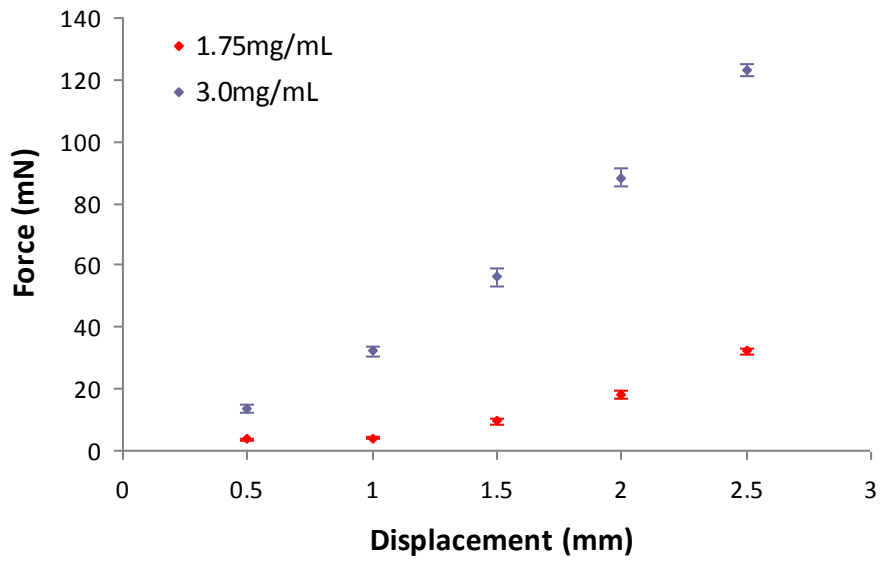
Figure 19: a) Average force versus displacement for 3/31/08-4/1/08 testing b) Average force versus displacement for 4/4/08 testing. Note the low standard deviation in both trials.

6.2   Determining Stiffness

In addition to showing reproducibility, it was also important to show that the device was capable of determining the stiffness of gels with varying stiffness properties. These varying stiffness properties were achieved by using different concentrations of collagen in the samples. The plot below contains representative data from a trial at each concentration of collagen.
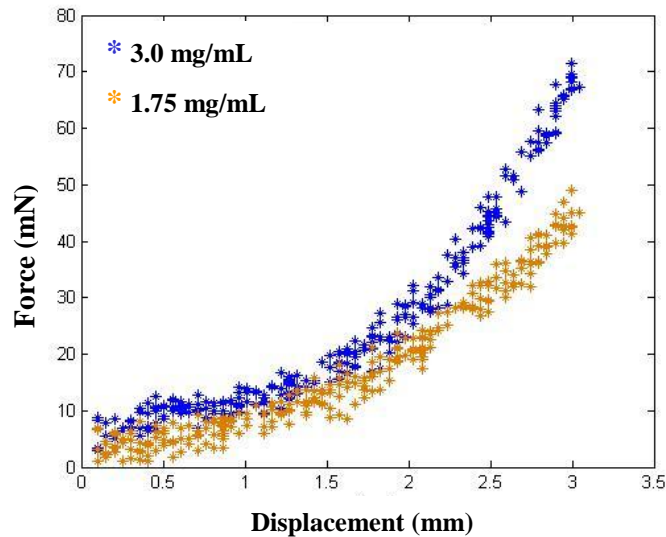
Figure 20: Representative plot of 3mg/ml and 1.75mg/ml of collagen gels.

As can be seen in the above plot, there is a clear visual difference between the 3.0mg/mL sample and the 1.75mg/mL sample.

Beyond establishing that higher forces were generated for the samples which had a higher concentration of collagen, stiffness values also had to be calculated. Stiffness values were calculated using three different equations to model the data in order to determine the equation which fit the data best. There are three different components of which some are present in each equation. The first component is the membrane deflection component which accounts for how much the membrane is deflected. This component was obtained from work completed on transverse loading by Scott et al. (2004) and can be seen below:

$$P = Eah\left(\frac{\delta}{f(v)a}\right)^3$$

The second component is the Hertz component. The purpose of this component is to account for how much the indenter tip indents to the gel and can be seen below:

$$P = \sqrt{\frac{16RE^2(k\delta)^3}{9}}$$

The third component is the bending stiffness component. The purpose of this component is to account for any bending stiffness which may be present in the gel and can be seen below:

$$P = \frac{4\pi\delta Eh^3}{3(1-v^2)a^2}$$

The variables in components one, two, and three are force (P), displacement (δ), span radius (a), film thickness(h), radius of indenter tip (R), initial force (Fo), displacement proportionality factor (k), and $f(v)$ which is equal to 1.049-.0146v-0.158v^2 for a v of 1/3.

It is important to note that only representative plots and tables of the stiffness values for data collected on 4/4/2008 can be found in this section. Due to inconsistencies in testing procedures and incubation periods the stiffness values from 3/31/08-4/1/08 were believed to be unreliable. Additionally, it is evident from the average force-displacement plots found in the last section, there were different forces present on the two days, further supporting the idea that data from the two days will not coincide. Modeled data and stiffness values for testing completed 3/31/08-4/1/08 can be found in Appendix XV.

6.3 Membrane Deflection Equation

The first equation used to analyze stiffness was the membrane deflection equation. This equation is made up of the membrane deflection component previously discussed as well as a tare load. Equation (1) can be seen below:

$$P = Eah\left(\frac{\delta}{f(v)a}\right)^3 + Fo \qquad\qquad (1)$$

A representative plot of the measured data and fitted data along with residuals can be seen below for both collagen concentrations (Figure 23).
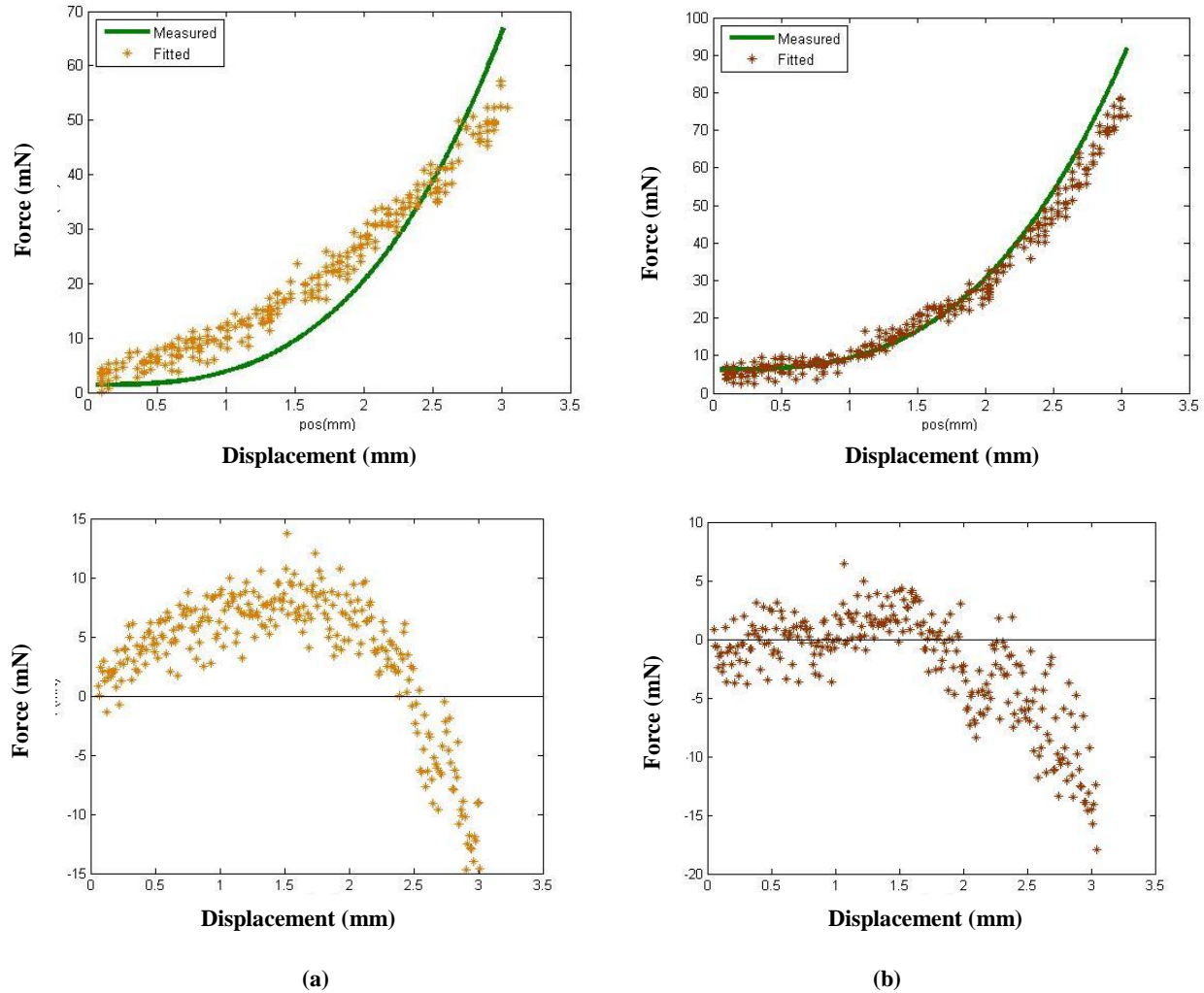
Figure 21: Collected data of collagen concentration of (a) 1.75mg/ml fitted with the membrane deflection equation and residual plots below (b) 3.0mg/ml fitted with the membrane deflection equation and residual plots below (a) has a R^2 value of 0.987 and a RMS value of 0.006mN while (b) has a R^2 value of 0.968 and a RMS value of 0.006mN

As can be seen on the representative plots, the model is not an ideal fit for the data as it apparent in the residuals that there is some systematic error. Comparatively, the fit for the 3.0mg/mL data is a better fit than that for the 1.75mg/mL data. Another way to evaluate the error of the fits is to analyze the R^2 and RMS values which can be seen below in Table 14.

Table 14: R² and RMS values for Equation 1 for both 3.0mg/mL and 1.75mg/mL collagen concentrations

Trial		1	2	3	4	5	Average
3.0mg/mL	R^2	0.992	0.981	0.990	0.992	0.982	0.987
	RMS (mN)	5.22	5.95	5.90	5.01	5.74	5.56
1.75mg/mL	R^2	0.965	0.979	0.970	0.959	0.970	0.968
	RMS (mN)	5.48	4.57	6.18	6.35	6.66	5.85

As can be seen in this table the R^2 value is very high for both concentrations (greater than 0.96 for both). However, the RMS value is high for both concentrations. The RMS value is above 5.5mN for both concentrations which is significant since the maximum force is 50-70mN.

In addition to observing the plots of the data, it is also important to determine how the determined stiffness values from the models vary between trials. A plot can be seen below of the stiffness values for the trials conducted on 4/4/08 (Table 15):

Table 15: Stiffness values determined using Equation 1 for 3.0mg/mL and 1.75mg/mL collagen gels

Trial		1	2	3	4	5	Average	Stdev	CV
3.0 mg/mL	E (MPa)	0.445	0.435	0.506	0.484	0.456	0.47	0.03	6.29%
1.75 mg/mL	E (MPa)	1.31	1.28	1.49	1.39	1.6	1.41	0.13	9.34%

Both stiffness values have a coefficient of variation of less than ten percent, indicating consistent stiffness value among trials.

6.4 Membrane Deflection and Hertz Equation

The second equation used to analyze stiffness was the membrane deflection and Hertz equation. This equation is made up of the membrane deflection component, Hertz component, and a tare load. The purpose of this equation is to account for both membrane deflection as well bending of the collagen gel. Equation (2) can be seen below:

$$P = Eah\left(\frac{\delta}{f(v)a}\right)^3 + \sqrt{\frac{16RE^2(k\delta)^3}{9}} + Fo \quad (2)$$

A representative plot of the measured data and fitted data along with residuals can be seen below for both collagen concentrations (Figure 23).

(a) **(b)**
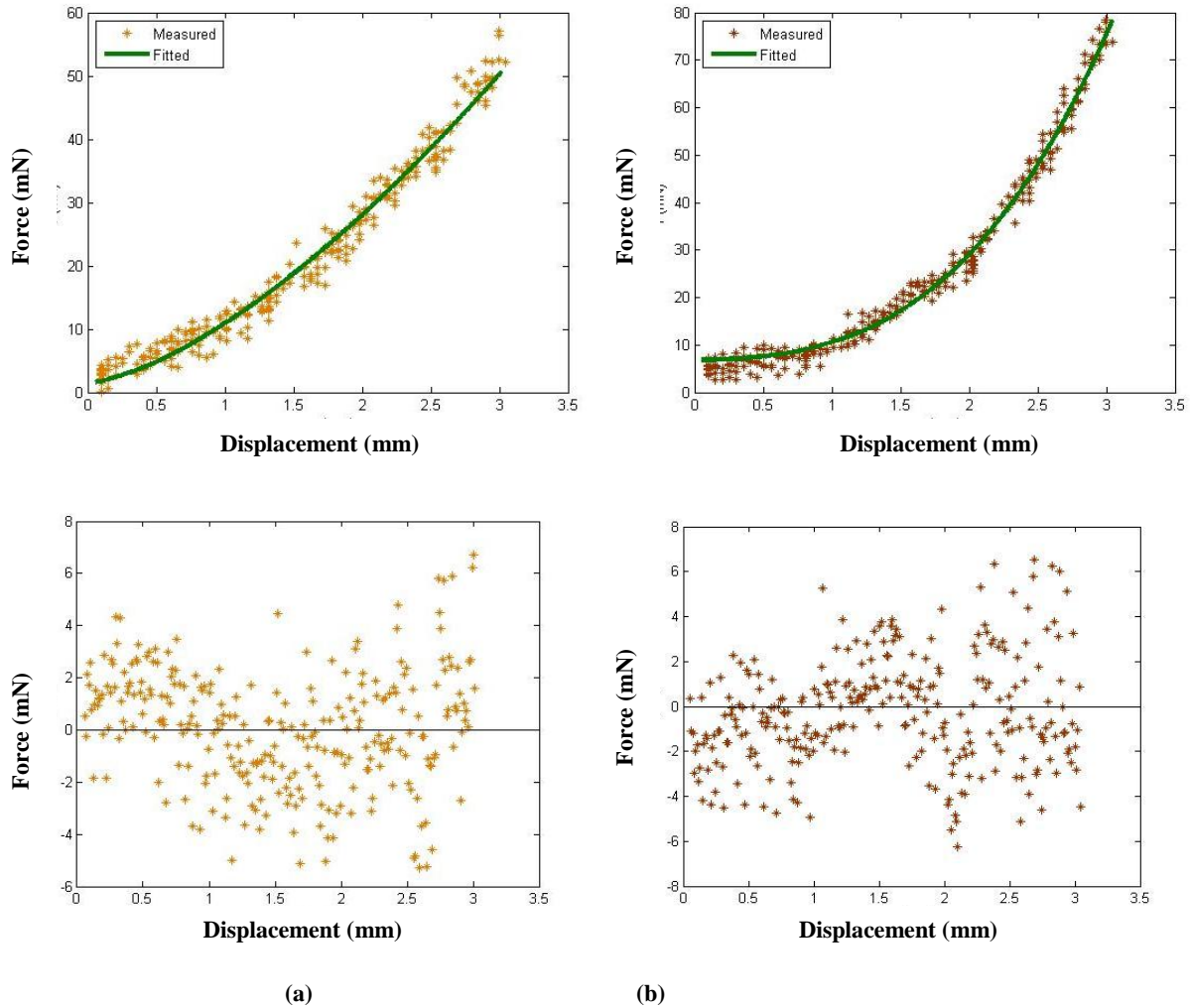
Figure 22: Collected data of collagen concentration of (a) 1.75mg/ml fitted with the membrane deflection and Hertz equation and residual plots below (b) 3.0mg/ml fitted with the membrane deflection and Hertz equation and residual plots below (a) has a R^2 value of 0.978 and a RMS value of 0.009mN while (b) has a R^2 value of 0.975 and a RMS value of 0.005mN

As can be seen on the representative plots, the model is a very good fit for the measured data for both concentrations. This is further supported by the residuals which show no systematic error. Also supporting this fit are the low R^2 and RMS values. A table can be seen below (Table 16) summarizing these values:

Table 16: R² and RMS values for Equation 2 for both 3.0mg/mL and 1.75mg/mL collagen concentrations

Trial		1	2	3	4	5	Average
3.0mg/mL	R^2	0.992	0.990	0.995	0.993	0.991	0.992
	RMS (mN)	0.002	0.002	0.002	0.002	0.003	0.002
1.75mg/mL	R^2	0.981	0.976	0.989	0.979	0.988	0.983
	RMS (mN)	0.003	0.003	0.002	0.003	0.002	0.002

The R^2 value is greater than 0.98 for all trials and the average RMS value for both concentrations is 0.002mN which is very low.

In addition to comparing how well the model fits the data, it is also important to compare the stiffness values among trials. A summary table can be seen below (Table 17) of these values:

Table 17: Stiffness values determined using Equation 2 for 3.0mg/mL and 1.75mg/mL collagen gels

Trial		1	2	3	4	5	Average	Stdev	CV
3.0 mg/mL	E (MPa)	0.297	0.201	0.263	0.360	0.243	0.273	0.060	22%
	k	0.027	0.056	0.048	0.019	0.045	0.039	0.016	40%
1.75 mg/mL	E (MPa)	0.00343	0.00054	0.00341	0.00052	0.00348	0.002	0.002	70%
	k	1.249	4.111	1.346	4.507	1.395	2.522	1.639	65%

It is important to note that despite the good fit of model to the data, the stiffness values generated are very inconsistent. The coefficient of variation for 3.0mg/mL is 22% and for the 1.75mg/mL is 75% which are both significantly higher than the generally accepted 105%, indicating inconsistency between trials. The reason for the inconsistency in the E and k values is due to the solving of this equation. Both variables were solved at the same time using MATLAB. Because of this sometimes a better fit was achieved by having a high k and low E and other times the better fit was achieved by solving with a low k and high E.

6.5 Membrane Deflection and Bending Stiffness Equation

The third equation used to analyze stiffness was the membrane deflection and bending stiffness equation. This equation is made up of the membrane deflection component, bending component, and a tare load. The purpose of this equation is to account for both membrane deflection as well bending stiffness of the collagen gel. Equation (3) can be seen below:

$$P = Eah\left(\frac{\delta}{f(v)a}\right)^3 + \frac{4\pi\delta Eh^3}{3(1-v^2)a^2} + Fo \qquad (3)$$

A representative plot of the measured data and fitted data along with residuals can be seen below for both collagen concentrations (Figure 23).
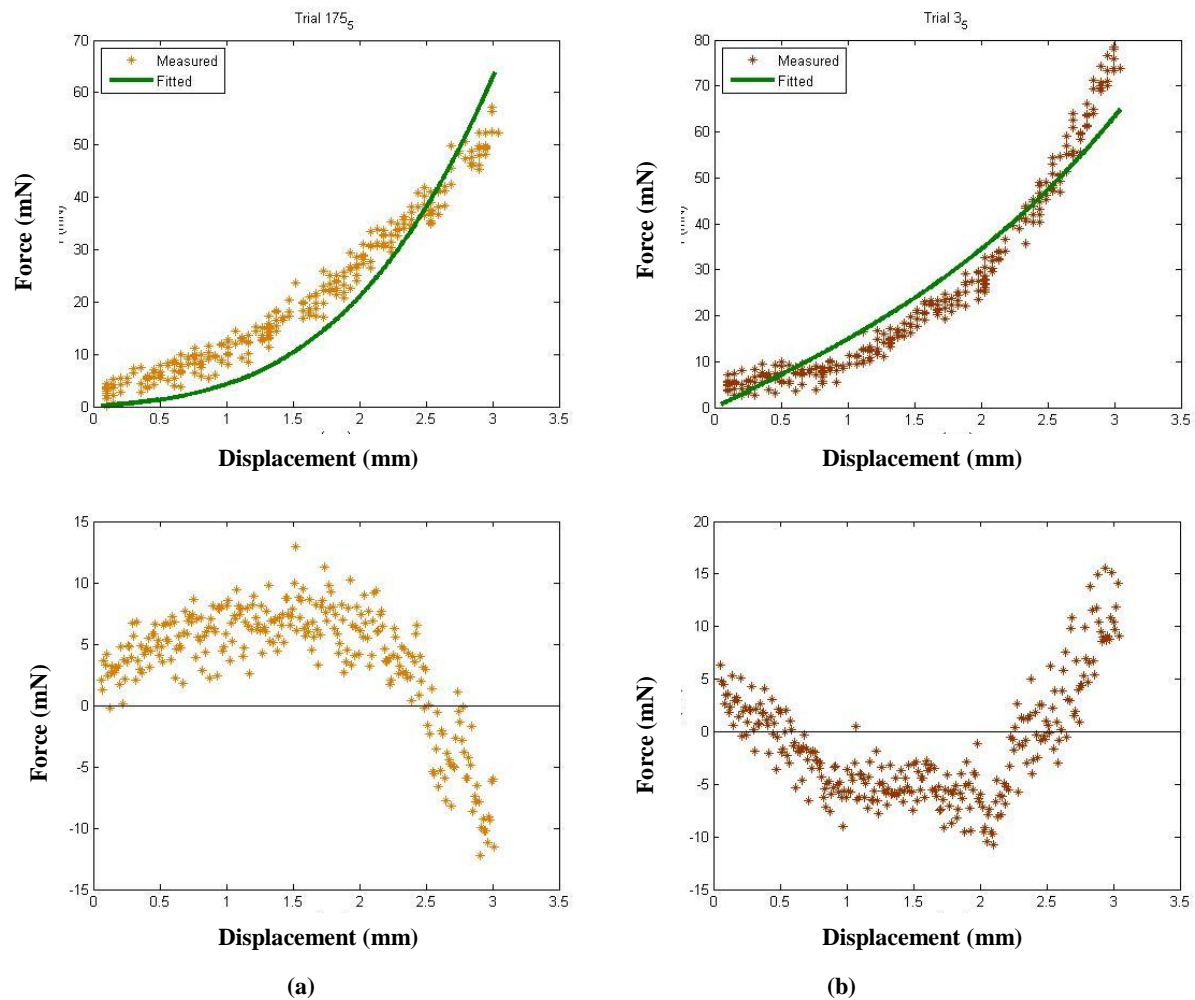
Figure 23: Collected data of collagen concentration of (a) 1.75mg/ml (b) 3.00mg/ml fitted with equation (5) and residual plots below. (a) has a R^2 value of 0.984 and a RMS value of 2.43mN while (b) has a R^2 value of 0.984 and a RMS value of 2.18mN

In observing the fit of the model to the measured data, in both cases it is apparent that the model is not a good fit for the data. There is systematic error for both concentrations, as can be seen by the plots of the residuals. The poor fit is also supported by the RMS values which can be seen below in Table 18.

Table 18: R^2 and RMS values for Equation 3 for both 3.0mg/mL and 1.75mg/mL collagen concentrations

Trial		1	2	3	4	5	Average
3.0mg/mL	R^2	0.964	0.983	0.982	0.972	0.987	0.978
	RMS (mN)	4.85	3.17	3.96	5.56	3.38	4.18
1.75mg/mL	R^2	0.971	0.983	0.977	0.966	0.977	0.975
	RMS (mN)	5.09	4.30	5.66	5.98	6.07	5.42

Despite this poor fit, there is however a low coefficient of variation among trials as seen below in Table 19.

Table 19: Stiffness values determined using Equation 3 for 3.0mg/mL and 1.75mg/mL collagen gels

Trial		1	2	3	4	5	Average	Stdev	CV
3.0 mg/mL	E (MPa)	1.15	1.14	1.32	1.24	1.18	1.21	0.07	6.19%
1.75 mg/mL	E (MPa)	1.16	1.12	1.31	1.22	1.41	1.24	0.12	9.42%

While the stiffness values among trials are consistent (less than ten percent variation) they do not make sense physically. The average stiffness value for each of the concentrations is approximately 1.2MPa and they should not be the same for the two concentrations. If the two gels were of the same stiffness they would produce the same force-displacement curve and they do not. The 3.0mg/mL concentration has a force of 80mN for a displacement of 3mm while the 1.75mg/mL concentration has a force of 60mN for the same displacement.

6.6 Selected Equation

The equation selected for use in this project was Equation 1, the membrane deflection equation. This equation provided a low coefficient of variation between the determined stiffness values among trials. The determined stiffness values for the second equation had a coefficient of variation among trials which was too high to allow that equation to be applied. Additionally, while Equation 3 had a low coefficient of variation among trials, the stiffness values were the same for both concentrations of collagen which should not be the case. Because of the aforementioned reasons, Equation 1 was selected for use. It is important to note that it is believed that there is indentation component to the equation but more research needs to be conducted to determine what this term is and how it changes as the gel is displaced and becomes thinner.

6.7   Comparison to Literature

In addition to comparing stiffness values among trials, it was also important to compare the stiffness values derived from testing to those found in literature. One past study on three-dimensional type I collagen extracellular was by Roeder et al., 2002 in which uniaxial mechanical testing on collagen gels was performed. In this testing collagen gel concentrations of 1.0mg/ml, 2.0mg/ml, and 3.0mg/ml were tested. The corresponding linear moduli were 10.7kPa, 16.6kPa, and 24.3kPa. The stiffness values in this trial were both higher than this range, 470kPa and 1.41MPa however this difference can be attributed to the gels in this studied being populated with human fibroblasts while the previously mentioned study used acellular gels which were not able to remodel. Another study conducted by Wakatsuki et al. (2000) found the stiffness of fibroblast-populated matrices to be 0.8 MPa. This value is within the range of the values that were found in this trial. These values from literature show that stiffness values found in this study are within a reasonable range when compared to similar studies. The variations are most likely due to different methods of testing and how the test samples are prepared.

6.8   Noise Testing

In order to determine the error associated with noise of the data gathered from the force transducer noise testing was completed. Noise testing was performed both on the bench top and on the air table (where testing was conducted for the collagen gels). Noise data was recorded both while the actuator was moving and while the device was still at both locations. The data was recorded for a period of fifty seconds at each of the locations at a sampling frequency of 1000Hz, resulting in 50,000 data points. For this testing the device was completely set up however no sample was used. The testing was completed in air.

