Mechanical Characterization of an External Fixator for Use in a Mouse Model

Thomas Albano

Union College '12

Bioengineering Department

Advisor: Jennifer Currey

Introduction:

Understanding the process of bone healing has become afundamental part of medical researchdue to the approximately one million fractures which occur annually in the United States. The current methods of fracture fixationwhich use intramedullary rods, external fixators, and fracture plates are effective but not ideal. These fracture fixation methods can lead to mal-union or non-union due to improper callus formation stemming from inadequatefixation and support. When mal-union and non-union occur, the structural integrity of the bone becomes greatly sacrificed and the patient is left to deal with continual pain.[1]

Previous studies have suggested that the mechanical environment surrounding the fracture site can have a profound influence on healing rate and efficiency. Studies have shown that mechanically stimulating the fracture site using intermittent tensile strains can increase the rate of healing and also increase the effectiveness of healing.[2]The amount of stimulation required to properly differentiate tissue formation and promote bone healing remains unknown. While some level of stimulation is known to have a positive influence on healing rates and efficiency, excess stimulation can inhibit healing and lead to improper tissue differentiation. Many qualitative theories seeking to characterize patterns of tissue differentiation have been established (figure 1).[3] These theories suggest that if the fracture site is over stimulated, tissues such as cartilage and fibro-cartilage may propagate and improperly overwhelm the fracture site.[4] Since cartilage and fibro-cartilage do not possess a mechanical strength equal to that of bone, differentiation of these tissues at a fracture site is not ideal.[5]

---

[1] *Mechanical Stimulation of a Healing Fracture Callus in a Mouse Model.*
[2] *Mechanical environment alters tissue formation patterns during fracture repair.*
[3] Carter's Theory of Tissue Differentiation
[4] Ibid
[5] *Mechanical Stimulation of a Healing Fracture Callus in a Mouse Model.*

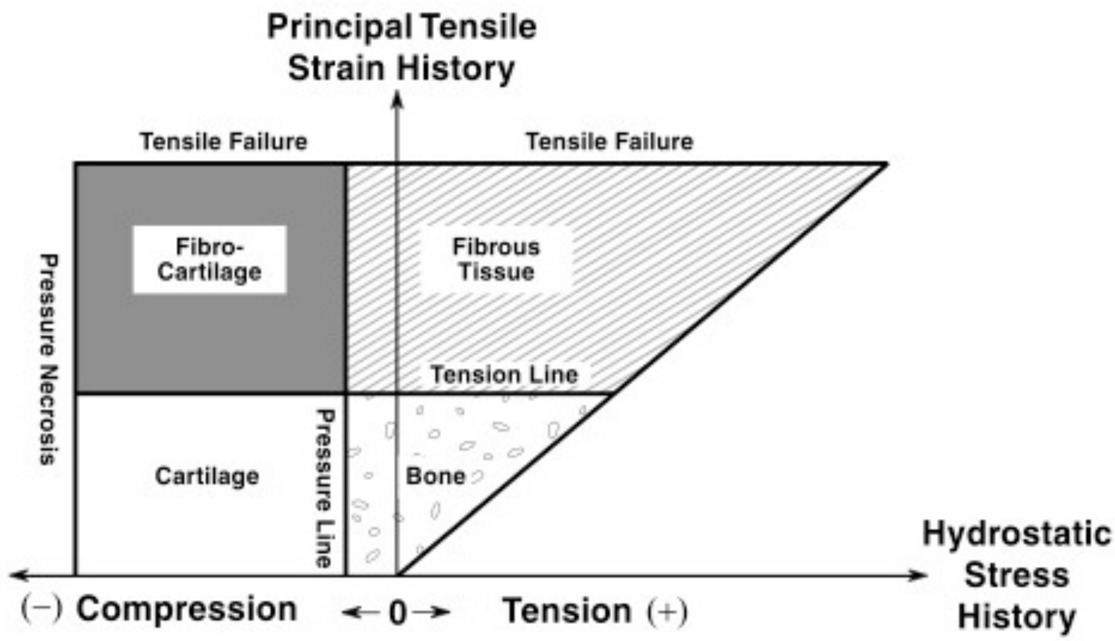

Figure 1: Carter's Theory of Tissue Differentiation.[6]

The goal of this research is to mechanically characterize the properties of wild-type mice tibias and mono-lateral external fixators which are attached to the tibias. External fixators were chosen over internal fixators in this study because external fixators do not interfere with the healing fracture callus.[7]The accessibility of external fixators also provides simpler way to monitor and control the mechanical strains across the fracture region.[8]The results of the mechanical testing will be used as a benchmark to compare future *in vivo* mechanically stimulated and healed tibia to standard intact tibia prior to fracture. By comparing the bones which have been stimulated at various rates, to standard bones we will work towards achieving

[6] Carter's Theory of Tissue Differentiation
[7] *An externally fixed femoral fracture model for mice.*
[8] Ibid.

an overall goal of "developing a better understanding of the relationship between the mechanical environment and healing mechanisms at a fracture site."[9]

In order to assess the healing response of fractures, mice were used as the test subject. Although mice are small in size, their healing rates are rapid and their healing response is similar to humans.[10]Mice are also an ideal model because of the ability to "knock out" specific genes if a gene is known to cause a certain response.[11] Thus, if a specific gene is known to influence bone healing, bone properties can be studied in the presence and in the absence of the specific gene.[12]

Three point bending mechanical tests were carried out using a tensile stage to determine the initial properties of excised mice tibias. The load-deformation data acquired using M-Test Quattro programming was used to calculate the modulus of elasticity and the ultimate allowable forces of the intact tibia during bending. These bone properties will be used as a benchmark for further examination of mice bones which will be fractured and healed *in vivo*. A variety of mechanical simulations will be applied to the fracture region through a mono-lateral external fixator in an effort to determine the ideal amount of stress needed to optimize the healing response of a fractured bone.

