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# Protocols For Preconditioning Of Patellar Tendon For Anterior Cruciate Ligament Reconstruction

Richard Lee Crawford

# PROTOCOLS FOR PRECONDITIONING OF PATELLAR TENDON FOR ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: STRESS RELAXATION VS. CREEP

By

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The ACL is one of the major ligaments in the knee connecting the femur to the tibia which provides stability by resisting shear in sagittal plane. ACL tears occur in 1 out of 3000, and due to inability to heal, reconstructive surgeries are performed at a rate of 200,000/year. Final graft fixation tension during surgery has been shown to wane due to stress-relaxation which has been correlated with negative clinical outcomes. Therefore, preconditioning, which currently is an isometric load (88N), is performed to remove stress-relaxation after the final tension has been applied *in vivo*.

Three preconditioning protocols, creep, stress relaxation, and none, were tested to show significant differences and variance in graft tension after 30 minutes. The results suggest that the current preconditioning protocol may not be efficient enough to remove stress-relaxation after final fixation, and that a creep protocol causes less variability than the other preconditions performed.

Key words: ACL reconstructive surgery, stress relaxation, preconditioning

DEDICATION

I would like to dedicate this work to my cousin Landon Michael Slavant and my niece Lillian Christine Crawford “Lily Pad”. The past year I have learned so much from both of them about who I am and what love really is about.

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# CHAPTER I

# INTRODUCTION

The Anterior Cruciate Ligament (ACL) is one of the four major ligaments in the knee connecting the femur to the tibia between the medial and lateral chondyles. The main function of the ACL is to provide stability by resisting shear between the femur and tibia in the sagittal plane (Woo 2006). ACL tears occur in 1 out of 3000 people per year in the US alone (Miyasaka 1991, Beaty 1999). Yet unlike many other tendons and ligaments in the body, the ACL is unable to repair or heal itself so that even small tears can result in very disabilitating effects (Frank 2004). These effects lead to an adverse change in the biomechanics of the knee resulting in instability which can further progress into the onset of osteoarthritis (Petrigliano 2006).

In order to correct ACL deficiencies, allograft or autograft reconstructive surgeries are performed at a rate of 200,000 annually, costing nearly 5 billion dollars in the US (Vunjak-Novakovic 2004). Autografts from the mid-section of the patellar tendon are most commonly used to replace the deficient ACL because of similar viscoelastic properties. Even though there is a high prevalence of these surgeries, there is no consensus among clinicians and scientists on the best surgical methods for preconditioning and final fixation load which is reflected in various clinical outcomes, in the short and long term, after reconstruction (Jomha 1999, Tomita 2001, O'Neil 2001,

Goldblatt 2005, Anne 2001, Nicholas 2004). Preconditioning of the graft before fixation is one of the methods that is under much scrutiny today since the preconditioning affects the viscoelastic properties of the graft after fixation, but very few studies have been done on the effects of preconditioning protocols on final fixation tension and moreover knee kinematics post-operatively. By preconditioning the graft the stress relaxation of the tissue is hopefully minimized, thereby having a more a controlled final fixation tensile load. Different types of preconditioning such as cyclic loading, creep, and stress relaxation result in varying degrees of minimization of load loss after fixation (Nurmi 2004). The current surgical preconditioning procedure holds the graft at a constant strain before final fixation at the graft site, which possibly does not minimize the viscous component resulting in more stress relaxation after placement than desired for normal knee biomechanics.

The current study will use an alternative method of preconditioning subjecting the graft to a creep protocol, instead of a stress relaxation protocol used currently The creep protocol is hypothesized to have a significant effect on the remaining stress after final fixation than the stress relaxation protocol and likewise no preconditioning. Furthermore the study hopes to show that in one type of preconditioning procedure, creep, stress relaxation or none, there is a greater ability to control the variance of the stress relaxation after final fixation, which could lead to better pretensioning and preconditioning protocols. Lastly, the results will be discussed and used to increase positive clinical outcomes by comparison of other clinical studies with varying preconditioning procedures.

CHAPTER II

BACKGROUND

## Anterior Cruciate Ligament

### Location

The ACL is located in the joint capsule of the knee connecting the femur and tibia. More specifically this ligament is attached in a fan-like pattern to the posteromedial aspect of the lateral femoral condyle and anterior medial part of the tibia with an approximate cross-sectional area of 44 $mm^2$ (Williams 1995, Noyes 1997).

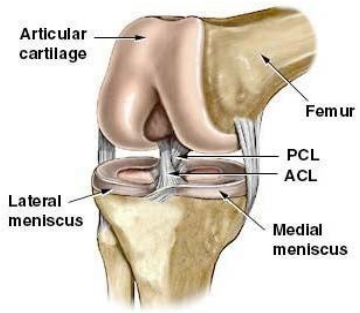


Figure 1: Schematic of the ACL with attachments to the femur and the tibia.

### Histology

A densely vascularized synovial tissue covers the majority of the ACL and is composed of loose connective tissue but is lacking where the ligament anterior impinges on the rim of the intercondylar fossa. In this particular region the ACL is covered by a

dense fibrous tissue consistent with that of fibrocartilage. The extracellular matrix is composed of collagen bundles separated by reticular fibers with each bundle varying in diameter from 70-150 μm (Peterson 1999). Embedded in the collagen bundles running longitudinally are fibroblast cells which are round to oval shaped, measure about 5-8 μm in diameter, and arranged longitudinally along the edge of the fascicles. The "crimp" period of the ACL collagen is 45-60 μm with a amplitude of less than 5 μm (Amiel 1988). The vascularization of the ACL consists mostly of vessels running longitudinally but is completely avascular in the region proximal to the tibial insertion, shown in Figure 3 (Peterson 1999).

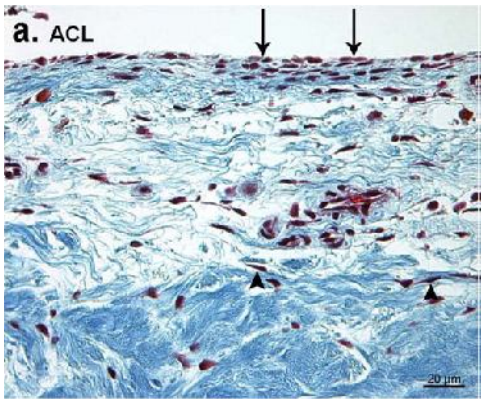


Figure 2: Native fibroblast cells located in the ACL (Brune 2007).

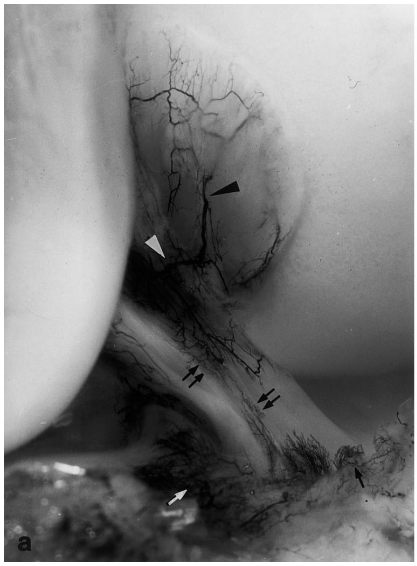

Figure 3: Vascularization of the ACL with vessels running longitudinally (Peterson 1999).

**Chemistry**

The collagen fibers located in the ACL have a lower dry collagen weight than most tendinous tissues, totaling 802.6 ± 9.8 mg/g of dry tissue. Furthermore, the collagen found in the ACL is primarily type 1 collagen (88 ± 2 %) and a smaller portion of type III collagen (12 ± 2 %). The total glycosaminoglycan (GAG) content has been found to be 9.89 ± 0.56 mg hexosamin/g dry tissue. These figures can also be found in Table 1 below for comparison with the patellar tendon (Amiel 1984).

Table 1: The total collagen, collagen type, and GAG amount present in the patellar ligament and ACL (Amiel 1984).

| | **Total Collagen** mg/g dry tissue | **Collagen** Type I | Type III | **GAG** mg hexosamine/g dry tissue |
|---|---|---|---|---|
| **Patellar Ligament** | 867.2 ± 8.9 | >95 % | <5% | 3.92 ± 0.56 |
| **Anterior Cruciate** | 802.6 ± 9.8 | 88 ± 2 % | 12±2 % | 9.89± 0.56 |

Amiel 1984

**Physiology**

The main functions of the ACL are to connect the femur to the tibia, provide stability, and resist shear in the saggital plane. The native ACL has an ultimate tensile load around 2,160 N with a stiffness of 242 N/mm (Woo 1991, Noyes 1984, Frank 1997). The ACL consists of the anteromedial bundle and the posterolateral bundle, which lengthens and tightens in flexion and extension respectively (Girgis et al 1975). These two bundles allow the ACL during weight-bearing flexion to elongate and twist to maintain proper knee stability throughout the full range of motion (Li 2005). On the other hand, the ACL was found to be slack when passively extended from 90 to 15 degrees of flexion (Roberts 1994). Furthermore, ACL functions have been quantified mostly by studying the effect of the knee deficient of the ACL. ACL deficiency was shown to increase valgus rotation under an isolated quadriceps load at 15 to 30 degrees of flexion, but all other degrees of flexion showed no such excess rotation compared to the intact ACL ( Li 2007). Anterior tibial translation also increased due to ACL deficiency at 0 to 30 degrees of flexion ( Li et al 2006). These flexion angles from 0 to 30 degrees correspond to the maximum loads in the flexion range for the ACL (Suggs 2004, Sakane

1997). In summation, the complex anatomy of the ACL limits excessive tibial translation, axial tibial rotation, and valgus knee rotations (Woo 2006).

The mechanics of the ACL have been studied to also show failure or injury occurrences. Such studies have reported that injuries occur most commonly when an excessive tensile force has been applied to the ACL (Yu 2007) which, as stated previously, occur at flexion angles from 0 to 30 degrees at the femoral insertion most frequently. The most common sports where this type of excessive load is likely to occur are football, basketball, and skiing (Zantop et al 2007).

## ACL Reconstructive Surgery

The ACL reconstructive surgery is performed on patients exhibiting ACL deficiencies caused by tears, rips, or ruptures resulting in knee laxity, excess rotation, and general knee instability. The surgery is performed to restore normal knee kinematics to that of the native joint by replacing the damaged ACL with a graft. A thorough explanation of the ACL reconstructive surgery procedures is not needed, but the basics are essential for understanding the current study. The general procedure calls for one incision made proximal to the knee joint on the anterior region of the femur to create a bone tunnel through the femur to the middle of the knee capsule. Another bone tunnel is created on the tibial side of the joint by way of the bone tunnel in the femur. The ACL graft is placed inside the tibial bone tunnel and secured; then spanning the knee capsule fastened into place in the femur bone tunnel (Hamner 1999, Beynnon 2002).

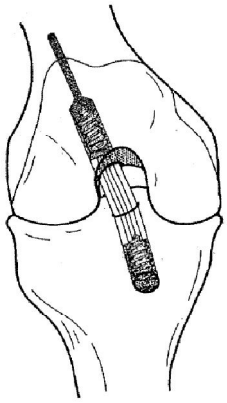

Figure 4: Schematic of bone tunnels and BPTB graft placement connecting the tibia to the femur (Numazaki 2002).

**Graft Selection**

After the clinician has decided that surgical intervention is needed for knee restoration, graft selection is determined by past medical history. The ideal graft should be harvested easily, have low harvest morbidity, have biomechanical properties equal to that of the natural ligament, have high initial strength and stiffness, have the ability for rabid incorporation into the surrounding tissue, and allow for immediate rehabilitation whilst maintaining native knee anatomy and function (Gladstone 2002, Miller 2002, Cain 2002, Robert 2007). Allografts can be used from the ACL, Achilles tendons, bone-patellar tendon-bone, hamstring tendon, fascia lata, tibialis anterior tendon, and posterior tibialis tendon of the donor. Allografts heal at a slower rate than the autografts, and possibly incite an immune response (Jackson 1993, Arnoczky 1982) and disease transmission (Tomford 1995) due to the matrix present in the allograft. Autograft choice is preferred to avoid these complications if preoperative diagnosis from radiographic images of the potential graft results in a positive assessment (Gladstone 2002, McAlister 2001).

The most common autografts are extracted from the hamstring tendon or patellar tendon which have been aggressively researched in the laboratory setting as well as clinically. The hamstring tendon graft comes in two forms: the two-strand graft and the four-strand graft as shown below in Figure 7 and Figure 8, respectively. Clinically

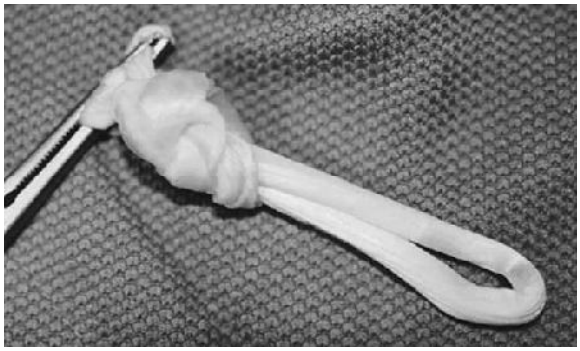

Figure 5: The two-strand hamstring graft used for ACL reconstructive surgery (Paessler 2003).

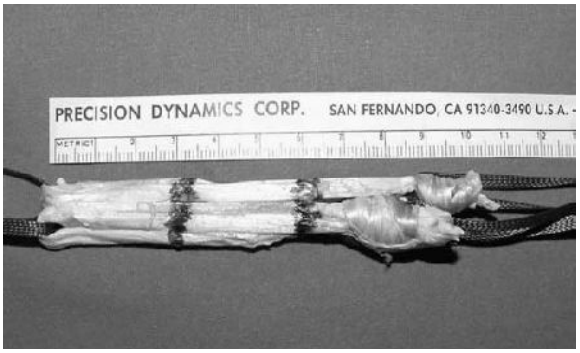


Figure 6: The four-strand hamstring graft used for ACL reconstructive surgery (Paessler 2003).

there is a consensus that reconstruction using the 2-strand hamstring autograft results in more negative outcomes that those using the Bone-Patellar Tendon-Bone (BPTB) and four-strand graft (Beynon 2005, O'Neil 1996, Anderson 2001, Beynnon 2002). Having an ultimate tensile load of 4,090 N, stiffness of 776 N/mm, and cross-sectional area of 53

$mm^2$, the four-strand graft biomechanical is similar to the native ACL and likewise the BPTB graft and is usually preferred over the two-strand hamstring graft (Hamner 1999, Staubli 1999, Harris 1997). Even though the four-strand hamstring graft does offer superior strength and stiffness over that of the BPTB (ultimate tensile load 2,977 N, stiffness 620 N/mm, and cross-sectional area of $35mm^2$), the major hindrance for the graft is the considerably longer healing time of the tissue-to-bone incorporation (12 weeks) than the bone-to-bone incorporation with the BPTB graft (6 weeks) (Rodeo 1993). The longer healing process usually results in slower rehabilitation and longer time period before complete return to daily activities than those with the BPTB graft (Gladstone 2002, Cain 2002, Jomha 1999). Even though early post-operative assessment of the four-strand graft is inferior to that of the BPTB, Roe et al. found that after a 7-year follow-up, the four-strand grafts showed no significant difference than the BPTB grafts, but the same study reported a higher graft rupture rate with the four-strand hamstring graft than the BPTB (Roe 2005). Furthermore, studies have shown that the use of the four-strand hamstring graft causes flexion deficits in the knee at various post-operative times (O'Neil 2001, Sherman 2004, Goldblatt 2005, Aune 2001). The BPTB graft is considered the gold standard for graft choice in ACL reconstructive surgery chiefly due to the ability for bone-to-bone healing which enables the patient to begin aggressive rehabilitation post-operatively in a relative short amount of time (Papageorgiou 2001, Tomita 2001, Gladstone 2002, Cain 2002, Jomha 1999). The BPTB graft also allows the surgeon to secure the graft firmly with greater ease than any other type of graft used (Schoderbek 2007). The most common complications found with using the BPTB graft

are extension deficits compared to the native knee, patella fracture, petalla baja, and general patellofemoral pain (Ejerhed 2003, Laxdal 2005, Laxdal 2007).

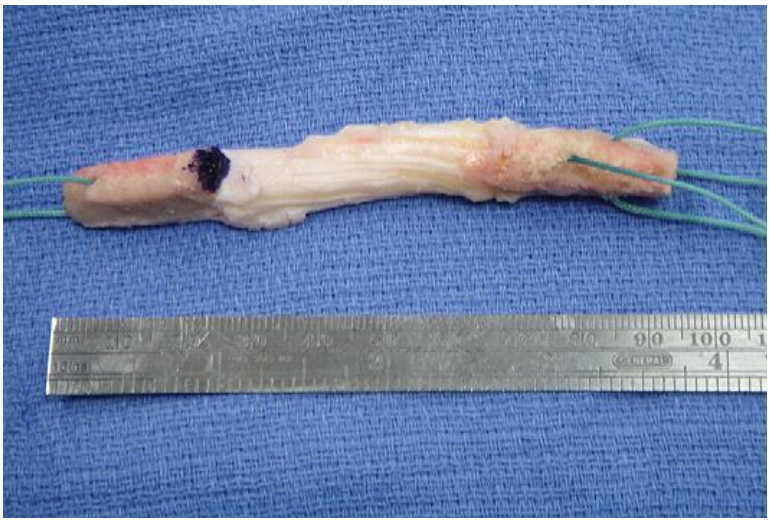

Figure 7: A BPTB graft with the bone block attachments at the proximal and distal ends (Schoderbek 2007).

### *Patellar Tendon*

The patellar tendon is also located in the knee, as shown in Figure 4, located dorsally outside the joint capsule. The patellar ligament is the continuous central band of the quadriceps tendon which descends distally from the patella to the tibial tuberosity. The strong and flat band is approximately 8 cm in length and attaches the rough areas of the distal posterior patellar to the smooth area of the tibial tuberosity. (Williams 1995)

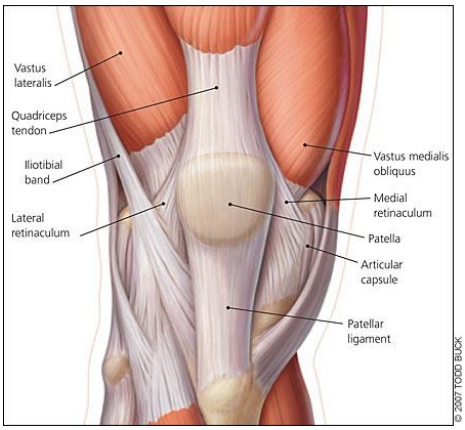


Figure 8: Schematic of the patellar ligament with attachments to the patella and tibia.

