ABSTRACT

Title of Document: BIOMECHANICS OF THE INTERVERTEBRAL DISC: THE EFFECTS OF LOAD HISTORY ON MECHANICAL BEHAVIOR

Adam Shabtai Gabai, Master of Science, 2007

Directed By: Professor Adam Hsieh, Fischell Department of Bioengineering

Degenerative disc disease is associated with back pain, and can be a debilitating disorder. In addition to the biological contributions of genetics and aging, mechanical factors have been implicated in accelerating the progression of disc degeneration.

Two studies were performed in order to explore the effects of various loading conditions on disc biomechanics. The first study explores the effects of compressive historical loads and disc hydration on subsequent creep loading and recovery. The second study investigates the restorative powers of creep distraction between compressive loading periods. In both cases three commonly applied mathematical models were employed to characterize disc behavior and the effectiveness of each model was validated.

The studies confirm that hydration level has a significant impact on disc stiffness and time dependent behavior. Distraction and conditioning phases are shown to have a significant impact on hydration level and thus subsequent mechanical behavior.

BIOMECHANICS OF THE INTERVERTEBRAL DISC: THE EFFECTS OF LOAD HISTORY ON MECHANICAL BEHAVIOR

By

Adam Shabtai Gabai

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2007

Advisory Committee:
Assistant Professor Adam Hsieh, Chair
Associate Professor David Bigio
Associate Professor Jeffrey Herrmann

Dedication

To my parents whose continual love and support have driven me to success as a student and *Ben Torah*

Acknowledgements

I would like to thank Boss Man Adam Hsieh, my advisor, for his continued support and guidance with my academic career. Thanks for always sharing your great knowledge, integrity, and time. A special thanks to Dr. Bigio for his constant support throughout my academic career as an undergraduate and graduate student, and specifically for helping out with the analysis and statistics. Thank you to Dr. Herrmann for participating as a member of my committee.

I am grateful to all of the members of the Orthopaedic Mechanobiology Laboratory for making themselves available whenever I was in need of a hand. I would like to give a special thank you to Alvin Yew for the countless hours spent aiding in my analyses.

Finally, I would like to thank my family and friends for continuously standing by me and supporting me.

Table of Contents

Chapter 1: Introduction

1.1 The Intervertebral Disc

The intervertebral disc (IVD) is found between two subsequent vertebral bodies allowing the spine to flex and twist while supporting gravitational and muscular loads. A motion segment is comprised of an intervertebral disc and its two neighboring vertebral bodies. The mechanical properties of the disc are imperative to its normal operation. The disc is comprised of several components that each contribute to the mechanical properties. Degradation of these components can lead to reduced mechanical performance as well as pain [1, 2, 3, 4, 5].

The disc degenerates naturally as a normal part of aging, but the relationship between degeneration and pain is not fully understood. Studies are focused on differentiating between natural aging and the debilitating effects of more extreme degeneration. The effects of degeneration on the mechanical behavior of the disc may be a contributing factor to pain. Degeneration can lead to degraded biomechanics in terms of increased flexibility, decreased fluid pressurization, and lower disc height. Severe disc degeneration involves the degradation of the components of the disc and can lead to herniation, spinal stenosis, and degenerative spondylolisthesis [2, 4, 6].

1.1.1 Degeneration Prevention

Currently, surgical procedures are oftentimes employed to counteract the symptoms of pain. However, there is emerging research which supports prophylaxis by prevention of the degeneration process. Through a thorough understanding of the degeneration process one could minimize the potential for degeneration through mechanical and/or biochemical stimuli.

By studying the mechanical behavior of the disc under normal as well as extreme loading conditions one can better understand the role of disc mechanics in normal operation and degeneration. It has been shown that a healthy disc is maintained through a safe window of mechanical behavior. Immobilization of the disc or overloading it can lead to injury or degeneration. Thus a comprehensive understanding of a range of loading conditions can aid in uncovering preventative measures. These techniques may lead to minimizing or even counteracting degeneration by altering the mechanical stimulus on the disc and the biological factors which react to disc biomechanics [2, 3, 4, 6, 7].

1.1.2 Disc Composition

The disc has a complex structure contributing to its non-linear time-dependent mechanical properties. The human intervertebral disc is comprised of an outer ring called the annulus fibrosis that surrounds an inner core of nucleus pulposus tissue. The annulus is made up of lamellar networks of collagen fibers which provide the disc with a stiffness to support mechanical loads. The nucleus has a high water content used to pressurize the disc. The annulus surrounds the nucleus along the sides but between the neighboring intervertebral bodies and the nucleus is a cartilage endplate. This endplate is porous and allows fluid flow between the disc and vertebral body. Though the endplate is believed to be the major pathway for fluid and nutrition transport, fluid flow occurs through the wall of the annulus as well (see Figure 1 and Figure 2) [2, 6, 7, 8].

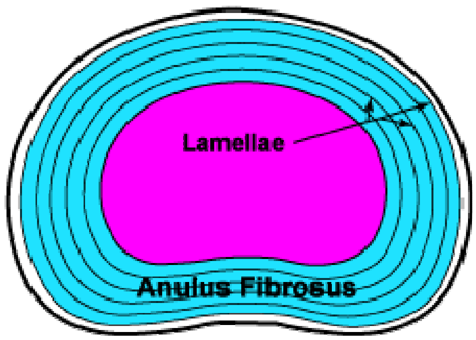


Figure 1: Top View of IVD from [9]

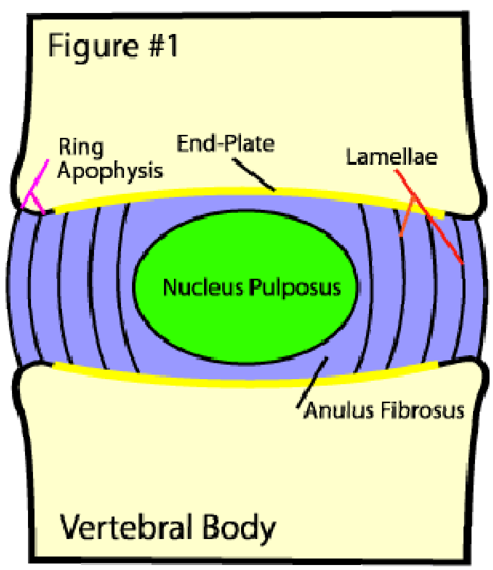


Figure 2: Side View of a Motion Segment from [9]

1.1.3 Basic Physiology

The materials that comprise the disc cause it to display viscoelastic behaviors; the fluid contained in the nucleus pulposus enters and exits the disc regularly causing variable intradiscal pressure and time dependant deformations. The swelling pressure of the disc is constantly regulated by this fluid flow to match the external pressures exerted during activity. The internal swelling pressure is maintained by a concentration of proteoglycans within the nucleus. These charged glycoproteins attract water to stabilize the disc. During loading the hydration level is continuously altered due to the imbalance of external and internal stresses. The pressurization of the nucleus causes fluid to flow out, increasing the proteoglycan concentration and thus creating a potential for fluid inflow when external stresses are removed.

During the day time in the normal human intervertebral disc the stresses due to gravity cause a net outflow of fluid which is replenished rapidly overnight while the disc is allowed to relax. Since overnight relaxation has a shorter duration than daytime loading, and hydration levels are restored during resting, it is believed that inflow occurs more rapidly than outflow and that the flow pathways (endplate and annulus pores) are responsible for this difference. These fluid properties along with the inherent stiffness of the collagen fibers that comprise the annulus fibrosis support the disc in a unique way causing the observed viscoelastic behavior [4, 10, 11, 12, 13].

1.1.4 Natural Loading Behavior

The human disc is under dynamic loading conditions throughout the day, but there is a constant minimal level of compression. Though static loading *in vitro* does not show the same mechanical behavior as *in vivo*, many insights into the mechanics of the disc can be obtained through creep *in vitro* testing [14]. Much can be learned from creep data though the disc is often subjected to dynamic loading.

An important aspect of disc mechanics is the ability of the disc to restore its mechanical properties between loading periods. In humans this occurs during non-load bearing states such as during sleep. Studying the relaxation and recovery behavior of the disc can lead insight into the effects on mechanical behavior during loading before and after relaxation. It has been shown that mechanical properties are not restored in the disc *in vitro*, even for relaxation durations that are twice as long as loading. Studies have investigated the effects of the endplate *in vitro* as to clues for why inflow is obstructed and why mechanical properties are restored only after very long periods of unloading [4, 14].

1.1.5 Interrelationship of Mechanics and Biology

The biological factors in disc degeneration are continuously studied as well. Mechanical behaviors can impact cell function and there are several methods employed to characterize the impact that mechanics and biology have on each other with regard to disc function. Not only do mechanical stimuli affect cell metabolism and protein synthesis, but the biological behavior of the disc cells leads to changes in extracellular protein contents thus impacting the mechanical properties of disc tissue. Therefore, it is important to learn the relationship between mechanical and biological behaviors.

Biological effects are especially interesting for intervertebral discs due to their unique form of nutrition exchange. Nutrients are brought into the disc during relaxation periods as fluid flows in and wastes are removed with outflow during active periods. Restriction of motion can be detrimental to disc health due to a decrease in nutrition availability as well as other mechanical and biological effects [10, 15].

It is clear that extreme mechanical exposure can lead to a breakdown of extracellular matrix or cell death, but the effect of moderate abnormal loading has not been determined. By studying disc biomechanical behavior during loading regiments that are within the physiological range one can gain insight into how the biological components will respond. Understanding the mechanical inner workings of intervertebral disc components can lead to insight on the resulting biochemical activity. Various studies have restricted disc motion to observe biological changes. Immobilization of the disc restricts nutrient flow, but abnormal loading at safe levels will maintain fluid flow and test the effects of such loading on disc behavior [1, 3].

1.1.6 Hydration and Load History Effects

As mentioned above, the mechanical behavior of the disc is determined by fiber interactions as well as a pressure gradient. Various studies have been performed to investigate the interrelationship of these mechanical factors [4, 5, 7, 8, 16]. Few studies however have explored the effect of the hydration level and historical loading on the disc [13].

1.2 Studies

The two studies performed seek to improve the understanding of intervertebral disc mechanics. The knowledge gained may lead to preventative therapeutic practices that minimize the risk of degeneration and thus minimize the risk of pain. Fluid flows out of the human disc over the course of the day due to mechanical stresses from gravity, muscle contraction, and spinal motions. The fluid is restored overnight while the disc is allowed to relax. The level of hydration and the height of the disc at a given point in time seem to have an effect on the mechanical behavior. These studies investigate the effect of hydration level on mechanical performance as well as how certain activities affect the hydration level.

1.2.1 Purpose

The nature of intervertebral disc degeneration is that it develops over extended periods of time. The present studies reported observations on subtle differences in behavior during healthy loading conditions. By studying these conditions accordingly we hope to provide insight into this healthy behavior so that precautionary activities can be developed in order to prevent degeneration before symptoms such as back pain or reduced hydration are present. Much like any biological system healthy and appropriate functioning is conducive to long term health [1, 2, 3, 6, 15].

1.2.2 Compressive Conditioning Experiment

The first study aims to adjust the hydration level of the disc through a conditioning phase and then test the effects of varying hydration levels on disc mechanics during an exertion loading period, followed by a recovery phase. The basic real life scenario this study addresses is whether there are relationships between prior mechanical exposure and subsequent load-bearing. For example, is there a range of acceptable hydration levels for heavy exercise, and should light exercise be used to adjust hydration level in preparation for more extreme lifting.

The experimental conditioning phase involves loading the disc in creep compression with varying load magnitude and duration to achieve various hydration levels and disc heights. Once these levels are achieved through application of loads that are normally felt by the disc during various activities, the disc is loaded in creep compression at a load that simulates a considerable strain on the disc while still being under what is considered a normal level. After a substantial amount of time the discs are allowed to relax at the natural resting pressure. The creep curves collected during the loading and relaxation phases are analyzed using mathematical models to determine what trends the model parameters display with regard to the various conditioning parameters. The results found show significantly different behavior during loading especially in regard to adjustments in conditioning phase duration.

1.2.3 Creep Distraction Experiment

The second study poses a different hypothesis, that putting the spine in tension between periods of heavy lifting aids in maintaining mechanical behavior over the course of several exercise periods. This hypothesis is driven by the thought that tension may facilitate the reimbibing of fluid into the disc to restore hydration. This study investigates the effects of distraction as compared to periods of compressive relaxation between loading cycles. Since tension could most reasonable be induced on the disc by suspending a person upside down by his legs, upper body weight percentage was used to calculate a level for applied distraction.

Discs were loaded in compressive creep just like in the conditioning phase of the first experiment with an exertion phase at a higher load after it. Then discs were subjected to either distraction or compressive relaxation, followed by a second exertion phase, a second distraction/relaxation phase, and then a third and final exertion phase. The discs were then allowed to recover in the same way as the first study. The loading phases were compared using the mathematical models to determine what effects distraction has on mechanical behavior. The recovery periods were analyzed as well, though minimal differences were found with regard to recovery. Distraction was shown to maintain certain aspects of mechanical behavior for successive loading phases suggesting that periods of tension aid in the rehydration process.

Chapter 2: Methods

2.1 Media

As described above, fluid flow is a major component of disc mechanical behavior. For this reason *in vitro* mechanical testing of the intervertebral disc must be performed in a liquid medium. Phosphate buffered saline (PBS) is used to maintain disc hydration in the following experiments. The specimens are kept in a bath of PBS since it has salt concentrations similar to those found near the *in vivo* disc. The PBS solution is mixed with protease inhibitors to form a protease inhibitor cocktail, allowing for preserved mechanical behavior. Proteases are enzymes that break down proteins, and a protease inhibitor cocktail is used to disrupt protease function in order to minimize tissue breakdown during testing. Such precautions lead to a more controlled experiment and a more valid model.

2.2 Characterization

Characterization of disc health can be a difficult task. X-ray images can show disc height but other characteristics are tough to distinguish; radiographing a motion segment (a specimen comprised of the disc and its two neighboring vertebral bodies) only gives a good picture of bone locations and does not show any physical features of the disc. X-rays were used in our studies to measure disc height as the distance between vertebral bodies (Figure 3). Magnetic resonance images (MRIs) can show much more detail. Since the disc has such a high water content, MRIs can show many types of irregularities in disc hydration.

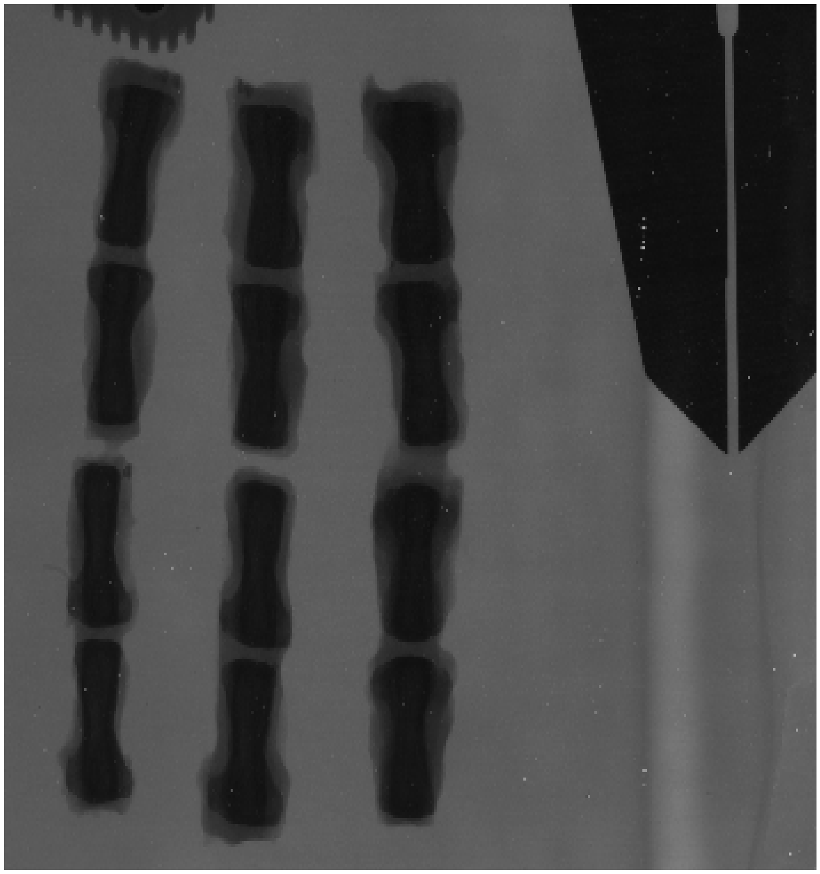


Figure 3: Radiographed Motion Segments and Calipers

The present studies characterize the disc using an indirect method. By modeling creep data with mathematical formulas one can characterize the disc's functional integrity in terms of physically significant parameters. There are several models currently used and our studies evaluate the effectiveness of three of them with regard to understanding specific mechanical processes. It seems that each model provides distinct insight into disc mechanics. One model may give a better picture for different types of mechanical testing or be more suitable depending on the research goals. Inclusion of more than one model can be beneficial in obtaining a more complete understanding of the complex interactions in the intervertebral disc during testing.

2.3 Rat Disc Model

Using an animal model to study disc mechanics can be informative for many reasons. Animal models are more financially convenient and *in vivo* experiments can be performed with animals instead of humans. Such approaches facilitate the generation of hypothesis that can eventually be translated to humans. Rat intervertebral discs have been used since they show similar mechanical and biological structures to human discs. Mouse lumbar discs have been shown to match the nonlinear behavior of human discs [7].

The studies presented use a caudal model because the discs of the tail are more round in shape (unlike humans). This simple geometry allows for straightforward mechanical analyses, and a more accurate and easier method of determining cross sectional area for normalization of forces. The discs of the tail are also more accessible for use in *in vivo* studies, in which biological consequences can be subsequently investigated.

Though the studies presented are performed *in vitro* they are designed to allow for future studies of the biological and biochemical interactions that would take place; related *in vivo* studies could be performed easily on the same level discs. If specimens from the lumbar spine were used, mimicking these studies would be spatially difficult using a live rat. Other studies have used rat caudal models *in vivo* already by fixing or loading a motion segment externally [3, 5, 6, 7, 15, 16].

Though this type of model is used more for the sake of convenience and is not as relevant as a human model, the nature of the study itself is comparative and seeks to pinpoint trends in mechanics due to varying conditions. If significant trends are established their relevance to the humans can be further investigated with *in vivo* animal models as well as cadaver experimentation. Of course a better understanding of the mechanics does not necessarily imply a better understanding of the causes of pain induced or even a full understanding of the degenerative process.

2.4 Preconditioning

In each of the two studies a preconditioning protocol was employed before testing. The specimens used were potted using polymethylmethacrylate (PMMA) bone cement to maintain position in the mechanical testing system. The bone cement was purchased from the Harry J. Bosworth Company under the product name “Fastray”, and was combined at a ratio of 2.5 parts powder to one part liquid to maximizing handling and setting time. This substance forms a hardened structure but does not bind chemically with metal or tissue. For this reason, screws were inserted in the pots to anchor the cement and pins were inserted in the ends of the vertebral bodies to anchor the motion segments in the cement. An image of a potted motion segment can be seen in Figure 4.

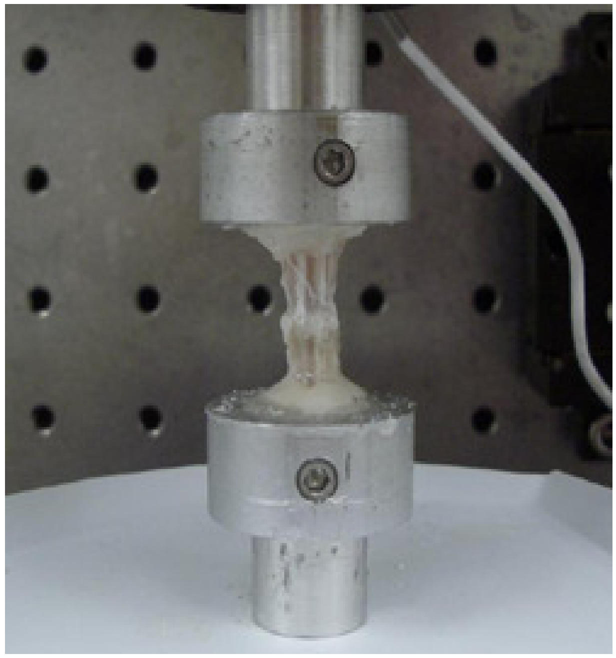


Figure 4: Potted Motion Segment

During polymerization air pockets form and the pins are not fully engaged prior to testing. In order to engage the pins a short preconditioning loading phase is employed which slightly compresses the discs. A component of this phase relaxes the disc to counteract the effects of this compression before testing is started. The procedure for this phase is documented in the articles for the studies.

2.5 Superhydration Consideration

Further preconditioning was considered to minimize the effects of superhydration. Due to the freezing and thawing process used during testing a condition termed “superhydration” has been thought to occur, meaning that the disc has a higher hydration level than at the time of tissue isolation from the animal.

Several studies employ protocols that load the disc in creep or cyclically (depending on the nature of the experiment itself) before testing to remove excess moisture and realign annulus fibers. A formal protocol was not employed in this study to reduce these effects since it is a comparison study and the control was at an identical hydration state. The benefit of such precautions has also not yet been fully validated. Additionally, the conditioning phases employed in both studies involve an extended duration of creep that should minimize superhydration effects before exertion [4].

Further experimentation to explore preconditioning protocols to minimize superhydration is being performed currently in our lab. Though the data observed is inconclusive, creep compression prior to testing seems to aid in maintaining mechanical behavior similar to that of a specimen tested immediately after being excised.

2.6 Protocol Methodology

The external stresses employed in the two studies were derived from the information from the cited MacLean study. For rat discs 1 MPa simulates the effects of 300% of body weight being exerted. This value was used for the exertion phases during experimentation. In order to simulate standing pressures 0.3 MPa was used. This value is between the 0.2-0.4 MPa recorded in the paper as sitting pressure and is close to body weight exertion. 0.5 MPa was used for walking since it is slightly higher while well below the exertion level. The resting pressure simulation is achieved using 0.1 MPa since in MacLean's study this pressure allowed for the disc to achieve initial thickness levels under this level of creep compression. The values chosen are evenly distributed, have physical significance, and resemble stresses employed in other studies [13, 15].

Phase durations were chosen based on pretesting data. As described, conditioning phases were based on the amount of time to reach stress equilibrium where negligible deformation rates were detected. The exertion curves employed 6,000 second durations since this level allowed for more precise model parameter calculations while maintaining healthy levels of deformation. The distraction phases in the tension study employed half of this duration since that was the level necessary to fully extend the disc during tension. Relaxation was performed over 20,000 seconds to allow for maximum understanding of equilibrium behavior without exceeding model calculation memory needs. The durations employed achieved desired calculation convenience while maintaining ample precision and physical significance. The experimental setup used can be seen in Figure 5.

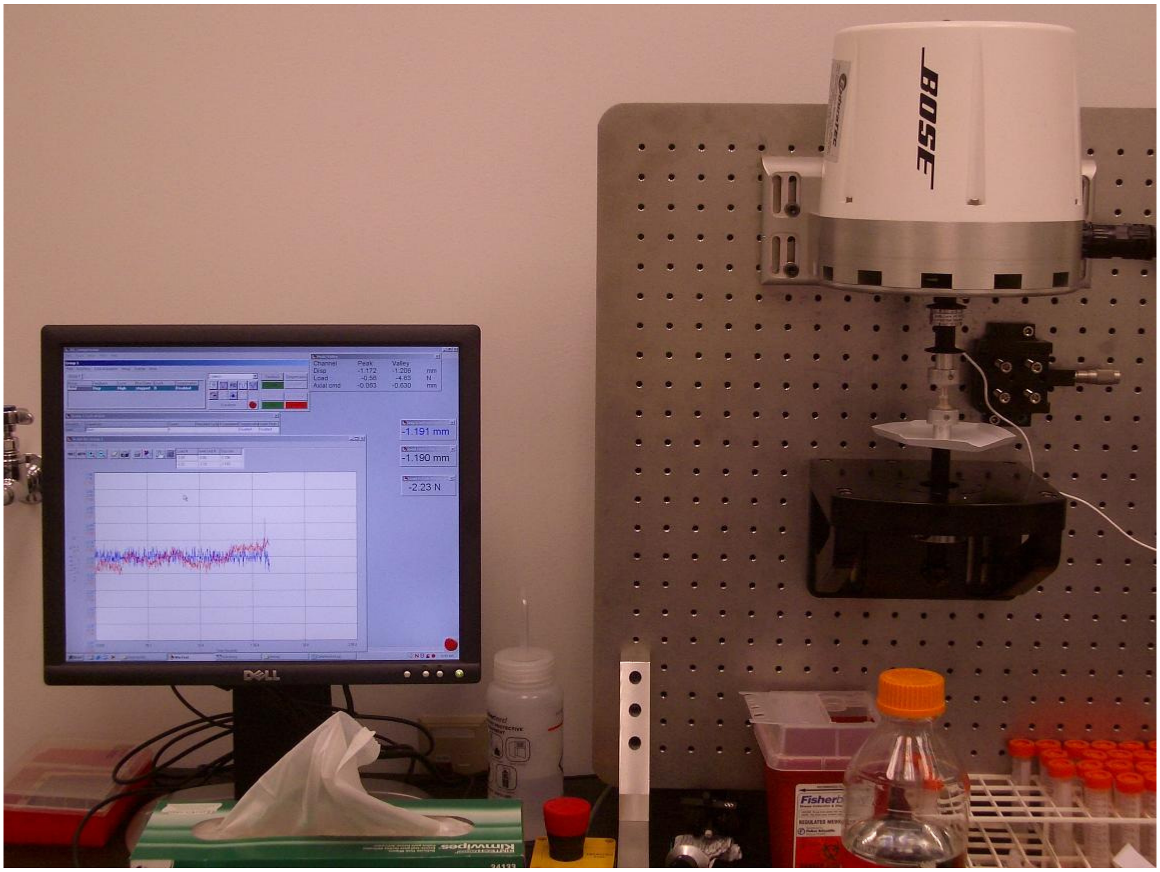


Figure 5: Experimental Setup

2.7 Mathematical Models

Three mathematical models were employed to characterize the mechanical behavior of the disc during loading (the equations used can be found in the methods sections of chapters 3 and 4). Creep data was recorded, normalized, and fit to each model. The model calculations were performed using MATLAB's curve fitting tool on the robust setting. Each model reported three parameters characterizing the behavior of the disc for each creep cycle. These parameters were analyzed to study the effectiveness in describing the behavior for the given studies.