Noise testing was completed using a Fast Fourier Transform or FFT was also performed on the data. The purpose of an FFT is to detect frequencies of noise which can be filtered out of the data. Generally, a FFT will produce a distinct peak, indicating the frequencies which should be filtered out. When an FFT is performed in MATLAB, the program will display a plot of different frequencies and also display how often each frequency appears. FFT's were performed on data

collected in four different testing conditions. First, noise testing was completed with the actuator moving on both the air table and bench top. FFT's for this testing can be seen below in Figure 24.
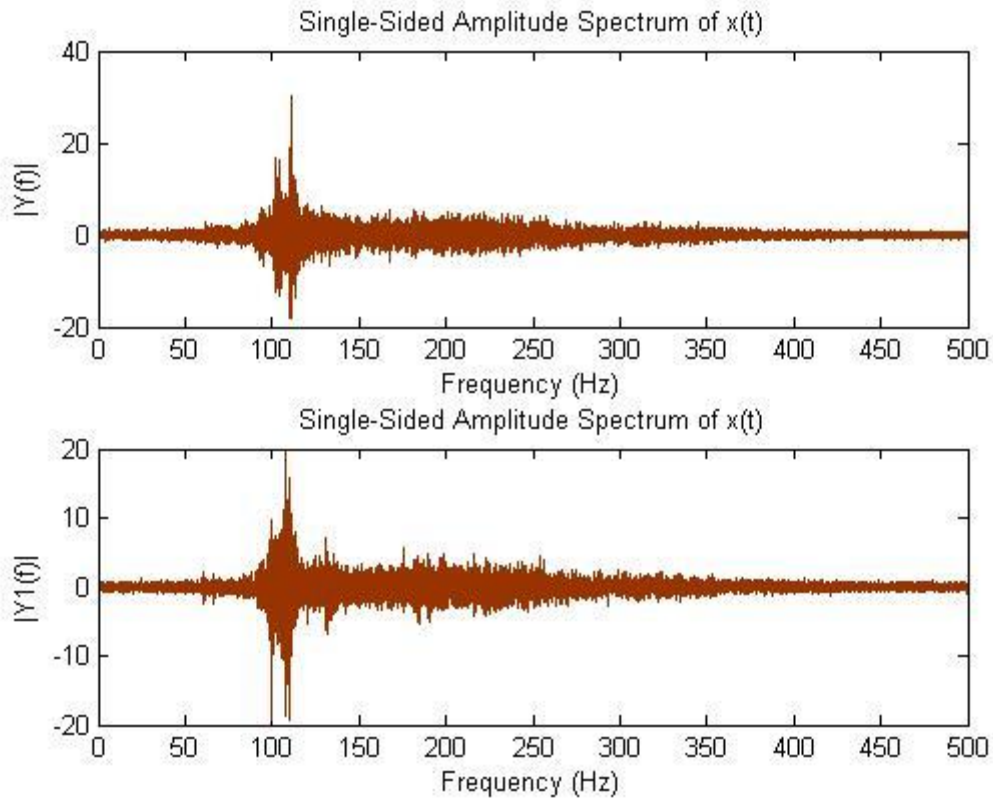
Figure 24: FFT for force transducer moving; Top: testing performed on air table; Bottom: Testing performed on bench top

For the moving force transducer on the air table there are two distinct peaks at frequency of 100Hz and 110Hz. There is no difference in the location of the peaks between testing surfaces however the magnitude of the peak frequency is increased for the air table.

The second set of noise testing was completed with the actuator not moving. This testing had the same setup as the testing completed with the actuator moving. The FFT for the second set of testing can be seen below in Figure 25.
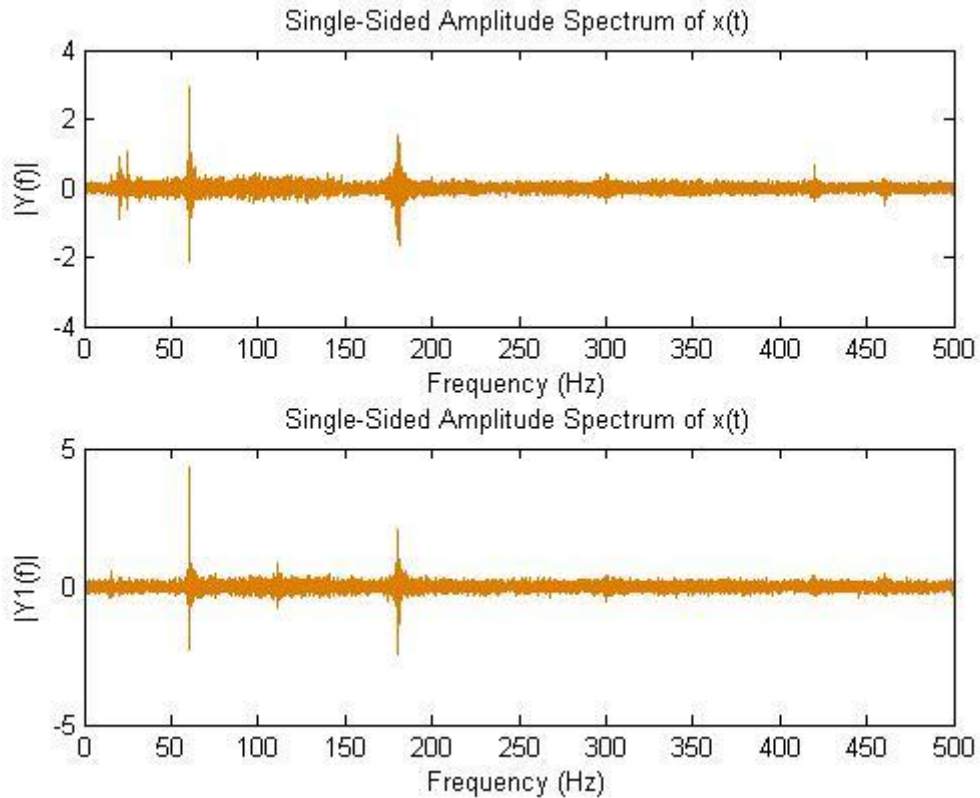
Figure 25: FFT for force transducer not moving; Top: testing performed on air table; Bottom: Testing performed on bench top

As compared to the transducer moving, the peaks are in different locations. This is because these peaks are a result of electrical noise rather than mechanical noise from the actuator. These peaks appear at 60Hz and every odd harmonic after that, representative of electrical noise. It should also be noted scale of the FFT graphs as for the moving data the range is from about -20 to 20 while for the still data the range is from about -2 to 4.

Based on the results of the FFT, filtering could be done post-data collection to filter out some of the noise present in the data which is a result of the actuator movement. To do so a band-stop filter would be used which would filter out frequencies of 100-110Hz.

7   Future Work

7.1   Varying Stiffness

The work completed in the project regarding the quantification of stiffness of circularly clamped gels provides an accurate and reliable means to measure gel stiffness. To build on this foundation, one of the next steps is to apply the device to testing samples in which stiffness has been varied by different methods. Stiffness is generally varied through one of two ways, either biochemically or mechanically. Gel stiffness can be altered through both chemical and physical crosslinking and by changing boundary conditions. Because crosslinking often results in a change in the structure of the collagen or cytotoxic byproducts, it is desirable to study the effects of purely mechanical changes on the cell environment. There are several different methods which currently exist for controlling stiffness mechanically. Methods used in previous studies include a free floating model, a restrained (fixed) model and a method which used weights to provide a variable boundary condition (Frederick Grinnell, 2000; Knezevic, Sim, Borg, & Holmes, 2002).

7.2   Methods for Mechanically Varying Stiffness

In the floating model, the collagen gel is released from its boundaries immediately following its polymerization. This immediate release prevents the matrix from developing tension during culture (Grinnell, 2000). Additionally, cells do not form stress fibers and mechanical properties of the gel soon diminish as the cells become apoptotic in this environment (Grinnell, 2000). The floating model results in the most compliant gels of the different models. This high gel compliance, along with the lack of stress fibers, contributes to an undetectable effect of TGF-β on fibroblasts. Because of the lack of mechanical stiffness and the ineffectiveness of TGF-β, the amount of differentiation seen in these highly complaint matrices is minimal (Arora, Narani, & McCulloch, 1999).

In the fixed, or restrained, model the collagen matrix is not removed from its boundaries after polymerization and tension is developed in the gel. This is the application which was used in this project with the circularly clamped collagen gels. This tension causes the collagen fibers to remodel and alter the structure of the gel. This isometric tension along with the formation of stress fibers and large cellular extensions is observed in all these fixed gels (Frederick Grinnell, 2000). The matrices under this tension have been shown to provide an environment in which TGF-β can be successfully used to induce fibroblast differentiation (Arora et al., 1999). It is important to note that once these fixed gels are released from their boundaries, the cells become apoptotic as they do in the free model (Frederick Grinnell, 2000). Tension is not only critical to the survival of the cells, as other studies have shown that mechanical tension is a crucial element in the differentiation of fibroblast cells (Garrett et al., 2004).

"Free" and "fixed" models make it possible to successfully investigate two extreme boundary conditions, which result in two very different phenotypes of cells. Because it has been shown that stiffness has a significant effect on myofibroblast differentiation, it is important to investigate the effects of a range of different stiffness beyond the free and fixed states. In order to obtain boundary stiffness levels that are between that of the free and fixed models, a method was developed in which different size weights were attached to the boundaries of gels to create a compliant boundary that provided varying degrees of resistance to the contractive forces caused by the collagen remodeling. This isotonic boundary model created inconsistencies in gel formation and did not allow for any method to determine the magnitude of the mechanical forces as seen by the cells. Also, this study did not investigate the relationship between boundary stiffness and fibroblast differentiation (Knezevic et al., 2002).
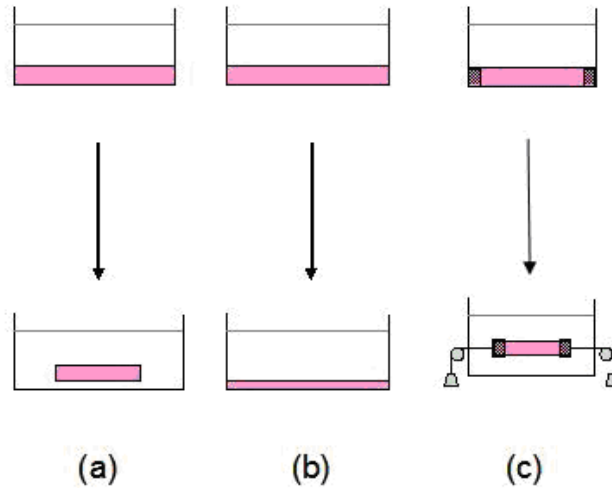
Figure 26: A depiction of the different types of collagen matrices.
(a) Free Floating (b) Fixed (c) Isotonic Boundary (Billiar)

Another investigation of intermediate stiffness values includes a study performed by Freyman et al (2002). In this study the effect of stiffness on contractile force is examined. In the experiment a gel seeded with fibroblasts was clamped at one end and attached to a compliant beam on the other end. Beams of different geometries were used to achieve different levels of stiffness and the total force, force of the beam and matrix, was measured. It was shown that an increase in stiffness decreased both displacement and rate of displacement. This study concluded that the forces per cell, as well as the rate at which these forces were generated, were not affected by stiffness. This is an important conclusion as it shows the effect of varying stiffness on forces generated by cells. However, this study fails to address completely the effects of stiffness on the cells. This research does not provide values of stiffness the cells are experiencing but rather quantifies a force per cell. The level of stiffness seen by cells is an important piece of information as it provides insight regarding how cells respond to varying stiffness.

7.3   A New Approach to Modulating Stiffness

In order to quantify the effects of varying stiffness without changing the biochemical environment, the Boundary Controlled Stiffness Device (BCSD), a novel device developed by Dr. Kristen Billiar of Worcester Polytechnic Institute, was built. The BCSD makes it possible to

study the effects of intermediate boundary stiffness by changing only mechanical properties. The device uses compliant springs, attached to edges of a gel to vary boundary stiffness. Springs of varying diameters are used to change the amount of stiffness. The ability to tailor stiffness is essential in order to develop biomaterials which meet specific needs *in vivo*. By developing a gel with a stiffness that causes the correct amount of differentiation of myofibroblasts it is possible to develop tissues which can avoid fibrocontractive diseases. Of additional importance is the fact that this novel device varies stiffness only by mechanical means.

With the development of the BCSD it is possible to measure the effects of boundary stiffness through image analysis. Beam stiffness can be calculating by measuring the force generated by given displacements. To do so, the beams are connected to a clamp and laid on a scale. The end of the beam connected to the clamp is then displaced incrementally to given displacements. Force versus displacement is then plotted and the slope of the line of best fit is the stiffness of the beam. By measuring the change in location of the springs based on the compaction of the gel it is possible to determine the force at which the cells remodeled. While this is valuable information, it is not the only information needed; also important is the stiffness of the gel. The stiffness the cells experience is a result of both boundary stiffness and gel stiffness.

7.4   Compatibility with the BCSD

The device produced in this project is unique as it was designed to also be compatible with the BCSD. To demonstrate that the device could be used in conjunction with the BCSD preliminary testing was conducted. Gels were poured at a concentration of 3.0mg/mL and populated with fibroblasts at a concentration of 0.5million cells/mL. One gel was poured in the BCSD with a compliant spring setup while another was poured with a stiff spring setup.

The first set of graphs shown below Figure 27, are plots of the raw data that was gathered by the device. Using only this data, there appears to be too much noise to make any conclusions about the differences between the two gels. This is due to the forces being lower than was expected based on the preliminary and circularly clamped gel testing.
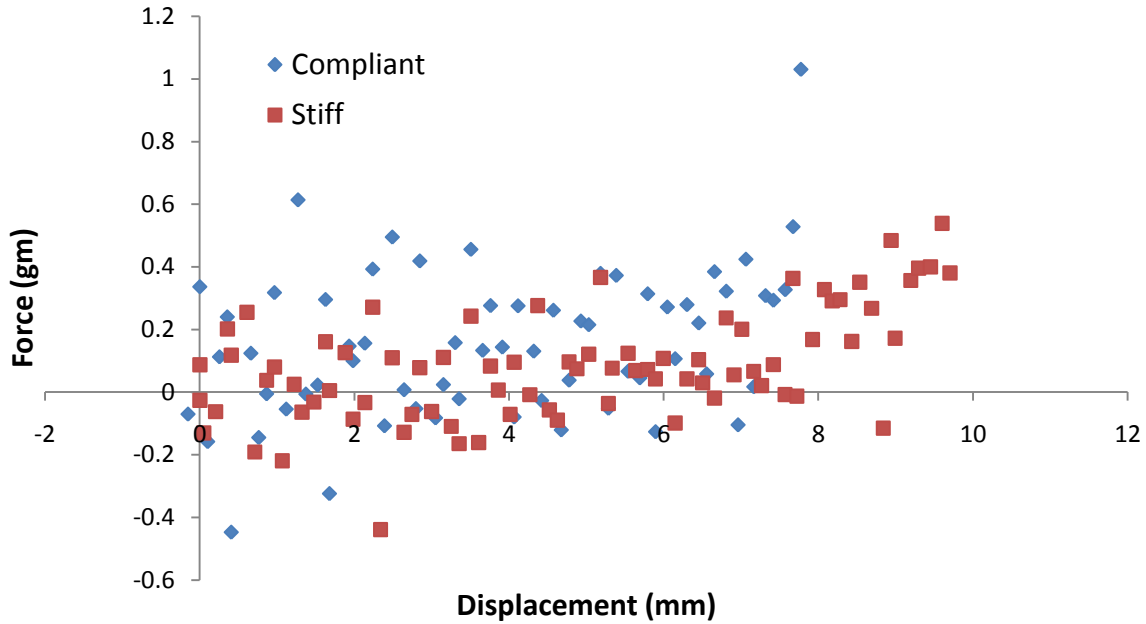
Figure 27: Raw data collected from BCSD testing; Note the large amount of noise present in the data

There is a small difference in the measured forces which can be seen for the two setups; the compliant setup has slightly higher forces. Overall however, the data is rather inconclusive. It is important to note that testing conditions may have affected the force-displacement curves for the samples. Both samples were removed from media for a period of time and then resubmerged. Additionally, one of the four springs in the stiff setup became disconnected from the sample and it had to be re-glued which may have affected testing. Furthermore, it is believed that in order to evaluate this data many other variables need to be taken into account which are not in the force-displacement curve. These include sample thickness, buoyancy, and amount of compaction which has occurred. Additionally, another important concern with testing using the BCSD comes from the notion of understanding the environment in which the gels are tested. Specifically, whether those gels are tested while submerged or tested while in the air. With air testing, the gel is taken out of its growth environment and there is a chance that the gels could dry out during the test period. However, if the gels were to remain submerged the existence of buoyant forces may affect the measured forces during the test.

8   Conclusions

The main outcome of this project was validation of the device. Two sets of testing were completed to validate the device: testing with PDMS and testing with circularly clamped collagen gels. The purpose of the PDMS testing was to validate the device against literature. Additionally, since PDMS is an elastic polymer, it is easier to work with than a biological substance for a first round of validation testing. The results of the PDMS testing show a high correlation between the measured data and data collected by Scott et al. (2004). Establishing this correlation was an important step in the development of the device as it showed that the device was able to output similar results to previously conducted research on transverse loading.

Following the PDMS testing, transverse loading on circularly clamped collagen gels was performed. Circularly clamped collagen gels were used because they are commonly used and it was necessary to show the device could be used in conjunction with this medium as the device will ultimately be used to test soft biological substances. Collagen gels of three different concentrations were tested in order to mimic different stiffness values of gels that could be encountered in the lab. It was determined that the lowest concentration of collagen gels (0.5mg/mL of collagen) could not be reliably tested as they were very fragile and prone to breaking upon handling. As this concentration is very low compared to the concentrations which are generally used, trials were not repeated to try and obtain more data on this concentration. However, data was successfully collected on the 1.75mg/mL and 3.0mg/mL concentration.

One of the main points which was important to illustrate was that the device produced repeatable results. To ensure this, the 6 samples at the same concentration within each round of testing were compared. To compare these samples, the force values at 6 different displacements (0.5, 1.0, 1.5, 2.0, 2.5, 3.0) were compared. This coefficient of variation was approximately 10% for most samples, demonstrating repeatability.

In addition to repeatability, it was also important to establish that the device could measure the stiffness of gels of varying stiffness. To determine the stiffness of the samples, the measured data of each of the samples was fitted to a model in MATLAB. As compared to literature the stiffness values were higher for the measured data. However, the stiffness values

determined by fitting Equation 1 to the data had a very low coefficient of variation between trials for both concentrations.

9   Recommendations

Although this project accomplished the goal of creating a device capable of determining the stiffness of collagen gels while meeting specifications, it is believed that this device has the potential to be used in an even greater manner. Due to constraints (time, money, scope, etc.) on this particular project, it was not possible to fully exploit the capabilities of this device and concept. With more time, more thorough and extensive testing with the BCSD is recommended. While the project did prove that the device is compatible in both theory and practice with the BCSD, testing did not go beyond proof of concept. Future testing could be done with the BCSD to determine the effect of boundary conditions on a number of different variables with the gels, including but not limited to cell differentiation, cell viability, and gel remodeling. The data could be collected with our device and then using this information along with an FEM model, the stiffness of the more intricately shaped BCSD gels could be determined. This project began to demonstrate how FE models could be used to accomplish this, but did not apply this theory to the BCSD.

Also, for future testing it is recommended that this device be validated for use with other similar materials such as fibrin gels, another commonly used wound healing model. This could be done easily but was not done in this project due to time constraints and the scope of the project.

In the future, changes could be made to this device in order to better suit it for different uses. Because of the ability of this device to accurately measure both force and displacement, it could be used for a variety of different applications other than gel stiffness. In order to accommodate this, simple changes could be made to the device. In this project, low strains on the gel were achieved due to the shape and size of the indenter tip. This tip, however, can be easily changed and different kinds of tips could be used for future applications.

Also, the actuator used in this project was thought to be the main source of the noise in the data. While this noise was found to be acceptable for this project, this might not hold true for future testing or applications. To counteract this, a less noisy, probably more expensive, actuator could be used. Where a stepper motor was used in this project because of its accuracy, cost and integration with available equipment, perhaps a less noisy type of actuator, such as a servo

motor, could be used to replace it. This would hopefully help to decrease the noise that is inherent with the current setup. Also to help reduce the noise level, the bracket holding the linear actuator and linear encoder should be remade out of aluminum or a similar metal, rather than the current acrylic material. This along with the addition of a rubber gasket between the actuator and bracket should help reduce the noise levels. A picture of how this would look can be seen in Figure 28 below.
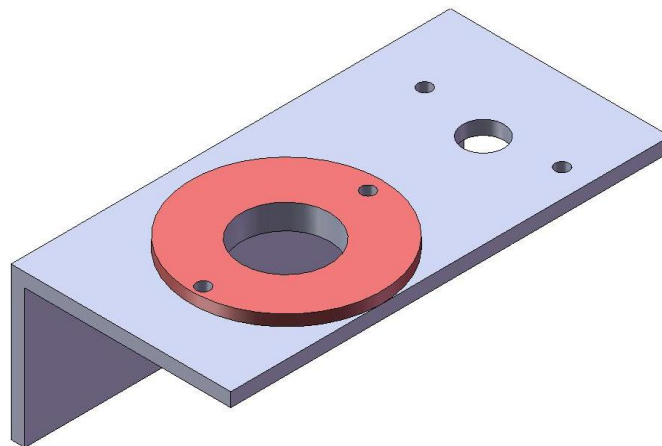
Figure 28: Modified bracket with rubber gasket

The compact size of the device gives it the ability to be easily moved and transported within the lab. This size could also allow it to be used within a sterile environment, which would create the potential for a whole new type of testing. Currently the device is not meant to be sterile, but this could be changed with little effort or a slight redesign. Those using this device for future applications may wish to consider their need to sterility and how they can integrate this device in a sterile environment.

Another addition that should be made to the device in order to aid in the BCSD testing process is an attached water bath for the BCSD to sit in. This would allow of the removal of the gel from the Petri dish that it is cultured in, with minimal disturbance. Also, the gel could then be tested in a controlled environment were the material properties would not change significantly. A concept of this additional water bath can be seen in Figure 29.
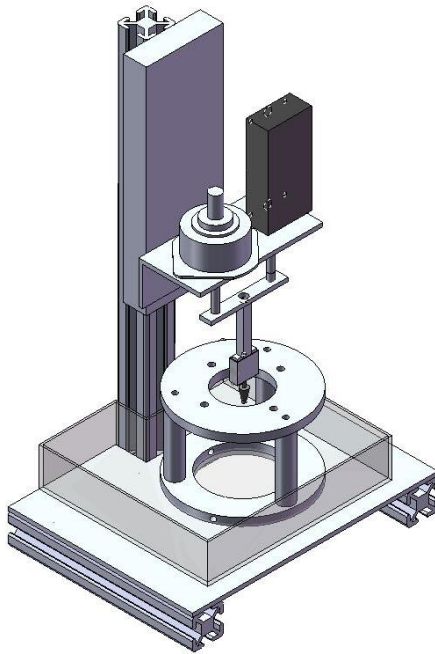
Figure 29: Device with additional water bath

In future testing, more research should be conducted in order to find an equation that better fits the complex transverse loading method done in these experiments. Initially, the membrane deflection equation was used as our first approximation of the stiffness of the gel sample. This model fit the data relatively well, but in order to obtain an even better fit, the Hertz indentation part of the equation was added. This addition to the equation, however, introduces some new issues to the validity of the equation. The Hertz component of the equation is based on the assumption that only indentation and not transverse deflection is taking place. Because of this, the Hertz equation assumes the stiffness value to be constant. This constant displacement and stiffness value does not hold true when the sample is being stretched. This is due to the changes in density and thickness of the sample during the stretching caused by transverse deflection. In simple terms, the Hertz component of the equation is not able to adapt to the changes in the sample during transverse deflection; the indenter tip does not deflect nearly as far into the gel once it has been significantly deflected as it would if the gel was not being deflected. This leads to a complex situation where the stretching value of stiffness, the indentation depth and indentation stiffness would all be changing at the same time. The goal of this project was to quantify stiffness, but in order to do so at as high a level as just previously mentioned is far

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beyond the scope of this project. This project was designed in order to provide a first level approximation of stiffness, but in order to obtain the best model possible, these factors should be taken into account for future testing.

Appendix I   Jell-O Stiffness Testing Protocol

Materials:

- Rectangular foam anchors with inner dimensions of 2cm X 0.5cm and outer dimensions of 2.4cm X 0.7cm
- Jell-O
- Paperclips
- Suture

Methods:

1. Cut foam anchor into rectangles with inner dimensions of 2cm X 0.5cm and outer dimensions of 2.4cm X 0.7cm
2. Lay foam anchors in the bottom of flat Tupperware containters.
3. Mix Jell-O using two Jell-O packages in 1¼ cups of boiling water. Pour Jell-O in containers to a height equal to that of the foam anchors.
4. Allow Jell-O to sit for four hours.
5. Cut around foam anchors and remove excess Jell-O.
6. Remove foam anchors with Jell-O inside.
7. Tape two binder clips to the bottom of two plastic beakers
8. Clip the foam anchor containing the Jell-O in-between the binder clips to achieve the setup shown below.

Figure 30: Setup for Jell-O mock testing

9. Guide suture between the collagen gel and foam anchor on one side of the sample and then loop it around the collagen gel and back in-between the foam anchor and gel being careful not to rip the gel.

10. Use a small piece of packaging tape to tape the two ends of the suture together. Incrementally add small paperclips to the suture until the gel fails. Record the number of paperclips added.
11. Repeat steps 8-10 for each gel.

Appendix II Collagen Gel Stiffness Testing Protocol

Materials:

- PDMS with 1cm X 2.5cm rectangular wells
- Pins lab device to clamp PDMS
- #10-32 X ¾" stainless steel screws
- Rectangular foam anchors with inner dimensions of 2cm X 0.5cm and outer dimenstions of 2.4cm X 0.7cm
- Collagen gels
- ¾" kidney wire
- Suture

Methods:

1. Prepare PDMS wells by making 4 rectangular pieces of PDMS and cutting out four 1cm X 2.5cm wells in two pieces.
2. Autoclave PDMS molds and Pins' Lab device to clamp PDMS
3. Sterilize the screws and foam anchors using isopropyl alcohol, then wash three times with PBS with each wash lasting 5 minutes.
4. Assemble the device by inserting into the device base the flat layer of PDMS, the PDMS with wells, the foam anchors and then the device lid.

Figure 31: Assembled device

5. Prepare collagen gels according to Appendix V.
6. Pipette collagen gel into wells placing 0.5mL in each well.
7. Allow gels to compact in the incubator for three days.
8. Remove screws and lid from the device. Take out the two layers of PDMS and peel off the top layer. Cut along the sides of the gel lengthwise to separate it from the foam anchor.
9. Tape two binder clips to the bottom of two plastic beakers. Tape a one-inch square piece of paper to one of the beakers to act as a reference point to measure displacement.

