Title
Mechanical Characteristics of a Polymer Spring Device used to Lengthen Small Intestine

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Mechanical Characteristics of a Polymer Spring Device used to Lengthen Small Intestine

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science in

Bioengineering

by

Douglas J. Steinberger

2014

ABSTRACT OF THE THESIS

Mechanical Characteristics of a Polymer Spring Device used to Lengthen Small Intestine

by

Douglas James Steinberger

Master of Science in Bioengineering

University of California, Los Angeles, 2014

Professor James C. Dunn, Chair

Short Bowel Syndrome (SBS) is a condition that occurs due to an insufficient amount of small intestine needed for nutrient absorption and water regulation of the body. A compression spring device is being developed in order to provide a mechanical stimulus to the tissue, as this type of force has been shown to promote lengthening of the tissue. The research completed in this thesis investigated the mechanical characteristics of the spring device and attempted to relate it to the functionality in rat and porcine intestinal tissue.

Results from the evaluation of the springs show that Poly(ε-caprolactone), or PCL, is a sufficient polymer to use for creating a biodegradable device as the spring dimensions can be adjusted through variations in the diameter, thickness, and band size in order to provide an adequate spring constant for multiple animal types. Design of the springs, however, need to take

into account the size of the gelatin capsule used, the amount of plastic deformation and creep behavior of the spring under compression for an extended time period, and the variation in the mechanical properties of the animal soft tissue that requires lengthening.

Integration of the spring in-continuity requires a feature that will provide a mechanical resistance to force that is greater than the force of the spring in the compressed state. The spring still requires further development and any design should also take into account the possibility of intestinal perforations or obstructions. The polymer spring device provides a good means towards developing a treatment option for SBS, and other potential soft tissue lengthening needs of the body.

The thesis of Douglas James Steinberger is approved.

Benjamin M. Wu

Min Lee

James C. Dunn, Committee Chair

University of California, Los Angeles

2014

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INTRODUCTION

Overview

The focus of regenerative medicine is to promote the growth or regeneration of human tissues in order to establish normal functionality. Scientists, engineers, and other medical professionals have utilized biomaterials, biomolecules, and cells in order to accomplish this for various areas of the body such as skin, bone, cardiac, renal, and hepatic tissues. Due to the intricacy of thicker tissues, mainly in terms of revascularization, cell quantities needed, and a variety of interconnected cell layers, it is nigh impossible to recreate complex functionality with current tissue engineering technology and techniques. To work through these issues, regenerative medicine often times uses processes within an organism to promote improving functionality for itself.

The small intestine is one such tissue that is made up of various small layers, which need to work together to provide adequate functionality. The small intestine is required for nutrient absorption and water regulation of the body, while providing peristaltic functionality to its contents. Loss of this functionality due to trauma, congenital defects, or other means is termed as Short Bowel Syndrome (SBS) as symptoms typically appear with greater than 70% bowel loss. (Vanderhoof & Langnas, 1997) Treatment consists mainly of parenteral nutrition, but can also include surgical options such as intestinal transplantation or procedures that increase length and slow down transit time. (Shekherdimian, Panduranga, Carman, & Dunn, 2010) These treatment methods, however, also provide a risk of complications such as immune system responses and liver failure.

Another SBS treatment option is being investigated in order to create lengthened intestinal tissue through means of mechanical enterogenesis. Similar to the concepts of distraction osteogensis being used to lengthen bone, various research groups have investigated lengthening via saline injections, screw advancement, hydraulic piston usage, and spring expansion. These methods show promise, but also have limitations including multiple surgical interventions, incorporating a device partly outside the abdominal cavity, unidirectional force application, and integration into the host tissue. (Shekherdimian, Panduranga, Carman, & Dunn, 2010) Solving the issues of a mechanical lengthening device may not provide a universal treatment for all patients experiencing SBS, but will provide an additional treatment method for patients that currently have options representing limited success rates.

Objectives

The objectives of this research project is to further advance the development of the spring device previously mentioned. The design of the spring has recently switched from utilizing a nickel titanium (Nitinol) material to using a biodegradable polymer material that is commonly used in sutures. This switch allows for the removal of the guide wire used to prevent buckling of the metal device in vivo, and also to prevent intestinal perforations that occurred due to sharp ends of the implanted items. The biodegradability of the polymer material provides a route to treatment through a single surgical procedure as it is expected for the device to be implanted in-continuity in the intestine, provide a force to lengthen the tissue, and pass through the gastrointestinal system after degradation of the polymer spring.

The mechanical properties will be evaluated on the polymer materials initially used to manufacture the spring device. Along with the material property information, evaluation of various spring dimensions and gelatin capsule deployment characteristics will be analyzed. Material properties of rat and pig intestinal tissue will be analyzed to understand similarities of various animals, and also to attempt to scale the spring design to an animal model similar to that of pediatric patients. Finally, integration of the spring into the host tissue will be investigated to better understand features needed for implantation in-continuity and any complications that may arise.

MATERIALS and METHODS

Polymer Material Property Testing

Materials:

PLLA or Poly(L-lactide) from Durect Lactel: Inherent Viscosity of 0.90-1.20

PCL or Poly(ε-caprolactone) from Durect Lactel: Inherent Viscosity of 1.00-1.30

$CHCl_3$ or Chloroform from Fisher Scientific

PTFE or Teflon mandrel from McMaster-Carr

LatheBot equipment run with Labview Program

VLS 2.30 laser cutter from Universal Laser Systems

Mechanical Load Frame from Instron: Model Number 5564

Methods:

1. Measure and combine the following materials into a sealable glass vial:

Mixture	Amount PLLA (g/100g mixture)	Amount PCL (g/100g mixture)	Amount $CHCL_3$ (g/100g mixture)
0% PLLA	0	3.5	96.5
25% PLLA	0.875	2.625	96.5
50% PLLA	1.75	1.75	96.5
75% PLLA	2.625	0.875	96.5
100% PLLA	3.5	0	96.5

Add paraffin film to the outside of the cap on the closed vial to ensure seal. Mixtures will be 3.5% wt. polymer to chloroform.

2. Place the polymer/chloroform mixture onto a rotator for at least 12 hours.
3. Spray a 10mm mandrel to a thickness of 0.3mm and a plateau length of at least 15mm. Complete spraying using LatheBot equipment and methods detailed in Appendix A.
4. After spraying, allow the polymer tube to dry for at least 6 hours.
5. Cut the tube from the mandrel with a razor along the long axis of the mandrel.
6. Flatten the tube by inserting the polymer between two smooth plates and placing in an oven at 50ºC for 1 hour. It is recommended that weight be added to ensure flattening.
7. Keep the plates (and weights) sandwiching the polymer and allow it to cool to room temperature for at least 30 minutes.
8. Set up the VLS 2.30 laser cutter to a power setting of 10%, speed of 5%, and pulses per inch (PPI) of 1000.
9. Laser cut “dog bone” samples from the flat polymer film to the dimensions shown in Figure 1. Dimensions shown are in millimeters.
10. Clamp the ends of the polymer “dog bone” samples into the Instron machine at an original separation distance of 10mm.
11. Apply a tensile load to the samples at a rate of 25 mm/min. Record the force and displacement of the samples during testing.
12. Calculate and graph the stress and strain of the samples. Correct for the toe region of the curve, if necessary.

13. Determine the yield stress, maximum load, and elastic modulus from the data obtained. Calculate the shear modulus using an estimated Poisson's ratio.

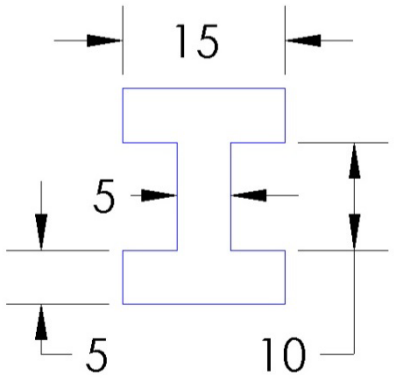

Figure 1 Dog Bone Dimensions (in mm)

Spring Mechanical Testing

Materials:

PCL or Poly(ε-caprolactone) from Durect Lactel: Inherent Viscosity of 1.00-1.30

$CHCl_3$ or Chloroform from Fisher Scientific

PTFE or Teflon mandrel from McMaster-Carr

Stainless steel mandrel from McMaster-Carr

LatheBot equipment run with Labview Program

Mini-LatheBot equipment run with Phidget Controller Program

VLS 2.30 laser cutter from Universal Laser Systems

Mechanical Load Frame from Instron: Model Number 5564

Methods:

1. Measure and combine 3.5% wt. polymer to chloroform into a sealable glass vial. Add paraffin film to the outside of the cap on the closed vial to ensure seal.
2. Place the polymer/chloroform mixture onto a rotator for at least 12 hours.
3. Spray a range of mandrels between 4mm and 8mm to various thicknesses between 0.2mm and 0.75mm and a plateau length of at least 20mm. Complete spraying using LatheBot equipment and methods detailed in Appendix A.

Note: A stainless steel mandrel may be used to spray smaller diameter tubes due to Teflon rod deflection or warping after repeating spraying cycles.

4. After spraying, allow the polymer tube to dry for at least 6 hours.
5. Slide the tube off of the Teflon mandrel and place onto a stainless steel mandrel. If a stainless steel mandrel was used for spraying, loosen the tube from the mandrel. A methanol soak for 20-30 minutes can be used to help remove or loosen the polymer tube.
6. Set up the VLS 2.30 laser cutter to a power setting of 50% for polymer tube thicknesses below 0.5mm and 100% for all other tube thicknesses. Set the pulses per inch (PPI) of 1000. The speed will be set to various rates based upon the desired spring band width.
7. Laser cut spring samples from the polymer tube. Laser cutting was completed using Mini-LatheBot equipment and methods detailed in Appendix B.
8. Lengthen the spring on the mandrel and place it in an oven at 50ºC for 1 hour in order to heat set to an expanded length.

9. Keep the spring on the mandrel and allow it to cool to room temperature for at least 30 minutes.
10. Remove the spring from the mandrel and place into the Instron machine.
11. Apply a compressive load to the samples at a rate of 10 mm/min. Record the force and displacement of the samples during testing.
12. Calculate the spring rate (or spring constant) as the slope of the linear force vs. displacement curve.
13. Graph the relationship of diameter to spring rate, thickness to spring rate, and band size to spring rate.

Encapsulation Deformation Testing

Materials:

PLLA or Poly(L-lactide) from Durect Lactel: Inherent Viscosity of 0.90-1.20

PCL or Poly(ε-caprolactone) from Durect Lactel: Inherent Viscosity of 1.00-1.30

$CHCl_3$ or Chloroform from Fisher Scientific

PTFE or Teflon mandrel from McMaster-Carr

Stainless steel mandrel from McMaster-Carr

LatheBot equipment run with Labview Program

Mini-LatheBot equipment run with Phidget Controller Program

VLS 2.30 laser cutter from Universal Laser Systems

Gelatin capsules from Torpac: Size #5 and Size #000

Methods:

1. Measure and combine 3.5% wt. polymer to chloroform into a sealable glass vial. Add paraffin film to the outside of the cap on the closed vial to ensure seal.
2. Place the polymer/chloroform mixture onto a rotator for at least 12 hours.
3. Spray 4mm and 7mm mandrels to thicknesses 0.3mm and 0.75mm, respectively. Use a plateau length of at least 20mm. Spraying was completed using LatheBot equipment and methods detailed in Appendix A.

Note: A stainless steel mandrel may be used to spray smaller diameter tubes due to Teflon rod deflection or warping after repeating spraying cycles.

4. After spraying, allow the polymer tube to dry for at least 6 hours.
5. Slide the tube off of the Teflon mandrel and place onto a stainless steel mandrel. If a stainless steel mandrel was used for spraying, loosen the tube from the mandrel. A methanol soak for 20-30 minutes can be used to help remove or loosen the polymer tube.
6. Set up the VLS 2.30 laser cutter to a power setting of 50% for polymer tube thicknesses below 0.5mm and 100% for all other tube thicknesses. Set the pulses per inch (PPI) of 1000. Set the laser speed to 2.9% for a 1mm band width on the 4mm mandrel and to 3.7% for a 2mm band width on the 7mm mandrel.
7. Laser cut half of the 4mm spring samples and all of the 7mm spring samples from the polymer tube. Laser cutting was completed using Mini-LatheBot equipment and methods detailed in Appendix B.

8. Use a threaded 4mm mandrel and a razor blade to hand cut the other half of the 4mm springs to a 1mm band width.
9. Freeze-dry half of the 4mm spring samples for 3 hours to remove any excess chloroform. An equal portion of laser and hand cut samples should be lyophilized.
10. Lengthen the spring on the mandrel and place it in an oven at 50ºC for 1 hour in order to heat set to an expanded length.
11. Keep the spring on the mandrel and allow it to cool to room temperature for at least 30 minutes.
12. Remove the spring from the mandrel and measure and record the length of the expanded spring.
13. Encapsulate the 4mm spring samples in in the Size #5 capsules. Remove the springs from the capsule, measure and record the length of the extended spring, and re-encapsulate the spring at time intervals of 3 hours, 6 hours, and 24 hours.
14. Encapsulate the 7mm spring samples in in the Size #000 capsules. Remove the springs from the capsule, measure and record the length of the extended spring, and re-encapsulate the spring at time intervals of 1 hour, 3 hours, 6 hours, 18 hours, 120 hours, 336 hours, and 672 hours.
15. Graph the measured extension length and associated time intervals of the samples.