In cases where applied stress is an input for the model equation, relative stress was used, where relative stress is the difference between the stress applied for a creep curve and the applied stress from the previous phase. In the case of the third model the osmotic pressure was assumed to be the same as the external stress from the previous curve since pressure equilibrium was assumed to be achieved. Thus, the same stress differential was employed for σ_o in the second model and σ_o-P_o in the third model. Also, the initial disc height used in the third model was taken from the disc height at the beginning of the current creep curve. The equations used for each model are found in the included articles.

Strains were calculated from the deformations reported and then corrected. The strain was calculated by normalizing with respect to the radiographed disc height, however, the strain recorded by the testing system is believed to be three times that of the actual deformation. As was presented in MacLean's study the deformation imposed on a motion segment is uniformly expressed along vertebral bodies and disc. Thus each vertebral body and the disc itself deforms a third of the amount of the overall deformation of the motion segment itself [7]. For this reason all calculated strain values were divided by three for accuracy.

2.7.1 Stretched Exponential Function

The first model employed is titled the stretched exponential function. It is used to characterize the actual shape of the creep curve itself. The τ and β values from this model are used to characterize its curvature. Studying the effects on curvature for loading after various processes allows one to see the effect these processes have on the behavior of the disc. This model has been used successfully multiple times to show a distinct difference in behavior between damaged discs (ones with degeneration induced by needle puncture or other mechanical stimuli) and healthy discs. In these studies the model was effective in showing these high level changes in behavior. However, it will be shown that in our studies, where the discs used are healthy, that this model is not sensitive enough to detect the more subtle changes in behavior caused by various normal historical loading conditions. The model is effective with regard to cases of detecting significantly altered mechanics but cannot detect differences from normal activity with an appropriate level of sensitivity [7, 17].

2.7.2 Kelvin-Type Standard Solid Linear Viscoelastic Model

The second model employed is the Kelvin-type standard solid linear viscoelastic model. This expression involves modeling a viscoelastic system using a spring and dashpot in series with another spring in parallel with them (depicted in Figure 6). Such a model is commonly used to study the behavior of cartilage and discs, but the physical significance of the behavior of the springs and dashpot is not obvious.

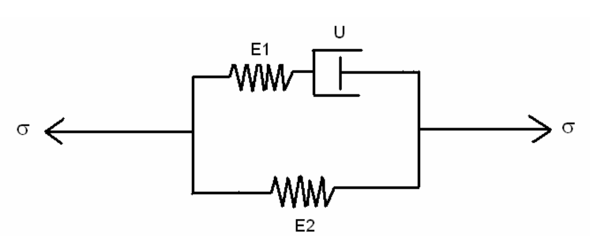


Figure 6: Kelvin-Type Standard Solid Linear Viscoelastic Model

For both studies this expression yielded significant results that had to be carefully analyzed in order to speculate on the physical meaning of the mathematical components with regard to the actual mechanics of the disc. This model was effective in detecting the more subtle differences in behavior characteristic of our studies, but further development is necessary to understand the value of this data. Combined with the third model, the results gained took form into a meaningful perspective on disc behavior during normal load level cycles.

2.7.3 Fluid Transport Model

The third and final model employed is a fluid transport model. As mentioned above the mechanics of the disc are dependant on the intradiscal pressure maintained by hydrophilic particles. The flow of fluid into and out of the disc allows for deformation and bulging of the disc determining its mechanical behavior. The parameters of this model are used to characterize these flow properties while displaying the impact of mechanical fiber interactions. Understanding the interrelationship between the annulus's fibrous structure and the flow of fluid through the disc is important to the understanding of the causes of degeneration [18, 19].

The model itself was designed under the assumption that the intervertebral bodies and cartilage endplates are incompressible. The permeability of the cartilage determines the flow rate of the fluid. The flow rate across the endplate is assumed to be laminar with a linear pressure gradient with homogenous hydrostatic pressure inside the disc. The model used acknowledges a strain and time dependant hydrostatic pressure. The resulting equation characterizes strain as being dependant on permeability, and strain and time dependent behavior [18].

The most telling parameter, the permeability of the disc can be a marker for healthy function. A decrease in permeability has been shown to be found in degenerated discs. The insights gained from the results of the fluid transport model coupled with those of the other two models afford a well rounded picture of the mechanical behavior of the disc during varying activities.

2.8 Statistics

A minimum of six specimens were employed for each group in both studies. This amount was validated using a power analysis employing the calculation:

$$n > 2\left(\frac{\sigma}{\delta}\right)^2 \left(t_{\alpha,\nu} + t_{2(1-P),\nu}\right)^2 \qquad (2.1)$$

Desired σ and δ values were determined from pilot test data (from the parameter for permeability, k, in the fluid transport model since it is the most physically significant). Then t values were assigned based on a guess for sample size n. A new n was calculated using the above equation and then input back in to recalculate the t values and get a new n. This iterative process was employed until a stable n of 4 or 5 was achieved (depending on starting value of n guess an n of 4 or 5 would result). The six-specimen per group amount was chosen as a valid sample size since it is above the calculated need.

The specimens used were excised 6-7 and 8-9 caudal motion segments but the difference in behavior between these levels seem to be negligible. In addition, the effect of disc size difference between spinal levels and rat ages are minimized by stress and deformation normalization. Mean and standard deviation data were calculated using Microsoft Excel and statistical significance was determined through SPSS software t-tests.

Chapter 3: Compressive Load History Effects on Mechanical Behavior of the Intervertebral Disc

3.1 Introduction

Degenerative disc disease is associated with back pain, and can be a debilitating disorder that has tremendous socio-economic impact [1, 2]. The factors that lead to disc degeneration and the mechanisms that link degeneration to pain are not fully understood. In addition to the biological contributions of genetics and aging, mechanical factors have been implicated in accelerating the progression of disc degeneration. Understanding the biomechanical and biochemical interactions may lead to preventative measures that reduce the risk of degeneration. In order to define quantitative relationships between such interactions, the mechanical behavior of the disc must be fully characterized [1, 2, 3, 4, 5].

Although chronic exposure to heavy loading has been shown to induce degenerative changes in animal models [2, 3, 4, 15], few studies have explored the effects of “normal” loading conditions on the intervertebral disc. In rats, low magnitudes of static compression at 0.15 MPa were found to have a stimulatory effect [3], but higher compressive stresses were required to elicit a marked response under short-duration dynamic compression [15]. *In vitro* studies found that long durations of free swelling after rigorous cyclic compression can restore transient disc mechanics [4]. While such uniform loading regimens provide insight into their individual roles in disc mechanics, physiologic spinal loading can span several different regimens and be consecutively applied.

How discs respond mechanically to external loads are dictated by several phenomena. The viscoelasticity of the collagenous annulus fibrosus and fiber-fiber sliding [2, 14, 15] imparts transient permanent deformation to the tissue. Fluid flow through the endplates and annulus fibrosus of the disc contributes to the disc's time dependent behavior, and is governed both by mechanical exposure and nucleus pulposus swelling pressure. Because of the complex relationship between the flow and the mechanical support of the extracellular matrix, the response of two discs to a given load may be dictated by their distinct load histories. One study that investigated this effect examined the role of tissue hydration and loading rate on disc mechanics [13]. They showed that disc stiffness was dependent on hydration level. It remains unknown how the transient mechanics of the disc can be impacted by disc hydration.

To investigate the role of load history on disc mechanics, this study examines an acute load stimulus and its subsequent relaxation response following a conditioning phase at one of several levels of physical activity. Three commonly used mathematical models for describing disc creep [17, 18, 19] were used to characterize mechanical response. We found that both the behavior of discs under a 1 MPa load and the relaxation response depended on a disc's prior load exposure. Importantly, our results suggest that the physical mechanisms involved in supporting compressive loads may be different, depending on the conditioning phase. These findings provide insight into the potential role that load history can have on cell function and on the mechanobiology of the intervertebral disc.

3.2 Methods

3.2.1 Specimen Preparation

Rat caudal motion segments c6-7 and c8-9 were removed from 6-12 month old Sprague-Dawley rats previously sacrificed and frozen. Muscle and tendon tissues surrounding discs were removed, and all specimens were radiographed. Initial disc heights were calculated using ImageJ software to plot and measure pixel intensity changes from the digitally scanned X-ray images. Disc diameters were measured using digital calipers and a 56x dissecting microscope (SZX7 Zoom Stereo Microscope, Olympus, NY). Two perpendicular wires were inserted into vertebral bodies close to the ends opposite the disc. Discs were allowed to free-swell in a protease inhibitor cocktail PBS bath and potted using PMMA bone cement into custom grips attached to a Bose Electroforce materials testing system (LM-1, Bose Corp., MN). All mechanical testing was performed with specimens submerged in the PBS-protease inhibitor bath.

3.2.2 Mechanical Testing

Each motion segment was loaded in creep compression using a four phase regimen involving (1) preconditioning, (2) conditioning, (3) exertion, and (4) relaxation. During the preconditioning phase, an external compressive stress of 0.04 MPa was applied for two seconds to ensure proper seating of the specimen within the bone cement, and then the displacement was held fixed for 500 seconds in order to equilibrate the external and internal stresses of the disc. Stress relaxation to an equilibrium force was confirmed. The conditioning phase was used in order to adjust disc hydration levels prior to exertion and relaxation. Four different stresses were used, each corresponding to some value associated with normal activity levels. Zero MPa was used as an unloaded control level, 0.1 MPa as the resting pressure of a rat intervertebral disc, 0.3 MPa as the stress imposed while standing, and 0.5 MPa approximating walking or light exercise [15]. All applied loads were calculated from the desired stress levels and disc diameters measured, under the assumption that the discs have a circular cross-section. Loads were applied for either 10,000 seconds (full conditioning) or 2,000 seconds (partial conditioning) and six specimens were used for each load and duration (with the exception of the 0 MPa unloaded controls for which only 10,000 seconds was used). These conditioning durations were selected based on pilot experiments that found 10,000 seconds was the duration required for the intradiscal pressure to equilibrate sufficiently with the applied loads, and 2,000 for the disc to attain about half the strain of equilibrium values under the same loads.

The second phase mimicked the effects of heavy exertion on the intervertebral disc. A compression of 1.0 MPa, approximately 3X body weight, was applied for 6,000 seconds. The third and final phase allowed the disc to relax to 0.1 MPa of compression for 20,000 seconds. An illustration of the testing protocol is depicted in Figure 7.

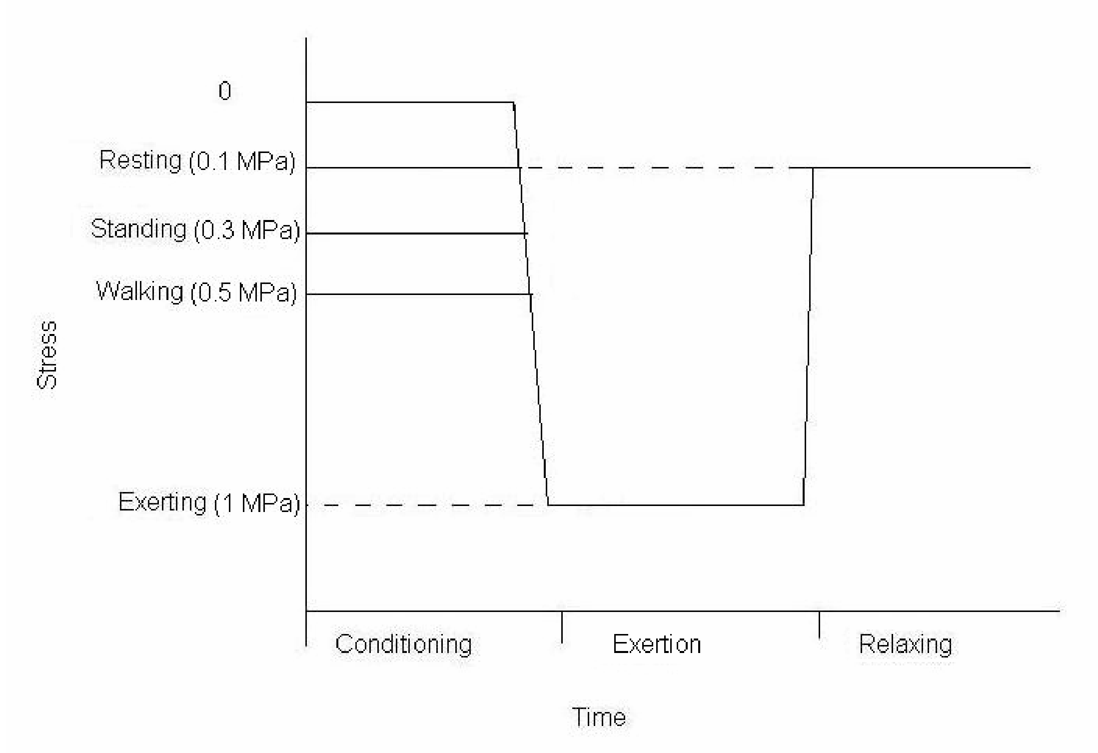


Figure 7: Study 1 Loading Protocol

To ensure a minimum of six data curves per analysis phase, a seventh specimen was added to the 0.5 MPa full conditioning group. This seventh specimen provided an additional data curve to the exertion phase, and replaced a set of missing data from the relaxation phase. Also, three additional specimens were used in the 0.3 MPa full conditioning group to ensure that certain observed behaviors were indicative of loading history effects and not human measurement errors.

3.2.3 Data Analyses

Load, displacement, and time data were collected for the exertion and relaxation phases with a 5 Hz sampling rate. The data was then fit to three different mathematical models that have been previously used to describe transient disc behavior (R^2 values for fit were above .996). Before analyzing the data load and displacement values were converted into stress and strain, respectively, and disc strain was adjusted to be one-third of the overall strain due to the equal strain experienced by each of the vertebral bodies [7]. The first mathematical fitting performed was a stretched exponential model (MOD1) [17], which describes the shape of the creep curve, but which has no mechanistic basis. Change in strain with respect to time for the stretched exponential model is represented as:

$$\varepsilon(t) = \varepsilon_\infty + (\varepsilon_0 - \varepsilon_\infty) \times \exp\left[-\left(\frac{t}{\tau}\right)^\beta\right] \tag{3.1}$$

Where ε_∞ is the equilibrium strain, ε_o is the initial strain, τ is the time constant, and β is the stretch parameter.

We also used a Kelvin-type standard solid linear viscoelastic model (MOD2), based on conceptual framework of a spring-dashpot series in parallel with a second spring. Change in strain with respect to time for the standard linear solid model is represented as:

$$\varepsilon(t) = \sigma_0\left[\frac{1}{E_2} + \left(\frac{1}{E_1 + E_2} - \frac{1}{E_2}\right) \times \exp\left(-\frac{E_1 E_2}{\mu E_1 + \mu E_2} t\right)\right] \tag{3.2}$$

Where σ_0 is the applied creep stress, E_1 is the spring constant in series with the dashpot, μ is the viscous damping coefficient of the dashpot, and E_2 is the parallel spring constant.

The third model is a fluid transport model derived from the transient behavior due to interstitial fluid flux into and out of the disc (MOD3) [18, 19]. While this model does not explicitly account for the interactions among disc subregions, it does provide some insight into the mechanisms of time-dependent behavior. Change in strain with respect to time for the Cassidy model is represented as:

$$\varepsilon(t) = \varepsilon_0 + \left(\frac{\sigma_0 - P_0}{D} - \frac{h_i G}{2kD^2}\right) \times \left[1 - \exp\left(-\frac{2kDt}{h_i}\right)\right] + \frac{G}{D}t \tag{3.3}$$

Where h_i is the starting disc height and ε_o the initial strain for each phase of creep loading; σ_o is the applied increment in creep stress and P_o, is the initial nuclear swelling pressure. The strain-dependence of swelling pressure (D), time-dependence of annular deformation (G), and endplate permeability (k) represent the factors contributing to fluid transport.

Three parameters from each of these models were obtained, using Microsoft Excel (Microsoft Corp., WA) to organize and process the data, and MATLAB (The Mathworks, MA) to perform curve fits using a trust-region algorithm. The parameters were then analyzed in order to interpret differences in the mechanical behavior of the intervertebral disc among treatments for each loading phase.

3.2.4 Statistical Analyses

Based on pilot test data, the necessary sample size was determined using an iterative power analysis. Using the data collected and desirable mean and standard deviation differences the test showed a minimum of four to five samples per group. Therefore, a minimum of six samples was maintained. The calculations can be found in Appendix A.

Results of the above mentioned analyses were then validated statistically with SPSS 14.0 software using one way ANOVA with Fisher's PLSD post-hoc tests, and a critical significance level of $\alpha = 0.05$.

3.3 *Results*

The values obtained from our analyses provide some insight into the behavior of the disc during testing. The final strain achieved during the exertion and relaxation phases of testing showed no differences between any of the different conditioning groups implying that equilibrium strain is a function only of creep load magnitude and not of compression history (Figure 9). This observation is important since maximum strain is one factor that can be discarded in our analyses of differences in model parameter values.

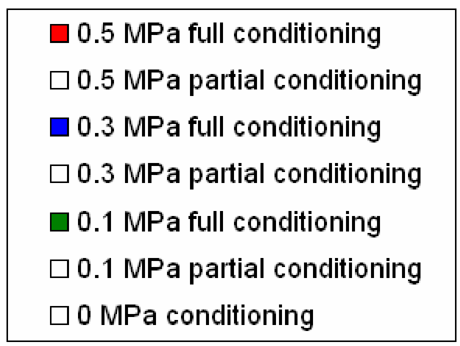


Figure 8: Results Key

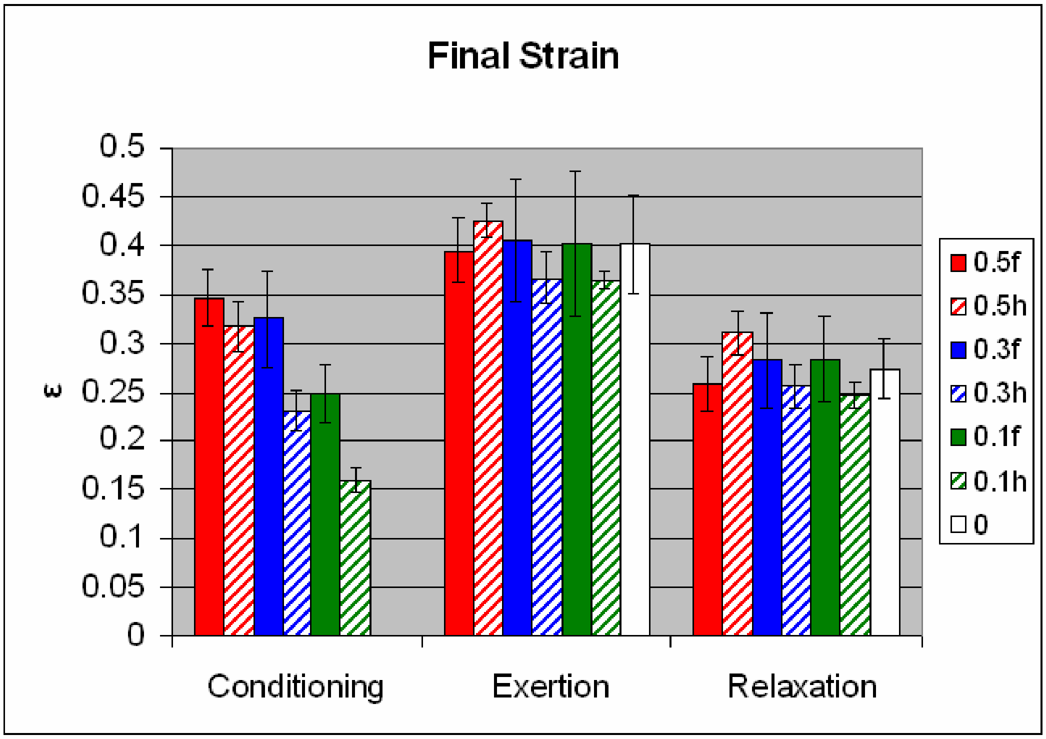


Figure 9: Final Phase Strains

For the β, ε_∞, and τ values of MOD1 there were few statistically significant differences detected by post-hoc tests and no apparent trends in those differences (Figure 10). The low variation between data sets suggests that the behavior of the various specimen groups is similar to each other during both exertion and relaxation. Of the minimal differences present, there appeared to be no correlations that can be made for any of the MOD1 parameters. Thus, the curvatures of the exertion and relaxation curves were unaffected by prior conditioning load regimens.

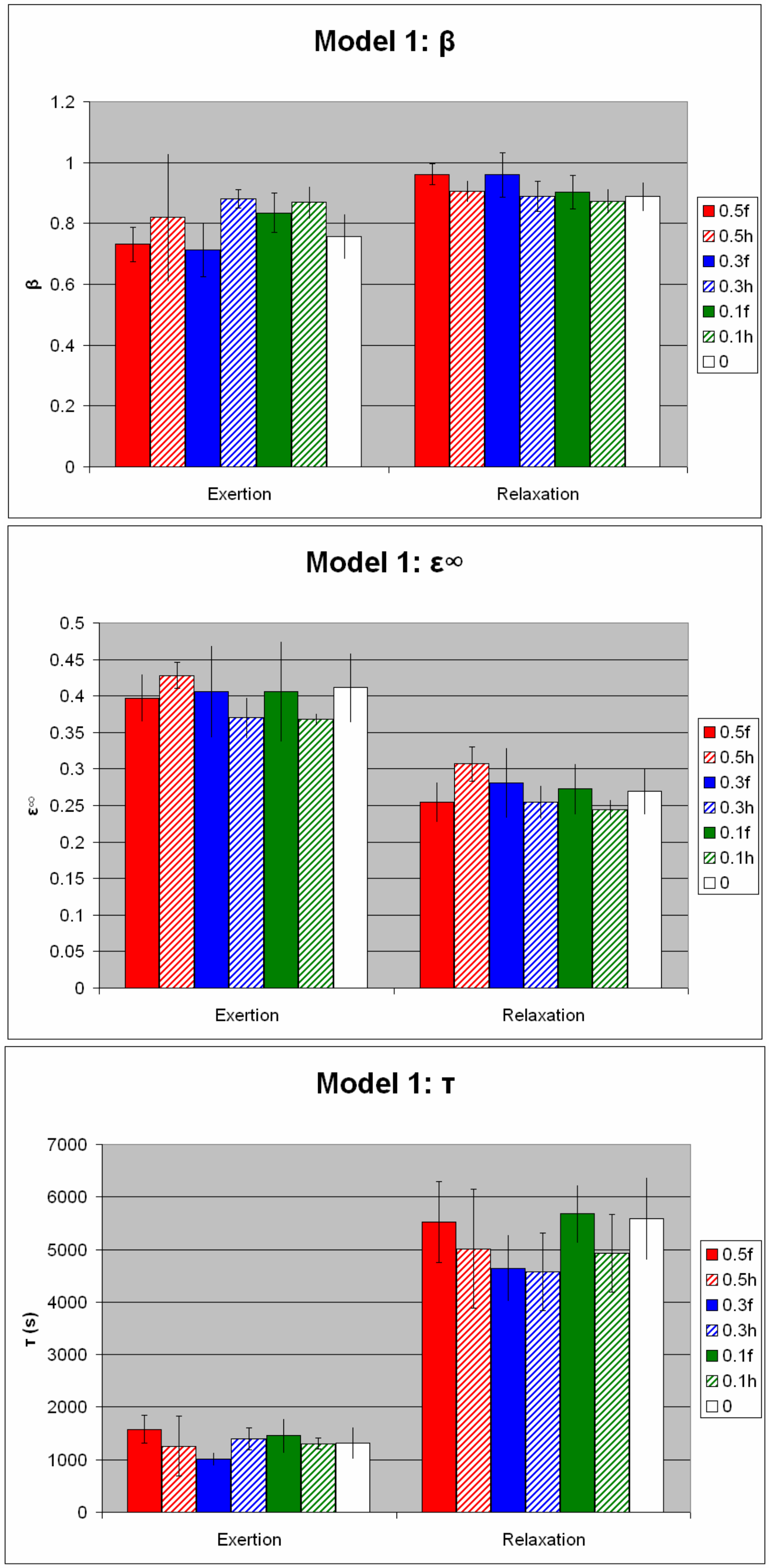


Figure 10: MOD1 Parameters

The parameters for MOD2 exhibited the most significant changes when the conditioning regimen was varied (Figure 11). During the exertion phase the value for E1 decreased with increased conditioning load and duration. E2 and U parameter values behaved similarly, but the differences between groups were less dramatic. The trends identified from these data indicate that all three model parameters during the exertion phase are tightly linked with conditioning-adjusted hydration levels. Just as for MOD1, relaxation phase data for MOD2 parameters were similar among all groups.