10. Clip the foam anchor containing the gel in-between the binder clips to achieve the setup shown below.

Figure 32: Setup for collagen gel testing

11. Setup the camera on a tripod and connect it to remote capture on the computer screen.
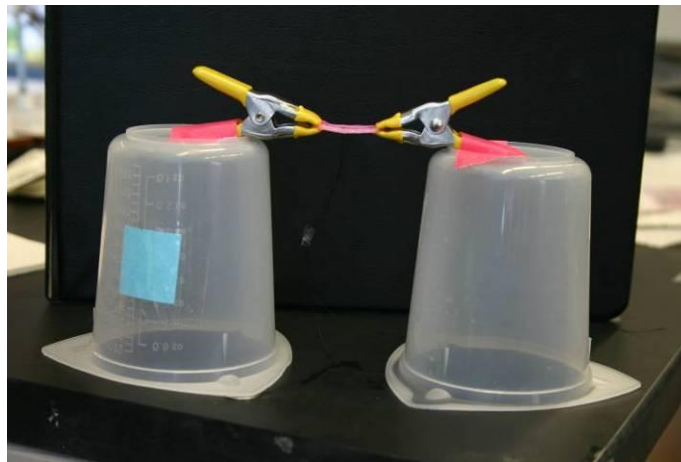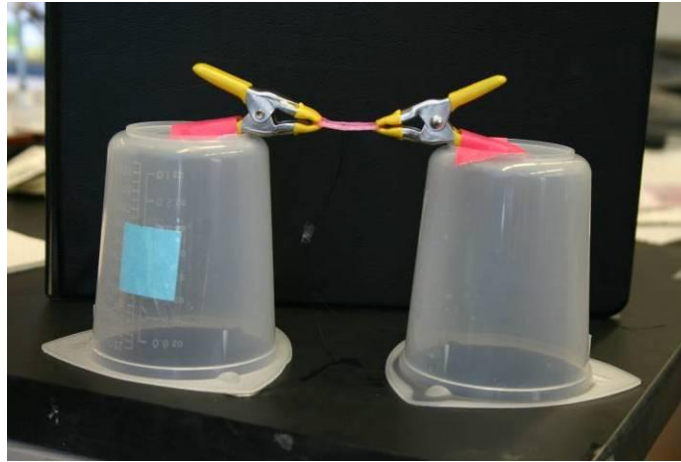12. Guide suture between the collagen gel and foam anchor on one side of the sample and then loop it around the collagen gel and back in-between the foam anchor and gel being careful not to rip the gel.
13. Use a small piece of packaging tape to tape the two ends of the suture together. Incrementally add pieces of ¾" wire to the suture and take a picture after adding each weight as seen below.
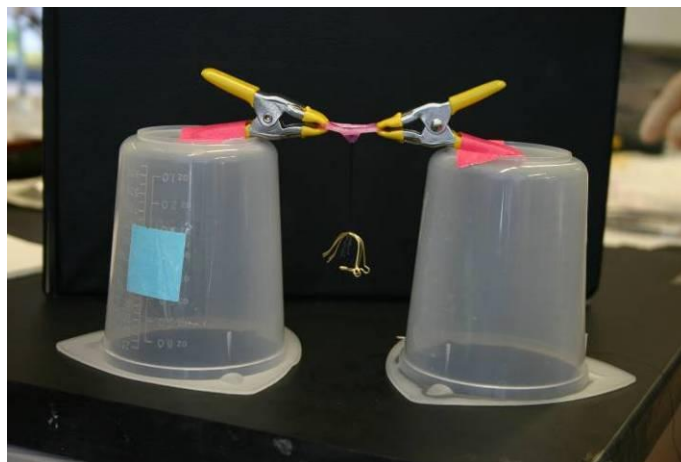
Figure 33: Collagen gel with weight added to create displacement of the gel

14. Repeat steps 10-13 for each gel.

15. Analyze results using ImageJ to determine the displacement of the each gel after each weight is added.
16. Calculate the stiffness of each gel based on ImageJ analysis.

Appendix III Latex Mock Testing Protocol

Materials:

- Sensotec 50g force transducer
- NI signal conditioning box
- 10V strain guage
- Billiar lab linear actuator
- Metal rod (1.55mm in diameter)
- Latex glove
- Plastic beaker
- Packaging tape and scotch tape
- Dell Optiplex GX280 with Microsoft Windows XP Professional Edition and National Instruments' LabVIEW v 7.1

Methods: Testing Protocol

1. Connect the force transducer to the signal conditioning box/strain gauge, set up LabVIEW by loading the appropriate .vi so the computer recognizes the additional strain gauge in the signal conditioning box.

Table 20: Wire Connections, Functions, and Colors

| Wire Color	Function	Connected to
Red	+ Excitation	**14 of signal conditioning box**
Black	- Excitation	**3 of signal conditioning box**
Green	- Output	**5 of strain gauge**
White	+ Output	**6 of strain gauge**

Calibrate the force transducer (see

Methods: Calibrating 50g Force **Transducer**)

2. Secure the force transducer in the holding device on the linear actuator. The tip of the force transducer should have the flat face oriented down.
3. Prepare the mock latex sample. Cut out the largest possible portion of a latex glove, secure glove in place over the mouth of the plastic beaker with tape.

4. Insert the indenter tip (metal rod) into the hole in the bottom of the force transducer. To ensure the tip does not get lodged within the force transducer, also insert a small piece of metal rod of the same diameter into the hole perpendicular to the rod and tape into place.
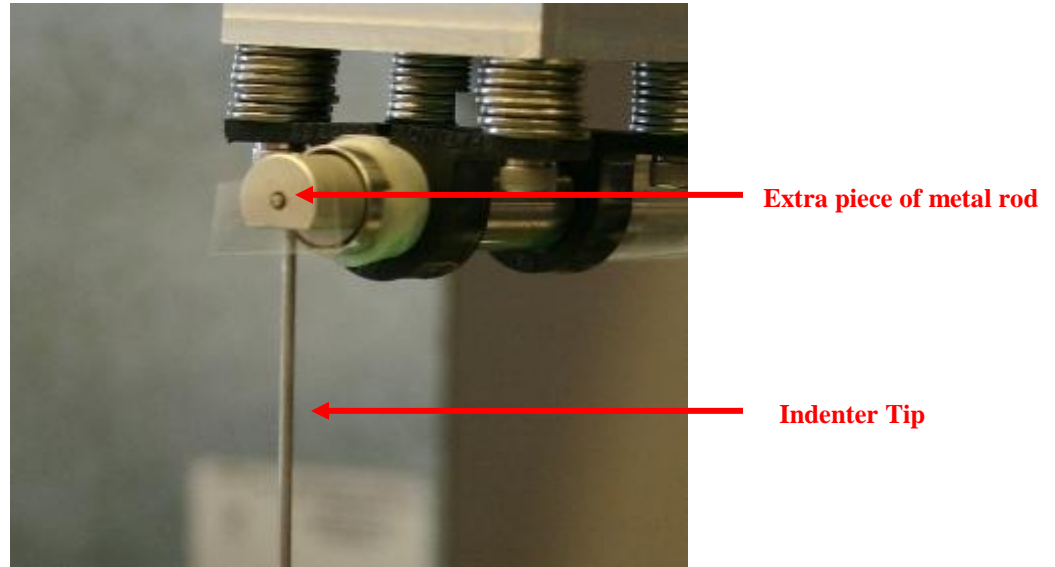
Figure 34: Setup of Force Transducer and Indenter Tip
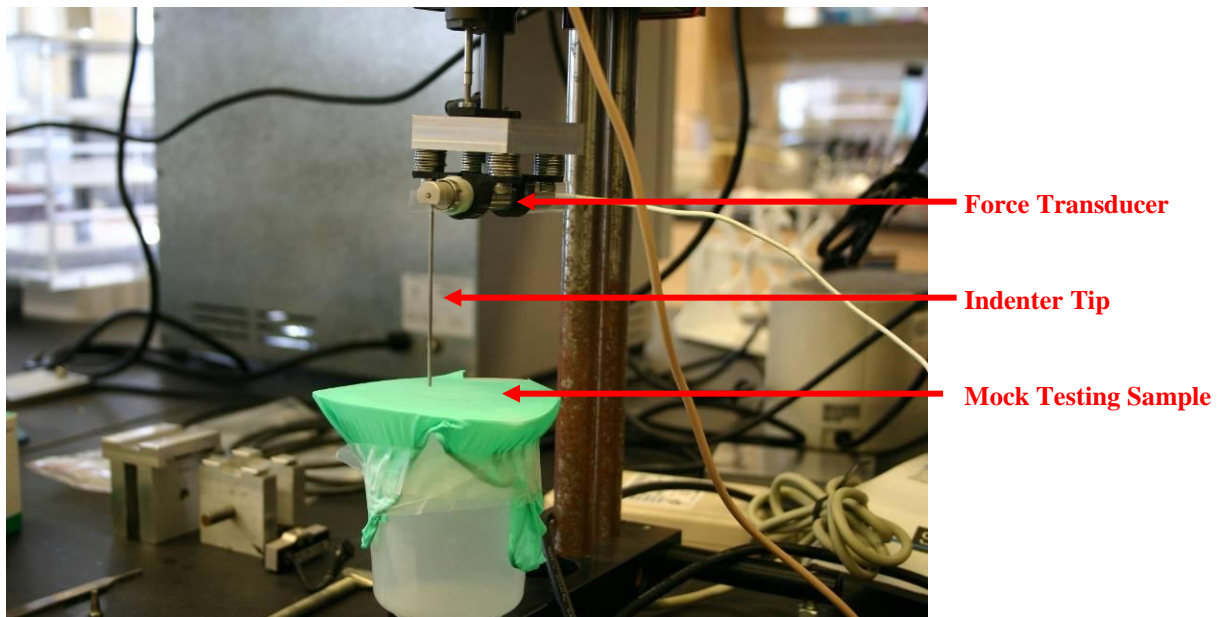
Figure 35: Mock Testing Setup

5. Open the uniaxial test program from the folder on the desktop called 'Lynn's Program'.

6. Select a Base Path of: Documents and Settings → All Users → Shared Documents → Lab Notebooks → SGS MQP. Select the testing folder as the location to save the data. Name the test 'Test 1'.
7. Ensure that there is at least 0.5 inches of clearance between the indenter tip and the mock test sample.
8. Start the actuator, set it to move down, and start recording with LabVIEW.
9. Allow the force to approach 50g, then turn off the actuator, reverse the direction, and turn the actuator back on to remove the tip from the sample.
10. Repeat steps 7-9 for all samples, changing the name of the test each time to avoid overwriting any of the previous tests' data (e.g. Test 1, Test 2, etc..).
Note: For mock testing trials, SGS tested different areas of the latex for each test (i.e. different areas of the mock material were tested)

Methods: Calibrating 50g Force Transducer

1. Secure the force transducer in the holding device on the linear actuator. The tip of the force transducer should have ⌣ flat face oriented up.
2. Open the LabVIEW program called 'Test' which simply outputs a reading of the force on the transducer.
3. Record the force when no weight is added to the transducer.
4. Weigh four paperclips and add the paperclips to the force transducer, recording the force reading after the addition of each paperclip.
5. Graph the weight and force measurements with the force reading on the x-axis and the weight reading on the y-axis. Fit a linear trend line to the data as seen below in Figure 36.
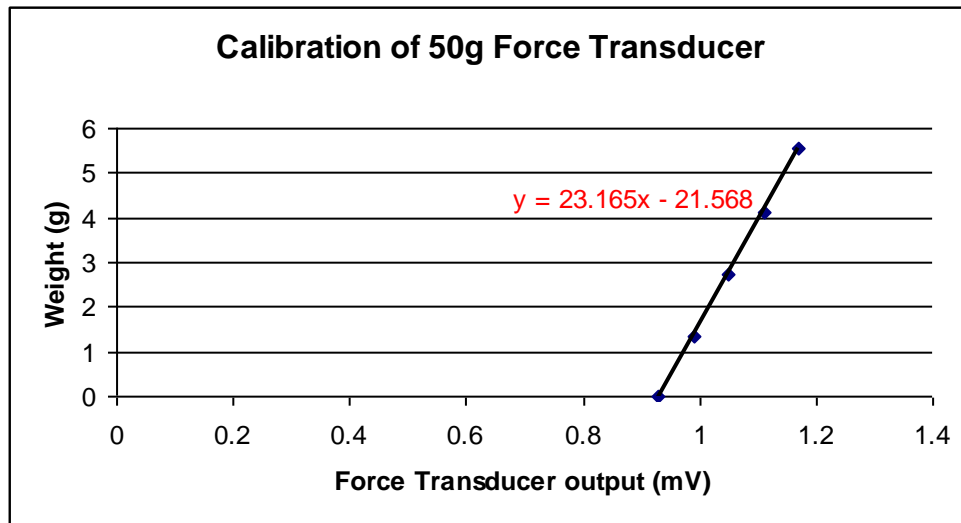

Figure 36: Graph used to determine calibration factor for force transducer

6. Modify the uniaxial test program so that the multiplication factor is the slope of the line multiplied by 1000. Modify the offset factor so that it is equal to the y-intercept.
7. Your Force Transducer should now be calibrated.

Appendix IV Circular Jell-O Mock Testing Protocol

Materials:

- Sensotec 50g force transducer
- NI signal conditioning box
- 10V strain guage
- Billiar lab linear actuator
- Metal rod (1.55mm in diameter)
- $\frac{1}{16}$" porous polypropylene
- 3 large plastic beakers
- 3 binder clips
- Scalpel
- Packaging tape and scotch tape
- Dell Optiplex GX280 with Microsoft Windows XP Professional Edition and National Instruments' LabVIEW v 7.1

Methods: Testing Preparation

1. Cut circular foam anchors from $\frac{1}{16}$" porous polypropylene diameter to the dimensions found in the table below:

Table 21: Foam Anchor Dimensions and Sizes

Number Cut	Size	Inner Diameter	Outer Diameter
6	Large	55 mm	**70 mm**
8	Medium	33 mm	**55 mm**
4	**Small**	**20 mm**	**28 mm**

2. Divide the foam anchors into two testing groups.
3. Lay the foam anchors out in the bottom of flat Tupperware containers. Pour Jell-O into the containers to a depth approximately equal to that of the height of the foam anchors. For half of the foam anchors use red Jell-O at a concentration of 3 packets/cup boiling water. For the other half of the foam anchors use blue Jell-O at a concentration of 2 packets/cup boiling water. Allow Jell-O to sit in fridge for at least 4 hours.
4. Carefully cut around the foam anchors using a scalpel and remove excess Jell-O. Carefully remove the Jell-O samples from the containers.
5. Invert 3 large plastic beakers and tape a binder clip to the bottom rim of each beaker.
6. Enough samples have been prepared to do a test run with a medium sized blue and red sample. To complete the test run load the sample by attaching the three binder clips equidistantly around the border of the foam ring. Be careful not grip the Jell-O as that will rip the sample.
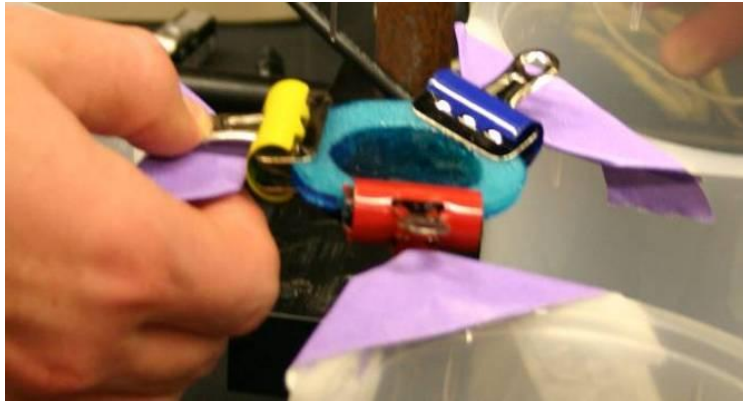
Figure 37: Mounting a sample with three binder clips

Methods: Testing Protocol

1. Connect the force transducer to the signal conditioning box/strain gauge, set up LabVIEW by loading the appropriate .vi so the computer recognizes the additional strain gauge in the signal conditioning box.

Table 22: Wire Connections, Functions, and Colors

Wire Color	Function	Connected to
Red	+ Excitation	**14 of signal conditioning box**
Black	- Excitation	**3 of signal conditioning box**
Green	- Output	**5 of strain gauge**
White	**+ Output**	**6 of strain gauge**

2. Secure the force transducer in the holding device on the linear actuator. The tip of the force transducer should have the flat face oriented down.
3. Insert the indenter tip (metal rod) into the hole in the bottom of the force transducer. To ensure the tip does not get lodged within the force transducer, also insert a small piece of metal rod of the same diameter into the hole perpendicular to the rod and tape into place.
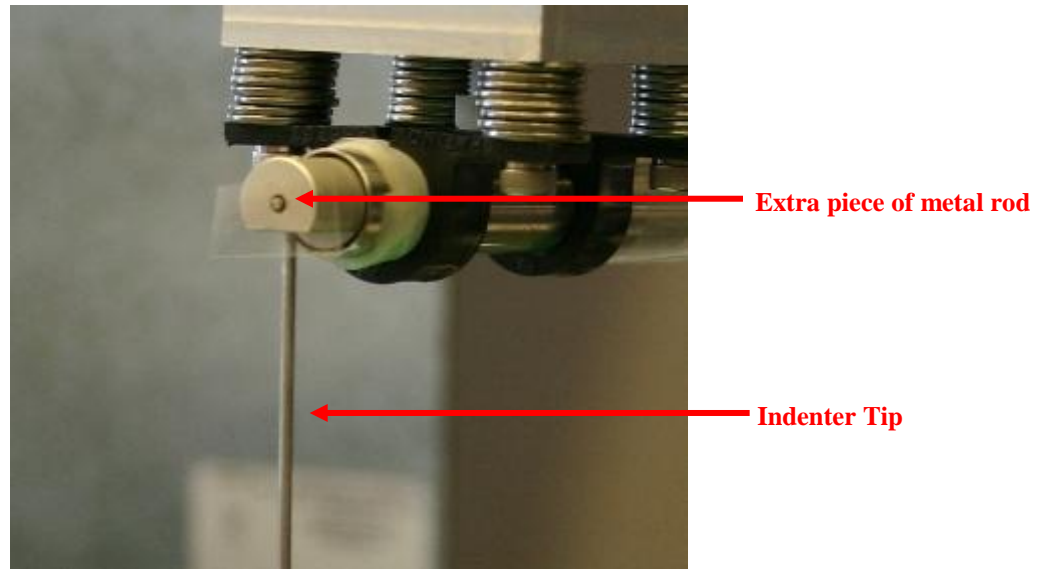
Figure 38: Setup of Force Transducer and Indenter Tip
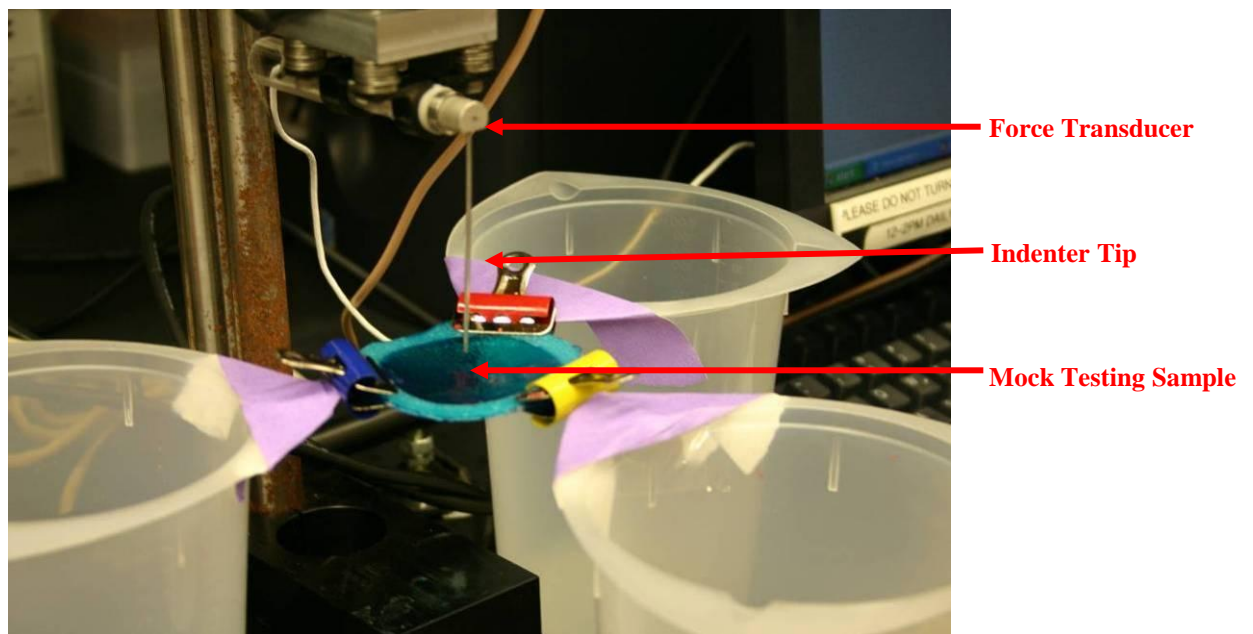
Figure 39: Mock Testing Setup

4. Open the uniaxial test program from the folder on the desktop called 'Lynn's Program'.
5. Select a Base Path of: Documents and Settings → All Users → Shared Documents → Lab Notebooks → SGS MQP. Select the testing folder as the location to save the data.
6. Record the thickness of each of the samples being tested and give each sample a sample ID as seen in the tables below. Note this chart will also be used to record other observations made during testing.

Blue Samples ("Jell-O" Brand)

Sample ID	Size	Thickness (mm)	Other notes:
B1	Large	3.41	
B2	Large	3.41	
B3	Large	3.80	
B4	Medium	4.25 (avg)	Jell-O not evenly poured
B5	Medium	2.40	
B6	Medium	3.26	
B7	Small	3.26	
B8	Small	3.60	Jell-O not evenly poured

Red Samples: ("Shaws" Brand)

Sample ID	Size	Thickness (mm)	Other notes:
R1	Large	5.65	
R2	Large	4.40	
R3	Large	4.40	
R4	Medium	4.40	
R4	Medium	5.65	
R6	Medium	5.65	
R7	Small	5.65	
R8	Small	5.65	

7. The blue samples will be tested first.
8. To load a large or medium sample, three binder clips should be used, spaced equidistantly around the rim of the foam anchor. To load the small samples, two binder clips should be used for each sample. The samples should be loaded and then moved under the indenter tip.
9. Center the sample under the indenter tip and ensure that there is at least 0.5 inches of clearance between the indenter tip and the mock test sample.

10. In the LabVIEW screen change the Test Name to match the sample ID of the test.
11. Start the actuator, set it to move down, and start recording with LabVIEW.
12. Once the indenter tip has pierced gel, hit stop in the LabVIEW screen, turn off the actuator, reverse the direction, and turn the actuator back on to remove the tip from the sample.
13. Repeat steps 9-13 for all samples, changing the name of the test each time to avoid overwriting any of the previous tests' data (e.g. B2, B3, etc..).
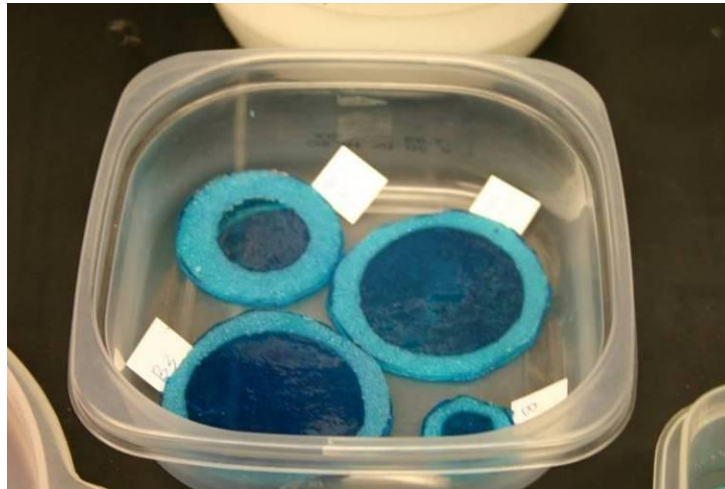
Figure 40: Blue mock samples
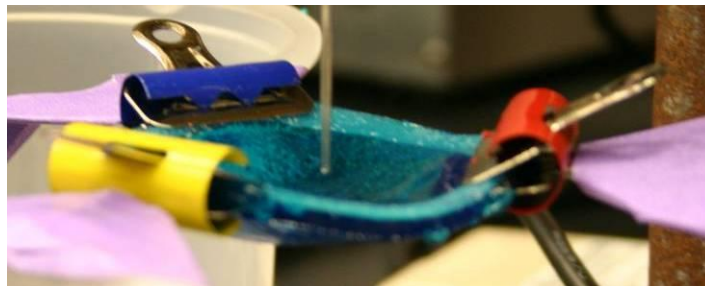
Figure 41: Loading of a large blue sample

Figure 42: Testing to failure of a small red sample

Appendix V Fabrication of Fibroblast Populated Collagen Gels Protocol

Materials:

- 5 mg/ml collagen solution
- 5x DMEM (Dulbuccos Modified Eagles Medium)
- 0.1 M NaOH
- ATCC fibroblasts (Passage 5-9)
- DMEM with 10% FBS
- Trypsin
- Culture plate (specific type depends on experiment)

Aliquot and label the proper amounts (see spreadsheet) of the following, place in ice:

1. To figure proper volumes of solution needed for one experiment, use the excel spread sheet (Collagen gel calculation spreadsheet).
   a. Collagen at 5 mg/ml (see preparation of collagen solution protocol) measure the necessary volume using a syringe
   b. 5X DMEM (see preparation of 5x DMEM protocol)
   c. 0.1 M NaOH

To obtain fibroblasts cells from T-150 flasks:

1. Remove T-150 plates from incubator, look at under microscope to ensure viability and 80-90% confluency, then perform the following in sterile hood.
   > Note: if cells are over 90% confluent, they should not be used. Cells change morphology at this density, which will alter the behavior of the cells.