The collagen bundles of the patellar ligament are approximately 20 μm in width similar to that of the ACL, but on the other hand the crimp period is almost double that of the ACL at 120 μm, and the amplitude is triple that of the ACL at about 15μm. The spindle-shaped fibroblast cells in the patellar ligament are approximately 25 μm and longitudinally aligned on the sides of the bundles or fascicles. Furthermore, these cells have no distinct cytoplasmic space with only nuclei observed (Amiel 1988).

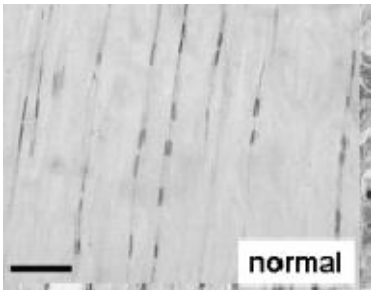


Figure 9: Fibroblast cells seen in the normal patellar ligament (Lui 2007).

The total dry collagen weight found in the patellar ligament is approximately 867.2 ± 8.9 mg/g dry tissue. The collagen present is greater than >95 % type I and less that <5% type III, which is a higher ratio of type I to type III than that found in the ACL. Lastly, the GAG content is much lower than the ACL at 3.92 ± 0.56 mg hexosamine/g dry tissue (Amiel 1984).

**Graft Extraction**

The BPTB is harvested by careful extraction from the tibial tubercle to the patella measuring 20 to 25 mm in length and 10 mm in width in the center of the whole patellar ligament as shown below in Figure 10. Each end of the BPTB graft is left with bone

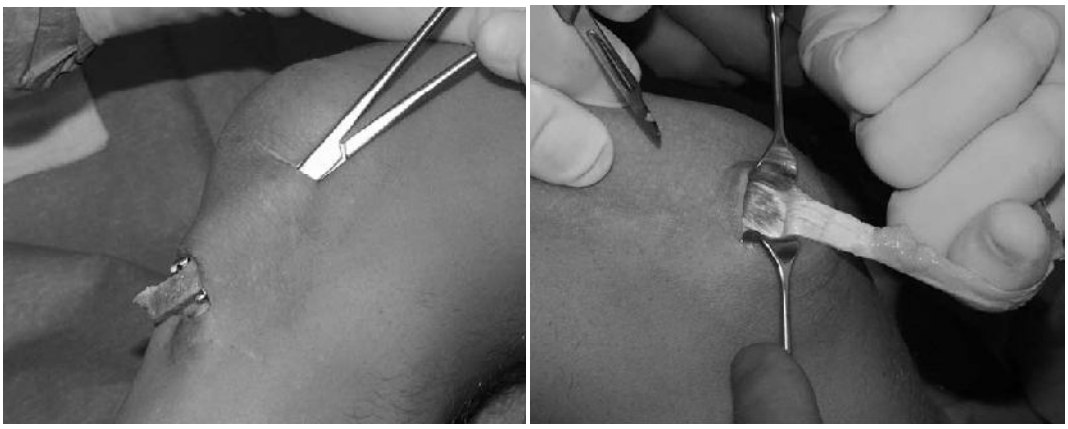

Figure 10: The two-incision method of graft harvest shown in the photo at the left. The photo on the right showing graft extraction (Paessler 2003).

blocks at the proximal and distal ends which are used as attachment points as seen in the above Figure 8, which as discussed earlier shorten healing time and quality of fixation (Schoderbek 2007). Graft harvest has shown to have negative clinical outcomes due to the missing segment of the patellar ligament. Patients complain of kneeling pain after

surgery due to the graft extraction, and patella tendonitis is usually present in 10 percent of patients within the first 3 to 6 months (Gladstone 2002, Yunes 2001, Sherman 2004). A most recent study compared the histology of the normal native central one-third of the patellar tendon to the 10 years of healing after graft harvesting. The images below show that there is an increased vascularization and cellular components present after 10 years of healing (images B and C compared to image A in Figure 10). Furthermore, image C

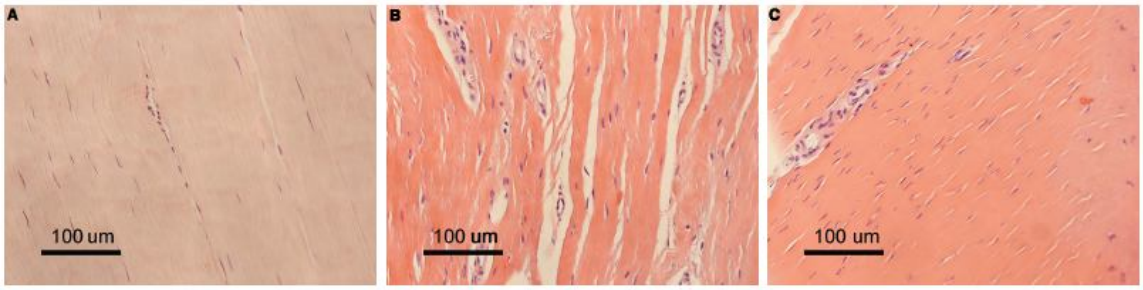


Figure 11: A, light-microscopic view of normal patellar tendon tissue obtained in ACL surgery. The fibers are parallel and densely packed with flattened nuclei between. B, light-microscopic view of patellar tendon tissue obtained by needle biopsy from the lateral part of the patellar tendon 10 years after reharvesting its central third. The cellularity and vascularity are slightly increased. C, light-microscopic view of patellar tendon tissue obtained by needle biopsy from the central part of the patellar tendon 10 years after reharvesting its central third, showing nonparallel collagen fibers with a vessel in the upper left quadrant.

shows more nonparallel collagen fibers than the native patellar tendon, leading to the principle finding that 10 years after harvesting significant histological abnormalities still exist (Liden 2008). The most serious side effect of extraction of the BPTB graft is indirect patella fractures post-operatively which is only reported within zero-two percent of all cases (Tay 2006).

**Graft Preparation**

All tendons and ligaments in the body are considered to exhibit viscoelastic properties due to the internal structure when undergoing tensile loading. Viscoelastic materials have a non-linear stress vs. strain curve because of viscous effects of the material as shown below in Figure 12. The stress strain curve can furthermore be broken down into individual regions: toe, linear, and failure. The toe region represents the un-crimping of the collagen which causes a large amount of deformation with very little load applied. Further load then causes the specimen to have a more linear curve which occurs due to inter-fibril sliding and/or intra-fibril deformation such as molecular elongation, increase in the gap region between the ends of two collagen molecules, and sliding by adjoining molecules. The failure region occurs when the specimen can no longer hold anymore load and tissue ripping occurs. When a constant displacement is applied to a tendon or ligament, such as the patellar tendon, the tissue initially deforms to the current

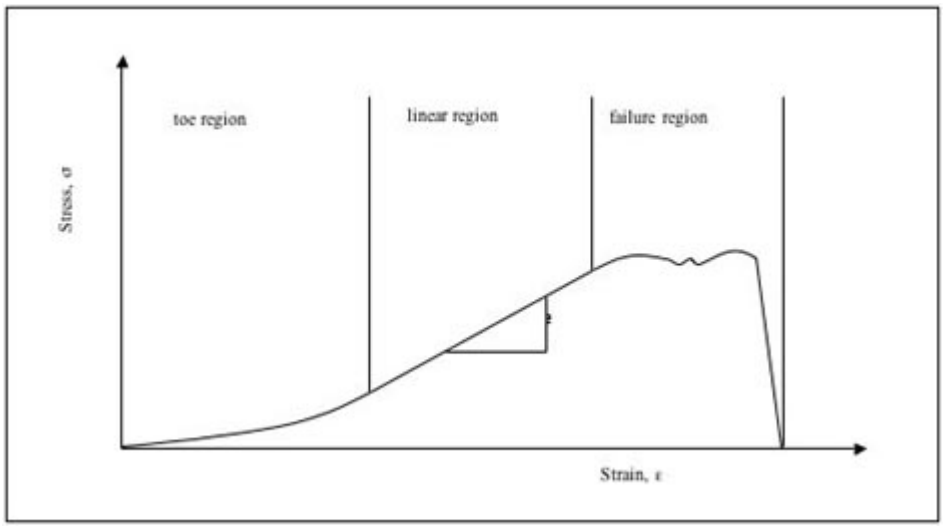


Figure 12: Stress vs. strain diagram showing the toe, linear, and failure region.

displacement point creating a tensile force within the tissue. Holding at a constant displacement causes un-crimping, inter-fibril sliding and/or the same intra-fibril mechanisms, but since no more total deformation is taking place the tensile force originally on the tissue slowly reduces resulting in stress relaxation. On the other hand, a constant load applied to the tendon or ligament causes immediate deformation similar to constant displacement through un-crimping, but as the tissue elongations through inter-fibril and intra-fibril mechanisms the force remains the same, not allowing the tissue to undergo stress relaxation but rather creep by further deformation (Sasaki 1996, Ozkaya 1999).

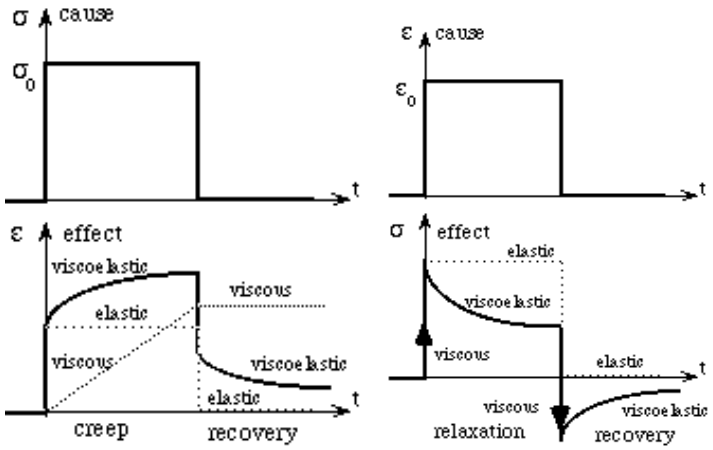


Figure 13: Idealized curves representing creep (left) and stress-relaxation (right). The top left graph represents a constant load with the bottom left graph representing the increase in deformation under that load. The top right graph represents a constant deformation with the bottom right graph representing the diminishing load due to the deformation.

After BPTB extraction, the graft undergoes preparation that essentially is performed to reduce the amount of stress relaxation in the tendon; this type of preparation in the current study will be known as preconditioning. The preconditioning is usually

performed on a specialized board such as the GraftMaster II (Smith & Nephew or Acufex). The preconditioning boards stretch the graft to a constant displacement corresponding to an 88 N load on the tendon initially. The preconditioning procedure allows the graft to undergo stress relaxation, but no further elongation through creep than the initial deformation placed on the graft.

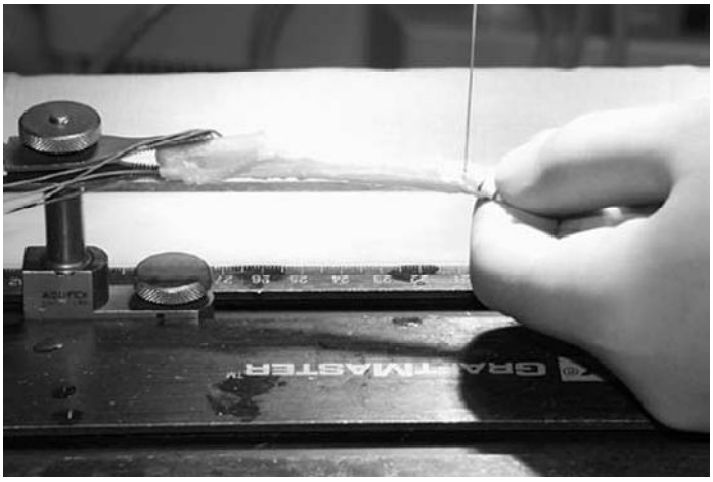

Figure 14: A graft held and being stretched on the GraftMaster board to undergo preconditioning (Paessler 2003).

The current preconditioning protocol used in reconstructive surgeries could not efficiently minimize the stress-relaxation. The study by Numazaki et al showed that the preconditioning has a long term effect on the tension for the BPTB graft. By varying the preconditioning procedures ( 20 N, 80 N, 140 N tensile loads for 2 minutes), then performing a cyclic force-relaxation test for 5,000 cycles where the graft was stretch 2 mm, the results showed a significant increase between all three preconditioning loads at the 5,000$^{th}$ cycle as shown in Figure 15 (Numazaki 2002). Shaztmann et al. has shown

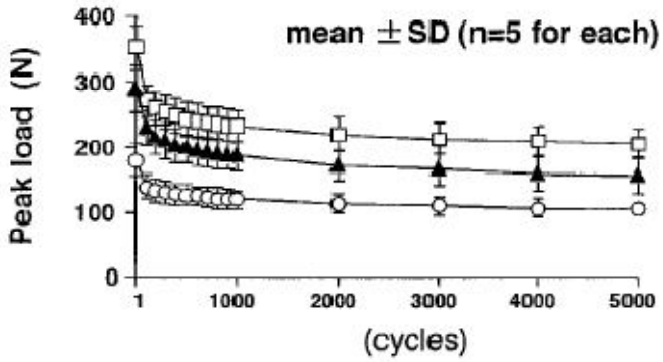


Figure 15: Peak load values at each of the 5,000 cycles performed (Numazaki 2002).

that 150 cycles of uniaxial loading from 75 to 800 N at 0.5 Hz are needed to completely remove all creep from the patella ligament (Shaztmann 1998). While this is completely unnecessary and very time consuming during surgery, others have performed the opposite extreme by performing no preconditioning before final fixation. The study concluded that there was significant increase in anterior tibial translation at full extension, 15°, and 30° of flexion when no preconditioning procedure was performed on the graft (Li 2006). Another study showed the difference between three different types of preconditioning procedures. Initially each group was placed on the Graftmaster II board for 15 minutes before final fixation, the first group did not undergo any additional preconditioning, the second group had 25 cycles from 0 to 80 N in 100 seconds, the third group was held at a constant load of 80 N for 100 seconds. The results are summarized in Table 2.

Table 2: Preconditioning results between the three groups immediately after screw insertion and 10 minutes after screw insertion (Nurmi 2004).

Preconditioning—Results[a]

| Group | Graft Tension Before Screw Insertion (N) | Graft Tension Immediately After Screw Insertion (N) | Graft Tension 10 Minutes After Screw Insertion (N) |
|---|---|---|---|
| 1 | 80 N | 79 ± 19 N | 49 ± 16 N |
| 2 | 80 N | 100 ± 17 N[b] | 60 ± 11 N |
| 3 | 80 N | 102 ± 15 N[b] | 64 ± 12 N[c] |

[a] Mean ± SD.
[b] Significantly different from group 1 ($P < .01$).
[c] Significantly different from group 1 ($P < .05$).

(Nurmi 2004)

The study concluded that these preconditioning protocols were not sufficiently able to eliminate the viscoelastic creep in the tendon grafts, and also no significant difference was found between cyclic and isometric preconditioning protocols (Nurmi 2004). Another study by Elias et al. specifically showed the effects of preconditioning of the patellar tendon graft ten minutes after final fixation. The graft was deformed to a 105 N load and held for 30 min to represent preconditioning then placed under a final fixation load of 105 N, resulting in an 81 ± 13 N force after 10 min (Elias 2008). A randomized double-blind clinical study also found that using no preconditioning produces a significant increase in knee laxity when decreasing the final fixation force of 90 N to 45 N twenty months after surgery. The same study reported that the 45 N final fixation force allowed the patients a greater decrease in extension loss post-operatively (Nicholas 2004).

**Graft Final Fixation**

After preconditioning, the graft is placed in the bone tunnels created by the surgeon as shown and fixed at one end in Figure 6. There are many types of fixation methods used in today's ACL reconstructive procedures which include interference screws, buttons, washers, staples, cross-pins, polyester tape-titanium buttons, and suture posts (Martin 2002). After the femoral side of the graft has been fixed, a final load is placed on the graft just before tibial fixation occurs. The final tension, which ranges from 20N to 100N, applied has been shown to have a great impact on total knee biomechanics (Jomha 1999, Tomita 2001, O'Neil 2001, Goldblatt 2005, Anne 2001, Nicholas 2004). A specific *ex vivo* experiment correlated tibiofemoral compressive forces increasing with increasing initial graft final fixation from 1, 15, 30, 60, and 90 N when undergoing the same preconditioning protocol. The increase of tibiofemoral compressive force was significant at every level of final fixation tensions and all flexion angles tested. The same study also reported that the neutral joint position of the joint was affected by the final tension placed on the graft (Brady 2007).

CHAPTER III

MATERIALS AND METHODS

## Specimens

The specimens used in the current study were all harvested from slaughter hogs from Sansing Meats Service (Maben, MS 39750). The male pigs were approximately 22-26 weeks old weighing 240-270 lbs at slaughter. The female pigs had a body weight approximately double that of the male pigs and were all over 32 weeks of age.

## Specimen Extraction

Each pig was sacrificed using a .22 caliber firearm shot on top of the head causing the bullet to penetrate through the skull to the brain. After sacrifice, each animal was gutted and stripped of all the internal organs and sliced completely down the sagittal plane through the spine from the posterior to the base of the skull. The skull was extracted by slicing through the soft tissue circumferentially. Each specimen was stored hanging from the Achilles tendon to allow blood drainage overnight. The following morning each animal was taken out of storage one at a time to specifically extract the patellar ligament.