Methods:

In order to examine bone properties before and after healing, intact tibiae wereexcised from wild-type mice cadavers. During the excision process all skin, muscle tissue, ligaments, joints, and periostium were removed so mechanical examination would reflect the true mechanical properties of the bones. Prior to testing, the fibula which attaches to the posterior

---

[9] *Mechanical Stimulation of a Healing Fracture Callus in a Mouse Model.*
[10] Ibid
[11] *An externally fixed femoral fracture model for mice.*
[12] Ibid

side of the tibia was removed in order to isolate the material properties of the tibia during testing and prevent the mechanical testing from prematurely stopping.[13]In order to position the mice bones consistently and limit any preload placed on the bones, an alternate base was designed to reposition the tensile stage vertically and allow the bones to rest on the bottom two supports (Figure 2). This repositioning of the tensile stage also significantly increased the consistency of bone placement for testing. Components for three point testing were designed and fabricated to account for the small size of the mice tibiae. The span between the bottom supports was reduced to 0.5 inches to prevent the tibiae from slipping between the supports. Three point bending was conducted using an MTI/FullamTensile Stage, M-Test Quattro Mechanical Testing Data Collection Software,and a 100 Lb compressive load cell (Figure 2). During testing, a strain monitoring LVDT measured the deformation of the bone throughout the bending process. Tests were conducted until break and the load-deformation data was used to calculate the modulus of elasticity.Bone geometry was determined using a Micro-CT scanner, and the diameter of the intramedullary cavity was estimated using undecalcified histological sections and imageJ (NIH).Equations from Schrieferet al.were used to calculate the modulus of elasticity for each of the bones from the collected load-displacement data (equation 1; where F = applied force, D = displacement, L = length, and I_x = moment of inertia).[14] The resulting calculations were compared to the results calculations performed by the M-Test Quattro program itself.

$$E = (F/D)*(L^3/48I_x) \qquad \text{(equation 1)}$$

In order to determine the effect drilling has on mechanical bone properties, four holes were drilled into intact bones and the bones were tested under three point bending and their

[13]*A comparison of mechanical properties derived from multiple skeletal sites in mice.*
[14] Ibid

properties were analyzed. External fixator mechanical characterization followed drilled bone mechanical testing. Four holes were drilled into the bone and specially designed mono-lateral external fixators (stainless steel grade 303) were attached to excised tibiae using0.6 mm in diameter, threaded, titanium “Filpins” (S.J. Filhol Dental, West Cork, Ireland). Since the location of the second pin hole from the distal end of the bone is in the region of the bone where bone diameter is smallest, a 0.3 mm pin was used in this location. Mechanical testing demonstrated premature bone fracture during mechanical testing at this pin hole, as opposed to the middle of the bone when 4 x 0.6 mm pins were used. Thus, the drill size was reduced and a smaller retention pin was used at this location. Using a smaller pin hole at this location shifted the fracture point back to the center of the bone, and premature bone fracture ceased. The pins were inserted into the bones and locked into place using medical grade instant adhesive (Loctite, Rocky Hill, CT) and the tibiae-fixator complexes were mechanically tested using the tensile stage. The raw data from the trials was used to calculate the modulus of elasticity of the complex and the results were compared to the mechanical properties of the tibiae alone. (A guide to syncing the MTI/Fullam Tensile State with the M-Test Quattro Mechanical Testing Data Collection Software can be found in the Appendix.)

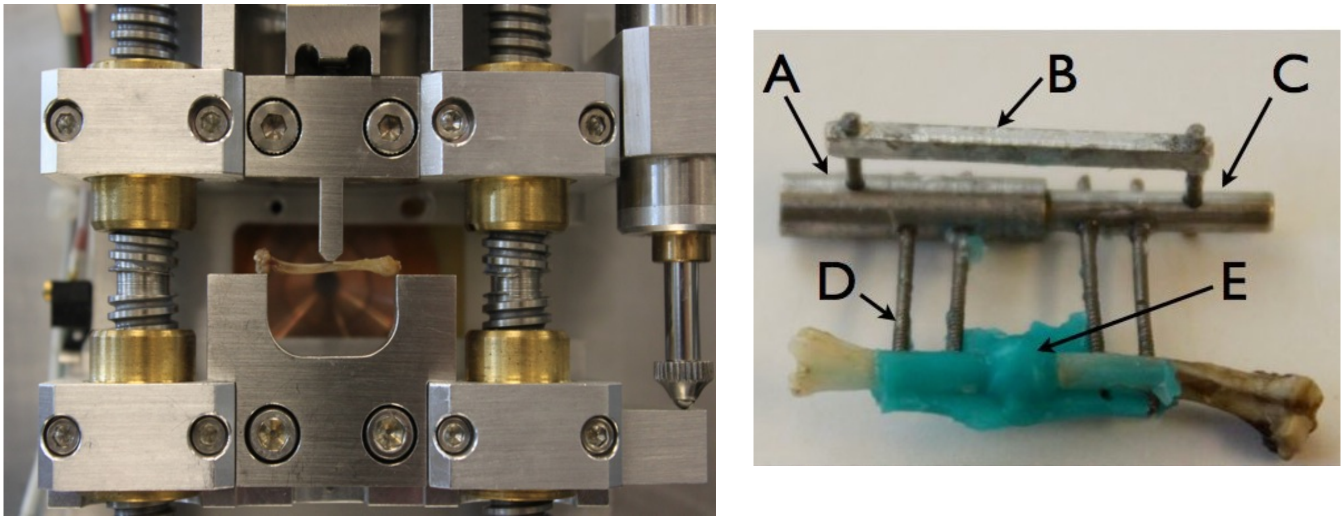

Figure 2: Figure 2a presents an image of the upright tensile stage with a tibia loaded for three point bending testing. Figure 2b is an image of an external fixator secured to a mouse tibia via 4x0.6 mm threaded pins and medical grade adhesive.