2. Aspirate media.
3. Add 8 ml Trypsin with EDTA.
4. Place each plate in the incubator, let sit for 5-10 minutes until cells have become unattached (there should be a yellowish cloudy appearance to the solution).
5. Add 8 ml of warm media (1X DMEM, 10% FBS with P/S) to each plate to deactivate the trypsin.
6. Remove the liquid from the flask with a pipette, place into a 50 ml conical tube and centrifuge the cell solution at 1200 rpm for 6 minutes.
7. Aspirate supernatant from tubes being careful not to disturb the pellet of cells in bottom.
8. Resuspend the cells in a small volume (1-2 ml) of DMEM with 10% FBS noting the volume of liquid in ml for cell count.
9. Mix the solution well and remove 100 μl of the cell-media solution from the center of the conical tube (this ensures an appropriate representation of the cell solution), and place in a 1-2 ml microcentrifuge tube. Add 100 μl of Trypan blue dye to the microcentrifuge

tube and mix well (do not vortex, this will kill the cells). Place ~10 □ l of this solution into the hematocytometer and perform a cell count.
10. Add media to the tube in order to bring the cell concentration (cells/ml) to that listed on the Collagen Gel Calculation Sheet.
11. Place the cells on ice immediately, and aliquot proper amounts for gel fabrication (per calculation spreadsheet). Proceed to next header OR
    a. For continued cell culture:
        i. Resuspend cells with 5 ml of DMEM with 10% FBS
        ii. Place cells in a T-150 flask. For cell passage, each T-150 flask should have 25 ml of DMEM with 10% FBS, p/s, and amp B and $1x10^6$ cells
        iii. Slide flask in a figure 8 motion
        iv. Place in incubator.

To fabricate gels:

NOTE: If a large volume of collagen gel solution is required, it may be advantageous to make 2 batches of gels to prevent the solution from polymerizing before all of the gels are plated.

1. First add 5X DMEM, 0.1M NaOH, and FBS to an appropriate sized conical tube mix well and then add the appropriate cell volume last (refer to calculation spreadsheet). Note: Since the DMEM-NaOH solution is very basic, it is important that cells are added last to minimize the time in this solution.
2. Using a syringe, add the collagen to the aliquot of cell-media-5X DMEM-NaOH mixture, mix well with pipette until color is homogonous throughout, taking care not to add air bubbles to the mixture.
3. Quickly add the desired volume of the collagen-cell solution to each well. Aspirate any bubbles off the top and swirl gently to assure solution is level and covers entire plate.
4. Place gels in 37° incubator for 1 hour.
5. Add 2 ml of media to each well (for 6-well plate) and place in incubator.
6. Replace media every other day.

Appendix VI **Collagen Gel Testing Data Analysis**

Below is the data analysis for the collagen gel testing. This testing was on collagen gels which were poured in rectangular foam anchors. In each test the displacement was measured through image analysis and the stress and strain were calculated. Based on the stress and strain it was then possible to calculate the stiffness. The stiffness values for each test were determined by the slope of the line of best fit from the stress vs strain graphs.
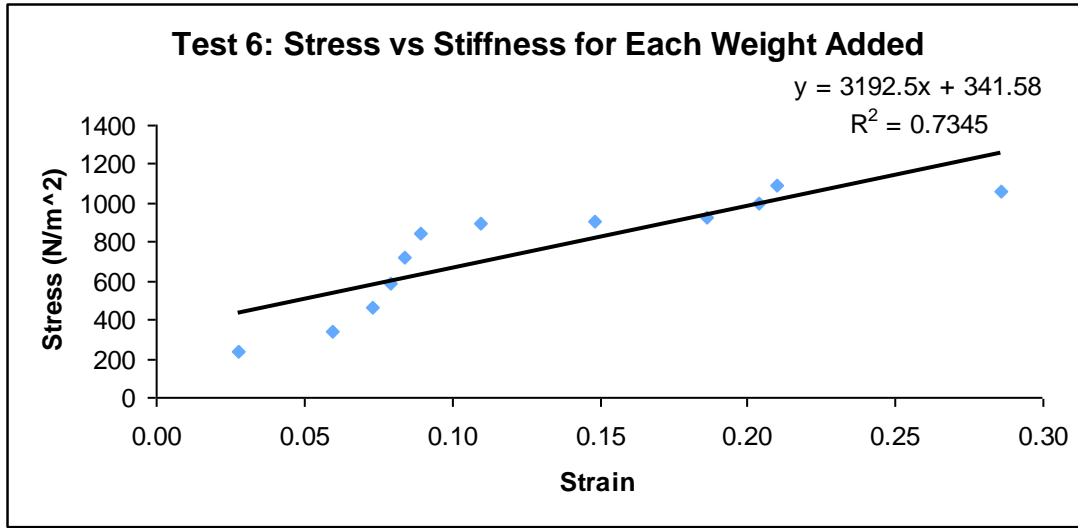
Test 2

Amount Weight (g)	Distance between Gel and Anchor (in)	Angle (rad)	Tensional Force (g)	Stress (gf/cm^2)	Stress (N/m^2)	Strain	Stiffness (N/m^2)
0.09	0.06	0.15	0.29	3.70	363	0.01	3.04E+04
0.18	0.08	0.21	0.43	5.39	529	0.02	2.31E+04
0.27	0.09	0.24	0.58	7.25	711	0.03	2.48E+04
0.36	0.11	0.27	0.67	8.40	824	0.04	2.14E+04
0.45	0.11	0.27	0.85	10.7	1051	0.04	2.84E+04
0.54	0.12	0.30	0.92	11.6	1135	0.05	2.46E+04
0.63	0.13	0.33	0.98	12.4	1216	0.06	2.19E+04
0.72	0.14	0.35	1.05	13.2	1292	0.07	1.99E+04
0.81	0.16	0.38	1.11	13.9	1366	0.07	1.83E+04
0.90	0.16	0.39	1.18	14.9	1461	0.08	1.80E+04
0.99	0.17	0.40	1.27	16.1	1574	0.09	1.85E+04
1.08	0.17	0.42	1.33	16.8	1648	0.09	1.76E+04
1.17	0.19	0.46	1.33	16.8	1644	0.11	1.45E+04
1.26	0.21	0.49	1.35	17.0	1666	0.13	1.27E+04
						Stiffness	**21 kPa**
						Stdev Stiffness	**4.398 kPa**

Test 2: Stress vs Stiffness for Each Weight Added

$$y = 11418x + 455.4$$
$$R^2 = 0.8699$$
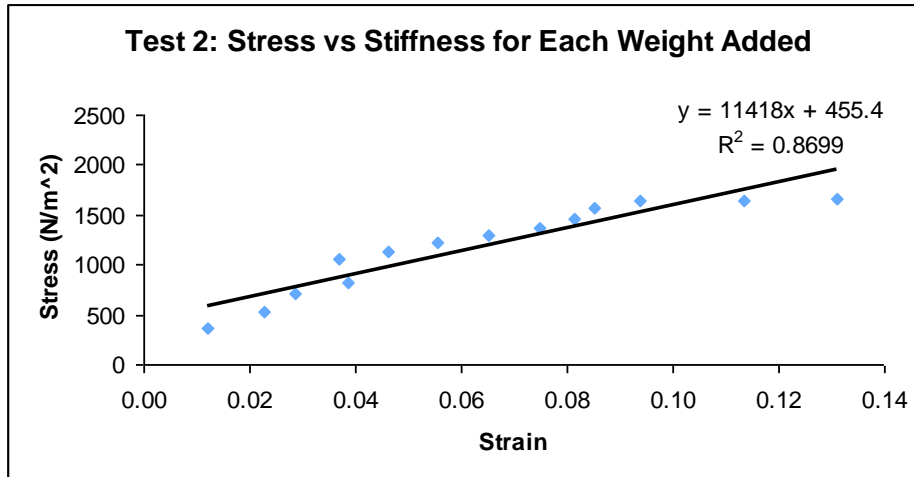
Test 6

Amount Weight (g)	Distance between Gel and Anchor (in)	Angle (rad)	Tensional Force (g)	Stress (g/cm^2)	Stress (N/m^2)	Strain	Stiffness (N/m^2)
0.09	0.09	0.23	0.19	2.45	240	0.03	8605.93
0.18	0.14	0.34	0.27	3.43	336	0.06	5.64E+03
0.27	0.15	0.37	0.37	4.70	460	0.07	6.32E+03
0.36	0.16	0.39	0.48	6.02	591	0.08	7.44E+03
0.45	0.17	0.40	0.58	7.33	719	0.08	8.53E+03
0.54	0.17	0.41	0.68	8.58	841	0.09	9.43E+03
0.63	0.19	0.45	0.73	9.16	898	0.11	8.19E+03
0.72	0.22	0.51	0.73	9.23	906	0.15	6.12E+03
0.81	0.25	0.57	0.75	9.48	930	0.19	4.99E+03
0.90	0.26	0.59	0.81	10.2	998	0.20	4.89E+03
0.99	0.27	0.60	0.88	11.1	1087	0.21	5.18E+03
1.08	0.32	0.68	0.86	10.8	1062	0.29	3.72E+03
						Stiffness	6.6 kPa
						Stdev Stiffness	1.81 kPa

Test 7

Amount Weight (g)	Distance between Gel and Anchor (in)	Angle (rad)	Tensional Force (g)	Stress (g/cm^2)	Stress (N/m^2)	Strain	Stiffness (N/m^2)
0.09	0.03	0.07	0.68	8.60	844	0.00	3.873E+05
0.18	0.11	0.26	0.35	4.40	431	0.03	1.23E+04
0.27	0.13	0.31	0.44	5.50	539	0.05	1.04E+04
0.36	0.15	0.35	0.52	6.56	643	0.07	9.79E+03
0.45	0.16	0.38	0.61	7.74	759	0.07	1.01E+04
0.54	0.17	0.41	0.68	8.62	846	0.09	9.58E+03
0.63	0.17	0.41	0.79	9.91	972	0.09	1.07E+04
0.72	0.19	0.45	0.83	10.51	1031	0.11	9.49$+03
0.81	0.18	0.44	0.96	12.03	1180	0.10	1.13E+04
0.90	0.20	0.46	1.01	12.69	1244	0.12	1.06E+04
0.99	0.20	0.47	1.10	13.84	1358	0.12	1.13E+04
1.08	0.21	0.49	1.15	14.55	1426	0.13	1.08E+04
1.17	0.22	0.51	1.21	15.21	1492	0.14	1.04E+04
						Stiffness	**42 kPa**
						Stdev Stiffness	**10.8 kPa**

Appendix VII PDMS Testing Protocol

Materials:

- #10-32 X ¾" stainless steel screws
- PDMS
- Rubber O-rings
- 100mm Petri dish
- Device

Methods:

1. Using a hole saw, drill the top and bottom of the Petri dish with the proper hole size (one with 64mm and the other with 70mm).
2. About ¼" from the center hole, drill 6 evenly spaced through holes for the size #10 screws.
3. Around the center hole of each Petri dish, glue a properly fitting O-ring.
4. Once the O-rings are secure, the PDMS sample is loaded between the two Petri dishes, with the O-rings being used to add extra grip to prevent slipping.
5. Place the clamping system centered under the indenter tip in the device.
6. Using LabVIEW, run the device program at a speed of -1 rev/sec. Run the program until the encoder reads a force of 10g and then reverse the speed of the actuator to remove the indenter tip from the sample.
7. Remove the PDMS from the clamping system and measure and record the thickness using a digital caliper.

Appendix VIII PDMS Testing Data Analysis

For this series of testing, two samples of PDMS were tested 5 times each using the device. In each trial the indenter tip was not in contact with the PDMS prior to the start of recording force. In order to zero the data, data was not analyzed till a negative slope in the force readings was maintained. Additionally, the program was still running when the actuator movement was reversed so data recorded after the maximum force was reached was also not analyzed. Below plots from each test can be seen which contain the test data, displacement calculated using Equation 4, and points from the Scott article. The data from sample 1 has a higher load recorded for each displacement as compared to the Scott article whereas the data for the sample 2 coincides with the data from the Scott article. The reason for this is the Scott article assumes an elastic modulus of 1.15MPA. Sample 1 has an elastic modulus of 2MPa which is greater while the elastic modulus for sample 2 is the same as the elastic modulus for the sample used by Scott et al.

Sample 1, Trial 1
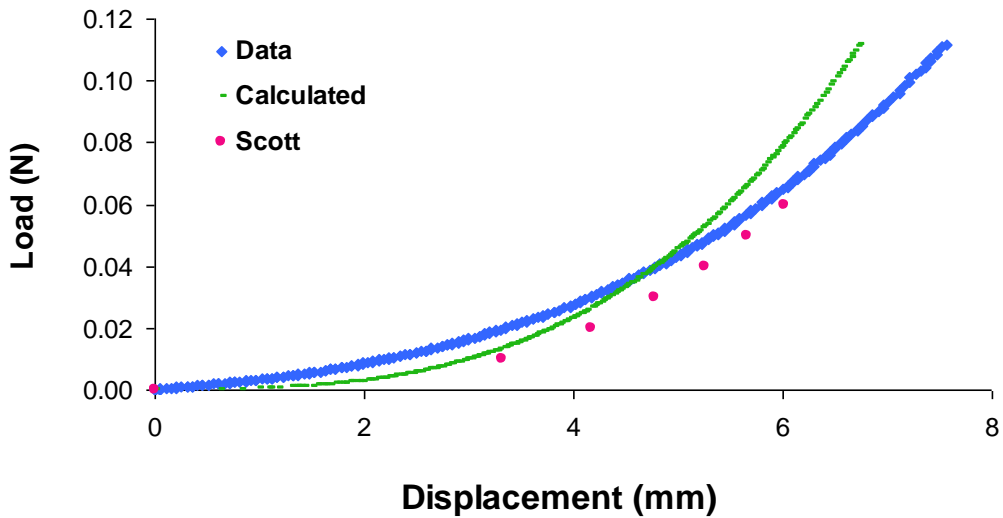**Load versus Displacement**
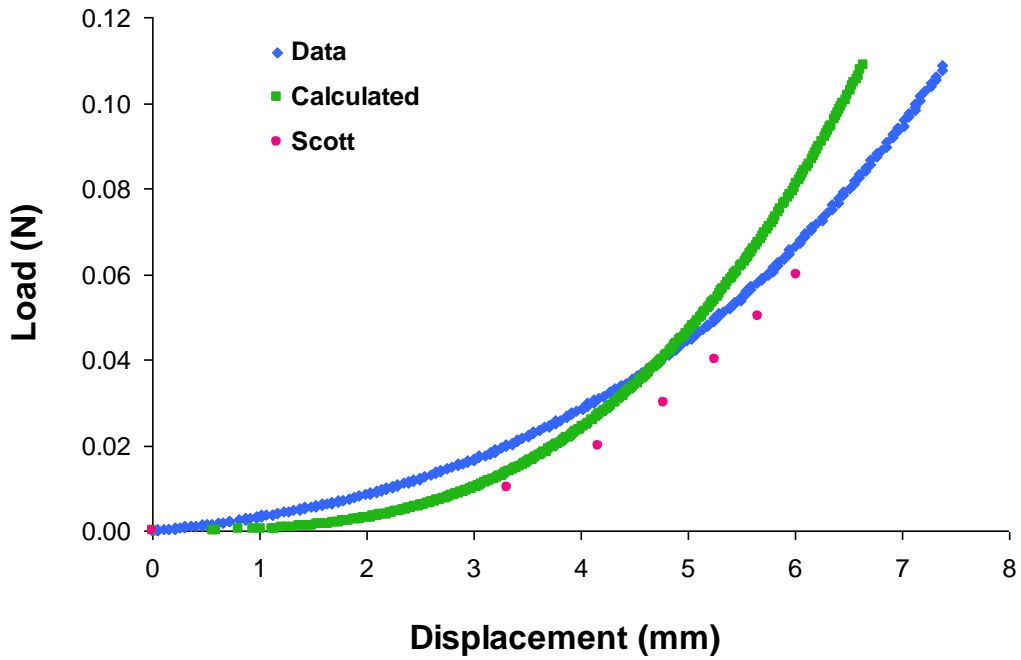
Sample 1, Trial 2
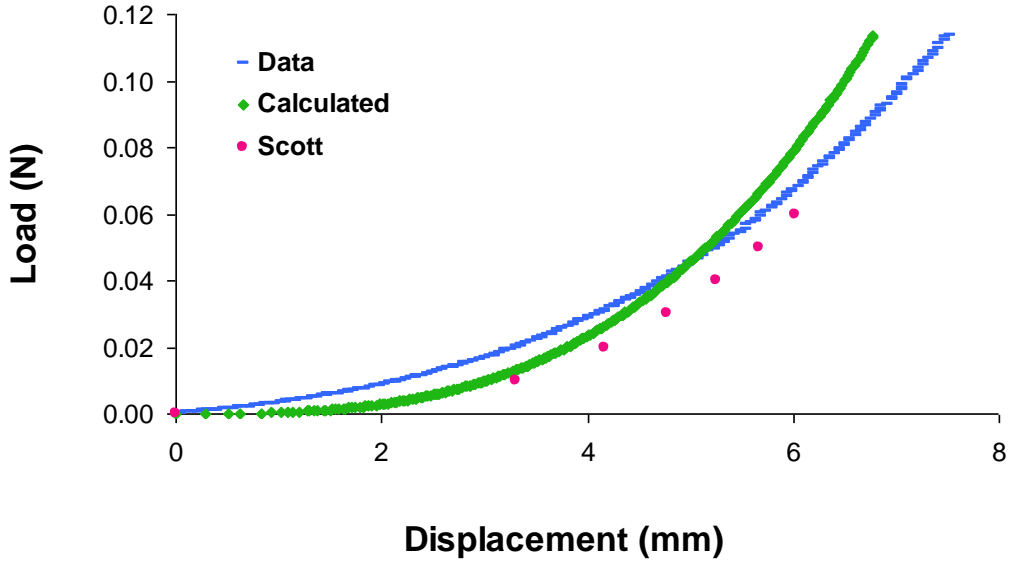**Load versus Displacement**
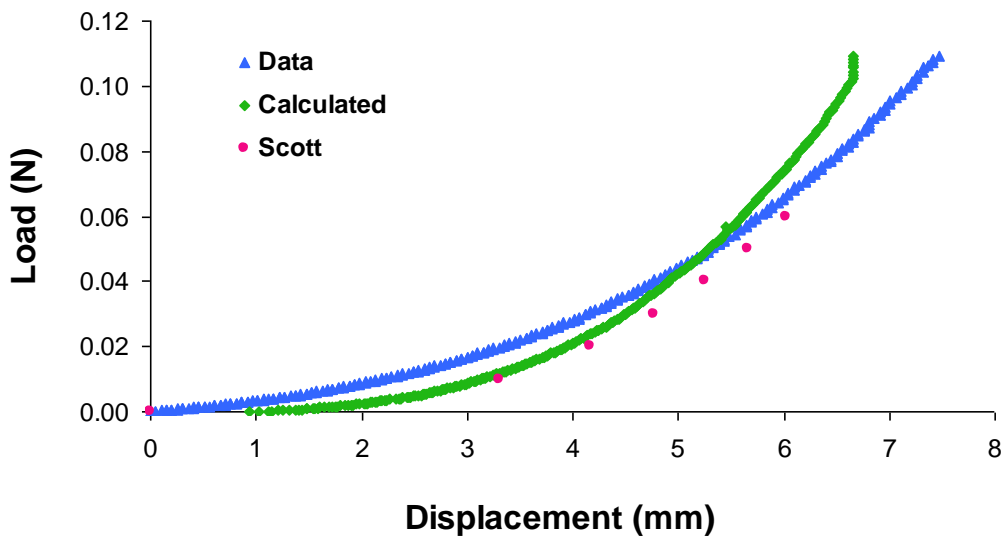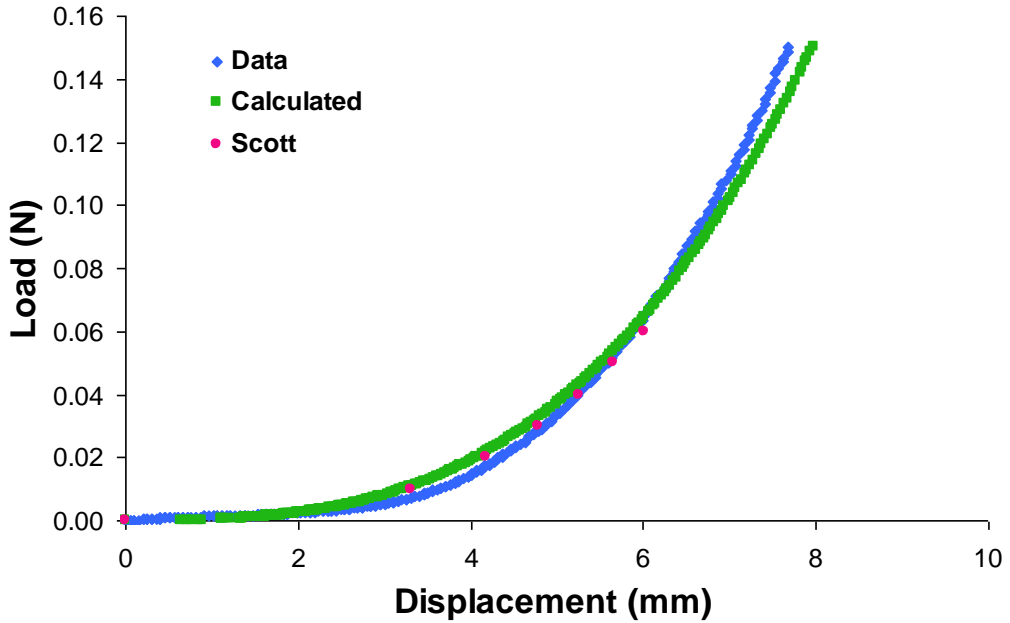
Sample 1, Trial 3
Load versus Displacement
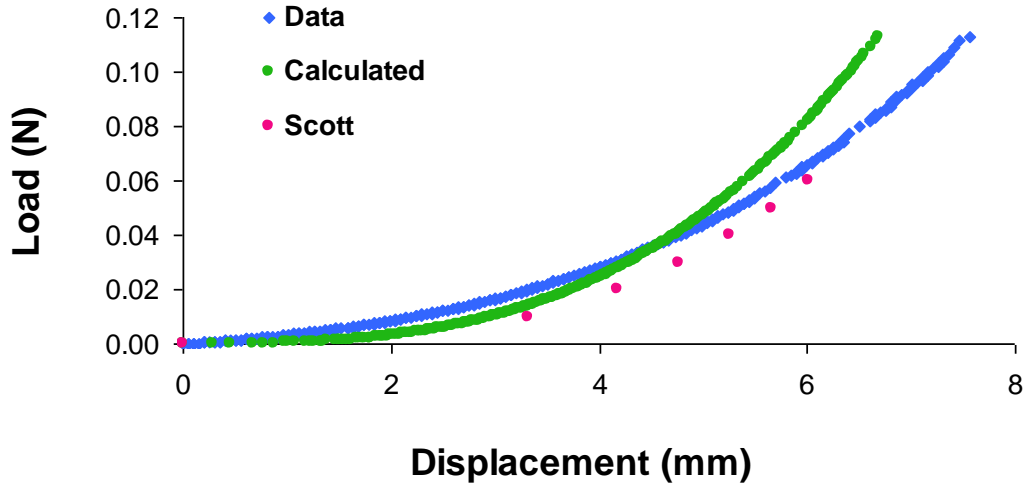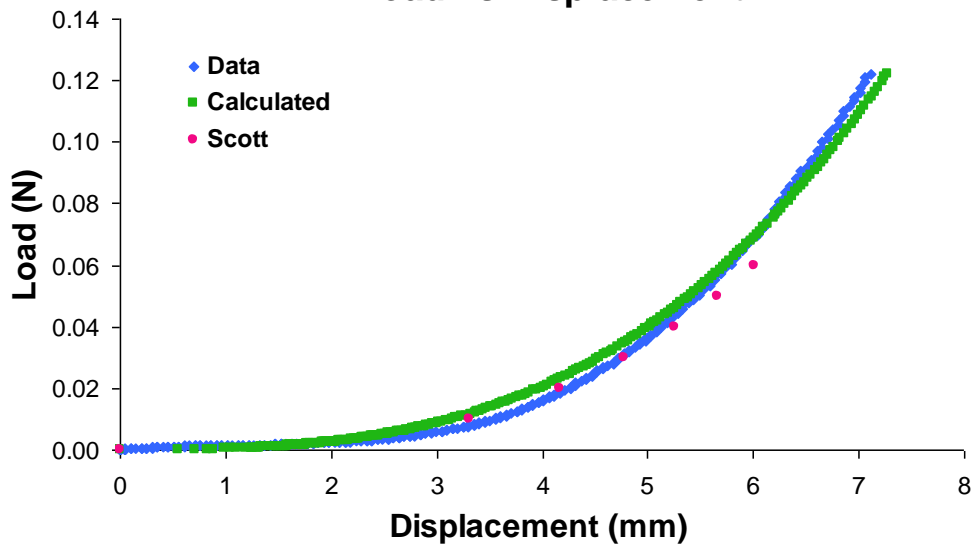

Sample 1, Trial 4
Load versus Displacement
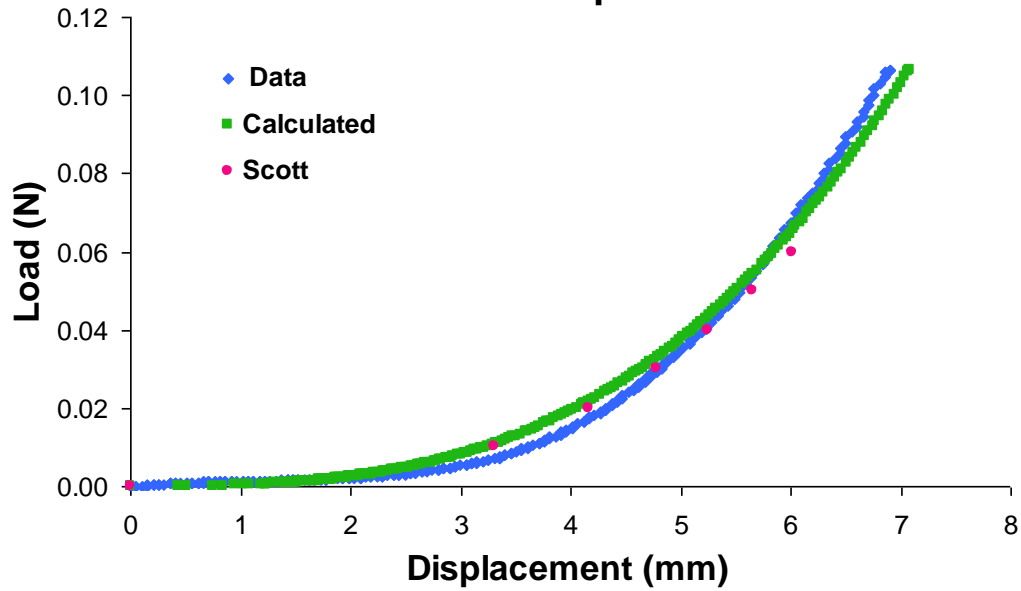
Sample 1, Trial 5
Load versus Displacement
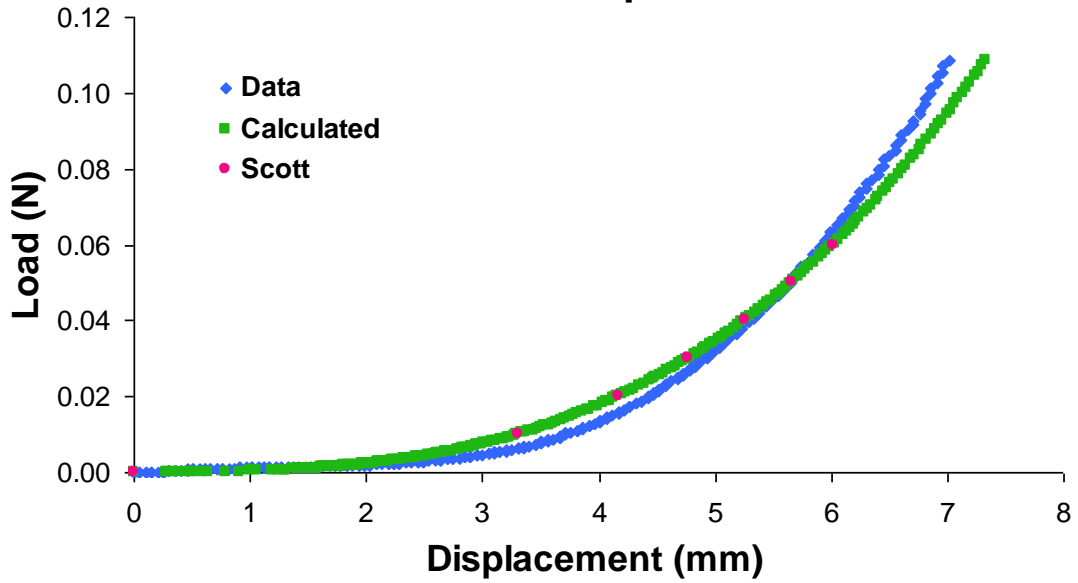