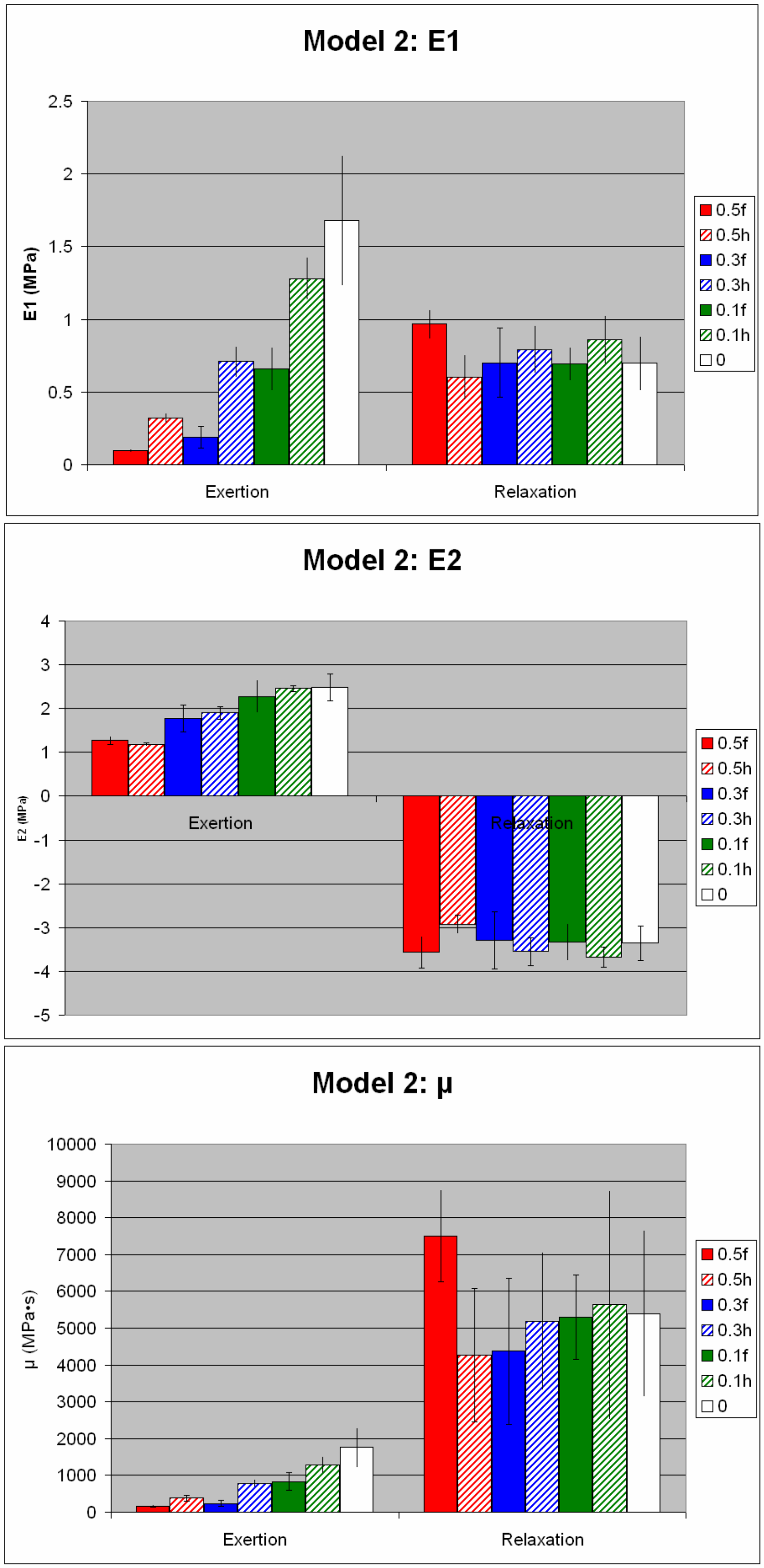


Figure 11: MOD2 Parameters

The fluid transport model, which is the most physically mechanistic of the three, provided the most insightful interpretation of the exertion curve but also demonstrated minimal differences during relaxation (Figure 12). Unlike MOD2, each of the parameters characterizing MOD3 exhibited different behavioral trends for each of the two loading durations. For MOD2 a combination of load magnitude and duration seem to characterize an overall trend during exertion. But for MOD3 the data appeared to fall into two categories, corresponding to the conditioning load durations (2,000 seconds and 10,000 seconds); in addition, the data in these two categories possessed completely different relationships to conditioning load magnitude. For the partial conditioning regimens, D, G, and k consistently showed similar values during exertion, whereas for the full conditioning groups D, G, and k were significantly different and depended on creep conditioning load. Exertion phase D and G values increased with increased conditioning load, while k values decreased.

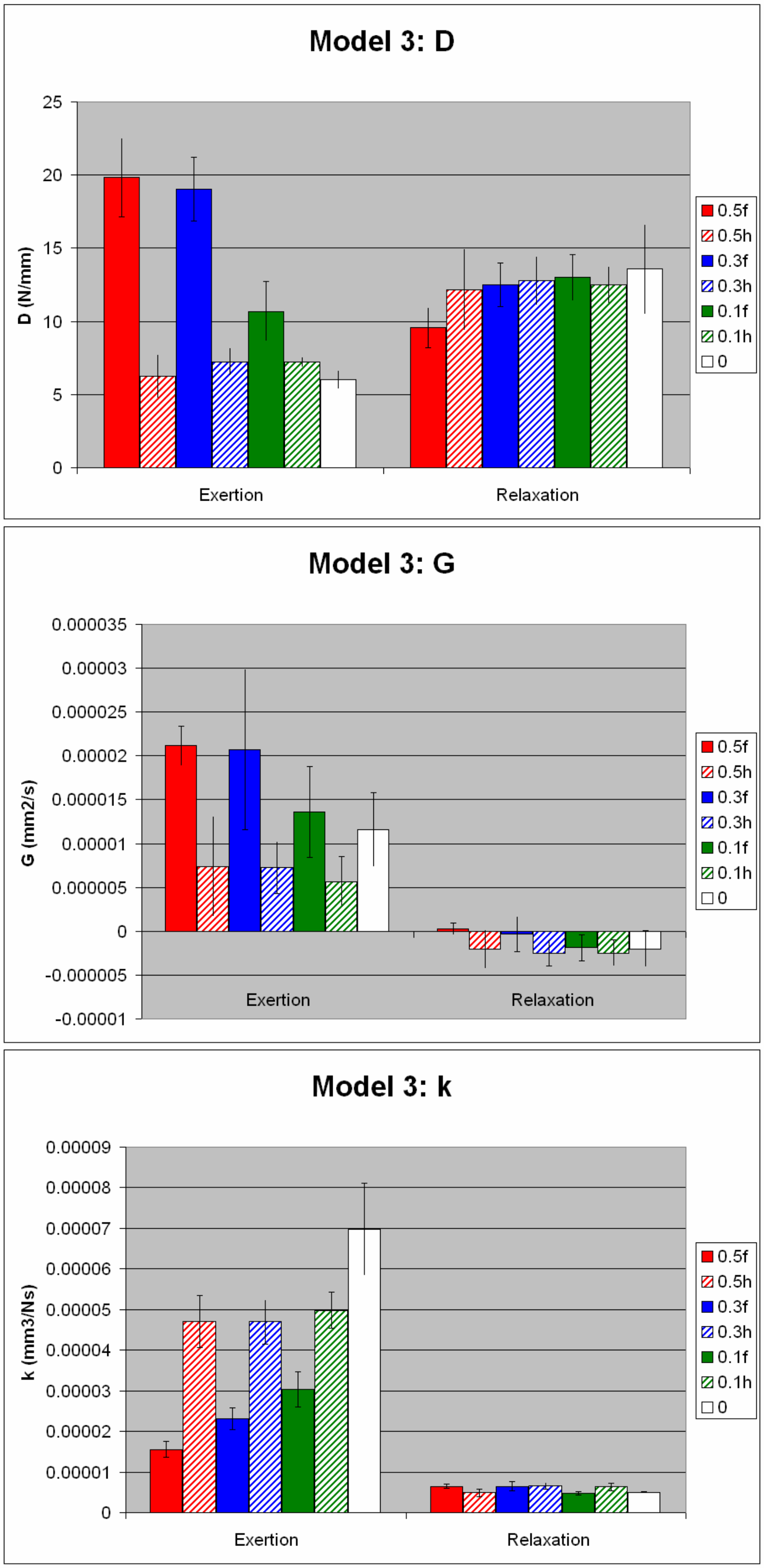


Figure 12: MOD3 Parameters

In addition to specific model trends, there were several overall observations. For many of the parameters, the 0.5 MPa full conditioning level showed significant differences in behavior during relaxation. These relaxation phase differences are present even though in several cases this group behaved similarly to others during conditioning and/or exertion. While minimal trend was observed for the relaxation phase in general, it seems that high levels of compressive conditioning, 0.5 MPa, have a significant impact on the relaxation behavior of the disc.

3.4 Discussion

The intervertebral disc is a complex biomechanical system and performs its mechanical function through two basic physical mechanisms: support of loads by the solid phase of extracellular matrix proteins, notably annular collagen, and the flow of interstitial fluid through the endplates and annulus due to differences between internal and external disc pressures. It is known that these two mechanisms govern disc biomechanics, but characterizing their relative contributions to a healthy disc is difficult [3, 4, 13, 14]. This study examines three models that have been previously been used to describe the disc's transient behavior. Most importantly, analyses found that load history is important in determining the subsequent load-bearing mechanisms of the disc during exertion, and that the exertion load-bearing mechanisms after short durations of loading, regardless of load magnitude, compare favorably with those discs that had no prior loading. Our results provide insight into how these three models together can be used to interpret different aspects of the mechanisms of load support and potentially be used to identify mechanobiologic phenomena.

Because the three models that were used in this study differ in their fundamental bases, distinct conclusions can be drawn from each of them. MOD1 is purely an exponential curve fit that has been used to describe creep behavior. Lack of any significant differences observed for MOD1 parameters among the different conditioning groups demonstrates that the overall creep behavior of the disc is not altered. This model is most appropriate for detecting injury or degeneration, or any other event that compromises the disc's load-bearing function, during which more drastic changes in parameter values occur. However, for our experiments in which healthy discs and physiologic loading conditions are employed, the parameter distinctions are not clear [17].

MOD2, the Kelvin-type standard solid linearly viscoelastic model, exhibits statistically significant differences between conditioning groups during the exertion phase, but it is difficult to interpret how these differences relate to physical mechanisms of disc deformation. Some observations we made, though, are intuitive and indicate that our analyses are valid. For instance, the spring E_1 in series with the dashpot, which partially mediates the instantaneous strain upon load application, is characterized by higher values for partial conditioning than full conditioning. Partial conditioning discs maintain greater water content at the start of the exertion phase, and are, therefore, mechanically stiffer. The second spring E_2 additionally governs long-term stiffness. Regardless of duration of conditioning, both groups undergo the same change in external stress during the exertion phase with small differences in strain. Thus, partial and full conditioning loads of equal magnitude show similar E_2 values during exertion. Because the discs subjected to lower conditioning loads are more hydrated and have deformed less, the energy dissipation parameter μ, which represents both intrinsic and flow-dependent viscoelasticity, is higher for lower conditioning loads. Though MOD2 does not directly describe the physical properties of the disc it gives a useful illustration of the general and time dependant effects of the two basic mechanisms involved as well how they balance and individually impact disc function.

The results of MOD3 were the most insightful in this study since the model is able to attribute specific physical mechanisms to the disc's transient response. Specifically, the three parameters – D, G, and k – explicitly predict the relative contributions of each mechanism to the overall creep behavior. For example, larger values of D indicates a stronger role of nuclear swelling in prolonging creep, while lower values of G and k indicate stronger roles of annular deformation and permeability, respectively. Most importantly, MOD3 demonstrated that partial conditioning, or short duration creep loading, resulted in load-bearing distributions that were very similar to those in discs that had not been subjected to a conditioning load. Specifically, the benchmark that was used for comparison was the group of discs subjected to no conditioning load prior to exertion. These discs exhibited low D, low G, and high k parameters, suggesting that the annulus was the predominant mechanism by which discs resisted creep. The contributions of D and k were both small. Conversely, full conditioning durations resulted in increasing D, increasing G, and decreasing k in a load-dependent manner. These trends indicate that the relative roles of disc subregions in bearing compression are altered, with greater contributions from the nucleus and permeability for limiting creep [18, 19].

One can see from the plot in Figure 9 that the final strain for the conditioning phase of the 0.5 MPa full, 0.5 MPa partial, and 0.3 MPa full conditioning units were similar, as were the 0.3 MPa partial and 0.1 MPa full conditioning units. This indicates firstly that at higher loads, near 0.3-0.5 MPa, the disc achieves relatively similar strains during conditioning. Additionally, one might infer from displacement data that the discs within each of these two subsets have reached similar mechanical stress states. Model parameters obtained for the exertion phase, however, suggest that the physical mechanisms of subsequent load-bearing may actually depend on prior conditioning. This is evident from the different perceived values for nuclear swelling, annular shear, and permeability among conditioning groups.

These significant differences during exertion in the face of similar axial strains may be due to both flow-dependent and intrinsic viscoelastic effects. In terms of flow-dependent effects, the conditioning protocols may differentially alter hydration levels, while producing similar axial strains. Nuclear swelling and strain-dependent permeabilities determine the interstitial fluid flow rate across disc boundaries, and some of the pressurization can be manifest as increased annular bulging. It is also possible that intrinsic viscoelasticity of the collagenous solid matrix maintains both similar hydration levels and similar axial strain across different loading groups. The latter scenario does not seem to be the case, as the changes in flow-dependent viscoelastic parameters from MOD3 were much more impacted by changes in conditioning protocols.

As mentioned above the 0.5 and 0.3 MPa full conditioning groups reached similar strain during the conditioning phase. Since these groups were tested for the same duration and reached similar strain their hydration levels could be assumed to be similar. From exertion phase data one can see that these two groups share similar characteristics except for τ of MOD1 and E_1 and E_2 from MOD2. Since MOD3 is designed to illustrate fluid flow behavior it seems evident that the hydration level achieved in 0.5 and 0.3 MPa full conditioning groups are similar from exertion phase data. These differences suggest areas where collagen fiber interactions play a significant role in disc mechanical behavior. Also during the relaxation phase significant differences are found between these two units.

Across the different models employed one can see a difference in relaxation behavior between the 0.5 MPa full conditioning group and the others. In most cases the other groups show similar data suggesting that above a certain conditioning load threshold relaxation behavior can be altered. Only some of the differences are significant, but in most cases the differences are present. This effect seems to be a result of some sort of change in the mechanical properties of the collagen fiber system of the annulus pulposus. As mentioned above the hydration effects between the 0.5 and 0.3 MPa full conditioning groups seem to be similar implying that this trend should extend into relaxation phase behavior. But, they differ in relaxation behavior supporting the idea of mechanical property changes. Further study should be made to explore the effects of higher loading conditions on relaxation in order to determine if the effects mentioned above are due to some form of plastic deformation to the annulus. However, there does not seem to be signs of extreme loading since the strains achieved during exertion and relaxation phases are normal for the 0.5 MPa full conditioning group as compared to the others.

The results of this experiment raise many questions for further study. As mentioned before, there seems to be specific loading levels and durations that can have a significant impact on future disc behavior. Through further exploration into these levels one can better understand these mechanisms. Also, many assumptions have been made with respect to intradiscal pressure levels throughout the duration of testing based on the mechanical data provided. Insertion of a real time pressure sensor can aid in the understanding of the mechanical relationships that determine disc pressure behavior.

This experiment also approached disc behavior from a purely mechanical viewpoint. By performing animal studies with similar protocols one can observe how the actual intervertebral disc cells are impacted and how they react to these stresses. One can see how the biological factors are related to the mechanical behaviors observed. *In vivo* testing can always add to the understanding of the complexity of biological systems.

Chapter 4: The Effects of Distraction on the Loading and Recovery Behavior of the Intervertebral Disc

4.1 Introduction

The degeneration of the intervertebral disc can lead to debilitating back pain. Exploration of the factors that lead to degradation can lend insight into which part of the degenerative process induces pain and a more comprehensive understanding of the process itself. It is known that mechanical and biochemical factors contribute to the health of the intervertebral disc but their actual role in degeneration is not certain. Studying the mechanics of the intervertebral disc may lead to preventative measures designed to minimize the risk of disc degeneration [1, 2, 3, 5].

Most studies of the influence of mechanical stimuli on the intervertebral disc impose an extreme stress level in order to visualize which types of conditions induce degeneration. However, there are few studies that focus on the behavior of the disc under normal loading conditions to observe the more subtle effects of loading. In humans, degeneration usually takes place over several decades and experiments that induce degeneration traditionally accelerate this process. By exploring the nature of the disc under normal loading conditions one may be able to discover behavioral trends that can result in a healthy or degenerated disc in the future [4].

The mechanical behavior of the disc can be attributed to two basic mechanisms: the mechanical support of disc annulus fibrosus fibers and the pressure potential between the inside and outside of the disc induced by the hydrating effects of nucleus pulposus proteoglycans. In an effort to better understand the effects of both of these factors and their interrelationship, studies have been performed using various mechanical stimuli [7, 13, 14].

Due to the integral impact of fluid motion on the overall mechanical behavior of the disc, flow behavior has been studied in various ways. Since compression causes an outflow of fluid from the disc, it is believed that distraction can allow for improved and accelerated rehydration of the disc. Guehring has shown significant results that demonstrate the restorative powers of disc distraction. Further exploration of the effects of distraction on the mechanical behavior of the disc may lend insight into therapeutic practices to improve disc health and minimize the potential for degeneration [10, 20].

Several mathematical models have been developed to characterize the mechanical behavior of the disc under creep loading conditions. Three of these models are employed and analyzed in this study to gain a comprehensive depiction of the effects of distraction in between loading phases.

4.2 Methods

4.2.1 Specimen Preparation

Specimens were prepared as previously described. Briefly, six c6-7 and six c8-9 rat caudal motion segments were isolated from 7-9 month old Sprague-Dawley rats previously sacrificed and frozen. Soft tissues surrounding discs were removed, and all specimens were radiographed. Initial disc heights were computed from image analysis of radiographs, and disc diameters were measured using digital calipers.

Two perpendicular wires were inserted into vertebral bodies close to the ends opposite the disc, and discs were allowed to free-swell in a PBS bath containing protease inhibitors. Specimens were potted into custom grips attached to a Bose Electroforce materials testing system (LM-1, Bose Corp., MN). All mechanical testing was performed with specimens submerged in the PBS-protease inhibitor bath.

4.2.2 Mechanical Testing

Each motion segment was loaded in creep through an eight phase regimen: (1) Preloading, (2) Conditioning, (3) Exertion I, (4) Relaxation/Tension I, (5) Exertion II, (6) Relaxation/Tension II, (7) Exertion III, and (8) Recovery. All applied loads were calculated from the desired stress levels and disc diameters measured, under the assumption that the discs have a circular cross-section. The six c6-7 and six c8-9 specimens were divided equally into two groups according to the loads experienced in Phases 4 and 6: one receiving relaxation loads, and the other receiving tensional loads.

During the preloading phase, a nominal compressive stress of 0.04 MPa was applied for two seconds to ensure proper seating of the specimen within the bone cement, and then the displacement was held fixed for 500 seconds in order to equilibrate the external and internal stresses of the disc. Stress relaxation to an equilibrium force was confirmed.

The conditioning phase was used in order to adjust disc hydration levels consistent with resting pressures prior to exertion and relaxation. Resting pressures of rat intervertebral discs were estimated to be 0.1 MPa [15]. Our prior studies found that standardizing the conditioning load to prime the hydration level is important for repeatability of subsequent mechanical behavior. Conditioning loads were applied for 10,000 seconds based on pilot experiments that found 10,000 seconds was the duration required for discs to reach equilibrium under creep compression.

The exertion phases were identical and mimicked the effects of heavy loading on the intervertebral disc. A compression of 1.0 MPa, approximately 3x body weight, was applied for 6,000 seconds. The relaxation/tension phases involved either a return to the resting stress of 0.1 MPa compression or a distraction (tension) load at 0.1 MPa corresponding to 0.6x body weight. The distraction load of 0.6x body weight was selected based on estimates that naturally occurring tension can occur through an inverted posture with a weight distribution of 60% body mass in the upper body (from the hips up) [21].

The eighth and final phase involved all discs returning to the resting stress (0.1 MPa compression) for 20,000 seconds. An illustration of the testing protocol is depicted in Figure 13.

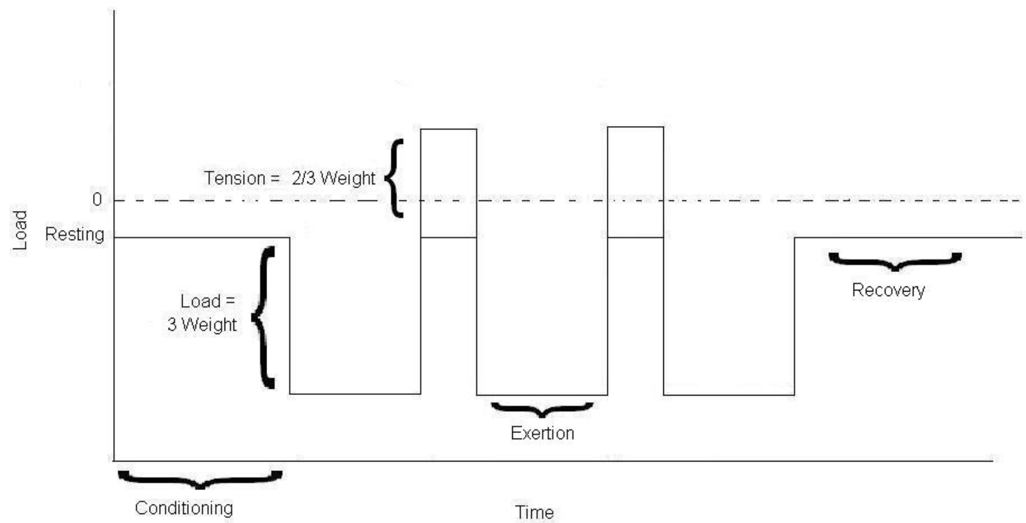


Figure 13: Study 2 Loading Protocol

4.2.3 Data Analyses

Load, displacement, and time data were collected for three exertion phases and recovery phase with a 5 Hz sampling rate. As had been done previously, load and displacement values were converted into stress and strain, respectively, and disc strain was adjusted to be one-third of the overall strain [7]. The data were then fit to three different mathematical models that had been used to describe transient disc behavior (with a minimum R^2 value of 0.9945). The first is a stretched exponential model (MOD1) [17], which has no mechanistic basis:

$$\varepsilon(t) = \varepsilon_\infty + (\varepsilon_0 - \varepsilon_\infty) \times \exp\left[-\left(\frac{t}{\tau}\right)^\beta\right] \tag{4.1}$$

Where ε_∞ is the equilibrium strain, ε_o is the initial strain, τ is the time constant, and β is the stretch parameter.