Biological Tissue Mechanical Testing

Materials:

Rat and Porcine jejunal tissue samples

Formalin 10% buffered from Fisher Scientific

HBSS (1X) or Hank's Balanced Salt Solution from Gibco

ABAM or Antibiotic-Antimycotic from Invitrogen

Mechanical Load Frame from Instron: Model Number 5564

Methods:

1. Resect a length of jejunal small intestine from a deceased animal specimen. Cut away and discard the mesentery tissue.
2. Lay the tissue flat and estimate the circumference by measuring the width of the flat tissue.
3. Cut away a portion of the tissue and prepare for Hematoxylin and Eosin (H&E) staining by inserting into "cassettes" and submerging in formalin for at least 24 hours.
4. Place the rest of the tissue sample in a vial containing 1:100 ABAM in HBSS (1X). Tissue may be stored up to 5 days and must be stored in a refrigerated environment.
5. Send tissue samples for H&E staining and slide preparation after required formalin soaking period.
6. Set the tip of a wide jaw soft tissue forceps at an original separation distance of 10mm from the surface of the pneumatic clamping end of the Instron machine.

7. Clamp one end of the tissue sample not sent for staining into the forceps and the other end into the pneumatic clamp. The tissue should be secured between the clamps with limited slack in the tissue.
8. Apply a tensile load to the samples at a rate between 1-50 mm/min. Record the force and displacement of the samples during testing. See Figure 2 for reference to a tissue sample undergoing testing.
9. Estimate the thickness of the H&E stained tissue samples. Use this information along with the estimated circumference value to determine the cross-sectional area of each animal type.
10. Calculate and graph the stress and strain of the samples. Correct for the toe region of the curve, if necessary.
11. Determine the elastic modulus from the data obtained.

Figure 2 Tissue Mechanical Testing

In Vitro Spring End Tissue Retention Testing

Materials:

PCL or Poly(ε-caprolactone) from Durect Lactel: Inherent Viscosity of 1.00-1.30

$CHCl_3$ or Chloroform from Fisher Scientific

PTFE or Teflon mandrel from McMaster-Carr

Stainless steel mandrel from McMaster-Carr

LatheBot equipment run with Labview Program

bFGF from National Institutes of Health

EDC or *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide from Sigma Aldrich

NHS or *N*-Hydroxysuccinimide from Sigma Aldrich

PAA or Poly(acrylic acid) from Sigma Aldrich

0.008 inch diameter Nickel Titanium (Nitinol) wire from McMaster-Carr

1M NaOH or Sodium Hydroxide solution

NaCl or Sodium Chloride from Sigma Aldrich

Rat jejunal tissue samples

HBSS (1X) or Hank's Balanced Salt Solution from Gibco

ABAM or Antibiotic-Antimycotic from Invitrogen

Mechanical Load Frame from Instron: Model Number 5564

Methods:

1. Measure and combine 3.5% wt. polymer to chloroform into a sealable glass vial. Add paraffin film to the outside of the cap on the closed vial to ensure seal.
2. Place the polymer/chloroform mixture onto a rotator for at least 12 hours.
3. Spray 4mm mandrels to thicknesses of 0.6mm, 0.3mm, or 0.15mm. Use a plateau length of at least 20mm. Spraying was completed using LatheBot equipment and methods detailed in Appendix A.

Note: A stainless steel mandrel may be used to spray tubes due to Teflon rod deflection or warping after repeating spraying cycles.

4. After spraying, allow the polymer tube to dry for at least 6 hours.
5. Using a razor, cut the tube into 5mm long segments and slide off of the Teflon or stainless steel mandrel. A methanol soak for 20-30 minutes can be used to help remove or loosen the polymer tube.
6. Process and label the tube segments according to the following table:

Label	Thickness	Instructions
Control	0.3 mm	
bFGF	0.3 mm	Conjugate with NHS/EDC and add bFGF per protocol detailed in Appendix C.
PAA	0.3 mm	Add 1:40 PAA in a 3.5% wt. polymer solution. Spray coat 250 layers onto tube segments using LatheBot equipment.
Thick	0.6 mm	
ESpin	0.3 mm	Make an 11% wt. polymer solution using similar methods as the

		3.5% solution. Put tube segments onto a stainless steel mandrel and cover exposed metal with tape. Electrospin fibers onto surface of tube segments per protocol detailed in Appendix D.
Stent	0.3 mm	Create a Nitinol spring on a 4mm stainless steel mandrel by wrapping the wire around the mandrel in a helical fashion and heat setting the Nitinol wire to 500ºC for 10 minutes. Slide the Nitinol spring onto the outside of the polymer tube segment. Dip the spring/tube construct into $CHCl_3$ for 1-2 seconds and allow the PCL to dry.
NaOH	0.3 mm	Slide tube segment onto a 4mm stainless steel mandrel and then soak tube segment in 1M NaOH for 3 days
Salt-Thin	0.15 mm	Create a 5:4 NaCl in $CHCl_3$ solution. Slide tube segment onto a 4mm stainless steel mandrel and then roll tube segment in NaCl solution for 1-2 seconds. Allow to dry, then soak in de-ionized water for 24 hours.
Salt-Thick	0.3 mm	Create a 5:4 NaCl in $CHCl_3$ solution. Slide tube segment onto a 4mm stainless steel mandrel and then roll tube segment in NaCl solution for 1-2 seconds. Allow to dry, then soak in de-ionized water for 24 hours.
Wedge	0.3 mm	Cut 4 equal-distant 1mm deep slits into one end of tube segment. Fold “flaps” created from slit cuts, outward
C-Ring	0.3 mm	Cut slit along the length of the tube segment. Slide “c-ring” tube segment onto 6.5mm stainless steel mandrel and place in oven at 50ºC for 1 hour.
Holes	0.3 mm	Puncture holes through tube segment using an 18gauge needle.

Crown	0.3 mm	Cut 8 equal-distance triangles into one end of tube segment. Triangles should be about 1mm in height and at a 60º angle. Fold "teeth" created from triangle cuts, outward

7. Resect a length of jejunal small intestine from a deceased animal specimen. Cut away and discard the mesentery tissue.
8. Place the tissue sample in a vial containing 1:100 ABAM in HBSS (1X). Tissue may be stored up to 5 days, and must be stored in a refrigerated environment.
9. Insert the tube segment onto a threaded cap screw, and pull one end of the tissue sample over the screw head and tube segment. Note that the screw head should not be larger than the outside diameter of the tube, but needs to be larger than the tube inside diameter of 4mm.
10. Secure the screw into the vice, and clamp the opposite end of the tissue sample with a soft tissue forceps. See test setup in Figure 3 below.
11. Apply a tensile load to the samples at a rate of 50 mm/min. Record the force and displacement of the samples during testing.
12. Plot the average peak load on a graph for comparison.

Figure 3 In Vitro Tissue Retention Testing

In Vivo Intestinal Integration Testing

Materials:

PCL or Poly(ε-caprolactone) from Durect Lactel: Inherent Viscosity of 1.00-1.30

$CHCl_3$ or Chloroform from Fisher Scientific

PTFE or Teflon mandrel from McMaster-Carr

Stainless steel mandrel from McMaster-Carr

LatheBot equipment run with Labview Program

NaCl or Sodium Chloride from Sigma Aldrich

Mini-LatheBot equipment run with Phidget Controller Program

VLS 2.30 laser cutter from Universal Laser Systems

Mechanical Load Frame from Instron: Model Number 5564

Gelatin capsules from Torpac: Size #4 and Size #5

CAP or Cellulose Acetate Phthalate from Eastman Chemicals

Sprague dawley or Lewis rats

Methods:

1. Create spring or spring end prototypes according to the above protocols Spring Mechanical Testing or In Vitro Spring End Tissue Retention Testing, respectively.
2. Place prototypes into size (4 or 5) gelatin capsules and coat with 1-2 layers of C-A-P] (cellulose acetate pthalate) for mucosal protection during prototype placement.

3. Spring End prototype in vivo testing:
    a. Roux-En-Y model (not continuous)
        i. Surgically place a spring end prototype into the blind end of a roux-en-y bypass segment of rat small jejunum for 7 days.
        ii. At euthanasia, collect both experimental tissue and normal jejunum.
        iii. Subjectively evaluate the experimental tissue for spring end prototype integration and required removal force by pulling the spring end prototype out of the jejunal segment.
        iv. Cut away a portion of the tissue and prepare for Hematoxylin and Eosin (H&E) staining by inserting into “cassettes” and submerging in formalin for at least 24 hours.
        v. Send tissue samples for H&E staining and slide preparation after required formalin soaking period
    b. In continuity model
        i. Surgically insert a spring end prototype into a segment of rat jejunum that is continuous with the flow of enteric contents for 1 day.
        ii. At euthanasia, subjectively evaluate the experimental tissue for spring end prototype integration by visually determining if the spring end remained in the originally placed intestinal position.
4. Spring prototype in vivo testing:
    a. Roux-En-Y model (not continuous)

i. Surgically place a spring device with the specified prototype end design into the blind end of a roux-en-y bypass segment of rat small jejunum for 7 days.

ii. At euthanasia, subjectively evaluate the experimental tissue for spring integration by visually determining if the spring remained in the originally placed intestinal position.

b. In continuity model

i. Surgically place a spring device with the specified prototype end design into a segment of rat jejunum that is continuous with the flow of enteric contents for 7 days.

ii. At euthanasia, subjectively evaluate the experimental tissue for spring integration by visually determining if the spring remained in the originally placed intestinal position.

5. Record subjective evaluations and any additional observations of animal integration tests. Review H&E slides for tissue damage and architecture compared to normal tissue and control samples.

RESULTS and DISCUSSION

Material Testing

Poly(ε-caprolactone) and Poly(L-lactide) are two materials used for biodegradable sutures in various surgical procedures. These materials were selected for use in the intestinal spring device due to their ability to degrade in the body and material property differences. The materials were spray coated onto mandrels and further flattened to allow tensile testing and analysis to be performed. Figure 4 thru Figure 8 shows the stress/strain profile of Poly(ε-caprolactone), Poly(L-lactide), or blends of these two polymers.