Sample 2, Trial 1
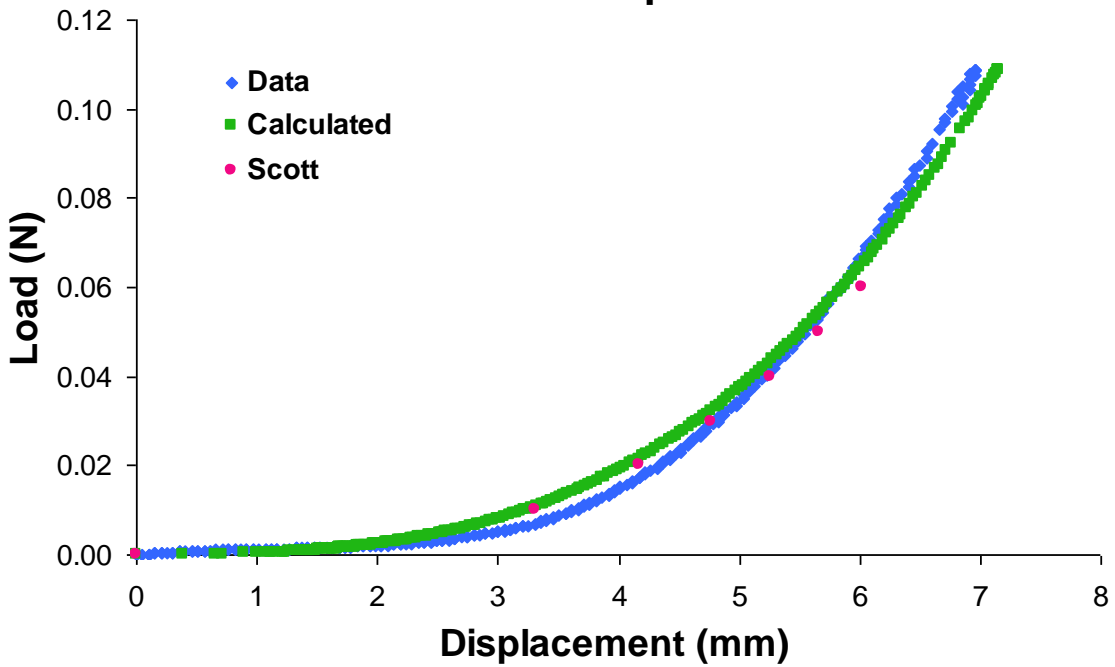## Load versus Displacement

Sample 2, Trial 2
Load vs Displacement

Sample 2, Trial 3
Load vs Displacement

Sample 2, Trial 4
Load vs Displacement
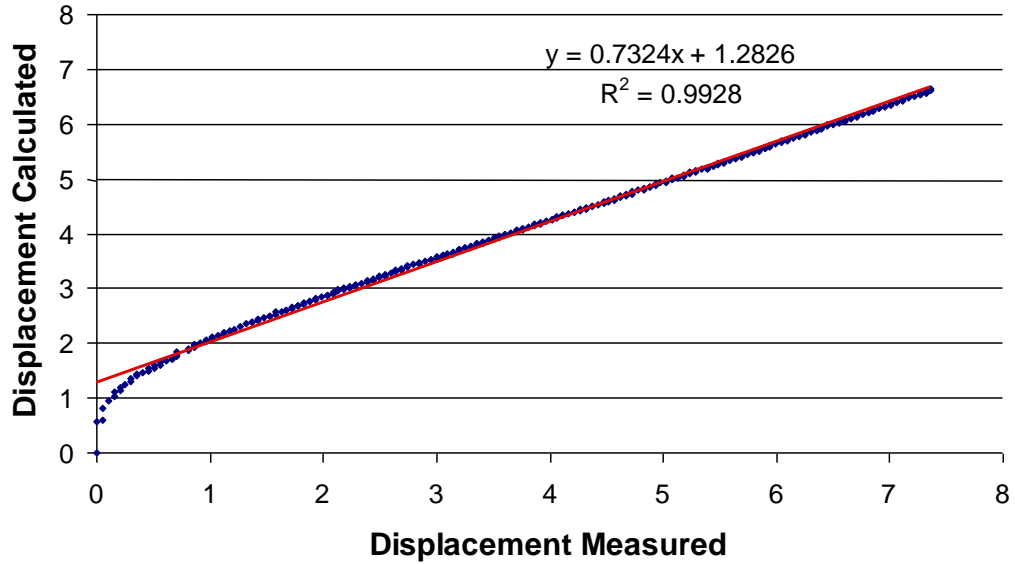

Sample 2, Trial 5
Load vs Displacement

To ensure that Equation (1) with v = 0.33 was a good fit, the measured and calculated displacements were plotted against each other. Plots of this for each sample can be seen below. If
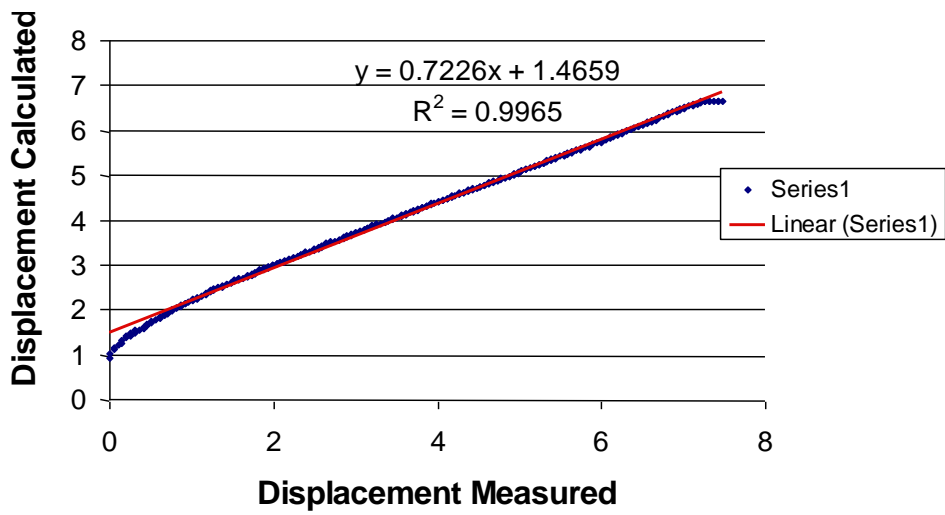
the model is a good fit then the graph is linear. While the model fit with a very high R^2 value, there was some discrepancy at the initial part of the graphs. It is to be expected, however, that a simple model will never fit perfectly and it was determined that for our purposes, this fit was acceptable. Below plots of calculated vs measured displacements with lines of best fit can be seen for the five trials completed with each sample.
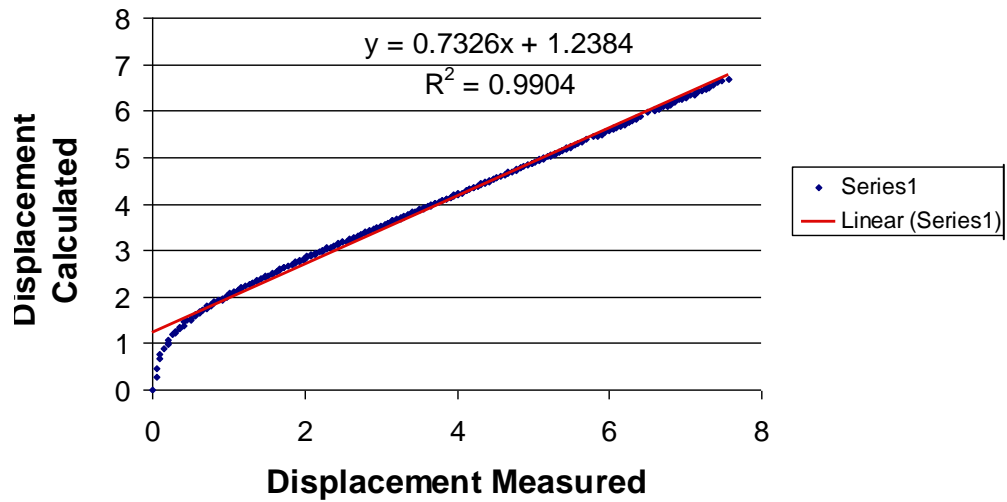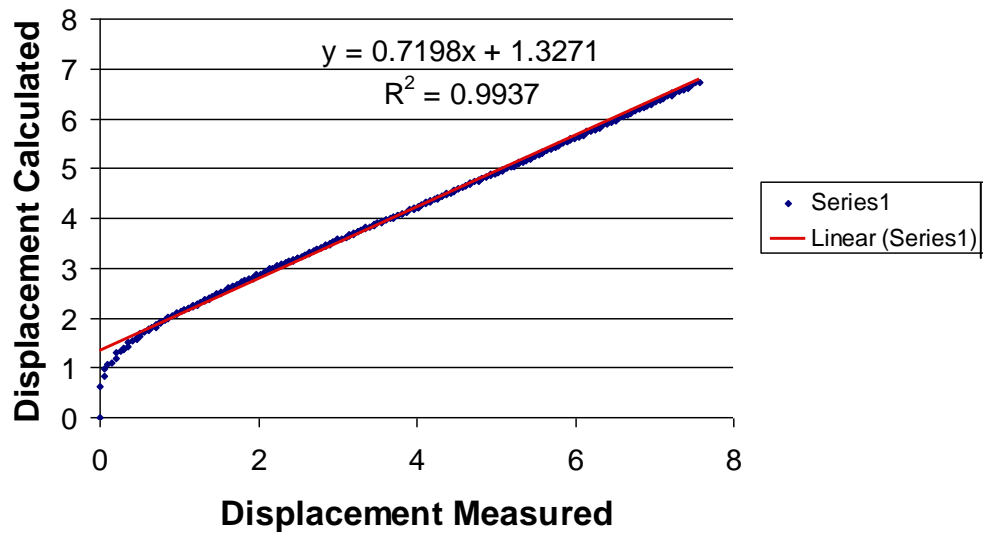
Sample 1, Trial 1
Calculated vs Measured Displacement

$y = 0.7324x + 1.2826$

$R^2 = 0.9928$

Sample 1, Trial 2
Calculated vs Measured Displacement

$y = 0.7226x + 1.4659$

$R^2 = 0.9965$

Series1
Linear (Series1)

Sample 1, Trial 3
Calculated vs Measured Displacement

$y = 0.7326x + 1.2384$

$R^2 = 0.9904$

Sample 1, Trial 4
Calculated vs Measured Displacement

$y = 0.7198x + 1.3271$

$R^2 = 0.9937$

Sample 1, Trial 5
Calculated vs Measured Displacement

$$y = 0.7426x + 1.2681$$
$$R^2 = 0.9918$$

Displacement Calculated (y-axis)

Displacement Measured (x-axis)

Legend: Series1, Linear (Series1)
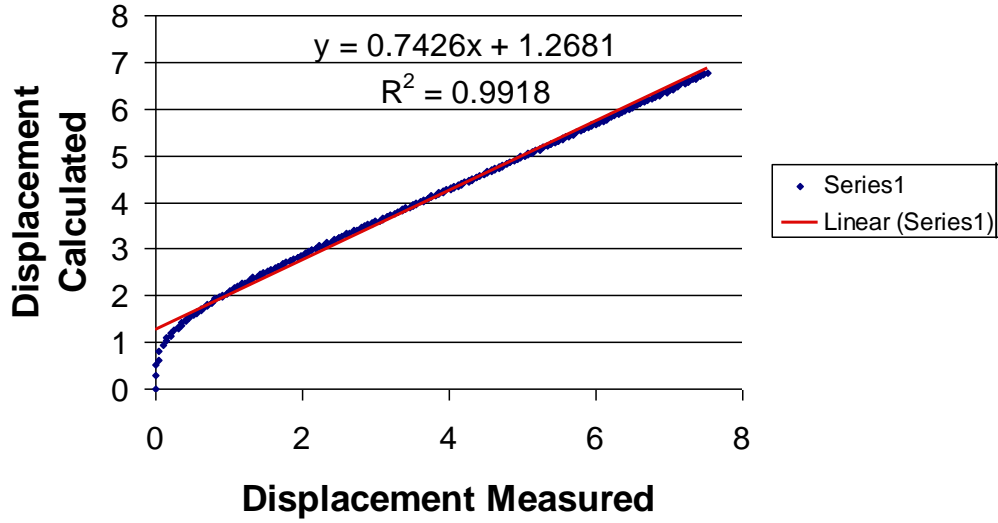
Sample 2, Trial 1
Calculated vs Measured Displacement

$$y = 0.9335x + 0.3255$$
$$R^2 = 0.9717$$

Displacement Calculated (y-axis)

Displacement Measured (x-axis)

Legend: Series1, Linear (Series1)
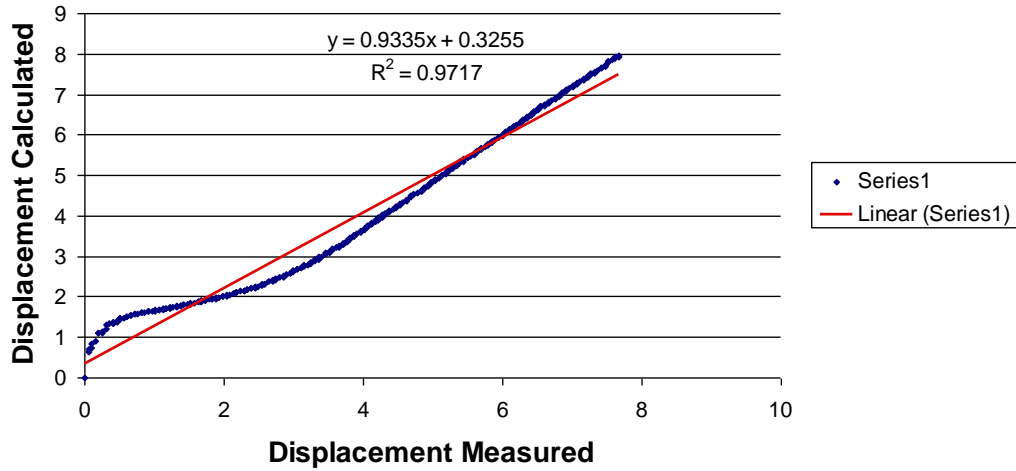
Sample 2, Trial 2
Calculated vs Measured Displacement

$y = 0.8946x + 0.44$
$R^2 = 0.9644$

- Series1
- Linear (Series1)
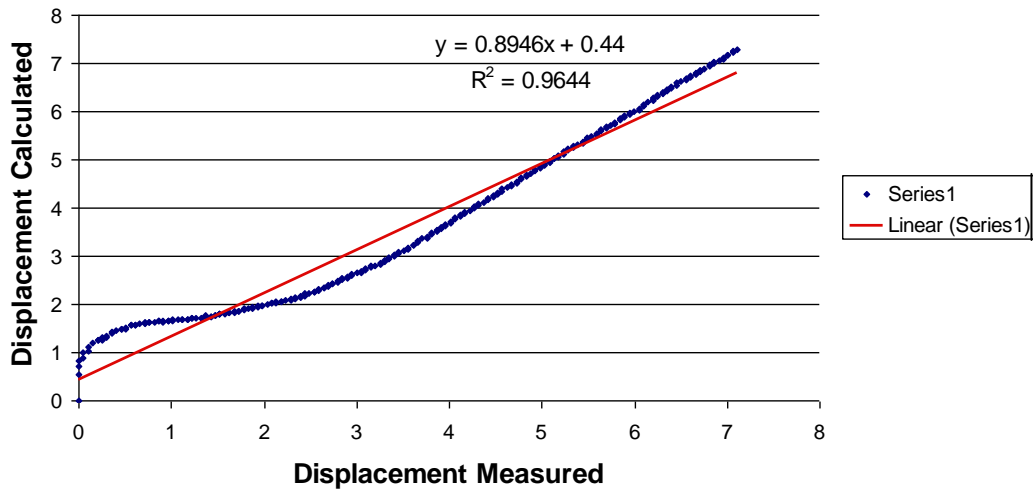
Sample 2, Trial 3
Calculated vs Measured Displacement

$y = 0.9103x + 0.3514$
$R^2 = 0.9647$

- Series1
- Linear (Series1)
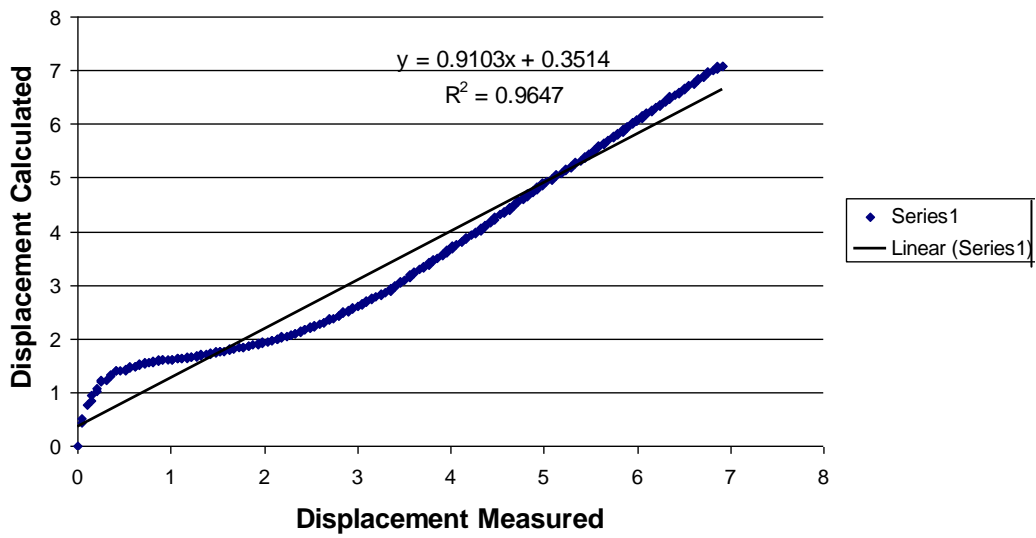
Sample 2, Trial 4
Calculated vs Measured Displacement

$y = 0.9377x + 0.2514$
$R^2 = 0.967$
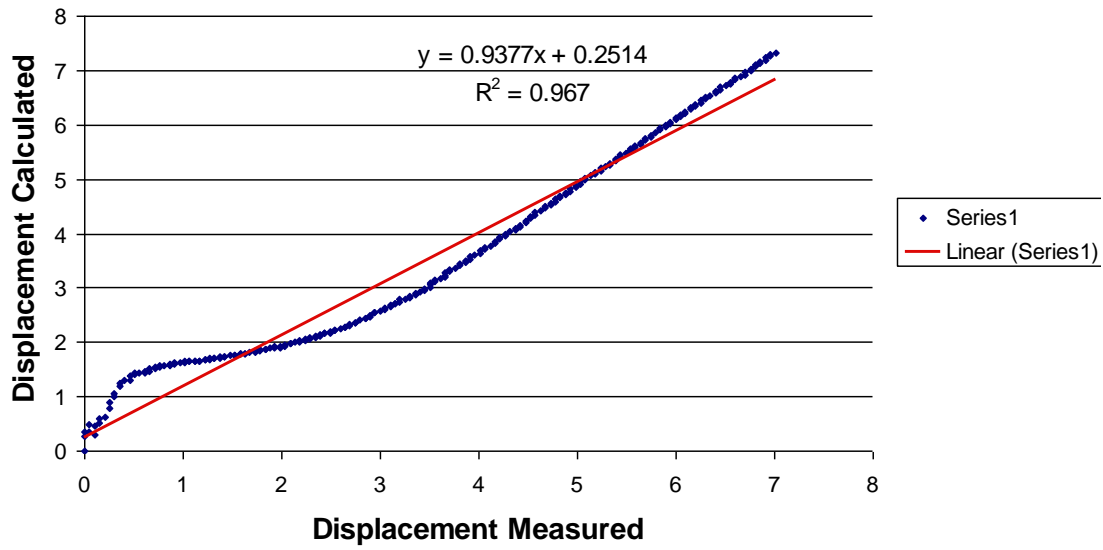
Sample 2, Trial 5
Calculated vs Measured Displacement

$y = 0.9271x + 0.2975$
$R^2 = 0.9677$
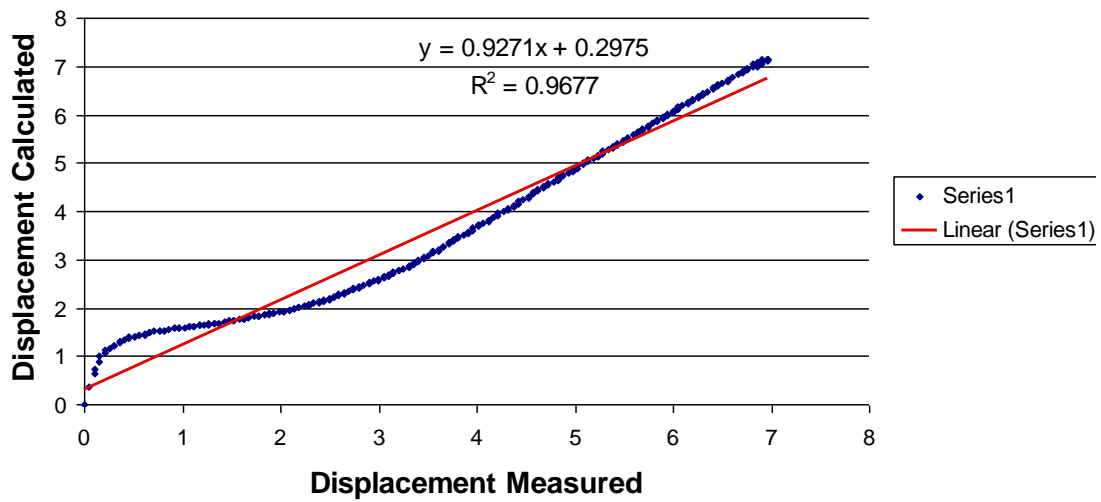
Appendix IX Circularly Clamped Collagen Gel Testing Protocol

Materials:

- #10-32 X ¾" stainless steel screws
- Circular clamping device
- Circular foam anchors with an inner diameter of 43mm and an out diameter of 60mm
- 60mm Petri dish
- Collagen gels
- Device

Methods:

8. Cut 5 foam anchors with an inner diameter of 43mm and an out diameter of 60mm from 1/16" porous polyethylene.
9. Assemble the circular clamping device made of two metal rings.
10. Use 4 small dots of vacuum grease on each foam anchor to secure it down to the bottom of the Petri dish.
11. Prepare collagen gels according to Appendix V.
12. Pipette collagen gel into wells placing 4.5mL in each well.
13. Allow gels to sit for 1 hour.
14. Use a scalpel to cut around the edge of the foam anchor and forceps to remove the sample. Place the sample on top of the bottom metal ring. Add the second metal ring on top of the sample and secure into place by tightening the nuts.
15. Place the circular clamping system centered under the indenter tip in the device.
16. Using LabVIEW, run the device program at a speed of -1. Run the program until the encoder reads a displacement of 3mm and then reverse the speed of the actuator to remove the indenter tip from the sample.
17. Remove the collagen gel from the clamping system and measure and record the thickness of the gel using a digital caliper.

Appendix X Circularly Clamped Acellular Collagen Get Testing Data Analysis

Five collagen samples were created according to the Circularly Clamped Collagen Gel protocol. These samples were then tested using the device with the indenter tip moving a speed of 1 revolution per second (.0254 mm/sec). Each sample was tested 3 times. Initially, the gels were to be tested to 5mm of displacement, but after the first sample was pierced by the indenter tip before reaching this displacement, the target displacement was reduced to 3 mm for the first two trials and then tested to failure for the last trial.

In each trial since the indenter tip was not in contact with the collagen gel prior to the start of recording force, the data prior to a downward slope in the force readings was not analyzed. Additionally, the program was still running when the actuator movement was reversed so data recorded after the maximum force was reached was also not analyzed. For each trial, LabVIEW outputted readings of time, force and encoder readings. Both the force readings and encoder readings (displacement) were zeroed at the determined point of contact.

Displacements were then calculated using the following equations with a v of both 0.5 and 0.33 for Equation 1:

$$\delta = f(v)a\left(\frac{P}{Eah}\right)^{1/3}, \qquad\qquad \frac{\delta}{R} = \left(\frac{16}{9\pi}\right)^{1/3}\left(\frac{a}{R}\right)^{3/4}\left(\frac{P}{EhR}\right)^{1/3}.$$

$$(1) \qquad\qquad\qquad\qquad\qquad (2)$$

The calculated displacements were then compared to the measured displacements and the difference was minimized by altering the stiffness value by using the Equation Solver function in Microsoft Excel. The results of tests 3-5 can be seen below in Table 23. **E4a** refers to Equation (1) with a v = 0.5, **E4b** refers to Equation (1) with a v = 0.33 and **E5** refers to Equation (2).