The second is a Kelvin-type standard solid linear viscoelastic model (MOD2), based on conceptual framework of a spring-dashpot series in parallel with a second spring:

$$\varepsilon(t)=\sigma_0\left[\frac{1}{E_2}+\left(\frac{1}{E_1+E_2}-\frac{1}{E_2}\right)\times\exp\left(-\frac{E_1E_2}{\mu E_1+\mu E_2}t\right)\right] \tag{4.2}$$

Where σ_0 is the applied creep stress, E_1 is the spring constant in series with the dashpot, μ is the viscous damping coefficient of the dashpot, and E_2 is the parallel spring constant.

The third is a fluid transport model derived from the transient behavior due to interstitial fluid flux into and out of the disc (MOD3) [18, 19]:

$$\varepsilon(t)=\varepsilon_0+\left(\frac{\sigma_0-P_0}{D}-\frac{h_iG}{2kD^2}\right)\times\left[1-\exp\left(-\frac{2kDt}{h_i}\right)\right]+\frac{G}{D}t \tag{4.3}$$

Where h_i is the starting disc height and ε_o the initial strain for each phase of creep loading; σ_o is the applied increment in creep stress and P_o, is the initial nuclear swelling pressure. The strain-dependence of swelling pressure (D), time-dependence of annular deformation (G), and endplate permeability (k) represent the factors contributing to fluid transport.

Three parameters from each of these models were obtained, using Microsoft Excel (Microsoft Corp., WA) to organize and process the data, and MATLAB (The Mathworks, MA) to perform curve fits using a trust-region algorithm. The parameters were then analyzed in order to interpret differences in the mechanical behavior of the intervertebral disc among treatments for each relaxation phase. Data were compared across exertion phases and between the two groups.

4.2.4 Statistical Analyses

Based on pilot test data, the necessary sample size was determined using an iterative power analysis. Using the data collected and desirable mean and standard deviation differences the test showed a minimum of four to five samples per group. Therefore, a minimum of six samples was maintained.

Results of the above mentioned analyses were then validated statistically with SPSS 14.0 software using one way ANOVA with Fisher's PLSD post-hoc tests, and a critical significance level of $\alpha = 0.05$ for differences and 0.95 for similarities.

4.3 Results

Overall, the values obtained from analyses showed that application of tension affects specific aspects of disc mechanics when subjected to subsequent exertion loads. As we found previously in a separate study, the final strain achieved during a 1 MPa exertion load and after recovery phases of testing was identical regardless of prior load exposure, indicating that equilibrium strain is minimally affected by load history (Figure 14).

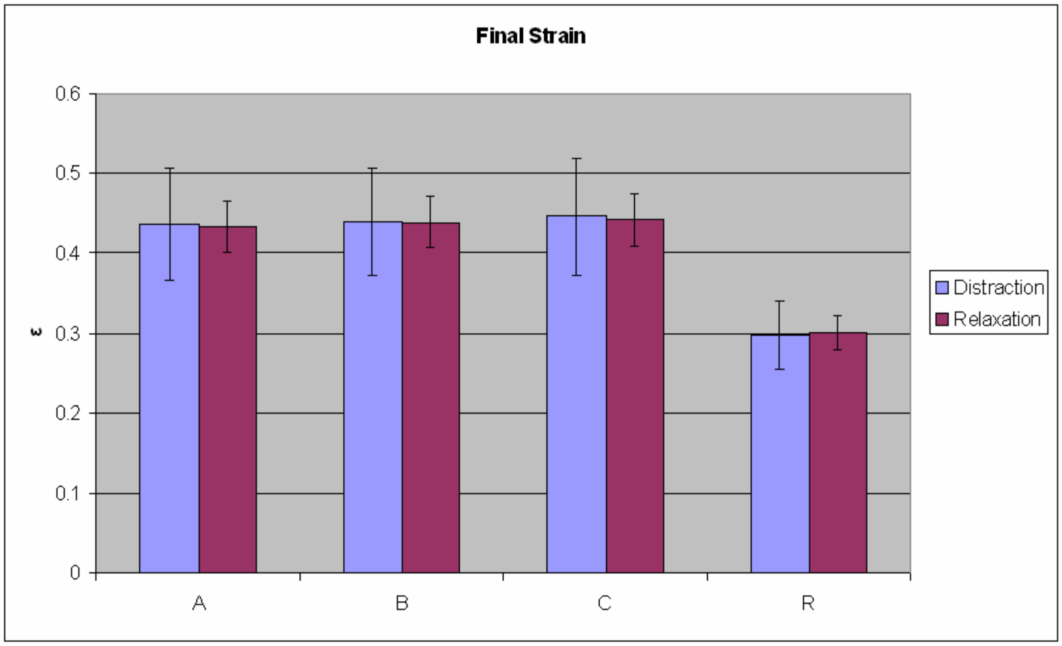


Figure 14: Final Phase Strains

For MOD1 the β parameter was the only one to possess significant differences between distracted and non-distracted discs (Figure 15). In agreement with strain measurements, d_∞ values were the same across exertion phases and between groups. Likewise, τ also did not exhibit any dependence on tension or repetition of loading. In the case of β, however, the distracted group maintained values with repetitive exertion loading, but values for the non-distraction group decreased dramatically over the last two exertion phases. This difference is accentuated when the data is plotted as change in β for the second and third exertion phases relative to that of the first (Figure 16).

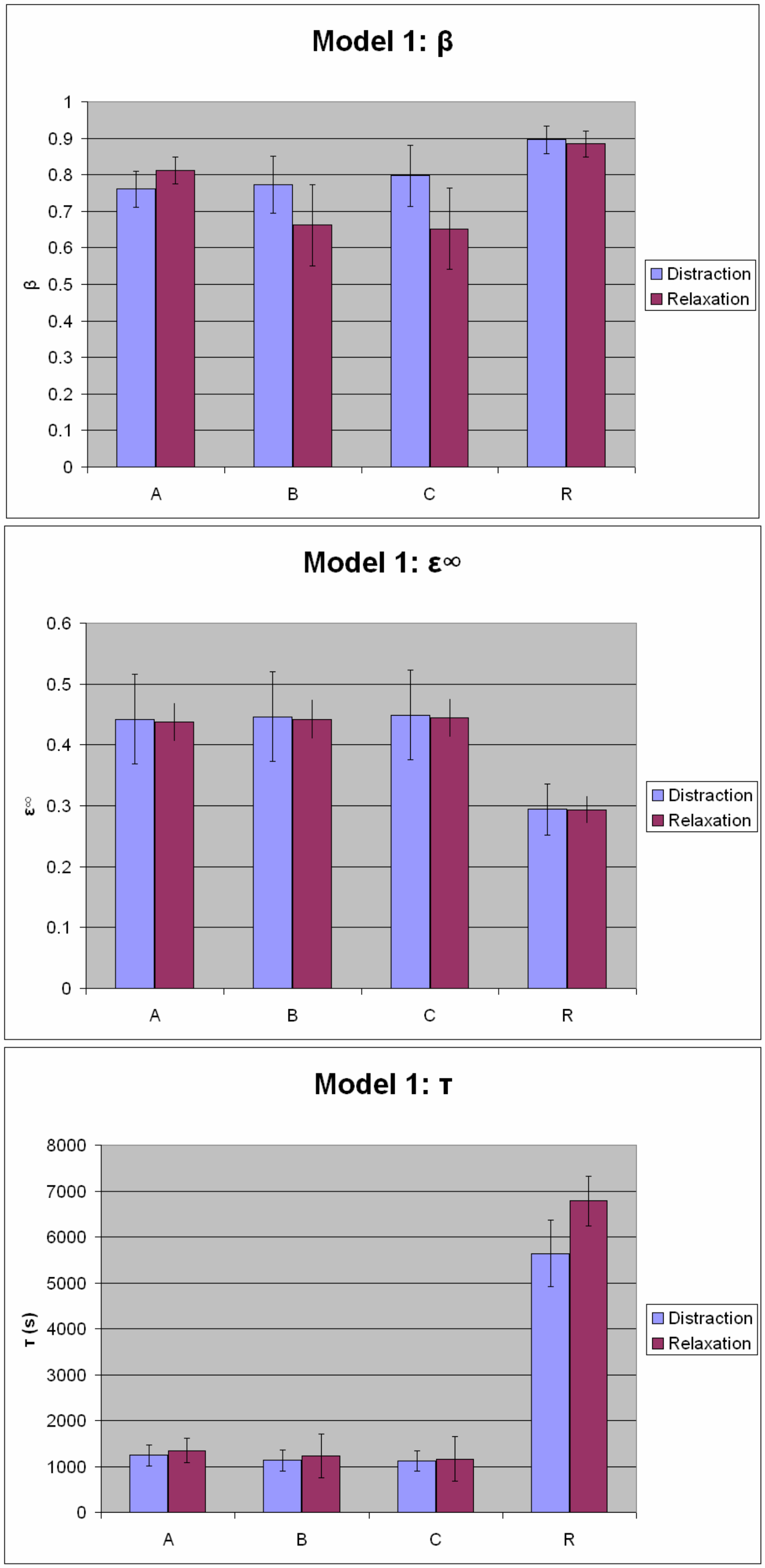


Figure 15: MOD1 Parameters

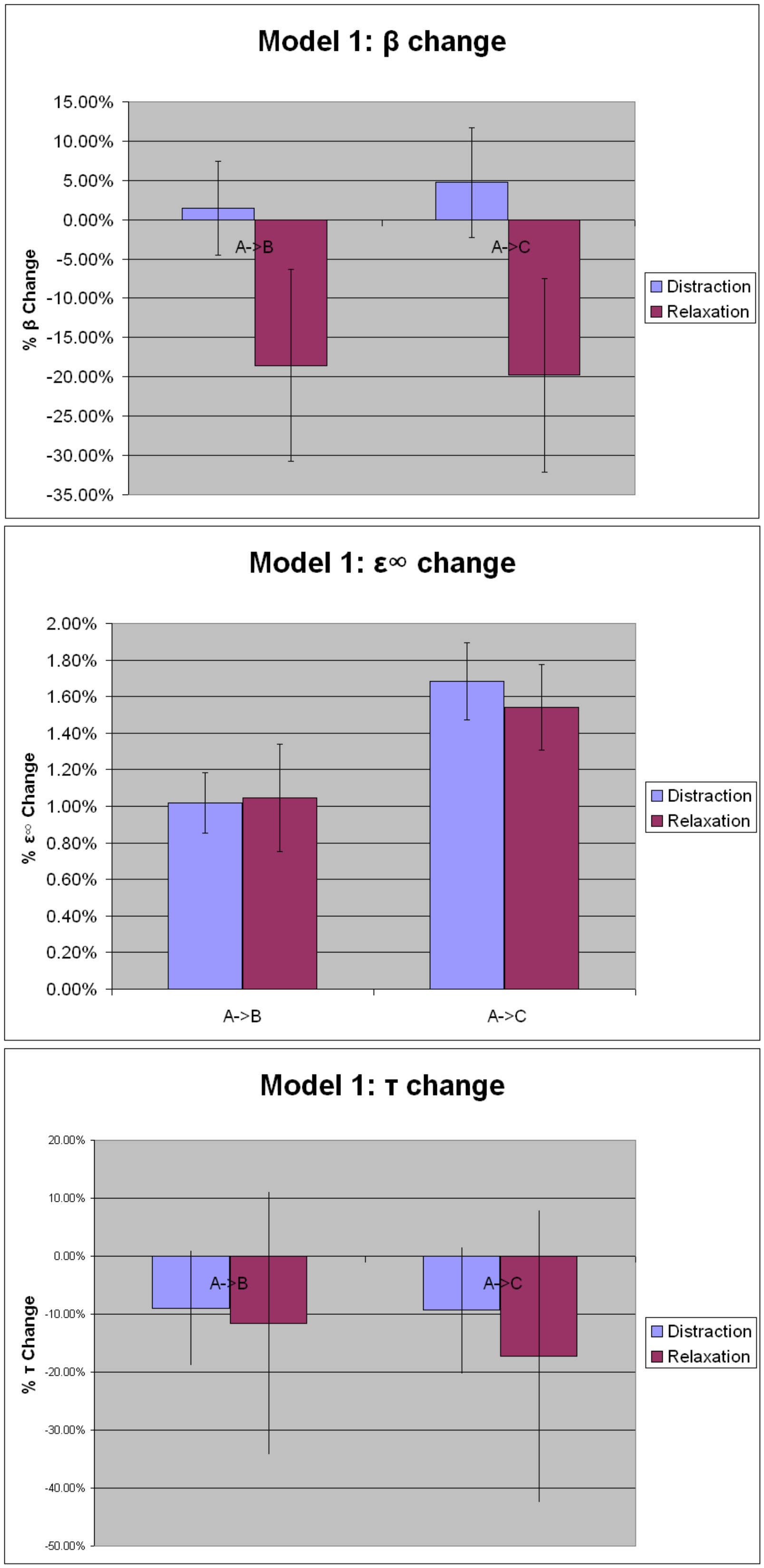


Figure 16: MOD1 Parameter Differences

For the second model, distraction led to a slight increase in stiffness parameter E_1 for the second and third exertion, while relaxation to resting pressures resulted in significant decreases in E_1 during the latter two exertion phases. There was a trend of increasing E_2 values with exertion cycles for the distraction group, and no change for those of the non-distraction group. Distraction allowed the value of viscosity, μ, to be maintained with each exertion load, but discs that were not placed in distraction exhibited decreasing μ values with subsequent load cycles. This model demonstrates that both the elastic and viscous contributions to disc mechanics are influenced by tension prior to loading (Figure 17).

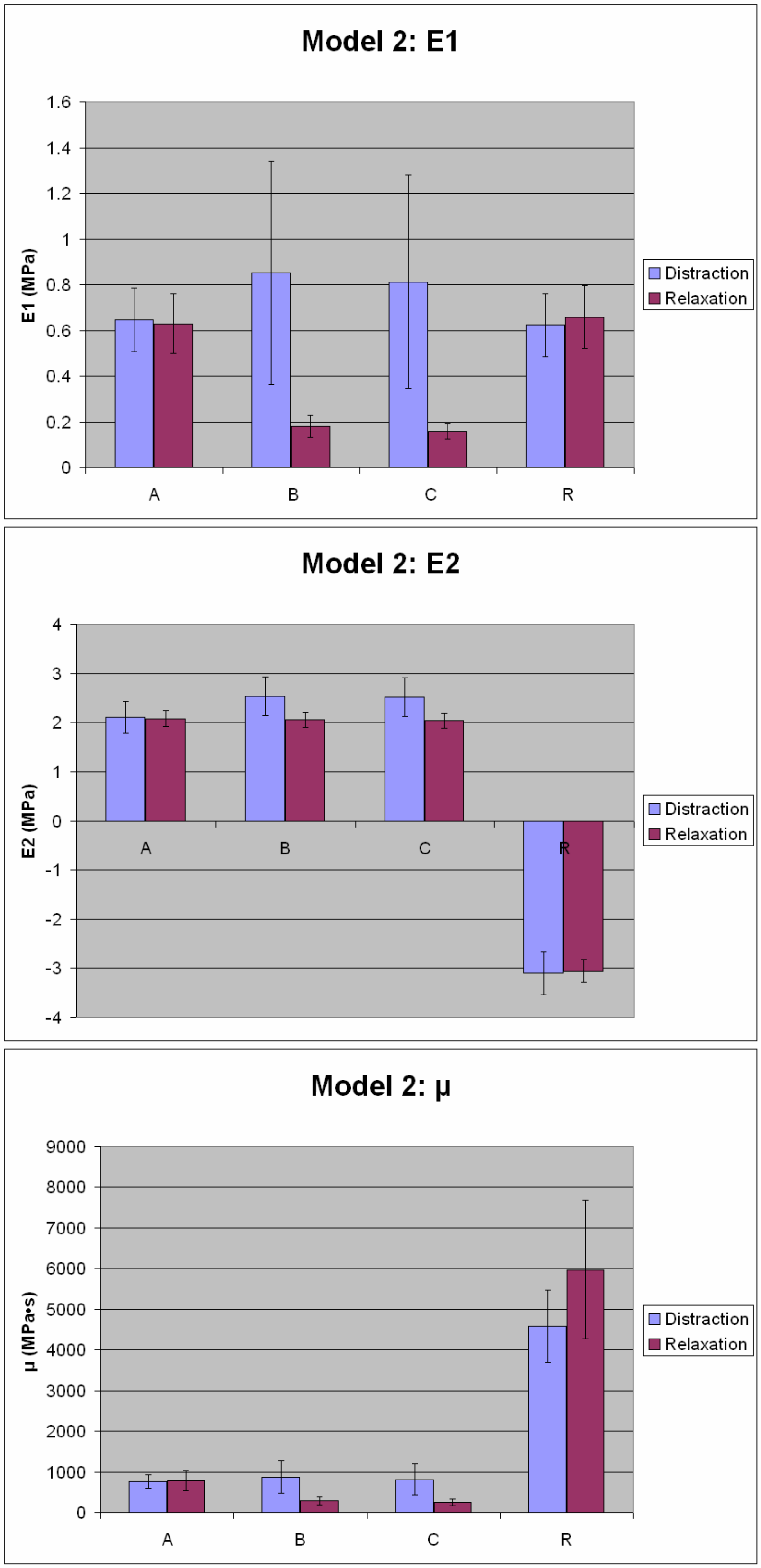


Figure 17: MOD2 Parameters

In MOD3, the distraction group had fairly stable D values across exertion phases. However, the relaxation group had a dramatic initial increase in D followed by a smaller while significant, increase in the third phase. This indicates a much greater role of nucleus pulposus swelling to transient behavior. The G parameter for the distraction group did not change significantly across exertion phases but did increase in variability. The relaxation group's G value increased significantly with repetitive exertion loading, indicating a less impact by the annulus in governing transient behavior. Distraction resulted in small increases in the permeability parameter, k, with loading cycles, whereas the relaxation group possessed lower permeabilities. For all MOD3 parameters significant changes were observed (Figure 18).

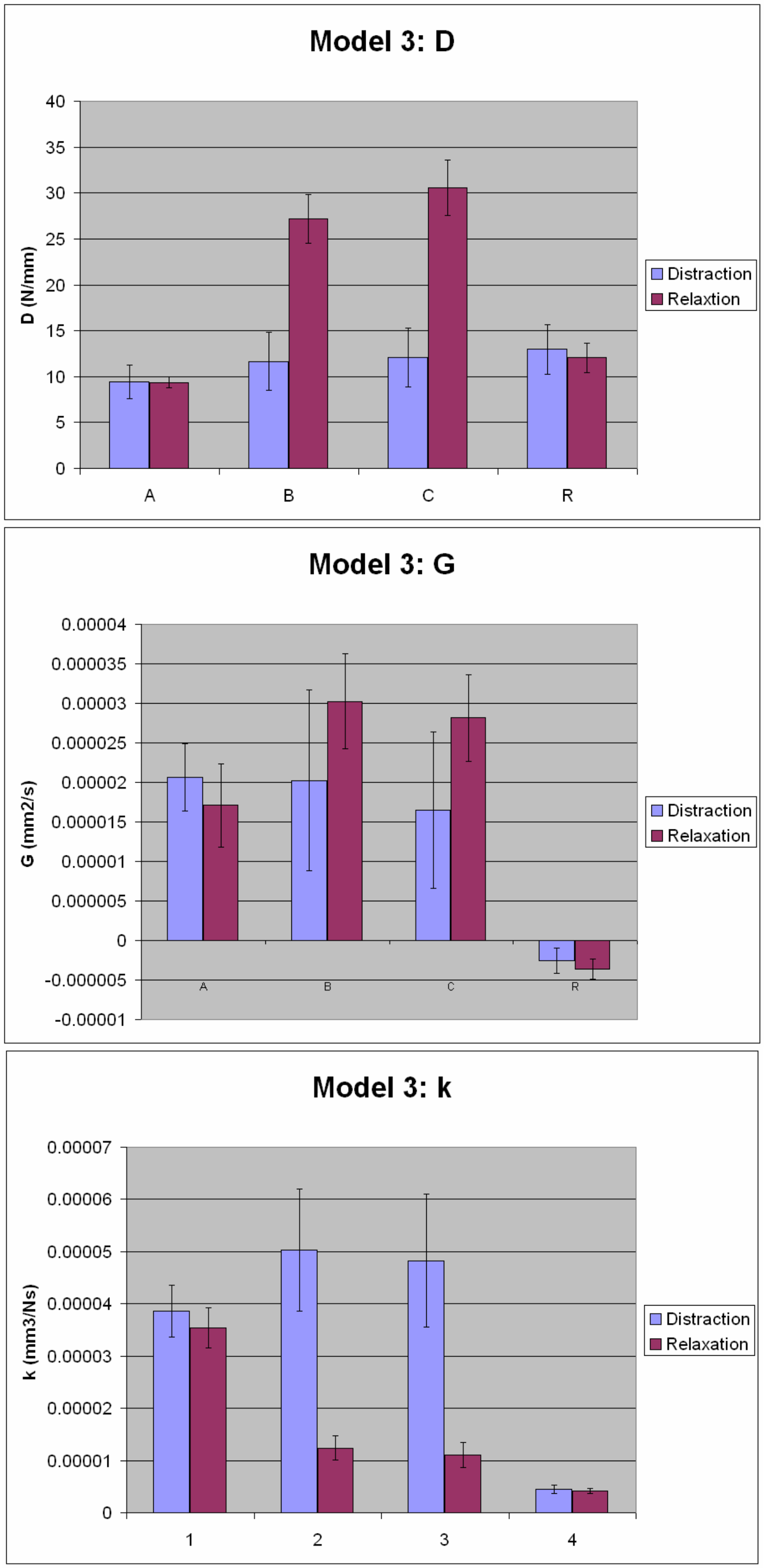


Figure 18: MOD3 Parameters

The final recovery phase showed no significant differences between relaxation to resting pressure and distraction across all model parameters except for in the case of τ of MOD1. These results demonstrate that though distraction affects viscoelastic behavior during subsequent exertion phases, recovery behavior from exertion loading remains virtually unchanged.

4.4 Discussion

Disc distraction has been shown to aid in disc rehydration observed through MRI, an increase in extracellular matrix gene expression, and a higher concentration of protein expressing cells [10]. Dynamic distraction has also been shown to aid in the remodeling of disc tissue through an increase in nutrition flow to the disc [20]. Such studies illustrate the impact of distraction on biological factors and the MRIs provided gave a clear picture of resulting disc hydration. However, the models presented in our study provide an overall picture of the effects distraction can cause on disc biomechanics. Each model provides different but important information regarding behavior.

The first model used provides insight into the creep curves that characterize loading. The distracted group shows stable behavior across exertion phases for all parameters suggesting that distraction aids in maintaining mechanics for subsequent loading. However, the resting groups seem to have maintained behavior for all parameters except for β, showing that there is a slight change in curvature between loading phases. Very little behavioral differences are detected through this model since it is less sensitive and more useful in expressing signs of severely reduced mechanical properties as compared to the more subtle differences explored in this study [17].

Distraction slightly increases spring constant values, E_1 and E_2, for exertion in MOD2 and maintains the value of the damping coefficient, μ. Relaxation behavior is only maintained for the E_2 parameter during exertion. The increased value of the spring constants for the exertion phases implies an increase in disc stiffness. This increase in stiffness supports the notion that greater disc hydration is achieved from a short period of distraction, even as compared to the post-conditioning load. A reduction in values for the relaxation groups seems to show that rehydration is not achieved as rapidly and that mechanical support properties are lost over time due to loading.

The third parameter shows the most physically significant results. The three parameters – D, G, and k – explicitly predict the relative contributions of each mechanism to the overall creep behavior. Larger values of D indicates a stronger role of strain to nuclear swelling, while lower values of G and k indicate stronger roles of annular deformation and permeability, respectively. There is a large and significant change in D values across exertion curves for the resting group while the distraction group shows a much smaller shift. This suggests that distraction aids the disc in maintaining nuclear swelling levels due to deformation. The G value is also better maintained by the distraction group suggesting that the annulus has recovered deformations prior to each exertion phase, whereas resting phases result in increased deformation effects across exertion creep. Permeability, k, is also increased by distraction as compared to relaxation. Since compression of the disc squeezes endplate pores and reduces permeability it seems clear that distraction maintains pore size and most likely aids in restoration of deformation effects before each exertion phase [18, 19].

It is interesting to note that in most cases where distraction or relaxation results in different behavior between the first two exertion phases, that the behavior of the second exertion is mimicked in the third exertion phase; the change in behavior between the first and second exertion curves is not observed between the second and third.

The τ value is the only parameter that shows a significant change in recovery behavior between distraction and relaxation groups. Though the τ value, which characterizes the curvature of the creep during this period, shows a change in creep behavior, little physical significance can be attributed by the other models with respect to such a change. Further study may be able to determine the source of this discrepancy in recovery behavior characterization. It may be possible that annulus fiber sliding effects are restored through distraction. A change in collagen fiber behavior may not be easily observed through MOD3, since it is designed to specifically characterize fluid flow behavior.