Extraction began by removing the muscular tissue surrounding the knee joint. After most of the tissue was removed, the knee capsule was opened posteriorly. Carefully, the cruciate ligaments were severed internally and surrounding the knee until

complete separation between the femur and tibia was achieved. Removal the femur from the ligament was performed by slicing ventral to dorsal behind the fat pad and the back of the patellar. The patellar ligament was further isolated by trimming the fatty tissue and the sheath that surrounds the ligament. The patellar was left completely intact, as was the tibia. The specimens were then placed in a cooler filled with ice for transportation to Mississippi State University's Department of Agriculture and Biological Engineering. To fit inside the specially designed pots discussed later, upon arrival, the tibia was further trimmed carefully as not to severe any attachment points of the patellar ligament using a circular saw. Each specimen was then measured for length, width, and thickness (see APPENDIX A) using a ruler and digital calipers for the thickness in the center of the specimen and placed in a freezer at -20° C in separate small bags until date of testing.

**Specimen Fixation**

At date of test the specimen is taken out of the freezer and allowed to unthaw to the temperature of the testing room (20° C) which closely resembles operating room temperatures. The tibial section was placed in the specially designed pot as shown below in Figure 15 and, using a Black & Decker® 3/8" Reversible Drill, pinned through the bone inside the pot (Figure 16 below) with a 3/16 inch drill bit. After inspection of the pin, the tibial section was further fixed using methyl methacrylate (Jorgensen Laboratories, INC. J-61PB Loveland, CO 80538) and allowed to harden. The patellar side was left unfixed until immediately prior to testing when it was clamped, allowing the patellar tendon free passage since the patellar became self-locked without crushing.

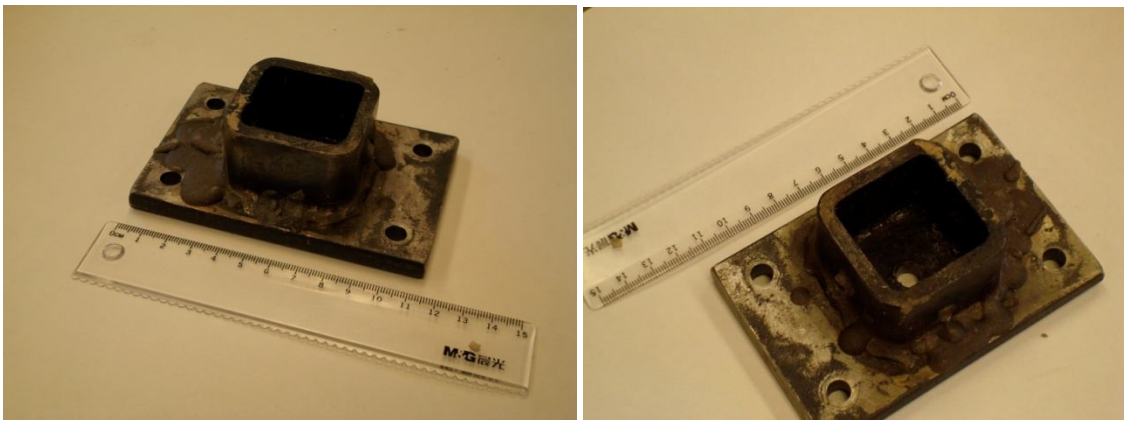

Figure 16: The specially designed pots in which the tibial section of the specimen was held in place. The pot also was able to be bolted down to the specialized water chamber as seen below in Figure 19.

Figure 17: The specially designed pots with the 3/16 drill bit passing through representing how the bone was pinned before methyl methacrylate was added.

## Testing Setup

All specimens were randomly assigned into each preconditioning protocol (creep, stress relaxation). The no preconditioning was not randomly assigned because the data was collected from the beginning part of the test from the stress relaxation protocol. The

table below summarizes the amount of males and females and left and right sides in each test group and the average cross-sectional area of the specimens in that given test.

Table 3: The location and sex amounts which were randomly assigned in each group with the average cross-sectional area of corresponding group.

| | | Location | | Sex | | Average Cross-sectional Area ($mm^2$) |
|---|---|---|---|---|---|---|
| | | R | L | Males | Females | |
| Precondition | Creep | 4 | 5 | 6 | 3 | 59.56 |
| | Stress Relaxation | 4 | 5 | 4 | 5 | 59.39 |
| | None | 4 | 5 | 4 | 5 | 59.39 |

Each morning before testing on the MTS® (Eden Prairie, MN 55344), a specialized chamber (20 cm cube) was fixed to the bottom testing plane in which the specimen is housed. A specific designed aluminum plate was bolted to the internal base of the chamber. A clamp was affixed to the actuator rod, which holds the patellar side of the ligament in the chamber.

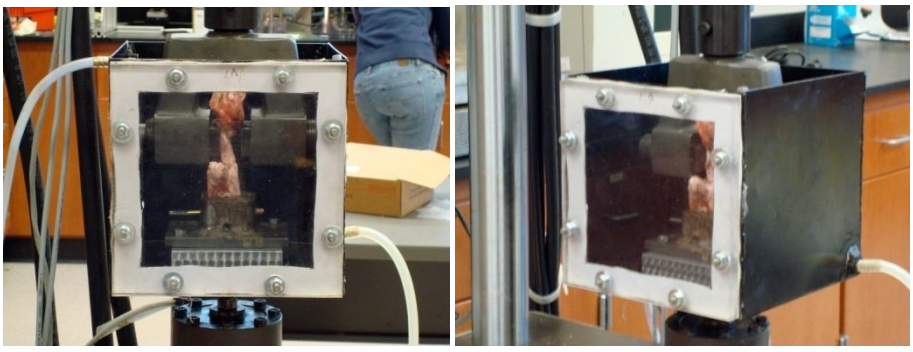

Figure 18: The aluminum plate attached to the bottom of the specialized chamber.

Figure 19: The MTS machine with the specialized chamber attached to the bottom of the testing plane.

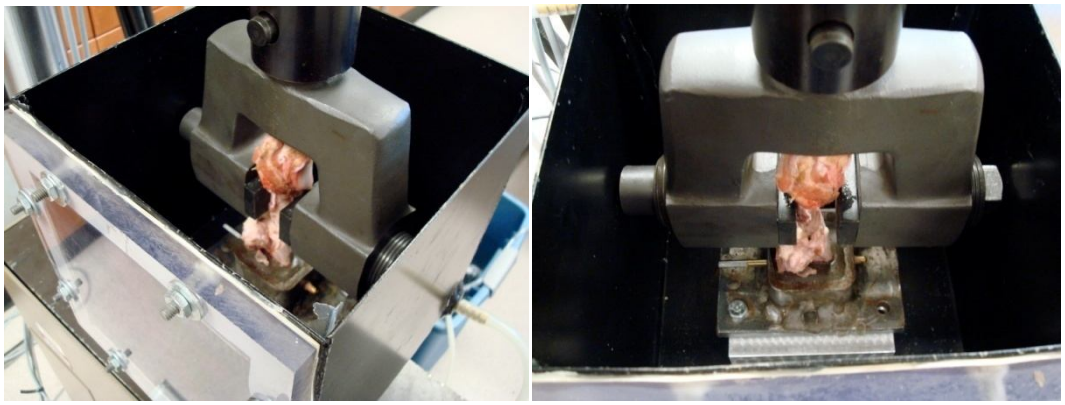

Figure 20: A close up of the clamp which "self-locks" the specimen in place.

The specialized chamber allowed a circulator water bath from a separate reservoir to be pumped through it using a Pump-Maxi-Jet 900 (Marineland® Moorpark, CA 93021). The water reservoir was set up every morning and maintained at 37°C with a

heat source (ULANET Model # 306 Newark, NJ 07105) and temperature controller monitor ( Omega® CSC32 Stamford, CT 06907-0047) to represent body temperature.

Figure 21: The water bath seen on the left circulates the water until the specialized chamber shown by the tubing connections on the photo on the right. The water is pumped in through the base of the chamber and returns to the reservoir through the top tubing.

**Testing Procedure**

After the specimen had been loaded into the specially designed equipment on the MTS in the way described above, TestResources (Shakopee, MN 55379) software was used for exact control of the MTS for all tests performed. The preconditioning of the graft was done at nearly operating room temperature 20° C. Using the TestResources interface a negligible load was placed on the specimen (>5N) while in stroke control. A visual inspection was performed on the entire chamber to ensure proper fixation of all components and no specimen pullout from the pot or slipping from the clamp (Figure 21). After data recording was turned on using the interface controls in stroke control, the specimen was ramped up slowly, for approximately 30 to 35 seconds, until a load of 100 N was reached. Depending on the type of preconditioning, stress relaxation or creep, the

machine was switched to the respective control mode once 100 N was reached, stroke or load respectively. Simultaneously at the beginning of the ramped load, a timer was manually started. The preconditioning was allowed to run for 30 minutes exactly before the machine was ramped back down in stroke control to zero load on the specimen.

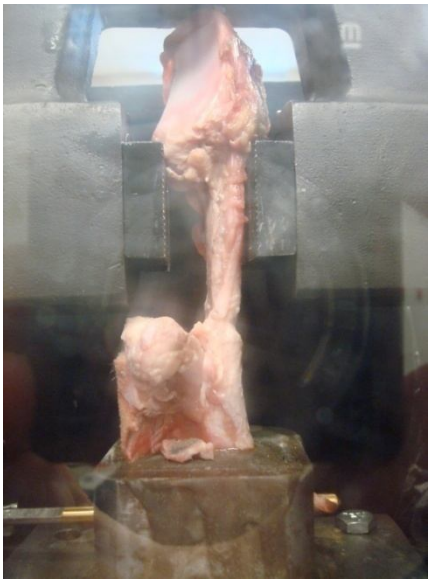

Figure 22: The specimen was visually inspected at the beginning of each precondition procedure and throughout the test.

At the end of the preconditioning procedure with the MTS in stroke control, before the final pretensioning of the specimen, the custom designed chamber was flooded with the 37° C water bath to simulate body temperature and allowed to circulate from the reservoir to the chamber back to the reservoir while holding the temperature constant through the temperature controller. Once the chamber was completely filled, the load cell was re-zeroed, and the specimen was again inspected visually for fixation integrity. The specimen had no actual force applied during this time and was allowed to "rest" for 6 minutes, representative of the time the surgeon places the graft in the bone tunnels for

proper positioning. At the end of the 6 minutes, the final pretensioning was performed again by ramping in stroke control to 100 N for approximately 30 to 35 seconds. The specimen was then run for an additional 30 minutes in which data collection ran continuously. The final tensioning was held at a constant displacement for only 30 minutes, resulting in the highest slope of -0.07N/min which fell under -0.1N/min as the cutoff for stress-relaxation slope for the current study.

At the end of each pretensioning procedure, the chamber was drained, and the specimen was removed from the chamber. The pin was taken out of the pots, and the specimen was removed from the pot. The specimen was then re-measured for thickness, width, and length (see APPENDIX A) and placed back into the freezer until the conclusion of all testing.

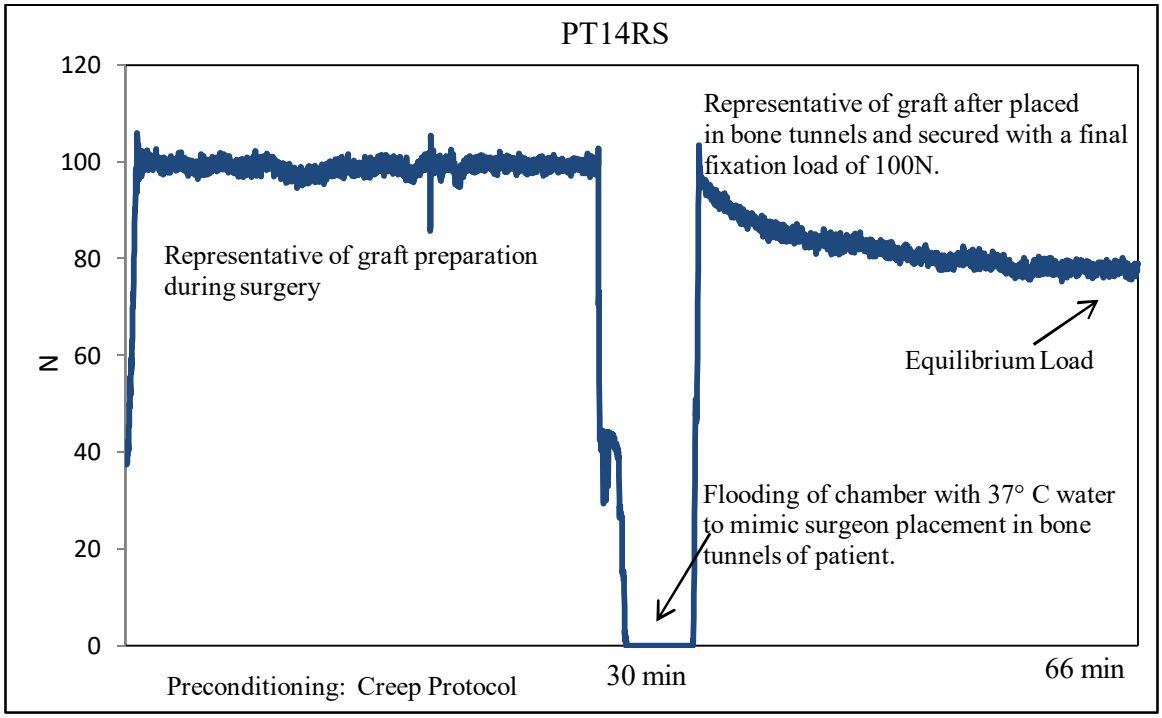


Figure 23: The specimen PT14LS force vs. time curve. The first half of the graph represents creep preconditioning with a 6 minute-period of rest followed by a

final tensioning to a constant deformation.

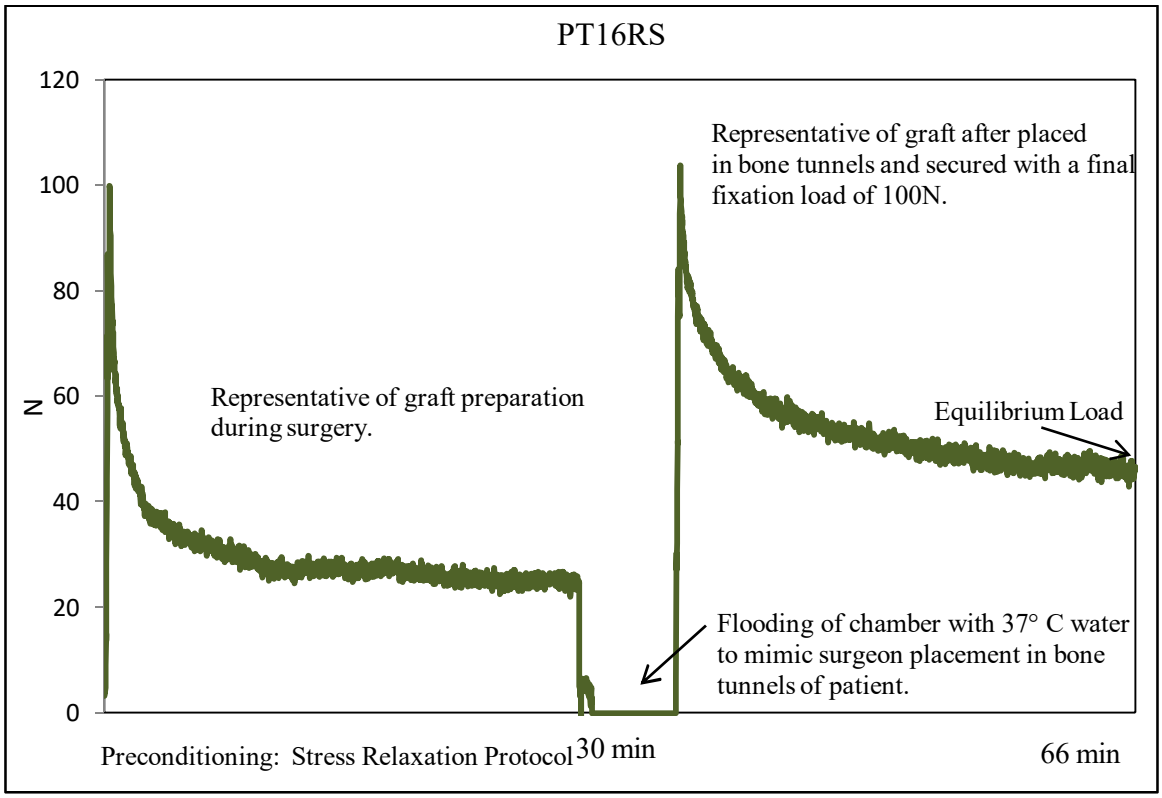


Figure 24: The specimen PT16RS force vs. time curve. The first half of the graph represents stress relaxation preconditioning with a 6 minute-period of rest followed by a final tensioning to a constant deformation.

**Data Collection**

Data was collected by TestResources starting before the first ramped stroke on each test specimen which scanned at a rate of 98.443802 Hz for time, load, and stroke. The files at the conclusion of each part of the test were extracted into Microsoft Excel® and saved as the raw data. Due to the extreme amount of data attained in one part of the test (approximately 60,000 data points), the data was trimmed down by picking only every tenth point using MathCad 14 coding file (see APPENDIX B). After the data was

thinned to approximately 6,000 points, it was once again extracted into Microsoft Excel®.

The data was analyzed by averaging the load from 3,955 to 3,960 seconds (corresponding to the last five seconds of the test) and dividing by the cross sectional area of the given specimen to attain the stress value. Furthermore, the same procedure was performed at 2,760 and 3,060 (10 min and 15 min after final fixation, respectively). The stress ratio was calculated by dividing the final stress over the initial stress (load initially applied over the cross sectional area). By using the stress ratio the data was normalized around the cross sectional area and peak load applied. The total strain was recorded during preconditioning for each specimen by subtracting the peak stroke value from the stroke value with an initial minimal load applied.

All stress ratios and measured data was placed in SAS 9.1 for statistical testing. The SAS code used for each is posted in APPENDIX C. The measurement data was analyzed for significant differences between left and right orientation, males and females, and each preconditioning procedure against the cross-sectional area. The stress data was also analyzed for significant differences between each preconditioning procedure (stress relaxation, creep, or none) against the stress ratio at 30 minutes. Furthermore, testing for significance in variances between each preconditioning group and measured group was performed.