Table 23: Circularly Clamped Collagen Data

	E4a (Pa)	E4b (Pa)	E5 (Pa)
E2,T1	4.77E+05	5.51E+05	9.03E+05
E3,T1	1.48E+06	1.71E+06	2.80E+06
E3,T2	3.18E+05	3.67E+05	6.02E+05
E3,T3	9.16E+05	1.06E+06	1.73E+06
E4,T1	1.40E+06	1.61E+06	2.64E+06
E4,T2	1.34E+06	1.55E+06	2.54E+06
E4,T3	8.68E+05	1.00E+06	1.64E+06
E5,T1	1.42E+06	1.64E+06	2.69E+06
E5,T2	1.33E+06	1.54E+06	2.52E+06
E5,T3	2.17E+06	2.51E+06	3.81E+06
Mean:	1.17E+06	1.35E+06	2.19E+06
Stdev:	5.40E+05	6.24E+05	9.65E+05
Coefficent of Variation:	46.08%	46.08%	44.08%

By looking at all of the data as a whole, there is a high standard deviation, but analyzing the data by the trial number, as seen in Table 24 provides much more significant data.

Table 24: Circularly Clamped Collagen Data Organized by Test Number

Test 1

E4a	E4b	E5
1.48E+06	1.71E+06	2.80E+06
1.40E+06	1.61E+06	2.64E+06
1.42E+06	1.64E+06	2.69E+06
1.43E+06	1.66E+06	2.71E+06
4.34E+04	5.01E+04	8.21E+04
3.03%	3.03%	3.03%

Test 2

E4a	E4b	E5
3.18E+05	3.67E+05	6.02E+05
1.34E+06	1.55E+06	2.54E+06
1.33E+06	1.54E+06	2.52E+06
9.98E+05	1.15E+06	1.89E+06
5.89E+05	6.81E+05	1.11E+06
59.02%	59.02%	59.02%

Test 3

E4a	E4b	E5
9.16E+05	1.06E+06	1.73E+06
8.68E+05	1.00E+06	1.64E+06
2.17E+06	2.51E+06	3.81E+06
1.32E+06	1.52E+06	2.40E+06
7.37E+05	8.52E+05	1.23E+06
55.97%	55.97%	51.20%

Table 24 shows that the data is consistent and reliable for only the first test of each gel. After the first indentation test, sample 3 began to show a decrease in stiffness while sample 4 and 5 did not. Again after the third test, samples 3 and 4 began to show a large decrease in stiffness while sample 5 actually increased. This is unusual and could be due to the fact that in the third trial the gel was tested to failure. The information learned in this study, will help in future trials as we will limit each sample to only one test.

Appendix XI Transverse Loading Device Test Protocol

Materials:

- Transverse Loading Device
- Desktop Computer with NI-DAQ and NI PCI-7334 cards
- NI MID-7604 motion power drive with terminal blocks
- NI SC-2345 signal conditioning connector unit
- NI SCC-SG24 strain gauge
- Power strip
- FUTEK Load Cell
- Indenter tip
- External power supply capable of 5V output
- Collagen samples
- Circular foam anchors (25 mm inner dia. and 32 mm outer dia.)

Methods: Device Set up and Preparation

1. Prepare test environment by plugging in appropriate power connectors of all electronic devices into the power strip (**do NOT turn the power strip on**).
2. Insert the green connector blocks attached to the encoder and actuator into axes 3 and 1, respectively, of the motion power drive.
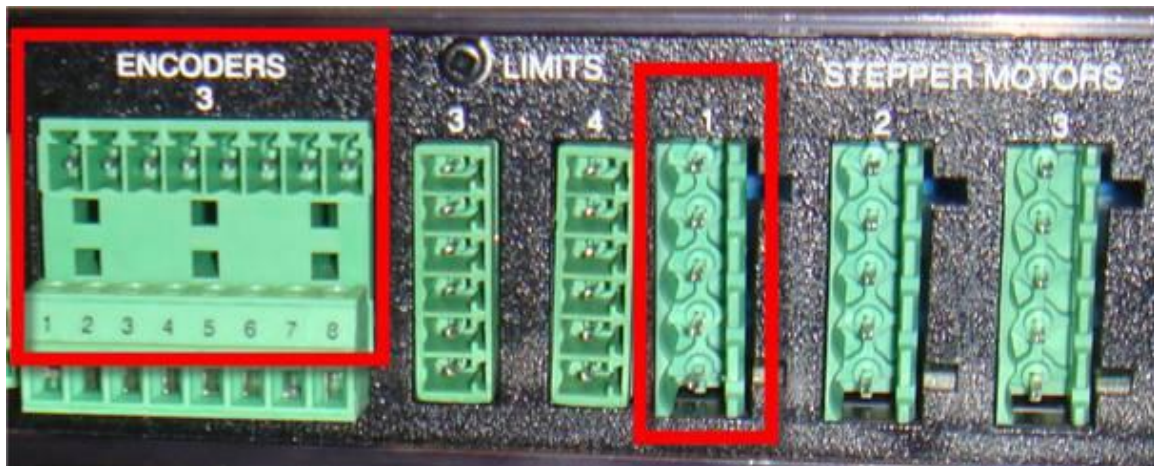

Figure 43: Encoder axis 3 and actuator axis 1

3. Wire the force transducer's excitation inputs (red and black wires) into their respective terminals of the 5V power supply (ensure that the power supply is indeed providing 5V of power).

4. Wire the force transducer's voltage outputs (green and white wires) into the **V+** and **V-** (positive and negative, respectively) terminals of the SCC-SG24 strain gauge (these terminals have input numbers 5 and 6).
5. Affix the strain gauge to the first terminal in the signal conditioner connector unit.

Figure 44: The first terminal in the signal conditioner

6. Screw the force transducer onto the indenter tip, ensuring that the text on the transducer unit is "upside down" in relation to the user.
7. In this order, power on the:
    a. Power strip
    b. External power supply
    c. Motion power drive
    d. Computer
8. On the computer, navigate and open the *SGSProgram.vi* LabVIEW virtual instrument.
9. On the virtual instrument, set the **Directory Path** to a writeable directory (e.g. C:\TestData\).

Method for Force Transducer Calibration

Materials:

- Transverse Loading Device
- Power supply
- Multimeter

Methods:

1. The force transducer was supplied with a 5V excitation by an external power supply while the output of the force transducer was connected to a multimeter.
2. The indenter was screwed into the force transducer and a voltage reading was recorded. Following this other measured weights (paperclips) were added incrementally and voltage readings can be seen below:

Mass (g)	Voltage (mV)
Indenter tip	0.634
+1.028	0.941
+1.014	1.246
+1.027	1.553
+1.062	1.870
+1.043	2.188
+1.042	2.498
+1.038	2.808
+1.034	3.118

3. This data was plotted with voltage on the x-axis and mass on the y-axis. A trendline was fitted to the data to determine the calibration equation:

$$Load = 3.349 \times (mVreading) - 2.1124$$

4. This equation was incorporated into the LabVIEW program.

Methods: Sample set up and preparation

1. Load gels into clamping device by removing the upper plate, placing the sample in the center of the circular gap, re-loading the upper plate and screwing it into place using the provided thumbscrews.
2. Place the sample under the indenter tip and orient the clamping device so that the indenter tip is aimed for the center of the gel.

Methods: Testing

3. Position the indenter tip as close to the gel as possible (without contact) by twisting the adjustable slide mount.
4. On the computer, create a new folder in the **Directory Path** mentioned in **Device Set up and Preparation** previous step and name it (e.g. "Sample01"). This folder is your Sample ID
5. Type the name of the Sample ID into the **Experiment ID Folder** section of the program.
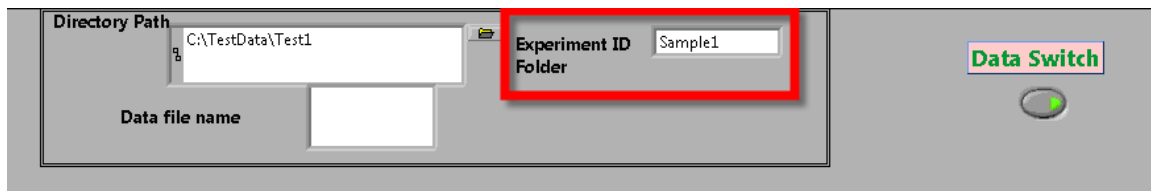
Figure 45: Experiment ID Folder

6. Enter the name of the trial in the **Data file name** section (this will be the name of the data file that LabVIEW exports).
7. Enter the force threshold that will be used to determine contact with the gel, once the transducer reads this threshold the program will begin taking data from the linear encoder (a good value to start with is 0.2 g).
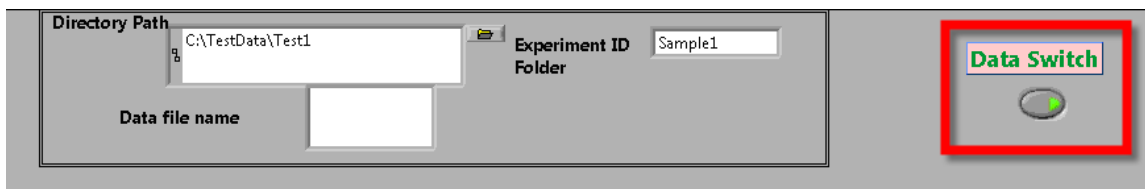8. Ensure that the "Data Switch" is flipped into the *on* position.

Figure 46: Data Switch set in the on position

9. Run the program by clicking on the **run** arrow on the LabVIEW menu bar.
10. The program is running and you should be reading a force of around zero grams.
11. Set the **Velocity (Before Distance Limit)** control to a negative number, the more negative the number, the faster the indenter will run. Most testing should be done using a velocity of -1.
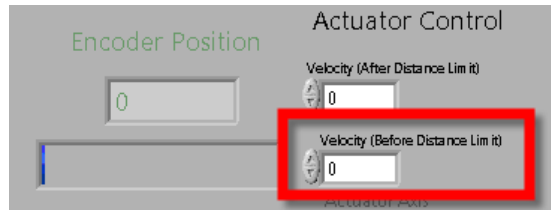
**Figure 47: The Velocity (Before Distance Limit) control**

12. The test will then go until the actuator reaches a distance of 3 mm. When this distance is reached, the program will set the actuator velocity to the **Velocity (After Distance Limit)** control (which defaults at zero).
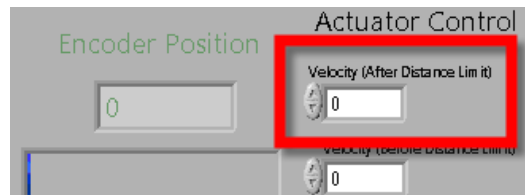
Figure 48: Velocity (After Distance Limit)

13. Set the **Velocity (Before Distance Limit)** control to a positive number (e.g. +1).
14. Set the **Velocity (After Distance Limit)** control to a positive number (e.g. +1). The actuator well then move up at the **After Distance Limit** velocity, reach past the encoder threshold, and continue at the **Before Distance Limit** velocity.
15. Once the actuator reaches a suitable position, set both velocities to zero and terminate the program by flipping the **Data Switch**.
16. The data file will now be available in your Sample ID folder.
17. Repeat steps 1 through 18 for each sample and trial, ensuring that you change the **data file** for each new trial and the **Sample ID** for each new sample

## Appendix XII LabVIEW Overview
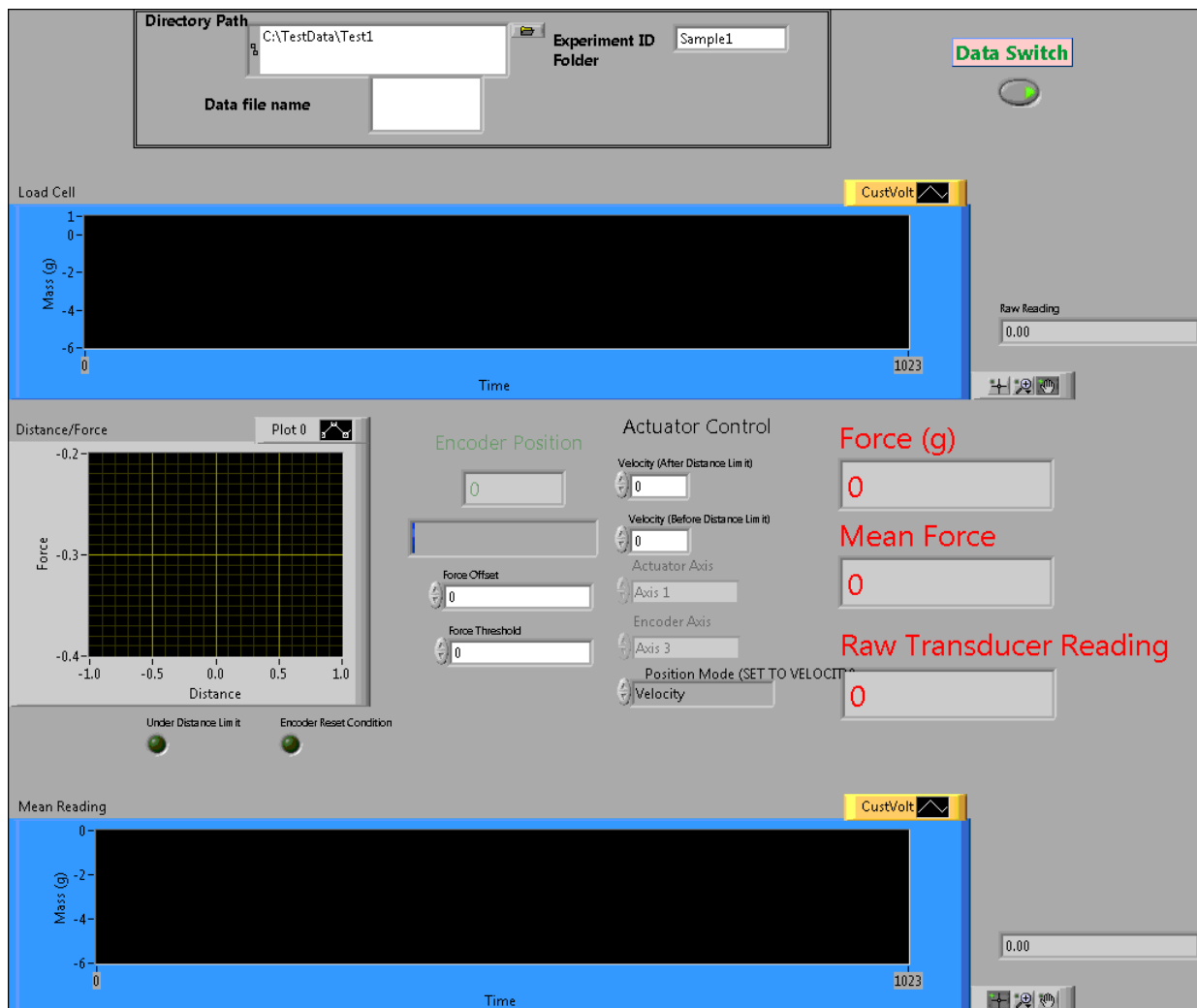
Figure 49: The LabVIEW Front Panel

Data collection and manipulation of all of the device's mechanical functions was performed through a custom virtual instrument (VI) coded with National Instruments' LabVIEW language and programming suite. All of these program operations were conducted on a Dell Optiplex desktop computer. The program was essentially tasked to perform four functions:

1. Control the motion of the linear actuator
2. Collect data from the linear encoder and force transducer
3. Provide a feedback mechanism so that the motion of the actuator is modulated by the data from the encoder and transducer.

4. Organize and export the data in a tab delimited text file that can later be read and analyzed with other software (e.g. MATLAB and Microsoft Excel).

Function # 1 was accomplished through the use of National Instrument's stepper motion control package. This package involved two components: a motion controller and an external power drive. With this design, an NI PCI-7334 was used as the motion controller while an NI MID-7604 was used as the power drive. Using the built in functionality of the NI Motion package, motion of the actuator can be controlled within LabVIEW and this control is conducted through two velocity inputs on the VI. The two inputs provide cases for when the device has traveled under and over 3 mm. With this "dual velocity" configuration, users are able to customize how fast the device indents as well as how fast it retracts after the 3 mm mark has been reached.
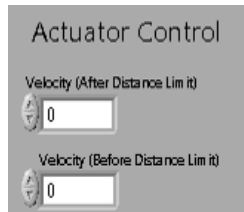
Figure 50: The two velocity controls on the LabVIEW VI

Function #2 was accomplished through both the motion package (in the case of reading encoder data) and a data acquisition package in the case of the force transducer. The NI-DAQ PCI card was used to read parse data from an SC-2345 connector block outfitted with an SCC-SG24 strain gauge. With this data acquisition system, data from the force transducer was received by the strain gauge, conditioned through the SC-2345 signal conditioner, and read by the VI through the PCI card.

Function #3 was performed entirely through custom coding the LabVIEW VI. Using a series of loops and conditional statements (e.g. If distance is greater than 3, switch velocity modes), a complete feedback system was established that allows the user to use the program in a way that best suits the needs of their study. Finally, it is important to note that since there was a significant amount of signal noise in the data from the force transducer, a "mean" operation was applied to minimize noise and that signal was used in each of the conditional statements (data from the transducer was still recorded without this mean operation being applied).

Finally, Function #4 was accomplished through LabVIEW's own data array and export tools. Data parameters including time, encoder position, and force reading were parsed into an array which divided each value in a tab-separated list. This data collection would continue until the program is cancelled by the user.

Appendix XIII Protocol for Fabrication of Fibroblast Populated Collagen Gels

Materials:

5 mg/ml collagen solution
5x DMEM (Dulbuccos Modified Eagles Medium)
0.1 M NaOH
ATCC fibroblasts (Passage 5-9)
DMEM with 10% FBS
Trypsin
Culture plate (specific type depends on experiment)

CHECK ALL VOLUMES BEFORE START (MEDIA, NaOH, TRYPSIN, ETC!!!!)

Set up sterile petri dishes with foam anchors, pre-wet foam anchors, aspirate off media.

Aliquot and label the proper amounts (see spreadsheet) of the following, place in ice:

To figure proper volumes of solution needed for one experiment, use the excel spread sheet (Collagen gel calculation spreadsheet).

    a.   Collagen at 5 mg/ml (see preparation of collagen solution protocol) measure the necessary volume using a syringe **- syringe**
    b. 5X DMEM (see preparation of 5x DMEM protocol) – **15mL conical tube**
    c. 0.1 M NaOH **– sterile eppendorf**

To obtain fibroblasts cells from T-150 flasks:

-   **Put DMEM in bath so that it begins to warm**

1. Remove T-150 plates from incubator, look at under microscope to ensure viability and 80-90% confluency, then perform the following in sterile hood.
Note: if cells are over 90% confluent, they should not be used. Cells change morphology at this density, which will alter the behavior of the cells.

2. Aspirate media of **all 8 flasks (note, should not be left more than 3 minutes before trypsin added)**
3. Add **10 mL** Trypsin with EDTA **to all 8 flasks**

4. Place each plate in the incubator, let sit for 5-10 minutes until cells have become unattached (there should be a yellowish cloudy appearance to the solution), **swirl every few minutes to make sure cells are detached**

5. Add **10 mL** of warm media (1X DMEM, 10% FBS with P/S) to each plate to deactivate the trypsin.
6. Remove the liquid from the flask with a pipette, place into a 50 ml conical tube and centrifuge the cell solution at 1200 rpm for 6 minutes.
- There will be 8 flasks. There should be 20mL liquid per flask (trypsin + media). Put 2 flasks worth of liquid in each 50mL conical tube, this will make 4 conical tubes.
7. Aspirate supernatant from tubes being careful not to disturb the pellet of cells in bottom.
- **Turn tube on side and do not cross line towards bottom, this ensures that no cells will be sucked in.**
8. Resuspend the cells in **2mL DMEM with 10% FBS** noting the volume of liquid in ml for cell count.
- **To do this, add 2mL to the first tube, mix VERY VERY VERY well with a micropipette. Remove from first tube and add to second. Mix VERY VERY VERY well and repeat until there is just one tube containing all the cells.**
9. Mix the solution well and remove **100 ul of the cell-media solution from the <u>center</u> of the conical tube** (this ensures an appropriate representation of the cell solution), and place in a 1-2 ml microcentrifuge tube. **Add 100 ul of Trypan blue** dye to the microcentrifuge tube and **mix well with micropipette** (do not vortex, this will kill the cells). **Place ~10 □ l of this solution into the hematocytometer and perform a cell count.**
- **Should count approximately 200 cells. Should have 200 cells in the first row. Make sure there is no overflow when loading!! (will cause movement of cells which is NOT good for cell count)**

Cell Count Calculations

cells/ # squares x 2 x 10^4 = **cells/mL**

- At this point evaluate # (multiply by mL) Do you have enough cells?

Go to spreadsheet. Want 1.07E7 cells. Divide by **cell/mL =** mL cell solution.

10. Add media to the tube in order to bring the cell concentration (cells/ml) to that listed on the Collagen Gel Calculation Sheet.

- **Bring cells to "Volume DMEM w/10% FBS +cells" from spreadsheet**

11. Place the cells on ice immediately

Get Angie to replate the cells.

And aliquot proper amounts for gel fabrication (per calculation spreadsheet). Proceed to next header OR

b. For continued cell culture:
    i. Resuspend cells with 5 ml of DMEM with 10% FBS
    ii. Place cells in a T-150 flask. For cell passage, each T-150 flask should have 25 ml of DMEM with 10% FBS, p/s, and amp B and 1×10^6 cells
    iii. Slide flask in a figure 8 motion
    iv. Place in incubator.

To fabricate gels:

NOTE: If a large volume of collagen gel solution is required, it may be advantageous to make 2 batches of gels to prevent the solution from polymerizing before all of the gels are plated.

Do in three sets, one for each batch so gels do not polymerize

7. First add 5X DMEM, 0.1M NaOH, and FBS to an appropriate sized conical tube mix well and then add the <u>appropriate cell volume last</u> (refer to calculation spreadsheet). Note: Since the DMEM-NaOH solution is very basic, it is **important that cells are added last to minimize the time in this solution**. **Make sure cells do not sit or cells will die!! (aka add collagen and mix right away)**
8. Using a syringe, add the collagen to the aliquot of cell-media-5X DMEM-NaOH mixture, mix well with pipette until color is homogonous throughout, taking care not to add air bubbles to the mixture.
9. Quickly add the desired volume of the collagen-cell solution to each well. Aspirate any bubbles off the top and swirl gently to assure solution is level and covers entire plate.
10. Place gels in 37° OVEN for 1 hour.
11. Add 2 ml of media to each well (for 6-well plate) and place in incubator. Make sure it covers the gels.
12. Check gels on Saturday, after that they should be good til Monday.

Appendix XIV Thickness Testing Protocol

Materials:

- Collagen gels
- Membrane inflation device

1. The membrane inflation device was calibrated to take thickness measurements. To do so a thin metal disk was placed on the membrane inflation device and the height of device was adjusted. The following is a table of the calibration values:

Device Reading (mm)	Zeroed Displacement (mm)	Voltage (V)
15.86	0	0
15.36	0.5	0.172
14.86	1.0	0.347
14.36	1.5	0.520
13.86	2.0	0.691
13.36	2.5	0.858
12.86	3.0	1.010

2. The voltage vs zeroed displacement was plotted and the data was fitted by the trend line. The calibration equation had an R^2 value of 0.996 and is as follows:

$$Thickness = 29487 \times Voltage - 15.623$$

3. After samples were tested through transverse loading the sample was removed the circular clamping device, returned to media in the 6-well plate and transported to the main lab area where the membrane inflation device is located.

4. The small metal disk was placed on the center of the membrane inflation device with forceps to ensure the voltage reading was zero and then removed.
5. The gel was removed from the well and placed on the surface of the membrane inflation device. The small metal disk was placed with forceps on the center of the gel. The disk was allowed to equilibrate for 2 minutes and a reading was taken.
6. Steps 3-5 were repeated for all samples. The voltage readings were then converted to thickness readings using the calibration equation.

Appendix XV FEM Data

FEM was used to predict the displacements which would result from applying various forces to the gel. Forces of a step size of 1mN were used.