Experimental Model:

Schriefer et al.'s study was an important reference in developing the methodology for mechanical testing. The study sought to determine the strength and mechanical properties of bones in various locations throughout the mouse skeleton. The femur, humerus, third metatarsal, radius, and tibia from a genetically high bone mass and low bone mass mice groupwere loaded until failure using three point bending. The excision and testing methods used in this study were consistent and reliableand therefore were replicated in our own experimentation. Micro-CT scanned images suggested that the fibula was removed from the posterior side of the tibia prior to testing in order to isolate the true mechanical properties of the tibia. Equations for calculating ultimate force and modulus of elasticity from force-displacement data were presented in this study and are as follows: (where F = applied force, D = displacement, L = length, I_x = moment of inertia, F_u = ultimate force, and c = span length)

$$E = (F/D)*(L^3/48I_x) \qquad \text{(equation 1)}$$

$$\sigma = F_u * (Lc/4I_x) \qquad \text{(equation 2)}$$

Due to the constantly changing contour of the mouse tibia this study suggests that it is not an optimal bone to test mechanical properties. The mouse tibia can be estimated as a hollow cylinder to simplify calculations, but the contour and shape of the bone drastically changes over its length.[15]

Results:

Mechanical testing of intact mice tibia (n=18) demonstrated general consistency throughout testing. The modulus of elasticity ranged from 2.24 GPa to 12.9 GPa with an average of 6.25 GPa and a standard deviation of 2.82 GPa. All of the extracted bones were similar in diameter with the maximum diameter of 1.27mm and a minimum diameter of 0.96mm. The maximal load experienced during testing ranged from 0.98N to 7.25 N. Maximal deformation in the bones during loading ranged from 0.13 mm to 0.69 mm. Variations in minimal and maximal loading combined with fluctuations in deformation to account for the variation in modulus of elasticity. In all intact tibiae testing trials, fracture occurred near the center of the bone at the central point of bending. The results from the intact tibiae mechanical testing can be found in table one.

[15] *A comparison of mechanical properties derived from multiple skeletal sites in mice.*

Table 1: Mechanical Characterization of intact mice tibiae.

Sample Number	Diameter (m)	Ultimate Force (N)	Displacement (m)	Calculated E (GPa)
1	0.0010922	3.64736	0.00044958	5.45
2	0.0010668	1.69024	0.00013462	9.36
3	0.0011684	6.8944	0.00065532	5.27
4	0.0009652	6.0048	0.00069088	1.02
5	0.0010922	2.75776	0.00032258	5.75
6	0.0010414	1.46784	0.0002286	5.33
7	0.0012446	4.2256	0.00034036	4.75
8	0.0011938	6.93888	0.00060198	5.27
9	0.001143	3.06912	0.00037084	4.56
10	0.001143	4.58144	0.00042672	5.92
11	0.001016	2.17952	0.00015748	1.29
12	0.00127	4.0032	0.0003683	3.82
13	0.0011176	3.42496	0.00043688	4.76
14	0.001143	3.02464	0.00037338	4.47
15	0.0010414	0.97856	0.00036322	2.24
16	0.0011938	1.28992	0.00016256	3.63
17	0.0009652	3.86976	0.00055626	8.19
18	0.001016	7.25024	0.000635	1.06
Average	**0.001106**	**3.73879**	**0.000404**	**6.25**
Standard Deviation				**2.82**

As expected, drilling through the bones altered the mechanical properties of the bone. The holes reduced the total area and created localized stress concentrations; these stress concentrations led to rapid fracture under limited loading. The use of the 0.3 mm pin at the second hole from the distal end of the bone led to the bones consistently breaking in the center, at the location of the 3rd point of contact. Since the diameter of the bone is larger at the location of the two proximal and the most distal pin holes, the 0.6 mm pins were adequate and did not lead to premature fracture.

The drilled bones demonstrated varied bone properties. Calculated modulus of elasticity ranged from 1.97 GPa to 9.87 GPa with an average of 4.67 GPa and a standard deviation of 2.36 GPa. All of the bones were similar in mid-diaphysis outer diameter which ranged from 0.96 mm to 1.14 mm. Testing demonstrated considerable variation in the maximal load in which the drilled bones could withstand. The maximal load ranged from 1.33 N to 8.45 N and averaged 3.78 N. Natural variations in the proximity of the pin holes in relation to the outer edge of the bone may have led to these variations in these bone properties.The results of the drilled bone testing can be found in table 2.

Table 2: Mechanical Characterization of tibiae drilled with 3x 0.5mm holes and 1x 0.3mm hole at the second pin location from the distal end of the bone.

Sample Number	Diameter (m)	Ultimate Force (N)	Displacement (m)	Calculated E (GPa)
1	0.0009906	4.2256	0.0008636	5.10
2	0.0009906	4.04768	0.00070612	5.90
3	0.0010922	1.69024	0.00037338	3.04
4	0.0010922	3.82528	0.00046228	5.56
5	0.0010922	3.15808	0.00044958	4.72
6	0.001016	3.82528	0.00040386	8.80
7	0.0012192	1.3344	0.00023876	2.34
8	0.0009652	4.98176	0.00059436	9.87
9	0.0011176	2.84672	0.0009017	1.92
10	0.001143	8.4512	0.00227076	2.05
11	0.001143	1.7792	0.0006096	1.61
12	0.0009652	5.24864	0.00134366	4.60
13	0.0010668	4.31456	0.00089662	3.59
14	0.0010668	2.35744	0.00035052	5.02
15	0.0011176	5.29312	0.00131064	2.45
16	0.001016	5.29312	0.0006858	7.17
17	0.001016	4.40352	0.000635	6.44
18	0.0010414	3.73632	0.00081026	3.83
Average		**3.78**		**4.67**
Standard Deviation				**2.36**

The attachment of the external fixator increased the overall mechanical properties of the bones. The modulus of elasticity of the bone-fixator complexes (n=9) ranged from 4.10GPa to 16.0 GPa and averaged 8.10 GPa with a standard deviation of 3.67GPa. Thus the average modulus of elasticity of the bone-fixator complex was 3.43 GPagreater than the average modulus of elasticity of the drilled bones and 1.85 GPa greater than the intact bones. The ultimate loads placed on the bone-fixator complex ranged from 2.93 N to 8.98 N and averaged 5.91 N. Thus the average ultimate load withheld by the bone-fixator complex was 2.18N greater than the average ultimate load withheld by the bone alone. The results from the bone-fixator complex testing can be found in table three.