Overall, distraction seems to maintain mechanical behavior across exertion phases as observed through all three models. These results agree with the findings of other studies regarding the therapeutic effects of distraction. The restorative potential of distraction has already been observed in other studies. Continued experimentation may lead to the discovery of therapeutic distraction procedures that minimize the risk of degeneration [10, 20].

Chapter 5: Conclusions

The intervertebral disc is made of a complex structure. The bulging of the annulus fibrosus results in tensional loads supported through a network of fibers, primarily collagen. And fluid flow into and out of the nucleus pulposus regulates internal pressures. The healthy behavior of these two mechanisms is imperative to proper disc functioning. Careful experimentation can contribute insight as to the contributions of each and their combination to overall disc function and behavior. The models employed in the two studies contribute different perspectives on this behavior allowing for a well rounded approach in determining the contributions of both fiber interactions and nucleus pressurization.

5.1 Results

5.1.1 Validity of Results

One can see from reported strain data that the variability within groups is relatively low. This observation supports the idea that disc width and height measurements were performed with a sufficient level of accuracy. Superhydration effects along with age and disc location differences seem to have had a minimal impact on variability. This level of precision was present, though a significant level of variability is characteristic of any biological tissue.

5.1.2 Disc Height Recovery

Though the parameters studied show a maintained biomechanical behavior during testing for certain protocols it should be noted that relaxation never fully restored disc height. This may be due to some level of cellular or structural degradation. Disc height was shown to recover after prolonged recovery by Johannessen [4, 14]. However in our studies, the trend of the creep curve does not seem to be approaching initial height at the necessary rate. It seems that *in vitro* studies face the challenge of maintaining mechanical conditions throughout testing. One should also keep in mind various biomechanical influences that act upon disc behavior *in vivo*, such as the impact of intra-abdominal pressure from respiration on disc height recovery. Such *in vivo* conditions are rarely accounted for during *in vitro* testing.

Fluid flow in and out of the disc is mostly time dependant and so recovery may take a specific amount of time regardless of loading magnitude or duration. *In vitro* testing may require long recovery periods for short loading durations, because relaxation time may not depend on loading time; full height recovery may require the same amount of time for short *in vitro* testing as the long term *in vivo* case. Further testing should investigate whether recovery time is dependant on and proportionate to loading conditions or simply a set value for the hydration levels achieved.

5.2 Discussion

Disc degeneration has been shown to be induced experimentally through compression and thus it is clear that certain mechanical effects are beneficial while others are damaging to disc health [3, 4, 15]. The two studies presented show a strong connection between hydration level and disc behavior. They show how behavior can be altered by compression through load magnitude as well as duration.

The studies also demonstrate how tensional loading can aid in the rehydration rate allowing for maintained biomechanical properties.

5.2.1 Model Validation

It has been shown that the first model, the stretched exponential function, gives a specific insight into creep behavior. It gives the simplest interpretation of the data, but allows for an overall picture of creep curvature. This curvature describes how quickly and to what extent the disc will deform under given loads which can be the first hint towards differing mechanical behavior. Such differences in curvature are usually more characteristic of disc damage.

During degeneration or injury, disc mechanics are greatly affected causing a shift in time dependant behavior. As expected in the present studies, minimal differences were found between creep curvatures despite varying loading conditions since the protocols were designed to load discs under relatively normal conditions. Due to the fact that discs were compressed to extreme levels characteristic of damage or degeneration, curvature differences were not expected nor did they result through experimentation.

This model can be insightful for experiments where the disc has undergone some sort of mechanical process and the user would like to test for significant damage that leads to reduced mechanical properties. These experiments are common to the study of disc degeneration; however, the present studies seek to determine behavioral differences during healthy procedures [17].

The second model employed, though it holds no inherent physical meaning, has shown insight into disc behavior for our studies. Initial hydration was shown to be comparative between groups through parameter values; a stiffer E_1 spring constant implies increased initial disc hydration whereas a larger E_2 implies maintained stiffness. Of course the damping coefficient, μ, is higher for increased hydration. Thus, this model proved to be insightful with regard to determination of relative disc hydration level as well as the impact hydration had on mechanical behavior during experimentation.

As characterized by a somewhat linear trend for E_2 and a somewhat exponential trend for E_1, the second model demonstrates reduced disc stiffness with an increase in conditioning load and duration. The damping factor is also characterized by a fairly linear behavior showing an increase in damping with a decrease in conditioning load magnitude and duration. The results during exertion are significant across most data sets.

As mentioned, distraction led to an increase in disc stiffness whereas relaxation resulted in a decrease in initial stiffness and maintained long-term stiffness. This can be attributed to the extra hydration acquired through distraction. Along the same lines, distraction proved to significantly maintain damping behavior as compared to the case of relaxation. Disc behavior seems restored to pre-exertion conditions in terms of fiber alignment as well as hydration level through distraction as compared to relaxation.

The third model proved to be the most physically significant since it was designed with disc mechanics in mind. Specific mechanisms are attributed to the individual parameters. The significance of these parameters allowed for the comparison between testing groups to describe differences in behavior in a way that illustrated the physical changes that had taken place within the disc. This model is powerful and led to various insights on the subtle effects of historical loads and distraction with regard to future disc behavior [18, 19].

Overall, the third model showed a constant relationship across all three parameters during exertion for the groups under short term conditioning duration. A linear trend was found during exertion for the longer duration groups. Disc swelling pressure and fiber deformation increases linearly with significance as load magnitude is increased during conditioning for the longer duration groups. However, above a minimum conditioning loading magnitude between 0.1 and 0.3 MPa the exertion results become constant. As expected, permeability decreases significantly with increased conditioning load magnitude, due to a reduction in endplate pore size caused by increased deformation. In several of the parameters from these three models, we see a significant difference in the behavior of the 0.5 MPa full duration group as compared to the others. There seems to be an extreme change in biomechanical properties due to this loading, which carries over to recovery. Further exploration into conditioning effects of higher loads may shed light onto this difference in recovery behavior.

Relaxation during the second study caused a significantly different behavior across all three parameters over the exertion phases, whereas distraction led to slight changes in swelling pressure, annulus deformation, and permeability. Permeability and swelling pressure slightly increased showing a recovery of hydration through distraction. Disc mechanics during exertion are maintained to some degree through the employment of intermittent distraction phases, though recovery behavior appears unaltered as compared to relaxation groups.

The results from these models provide a comprehensive picture of the changes in mechanical behavior induced through varying conditioning load magnitudes, durations and inducing disc distraction.

5.2.2 Future Study

As has been described in the articles the results observed raise more questions than answers. Differences in conditioning: employing lower loads, which simulate rest and sitting stresses, seem to have a significant impact on further loading behavior, whereas higher loads seem to impact subsequent relaxation behavior. Investigated of historical effects in our study gave a good view of lower load magnitude conditioning effects, although there is room to investigate load duration further. Relaxation was only impacted by the highest load induced during conditioning. Further study of greater loads may lead insight into relaxation behavior, which has not been sufficiently studied at present.

Investigation of the creep compression duration necessary to impact flow rates as well as the minimum conditioning load required to induce changes in relaxation behavior can shed further light on disc mechanics. Studying the effects of these more extreme conditions under distraction protocols can then better illuminate the ability of distraction to positively impact biomechanics. *In vivo* testing could also allow for a better understanding of the effects of distraction on mechanics as well as the biological response to such behavior. It has been shown that distraction can aid in nutrition intake by acting as a fluid absorbing pump [10, 20]. Dynamic distraction has been shown to contribute various biological benefits. The benefits of distraction combined with the mechanical benefits observed in the present studies form a strong case for the study and implementation of distraction therapy to minimize the risk of degeneration.

Additional insights on disc mechanics can be attained through coupling the presented results with intradiscal pressure data. Presently, only studies on large discs, such as that of humans, have been able to test intradiscal pressure using sensors [5]. Currently, pressure sensors are being developed for use in smaller disc such as caudal rat models using fiber optic technology. Coupling the results presented in these studies with disc pressure data can greatly improve the overall understanding of disc mechanics. The knowledge of internal disc pressure and external stresses along with deformation data, can present a fuller picture of disc material properties and interactions.

Further study with protocols similar to the ones employed here but applied to human tissue or an *in vivo* model, can lend further insight into disc behavior under historical loading as well as distraction conditions. Such studies can also lend insight into the biological interactions. It has been shown that distraction can be used to increase hydration and protein levels, so further study into these protocols would be beneficial as well [10, 20]. Investigation as to the actual impact of hydration level on biological processes could potentially shed light onto our findings as well as those from the other studies sited.

Long term studies could also be employed to show how precautionary measures such as compressive conditioning or intermittent distraction may lead to minimized degeneration potential or possibly even rehabilitative impacts. By minimizing the risk of degeneration one could come one step closer to reducing incidents of back pain.

The observations from the presented studies demonstrate the mechanical impact that compression and distraction can have on immediate heavy lifting. It is believed that maintaining disc mechanics is an integral component in minimizing risk of degeneration. Distraction, as well as certain types of compressive conditioning, have shown to maintain biomechanics or alter hydration level respectively. Further exploration into the biological results of these altered hydration levels can describe which conditioning protocols have a more beneficial impact on disc health. Distraction has already been shown to improve hydration level and biological conditions. These results are now further validated as a result of maintained biomechanics. The current studies present insight into the effects on immediate loading but more long term mechanical effects have yet to be investigated. The impact of these processes on mechanics should be further explored over longer durations.

Appendix A

Sample Size Calculations

k values

	Means	A	Stdev's		Means	B	Stdev's
	8.81383E-05		8.04628E-06		1.54583E-05		2.13526E-06
	0.000152832		3.68724E-05		2.30822E-05		2.68989E-06
	0.000256267		3.73176E-05		3.26833E-05		3.97623E-06
					0.000070646		1.24831E-05
diff	6.47E-05						
	1.03E-04			Diff	7.62389E-06		
					9.60111E-06		
avg	8.40642E-05		2.74121E-05		3.79627E-05		
				Avg	1.83959E-05		5.32113E-06
sigma/delta		0.326085		Sigma/delta		0.289256	
			Avg	**0.307671**			
alpha	0.05		n>2(sig/delt)^2(talpha,new+t2(1-P)new)^2				
P	80%						

		P=90%		P=95%		P=99%	
assume n=15	4 subgroups	assume n=15		assume n=11		assume n=11	
v	56	V	56	V	40	v	40
tav	2.0042	tav	2.0042	tav	2.021	tav	2.021
2(1-P)	0.4	2(1-P)	0.2	2(1-P)	0.1	2(1-P)	0.02
t2(1-P)v	0.8486	t2(1-P)v	1.2974	t2(1-P)v	1.684	t2(1-P)v	2.423
n	1.5408	N	2.0637	N	2.5989	n	3.7390
n=2		n=3		n=3		n=4	
v	4	V	8	V	8	v	12
tav	2.776	tav	2.306	tav	2.306	tav	2.179
2(1-P)	0.4	2(1-P)	0.2	2(1-P)	0.1	2(1-P)	0.02
t2(1-P)v	0.941	t2(1-P)v	1.397	t2(1-P)v	1.86	t2(1-P)v	2.681
n	2.6157	N	2.5960	N	3.2858	n	4.4717
n=3				n=4		n=5	
v	8			V	12	v	16
tav	2.306			tav	2.179	tav	2.12
2(1-P)	0.4			2(1-P)	0.1	2(1-P)	0.02
t2(1-P)v	0.889			t2(1-P)v	1.782	t2(1-P)v	2.583
n	1.9326			N	2.9704	n	4.1875
	3	**3**			**4**		**5**

Appendix B

Protocol Logs

Study 1

Date	Test #	Disc D (mm)	Disc H (mm)	P (MPa)	P (N)	Pl t (s)	Pd t (s)	C (MPa)	C (N)	C t (s)	E (MPa)	E (N)	E t (s)	R (MPa)	R (N)	R t (s)
06/12/06	1A	3.65	1.1	-0.05	-0.52	2	500	-0.50	-5.23	10000	-1.00	-10.46	6000	-0.10	-1.05	20000
06/13/06	1B	3.66	1.07	-0.05	-0.53	2	500	-0.50	-5.26	10000	-1.00	-10.52	6000	-0.10	-1.05	20000
06/14/06	2A	3.85	1.09	-0.05	-0.58	2	500	-0.50	-5.82	10000	-1.00	-11.64	6000	-0.10	-1.16	20000
06/15/06	2B	3.38	1.18	-0.05	-0.45	2	500	-0.50	-4.49	10000	-1.00	-8.97	6000	-0.10	-0.90	20000
06/16/06	3A	3.88	1.17	-0.05	-0.59	2	500	-0.50	-5.91	10000	-1.00	-11.82	6000	-0.10	-1.18	20000
06/18/06	3B	3.85	1.37	-0.05	-0.58	2	500	-0.50	-5.82	10000	-1.00	-11.64	6000	-0.10	-1.16	20000
06/19/06	4A	3.68	1.31	-0.05	-0.53	2	500	-0.30	-3.19	10000	-1.00	-10.64	6000	-0.10	-1.06	20000
06/20/06	4B	3.79	1.27	-0.05	-0.56	2	500	-0.30	-3.38	10000	-1.00	-11.28	6000	-0.10	-1.13	20000
06/21/06	5A	3.81	1.36	-0.05	-0.57	2	500	-0.30	-3.42	10000	-1.00	-11.40	6000	-0.10	-1.14	20000
06/25/06	5B	3.81	1.40	-0.05	-0.57	2	500	-0.30	-3.42	10000	-1.00	-11.40	6000	-0.10	-1.14	20000
06/26/06	6A	4.72	1.37	-0.05	-0.87	2	500	-0.30	-5.25	10000	-1.00	-17.50	6000	-0.10	-1.75	20000
06/27/06	6B	4.23	1.27	-0.05	-0.70	2	500	-0.30	-4.22	10000	-1.00	-14.05	6000	-0.10	-1.41	20000
06/28/06	7A	4.47	1.13	-0.05	-0.78	2	500	-0.10	-1.57	10000	-1.00	-15.69	6000	-0.10	-1.57	20000
06/29/06	7B	4.03	1.09	-0.05	-0.64	2	500	-0.10	-1.28	10000	-1.00	-12.76	6000	-0.10	-1.28	20000
~~07/02/06~~	~~8A~~	~~4.02~~	~~0.99~~	~~-0.05~~	~~-0.63~~	~~2~~	~~500~~	~~-0.10~~	~~-1.27~~	~~10000~~	~~-1.00~~	~~-12.69~~	~~6000~~	~~-0.10~~	~~-1.27~~	~~20000~~
07/06/06	8B	3.93	1.10	-0.05	-0.61	2	500	-0.10	-1.21	10000	-1.00	-12.13	6000	-0.10	-1.21	20000
07/07/06	9A	4.18	0.95	-0.05	-0.69	2	500	0.00	-0.13	10000	-1.00	-13.72	6000	-0.10	-1.37	20000
07/10/06	9B	4.04	1.01	-0.05	-0.64	2	500	0.00	-0.09	10000	-1.00	-12.82	6000	-0.10	-1.28	20000
07/11/06	10A	4.23	1.07	-0.05	-0.70	2	500	0.00	-0.14	10000	-1.00	-14.05	6000	-0.10	-1.41	20000
08/17/06	18A	5.01	1.49	-0.05	-0.99	2	500	0.00	0.00	10000	-1.00	-19.71	6000	-0.10	-1.97	20000

08/21/06	18B	4.61	1.41	-0.05	-0.83	2	500	0.00	0.00	10000	-1.00	-16.69	6000	-0.10	-1.67	20000
08/22/06	19A	5.27	1.46	-0.05	-1.09	2	500	0.00	0.00	10000	-1.00	-21.81	6000	-0.10	-2.18	20000
08/23/06	19B	4.69	1.30	-0.05	-0.86	2	500	-0.10	-1.73	10000	-1.00	-17.28	6000	-0.10	-1.73	20000
08/24/06	20A	5.03	1.11	-0.05	-0.99	2	500	-0.10	-1.99	10000	-1.00	-19.87	6000	-0.10	-1.99	20000
08/25/06	20B	4.57	1.24	-0.05	-0.82	2	500	-0.10	-1.64	10000	-1.00	-16.40	6000	-0.10	-1.64	20000
08/27/06	21A	4.10	1.06	-0.05	-0.66	2	500	-0.50	-6.60	**2000**	-1.00	-13.20	6000	-0.10	-1.32	20000
08/28/06	21B	3.86	0.99	-0.05	-0.59	2	500	-0.50	-5.85	**2000**	-1.00	-11.70	6000	-0.10	-1.17	20000
08/29/06	22A	4.12	0.99	-0.05	-0.67	2	500	-0.50	-6.67	**2000**	-1.00	-13.33	6000	-0.10	-1.33	20000
08/30/06	22B	4.01	1.00	-0.05	-0.63	2	500	-0.50	-6.31	**2000**	-1.00	-12.63	6000	-0.10	-1.26	20000
08/31/06	23A	4.22	0.92	-0.05	-0.70	2	500	-0.50	-6.99	**2000**	-1.00	-13.99	6000	-0.10	-1.40	20000
09/01/06	23B	4.01	0.87	-0.05	-0.63	2	500	-0.50	-6.31	**2000**	-1.00	-12.63	6000	-0.10	-1.26	20000
09/03/06	24A	4.18	1.19	-0.05	-0.69	2	500	-0.30	-4.12	**2000**	-1.00	-13.72	6000	-0.10	-1.37	20000
09/04/06	24B	3.56	1.21	-0.05	-0.50	2	500	-0.30	-2.99	**2000**	-1.00	-9.95	6000	-0.10	-1.00	20000
09/05/06	25A	4.06	1.13	-0.05	-0.65	2	500	-0.30	-3.88	**2000**	-1.00	-12.95	6000	-0.10	-1.29	20000
09/06/06	25B	3.89	1.15	-0.05	-0.59	2	500	-0.30	-3.57	**2000**	-1.00	-11.88	6000	-0.10	-1.19	20000
09/07/06	26A	4.00	1.29	-0.05	-0.63	2	500	-0.30	-3.77	**2000**	-1.00	-12.57	6000	-0.10	-1.26	20000
09/08/06	26B	3.72	1.23	-0.05	-0.54	2	500	-0.30	-3.26	**2000**	-1.00	-10.87	6000	-0.10	-1.09	20000
09/10/06	27A	4.15	1.08	-0.05	-0.68	2	500	-0.10	-1.35	**2000**	-1.00	-13.53	6000	-0.10	-1.35	20000
09/14/06	27B	3.77	1.03	-0.05	-0.56	2	500	-0.10	-1.12	**2000**	-1.00	-11.16	6000	-0.10	-1.12	20000
09/18/06	28A	4.07	1.36	-0.05	-0.65	2	500	-0.10	-1.30	**2000**	-1.00	-13.01	6000	-0.10	-1.30	20000
09/25/06	28B	3.92	1.23	-0.05	-0.60	2	500	-0.10	-1.21	**2000**	-1.00	-12.07	6000	-0.10	-1.21	20000
10/03/06	29A	3.96	1.29	-0.05	-0.62	2	500	-0.10	-1.23	**2000**	-1.00	-12.32	6000	-0.10	-1.23	20000
10/09/06	29B	3.91	1.22	-0.05	-0.60	2	500	-0.10	-1.20	**2000**	-1.00	-12.01	6000	-0.10	-1.20	20000
~~10/10/06~~	~~30A~~	~~4.13~~	~~1.08~~	~~-0.05~~	~~-0.67~~	~~2~~	~~500~~	~~-0.30~~	~~-4.02~~	~~10000~~	~~-1.00~~	~~-13.40~~	~~6000~~	~~-0.10~~	~~-1.34~~	~~20000~~
10/11/06	30B	3.71	1.04	-0.05	-0.54	2	500	-0.30	-3.24	10000	-1.00	-10.81	6000	-0.10	-1.08	20000
10/12/06	31A	4.19	1.17	-0.05	-0.69	2	500	-0.30	-4.14	10000	-1.00	-13.79	6000	-0.10	-1.38	20000
10/16/06	31B	3.68	1.01	-0.05	-0.53	2	500	-0.30	-3.19	10000	-1.00	-10.64	6000	-0.10	-1.06	20000
10/18/06	32A	3.94	1.14	-0.05	-0.61	2	500	-0.30	-3.66	10000	-1.00	-12.19	6000	-0.10	-1.22	20000
11/30/06	32B	3.23	0.98	-0.05	-0.41	2	500	-0.50	-4.10	10000	-1.00	-8.19	6000	-0.10	-0.82	20000

Study 2 Log

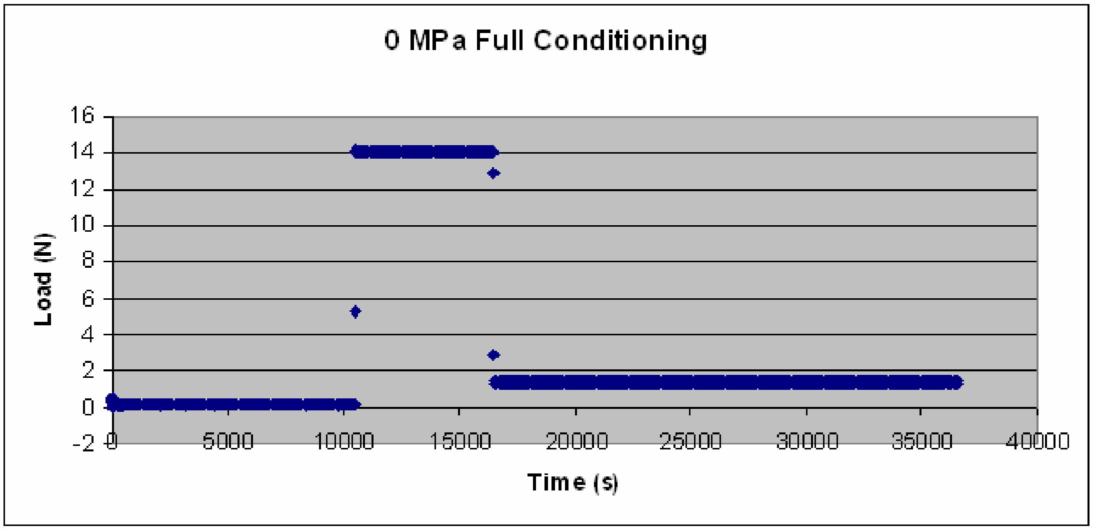
Test Date	Test #	Disc D (mm)	Disc H (mm)	P (MPa)	P (N)	Pl t (s)	Pd t (s)	C (MPa)	C (N)	C t (hrs)	C t (s)
07/12/06	10B	4.29	1.02	-0.05	-0.72	2	500	-0.10	-1.45	2.78	10000
07/14/06	12A	5.33	1.30	-0.05	-1.12	2	500	-0.10	-2.23	2.78	10000
07/21/06	12B	4.89	1.34	-0.05	-0.94	2	500	-0.10	-1.88	2.78	10000
07/23/06	13A	5.21	1.05	-0.05	-1.07	2	500	-0.10	-2.13	2.78	10000
07/24/06	13B	5.05	1.16	-0.05	-1.00	2	500	-0.10	-2.00	2.78	10000
07/25/06	14A	5.00	1.06	-0.05	-0.98	2	500	-0.10	-1.96	2.78	10000
07/26/06	14B	4.68	0.99	-0.05	-0.86	2	500	-0.10	-1.72	2.78	10000
07/27/06	15A	5.40	1.27	-0.05	-1.15	2	500	-0.10	-2.29	2.78	10000
08/02/06	15B	4.68	1.24	-0.05	-0.86	2	500	-0.10	-1.72	2.78	10000
08/04/06	16A	5.31	0.96	-0.05	-1.11	2	500	-0.10	-2.21	2.78	10000
08/07/06	16B	5.03	1.02	-0.05	-0.99	2	500	-0.10	-1.99	2.78	10000
08/08/06	17A	5.42	1.17	-0.05	-1.15	2	500	-0.10	-2.31	2.78	10000
08/09/06	17B	4.76	1.02	-0.05	-0.89	2	500	-0.10	-1.78	2.78	10000