Table 25: FEM Data calculated for 3.0 mg/ml Circularly Clamped Collagen Gel

Force (mN)	Dispacement (mm)
1	0.094264
2	0.18808
3	0.28102
4	0.3727
5	0.46277
6	0.55097
7	0.63707
8	0.72092
9	0.80242
10	0.88152
11	0.95822
12	1.0325
13	1.1044
14	1.1741
15	1.2415
16	1.3068
17	1.37
18	1.4313
19	1.4907
20	1.5484
21	1.6043
22	1.6587
23	1.7115
24	1.7628
25	1.8128
26	1.8614
27	1.9089
28	1.9551
29	2.0001

30	2.0441
31	2.0871
32	2.1291
33	2.1701
34	2.2103
35	2.2496
36	2.2881
37	2.3257
38	2.3627
39	2.3989
40	2.4344
41	2.4693
42	2.5035
43	2.5371
44	2.5701
45	2.6025
46	2.6344
47	2.6658
48	2.6967
49	2.727
50	2.7569
51	2.7863
52	2.8153
53	2.8439
54	2.872
55	2.8997
56	2.9271
57	2.9541
58	2.9807
59	3.0069

Appendix XVI Circularly Clamped Collagen Gel MATLAB Data
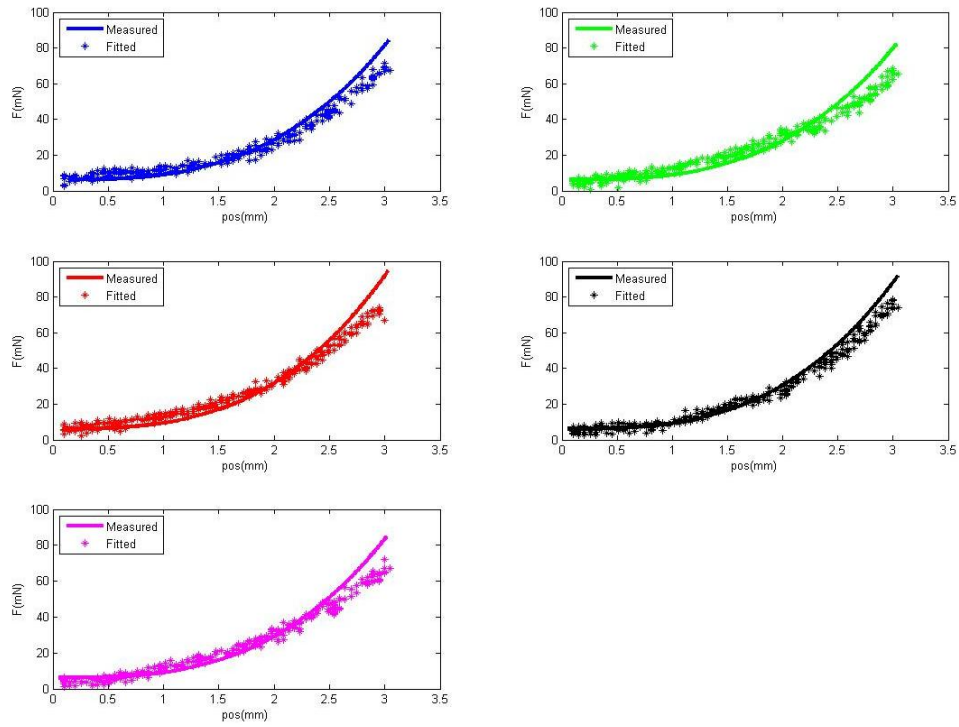
Figure 51 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 1
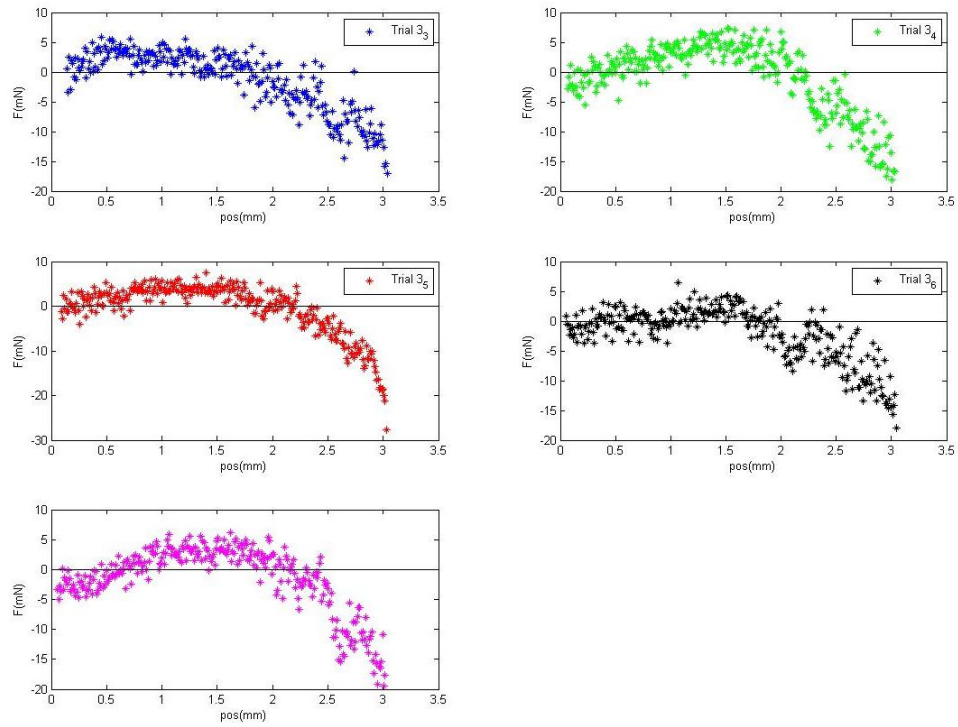
Figure 52 Residuals of 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 1
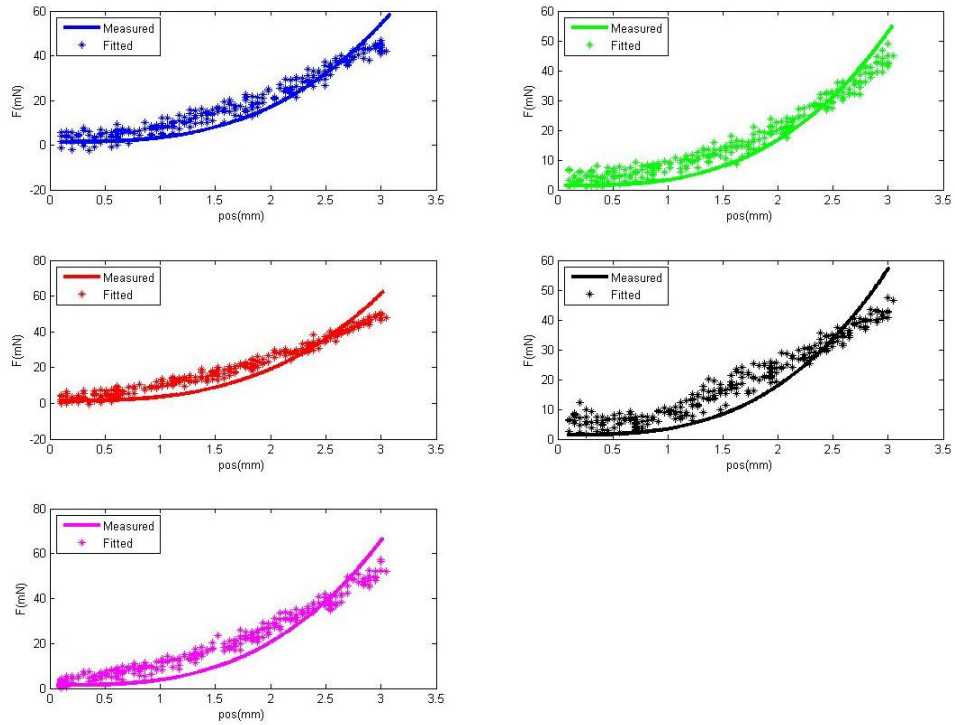
Figure 53 1.75 mg/ml collagen concentration data (04/04/08) fitted with Equation 1
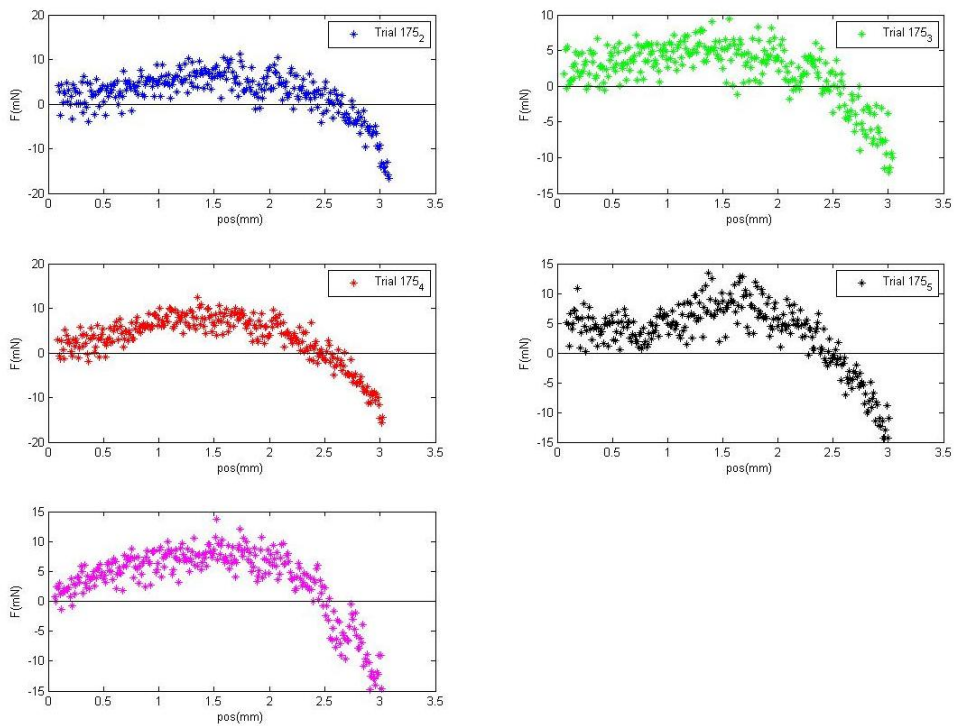
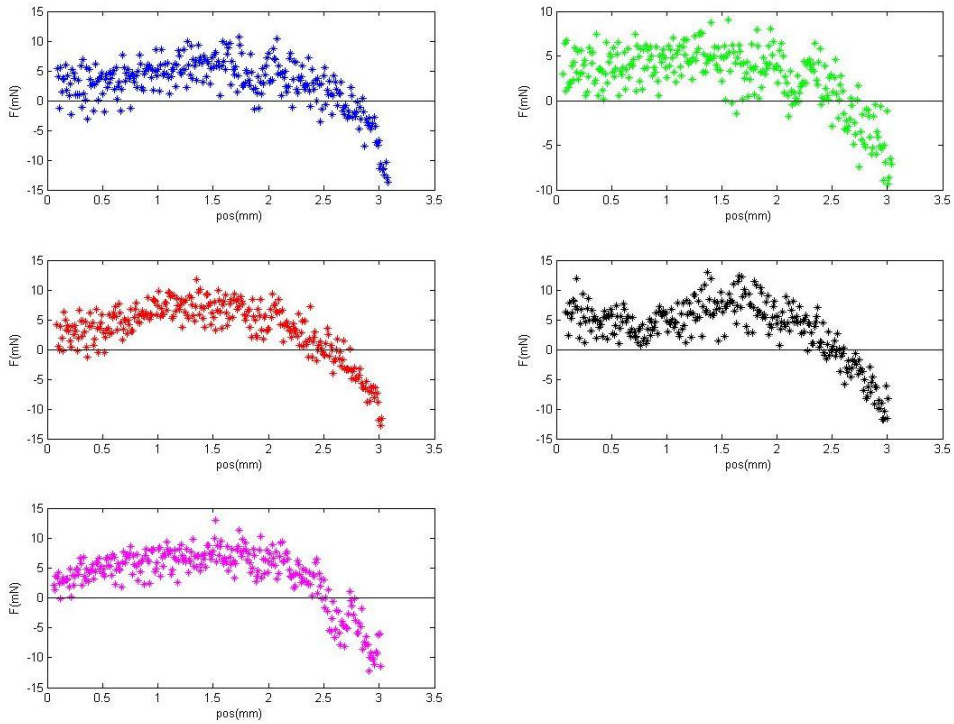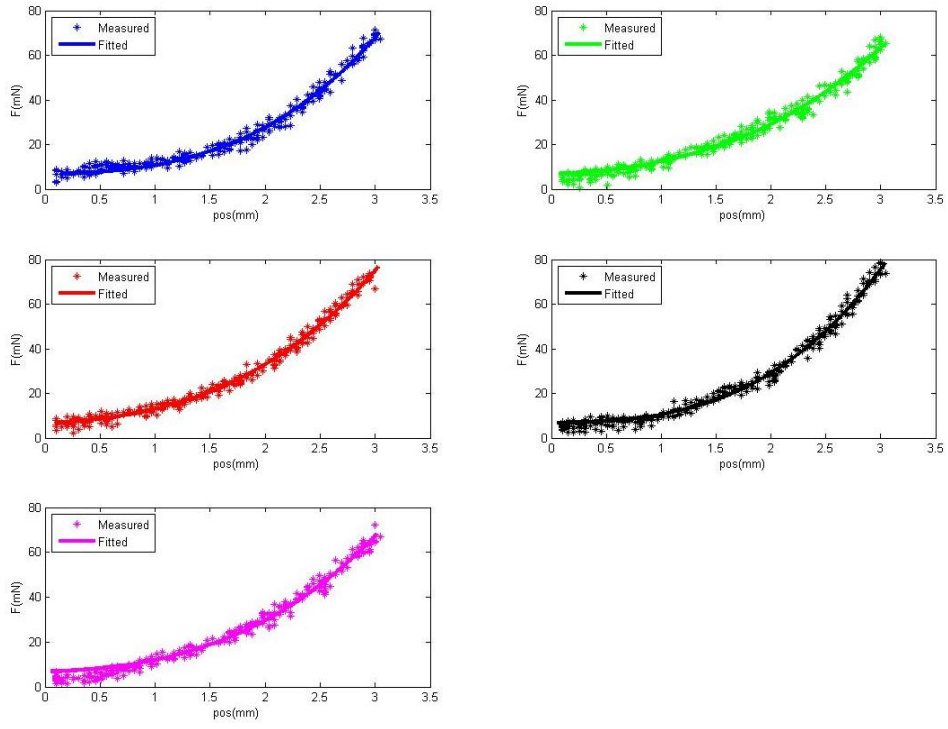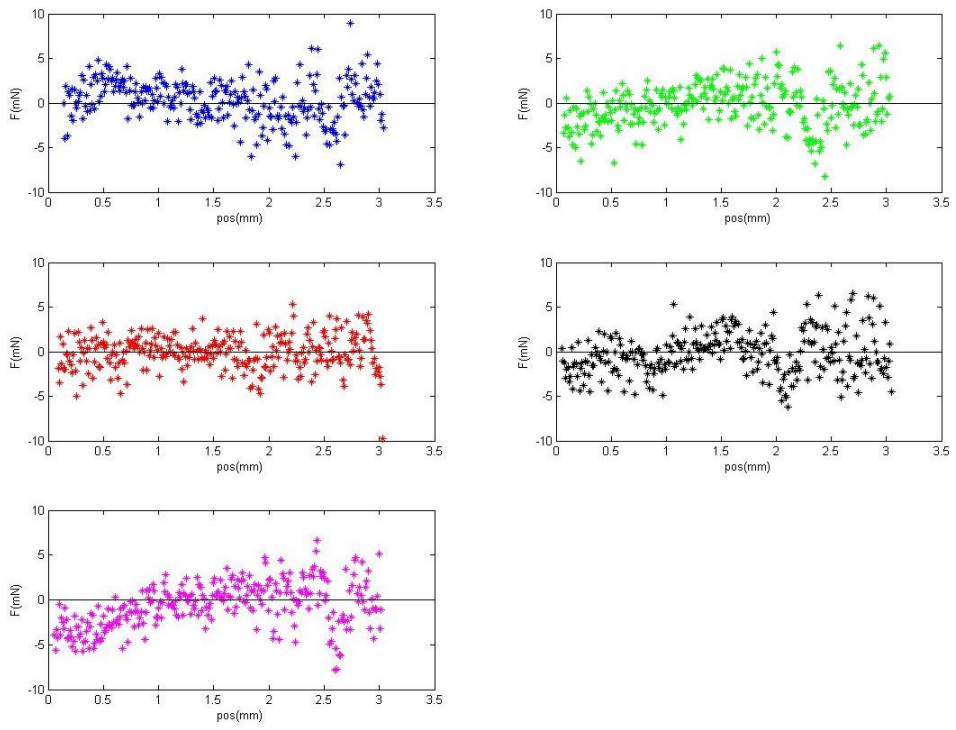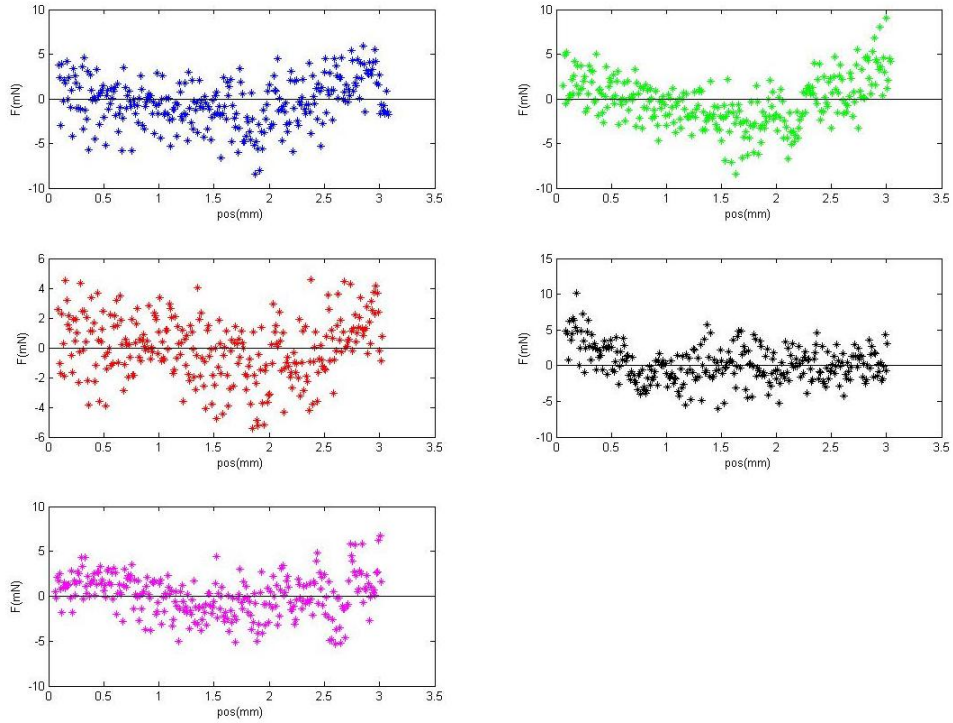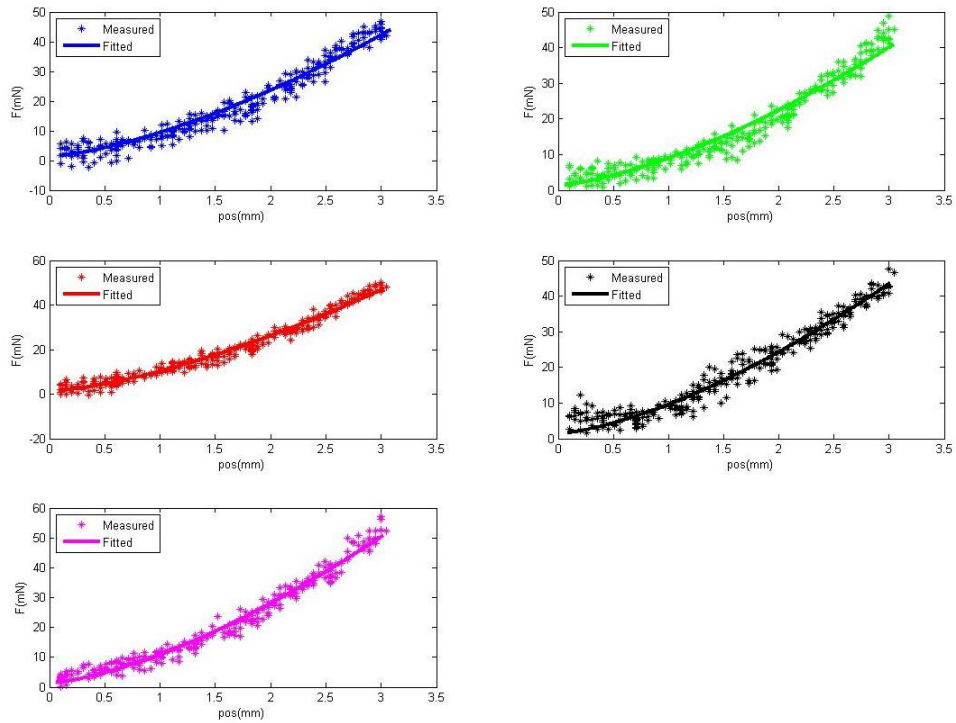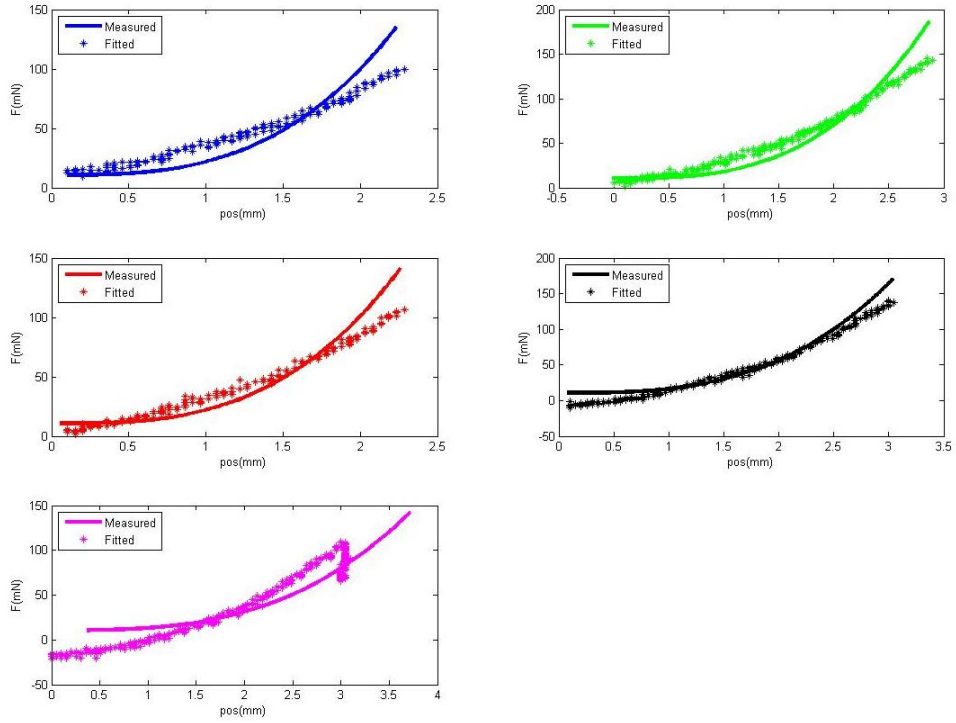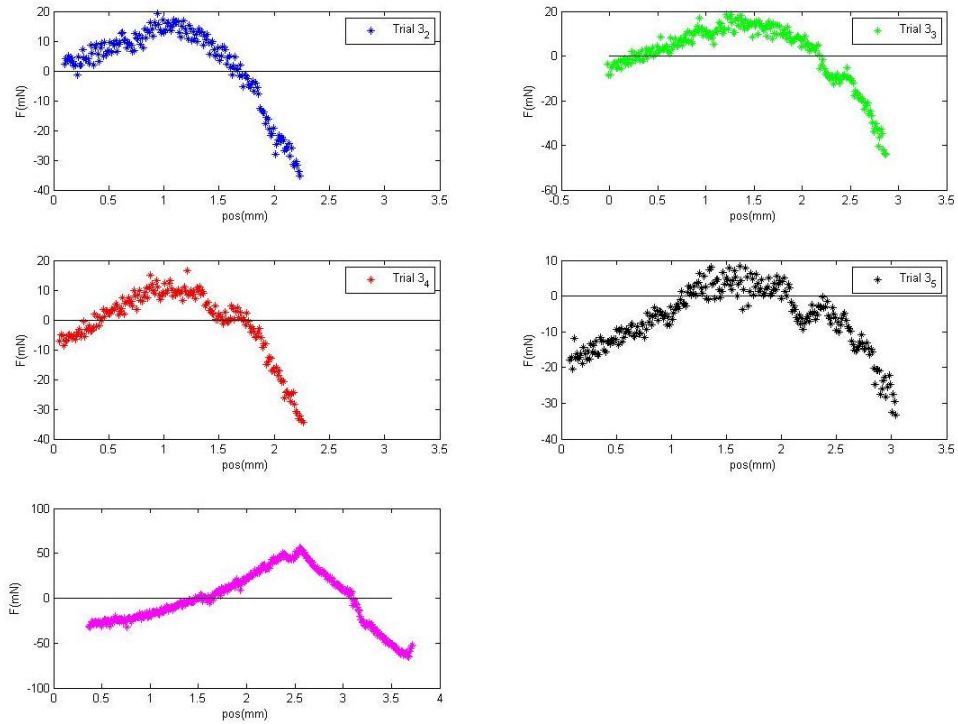**Figure 54 Residuals of 1.75 mg/ml collagen concentration data (04/04/08) fitted with Equation 1**
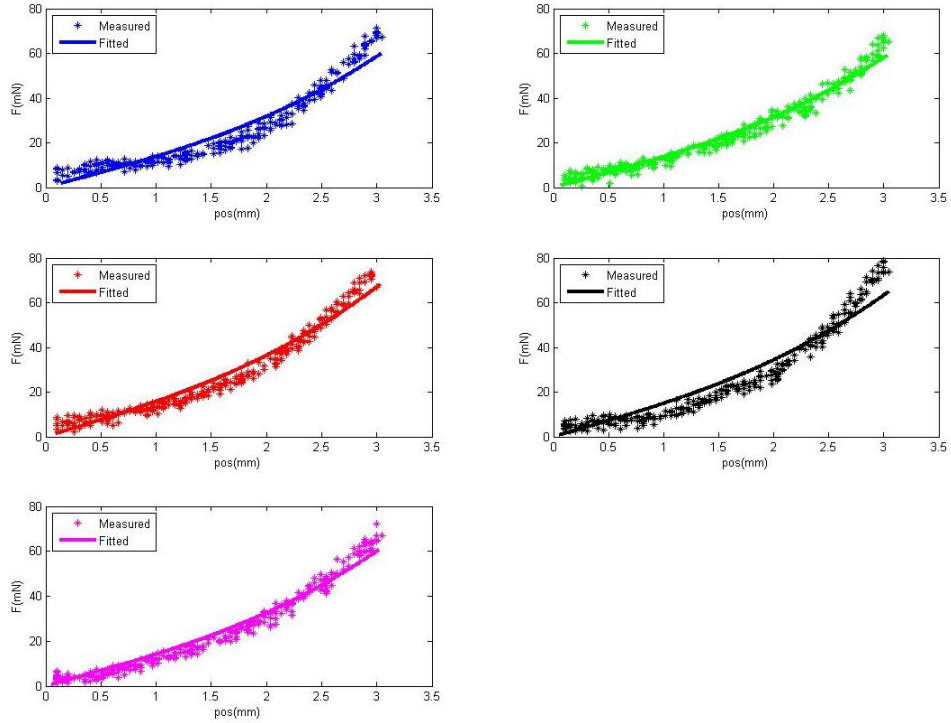
Figure 55 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 3
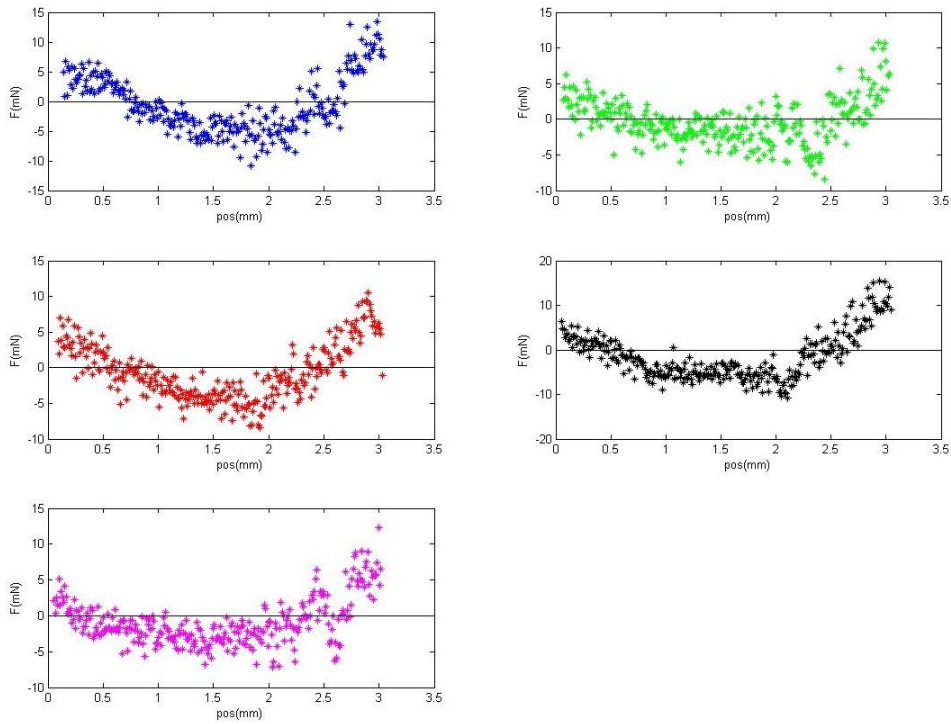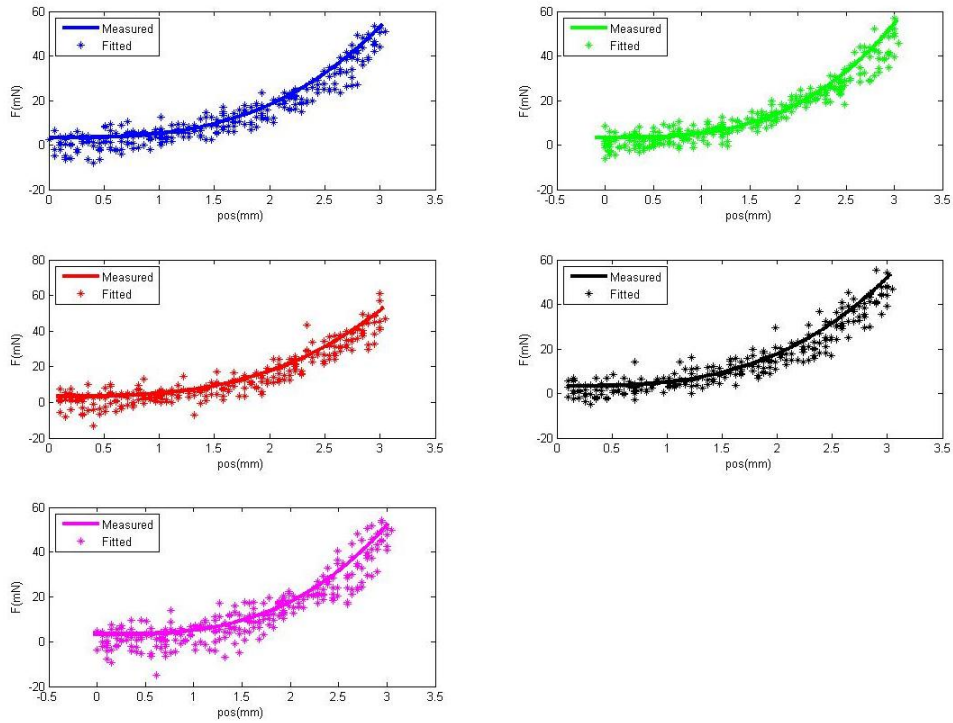