Table 3: Mechanical testing data and modulus of elasticity calculations of the bone-fixator complexes is shown here. The modulus of elasticity was calculated using equation 1. The asterisk marks a trial which was incomplete due to slippage during testing and is not included in the averages.

Sample Number	**Ultimate Force (N)**	**Displacement (m)**	**Calculated E (GPa)**
1	5.24864	0.00022098	16.0
2	5.64896	0.00037338	10.2
3	5.56	0.00075184	6.87
4	2.93568	0.00022352	10.9
5	4.53696	0.00079248	5.97
6	6.98336	0.00103632	4.10
7	8.98496	0.0006096	6.16
8	5.56	0.00084582	5.46
9	7.784	0.00050038	7.26
Average	**5.91**	**0.00059**	**8.10**
Standard Deviation			**3.67**

Statistical analysis was conducted to analyze the relationships between the three testing groups. A single tailed T-test between the intact and drilled groups produced a P-value of 0.06. Typical statistical analysis uses P-values< 0.05 as the threshold for statistical variance. Our P-value is 0.01 above this standard value thus the mechanical properties of the drilled tibiae and the intact tibiae are not statistically different. Therefore drilling into the tibiae did not significantly reduce the properties of the bones. A t-test between the drilled tibiae and the fixator attached tibiae was also conducted. The resulting P-value of 0.10 suggests that there is no statistical significance between the drilled and fixator attached tibial properties.

Discussion:

The methodology used to extract the bones and attach the fixators was reliable and repeatable. The design of the upright tensile stage base and the fabrication of the smaller three point bending pieces make the mechanical testing process more consistent. Limiting the time between bone extraction and mechanical testing prevented the bones from drying out and helped to reduce variance in bone properties.

The attachment of the mono-lateral external fixator was intended to significantly increase the mechanical stability and mechanical properties of the bones. From a numerical standpoint, the mechanical testing data shows that the modulus of elasticity of the bone-fixator complex is 1.85GPa greater than the modulus of elasticity of the tibiae alone. Although drilling into the bones reduced the mechanical properties of the bones, the rigidity of the external fixator was able to compensate for the reduction in bone properties caused by drilling.

Now that the mechanical properties of wild-type mice tibiae have been defined, these properties will be used as a benchmark for further testing (Table 4). In an effort to define an acceptable rate and amount of mechanical stimulus which could potentially enhance and increase bone healing, mechanical testing of bones post *in vivo* mechanical stimulus will follow.The mechanical properties of the bones which have completed *in vivo* mechanical stimulation will be compared to the established benchmark. Comparison of bone properties based on the rate in which the bones were mechanically stimulated will help to establish a threshold for the ideal amount of compression and tension needed to enhance bone healing. Continually altering the rate and amount of mechanical stimulus will help to develop a better understanding of the relationship between the mechanical environment and healing mechanisms so clinical treatment of non-unions can be improved.

Table 4: Mechanical Characterization of Intact Tibia, Drilled Tibiae, and Tibiae with Fixators Attached.

	Sample Size	**Average Modulus of Elasticity (GPa)**	**Standard Deviation (GPa)**
Standard Intact Tibiae	**18**	**6.25**	**2.82**
Drilled Tibiae	**18**	**4.66**	**2.30**
Tibiae with Fixators Attached	**9**	**8.10**	**3.67**

Appendix:

MTEST Quattro set up for three point bending tests:

Step 1: **Select File→New→Test Procedure**: To open a new test procedure

Step 2: Under the **Specimen Tab** Select the appropriate test (Figure 3)

- Enter a name for the sample in the **identifier space**. (the number will automatically increase as multiple tests are run if **auto increment** is selected)

- Enter the **dimensions** and **properties** of the material being tested

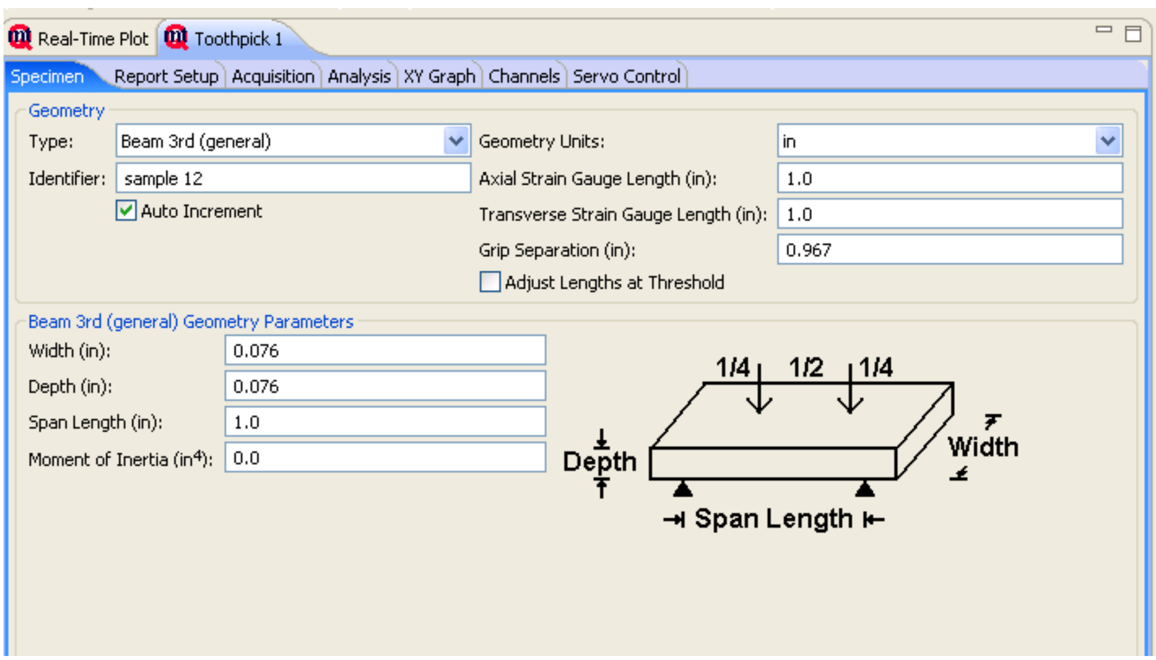

Figure 3: The Specimen Tab display.