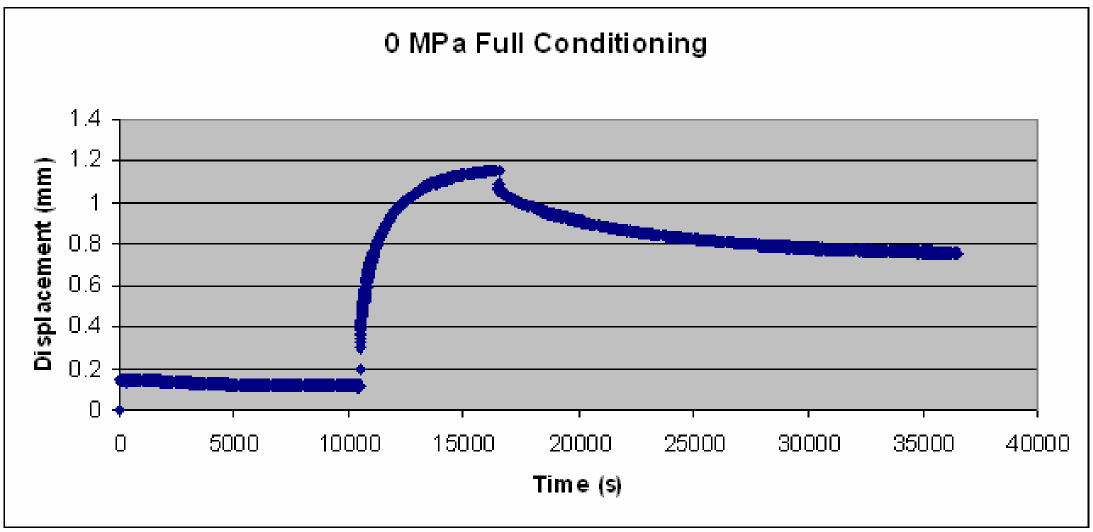
Test Date	Test #	E (MPa)	E (N)	E t (hrs)	E t (s)	S (MPa)	S (N)	S t (hrs)	S t (s)	R (MPa)	R (N)	R t (hrs)	R t (s)
07/12/06	10B	-1.00	-14.45	1.67	6000	0.1	1.45	1.666667	6000	-0.10	-1.45	5.56	20000
07/14/06	12A	-1.00	-22.31	1.67	6000	0.1	2.23	0.833333	3000	-0.10	-2.23	5.56	20000
07/21/06	12B	-1.00	-18.78	1.67	6000	0.1	1.88	0.833333	3000	-0.10	-1.88	5.56	20000
07/23/06	13A	-1.00	-21.32	1.67	6000	0.1	2.13	0.833333	3000	-0.10	-2.13	5.56	20000
07/24/06	13B	-1.00	-20.03	1.67	6000	-0.1	-2.00	0.833333	3000	-0.10	-2.00	5.56	20000
07/25/06	14A	-1.00	-19.63	1.67	6000	-0.1	-1.96	0.833333	3000	-0.10	-1.96	5.56	20000
07/26/06	14B	-1.00	-17.20	1.67	6000	-0.1	-1.72	0.833333	3000	-0.10	-1.72	5.56	20000
07/27/06	15A	-1.00	-22.90	1.67	6000	-0.1	-2.29	0.833333	3000	-0.10	-2.29	5.56	20000
08/02/06	15B	-1.00	-17.20	1.67	6000	-0.1	-1.72	0.833333	3000	-0.10	-1.72	5.56	20000
08/04/06	16A	-1.00	-22.15	1.67	6000	-0.1	-2.21	0.833333	3000	-0.10	-2.21	5.56	20000
08/07/06	16B	-1.00	-19.87	1.67	6000	0.1	1.99	0.833333	3000	-0.10	-1.99	5.56	20000
08/08/06	17A	-1.00	-23.07	1.67	6000	0.1	2.31	0.833333	3000	-0.10	-2.31	5.56	20000
08/09/06	17B	-1.00	-17.80	1.67	6000	0.1	1.78	0.833333	3000	-0.10	-1.78	5.56	20000

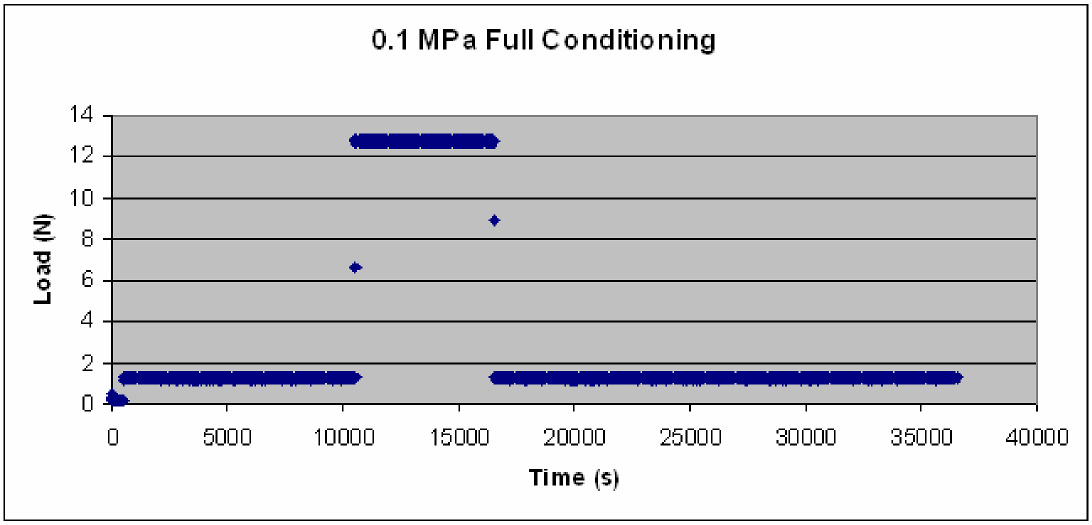
Appendix C

Sample Creep Curves

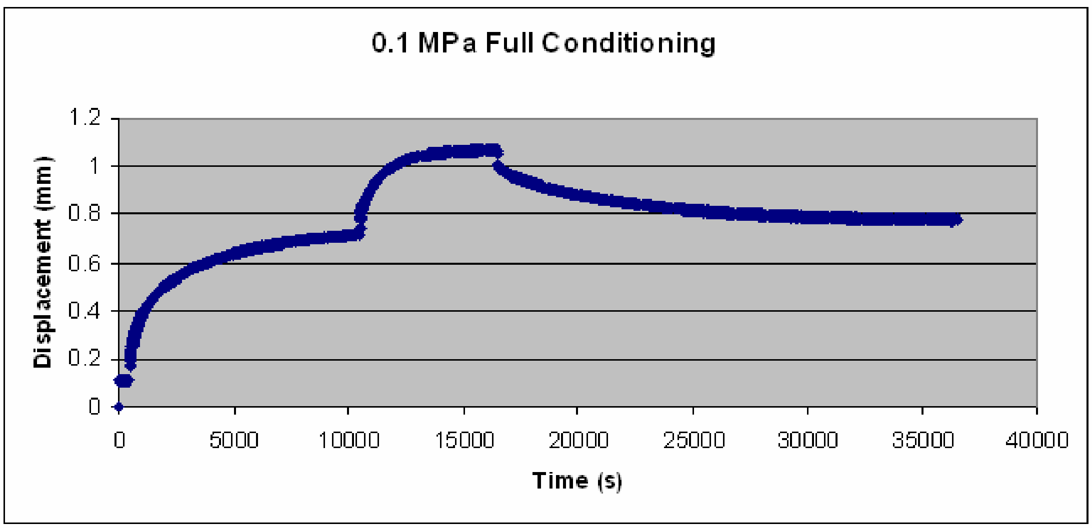
Study 1



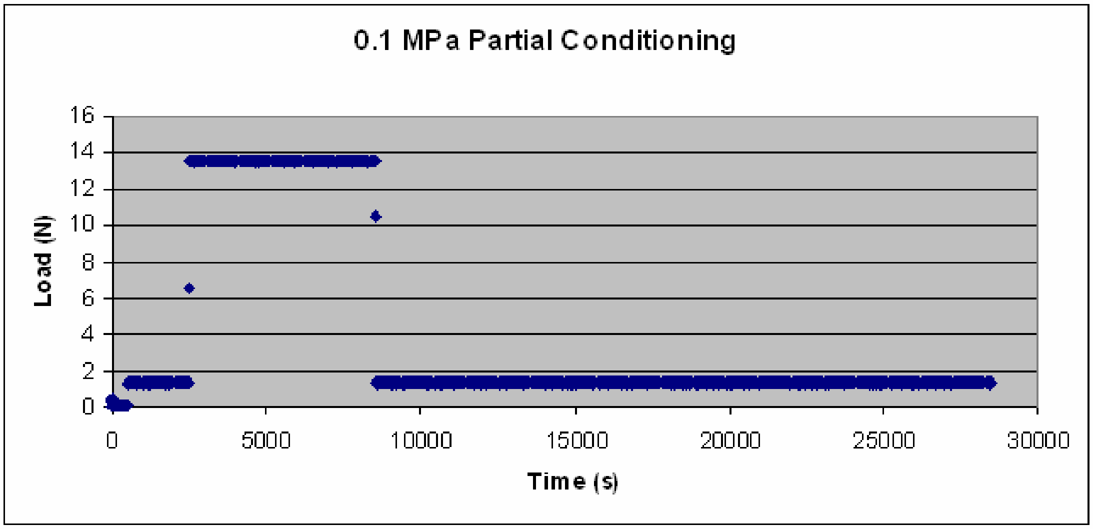


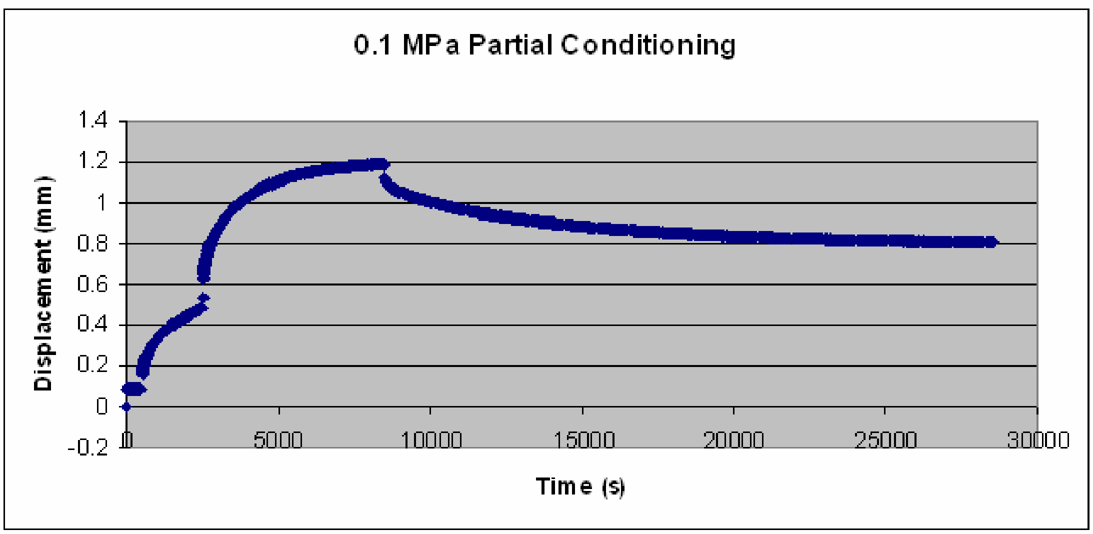


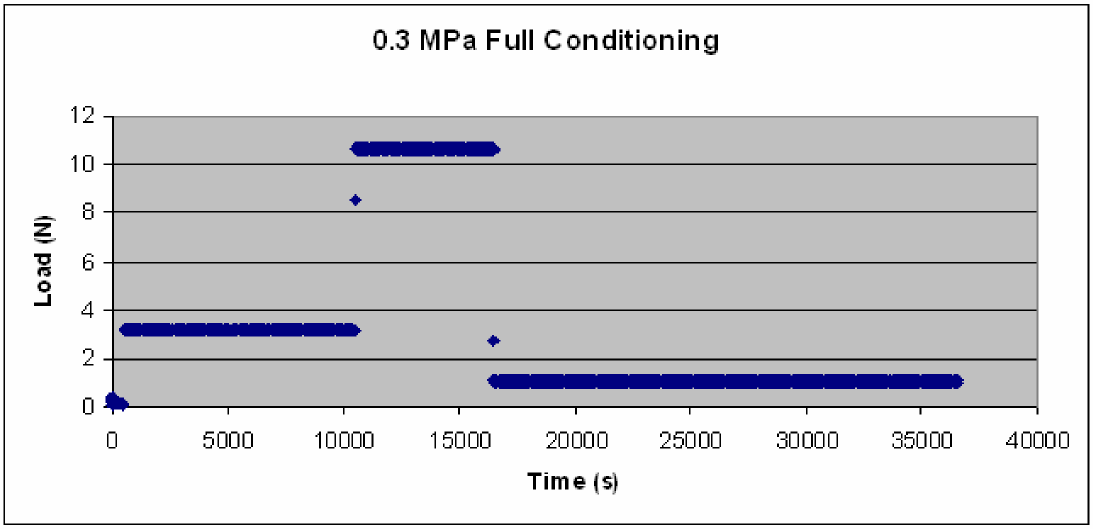
0.1 MPa Full Conditioning
Load (N)
14
12
10
8
6
4
2
0
0
5000
10000
15000
20000
25000
30000
35000
40000
Time (s)

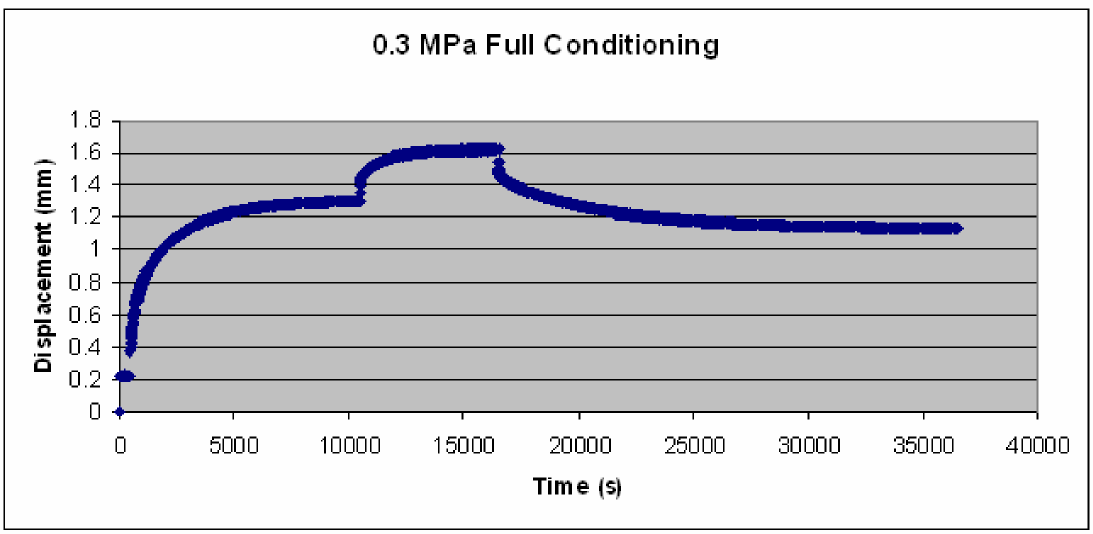


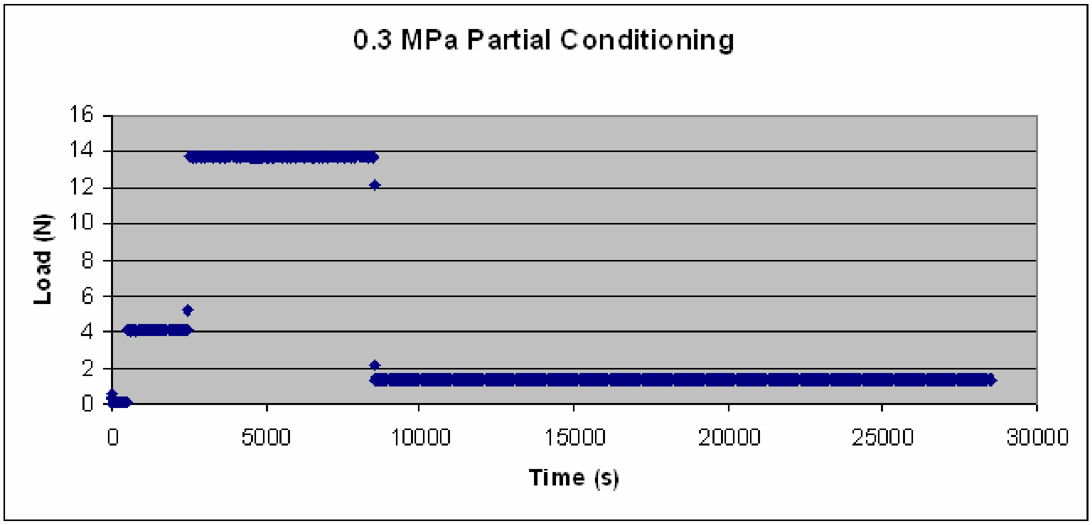
0.1 MPa Full Conditioning
Displacement (mm)
1.2
1
0.8
0.6
0.4
0.2
0
0
5000
10000
15000
20000
25000
30000
35000
40000
Time (s)

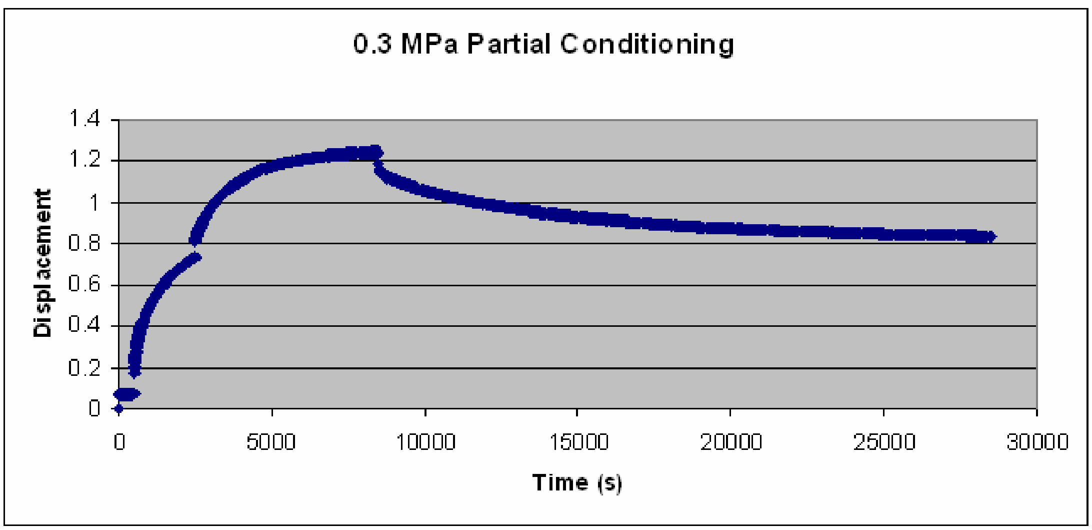

0.1 MPa Partial Conditioning
Load (N)
16
14
12
10
8
6
4
2
0
0
5000
10000
15000
20000
25000
30000
Time (s)

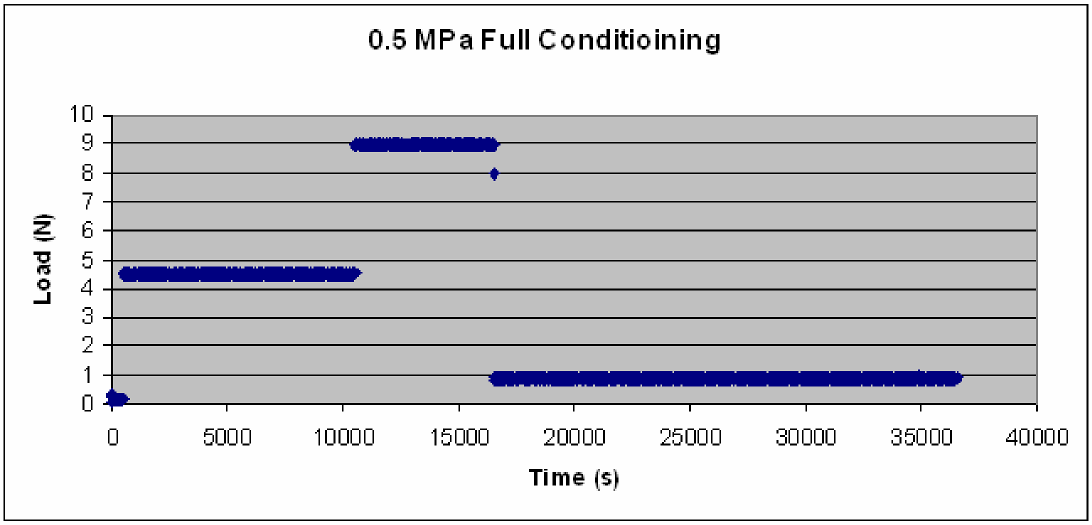

0.1 MPa Partial Conditioning
Displacement (mm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
5000
10000
15000
20000
25000
30000
Time (s)

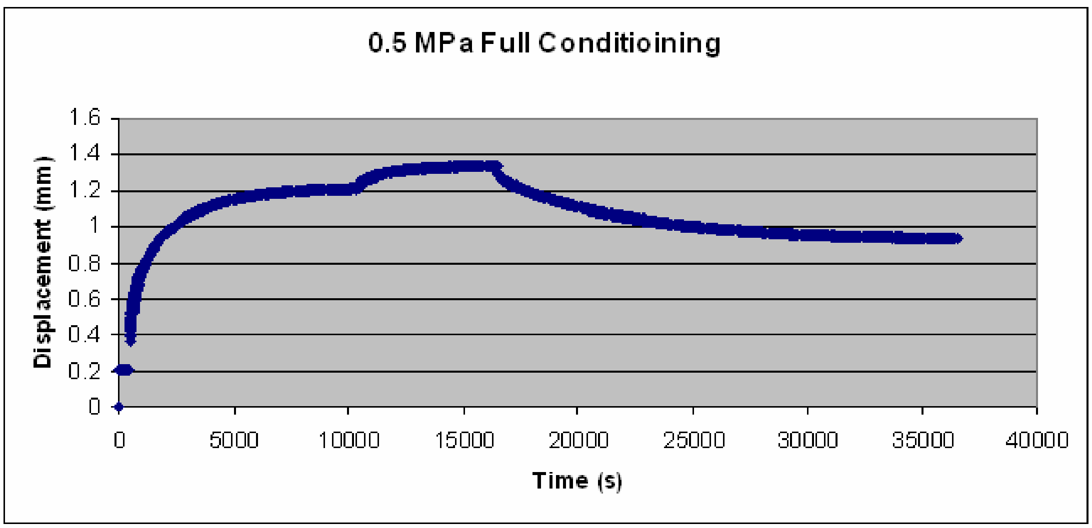

0.3 MPa Full Conditioning
Load (N)
0
2
4
6
8
10
12
0
5000
10000
15000
20000
25000
30000
35000
40000
Time (s)

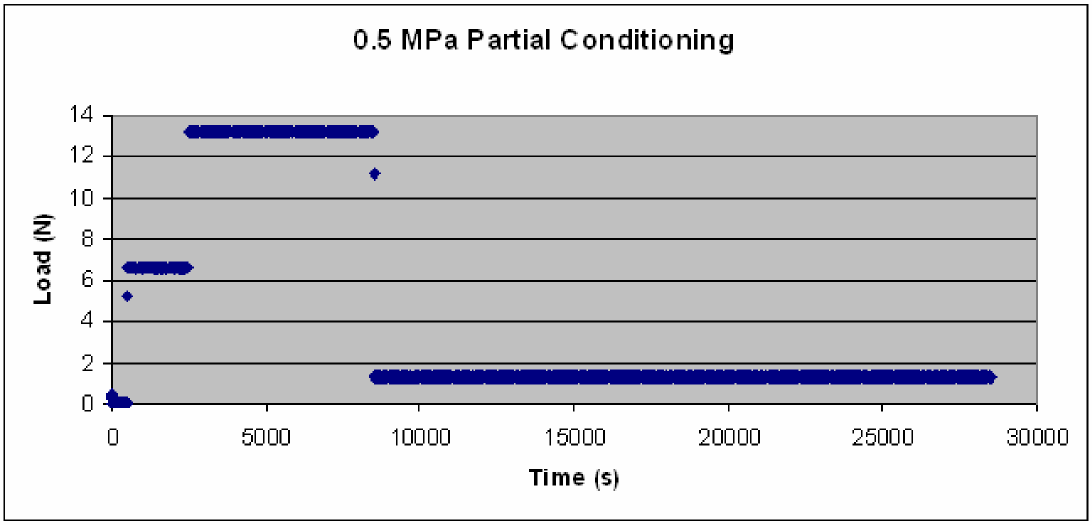

0.3 MPa Full Conditioning
Displacement (mm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0
5000
10000
15000
20000
25000
30000
35000
40000
Time (s)