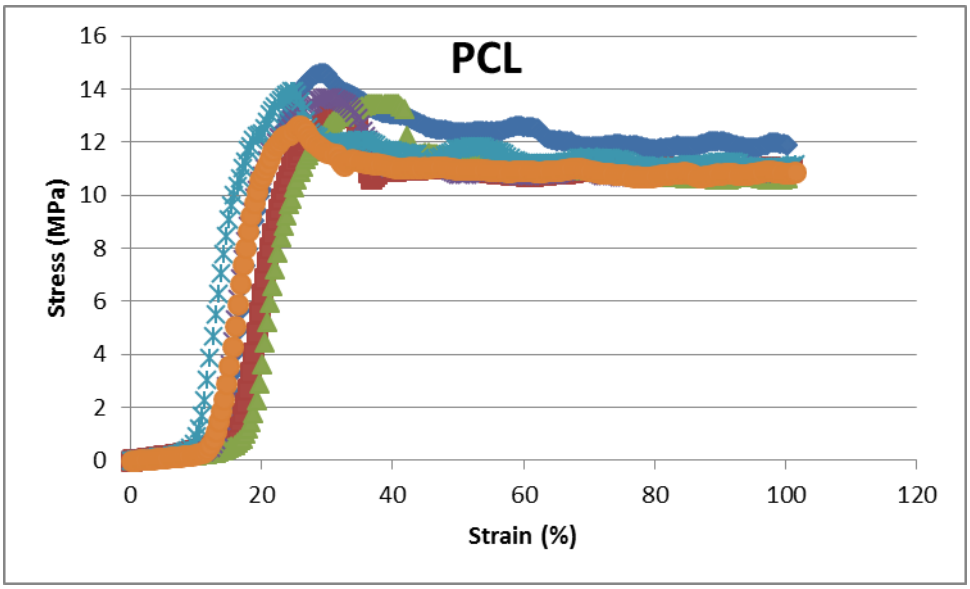

Figure 4 Poly(ε-caprolactone) Stress vs. Strain Profile

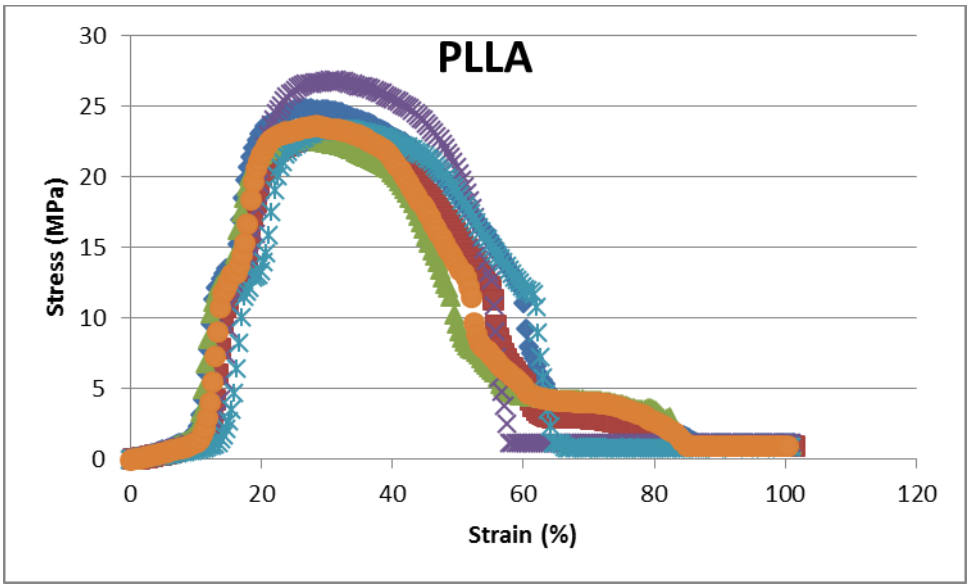

Figure 5 Poly(L-lactide) Stress vs. Strain Profile

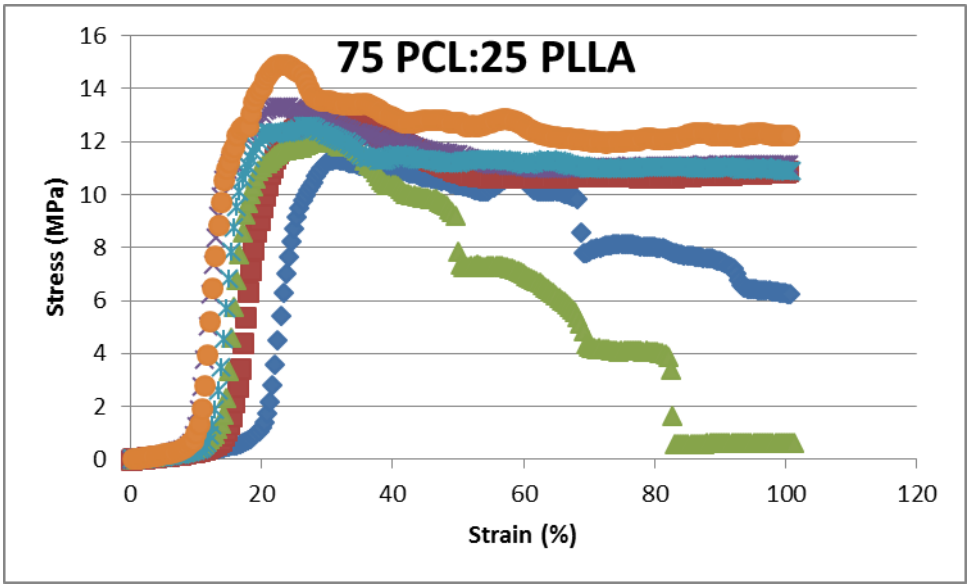

Figure 6 75% PCL / 25% PLLA blend Stress vs. Strain Profile

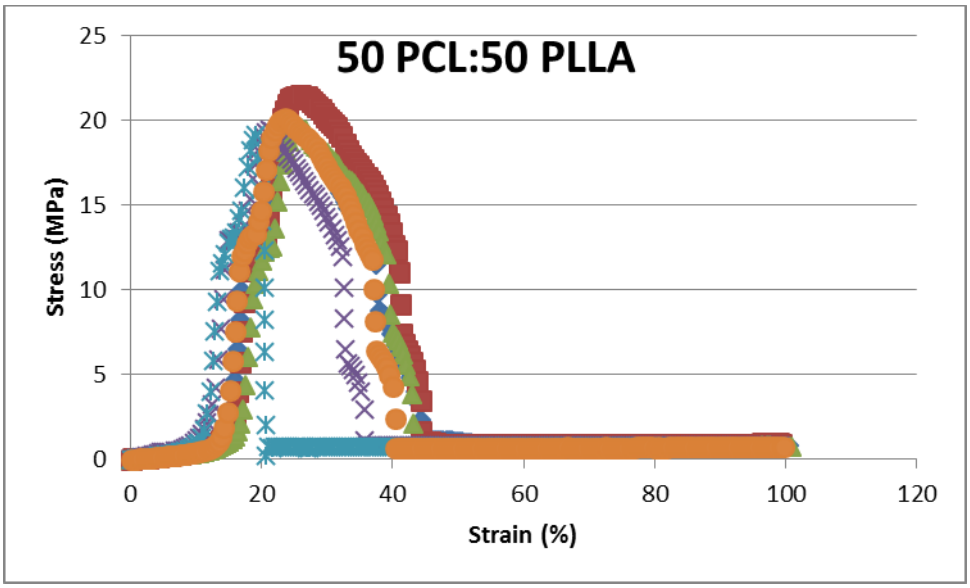

Figure 7 50% PCL / 50% PLLA blend Stress vs. Strain Profile

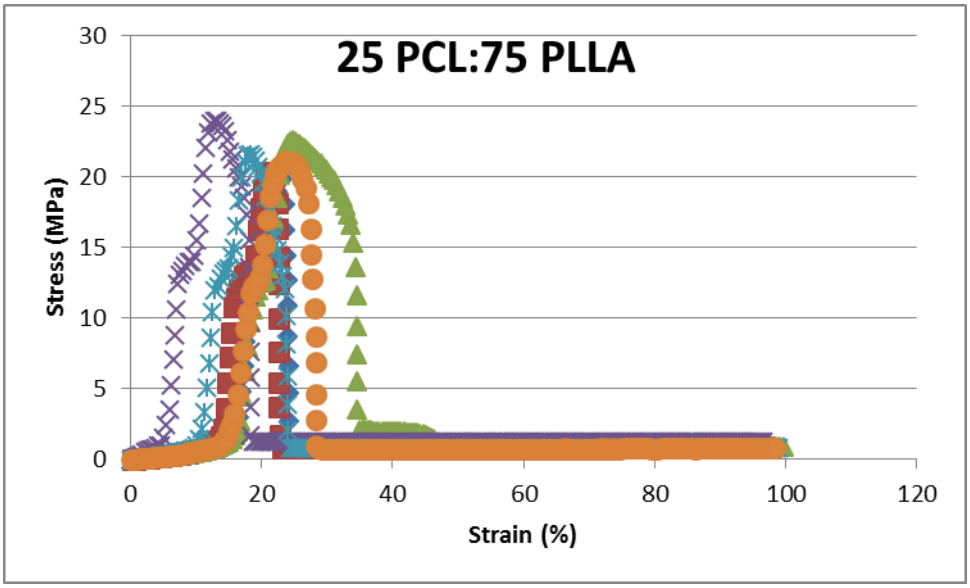

Figure 8 25% PCL / 75% PLLA blend Stress vs. Strain Profile

Results of this material testing show that both PCL and PLLA reach their ultimate stress value at a similar amount of strain, however, the PCL material shows more ductility due to the elongation of the material evident in the extended plastic region of the stress/strain curve. PLLA provides a stronger material due to the larger yield stress and elastic modulus. These material differences are due in large part to the variation in glass transition temperature. PCL has a T_g of -65°C to -60°C, and thus at room temperature is in a rubbery state, while PLLA has a T_g of 60°C to 65°C and is in a glassy state at room temperature.[1]

The ability to blend PCL and PLLA allow the advantage of combining the strength of PLLA and the ductility of PCL. Various mixtures of the two polymers were created and also tested in tension to determine materials properties. Results of the multiple variations of the polymers and polymer blends are shown below in Table 1. The yield stress was calculated from the stress/strain curve by drawing a parallel line to the linear portion of the curve that intersected at the 0.2% strain value. The maximum load was determined from the force obtained by the Instron load cell. The elastic modulus was obtained by determining the slope of the stress/strain curve. Finally, the shear modulus was calculated using a Poisson's ratio of 0.38 for both polymers, which was an average value obtain from literature. (Podichetty & Madihally, 2014), (Wan & Chen, 2012), (Kawamoto & Sugahara, 2005)

[1] According to material supplier's website: http://www.absorbables.com/technical/properties.html

Table 1 Material Properties

Sample	Yield Stress (Mpa)	Max. Load (N)	Elastic Mod. (MPa)	Shear Mod. (MPa)
100% PCL	11.67 ± 0.22	19.34 ± 0.98	169.87 ± 10.63	61.55 ± 3.85
75 PCL:25 PLLA	11.62 ± 0.75	18.41 ± 1.77	220.52 ± 30.52	79.90 ± 11.06
50 PCL:50 PLLA	16.96 ± 0.55	28.34 ± 1.18	361.08 ± 19.55	130.83 ± 7.08
25 PCL:75 PLLA	20.74 ± 1.44	30.77 ± 2.15	418.73 ± 91.18	151.71 ± 33.04
100% PLLA	20.44 ± 0.90	34.33 ± 2.22	366.22 ± 20.30	132.69 ± 7.36

**n = 6 samples for each type of polymer blend*

Additional material observations were found during tensile testing as the ductile characteristic of PCL disappeared after 25% of PLLA was added to the mixture. Also, the polymer blend at a 50:50 mixture, showed a statistically similar elastic modulus to polymer blends with a larger percentage of PLLA. It is assumed that at this 50% mixture point, the crystalline state of the PLLA overtakes much of the mechanical properties of the material as a whole. The information about the individual materials and also the blended properties allows for the spring devices to be created to take advantage of meeting a multitude of mechanical requirements.

Spring Testing

Evaluation of the spring device was completed by varying dimensions of the design to achieve various spring rates along with encapsulation of the spring device to better understand the characteristics of deployment from the gelatin capsule. Heat treated expansion of the springs was performed based upon the length of the helix, as the springs were lengthened to 60% of the helix length as calculated by the following equation:

$$Spring\ Length = \sum Length_{Spring\ End} + 0.6 * (n * \sqrt{B.S.^2 + (\pi * \emptyset_{mandrel})^2})$$

** n is the number of "active" turns on the spring & B.S. is the Band Size*

The above equation was based upon the standard mathematical equation for the curve length of one turn of a helix shown by the following equation:

$$Helix\ Length = \sqrt{Pitch^2 * Circumference^2}$$

The first analysis of the dimensions was to compare the diameter to the spring rate. Springs with a solid height of 20mm, a thickness of 0.75mm, and a band size of 2mm were created with diameters of 4, 5, 6, 7, and 8mm. As shown in Figure 9, an increase in the diameter leads to an exponential decrease in the spring rate. These results were based upon a sample size of three springs for each diameter tested.

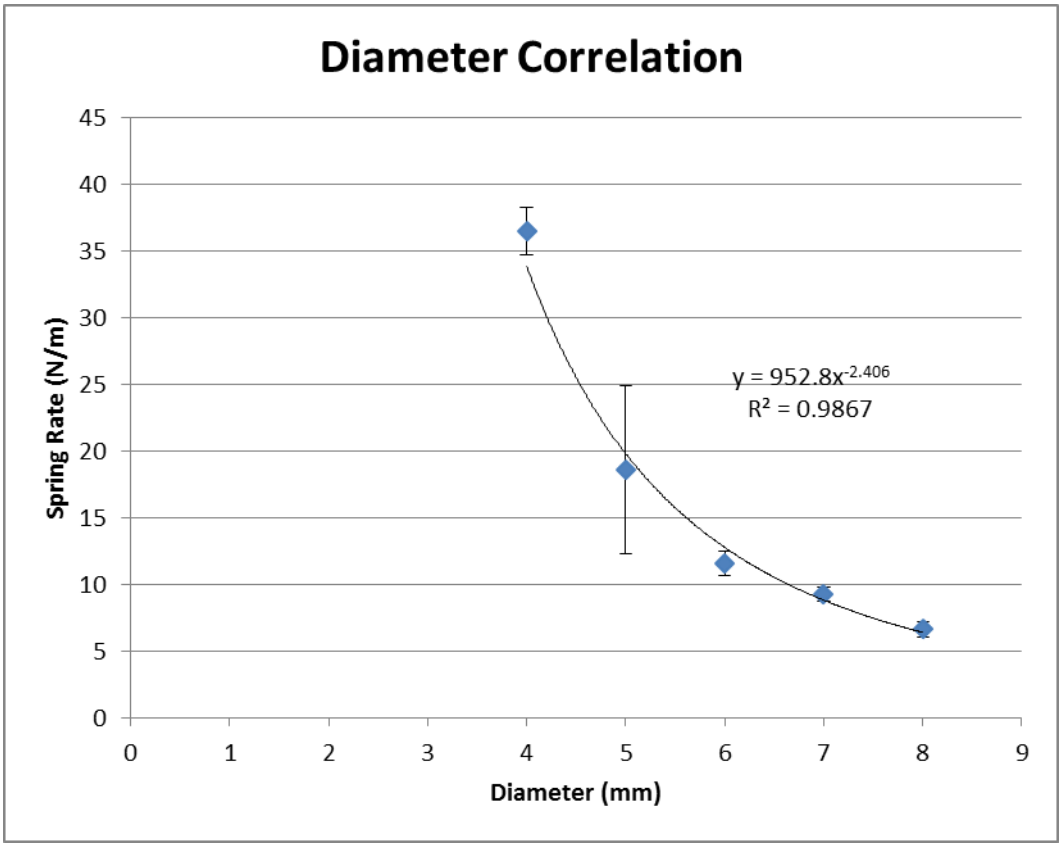

Figure 9 Spring Diameter Correlation

The second analysis of the dimensions was to compare the thickness and band size of various springs to the spring rate. Springs were created with a solid height of 1mm on a 4mm diameter mandrel. Thickness variations 0.2mm, 0.4mm, and 0.6mm were combined with band size variations of 1mm, 2mm, and 3mm to create a combination of nine possible spring designs. These designs and the corresponding spring rates were graphed as shown in Figure 10 and Figure 11. An increase in both of these parameters show an increase in the spring rate. These results were based upon a sample size of three springs for each parameter tested.