Step 3: Select the **Report Setup Tab**(Figure 4)

- Enter the **report title**

- Enter the **material** and **operator** in the boxes on the left.

- Other details pertaining to the report can also be entered here (ie. Temperature ect.)

- Select **include plot when reporting results** if a plot is desired with the results

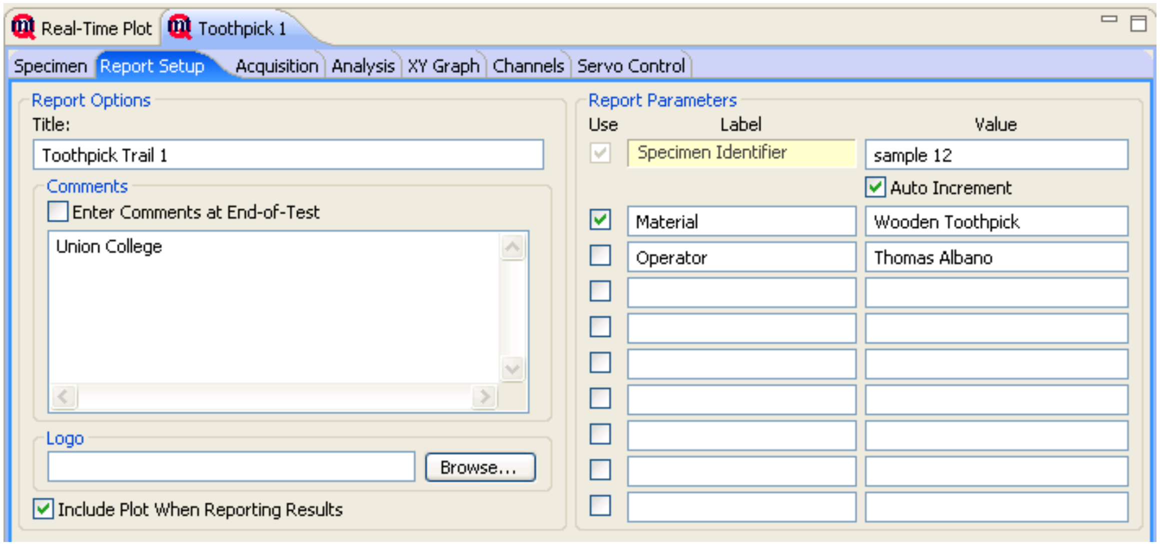

Figure 4: The Report Setup display.

Step 4: Select the **Acquisition Tab**(Figure 5)

- Chose **Load** as the threshold channel

- The **Threshold Value** should be 0.0 so that the test begins as soon as the third point of bending comes in contact with the bone

- **Break Threshold** should also be 0.0 so that the test stops as soon as the bone is fractured.

- Choose **stop sample at break** to stop the test as soon as the sample breaks

- Select **Auto Save Test Data**
-
- Enter an appropriate test result name

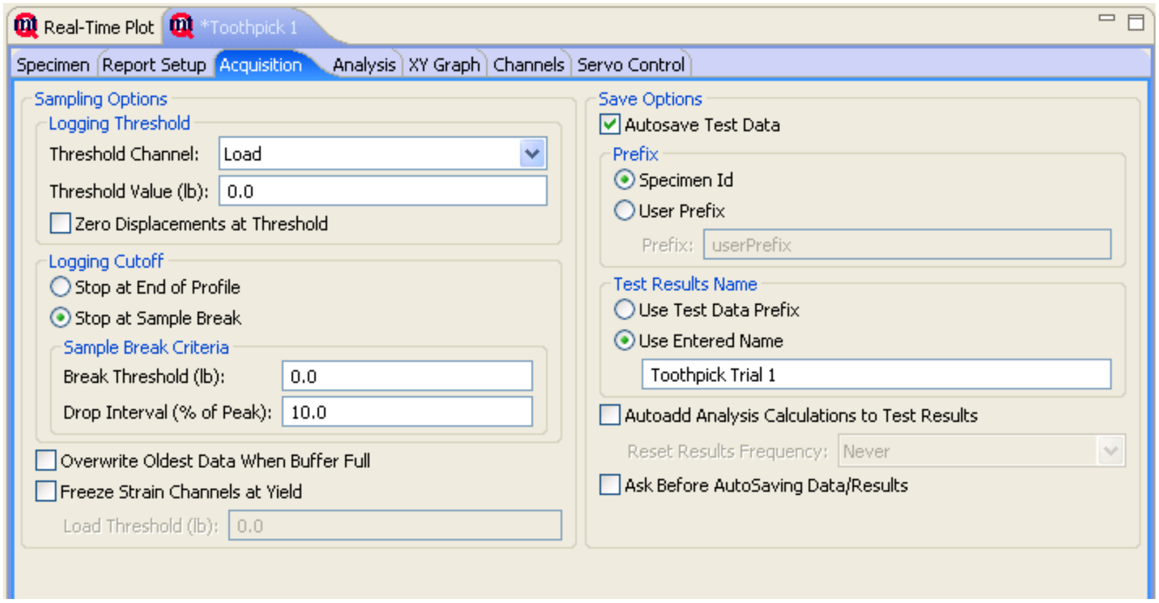

Figure 5: The Acquisition Tab display.