# CHAPTER IV

# RESULTS

Two actual experimental stress vs. time curves are plotted below for preconditioning under creep and stress relaxation. The rest of the individual test curves were placed in APPENDIX D.

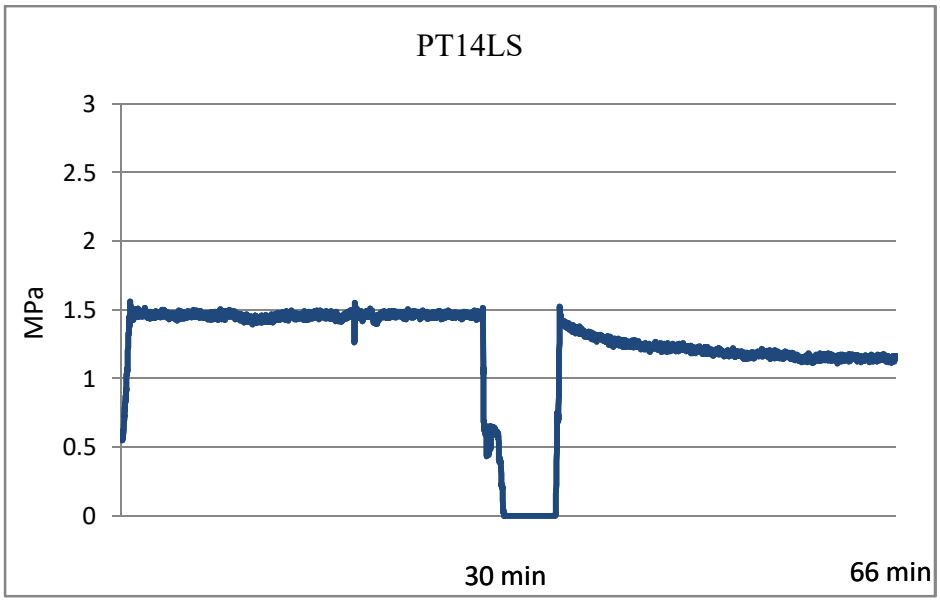


Figure 25: Stress vs, time curve for PT14LS performed under the creep protocol.

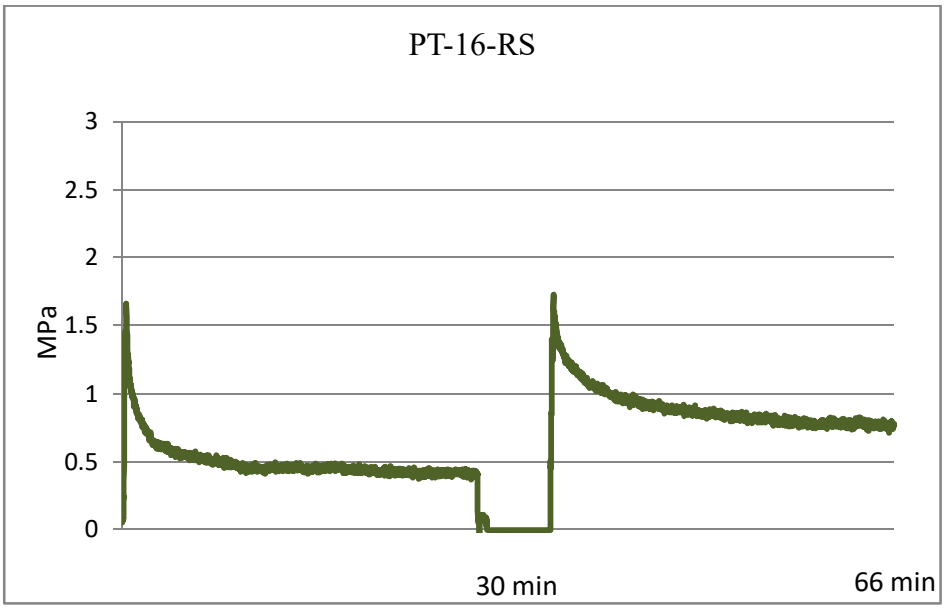


Figure 26: Stress vs. time curve for PT16RS performed under the stress relaxation protocol.

All average stress ratios data at 30 min after final fixation are placed in the table below for each corresponding precondition. The complete stress tables for each specimen are given in APPENDIX E. Also, the average stress relaxation curves after final fixation of each precondition are plotted. The 30 min average values are 0.58 ± 0.12, 0.45 ± 0.14, and 0.39 ± 0.16 MPa for the precondition of creep, stress relaxation, and none respectively.

Table 4: Average stress ratio values after final fixation at 30 min for each precondition with corresponding standard deviations

| | | Stress (MPa) | | |
|---|---|---|---|---|
| | | Initial | Final | Stress Ratio |
| Precondition | Stress Relaxation | 1.86 ± 0.59 | 0.79 ± 0.23 | 0.45 ± 0.14 |
| | Creep | 1.18 ± 0.39 | 1.05 ± 0.18 | 0.58 ± 0.12 |
| | None | 1.83 ± 0.57 | 0.68 ± 0.31 | 0.39 ± 0.16 |

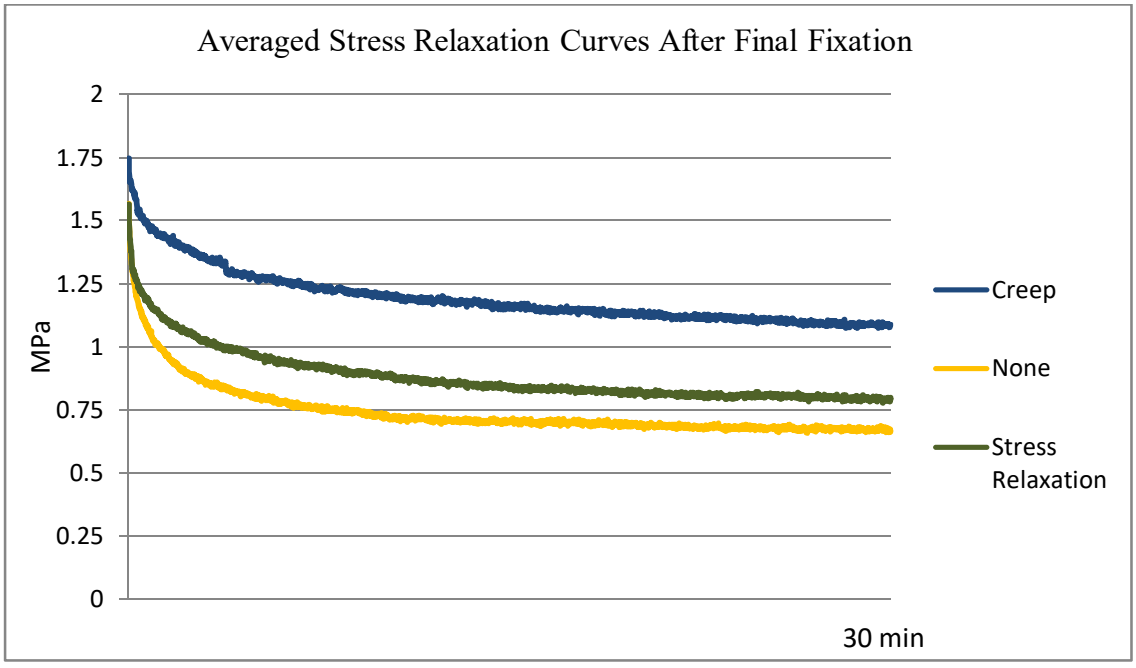


Figure 27: Average final fixation stress relaxation curves for each precondition procedure.

Each preconditioning procedure (creep, stress relaxation, none) was tested at $\alpha$=0.05 for significance using multiple comparison testing and homogeneity of variances. The final fixation stress ratio after 30 min did show a significant difference between the

three preconditions ($p<0.05$). Furthermore, a significant difference in the stress ratio after final fixation was present between the creep and stress relaxation protocols ($p<0.05$) in the least significant difference test, but interestingly no significant difference was found between stress relaxation and no precondition procedure stress ratios using the same significance test. A more conservative test, Student-Newman-Keuls, gave the same significance values to that least significant difference test. The most conservative multiple comparison test, Tukey, showed that there was no significant difference between the preconditioning undergoing stress relaxation or creep stress ratios ($p>0.05$), but there was a significant difference between using constant load and no preconditioning procedure on final fixation stress ratio after 30 min. Other testing was performed for homogeneity of the variances among each treatment. Levene's, Barlett's, and Brown and Forsythe's test all showing no significant difference in the variance between each treatment group, even though the upper and lower curve ranges for each precondition show a much smaller range for constant stress ratio than the other two preconditions (stress relaxation and none) as shown below.

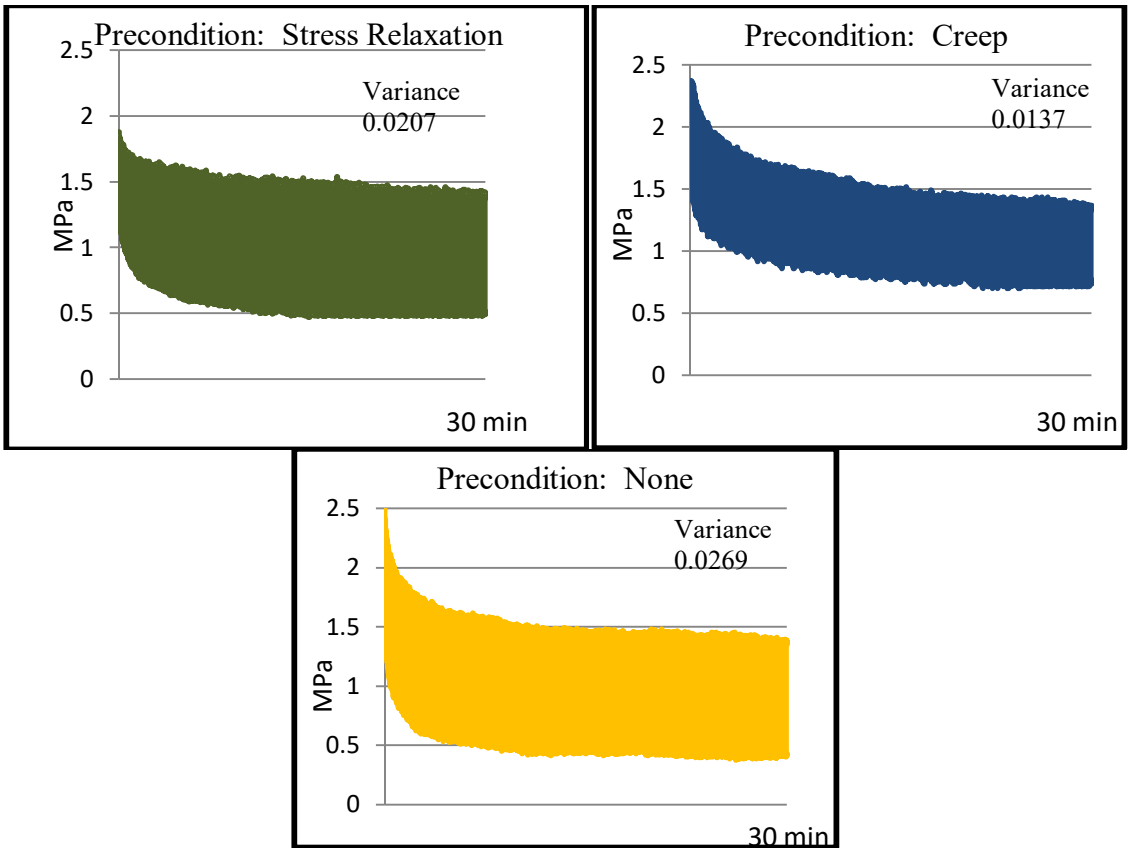


Figure 28: The upper and lower bounds with shaded in areas of the final fixation curves for each precondition with the corresponding variance values.

# CHAPTER V

# DISCUSSION

The results showed, with the more liberal LSD multiple comparison test for significance, that the creep preconditioning protocol to have a higher stress ratio 30 min after final fixation than the stress relaxation preconditioning and no preconditioning. The same multiple comparison test though was unable to show a significant difference between the stress-relaxation preconditioning and no preconditioning. The more conservative multiple comparison test, Student-Newman-Keuls, showed the exact same results of that of the LSD multiple comparison test. The creep preconditioning protocol had an average higher stress by 0.26 MPa and 0.37 MPa on final fixation force after 30 min than the stress-relaxation protocol and no preconditioning, respectively. Whereas the difference between stress relaxation protocol and no preconditioning averaged about 0.11 MPa not enough to show a significant difference. Even though no significant difference was seen, the no preconditioning group was not subjected to a 37°C water bath because the data was collected from the beginning of the stress relaxation protocol experimental procedure. Since preconditioning of the graft before final fixation is performed to remove stress relaxation, the current study clearly shows that the best method out of the three tested to remove the stress-relaxation is the creep protocol. Creep preconditioning was able to show a significantly higher stress ratio after 30 min from final fixation with

only a 42% stress loss, compared to 55% and 62% for the stress relaxation and no precondition protocols. The difference could be explained by a greater increase in stretch during the creep preconditioning protocol, on average 1.09 mm, than the stress relaxation protocol. The average max percentage strain of each preconditioning are outside the toe regions of the human patellar tendon which is reported to be at 2% strain (Johnson 1994), which effectively removed all crimp in the collagen wave-like pattern (8.3% for creep and 5.7% for stress relaxation). The creep protocol deforms the graft by a greater amount by the inter-fibril and intra-fibril mechanisms, which as discussed previously does not allow the tension on the graft to wane because the load remains the same no matter the displacement. On the other hand, the stretch in the stress-relaxation creates an initial tension in the graph but is lost due to the same mechanisms resulting in a greater degree of stress relaxation in subsequent deformations such as final fixation; whereas, the greater elongation as seen in the creep protocol in the tissue causes less stress relaxation in later deformations. Interestingly though the no preconditioning and the stress-relaxation preconditioning showed no significant difference in the stress ratio after 30 min from final fixation ($0.45 \pm 0.14$, and $0.39 \pm 0.16$). The current precondition protocol for the ACL reconstructive surgery calls for a stress relaxation procedure analogous to the one used in the current study to minimize stress-relaxation after final fixation of the graft once secured to the femoral bone tunnel, but these findings show this protocol possibly could not be efficient in removing the necessary stress relaxation of the graft which could result in knee laxity, excessive valgus rotation, and instability in the joint post-operatively. The current design of the preconditioning board could be revised easily to a new design which would hold the graft a constant load, thereby creating a creep protocol

like the one used in the current study that showed less stress-relaxation after final fixation.

Even though the current study used patellar tendon as opposed to the hamstring tendons in Nurmi et al., the results show surprising similarities (see Table 2). When undergoing an isometric preconditioning procedure for just 15 min, Nurmi et al. showed an approximate 37% tension loss after 10 min compared to the 49.5% loss in the current study with stress-relaxation protocol. Interestingly, the preconditioning procedure which placed stress-relaxation protocols, isometric load of 88N for 15 min followed by 80N isometric load for 100 s, and a cyclic protocol, isometric load of 88N for 15 min followed by 25 cycles between 0 and 80N, on the graft before final fixation of 100N showed roughly the same graft tension at 10 min as the creep protocol in the current study at approximately 60 to 68N (Nurmi 2004). Elias et al. also performed a very similar preconditioning protocol to the stress relaxation protocol used in the current study (105 N stress relaxation for 30 min and 105 N stress relaxation for 30 min, respectively), but the findings reported in the current study did show a lower average load on the grafts after final fixation, 51.61 ± 13.82 N compared to 81 ± 13 N. The differences in the methods used by each study could account for the deviations in the load values reported by each. The current study upon final fixation had a 37° C water bath compared to a 20° C used by Elias et al which caused more stress relaxation on the graft than the cooler temperature. Furthermore, the current study did create a hypotonic solution for the tissue during final fixation, whereas Elias et al. used saline solution for all testing procedures. Hypotonic solutions have been shown to have significant effects on the patellar tendon by causing more stress relaxation than testing performed in a hypertonic solution, which would have

additionally driven the load further down than the load reported by Elias et al (Haut 1996, Elias 2008).

Final fixation loads applied have been highly correlated with positive and negative clinical outcomes as discussed previously. A final fixation load which is too small results in knee laxity, excessive rotation, and knee instability with a larger range of motion (Nicholas 2004, Li 2007); whereas a final fixation load too great results in increased total tibiofemoral compressive force and displaced neutral joint position leading to the onset of early osteoarthritis (Brady 2007, Nicholas 2004). The variances in the final fixations stress ratios were also shown to be homogenous for all non-parametric and parametric variance tests, but the no preconditioning protocol and stress relaxation protocol did show a greater amount of variance than the creep preconditioning (0.0269, 0.0208, and 0.0137 respectively). The variance value for the stress relaxation protocol, and likewise the current surgical protocol, shows lesser ability to control within a desired range the stress relaxation after final fixation than the creep preconditioning. A high variance in final fixation could result in complications post-operatively due to an overly high tensile force in the graft to a low tensile force in the graft. On the other hand a smaller variance in stress relaxation could allow clinicians a much more accurate assessment of final force of the graft and moreover a better ability to adequately apply the proper amount of fixation force to the graft resulting in more positive clinical outcomes. The amount of variability in the current preconditioning protocol could be causing such an inadequate control of the final fixation stress relaxation resulting in these negative outcomes. A proper preconditioning procedure should not only be able to remove stress

relaxation and creep but decrease the range of variability in stress-relaxation after final fixation.

Onambele et al performed an *in vivo* study showing the differences in mechanical properties between males and females. The study concluded that females had significantly greater displacement values and likewise lower stiffness values than males causing an overall lower young's modulus (Onambele 2007). Since, the random sampling caused an unequal amount of males and females in each group these affects could have skewed the data to show more stress relaxation after final fixation in the stress relaxation and no preconditioning protocol because more females were in these two groups than in the creep protocol group. In the same manner, the creep protocol which had six males and three females in the group could have shown less stress relaxation if equal amounts of males and females were in the group.