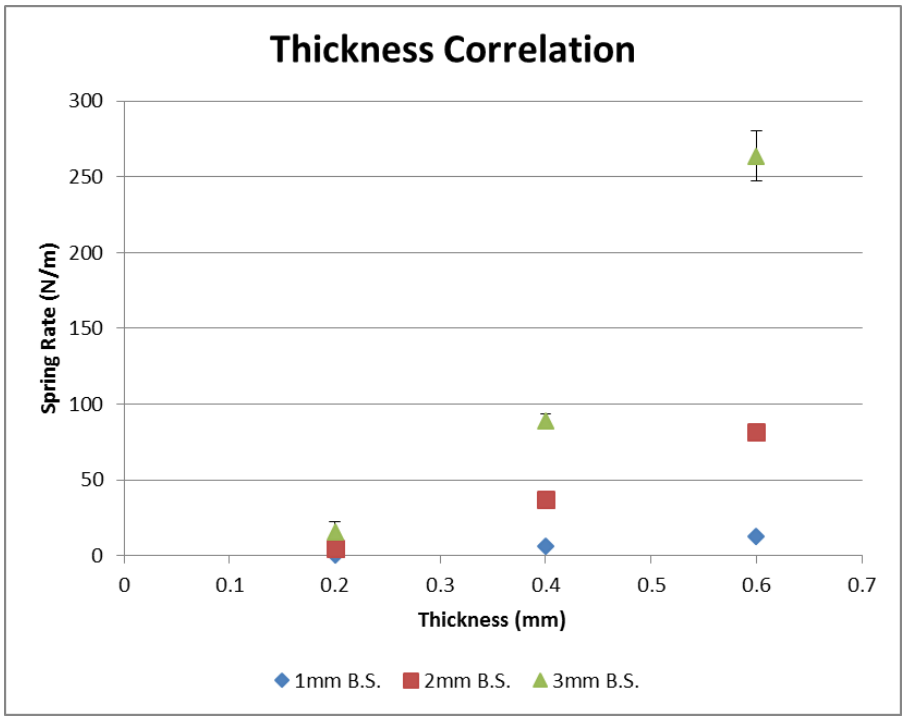

Figure 10 Spring Thickness Correlation

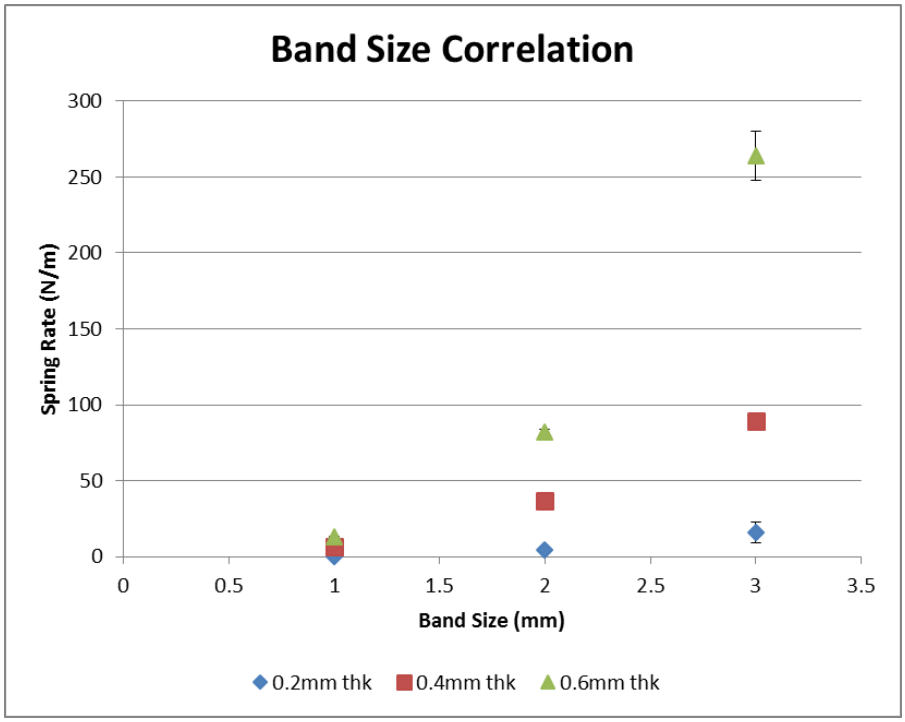

Figure 11 Spring Band Size Correlation

The spring diameter should be based upon the subject that is receiving the implant. For instance, rats have been implanted with springs made from a 4mm diameter mandrel, whereas the pigs have been implanted with springs made from a 7mm diameter. Thus the thickness and band size are the spring parameters that should mainly be considered when trying to create a spring. Note that the 4mm springs created for the rat, can reach a spring rate over 250 N/m, which is well above the minimum 1 N/m reported in prior research. (Stark, Panduranga, Carman, & Dunn, 2012)

The rates obtained from the creation of polymer springs from sprayed tubes, follows the rates calculated for springs with rectangular cross sections. (Swieskowski, Design of Helical Compression Springs, 1979) The following equations can be used to predict subsequent spring designs, within an 80% accuracy:

$$Rate\ (k) = \frac{K_1 * G * b * t^3}{D^3 * n}$$

$$Shape\ Factor\ Constant\ (K_1) = 0.202 * (b/t)^{0.451}$$

$$Mean\ Spring\ Diameter\ (D) = \emptyset_{mandrel} + t$$

$$Number\ of\ \text{Active}Turns\ (n) = LaserCut\ length\ \div b - 1.25$$

** G is the shear modulus, b is the band thickness, & t is the material thickness*

The last evaluation of the mechanical properties of the spring was to determine the effects of encapsulation within the gelatin capsules. Both 4mm rat-type springs and 7mm pig-type springs were evaluated for release from the capsules after various time periods. The lengths of

the spring were measured and graphed at each interval. A sample image of a spring after encapsulation is shown in Figure 12 below.

Figure 12 Spring Post-Encapsulation

All of the four samples of the 4mm springs were made from PCL and include manufacturing variations of laser cut springs [LC], hand cut springs [HC], lyophilized laser cut springs [LC (L)], and lyophilized hand cut springs [HC (L)]. The results in Figure 13 show a consistent reduction in length of the spring that occurs during the first few hours of encapsulation. This was determined to be due to plastic deformation of the material due to being compressed in the capsule, and may require steps to relieve manufacturing stresses similar to metal springs. (Swieskowski, Stress Relieving Procedures for Helical Compression Springs, 1974) Additionally, single samples of springs that were cut to an expanded length to avoid heat treating [No HT], a blended polymer spring [PCL/PLLA], and a PCL spring made with hydroxyapatite nanoparticles [HA Nanoprtcl] were evaluated to quickly evaluate if the plastic deformation issue could be resolved through other material modifications. Additional research into potential solutions was not completed at this time.

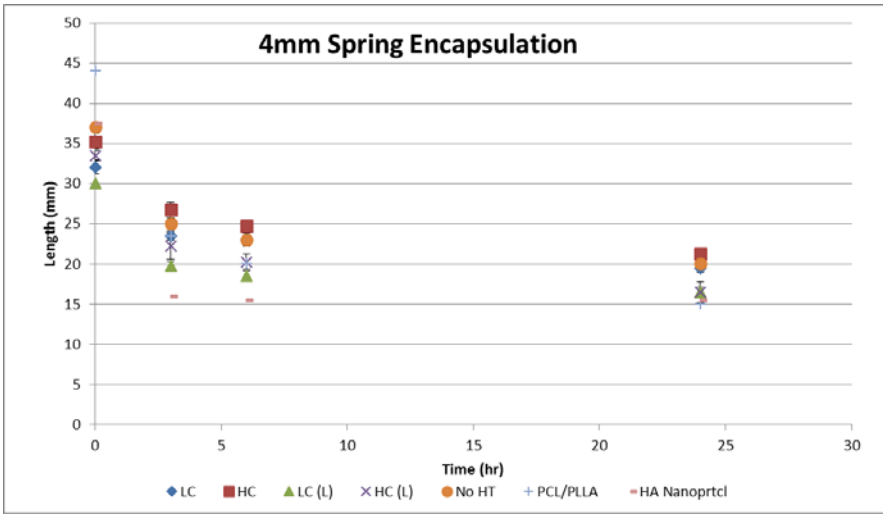

Figure 13 Spring Encapsulation (4mm)

Three samples each of the larger 7mm springs were encapsulated for a time period (28 days) to determine long term effects of encapsulation. These springs were made from both PCL and PLLA and show a reduction in length similar to that of the smaller 4mm springs. The results in Figure 14 show a continued reduction in length of the spring over the entire duration of encapsulation. This reduction can be related to a creep behavior that is typical in many plastics.

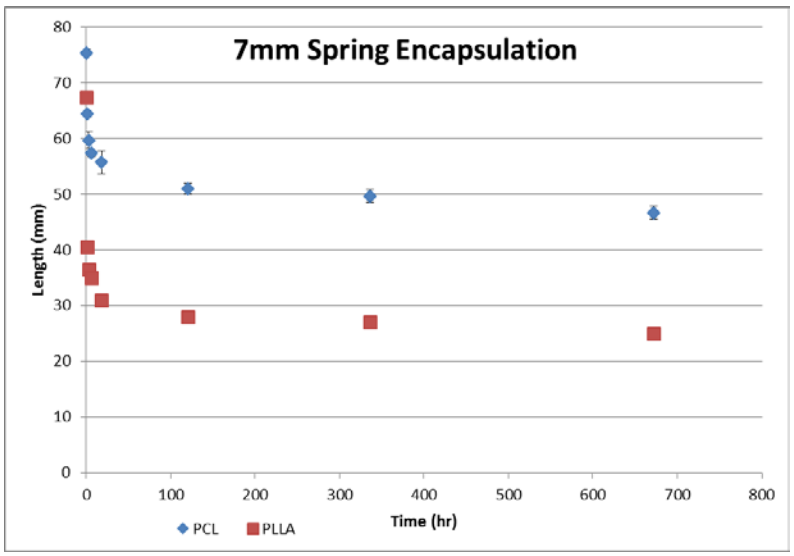

Figure 14 Spring Encapsulation (7mm)

Tissue Testing

In addition to evaluating the materials used in the spring designs and the actual springs themselves, the tissues of the animal subjects were evaluated to determine the elastic modulus. This evaluation was performed to attempt to relate the tissues of various animals and use this information to create a spring for an animal based upon the tissue mechanical properties. Sprague dawley or Lewis rat specimens ranged from 250-350g, while the Yucatan mini pigs ranged from 3-7.5kg in size.

In order to calculate the stress of the materials, the cross sectional area of the tissue was estimated by measuring the circumference and the thickness of the tissue. The average circumference and thickness for the rats was 8.4 ± 0.32mm and 0.08 ± 0.02mm, respectively. The average circumference and thickness for the pigs was 48 ± 5.7mm and 0.19 ± 0.02mm, respectively. Therefore, on average, rat intestinal area was about 0.67 mm^2 and pig intestinal area was about 9.0 mm^2, as calculated by the following equation:

$$Area = \pi r^2 - \pi(r - thk.)^2$$

** r is the radius calculated by circumference ÷ 2π & thk. is the tissue thickness*

Histological samples of each tissue type can be seen in Figure 15 for rat tissue and Figure 16 for porcine tissue. Histology was used to determine tissue thickness, while the circumference was measured with a ruler on tissue laid onto a flat surface.

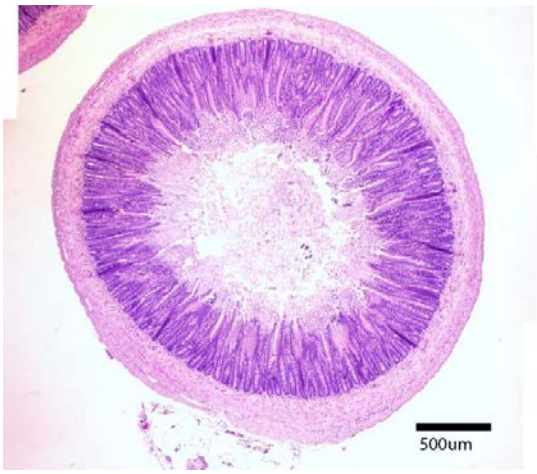

Figure 15 Rat Tissue H&E

Figure 16 Pig Tissue H&E

Results from tensile testing the intestinal tissue samples at various loading rates between 1-50 mm/min resulted in no significant differences in the stress/strain curve profiles. However, there was variation of the stress/strain curves due to manually loading the tissue into the clamps of the Instron machine. This did not affect the slope of the linear portion of the curve, but did show an extended toe region for some samples. This was remedied by correcting for the strain and aligning the linear portion of the curve to the y-intersect at a 0% strain value for each sample. A graphical representation of the stress/strain curve for both the rat (in green) and pig (in blue) tissue is shown in Figure 17. The results show an increased elastic modulus for the rat tissue (10.735 ± 2.67 MPa) compared to that of the pig tissue (1.539 ± 0.27 MPa), which might be due to the redundant projections of the muscularis mucosa evident in only the porcine tissue. Neither species demonstrated any differences in the toe region, therefore the linear region differences were used to help develop a spring for the porcine specimen. These results were based upon four samples from each of three rat specimens and upon two samples from each of the two porcine specimens.