Step 5: Select the **Analysis Tab**(Figure 6)

- Select the types of analyses needed for the test using the suite dropdown menu
- Add analysis types using the select button
- **Modulus of Elasticity**, **Load at Break**, **Load at Maximum Position** and **Extension atMaximum Load** were chosen for the Mechanical Characterization Trials

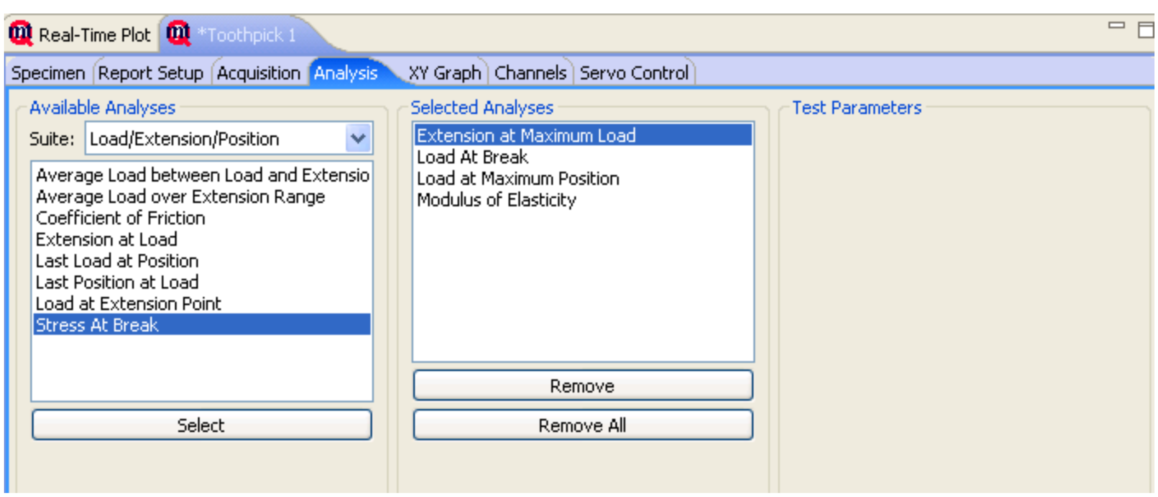

Figure 6: The Analysis Tab display.

Step 6: Select the **XY Graph Tab**(Figure 7)

- Choose appropriate X-Y Graph axes labels.
    - Typically **Load** (Y-Axis) and **Time** (X-Axis)
        - This will help determine when to stop the test if "stop at sample break" is not selected under the acquisition tab

- Select **Auto Scale** to auto scale the axes while the test is running

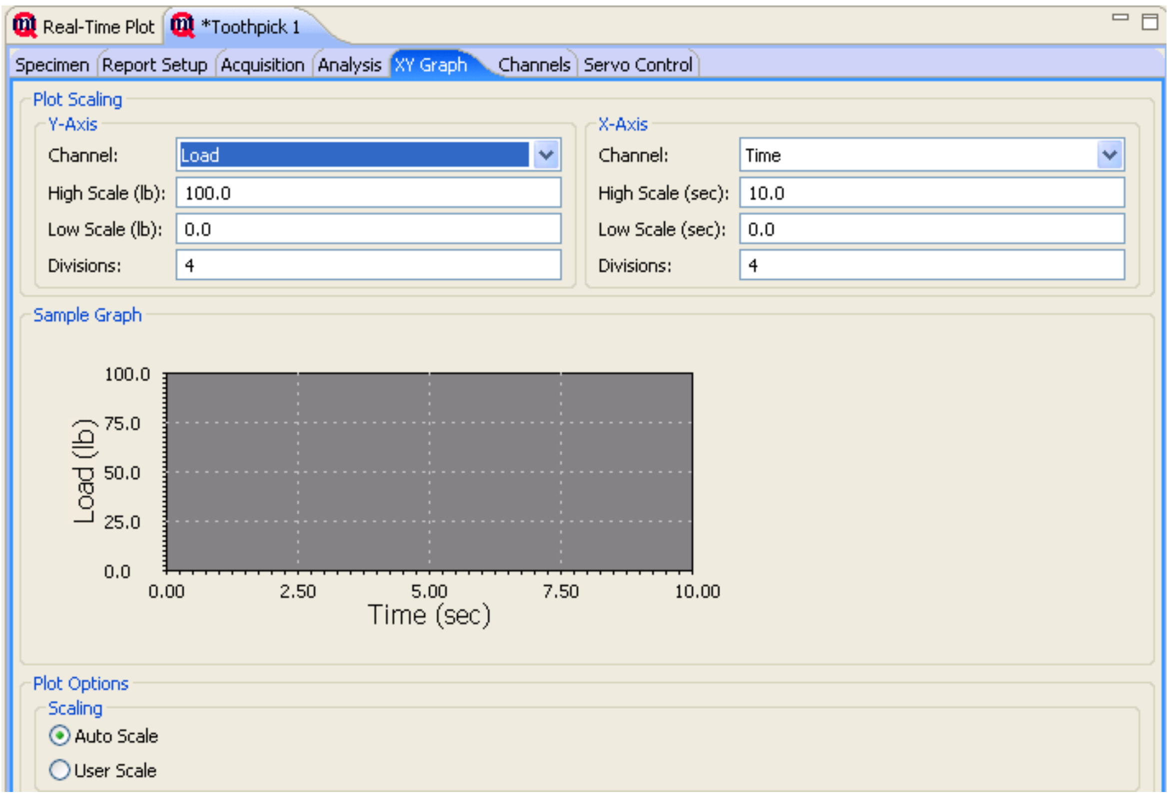

Figure 7: The XY Graph Tab display.