Furthermore, Tukey, the most conservative of multiple comparison tests, was unable to find a significantly higher stress ratio after 30 min for the creep preconditioning than for the stress-relaxation preconditioning but still showed a difference between creep preconditioning and no preconditioning. The non-significant difference shown by Tukey could partly be justified by the small sample size used in the current study. In order to have a 95 percent confidence for significance between the preconditioning protocols a sample size of 45 would be needed. The extreme amount is impractical for the current study, but the results here show that more in-depth studies should be performed with a larger sample population to have a higher confidence of significance. Also non-significant seen between the stress relaxation group and the no preconditioning group could be attributed to the difference in temperature. The temperature difference could

have caused the no preconditioning group to have more of a stress loss curve further separating it from the stress relaxation group.

The MTS machine used did show trouble in maintaining the 100 N load constant for 30 minutes which subjected the graft to inconsistent load levels. These loads could have caused a change in the final stress relaxation and therefore caused erroneous results, but only one test, PT11R, showed major deviations. The tissues could have become dehydrated, while undergoing testing, this was counteracted the best possible way by spraying the tendon with saline solution periodically until the chamber was flooded with water. This also could have caused another difference in the no preconditioning protocol causes the tissue to become stiffer showing less stress-relaxation. The chamber being flooded with water created a hypotonic environment for the tissue during testing which has shown to have significant effects on the biomechanical properties of tendons and ligaments. Haut et al. were able to show that the human patellar tendon did undergo a significantly greater amount stress relaxation and smaller stiffness value in a hypotonic solution (distilled water) than in the hypertonic solution (25% glucose) (Haut 1996). The results in the current study could have significantly lower stress relaxation values at 30 min if using a saline solution bath instead of water. The focus in the current study though was not to reach an ultimate final specific stress, but to show a difference in stress after 30 min from final fixation due to a specific preconditioning protocol. All tissues were subjected to the same hypotonic environment therefore the significant differences in the different protocols should still be valid even though the environment was not the best suited for the testing specimens.

# CHAPTER VI

# CONCLUSION

The current study was able to show how preconditioning protocols affect the remaining stress after stress-relaxation in the patellar graft used for ACL reconstructive surgery. The creep protocol showed a significantly higher stress ratio on the graft after 30 minutes than the stress-relaxation and no preconditioning protocols. Furthermore, the stress-relaxation protocol effect on final fixation tension was not significantly different than that of the no preconditioning effect. Due to this finding, the current surgical procedures which subjects the graft to a stress-relaxation preconditioning protocol are not able to sufficiently minimize graft relaxation after the fixation. Moreover, the results showed a greater amount of variance in final stress-relaxation curves when using no preconditioning protocol and stress-relaxation protocol. The large variance found in the load after final fixation of the graft could cause an excessive or negligible amount of tensile force which results in poor clinical outcomes. The creep protocol did have a smaller variance than the other protocols testing and could allow clinicians to have a better ability to apply the proper load to a given graft to return normal knee kinematics.

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# APPENDIX A

# SPECIMEN DIMENSIONS

Table 5: Measurements taken from each specimen on the date of extraction.

| Specimen Name | Width | | | Thickness | Length | Cross Sectional Area |
|---|---|---|---|---|---|---|
| | Proximal | Center | Distal | | | |
| ***PT-01-L*** | ***18.00*** | ***18.00*** | ***18.00*** | ***3.00*** | ***43.00*** | ***54.00*** |
| PT-02-R | 20.00 | 20.00 | 20.00 | 3.00 | 40.00 | 60.00 |
| ***PT-04-R*** | ***15.00*** | ***15.00*** | ***15.00*** | ***3.00*** | | ***45.00*** |
| PT-05-L | 15.00 | 15.00 | 15.00 | 3.00 | | 45.00 |
| ***PT-06-R*** | ***18.00*** | ***16.00*** | ***14.00*** | ***3.00*** | ***51.00*** | ***48.00*** |
| PT-07-L | 17.00 | 17.00 | 16.00 | 3.00 | 52.00 | 51.00 |
| ***PT-08-R*** | ***18.00*** | ***17.00*** | ***15.00*** | ***3.00*** | ***55.00*** | ***51.00*** |
| PT-09-L | 18.00 | 17.00 | 16.00 | 3.00 | 52.00 | 51.00 |
| ***PT-10-L*** | ***18.00*** | ***16.00*** | ***15.00*** | ***3.50*** | ***53.00*** | ***56.00*** |
| PT-11-R | 15.00 | 14.00 | 14.00 | 3.00 | 57.00 | 42.00 |
| ***PT-12-R*** | ***18.00*** | ***16.00*** | ***15.00*** | ***3.00*** | ***51.00*** | ***48.00*** |
| PT-13-RS | 18.00 | 17.00 | 15.00 | 4.00 | 67.00 | 68.00 |
| ***PT-14-LS*** | ***17.00*** | ***17.00*** | ***16.00*** | ***4.00*** | ***63.00*** | ***68.00*** |
| PT-15-LS | 15.00 | 15.00 | 14.00 | 4.00 | 67.00 | 60.00 |
| ***PT-16-RS*** | ***16.00*** | ***15.00*** | ***15.00*** | ***4.00*** | ***59.00*** | ***60.00*** |
| PT-17-RS | 20.00 | 18.00 | 16.00 | 5.00 | 79.00 | 90.00 |
| ***PT-18-RS*** | ***18.00*** | ***17.00*** | ***16.00*** | ***4.00*** | ***70.00*** | ***68.00*** |
| PT-19-R | 20.00 | 18.50 | 18.00 | 4.00 | 41.00 | 74.00 |
| ***PT-20-R*** | ***11.00*** | ***11.50*** | ***12.50*** | ***3.00*** | ***42.00*** | ***34.50*** |
| PT-21-LS | 18.50 | 15.00 | 15.00 | 4.00 | 62.00 | 60.00 |
| ***PT-22-RS*** | ***18.50*** | ***16.00*** | ***15.00*** | ***5.00*** | ***67.00*** | ***80.00*** |
| PT-23-LS | 18.00 | 15.00 | 15.00 | 5.00 | 67.00 | 75.00 |
| **AVERAGE** | **17.33** | **16.00** | **15.14** | **3.75** | **58.61** | **60.25** |
| STD | 2.09 | 1.77 | 1.58 | 0.72 | 10.82 | 13.58 |

APPENDIX B

MATHCAD CODING FILE FOR DATA THINNING

**Downloading the experimental data:**

Data :=

Time := $Data^{\langle 0 \rangle}$ RawLoad := $Data^{\langle 2 \rangle}$ RawStroke := $Data^{\langle 1 \rangle}$

**Thinning the experimental data by picking every 10th data point:**

$$\begin{aligned}
&\text{ThinData}(T, S, L, \text{interval}) := \\
&\quad \text{upperbound} \leftarrow \text{length}(T) \\
&\quad \text{newArrayLength} \leftarrow (\text{upperbound} \div \text{interval}) \\
&\quad \text{for } k \in 0..\text{newArrayLength} - 1 \\
&\qquad \text{newT}_k \leftarrow T_{k \cdot \text{interval}} \\
&\quad \text{for } j \in 0..\text{newArrayLength} - 1 \\
&\qquad \text{for } k \in 0..\text{interval} - 1 \\
&\qquad\quad \text{newS}_j \leftarrow S_{j \cdot \text{interval}} \\
&\quad \text{for } i \in 0..\text{newArrayLength} - 1 \\
&\qquad \text{for } k \in 0..\text{interval} - 1 \\
&\qquad\quad \text{newL}_i \leftarrow L_{i \cdot \text{interval}} \\
&\quad \text{newArray} \leftarrow \text{augment}(\text{newT}, \text{newS}, \text{newL}) \\
&\quad \text{newArray}
\end{aligned}$$

AssignedInterval := 10

ThinResult := ThinData(Time, RawStroke, RawLoad, AssignedInterval )

ThinResult =

| | 0 | 1 | 2 |
|---|---|---|---|
| 0 | 106.9 | 119.6 | 100 |
| 1 | 80.288 | 108 | 78.18 |
| 2 | 72.62 | 104.5 | 71.86 |
| 3 | 66.08 | 100.4 | 63.05 |
| 4 | 61.569 | 99.2 | 61.17 |
| 5 | 59.539 | 95.03 | 58.96 |
| 6 | 56.156 | 93.71 | 56.82 |
| 7 | 55.029 | 94.16 | 54.14 |
| 8 | 51.646 | 91 | 52.47 |
| 9 | 51.646 | 89.75 | 51.56 |
| 10 | 50.744 | 90.25 | 49.21 |
| 11 | 48.488 | 90.06 | 50.22 |
| 12 | 46.233 | 88.97 | 45.61 |
| 13 | 47.361 | 88.61 | 44.75 |
| 14 | 43.978 | 85.25 | 46.97 |
| 15 | 43.978 | 86.65 | ... |

ThinTime := ThinResult$^{\langle 0 \rangle}$ ThinStress := ThinResult$^{\langle 1 \rangle}$ ThinLoad := ThinResult$^{\langle 2 \rangle}$

**Outputting the thinned results to a text file:**

file0 := "C:\Users\Lee Crawford\Desktop\thinneddata1.txt"

mat0 := ThinData(Time, RawStroke, RawLoad, AssignedInterval )

APPENDPRN(file0) := mat0

(1) Right-click in the open space
(2) Select "Insert"
(3) Click on "File Input"
(4) Under File Format, select "Text"
(5) Browse to find the text file with the original experimental data
(6) Click on "Finish"
(7) Type "Data" into the black box located to the left of the := symbol
(8) Increase the size of the floopy disk icon to make it easier to read where the file originates
(9) Delete the data file in this original document and replace it with the one just created

APPENDIX C

SAS STATISTICAL CODE

SAS Code For Load Statistics:

```
OPTIONS PS=55 LS=85 NODATE;
DATA LOAD_30MIN;
INFILE 'C:\Users\Lee Crawford\Desktop\30MIN.dat';
INPUT PRECONDITION $ LOAD;
RUN;
PROC MEANS SUM MEAN CSS VAR;
VAR LOAD;
CLASS PRECONDITION;
WAYS 1;
RUN;
PROC GLM;
CLASS LOAD;
MODEL PRECONDITION;
MODEL LOAD = PRECONDITION;
MEANS PRECONDITION/LSD LINES;
RUN;
PROC ANOVA;
CLASS PRECONDITION;
MODEL LOAD = PRECONDITION;
RUN;
PROC GLM;
CLASS PRECONDITION;
MODEL LOAD = PRECONDITION;
MEANS PRECONDITION/ LSD BON DUNCAN SNK TUKEY SCHEFFE WALLER
REGWQ LINES;
MEANS PRECONDITION/ DUNNETT ('CONTROL');
RUN;
PROC GLM;
CLASS PRECONDITION;
MODEL LOAD = PRECONDITION;
MEANS PRECONDITION/HOVTEST=LEVENE(TYPE=ABS);
MEANS PRECONDITION/HOVTEST=LEVENE(TYPE=SQUARE);
MEANS PRECONDITION/HOVTEST=BARTLETT;
MEANS PRECONDITION/HOVTEST=BF;
RUN;
```

SAS Code For Measurements Statistics:

```
OPTIONS PS=55 LS=85 NODATE;
DATA PT_XSECTION;
INFILE 'C:\Users\Lee Crawford\Desktop\measurementDATA.dat';
INPUT LOCATION $ SEX $ XSECTION;
RUN;
PROC MEANS SUM MEAN CSS VAR;
VAR XSECTION;
```

```
CLASS LOCATION SEX;
WAYS 1;
RUN;
PROC GLM;
CLASS LOCATION SEX;
MODEL XSECTION = LOCATION;
MEANS LOCATION/LSD LINES;
RUN;
PROC GLM;
CLASS LOCATION SEX;
MODEL XSECTION = SEX;
MEANS SEX/LSD LINES;
RUN;
PROC ANOVA;
CLASS LOCATION SEX;
MODEL XSECTION = LOCATION SEX;
RUN;
PROC GLM;
CLASS LOCATION SEX;
MODEL XSECTION = LOCATION SEX;
MEANS LOCATION/ LSD BON DUNCAN SNK TUKEY SCHEFFE WALLER
REGWQ LINES;
MEANS LOCATION/DUNNETT ('CONTROL');
RUN;
PROC GLM;
CLASS LOCATION SEX;
MODEL XSECTION = LOCATION SEX;
MEANS SEX/LSD BON DUNCAN SNK TUKEY SCHEFFE WALLER REGWQ
LINES;
MEANS SEX/DUNNETT ('CONTROL');
RUN;
PROC GLM;
CLASS LOCATION;
MODEL XSECTION = LOCATION;
MEANS LOCATION/HOVTEST=BARTLETT;
RUN;
PROC GLM;
CLASS SEX;
MODEL XSECTION = SEX;
MEANS SEX/HOVTEST=BARTLETT;
RUN;
```