Figure 56 Residuals of 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 3
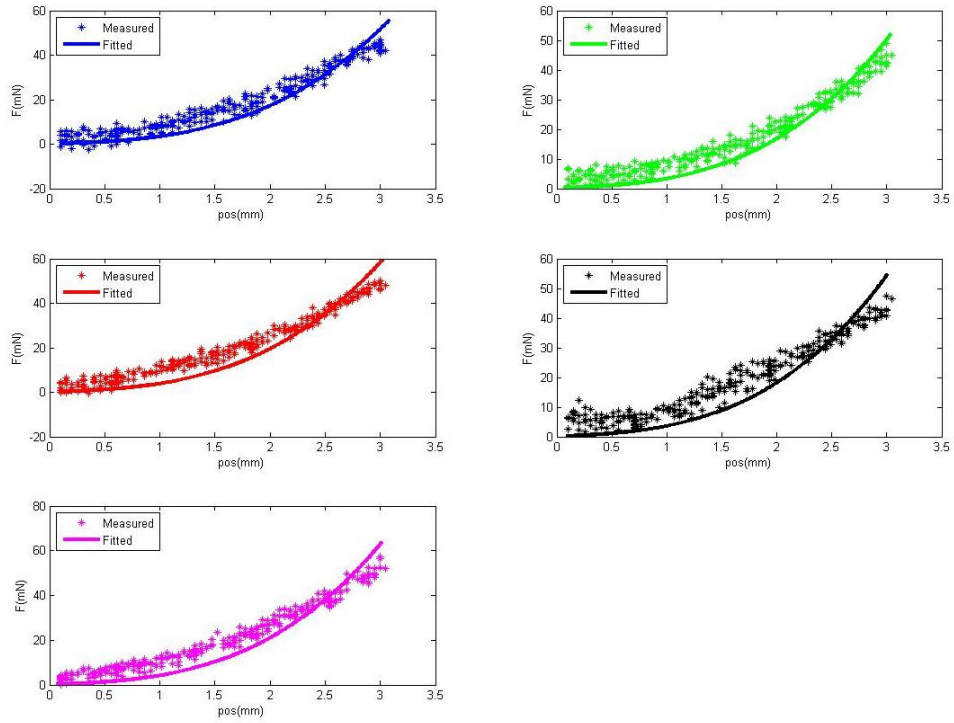
Figure 57 1.75 mg/ml collagen concentration data (04/04/08) fitted with Equation 3

Figure 58 Residuals of 1.75 mg/ml collagen concentration data (04/04/08) fitted with Equation 3
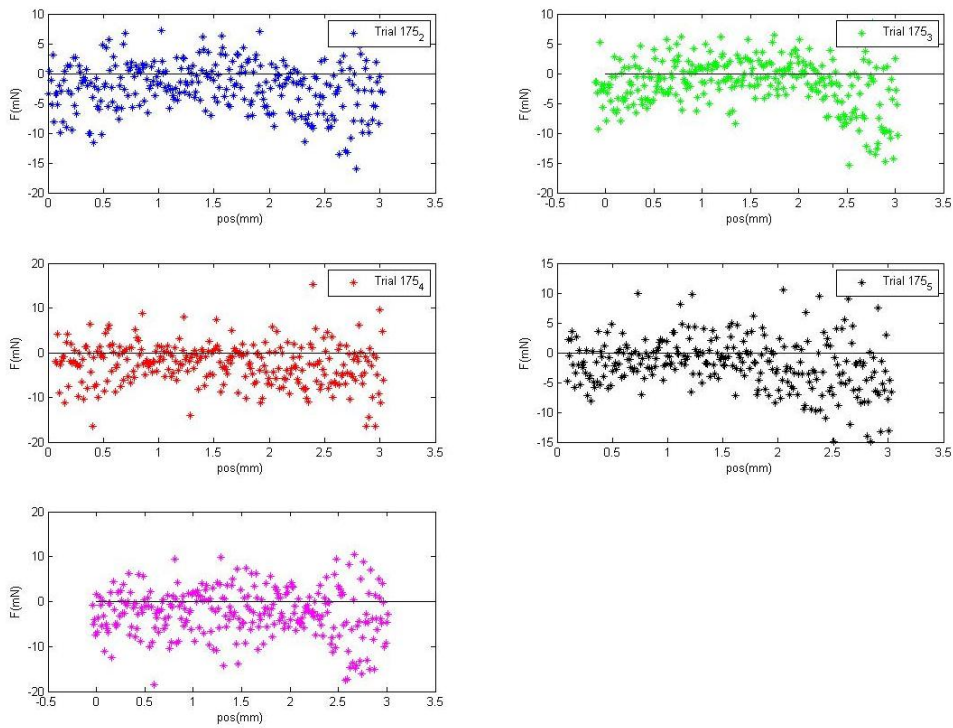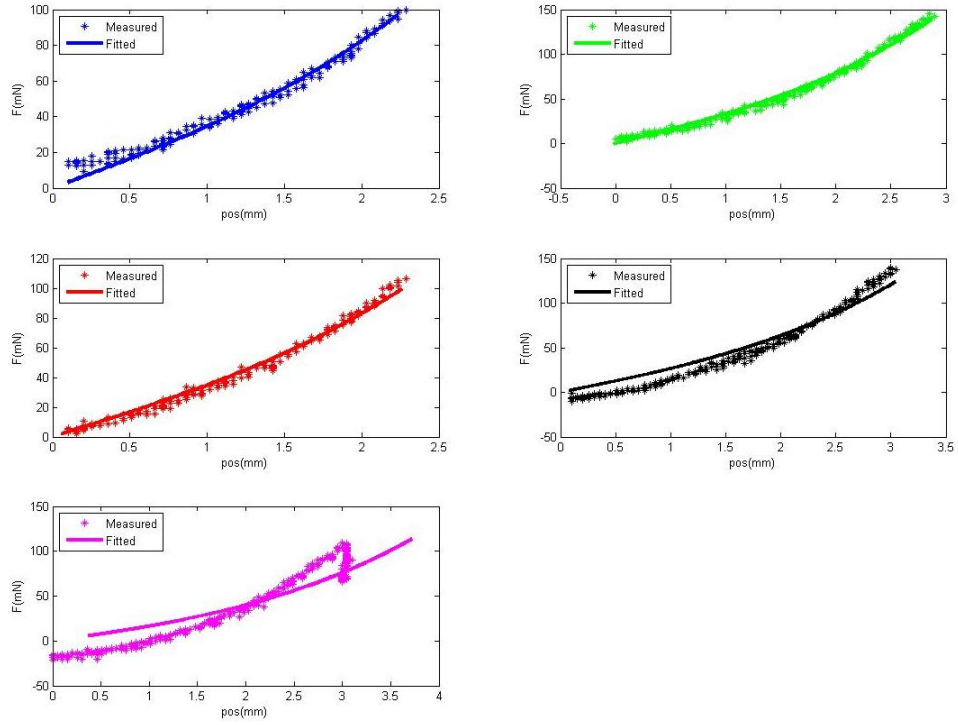
Figure 59: 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 2

Figure 60: Residuals of 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 2

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Figure 61: 1.75 mg/ml collagen concentration data (04/04/08) fitted with Equation 2

Figure 62: Residuals of 3.00 mg/ml collagen concentration data (04/04/08) fitted with Equation 2

Figure 63: 3.00 mg/ml collagen concentration data (04/01/08) fitted with Equation 1

Figure 64: Residuals of 3.00 mg/ml collagen concentration data (04/01/08) fitted with Equation 1
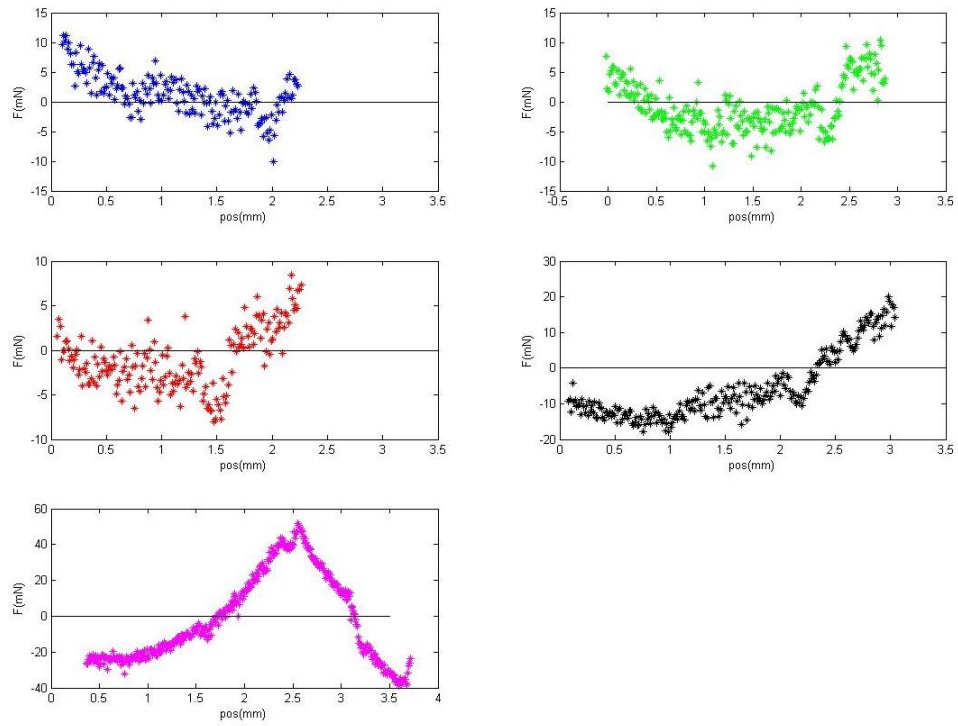
Figure 65: 1.75 mg/ml collagen concentration data (03/31/08) fitted with Equation 1

Figure 66: Residuals of 1.75 mg/ml collagen concentration data (03/31/08) fitted with Equation 1
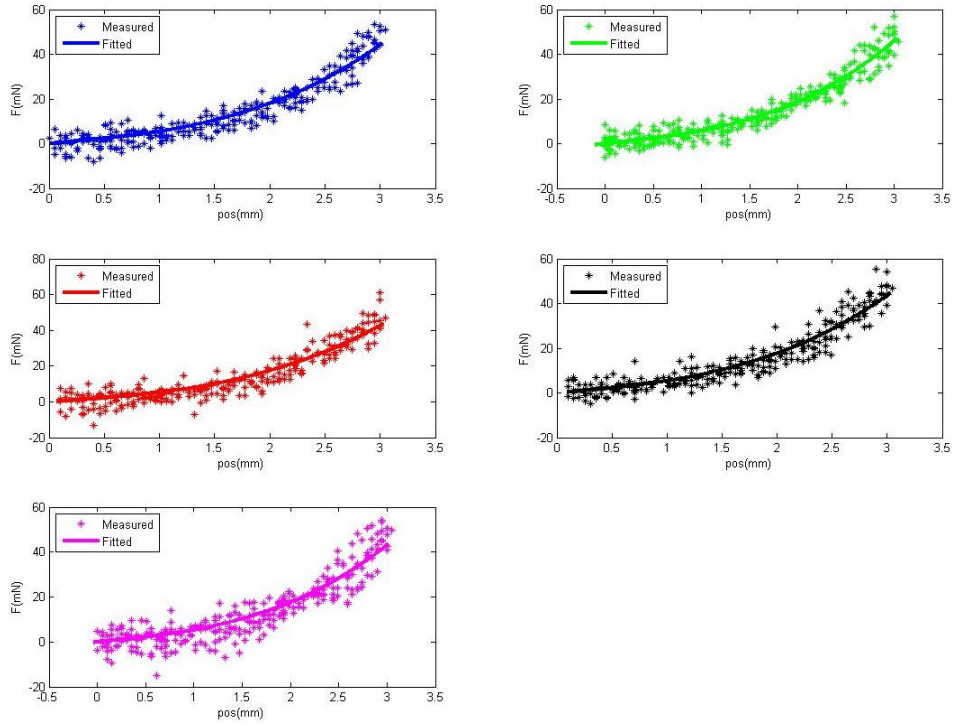
Figure 67: 3.00mg/ml collagen concentration data (04/01/08) fitted with Equation 3
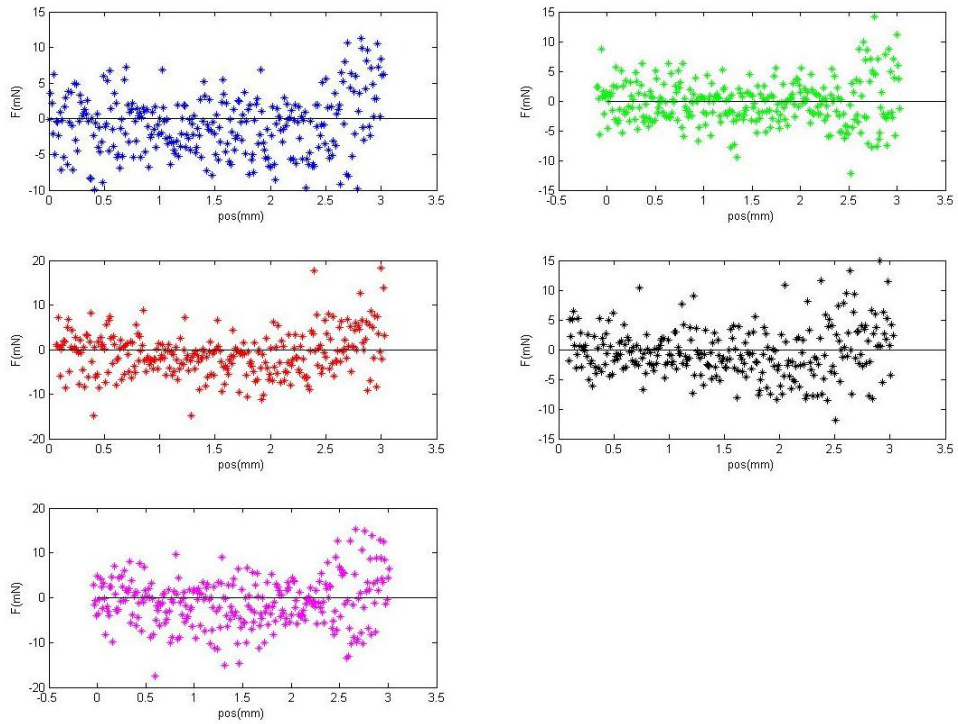

Figure 68: Residuals of 3.00mg/ml collagen concentration data (04/01/08) fitted with Equation 3

Figure 69: 1.75mg/ml collagen concentration data (03/31/08) fitted with Equation 3

Figure 70: Residuals of 1.75mg/ml collagen concentration data (03/31/08) fitted with Equation 3
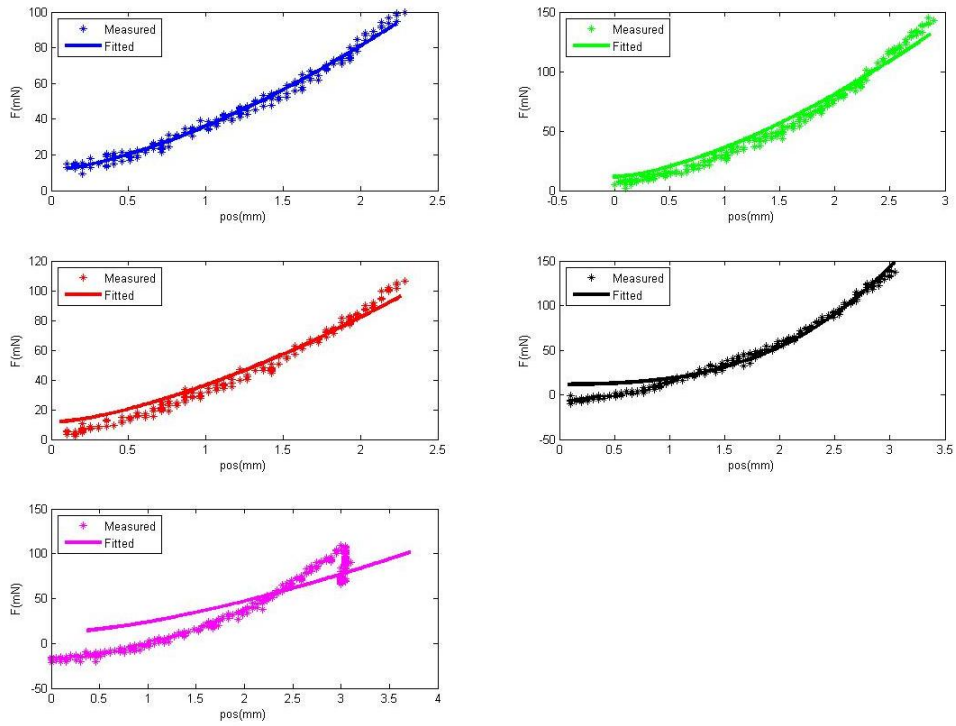
Figure 71: 3.00 mg/ml collagen concentration data (04/01/08) fitted with Equation 2
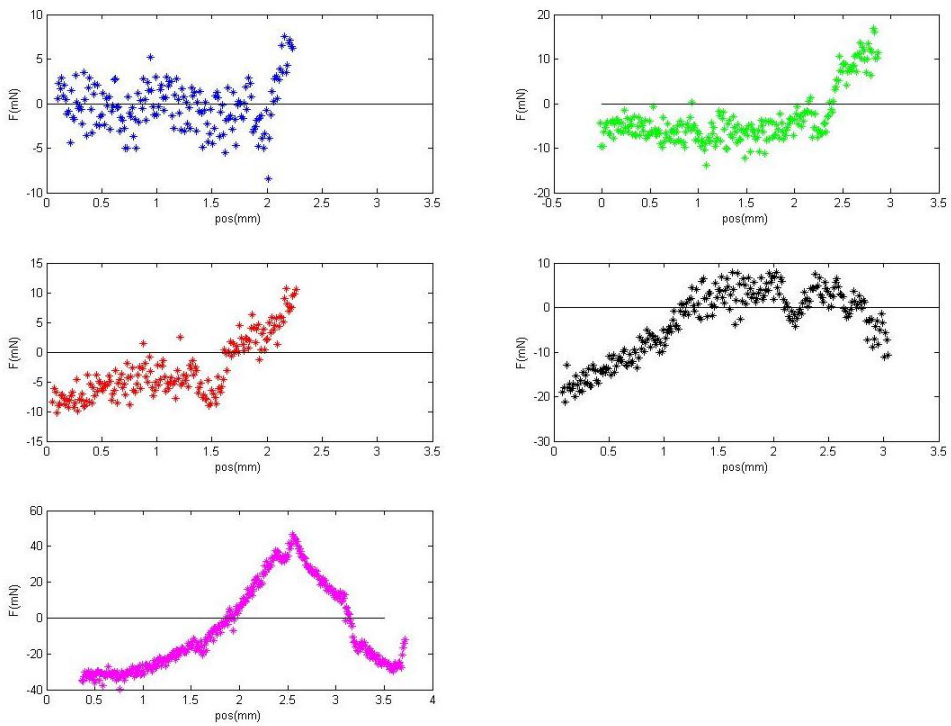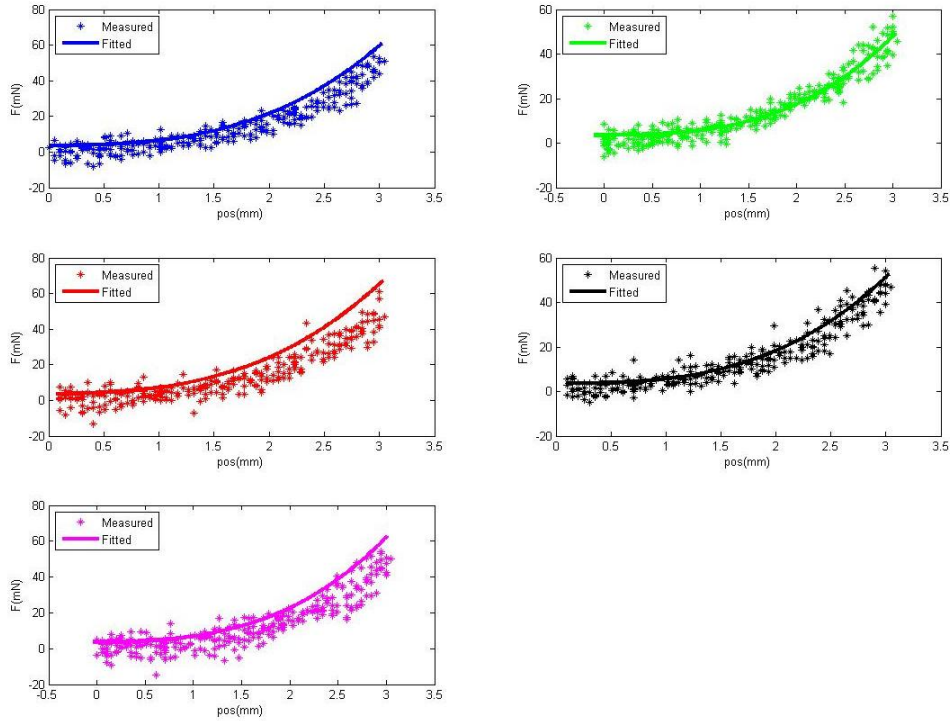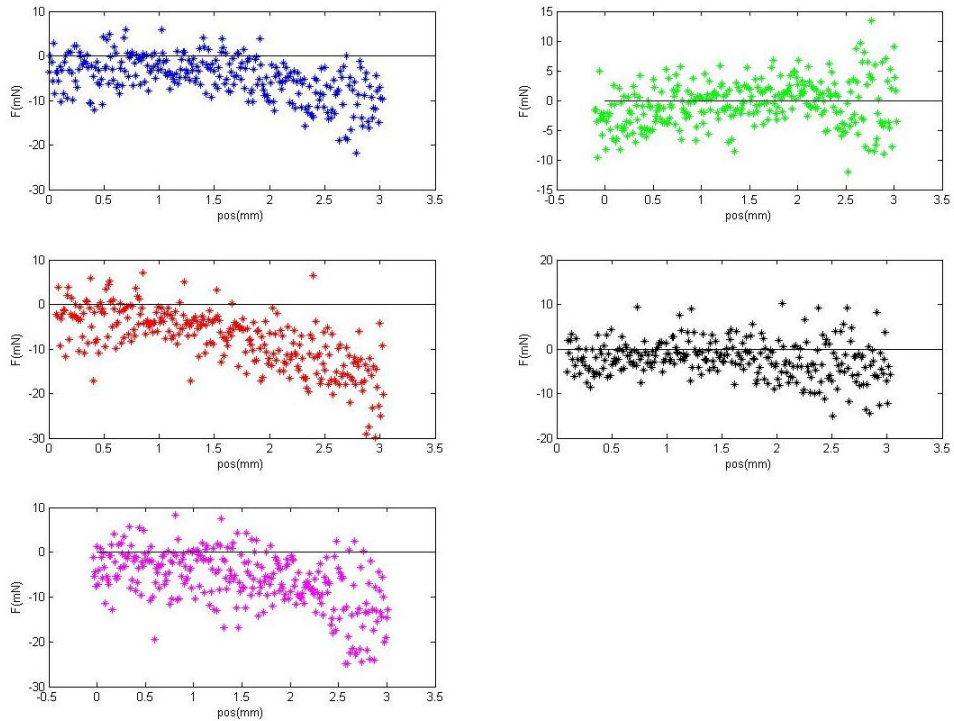

Figure 72: Residuals of 3.00 mg/ml collagen concentration data (04/01/08) fitted with Equation 2

Figure 73: 1.75 mg/ml collagen concentration data (04/01/08) fitted with Equation 2

Figure 74: Residuals of 1.75 mg/ml collagen concentration data (04/01/08) fitted with Equation 2

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