Step 7: Select the **Channels Tab**(Figure 8)

- Select the appropriate **transducer** from the drop down menu
    - 100 Lb Compression Transducer was used to Mechanical Characterization Trials.
    - Transducer selection must coincide with the load cell that is currently being used.

- Choose the desired units and rate from the drop down menus

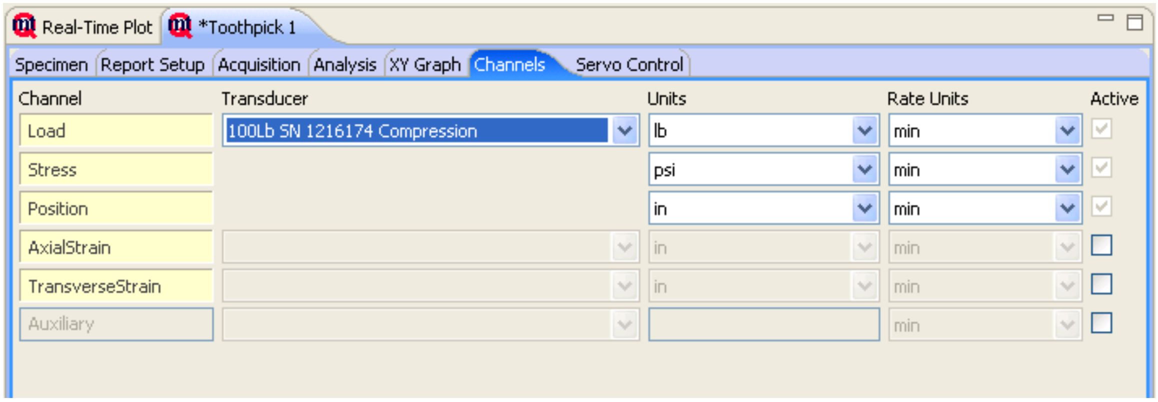

Figure 8: The Channels Tab display.

Step 8: Choose the **Servo Control Menu**(Figure 9)

- Choose the **General Tab**:

    - Adjust the **Jog Rate**: this controls how fast the stage manually moves using the jog arrows

    - Adjust the **Home Rate**: this determines how fast the stage returns to the home position following each trial

    - Set the **pre-load** to zero so that the testing begins as soon as the third point of bending comes in contact with the bone

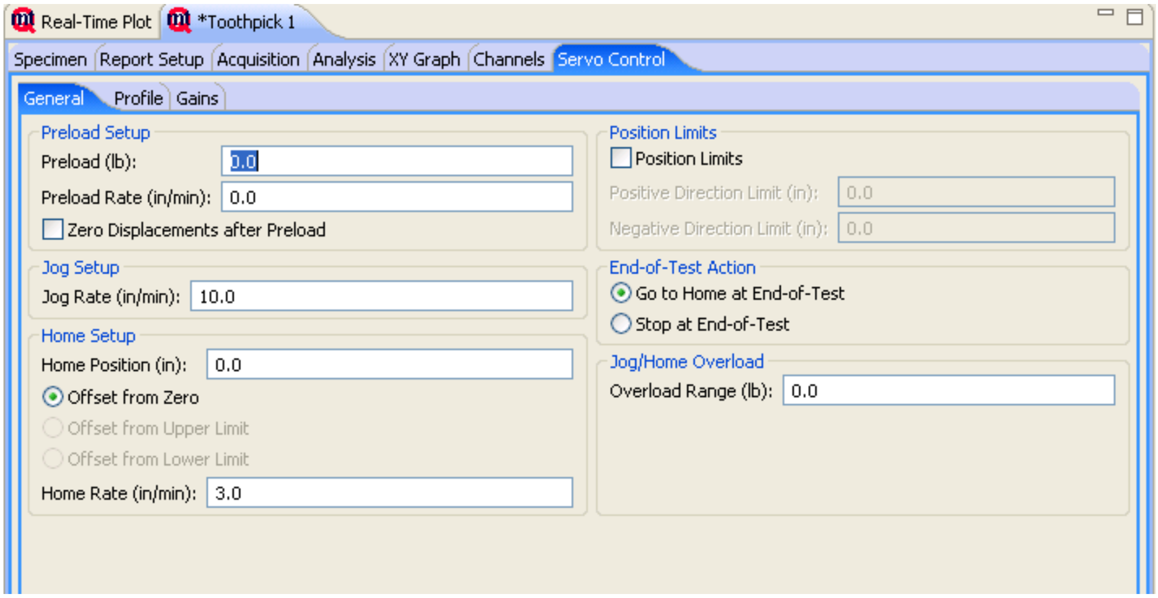

Figure 9: The Servo Control Tab → General display.

Step 9: Select the **Profile Tab**: (Figure 10)

- Choose "**Ramp**" under waveform

- Set the sampling rate to define how many data points are collected per second

- Choose **position** from the control menu
  - Set the desired rate of the control

- Choose **load** from the limit menu
  - Set the maximum load value and adjust the increment to determine how the load will be applied

- Select **insert segment** when parameters are set
  - The test segment should appear in the segment box