APPENDIX D

FORCE AND STRESS VS. TIME PLOTS FOR ALL SPECIMENS

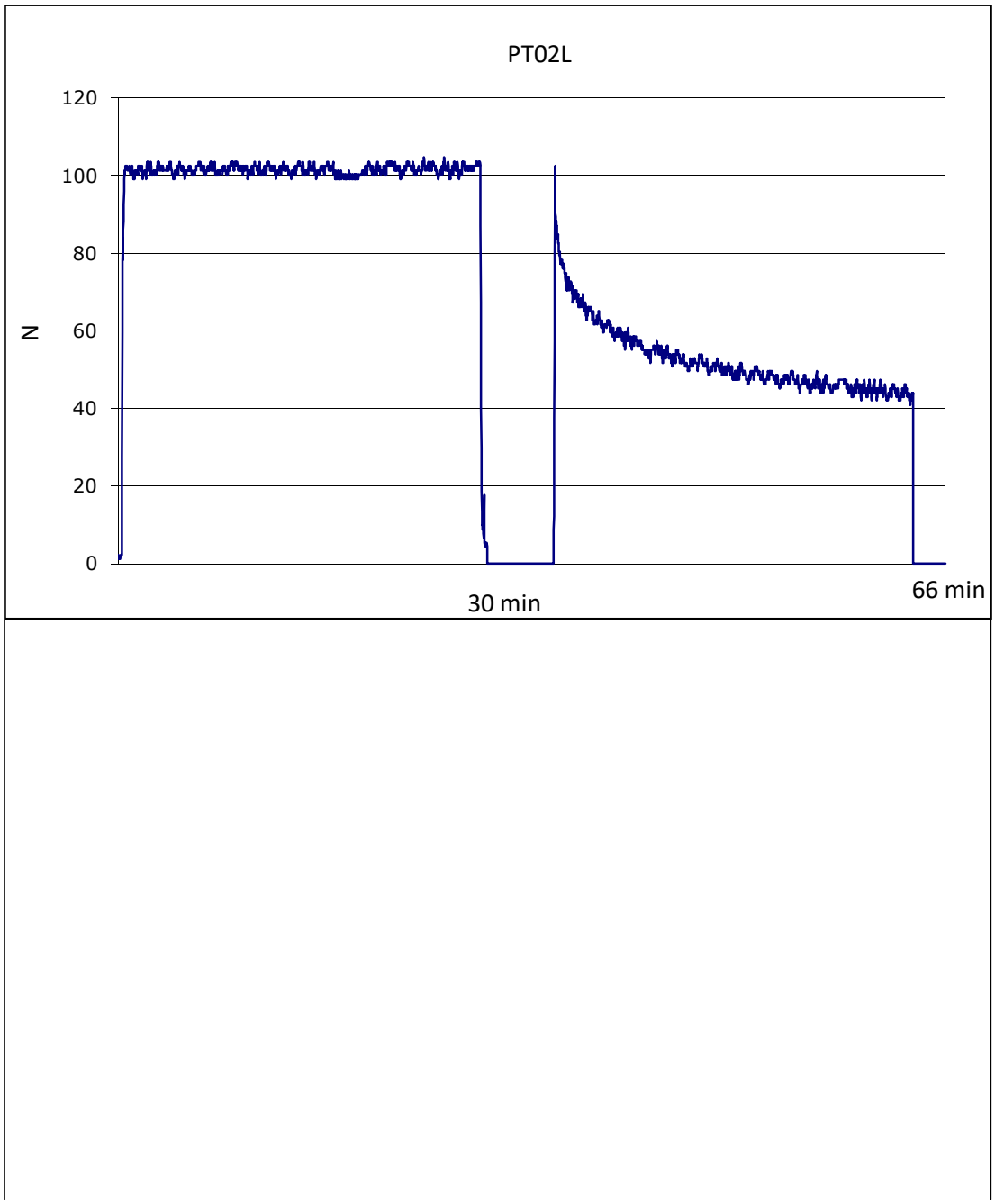


Figure 29: Force vs. Time and Stress vs. Time curve for PT02L

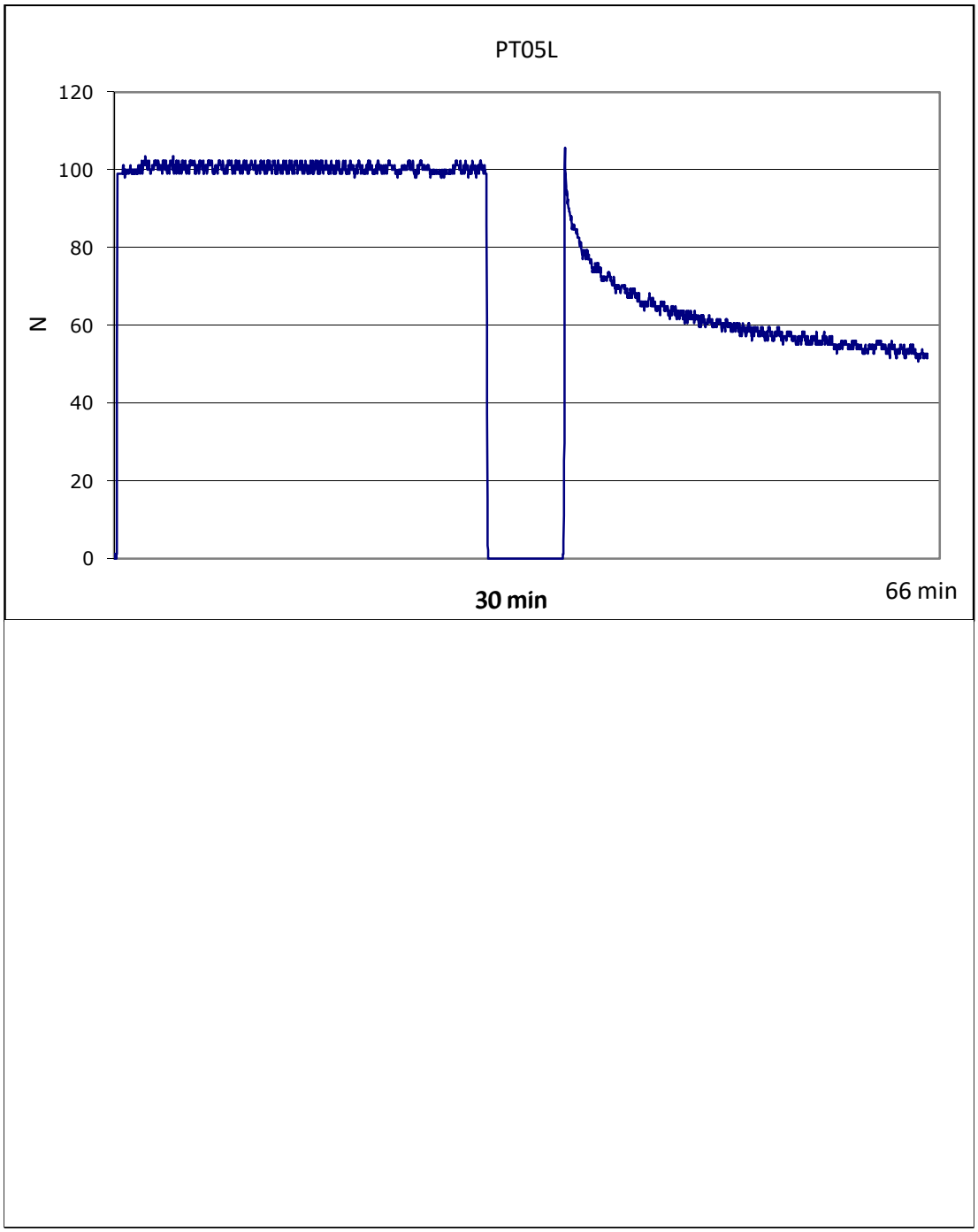


Figure 30: Force vs. Time and Stress vs. Time curve for PT05L

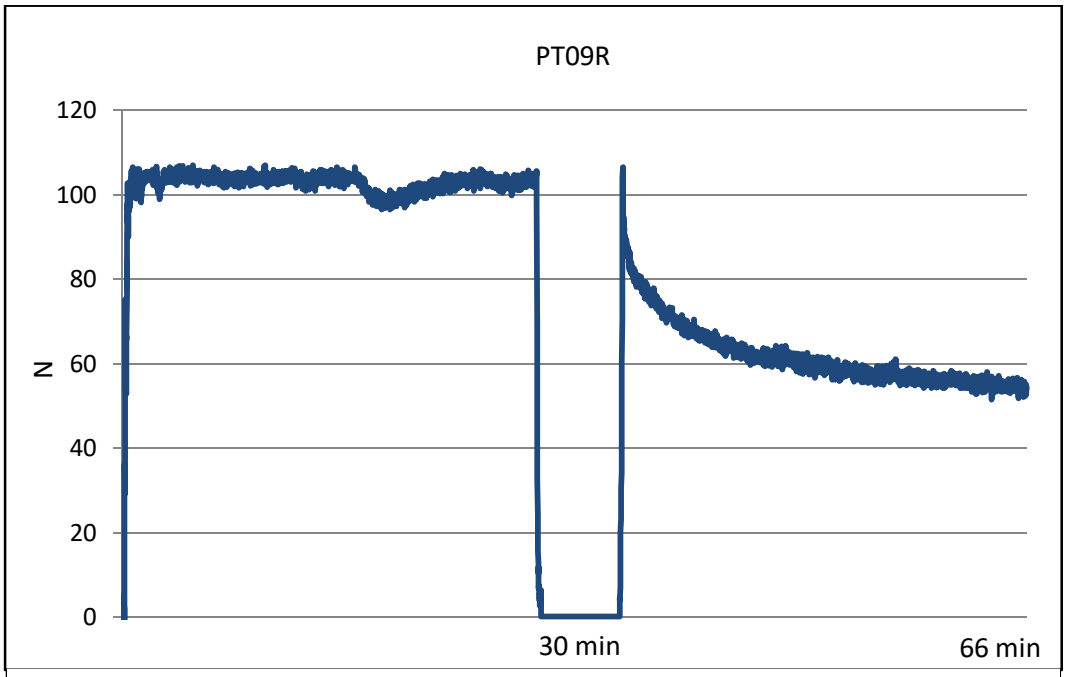


Figure 31: Force vs. Time and Stress vs. Time curve for PT00R

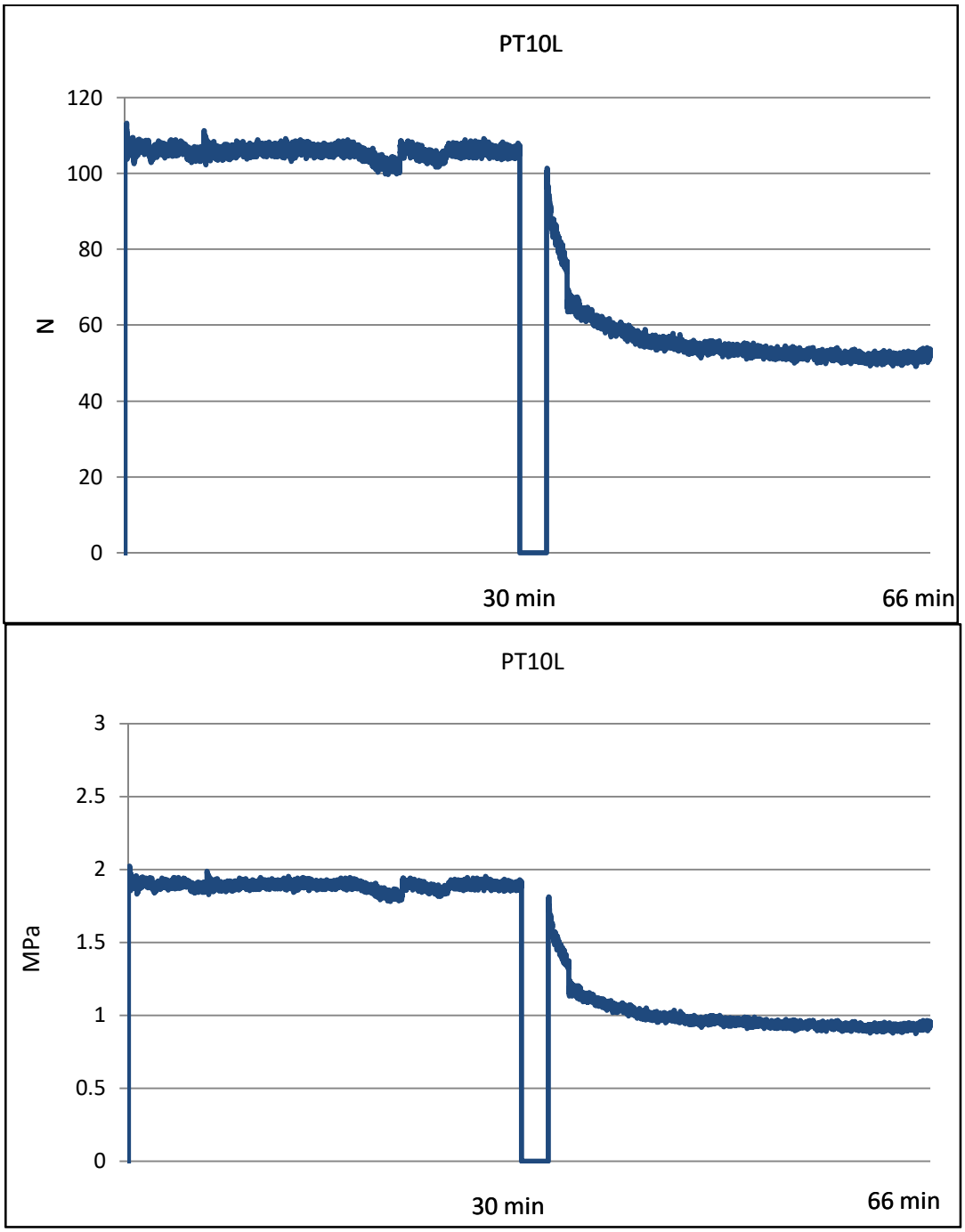


Figure 32: Force vs. Time and Stress vs. Time curve for PT10L

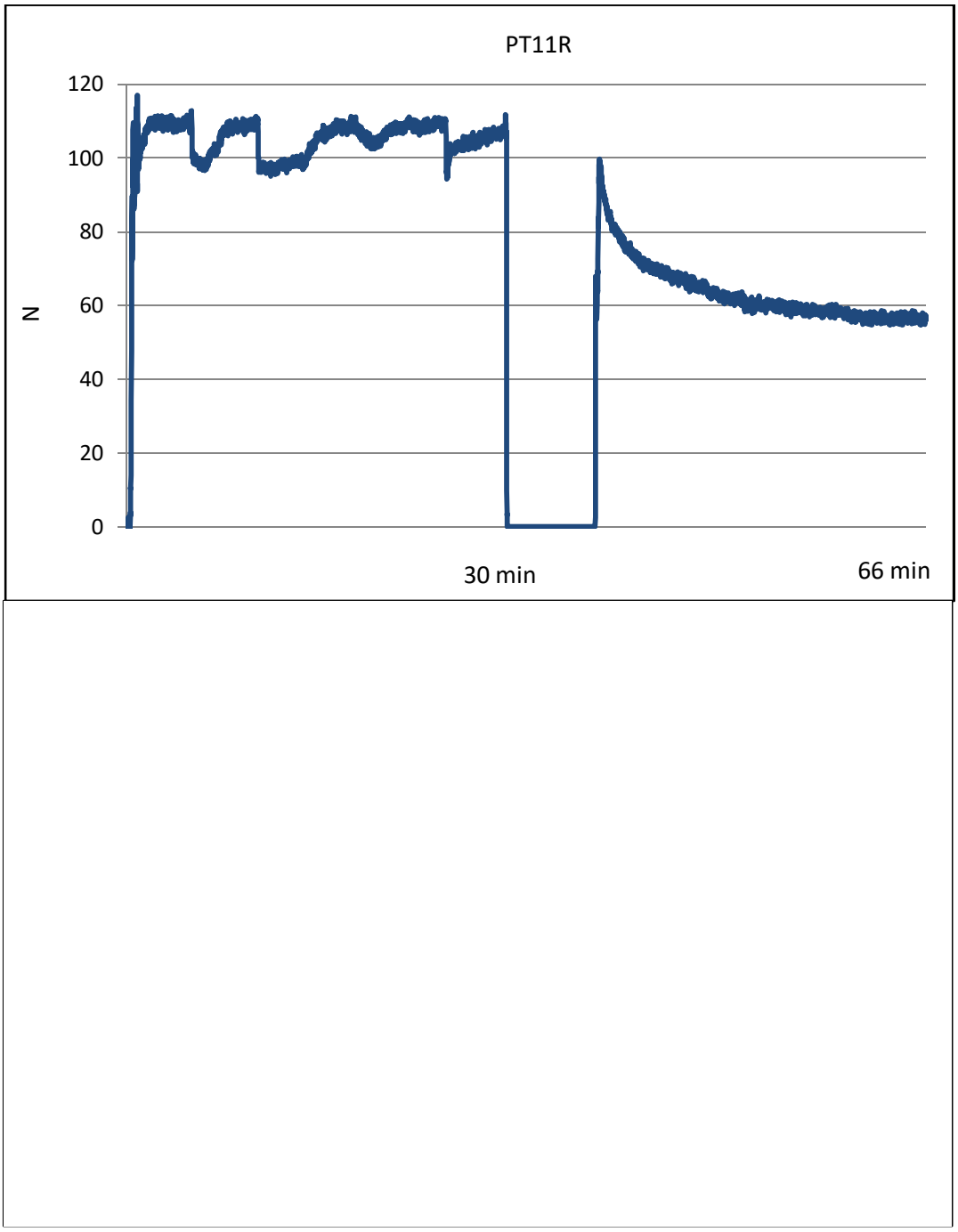


Figure 33: Force vs. Time and Stress vs. Time curve for PT11R

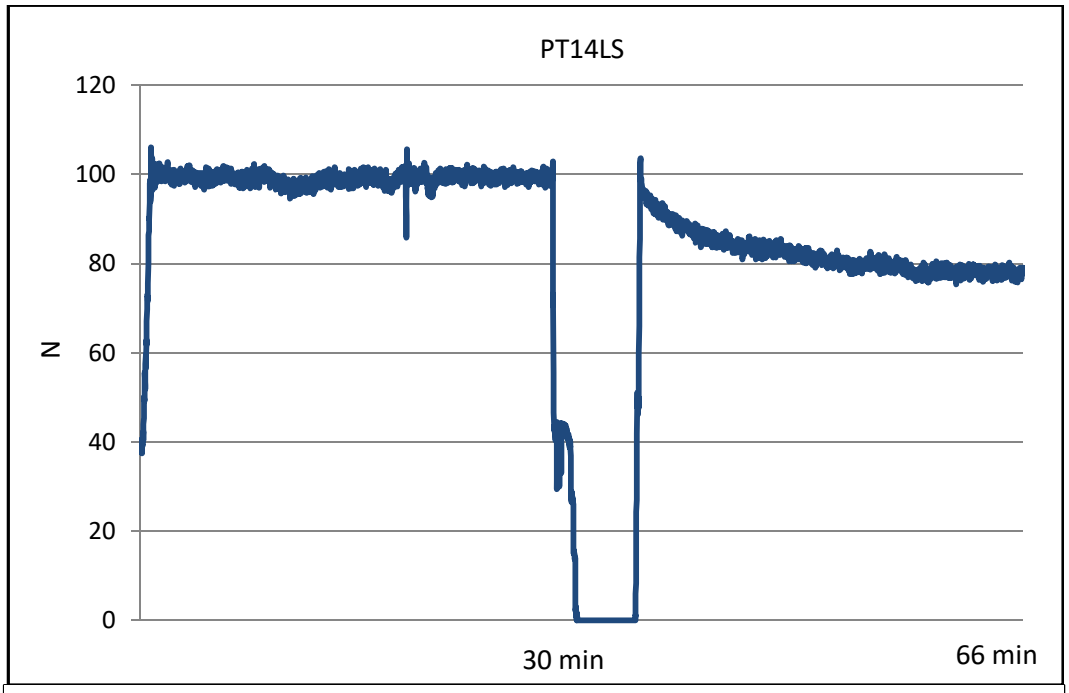


Figure 34: Force vs. Time and Stress vs. Time curve for PT14LS

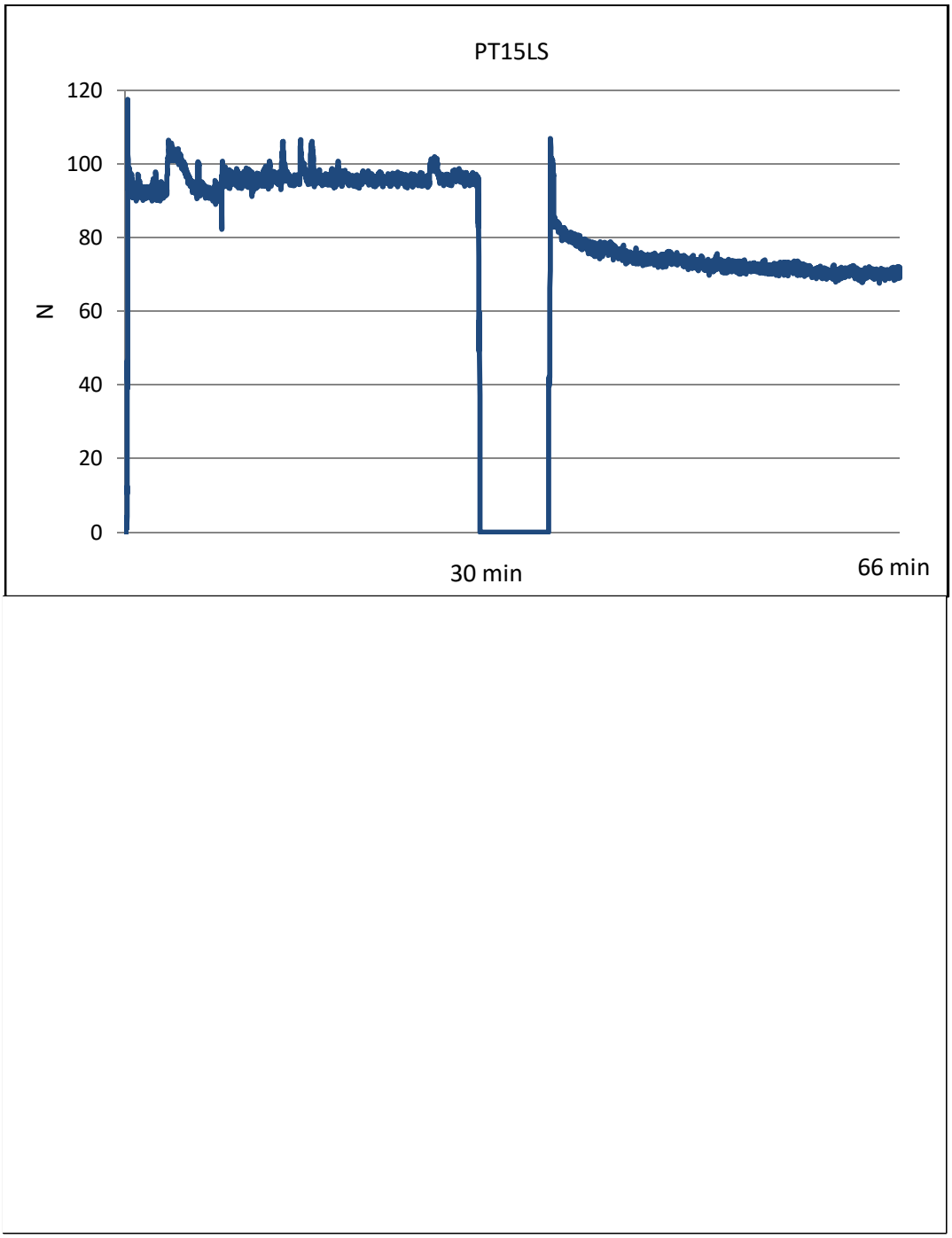


Figure 35: Force vs. Time and Stress vs. Time curve for PT15LS

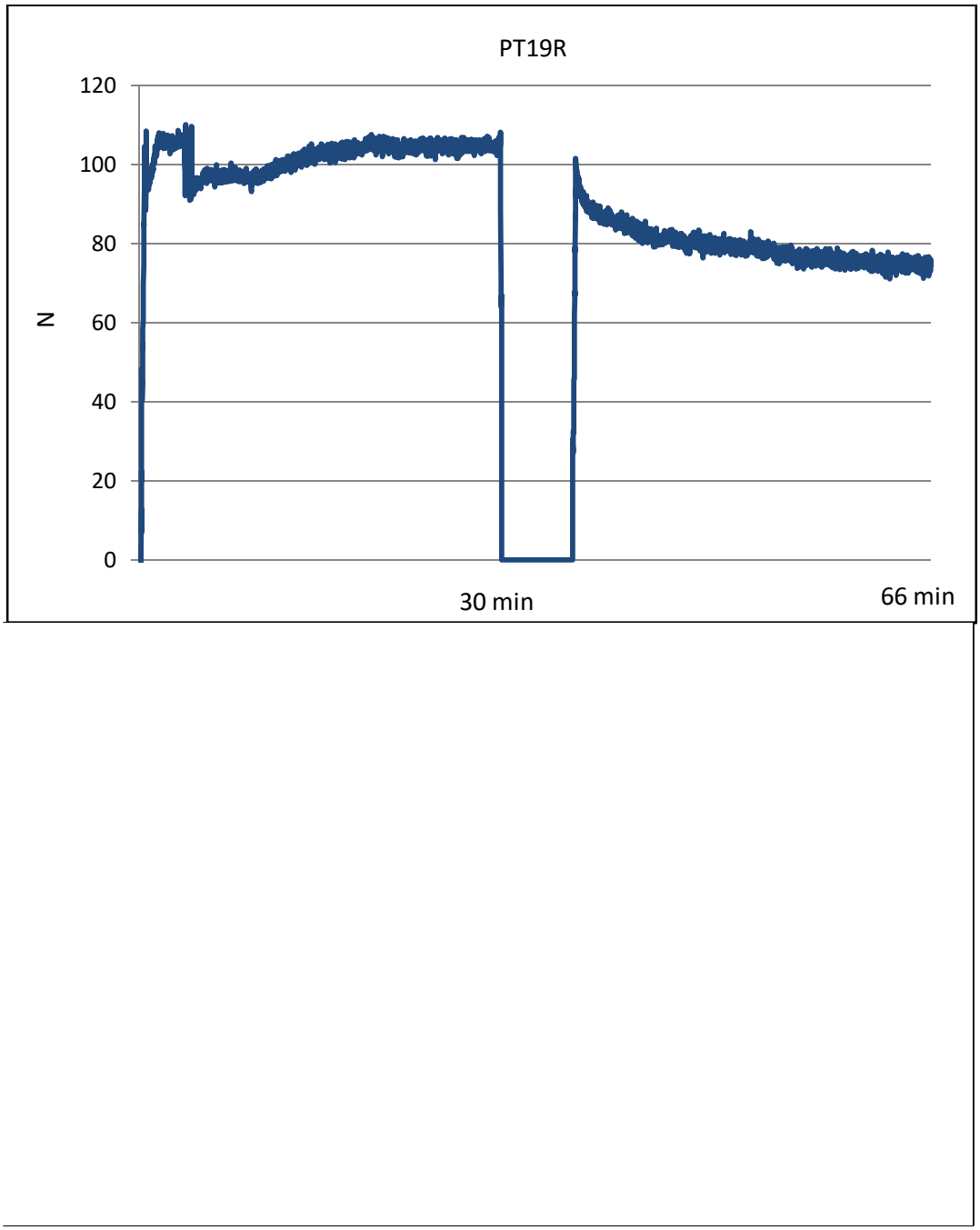


Figure 36: Force vs. Time and Stress vs. Time curve for PT19R

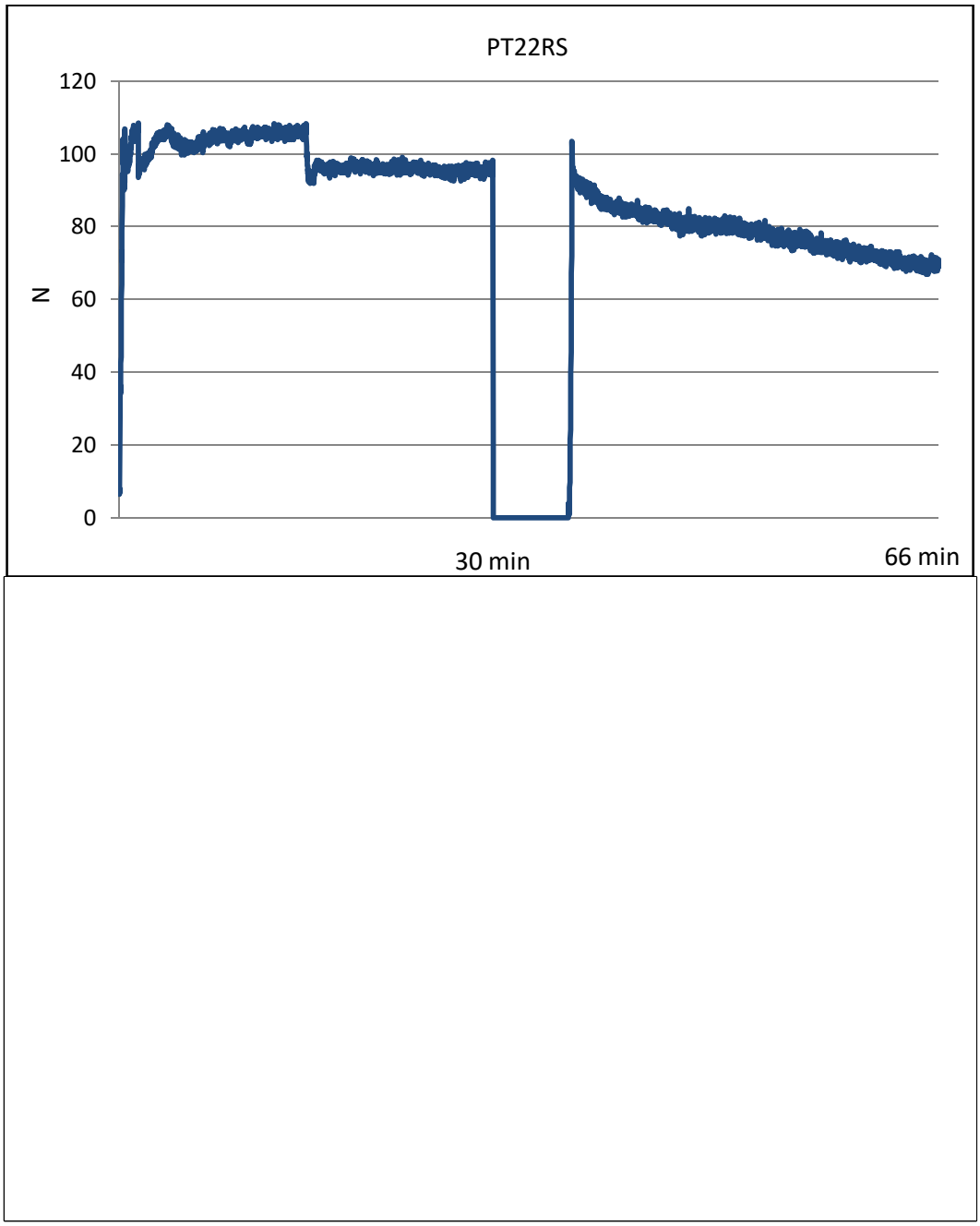


Figure 37: Force vs. Time and Stress vs. Time curve for PT22RS

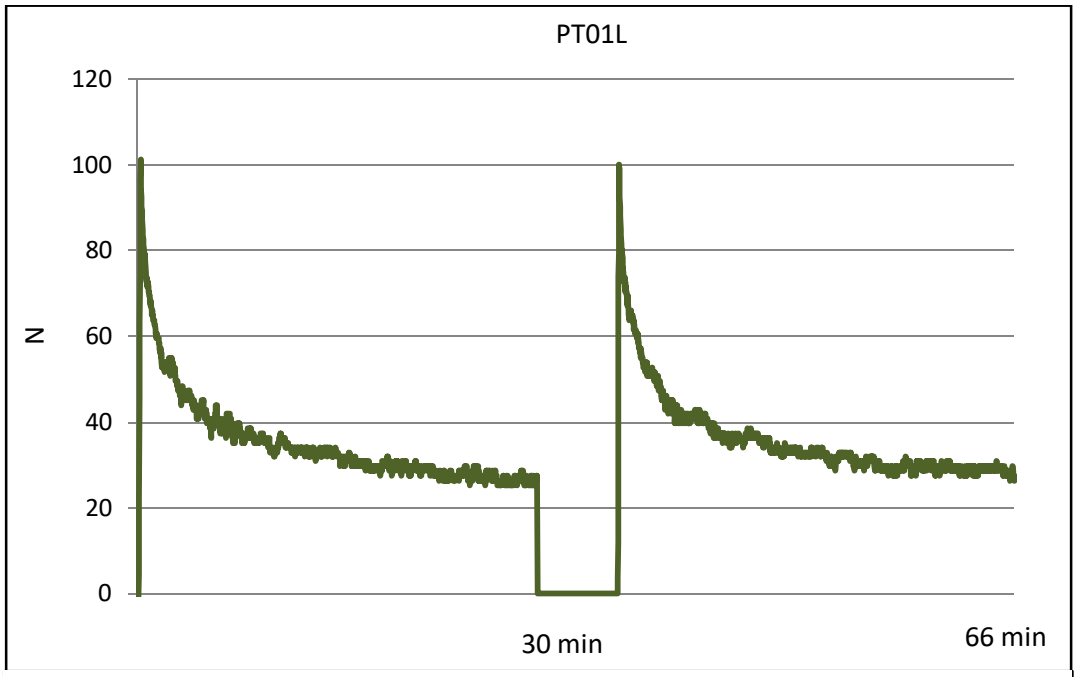


Figure 38: Force vs. Time and Stress vs. Time curve for PT01L

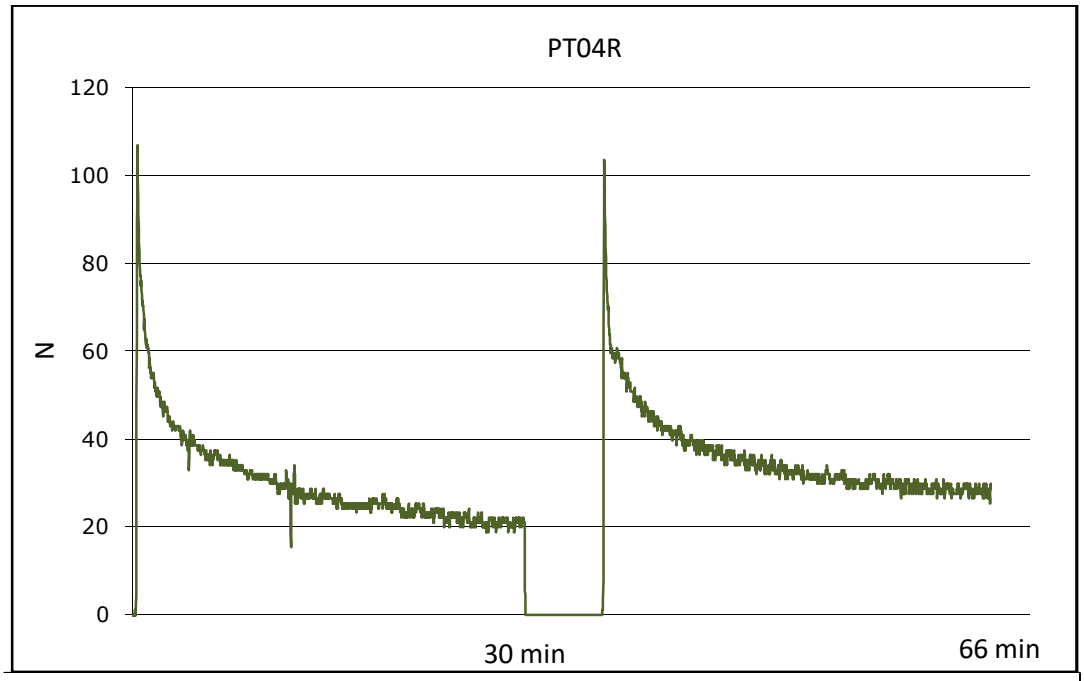


Figure 39: Force vs. Time and Stress vs. Time curve for PT04R

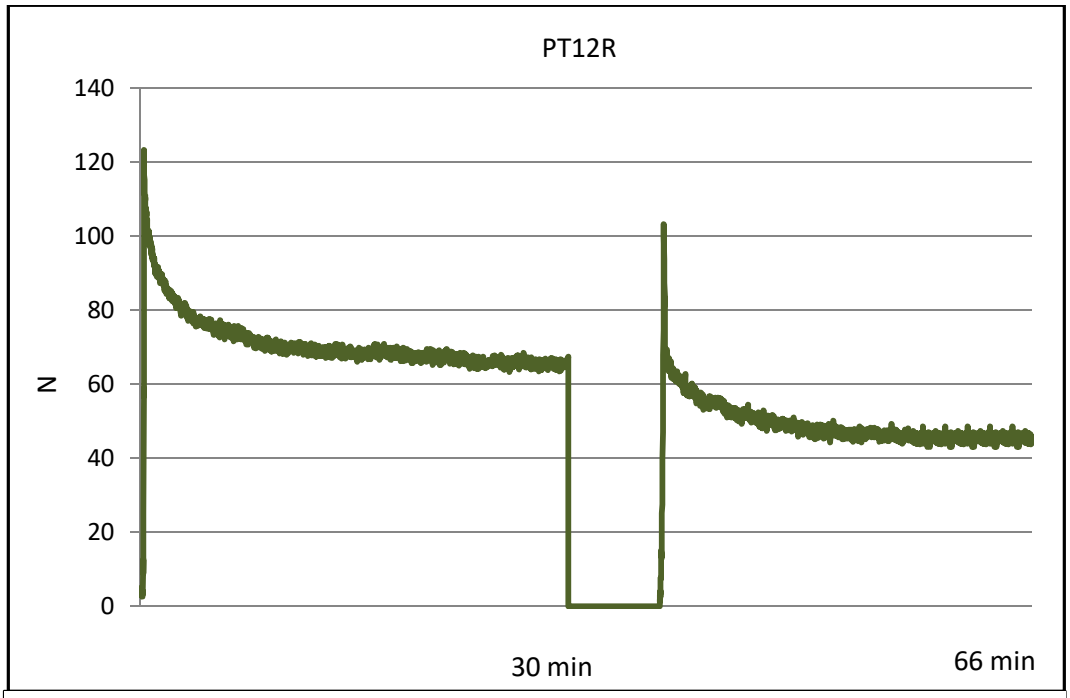


Figure 40: Force vs. Time and Stress vs. Time curve for PT12R

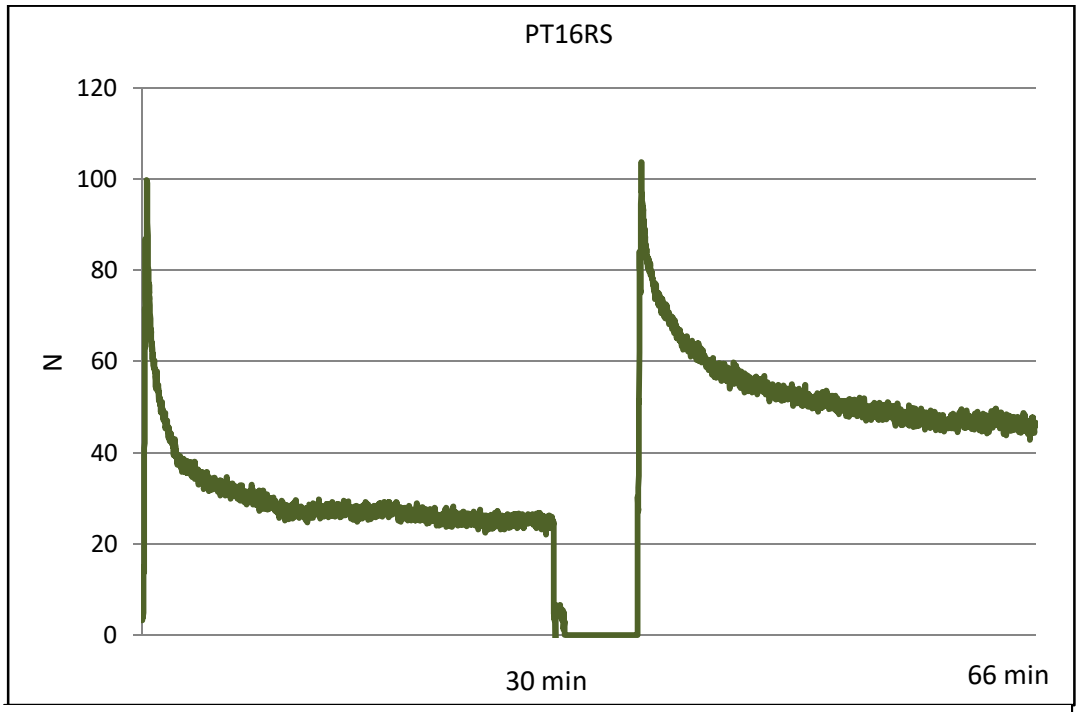


Figure 41: Force vs. Time and Stress vs. Time curve for PT16RS

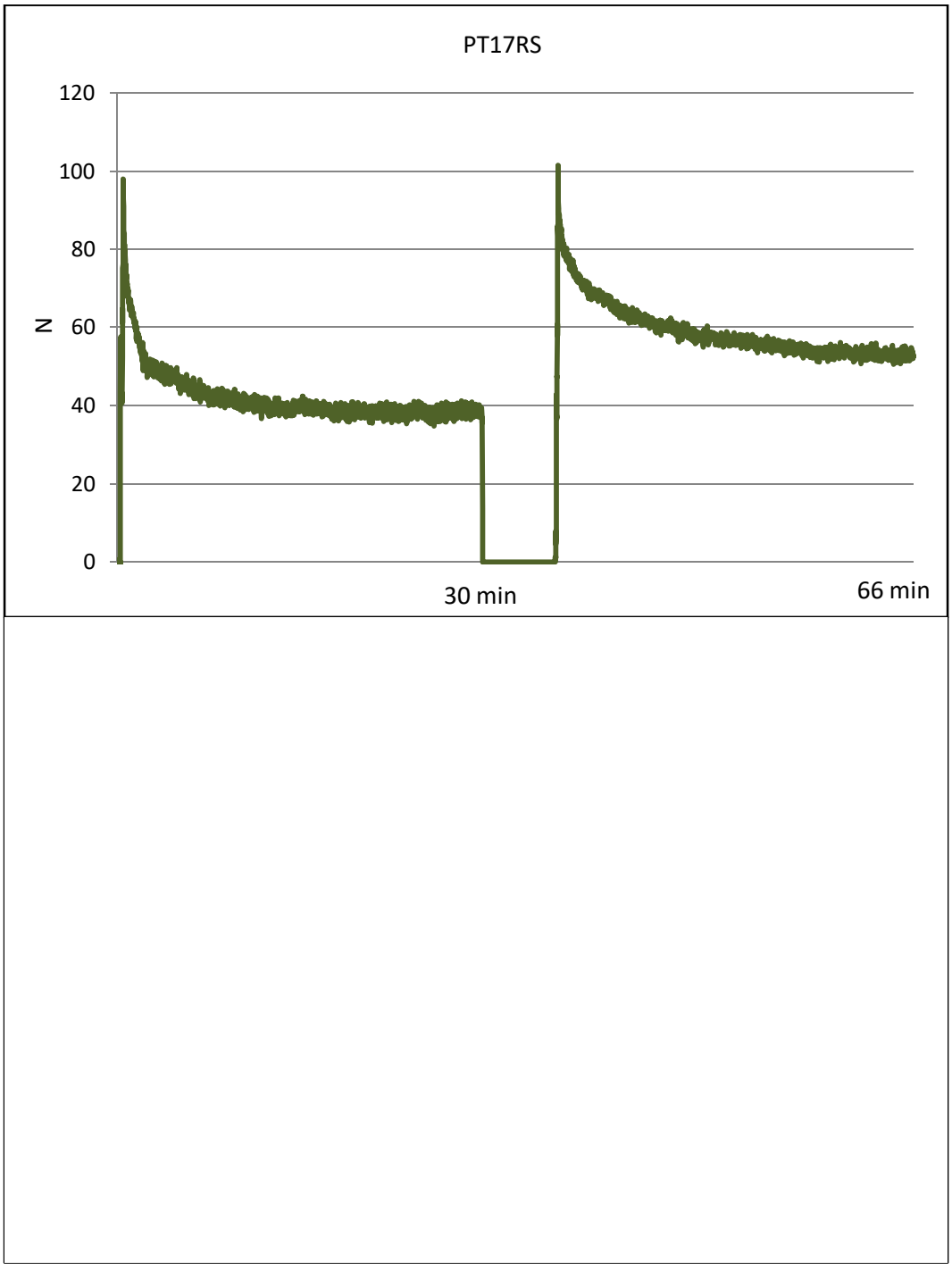