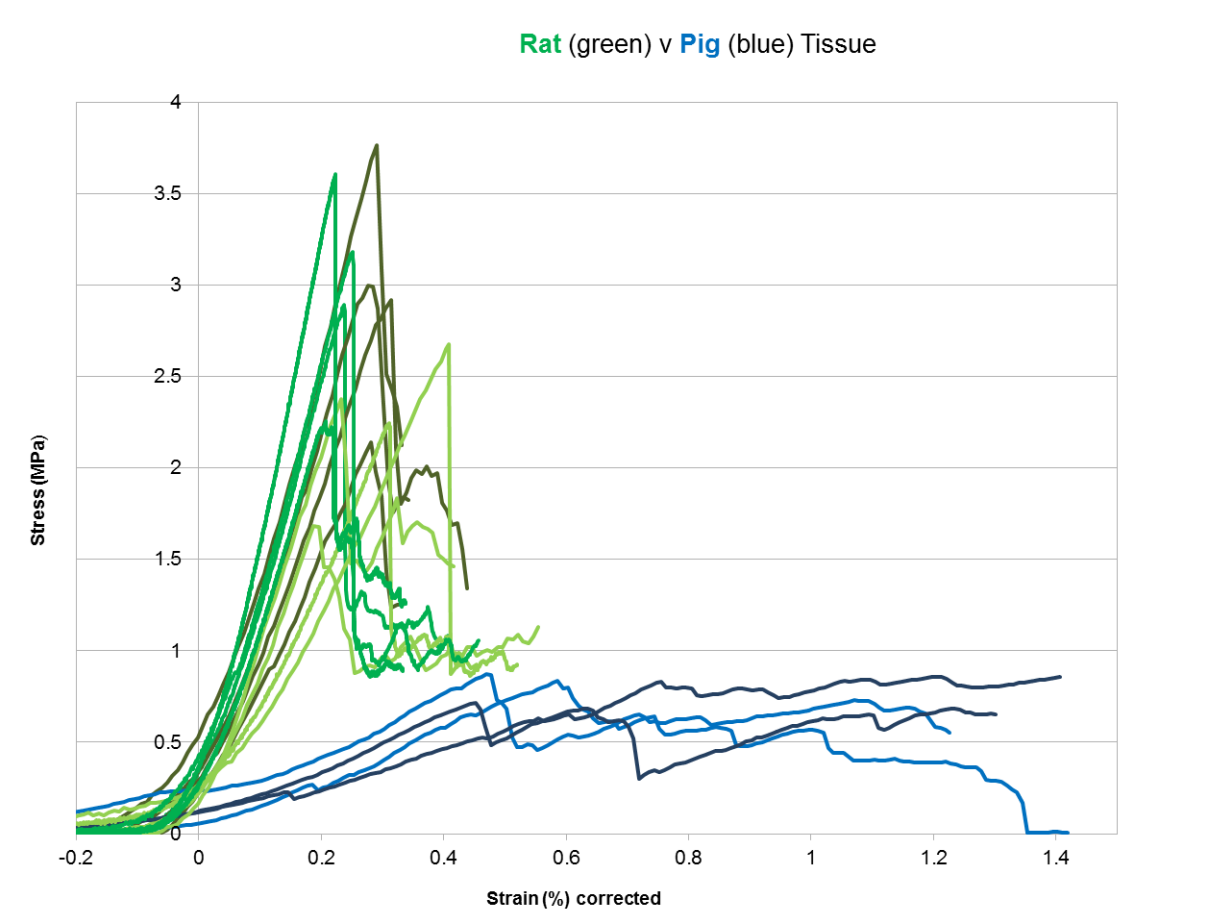

Figure 17 Animal Tissue Stress vs. Strain Profile

The rat lengthening was over an expansion length of 30mm which equates to a maximum estimated force of 0.045N, based upon the linear relationship of Hooke's Law show below and an actual spring rate of 1.5 N/m used to induce lengthening in rats in previous research. (Stark, Panduranga, Carman, & Dunn, 2012)

$$Force\ (F) = \ Rate\ (k) * Distance\ (x)$$

This calculated force relates to a stress of 0.0672 MPa in the rat tissue. An equivalent stress in the pig tissue would be due to a force of 0.6045N, based upon a greater cross-sectional area of the porcine tissue and calculations performed using the following relationship:

$$F_{Pig} = (F/A)_{Rat} * A_{Pig}$$

In addition, the elastic modulus for the rat tissue is 6.975 times larger than that for the porcine tissue, and the spring constant should account for this material elasticity difference. An equivalent spring rate for the porcine tissue should therefore be about 10.46 N/m as calculated using the following equation:

$$k_{Pig} = \frac{E_{Rat}}{E_{Pig}} * k_{Rat}$$

From these calculations, the spring rate of 10.46 N/m (adjusted for pig tissue), should allow for 57.8mm of expansion in porcine tissue to be able to match the stress point in both animals and provide theoretically equivalent lengthening profiles. This increased spring rate and expansion distance should also help to counteract the effects of the pig tissue stiffness differences compared to rat tissue as calculated by the following equation:

$$Stiffness\ (k) = \frac{A * E}{L}$$

** A is the cross-sectional area, E is the elastic modulus, & L is the element length (i.e. spring solid length)*

Calculations of the stiffness show a comparable value of about 0.7 N/mm when the properties of both the rat and pig spring designs were taken into consideration.

Intestinal Integration

Any design that is used must be able to maintain the position of the spring in the tissue immediately after spring deployment from the gelatin capsule, along with being able to withstand the forces of enteric fluids and peristalsis. Mechanical, biological, and chemical means of integration were considered as a means for initial and sustained integration into the tissue. An original in vitro evaluation of various prototype spring ends was perform to determine the potential of various designs to withstand the initial spring deployment forces. Results, in Figure 18, show that a more macroscopic mechanical feature would provide a better retention into the tissue initially, compared to any biological or chemical feature. These results were based upon a sample size of three spring ends for each prototype design tested.

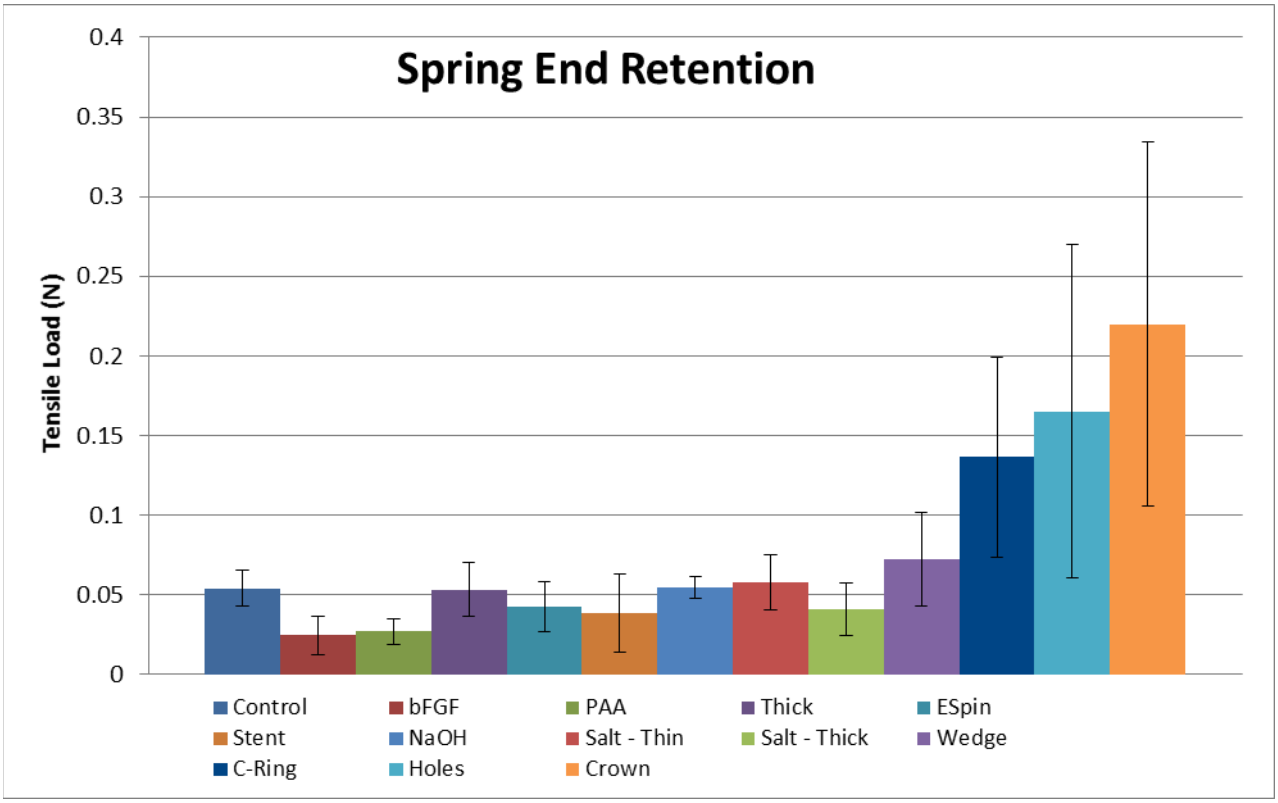

Figure 18 Spring End Retention - In Vitro

Further follow up in vivo experiments with various spring ends included evaluations of a crown design with protrude teeth, a salt leach design with a rough/porous surface architecture, and spring ends of larger than normal diameter. Histological evaluation of a 7-day Roux-En-Y implantation of the salt leach and crown spring end designs, shown in Figure 19 and Figure 20 respectively, did not show much difference compared to a control spring end sample as shown in Figure 21. Subjectively, the crown prototype was the most difficult to remove from the intestinal tissue after 7 days and was thus added to a complete spring design for implantation into a Roux-En-Y segment. This crown spring implantation did not provide lengthened tissue due to the lack of tissue integration. The reason for the lack of integration was due to the plastic deformation of the crown teeth during encapsulation. The teeth were no longer protruding outwards after deploying from the gelatin capsule, and thus could not stick into the tissue.