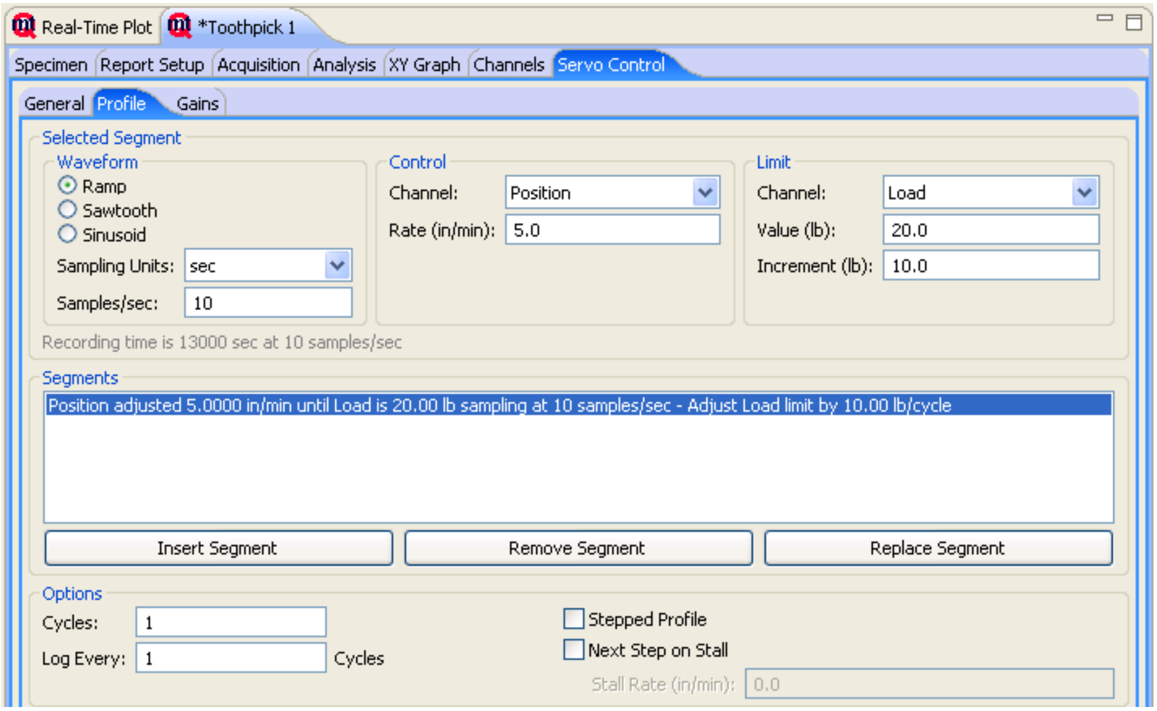

Figure 10: The Servo Control Tab → Profile display.

Step 10: **Start** the Test (Figure 11)

- Load the sample into the tensile stage
- The **yellow jog arrows** move the stage in and out to make loading the sample easier
- Make sure all input channels are zeroed by clicking the **zero all inputs**
- Select the Green **play** button from the control panel to start the test
- The test can be **stopped** and paused using the appropriate buttons

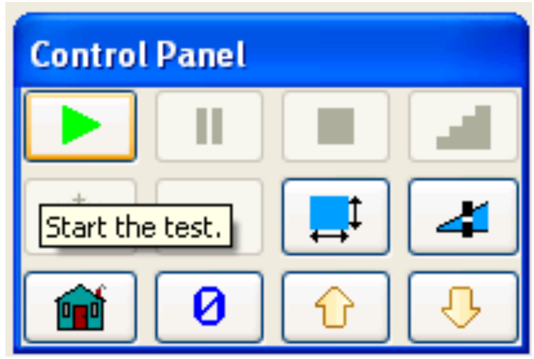

Figure 11: Control Panel Start Button display.

Step 11: **Results**(Figure 12)

- Following the test select "**view the test analysis/results**" icon at the top
- The results will appear on a new screen with all selected variables from the analysis tab calculated.

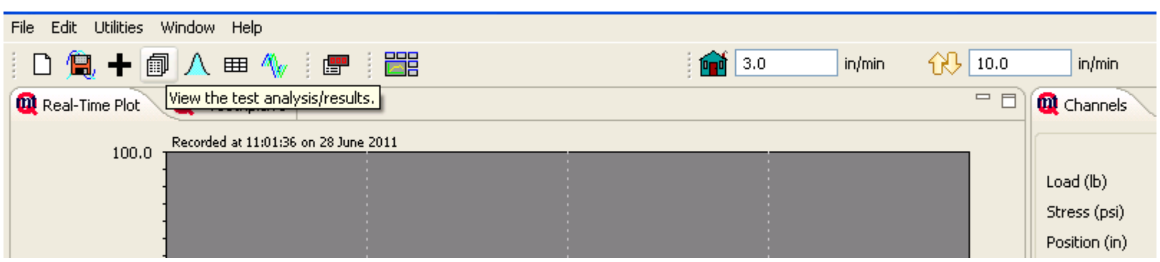

Figure 12: Viewing the Test Analysis and Results.

Results Example: (Figure 13)

Specimen Identifier:	sample 18
Material:	Wooden Toothpick
Geometry:	Beam 3rd (general)
Width:	0.076 in
Depth:	0.076 in
Span Length:	1.000 in
Moment of Inertia:	0.000 in^4
Area:	0.00 sq in

Analysis Results

Extension at Maximum Load	
Extension at Maximum Load	0.0449 in
Load At Break	
Load	3.61 lb
Load at Maximum Position	
Load at Maximum Position	N/A
Modulus of Elasticity	
Modulus	1995.511 MPa

Union College

Figure 13: An Example of Test Results.

References:

1. Project Description: BRIGE: Mechanical Stimulation of a Healing Fracture Callus in a Mouse Model.

2. E.A. Smith et al. "Mechanical Environment Alters Tissue Formation Patterns During Fracture Repair."Journal of Orthopaedic Research .2004. 22 (5):1079-1085.

3. Jennifer Currey. "Carter's Theory of Tissue Differentiation." Mechanobiology Class Notes.Union College. 2011.

4. KM Cheung et al. "An externally fixed femoral fracture model for mice." Journal of Orthopaedic Research. Jul 21, 2003. (4):685-90.

5. Jennifer L. Schriefer et al. "A comparison of mechanical properties derived from multiple skeletal sites in mice." Journal of Biomechanics. 2005. (38):467-475.

6. K. Kaspar et al. "A new animal model for bone atrophic non-union: fixation by external fixator." Journal of Orthopaedic Research. Dec 26, 2008. (12):1649-55.

7. B. Willie. "Mechanical Characterization of External Fixator Stiffness for a Rat Femur."Journal of Orthopaedic Research. May 27, 2009. (5):687-93.