Figure 42: Force vs. Time and Stress vs. Time curve for PT17RS

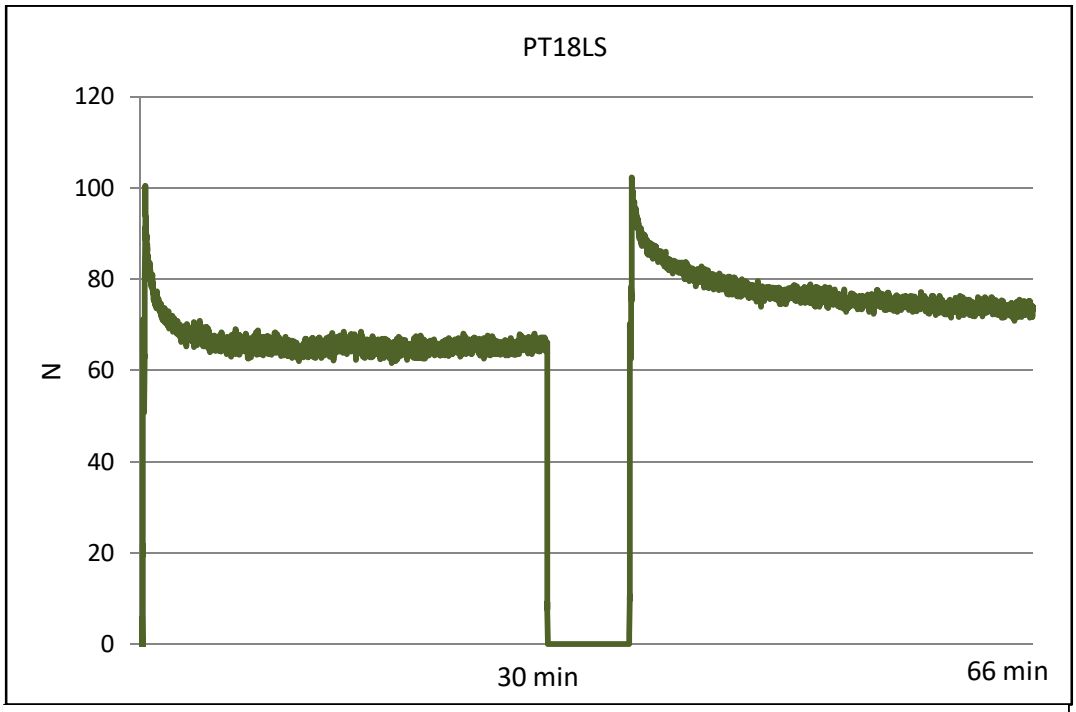


Figure 43: Force vs. Time and Stress vs. Time curve for PT18LS

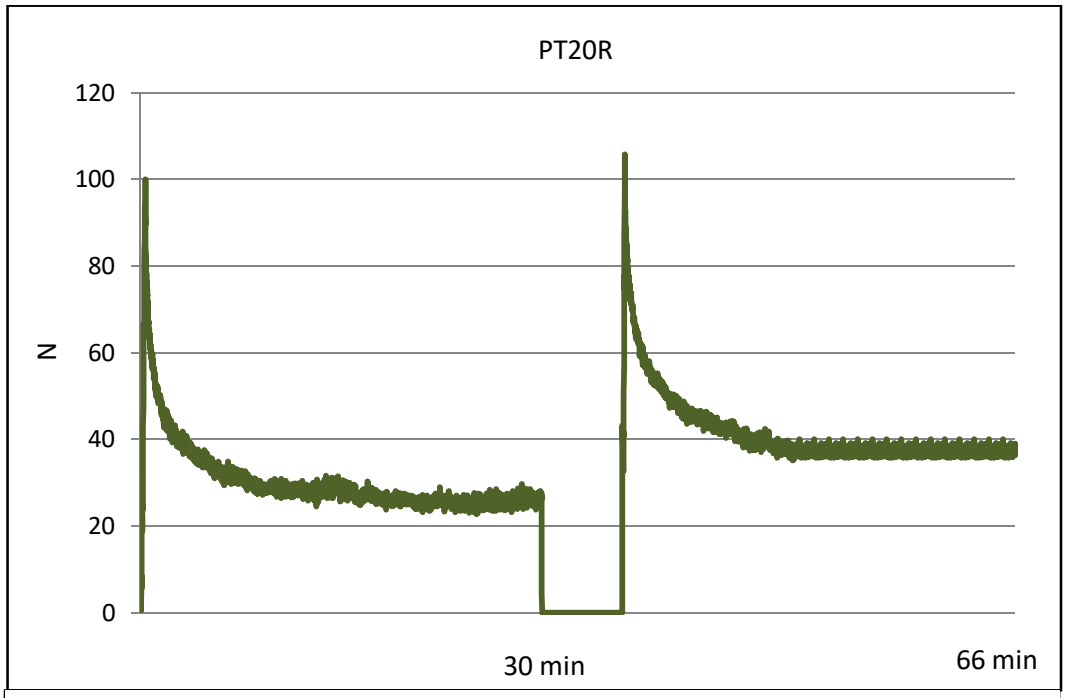


Figure 44: Force vs. Time and Stress vs. Time curve for PT20R

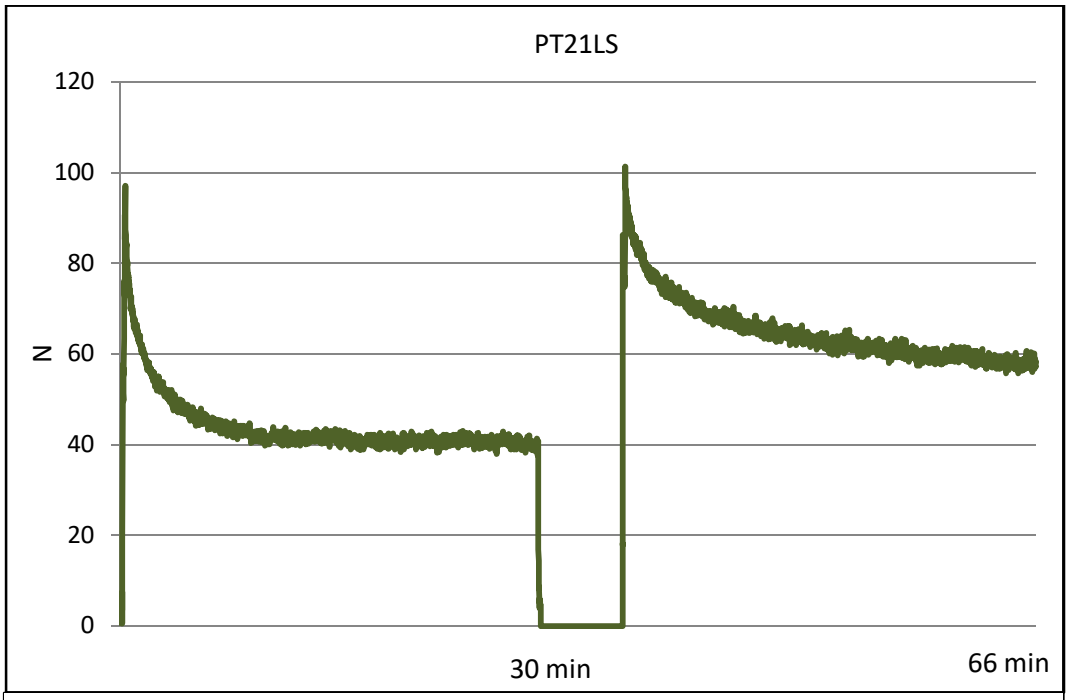


Figure 45: Force vs. Time and Stress vs. Time curve for PT21LS

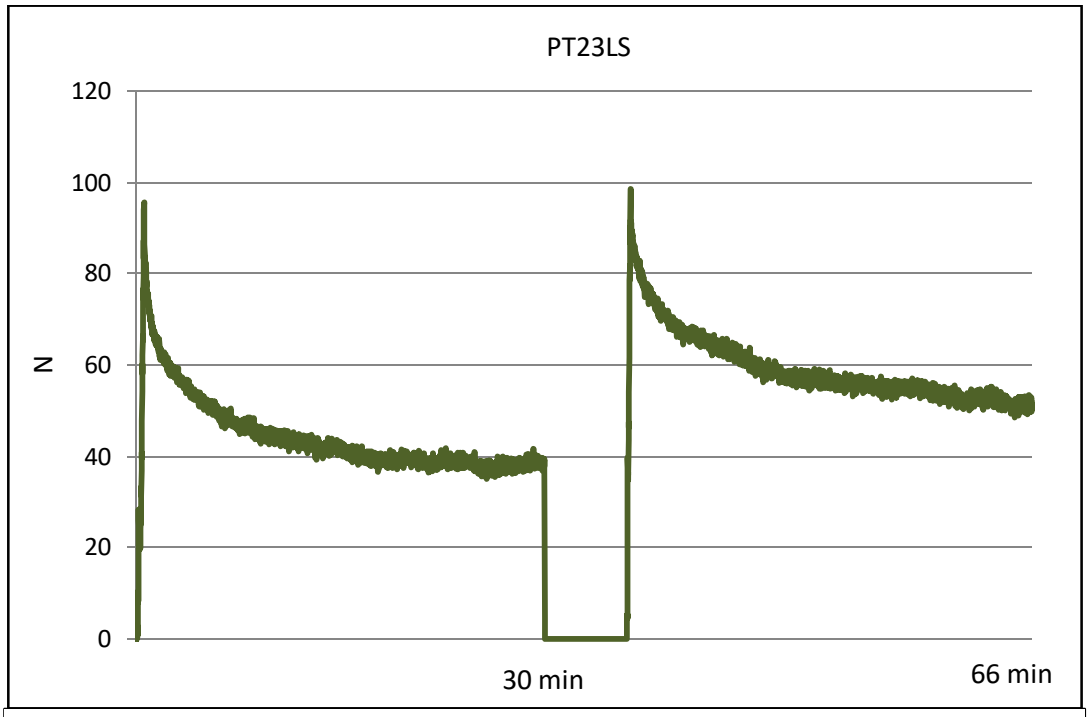


Figure 46: Force vs. Time and Stress vs. Time curve for PT23LS

APPENDIX F

LOAD, STRESS, AND STRESS RATIO FOR EACH SPECIMEN

Table 6: The average load and stress values with the corresponding stress ratio divided by preconditioned protocol.

| | | AVERAGE LOAD | | | STRESS (MPa) | | |
|---|---|---|---|---|---|---|---|
| PRECONDITION | Specimen | 10 min | 15 min | 30 min | Initial | Final | Stress Ratio |
| CREEP | PT-02-L | 62.03 | 59.86 | 54.10 | 1.71 | 0.73 | 0.43 |
| | PT-05-L | 55.82 | 53.23 | 52.53 | 2.35 | 1.16 | 0.49 |
| | PT-09-R | 65.01 | 59.75 | 56.37 | 2.09 | 1.06 | 0.51 |
| | PT-10-L | 83.19 | 81.30 | 78.35 | 1.80 | 0.94 | 0.52 |
| | PT-11-R | 74.06 | 71.73 | 70.34 | 2.37 | 1.34 | 0.57 |
| | PT-14-LS | 80.41 | 79.20 | 74.68 | 1.52 | 1.15 | 0.76 |
| | PT-15-LS | 80.59 | 78.65 | 69.38 | 1.78 | 1.17 | 0.66 |
| | PT-19-R | 53.34 | 50.00 | 43.79 | 1.37 | 1.01 | 0.74 |
| | PT-22-RS | 63.45 | 59.92 | 52.21 | 1.29 | 0.87 | 0.67 |
| | **AVERAGE** | **68.66** | **65.96** | **61.31** | **1.81** | **1.05** | **0.58** |
| | ***STD*** | ***11.21*** | ***11.91*** | ***12.04*** | ***0.39*** | ***0.18*** | ***0.12*** |