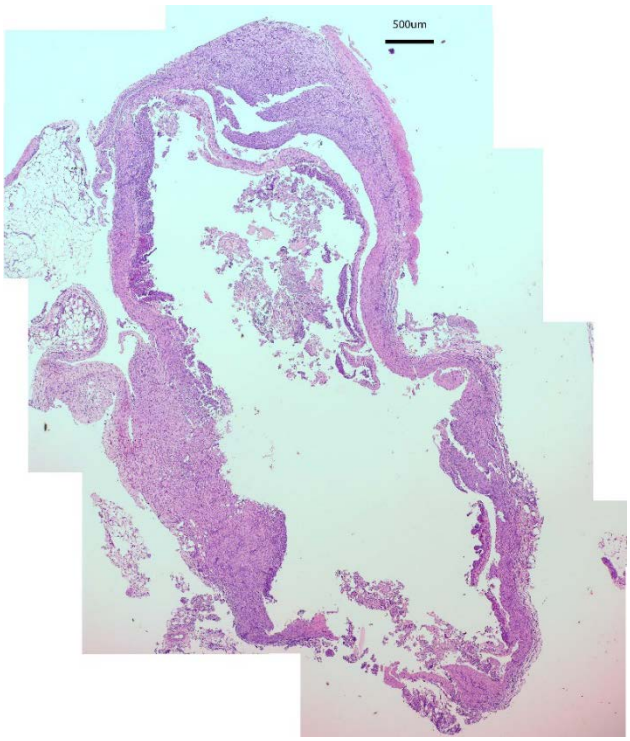

Figure 19 Salt Leach Spring End - 7 Day In Vivo

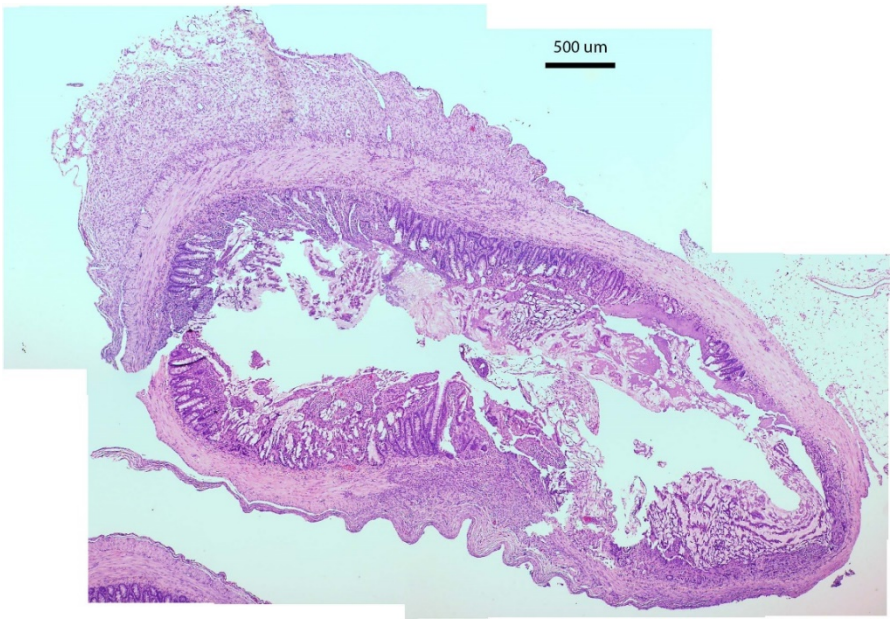

Figure 20 Crown Spring End - 7 Day In Vivo

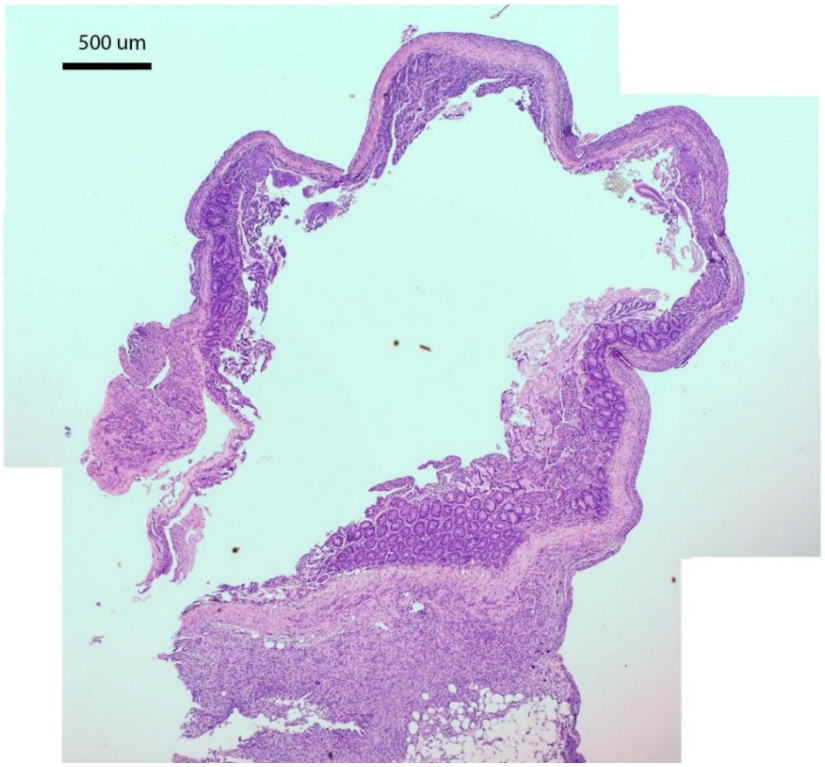

Figure 21 Control Spring End - 7 Day In Vivo

A mechanical means of tissue integration was desired, but another means besides using a crown design was needed. In order to determine the maximum diameter that is able to integrate into the tissue, spring ends of larger diameters were implanted in-continuity for a length of 1 day. The results from this implantation test showed that a spring end made with an outside diameter of 5.5mm was able to remain in the intestine. This is evident by the tissue shown in Figure 22, which was marked with blue suture to denote the original implantation location.

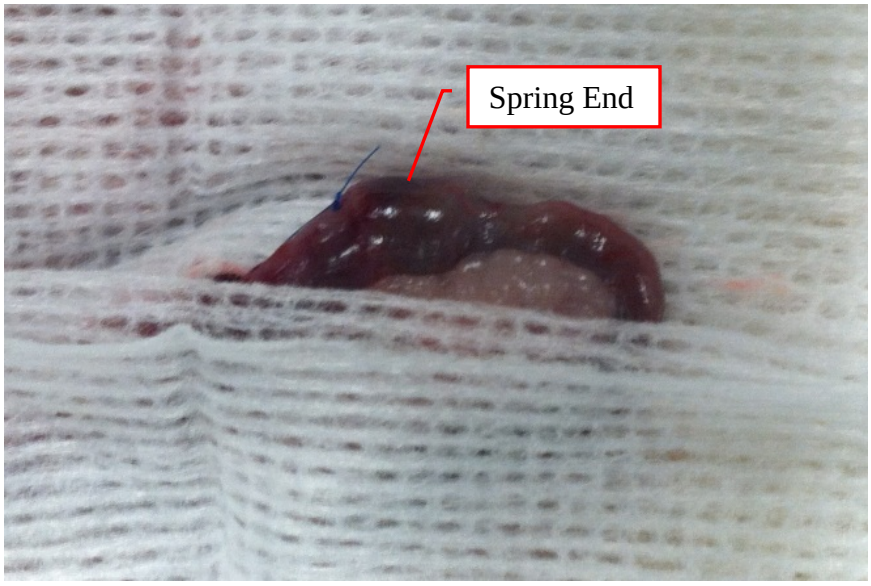

Figure 22 Spring End In-continuity - 1 Day In Vivo

Following the 1-day spring end evaluation results, a complete spring with 5.5mm outside diameter spring ends was implanted in-continuity. After implantation, the rat specimen appeared to be under great distress until the intact spring was excreted. Analysis of the intestinal tissue after the 7-day evaluation period showed an enlargement of the intestinal tissue proximal to the spring location. This evidence points to obstruction of the intestine due to the spring implantation.

CONCLUSION

The goal of this research was to further understand the mechanical properties of biodegradable polymers used to create the intestinal spring device along with obtaining a better understanding of the tissue properties. Experiments were conducted to determine the elastic modulus of the materials and animal tissues involved in this research. Further characterization of the functionality of the spring device was evaluated through experiments that attempted to determine spring rates through various dimensional adjustments, to understand deployment parameters from the gelatin capsule, and to realize potential design features that may allow for tissue integration.

The biodegradable materials, chosen for use in the intestinal lengthening springs, show promise for usage in multiple animal models. The spring design can be dimensionally adjusted to meet a wide range of spring constants based on adjustments to the thickness and band size of the spring. Additional research is required to solve issues that occur due to placement inside of gelatin capsules. The PLLA material demonstrated worse material "memory" when compared to the PCL material as 7mm springs made from PLLA showed a much greater plastic deformation within the first few hours of encapsulation. This effect may be due to the brittle nature of PLLA, and thus PCL should be used as the major material for spring creation.

Each new animal model used will need to be evaluated in order to determine an effective spring design for tissue lengthening. The spring designs should take into account the size of the animal tissue and available gelatin capsules to select the correct spring diameter. Additionally, the elastic modulus of the intestinal tissue should be used to determine the spring rate. The expansion length and spring forces need to be adjusted to apply comparable stresses and match tissue stiffness values for various intestinal tissue specimens.

Finally, tissue integration of the spring device shows promise through the route of using a feature or method that mechanically resists the combined forces of the spring and the tissue. Due to the thin walls of the intestine, designs should reduce any excessive radial tissue force or any features that may cause a tissue perforation. Additionally, designs should take in consideration the potential for obstruction of the enteric fluid flow. Experiments conducted through the research described here provides information on preliminary evaluation of tissue integration features that can be implemented onto the current spring designs.

The mechanical characteristics of the polymer spring device was evaluated and allowed for further comparison to the animal models used for current in vivo studies being performed. The information in this research should allow for better scaling of the spring device to other animal models, and potentially future human trials. The spring design still needs adjustments to provide better functionality and ease of use. The current design has the potential to reach the ultimate goal of a biodegradable mechanical lengthening device that can be implanted in continuity through a single surgical intervention.

LIMITATIONS OF THIS PROJECT

One of the main limitations of this project was the availability of tissue samples from porcine and other animal sources. In order to have a better understanding of a large range of material properties of the small intestine tissue, addition porcine specimens would have been beneficial along with other types of animals. In addition, it would have been advantageous to be able to obtain human intestinal tissue, either from a pediatric of adult patient.

Coincident with the lack of porcine tissue samples, another limitation was the lack of porcine specimens for implantation of the polymer spring device. In order to obtain sufficient experimental feedback upon the spring constants and forces needed for implantation into the pigs, additional samples are necessary before an exact determination of the spring device can be made. Issues with scaling up size from rats to pigs, also provides issues of size for determining the proper gelatin capsule. The current supplier, Torpac, provides various gelatin capsule sizes but has a large gap in outer diameter size between 10mm and 15mm, which is the range of the porcine intestinal tissue. The current porcine capsule size used, size #000, is on the smaller end of this range, and is the best available option known based on suppliers contacted and limited animal specimens evaluated.

Finally, a limitation of this project is the plastic deformation and creep behavior of the polymer springs after encapsulation. The Nitinol spring devices used in earlier research showed the ability to expand to a further distance and did not undergo similar changes in the material as did the polymer used in this research. The results from the material change to a polymer have had effects on the lengthened animal tissues and the future storage of the spring device within the capsule for an extended period of time.

FUTURE WORK

Additional studies onto larger animal models, especial porcine specimen, are expected to occur in the future. These studies will allow for the scaling of the spring device to occur, and will also allow for additional evaluation of the material properties of an animal that is similar to human pediatric intestinal tissue. Along with additional porcine tissue samples for mechanical property testing, human tissue may become available in the future. These future samples will allow for a better evaluation of the gelatin capsule size needed for implantation.

The integration of the spring device into the intestinal tissue is still in early stages of research. Future work will be done to investigate different surgical approaches and design features that will allow the device to function efficiently and be easy to use. Some proposed differences in the surgical approach include moving the spring external to the intestinal tissue, modification of the animal's post-surgical diet, and also implantation of a secondary set of devices designed to hold the spring in place. Design feature proposed changes include adding protruding spikes to the outside surface of the ends of the spring, adjusting the spring end to be able to ratchet open in order to apply an expanding radial force, and also a spring design with no ends that is originally a much larger diameter coil thus providing an outward radial force when implanted in-continuity.

Finally, in order to solve the issue of polymer deformation and creep behavior, future samples may undergo a stress relieving step prior to encapsulation. This will not remove the creep behavior of the material, but may allow for additional expansion of the spring in vivo if the deformation is accounted for in the original spring manufacturing process. In order to solve the issue of creep behavior, additives to the polymer such as nanoparticles or other polymer types will be investigated. (Zhang, Yang, & Friedrich, 2004) In addition, a biodegradable metal such

as a magnesium alloy could be used instead of a polymer. (Waizy H1, et al., 2014) However, an earlier attempt to obtain material samples of magnesium alloys was futile.

APPENDICES

Appendix A: LatheBot Protocol

Using a 3.5% polymer solution and the LatheBot equipment shown in Figure 23 below, polymer tubes can be created and used to cut springs for implantation into animal models. The LatheBot equipment should be positioned inside a fume hood to prevent inhalation of chloroform fumes and to contain excess polymer fibers.

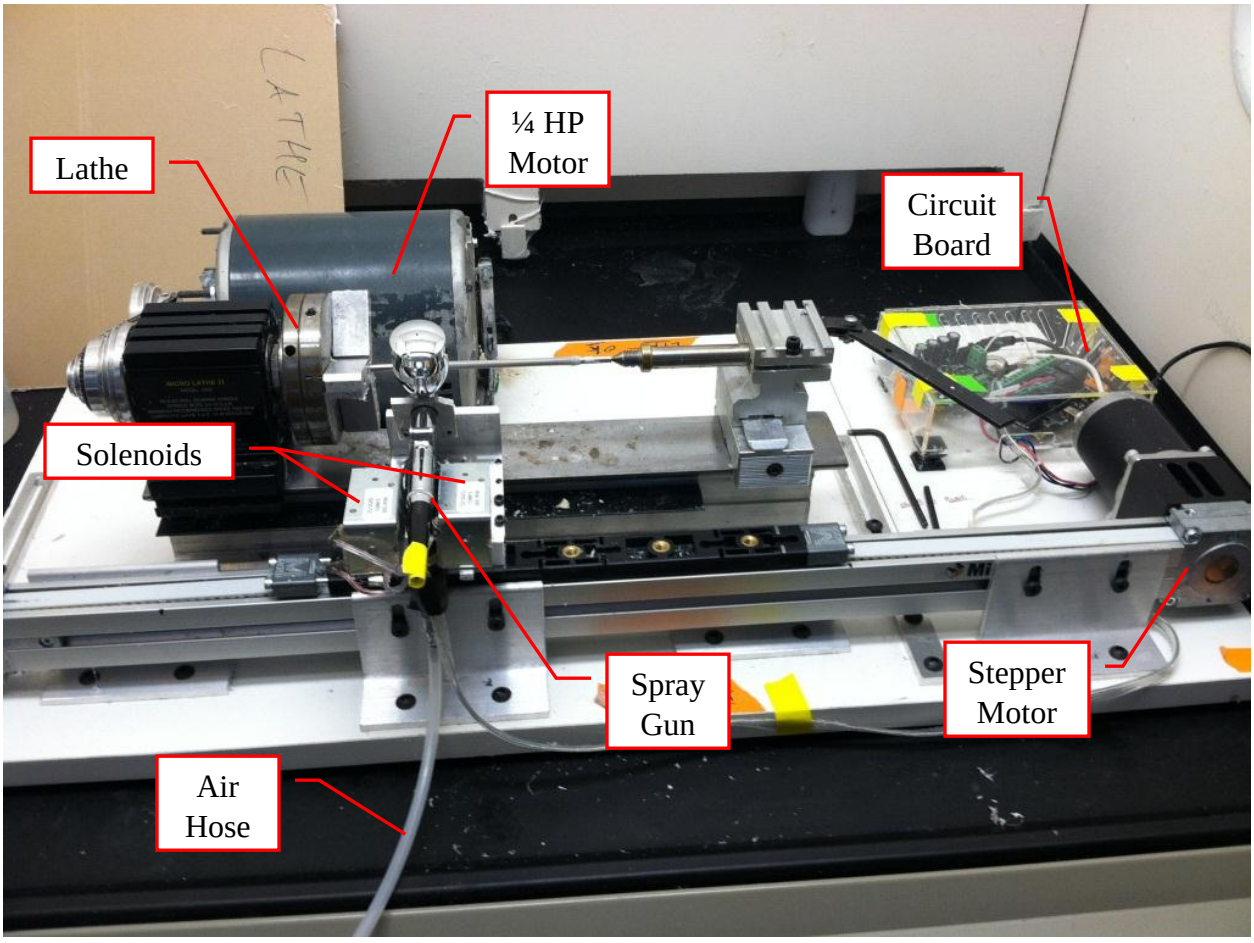

Figure 23 LatheBot Setup

Protocol:

1. Supply 20 psi of air pressure to the spray gun. Use an air gauge to ensure that adequate pressure is supplied when the spray gun trigger is pulled.
2. Cover the 1/4 HP motor with aluminum foil during spraying for safety.
3. Adjust the knob at the back end of the spray gun to a flow rate of about 2 g/min (correlates to a distance of 2.0 – 2.5 mm on Master airbrush model G79). Use tape to secure the position of the knob, as shown in Figure 24 below.
4. Adjust the sprayer separation distance according to the diameter of mandrel used in spraying. The surface of the mandrel should be about 8mm from the spray gun nozzle. Use the following equation to determine separation distance: (Mandrel Diameter) ÷ 2 + 8. The separation distance is shown from a top view of the LatheBot in Figure 25 below.