0.3 MPa Partial Conditioning
Load (N)
16
14
12
10
8
6
4
2
0
0
5000
10000
15000
20000
25000
30000
Time (s)

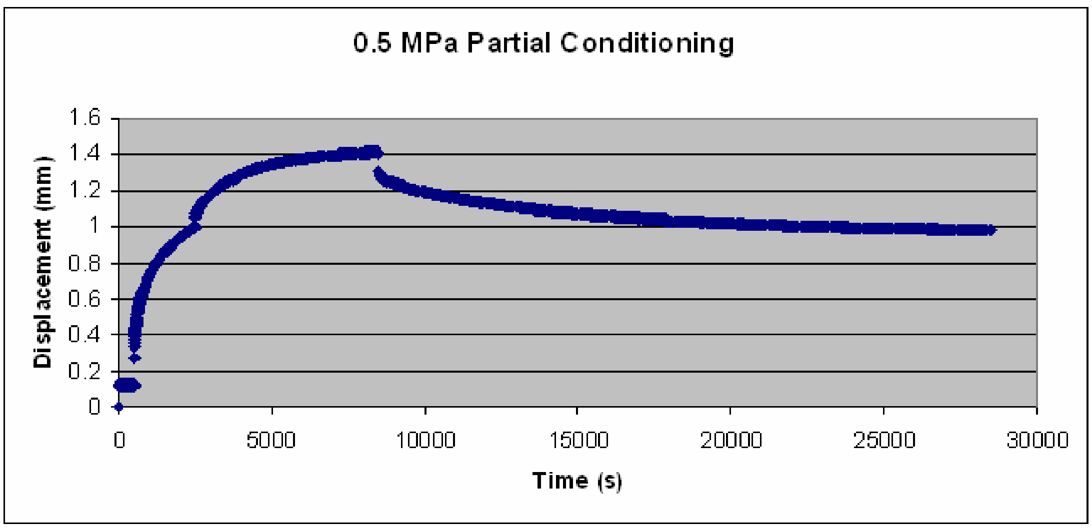

0.3 MPa Partial Conditioning
Displacement
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5000
10000
15000
20000
25000
30000
Time (s)


0.5 MPa Full Conditioining
Load (N)
10
9
8
7
6
5
4
3
2
1
0
0
5000
10000
15000
20000
25000
30000
35000
40000
Time (s)


0.5 MPa Full Conditioining
Displacement (mm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5000
10000
15000
20000
25000
30000
35000
40000
Time (s)

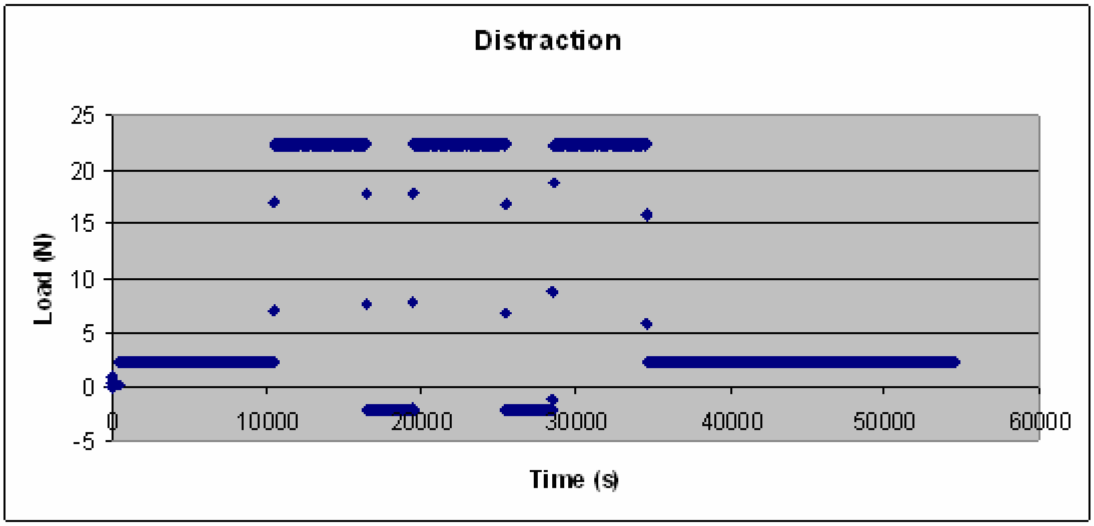


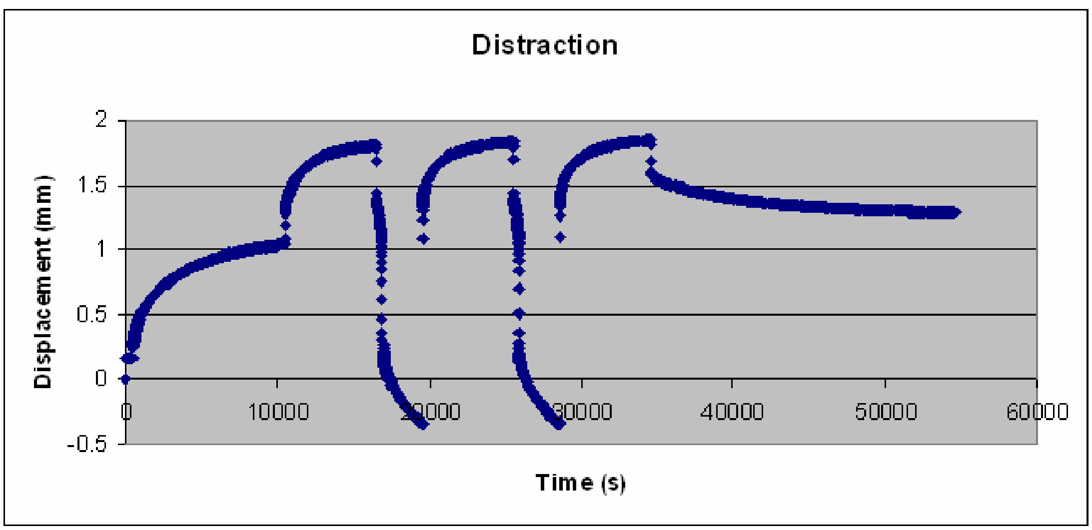
0.5 MPa Partial Conditioning
Load (N)
14
12
10
8
6
4
2
0
0
5000
10000
15000
20000
25000
30000
Time (s)

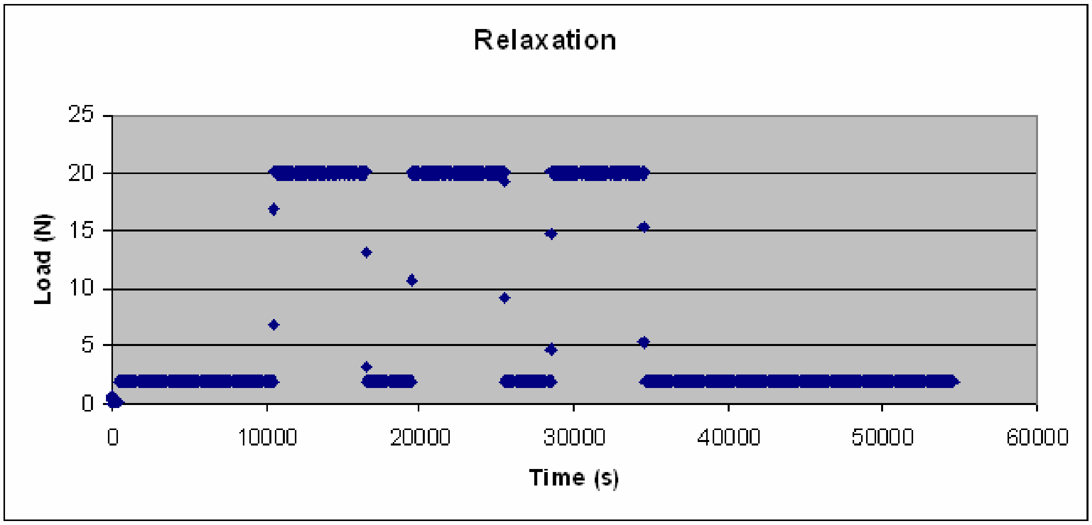


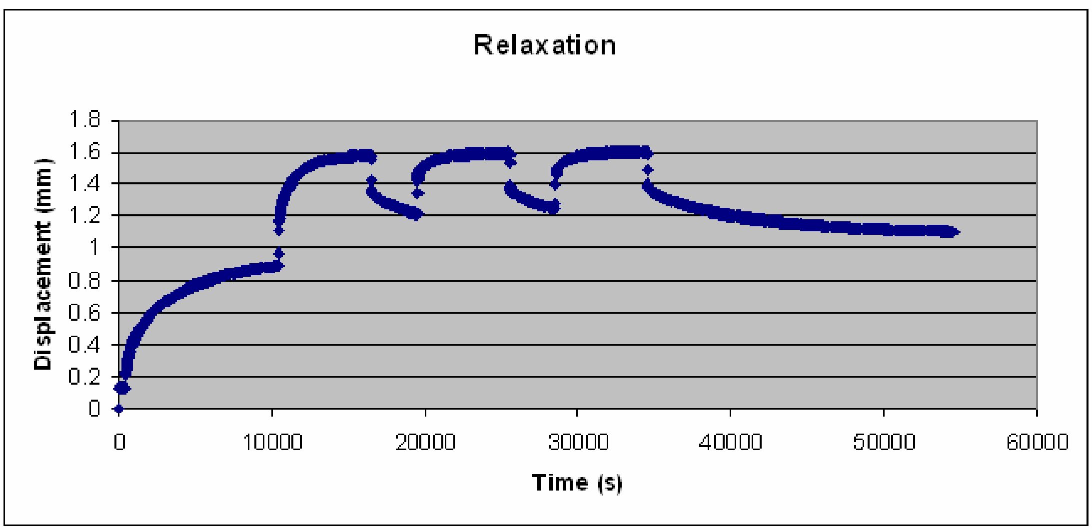
0.5 MPa Partial Conditioning
Displacement (mm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5000
10000
15000
20000
25000
30000
Time (s)

Study 2


Distraction
25
20
15
10
5
0
-5
Load (N)
0
10000
20000
30000
40000
50000
60000
Time (s)


Distraction
2
1.5
1
0.5
0
-0.5
Displacement (mm)
0
10000
20000
30000
40000
50000
60000
Time (s)


Relaxation
25
20
15
10
5
0
Load (N)
0
10000
20000
30000
40000
50000
60000
Time (s)


Relaxation
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Displacement (mm)
0
10000
20000
30000
40000
50000
60000
Time (s)

Appendix D

Final Strains and Parameters

Study 1: Final Strains (mm)

Specimen	C stress	C	E	R
1A	-0.50	0.363939	0.421818	
1B	-0.50	0.344548	0.390343	0.260436
2A	-0.50	0.399694	0.453517	0.306422
2B	-0.50	0.342938	0.377966	0.263559
3A	-0.50	0.335897	0.388319	0.250997
3B	-0.50	0.317762	0.36253	0.244039
4A	-0.30	0.332061	0.411959	0.289313
4B	-0.30	0.33937	0.420472	0.301575
5A	-0.30	0.372059	0.47451	0.326716
5B	-0.30	0.379286	0.474048	0.33381
6A	-0.30	0.307299	0.381265	0.274209
6B	-0.30	0.36378	0.450656	0.323622
7A	-0.10	0.279351	0.473746	0.325074
7B	-0.10	0.218654	0.3263	0.237615
8B	-0.10	0.249697	0.404242	0.285758
9A	0.00		0.463158	0.306316
9B	0.00		0.433663	0.312541
10A	0.00		0.358879	0.235826
18A	0.00		0.371812	0.263758
18B	0.00		0.33948	0.2487
19A	0.00		0.439954	0.275114
19B	-0.10	0.207692	0.378462	0.250256
20A	-0.10	0.245045	0.483183	0.308108
20B	-0.10	0.255108	0.341129	0.251075
21A	**-0.50**	0.314151	0.443711	0.308805
21B	**-0.50**	0.319529	0.427273	0.314478
22A	**-0.50**	0.293266	0.407744	0.284848
22B	**-0.50**	0.293333	0.401333	0.295667
23A	**-0.50**	0.32029	0.431159	0.308333
23B	**-0.50**	0.36092	0.440613	0.349425
24A	**-0.30**	0.206443	0.348179	0.234454
24B	**-0.30**	0.232507	0.335537	0.248485
25A	**-0.30**	0.246313	0.403835	0.271976
25B	**-0.30**	0.204348	0.346667	0.230145
26A	**-0.30**	0.248837	0.383463	0.284238
26B	**-0.30**	0.242547	0.38374	0.271816
27A	**-0.10**	0.149074	0.367593	0.248457
27B	**-0.10**	0.156634	0.365372	0.250809

28A	**-0.10**	0.165686	0.367892	0.258824
28B	**-0.10**	0.175339	0.363144	0.255556
29A	**-0.10**	0.139793	0.348062	0.221964
29B	**-0.10**	0.172678	0.374863	0.249727
30B	-0.30	0.238782	0.297436	0.201603
31A	-0.30	0.253846	0.317664	0.212251
32A	-0.30	0.334211	0.414327	0.280994
32B	-0.50	0.318367	0.368367	0.222789

Study 2: Final Strains (mm)

Date	Specimen	A	B	C	R
07/12/06	10B	0.40719	0.410131	0.413725	0.288562
07/14/06	12A	0.46359	0.47	0.473333	0.330513
07/21/06	12B	0.355224	0.360199	0.36393	0.25995
07/23/06	13A	0.564127	0.554603	0.576825	0.368254
07/24/06	13B	0.454023	0.458333	0.460632	0.318391
07/25/06	14A	0.476415	0.480818	0.483333	0.327358
07/26/06	14B	0.438384	0.444444	0.446801	0.308418
07/27/06	15A	0.413648	0.419948	0.422835	0.290814
08/02/06	15B	0.380108	0.386828	0.388978	0.273925
08/04/06	16A	0.436806	0.444444	0.447569	0.281597
08/07/06	16B	0.475163	0.485621	0.490523	0.313725
08/08/06	17A	0.41453	0.419658	0.422792	0.280342
08/09/06	17B	0.37549	0.380392	0.383333	0.243464

Study 1: Model 1 Parameters

			A			B			C	
Specimen	C stress	b	Dinf	Tau	b	Dinf	tau	b	Dinf	tau
1A	-0.50	0.7912	0.368	1780	0.7322	0.4255	1709			
1B	-0.50	0.7993	0.3478	1718	0.7747	0.3928	1693	0.942	0.2548	6176
2A	-0.50	0.8102	0.4015	1505	0.7733	0.4556	1543	0.9893	0.3034	5537
2B	-0.50	0.8312	0.3456	1768	0.7667	0.38	1621	0.9965	0.26	5734
3A	-0.50	0.8024	0.3403	1846	0.7461	0.3918	1789	0.9681	0.247	5748
3B	-0.50	0.775	0.3203	1532	0.7045	0.3653	1673	0.9701	0.2405	5932
4A	-0.30	0.7544	0.3348	1423	0.8355	0.4127	984.2	0.9793	0.2884	4436
4B	-0.30	0.8451	0.342	1869	0.5945	0.4219	762.4	1.002	0.3004	4518
5A	-0.30	0.8666	0.3752	1968	0.7575	0.4761	1139	1.034	0.3255	4775
5B	-0.30	0.8255	0.3807	1505	0.643	0.4759	908.2	1.078	0.3331	4075
6A	-0.30	0.8005	0.3102	1663	0.6348	0.3833	1000	0.9478	0.2717	5560
6B	-0.30	0.8252	0.3677	1917	0.6726	0.4529	1086	0.9247	0.3211	5496
7A	-0.10	0.6651	0.2983	2963	0.7973	0.4777	1310	0.8629	0.3218	5113
7B	-0.10	0.6516	0.2325	2465	0.9064	0.3263	982.9	0.9442	0.2352	5554
8B	-0.10	0.6628	0.2723	3248	0.8629	0.4066	1339	0.9364	0.2832	5319
9A	0.00				0.7219	0.4681	906.3	0.947	0.3044	4626
9B	0.00				0.8386	0.4363	1060	0.9267	0.3107	4554
10A	0.00				0.8261	0.3644	1238	0.8962	0.23	6230
18A	0.00				0.7345	0.386	1570	0.897	0.2601	6056
18B	0.00				0.6392	0.3585	1452	0.8316	0.2446	5918
19A	0.00				0.7802	0.4546	1670	0.8279	0.2695	6174
19B	-0.10	0.6319	0.2343	3586	0.7771	0.3889	1921	0.8826	0.2458	6091
20A	-0.10	0.7884	0.4938	1745	0.7884	0.4938	1745	0.8568	0.3005	6595
20B	-0.10	0.5942	0.246	3974	0.8792	0.3433	1426	0.9351	0.2489	5381
21A	**-0.50**				0.8532	0.4492	1614	0.8625	0.3022	6270
21B	**-0.50**				0.8792	0.43	1426	0.9351	0.3118	5381

22A	**-0.50**				0.8384	0.4128	1596	0.91	0.2805	5747
22B	**-0.50**				1.005	0.4036	1583	0.8866	0.2916	5421
23A	**-0.50**				0.9307	0.4316	1185	0.8793	0.3069	3899
23B	**-0.50**				0.4076	0.4424	157.8	0.9598	0.3492	3352
24A	**-0.30**				0.8372	0.3518	1363	0.8422	0.2282	6004
24B	**-0.30**				0.8512	0.3365	1.07E+03	9.23E-01	0.2471	4180
25A	**-0.30**				0.8895	0.4066	1364	0.9662	0.2705	4446
25B	**-0.30**				0.9061	0.3484	1308	0.8898	0.2289	3884
26A	**-0.30**				0.8813	0.3881	1608	0.8415	0.2817	4459
26B	**-0.30**				0.9193	0.388	1638	0.8652	0.2697	4446
27A	**-0.10**				0.8343	0.3731	1412	0.8376	0.2433	5450
27B	**-0.10**				0.8973	0.3679	1215	0.8322	0.2468	5132
28A	**-0.10**				0.8072	0.3739	1378	0.8573	0.2561	4881
28B	**-0.10**				0.885	0.3654	1225	0.8655	0.2537	4322
29A	**-0.10**				0.8346	0.3536	1398	0.9046	0.2168	5886
29B	**-0.10**				0.9528	0.376	1220	0.9376	0.2489	3866
30B	-0.30	0.7761	0.2451	2209	0.8483	0.2983	1208	0.89	0.1987	4935
31A	-0.30	0.8801	0.2559	1912	0.7362	0.3189	1076	0.8458	0.21	4439
32A	-0.30	0.869	0.3359	1671	0.693	0.4151	937	0.9335	0.2801	3583
32B	-0.50	0.8224	0.3188	1204	0.6184	0.3701	1001	0.903	0.2211	3998

Study 1: Model 2 Parameters

			A			B			C	
Specimen	C stress	E1	E2	U	E1	E2	U	E1	E2	U
1A	-0.50	0.9357	1.31	1303	0.09917	1.182	172			
1B	-0.50	1.082	1.391	1389	0.1036	1.278	173.6	0.9939	-3.518	8789
2A	-0.50	0.8683	1.205	1008	0.0869	1.102	135.9	0.7845	-2.965	5939
2B	-0.50	1.004	1.4	1310	0.09296	1.321	158.5	0.9455	-3.46	7470
3A	-0.50	1.268	1.423	1621	0.1183	1.284	209.6	1.064	-3.636	8783
3B	-0.50	0.983	1.522	1309	0.1018	1.376	188.2	0.9736	-3.736	7926
4A	-0.30	0.6627	0.862	813	0.1799	1.698	194.4	0.6648	-3.119	3799
4B	-0.30	0.5057	0.8274	725.6	0.132	1.667	160.9	0.538	-2.996	2959
5A	-0.30	0.5548	0.7624	744.5	0.171	1.476	214	0.6105	-2.767	3663
5B	-0.30	0.4903	0.7513	573.5	0.1237	1.476	175.2	0.5136	-2.704	2422
6A	-0.30	0.7584	0.9431	934.3	0.1683	1.835	247.3	0.6434	-3.307	4587
6B	-0.30	0.5059	0.7898	748.5	0.1409	1.552	203.4	0.4699	-2.797	3247
7A	-0.10	0.2232	0.3059	490.7	0.4336	1.893	558.1	0.5016	-2.785	3466
7B	-0.10	0.3545	0.3867	639.3	0.8602	2.762	699.2	0.8046	-3.819	5842
8B	-0.10	0.3421	0.3627	698.8	0.6986	2.221	808.5	0.6929	-3.171	4912
9A	0.00				1.426	2.137	1158	0.6425	-2.952	3969
9B	0.00				1.308	2.283	1106	0.555	-2.892	3332
10A	0.00				2.486	2.735	2017	1.074	-3.884	9735
18A	0.00				1.623	2.627	2069	0.6401	-3.446	5009
18B	0.00				1.886	2.871	2469	0.6307	-3.653	4939
19A	0.00				1.35	2.215	1730	0.6462	-3.309	5394
19B	-0.10	0.491	0.437	984.6	0.7511	2.343	1254	0.809	-3.64	6767
20A	-0.10	0.4729	0.3612	912.4	0.5822	1.841	895.2	0.656	-2.968	5907
20B	-0.10	0.5497	0.4229	1107	0.6306	2.63	789.2	0.7017	-3.607	4886
21A	**-0.50**				0.3186	1.118	435	0.6461	-2.957	5593
21B	**-0.50**				0.2793	1.164	349.5	0.5603	-2.881	3901

22A	**-0.50**				0.3557	1.216	489.7	0.8017	-3.193	6462
22B	**-0.50**				0.3669	1.238	446.6	0.6481	-3.072	4786
23A	**-0.50**				0.3015	1.164	2.96E+02	6.30E-01	-2.925	3524
23B	**-0.50**				0.3003	1.159	295.1	0.3279	-2.577	1302
24A	**-0.30**				0.8692	2.005	932.3	0.9997	-3.901	8801
24B	**-0.30**				0.6313	2.087	611.2	0.7765	-3.635	4399
25A	**-0.30**				0.695	1.73	743.8	0.8365	-3.324	5104
25B	**-0.30**				0.809	2.011	812.7	0.9248	-3.921	5219
26A	**-0.30**				0.6321	1.809	828.3	0.5762	-3.183	3657
26B	**-0.30**				0.6174	1.807	799.1	0.6409	-3.326	3987
27A	**-0.10**				1.311	2.424	1435	0.8941	-3.668	7233
27B	**-0.10**				1.319	2.453	1178	0.8011	-3.62	6028
28A	**-0.10**				1.223	2.429	1387	0.7322	-3.496	5042
28B	**-0.10**				1.132	2.47	1091	0.729	-3.536	901.9
29A	**-0.10**				1.534	2.564	1611	1.176	-4.125	1.02E+04
29B	**-0.10**				1.16	2.392	1001	0.8197	-3.612	4370
30B	-0.30	1.176	1.224	1725	0.3444	2.352	400.6	1.152	-4.507	8221
31A	-0.30	1.4	1.162	1413	0.2775	2.203	346.9	1.045	-4.264	7142
32A	-0.30	0.837	0.8814	853.3	0.1709	1.692	209.7	0.681	-3.21	3314
32B	-0.50	1.496	1.555	1186	0.08649	1.357	123.4	1.038	-4.061	6150