| | | | | | | | |
| NONE | PT-01-L | 34.05 | 32.78 | 27.76 | 1.88 | 0.51 | 0.27 |
| | PT-04-L | 31.65 | 26.76 | 21.73 | 2.38 | 0.48 | 0.20 |
| | PT-12-R | 70.23 | 68.01 | 65.72 | 2.15 | 1.37 | 0.64 |
| | PT-16-RS | 27.33 | 27.60 | 25.13 | 1.67 | 0.42 | 0.25 |
| | PT-17-RS | 41.08 | 39.74 | 38.36 | 1.09 | 0.43 | 0.39 |
| | PT-18-LS | 66.17 | 66.63 | 65.44 | 1.48 | 0.96 | 0.65 |
| | PT-20-R | 28.45 | 28.14 | 26.76 | 2.90 | 0.78 | 0.27 |
| | PT-21-LS | 41.46 | 41.44 | 38.85 | 1.63 | 0.65 | 0.40 |
| | PT-23-LS | 44.58 | 41.64 | 38.79 | 1.28 | 0.52 | 0.41 |
| | **AVERAGE** | **42.78** | **41.42** | **38.73** | **1.83** | **0.68** | **0.39** |
| | ***STD*** | ***15.62*** | ***15.82*** | ***16.51*** | ***0.57*** | ***0.31*** | ***0.16*** |
| | | | | | | | |
| STRESS RELAXATION | PT-01-L | 35.59 | 32.63 | 27.33 | 1.85 | 0.51 | 0.27 |
| | PT-04-L | 36.12 | 32.96 | 27.51 | 2.30 | 0.61 | 0.27 |
| | PT-12-R | 48.90 | 46.63 | 45.00 | 2.15 | 0.94 | 0.44 |
| | PT-16-RS | 53.97 | 50.83 | 45.95 | 1.73 | 0.77 | 0.44 |
| | PT-17-RS | 59.57 | 56.71 | 52.51 | 1.13 | 0.58 | 0.52 |
| | PT-18-LS | 76.41 | 76.26 | 73.08 | 1.50 | 1.07 | 0.72 |
| | PT-20-R | 39.66 | 37.51 | 37.60 | 3.07 | 1.09 | 0.36 |
| | PT-21-LS | 65.41 | 61.91 | 57.41 | 1.69 | 0.96 | 0.57 |
| | PT-23-LS | 48.90 | 46.63 | 45.00 | 1.32 | 0.60 | 0.46 |
| | **AVERAGE** | **51.61** | **49.12** | **45.71** | **1.86** | **0.79** | **0.45** |
| | ***STD*** | ***13.82*** | ***14.32*** | ***14.44*** | ***0.59*** | ***0.23*** | ***0.14*** |