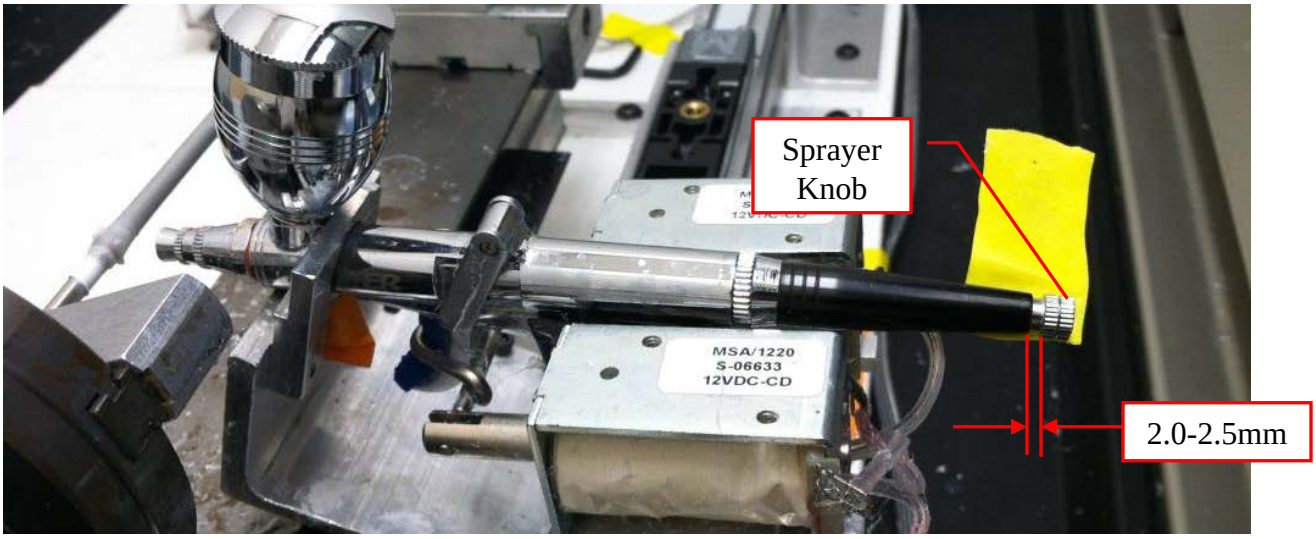

Figure 24 LatheBot Sprayer Knob

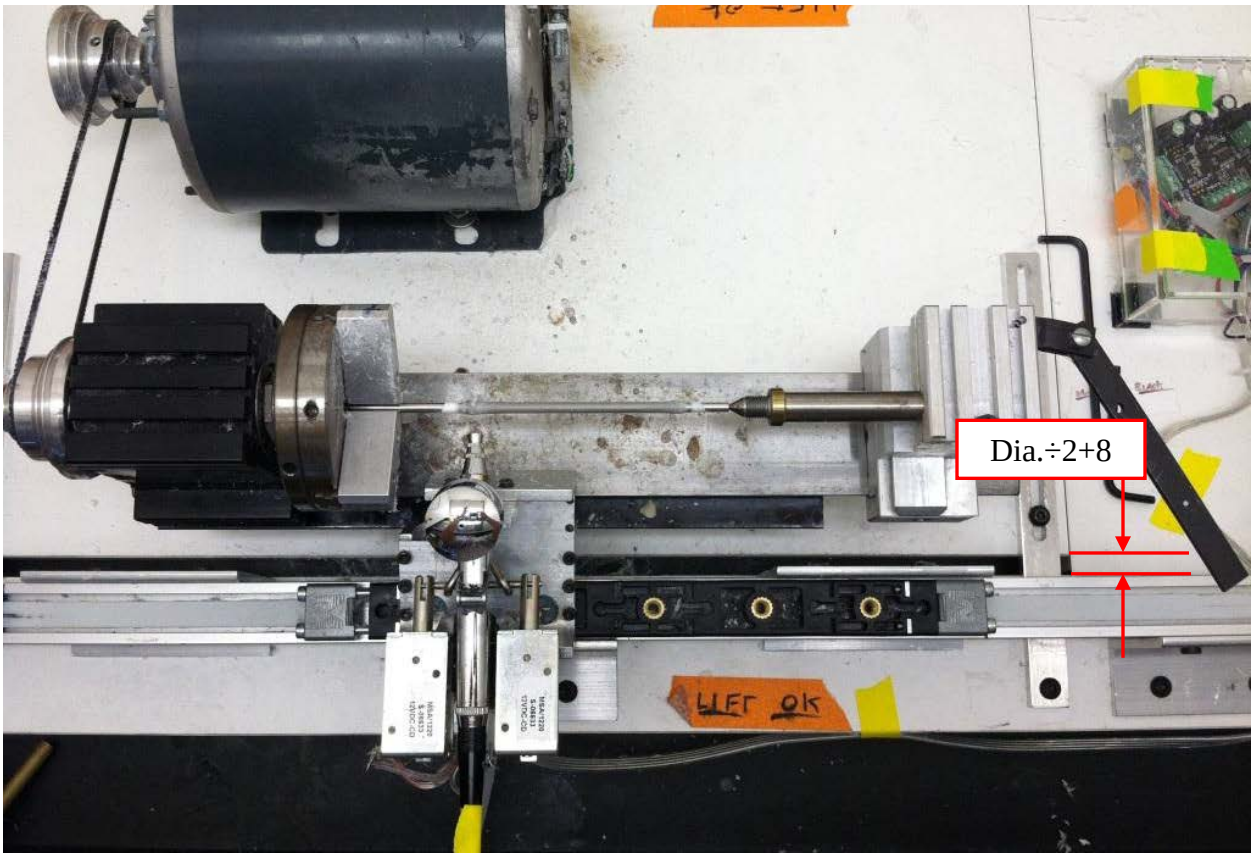

Figure 25 LatheBot Top View

5. Spray chloroform through the spray gun to clean any debris.
6. Insert mandrel into head stock and tighten chuck jaws. The tailstock may be used, but can be omitted for usage with the stainless steel mandrels due to excessive noise created.
7. Open LabView program as shown by front panel and block view in Figure 26 and Figure 27 below.
8. Set the Plateau Length to allow for creation of a tube. Note that the actual travel length of the stepper motor will be 20mm greater to allow for creation of thicker tube ends due to stepper motor acceleration.

9. Set the Number of Layers value. It is recommended that a lower value be used for the initial spraying cycle as a quick validation test run. After the initial cycle, layer ranges between 500-1500 cycles is preferred, and allows for refilling of the solution reservoir.
10. Fill the reservoir with the polymer solution and run the program. Monitor for any issues and verify flow. Refill the reservoir as necessary.
11. Between cycles, use a calipers to measure the thickness of the sprayed tube. The tailstock of the lathe can be used to ensure position of the mandrel, if needed.
12. Once the proper tube thickness is obtained, remove the mandrel from the lathe. Spray chloroform through spray gun to clean nozzle and clean up machine and fume hood from residual polymer fibers.

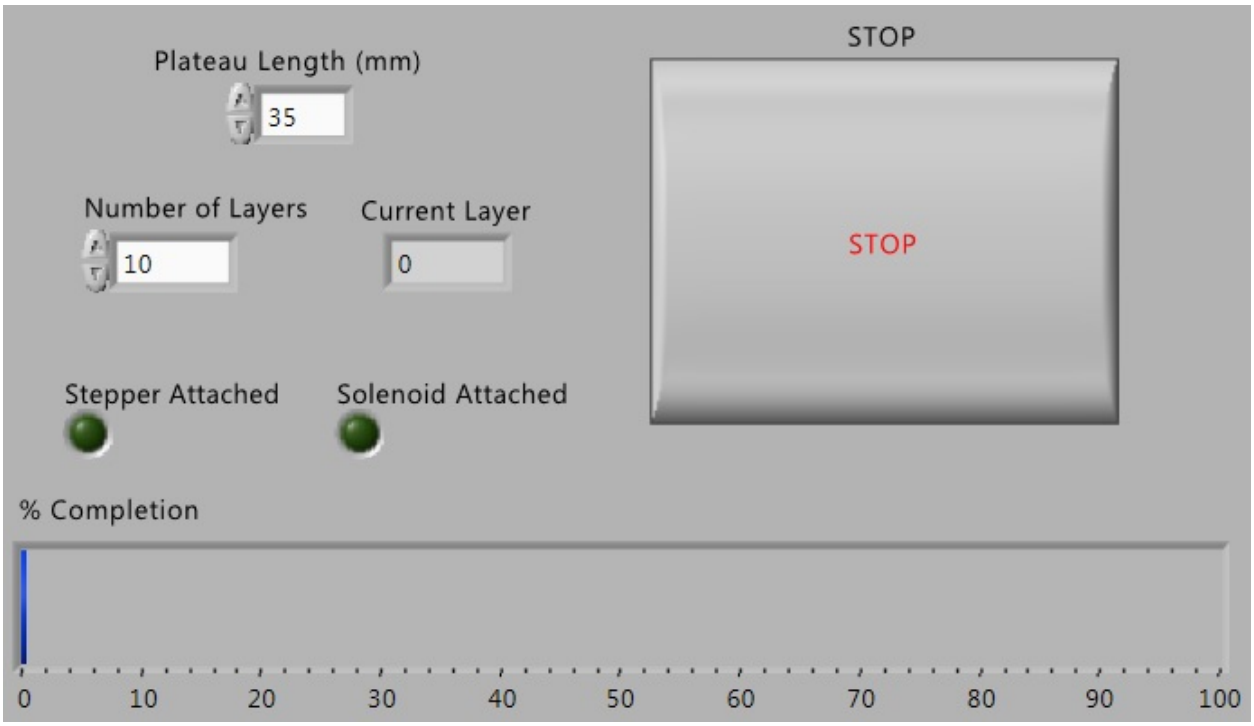

Figure 26 LabView Front Panel

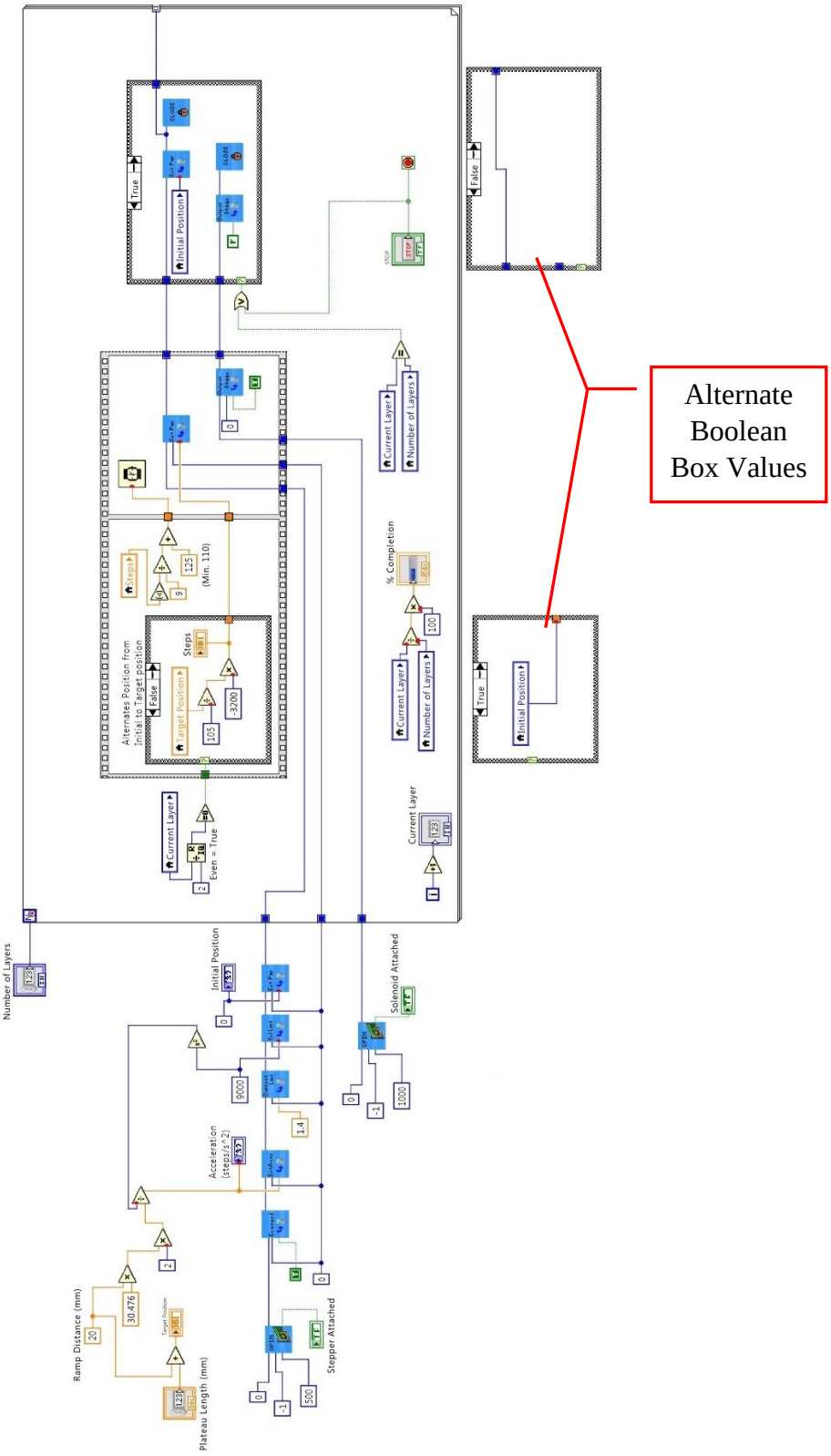

Figure 27 LabView Block Diagram

Appendix B: Mini-LatheBot Protocol

Using a polymer tube and the Mini-LatheBot equipment shown in Figure 28 below, springs can be created and then lengthened for implantation into animal models. The Mini-LatheBot equipment should be positioned inside the laser cutter (Universal Laser System model: VersaLaser 2.30) with the colorful wire protruding from under the front of the laser cutter lid, as all other wires are too thick and don't allow the lid to close enough for laser operation.