Study 1: Model 3 Parameters

			A				B				C		
Specimen	C stress	D	Eo	G	k	D	Eo	G	k	D	Eo	G	k
1A	-0.50	3.091	0.2028	2.95E-06	8.17E-05	17.5	0.3875	2.36E-05	1.65E-05				
1B	-0.50	3.137	0.1858	2.55E-06	8.06E-05	19.25	0.3603	1.86E-05	1.39E-05	8.98E+00	3.57E-01	-4.10E-07	5.81E-06
2A	-0.50	2.816	0.2226	2.26E-06	0.000101	16.82	0.4188	1.85E-05	1.60E-05	8.169	0.412	8.32E-07	6.36E-06
2B	-0.50	3.296	0.1931	1.97E-06	8.30E-05	22.21	0.3515	1.97E-05	1.19E-05	9.07E+00	3.57E-01	1.05E-06	6.82E-06
3A	-0.50	2.997	0.1702	2.64E-06	8.89E-05	17.46	0.3544	2.14E-05	1.69E-05	8.82E+00	3.50E-01	-1.94E-07	7.00E-06
3B	-0.50	3.714	0.182	2.82E-06	9.36E-05	22.26	0.3351	2.40E-05	1.76E-05	1.05E+01	3.25E-01	6.82E-07	6.74E-06
4A	-0.30	1.8	0.1651	1.82E-05	1.94E-04	17.73	0.3729	-4.68E-07	2.05E-05	1.16E+01	3.67E-01	-1.98E-07	7.47E-06
4B	-0.30	2.156	0.2063	9.47E-07	1.29E-04	22.36	0.3829	2.80E-05	2.41E-05	1.36E+01	3.65E-01	1.15E-06	5.75E-06
5A	-0.30	1.739	0.2098	1.21E-06	1.73E-04	14.9	0.421	2.06E-05	2.79E-05	9.61E+00	4.16E-01	1.76E-06	7.49E-06
5B	-0.30	1.865	0.2211	1.33E-06	1.98E-04	18.26	0.4294	2.37E-05	2.51E-05	1.14E+01	4.09E-01	2.32E-06	7.72E-06
6A	-0.30	2.073	0.1617	1.60E-06	1.62E-04	21.6	0.3423	2.89E-05	2.37E-05	1.37E+01	3.37E-01	3.10E-07	5.35E-06
6B	-0.30	1.999	0.216	1.33E-06	1.35E-04	18.78	0.4066	2.68E-05	2.22E-05	1.41E+01	3.88E-01	-1.72E-06	4.56E-06
7A	-0.10	0.7943	0.1563	1.65E-06	2.46E-04	10.48	0.3823	1.53E-05	2.93E-05	1.30E+01	3.96E-01	-3.13E-06	4.54E-06
7B	-0.10	0.8504	0.09852	2.13E-06	2.98E-04	11.75	0.2465	7.55E-06	3.70E-05	1.43E+01	2.99E-01	-6.57E-08	4.48E-06
8B	-0.10	0.8387	0.1231	2.00E-06	2.25E-04	9.439	0.3054	8.61E-06	3.18E-05	1.15E+01	3.64E-01	-1.16E-06	5.37E-06
9A	0.00					5.311	0.2638	1.50E-05	7.63E-05	1.06E+01	3.90E-01	3.00E-08	4.97E-06
9B	0.00					6.188	0.2695	6.16E-06	6.29E-05	1.22E+01	3.85E-01	1.10E-07	4.82E-06
10A	0.00					5.678	0.1795	8.99E-06	6.38E-05	1.04E+01	3.21E-01	-1.50E-06	5.30E-06
18A	0.00					7.106	0.2253	1.72E-05	6.02E-05	1.55E+01	3.22E-01	-1.84E-06	5.07E-06
18B	0.00					5.729	0.1795	9.07E-06	9.00E-05	1.83E+01	2.99E-01	-3.87E-06	4.53E-06
19A	0.00					6.035	0.2717	1.30E-05	6.56E-05	1.45E+01	3.40E-01	-4.86E-06	4.99E-06
19B	-0.10	0.9498	0.09385	2.24E-06	0.000232	10.35	0.286	2.00E-05	2.84E-05	1.32E+01	3.19E-01	-2.76E-06	5.20E-06
20A	-0.10	0.7656	0.1014	2.66E-06	0.000249	8.123	0.3656	1.85E-05	3.14E-05	1.10E+01	3.91E-01	-3.68E-06	4.24E-06
20B	-0.10	0.8865	0.08441	2.66E-06	0.000243	14.02	0.274	1.18E-05	2.41E-05	1.50E+01	3.10E-01	-4.96E-07	4.94E-06
21A	**-0.50**					5.319	0.3442	9.02E-06	4.62E-05	1.11E+01	3.93E-01	-4.80E-06	4.72E-06
21B	**-0.50**					6.208	0.3432	6.53E-06	3.97E-05	1.20E+01	3.88E-01	-4.96E-07	4.29E-06

22A	**-0.50**					5.708	0.3114	1.26E-05	4.47E-05	9.81E+00	3.78E-01	-2.74E-06	5.49E-06
22B	**-0.50**					5.337	0.312	-1.98E-06	5.90E-05	1.18E+01	3.73E-01	-2.77E-06	4.80E-06
23A	**-0.50**					5.785	0.3408	4.67E-06	4.75E-05	1.07E+01	3.96E-01	-2.75E-06	6.28E-06
23B	**-0.50**					9.221	0.3791	1.35E-05	4.56E-05	1.76E+01	3.99E-01	1.49E-06	3.81E-06
24A	**-0.30**					6.922	0.2393	1.30E-05	5.32E-05	1.20E+01	3.13E-01	-4.95E-06	5.91E-06
24B	**-0.30**					9.002	0.2541	7.32E-06	4.63E-05	1.35E+01	3.16E-01	-1.42E-06	7.11E-06
25A	**-0.30**					6.137	0.2857	6.77E-06	5.13E-05	9.92E+00	3.62E-01	-4.46E-07	7.59E-06
25B	**-0.30**					7.138	0.2452	6.62E-06	4.99E-05	1.28E+01	3.03E-01	-2.60E-06	7.60E-06
26A	**-0.30**					7.104	0.2844	5.00E-06	4.17E-05	1.45E+01	3.48E-01	-2.82E-06	6.00E-06
26B	**-0.30**					7.217	0.2867	4.76E-06	3.97E-05	1.41E+01	3.38E-01	-2.63E-06	5.94E-06
27A	**-0.10**					6.961	0.2361	7.43E-06	4.32E-05	1.17E+01	3.26E-01	-2.80E-06	5.28E-06
27B	**-0.10**					6.993	0.236	3.28E-06	4.73E-05	1.31E+01	3.23E-01	-4.26E-06	5.08E-06
28A	**-0.10**					7.323	0.2406	1.04E-05	5.22E-05	1.36E+01	3.28E-01	-4.08E-06	6.76E-06
28B	**-0.10**					7.834	0.2467	4.57E-06	4.91E-05	1.37E+01	3.23E-01	-1.88E-06	6.29E-06
29A	**-0.10**					6.883	0.2162	5.57E-06	5.16E-05	1.05E+01	3.06E-01	-1.34E-06	6.73E-06
29B	**-0.10**					7.33	0.2513	2.86E-06	5.57E-05	1.23E+01	3.23E-01	-5.30E-07	7.68E-06
30B	-0.30	2.512	0.1161	2.61E-06	8.22E-05	19.25	0.2581	1.35E-05	1.85E-05	1.34E+01	2.70E-01	-2.19E-06	5.55E-06
31A	-0.30	2.097	0.111	9.37E-07	0.000133	19.99	0.2772	2.34E-05	2.31E-05	1.32E+01	2.84E-01	-4.10E-06	7.05E-06
32A	-0.30	1.784	0.1647	1.10E-06	0.000171	18.46	0.3701	2.24E-05	2.27E-05	1.19E+01	3.56E-01	-2.98E-08	7.34E-06
32B	-0.50	3.111	0.154	2.21E-06	0.00011	23.28	0.3424	2.24E-05	1.63E-05	1.19E+01	2.98E-01	-3.62E-07	6.00E-06

Study 2: Model 1 Parameters

			A			B			C			R	
Date	Specimen	b	dinf	tau	B	dinf	tau	b	dinf	tau	b	dinf	tau
07/12/06	10B	0.8	0.4086	1028	0.903	0.4122	1126	0.9279	0.4151	1156	0.937	0.2876	4366
07/14/06	12A	0.7083	0.4717	1398	0.6803	0.4768	1258	0.6941	0.4795	1242	0.9006	0.3257	6419
07/21/06	12B	0.7145	0.3563	849.6	0.7346	0.3603	687.2	0.7845	0.3635	703.6	0.9089	0.2571	5903
07/23/06	13A	0.7374	0.5713	1234	0.7684	0.5766	1147	0.7933	0.5793	1116	0.8521	0.3617	6273
07/24/06	13B	0.8475	0.4552	1043	0.7105	0.459	885	0.7344	0.4611	850.9	0.8567	0.3125	6368
07/25/06	14A	0.7866	0.4778	1003	0.4548	0.4834	460.9	0.4415	0.485	337.8	0.9035	0.3236	6098
07/26/06	14B	0.8071	0.4432	1381	0.7415	0.4467	1356	0.7275	0.449	1309	0.9111	0.3023	6589
07/27/06	15A	0.8477	0.4204	1688	0.7016	0.4239	1640	0.6801	0.4269	1659	0.8457	0.281	7489
08/02/06	15B	0.8239	0.3846	1484	0.7414	0.3888	1370	0.7086	0.3911	1284	0.8605	0.2654	7319
08/04/06	16A	0.7536	0.4448	1483	0.6217	0.4517	1664	0.6197	0.4533	1507	0.9324	0.2753	6819
08/07/06	16B	0.7545	0.4863	1464	0.7363	0.4905	1327	0.7578	0.4944	1282	0.8363	0.3073	5963
08/08/06	17A	0.7619	0.4195	1297	0.7312	0.4234	1030	0.736	0.426	1013	0.9253	0.2767	5505
08/09/06	17B	0.8463	0.3794	1413	0.8548	0.3844	1327	0.8857	0.3866	1335	0.9102	0.241	5038

Study 2: Model 2 Parameters

			A			B			C			R	
Date	Specimen	E1	E2	U	E1	E2	U	E1	E2	U	E1	E2	U
07/12/06	10B	0.6527	2.213	627.1	1.501	2.673	1223	1.499	2.653	1207	0.648	-3.124	3706
07/14/06	12A	0.5148	1.929	757	0.5034	2.33	739.3	0.5234	2.316	717.6	0.4795	-2.751	3873
07/21/06	12B	0.6608	2.542	600.9	0.7631	3.065	583.1	0.752	3.033	548.2	0.5618	-3.489	4128
07/23/06	13A	0.4814	1.588	607.4	0.3629	1.915	459.7	0.3152	1.905	375.3	0.4695	-2.471	3919
07/24/06	13B	0.5012	1.982	490.9	0.146	1.965	166.4	0.146	1.965	166.4	0.5341	-2.859	4448
07/25/06	14A	0.4621	1.891	466.3	0.1016	1.871	155.6	0.09452	1.863	116.6	0.4679	-2.77	3557
07/26/06	14B	0.6091	2.043	760.9	0.1934	2.023	293.2	0.1675	2.012	252.8	0.652	-2.961	5694
07/27/06	15A	0.7099	2.154	1001	0.1914	2.136	340.5	0.1638	2.121	305.6	0.6959	-3.162	6910
08/02/06	15B	0.8035	2.357	1001	0.2254	2.325	344.1	0.1896	2.31	288.4	0.7593	-3.353	7416
08/04/06	16A	0.6901	2.043	972.3	0.217	2.017	396.9	0.1831	2.004	331.4	0.8375	-3.252	7826
08/07/06	16B	0.656	1.882	866.9	0.4402	2.26	605.3	0.3899	2.236	544.2	0.6123	-2.904	5064
08/08/06	17A	0.6451	2.163	801.7	0.8591	2.614	971.6	0.8151	2.597	902.1	0.7383	-3.243	5520
08/09/06	17B	0.9113	2.386	1049	1.537	2.875	1559	1.394	2.855	1405	0.8465	-3.723	5881

Study2: Model 3 Parameters

			A			B			C			R	
Date	Specimen	D	G	k	D	G	K	D	G	k	D	G	k
07/12/06	10B	9.945	1.63E-05	3.74E-05	7.403	3.95E-06	6.49E-05	7.33E+00	2.37E-06	6.50E-05	1.22E+01	-1.84E-06	5.76E-06
07/14/06	12A	9.639	2.56E-05	3.63E-05	13.64	2.80E-05	3.86E-05	1.32E+01	3.16E-05	4.31E-05	1.36E+01	-3.07E-06	4.18E-06
07/21/06	12B	12.71	2.42E-05	4.82E-05	15.43	2.01E-05	6.13E-05	1.53E+01	1.55E-05	6.18E-05	1.87E+01	-2.24E-06	3.98E-06
07/23/06	13A	7.019	1.96E-05	4.22E-05	11.62	3.25E-05	5.00E-05	1.37E+01	1.75E-05	3.67E-05	1.12E+01	-4.84E-06	3.51E-06
07/24/06	13B	9.841	1.05E-05	4.09E-05	28.61	2.50E-05	1.50E-05	3.08E+01	2.09E-05	1.39E-05	1.32E+01	-4.42E-06	4.08E-06
07/25/06	14A	9.799	1.55E-05	3.79E-05	29.99	3.79E-05	1.55E-05	3.42E+01	3.33E-05	1.41E-05	1.43E+01	-3.54E-06	3.40E-06
07/26/06	14B	9.235	1.64E-05	2.95E-05	24.249	2.43E-05	1.05E-05	2.78E+01	2.41E-05	9.19E-06	1.08E+01	-2.03E-06	3.99E-06
07/27/06	15A	9.098	1.57E-05	3.43E-05	28.72	3.04E-05	1.06E-05	3.24E+01	2.85E-05	8.93E-06	1.22E+01	-5.43E-06	4.65E-06
08/02/06	15B	9.783	1.80E-05	3.56E-05	27.9	2.67E-05	1.25E-05	3.21E+01	2.67E-05	1.10E-05	1.21E+01	-3.87E-06	4.72E-06
08/04/06	16A	8.347	2.66E-05	3.41E-05	23.43	3.71E-05	1.02E-05	2.63E+01	3.55E-05	9.34E-06	9.73E+00	-2.49E-06	4.26E-06
08/07/06	16B	7.745	2.43E-05	3.68E-05	14.81	3.16E-05	3.22E-05	1.55E+01	2.47E-05	3.00E-05	1.14E+01	-4.31E-06	4.06E-06
08/08/06	17A	9.811	1.98E-05	3.58E-05	10.35	1.86E-05	5.41E-05	1.08E+01	1.69E-05	5.19E-05	1.10E+01	-3.09E-07	5.35E-06
08/09/06	17B	8.943	1.45E-05	3.38E-05	8.282	7.16E-06	5.08E-05	8.75E+00	6.99E-06	4.95E-05	1.28E+01	-1.46E-06	4.83E-06

Appendix E

R^2 Tables

Study 1

			Model 1			Model 2			Model 3	
Specimen	C Stress	C	E	R	C	E	R	C	E	R
1A	-0.50	1	0.9995		0.9999	0.9986		0.9999	0.9994	
1B	-0.50	1	0.9995	0.9999	0.9999	0.9988	0.9999	0.9999	0.9994	0.9999
2A	-0.50	1	0.9994	0.9999	0.9999	0.9986	0.9999	0.9999	0.9995	1
2B	-0.50	1	0.9994	0.9999	0.9999	0.9988	0.9999	0.9999	0.9991	1
3A	-0.50	1	0.9996	1	0.9999	0.999	1	0.9999	0.9995	1
3B	-0.50	1	0.9996	0.9999	0.9999	0.9991	0.9999	0.9999	0.9993	0.9999
4A	-0.30	1	0.9995	0.9999	0.9999	0.9995	0.9999	0.9999	0.9995	0.9999
4B	-0.30	1	0.9992	0.9999	0.9999	0.9963	0.9999	0.9999	0.9993	0.9999
5A	-0.30	1	0.9997	0.9999	0.9999	0.9986	0.9999	0.9999	0.9996	0.9999
5B	-0.30	1	0.9997	0.9999	0.9998	0.9989	0.9998	0.9999	0.9995	0.9999
6A	-0.30	1	0.9996	0.9999	0.9999	0.9984	0.9999	0.9999	0.9995	0.9999
6B	-0.30	1	0.9996	0.9999	0.9999	0.9986	0.9999	0.9999	0.9995	0.9999
7A	-0.10	1	0.9999	0.9999	0.9998	0.9996	0.9999	0.9999	0.9997	0.9999
7B	-0.10	1	0.9998	0.9999	0.9998	0.9997	0.9999	0.9998	0.9999	0.9999
8B	-0.10	1	0.9999	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999
9A	0.00		0.9999	0.9999		0.9996	0.9999		0.9998	0.9999
9B	0.00		1	0.9999		0.9998	0.9999		0.9998	0.9999
10A	0.00		1	0.9999		0.9999	0.9999		0.9999	0.9999
18A	0.00		1	0.9999		0.9998	0.9999		0.9998	0.9999

18B	0.00		1	0.9998		0.9997	0.9998		0.9999	0.9998
19A	0.00		1	0.9999		0.9997	0.9998		0.9997	0.9998
19B	-0.10	1	0.9999	0.9999	0.9998	0.9998	0.9999	0.9999	0.9998	0.9999
20A	-0.10	0.9999	0.9999	0.9999	0.9998	0.9998	0.9999	0.9999	0.9997	0.9999
20B	-0.10	1	0.9999	0.9999	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999
21A	**-0.50**		0.9999	0.9999		0.9998	0.9999		0.9999	0.9999
21B	**-0.50**		0.9999	0.9999		0.9998	0.9999		0.9999	0.9999
22A	**-0.50**		0.9999	0.9999		0.9997	0.9999		0.9999	0.9999
22B	**-0.50**		0.9999	0.9999		0.9999	0.9999		0.9999	0.9999
23A	**-0.50**		0.9998	0.9999		0.9998	0.9998		0.9999	0.9999
23B	**-0.50**		0.9992	0.9995		0.9998	0.9995		0.9997	0.9996
24A	**-0.30**		0.9999	0.9999		0.9997	0.9999		0.9999	0.9999
24B	**-0.30**		0.9999	0.9999		0.9998	0.9999		0.9999	0.9999
25A	**-0.30**		1	0.9999		0.9999	0.9999		0.9999	0.9999
25B	**-0.30**		0.9999	0.9999		0.9999	0.9998		0.9999	0.9999
26A	**-0.30**		0.9999	0.9999		0.9999	0.9999		0.9999	0.9998
26B	**-0.30**		0.9999	0.9999		0.9999	0.9999		0.9999	0.9999
27A	**-0.10**		0.9999	0.9999		0.9999	0.9999		0.9999	0.9999
27B	**-0.10**		0.9999	0.9999		0.9999	0.9998		0.9999	0.9998
28A	**-0.10**		1	0.9999		0.9999	0.9998		0.9999	0.9999
28B	**-0.10**		1	0.9999		0.9999	0.9999		0.9999	0.9999
29A	**-0.10**		1	0.9999		0.9999	0.9999		0.9999	0.9999
29B	**-0.10**		0.9999	0.9999		0.9999	0.9999		0.9999	0.9999
30B	-0.30	1	0.9996	0.9999	0.9999	0.9991	0.9998	0.9999	0.9996	0.9999
31A	-0.30	1	0.9995	0.9999	0.9999	0.9984	0.9999	0.9999	0.9996	0.9999
32A	-0.30	1	0.9995	0.9999	0.9999	0.9982	0.9999	0.9999	0.9996	0.9999

Study 2

			Model 1				Model 2				Model 3		
Date	Specimen	A	B	C	R	A	B	C	R	A	B	C	R
07/12/06	10B	0.9998	0.9998	0.9998	0.9998	0.9993	0.9995	0.9995	0.9998	0.9998	0.9992	0.9992	0.9998
07/14/06	12A	0.9999	0.9984	0.9945	0.9999	0.9996	0.9983	0.9982	0.9999	0.9998	0.9976	0.9976	0.9999
07/21/06	12B	0.9996	0.9995	0.9996	0.9999	0.9977	0.9972	0.9975	0.9999	0.9998	0.9972	0.997	0.9998
07/23/06	13A	0.9999	0.9985	0.9963	0.9999	0.9996	0.9973	0.9974	0.9999	0.9997	0.9977	0.9992	0.9998
07/24/06	13B	0.9998	0.9993	0.9992	0.9999	0.9996	0.9977	0.9977	0.9999	0.9997	0.9988	0.9984	0.9999
07/25/06	14A	0.9997	0.9992	0.999	0.9999	0.9993	0.9977	0.9964	0.9998	0.9997	0.9983	0.9978	0.9998
07/26/06	14B	0.9999	0.9996	0.9995	0.9999	0.9997	0.9991	0.999	0.9999	0.9998	0.9992	0.9991	0.9999
07/27/06	15A	0.9999	0.9996	0.9994	0.9999	0.9998	0.9991	0.9991	0.9999	0.9998	0.9991	0.9989	0.9999
08/02/06	15B	0.9999	0.9996	0.9995	0.9999	0.9997	0.9992	0.9999	0.9999	0.9999	0.9993	0.9992	0.9999
08/04/06	16A	0.9999	0.9995	0.9994	0.9999	0.9997	0.9989	0.9989	0.9999	0.9998	0.999	0.9987	0.9999
08/07/06	16B	0.9999	0.9998	0.995	0.9999	0.9996	0.9974	0.9965	0.9999	0.9998	0.9995	0.9958	0.9999
08/08/06	17A	0.9999	0.9992	0.9991	0.9999	0.9996	0.9989	0.9988	0.9999	0.9998	0.9984	0.9983	0.9999
08/09/06	17B	0.9999	0.9993	0.9991	0.9999	0.9997	0.9993	0.9992	0.9999	0.9998	0.9989	0.9986	0.9999

Bibliography

[1] Lee CR, Iatridis JC, Poveda L, Alini M. *In Vitro* Organ Culture of the Bovine Intervertebral Disc: Effects of Vertebral Endplate and Potential for Mechanobiology Studies. Spine 31(5), 2006. 515-522

[2] Urban JPG, Roberts S. Review: Degeneration of the intervertebral disc. Arthritis Res Ther 5(3), 2003. 120-130

[3] Iatridis JC, Mente PL, Stokes IAF, Aronsson DD, Alini M. Compression-Induced Changes in Intervertebral Disc Properties in a Rat Tail Model. Spine 24(10), 1999. 996-1002

[4] Johannessen W, Vresilovic EJ, Wright AC, Elliott DM. Intervertebral Disc Mechanics Are Restored following Cyclic Loading and Unloaded Recovery. Ann Biomed Eng 32(1), 2004. 70-76

[5] Wilke HJ, Neef P, Caimi M, Hoogland T, Claes LE. New *In Vivo* Measurements of Pressures in the Intervertebral Disc in Daily Life. Spine 24(8), 1999. 755-762

[6] An HS, Anderson PA, Haughton VM, Iatridis JC, Kang JD, Lotz JC, Natarajan RN, Oegema TR, Roughley P, Setton LA, Urban JP, Videman T, Andersson GBJ, Weinstein JN. Introduction: Disc Degeneration: Summary. Spine 29(23), 2004. 2677-2678

[7] MacLean JJ, Owen JP, Iatridis JC. Role of endplates in contributing to compression behaviors of motion segments and intervertebral discs. J Biomech 40(1), 2007. 55-63

[8] Bruehlmann SB, Matyas JR, and Duncan NA. ISSLS prize winner: Collagen fibril sliding governs cell mechanics in the anulus fibrosus: an in situ confocal microscopy study of bovine discs. Spine 29(23), 2004. 2612-2620

[9] Gillard DM. "Disc Anatomy" in "ChiroGeek.com". Dr. Douglas M. Gillard DC: 2005. April 18, 2007. <http://www.chirogeek.com/000_Disc_Anatomy.htm>

[10] Guehring T, Omlor GW, Lorenz H, Engelleiter K, Richter W, Carstens C, Kroeber M. Disc Distraction Shows Evidence of Regenerative Potential in Degenerated Intervertebral Discs as Evaluated by Protein Expression, Magnetic Resonance Imaging, and Messenger Ribonucleic Acid Expression Analysis. Spine 31(15), 2006. 1658-1665

[11] Karakida O, Ueda H, Ueda M, and Miyasaka T. Diurnal T2 value changes in the lumbar intervertebral discs. Clin Radiol 58(5), 2003. 389-392

[12] Nachemson AL. Disc Pressure Measurements. Spine 6(1), 1981. 93-97

[13] Race A, Broom ND, Robertson P. Effect of Loading Rate and Hydration on the Mechanical Properties of the Disc. Spine 25(6), 2000. 662-669

[14] van der Veen AJ, van Dieën JH, Nadort A, Stam B, Smit TH. Intervertebral disc recovery after dynamic or static loading in vitro: Is there a role for the endplate? J Biomech, 2006.

[15] MacLean JJ, Lee CR, Alini M, Iatridis JC. The effects of short-term load duration on anabolic and catabolic gene expression in the rat tail intervertebral disc. J Orthop Res 23, 2005. 1120-1127

[16] Hsieh AH, Wagner DR, Cheng LY, and Lotz JC. Dependence of mechanical behavior of the murine tail disc on regional material properties - a parametric finite element study. J Biomech Eng 127, 2005. 1158-1167

[17] Berry GC, Plazek DJ. On the use of stretched-exponential functions for both linear viscoelastic creep and stress relaxation. Rheol Acta 36, 1997. 320-329

[18] Cassidy JJ, Silverstein MS, Hiltner A, Baer E. A water transport model for the creep response of the intervertebral disc. J Mater Sci: Mater Med 1(2), 1990. 81-89

[19] Palmer EI, Lotz JC. The compressive creep properties of normal and degenerated murine intervertebral discs. J Orthop Res 22, 2004. 164-169

[20] Kroeber M, Unglaub F, Guegring T, Nerlich A, Hadi T, Lotz J, Carstens C. Effects of Controlled Dynamic Disc Distraction on Degenerated Intervertebral Discs: An *in Vivo* Study on the Rabbit Lumbar Spine Model. Spine 30(2), 2005.

[21] de Leva P. Adjustments to Zatsiorsky-Seluyanov's Segment Inertia Parameters. J Biomech 29(9), 1996. 1223-1230

[22] Haughton V. Imaging Intervertebral Disc Degeneration. J Bone Joint Surg Am 88, 2006. 15-20

[23] Iatridis JC, Laible JP, and Krag MH. Influence of fixed charge density magnitude and distribution on the intervertebral disc: applications of a poroelastic and chemical electric (PEACE) model. J Biomech Eng 125(1), 2003. 12-24

[24] Setton LA, Chen J. Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration. J Bone Joint Surg Am 88, 2006. 52-57