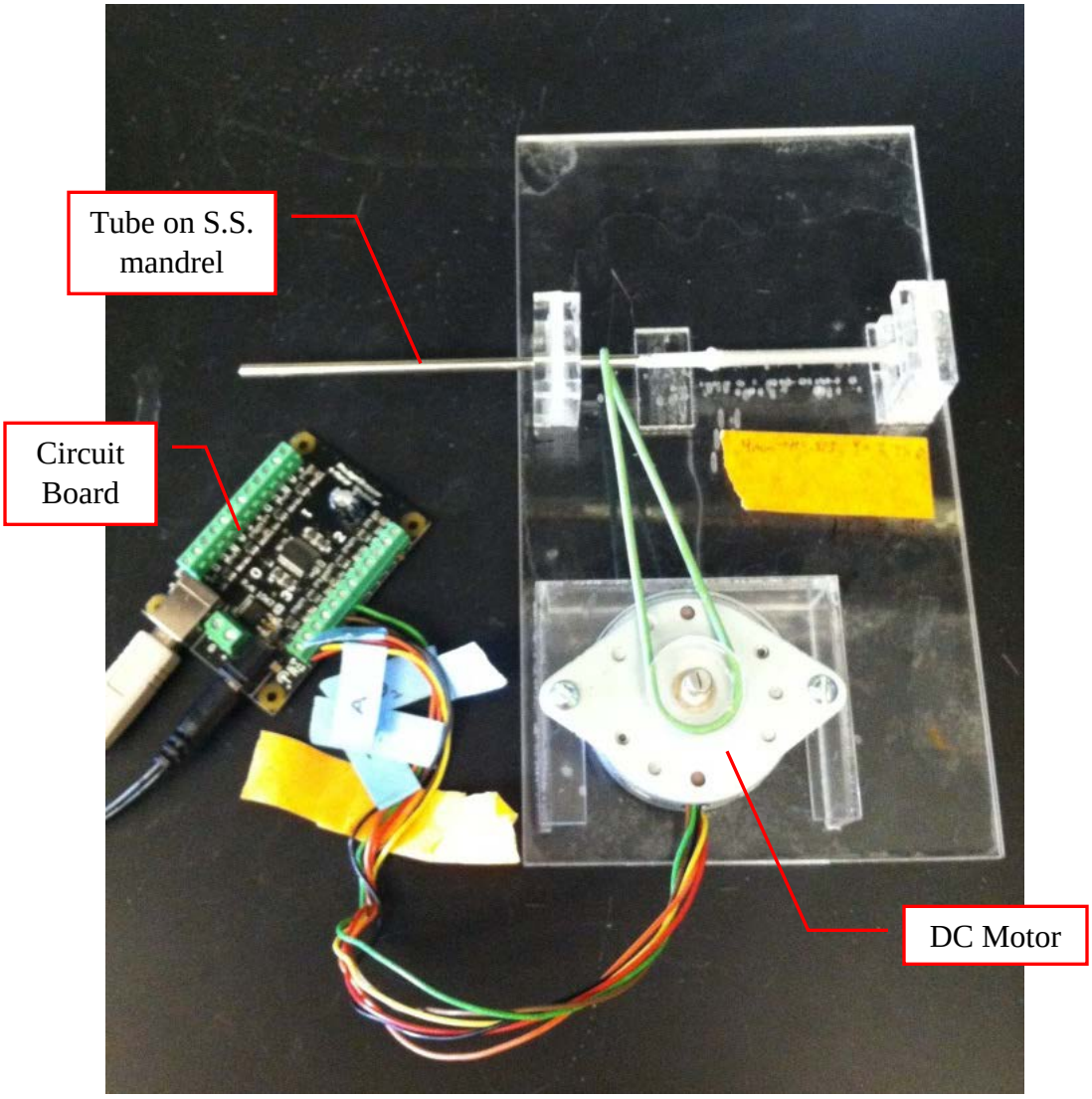

Figure 28 Mini-LatheBot Setup

Protocol:

1. Align edge (furthest from DC motor) to furthest tray edge inside laser cutter. Secure the base of the Mini-LatheBot to the laser cutter tray with tape.
2. Insert the mandrel into the base of the Mini-LatheBot. Guide one end of the mandrel through the drive belt prior to inserting the mandrel into the holding brackets.
3. Open the Phidget software used to operate the Mini-LatheBot and select the Phidget Unipolar Stepper Controller 4-motor option from the dialog box.
4. Select the stepper motor channel based on the colorful wire location as connected to the Mini-LatheBot circuit board (shown as location 3 in Figure 29 below).
5. Move the Velocity Limit slider to a value of 200. Move the Acceleration slider to the maximum value. Move the Current Position slider left to the most negative value, and the Target position slider right to the most positive value.
6. Click Engage and verify clockwise rotation of the motor. Click Engage again to stop DC motor.

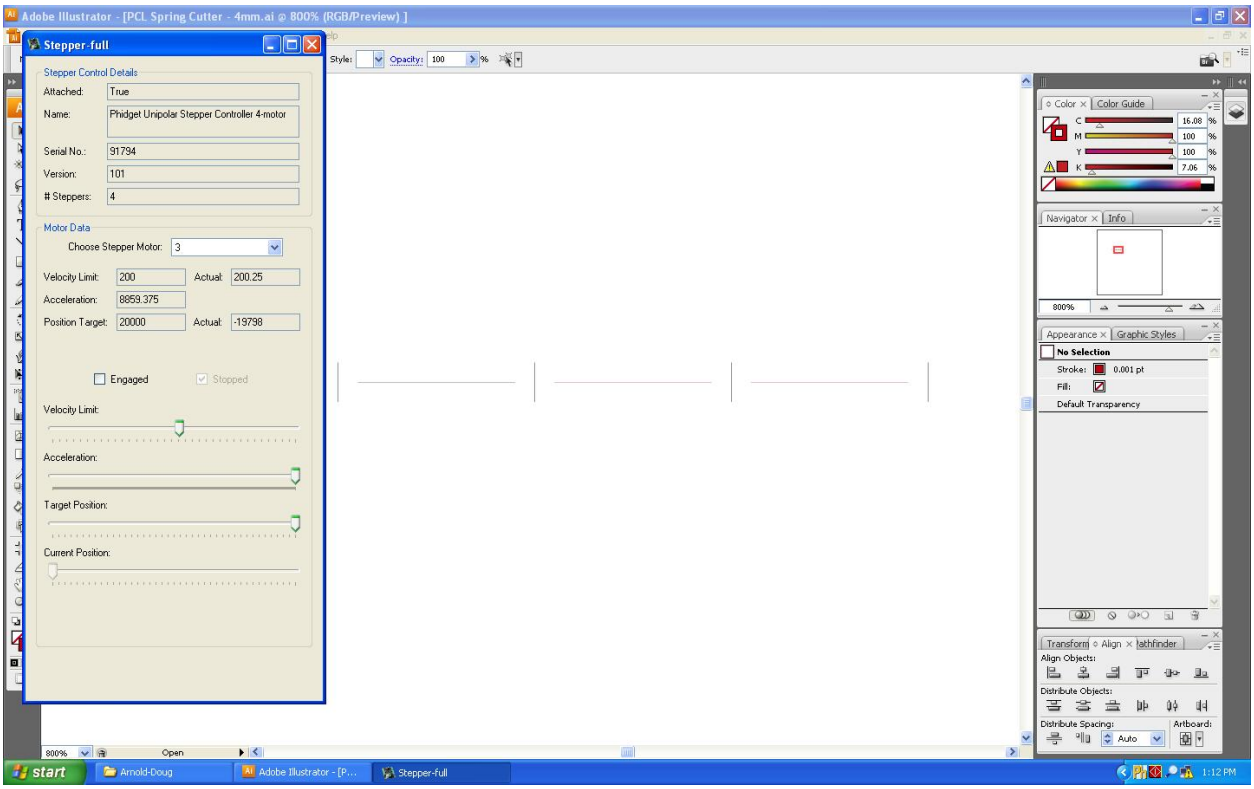

Figure 29 Mini-LatheBot Software

7. Create a laser cutting path profile using Adobe Illustrator. Spring helices are cut as the laser moves along the long axis of the mandrel while the mandrel is rotating. Laser path cuts that are perpendicular to the long axis of the mandrel are used to part individual springs.
8. Once the cutting path profile is created, hit Ctrl+P on the computer to print the image to the laser cutter. Adjust the z-axis, laser power, laser speed, and pulses per inch (PPI) printer settings as needed. Use the following equation for adjusting the laser speed to obtain a specific band size based upon usage of a certain mandrel diameter ($\varnothing_{mandrel}$) and a velocity setting of 200.

$$Laser\ Speed = \frac{Band\ Size}{0.0658 * \emptyset_{mandrel} + 0.0867}$$

9. Apply and accept all changes and click print once printer settings are adjusted properly.
10. Access the laser cutter printer software in the lower right corner of the computer taskbar (red icon near the clock). Using the focus and location settings on the laser cutter printer software, position the laser cutter pathway at the proper location on the tube.
11. Close the laser cutter lid and turn on the blower in the fume hood.
12. Click Engage on the Phidget software and ensure rotation of the mandrel.
13. Click the play button on the laser cutter software to begin laser cutting the tube.
14. Repeat cutting as necessary. Once laser cutting is complete, clean up and power off equipment as necessary.

Appendix C: Growth Factor Conjugation Protocol

Using a polymer tube segment on a stainless steel mandrel and the protocol detailed below, bFGF was conjugated to the surface of PCL using EDC and NHS. Protocol was based upon previous research that cross-linked heparin onto PCL scaffolds. (Singh, Wu, & Dunn, 2011)

Protocol:

1. Sterilize the polymer tube segment by submerging in 70% ethanol for 30 minutes.
2. Wash the tube segment with distilled water three times.
3. Saturate tube segments in 0.05M MES (2-(N-Morpholino)ethanesulfonic acid from Sigma Aldrich) buffer solution, adjusted to pH 5.5 with 0.1M NaOH, for 15 minutes.
4. Prepare a 0.5M solution of EDC and NHS. Add bFGF to obtain a 1% concentration of growth factor in solution (1 mg/mL).
5. Dissolve EDC, NHS, and bFGF in MES buffer and immediately strain through 0.4µm pore size filter in the fume hood.
6. Immerse tube segment in prepared solution and vortex briefly to get rid of small air bubbles on surface of the tube segments. Allow the reaction to sit overnight at room temperature.
7. Wash the tube segment with distilled water three times to remove byproducts.

Appendix D: Electrospinning Protocol

Using a polymer tube segment on a stainless steel mandrel and the protocol detailed below, PCL fibers were electrospun onto the surface of the tube segment. Protocol was based upon previous research that was used to create electrospun PCL scaffolds. (Joshi, Lei, Walthers, Wu, & Dunn, 2013)

Protocol:

1. Measure and combine 11% wt. PCL polymer to chloroform into a sealable glass vial. Add paraffin film to the outside of the cap on the closed vial to ensure seal.
2. Place the polymer/chloroform mixture onto a rotator for at least 12 hours.
3. Insert the mandrel into an acrylic containment box and connect to a 1/4 HP motor. Insert a vacuum hose into the containment box to assist in fiber deposition.
4. Pour the polymer solution into a syringe with an 18 gauge needle tip. Install the syringe into the automated infusion pump and set the infusion rate to 2.5 mL/hr. Insert the syringe tip into the containment box.
5. Connect the leads of the voltage supply to the syringe tip and motor terminals. Set the voltage supply to a power setting of 15-20kV DC power.
6. Turn on the vacuum and motor (set to run at 3450 rpm).
7. Turn on the voltage and start the infusion pump. Electrospin 0.250mL of the PCL solution onto the surface of the polymer tube segment.
8. Remove the tube segment and allow it to air-dry overnight. Turn off all equipment and clean up all surfaces in the fume hood to remove any excess fibers.

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