AN ABSTRACT OF THE THESIS OF

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Title: Mechanical Characterization of a Simple Gel in a Prototype Device that Models a Degenerative Intervertebral Disc.

Abstract approved:

Brian K. Bay

A large part of low-back pain occurrences are due to disc herniation or prolapse of the nucleus pulposus. An alternative technique to existing treatments of low back pain includes nucleus pulposus replacement by an artificial material to relieve pain and restore normal function. To date, there has been a deficiency in testing new materials in a physiologically accurate model of a degenerative intervertebral disc. The goals of this study were to use a simple gel system in a simple geometry simulating the disc space to identify how the extrudability of the gel changed as its characteristics were altered and compare those to the native tissue as well as determining the experimental reproducibility and relationship to measurable rheological properties. A prototype device modeling a degenerative intervertebral disc was constructed in Plexiglas^{TM}, which consisted of a circular

disc shape with a lateral channel to simulate an annular tear. Agarose gels of varying weight percent were made and samples were tested in compression in the degenerative disc device. The rheological properties of the gels were measured in a parallel-plate dynamic oscillatory shear experiment. The compressive moduli and rheological properties were compared with those of the native tissue. The elastic and viscous moduli of the agarose gel did not completely match those of nucleus pulposus, but the static compressive modulus was similar in the two materials. The compression experiments conducted showed reproducible results and a correlation to rheological properties of the simple polymer gel system. Since a test protocol was established, the extrudability of more complex polymer gel systems can be tested on the degenerative disc device and related to its measurable rheological properties.

MECHANICAL CHARACTERIZATION OF A SIMPLE GEL IN A PROTOTYPE DEVICE THAT MODELS A DEGENERATIVE INTERVERTEBRAL DISC

by

Yannick Cenar

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APPROVED:

Major Professor, representing Mechanical Engineering

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Yannick Cenar, Author

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It has been a very long journey for me and I don't think I could have gotten very far without the support of those people close to me. I want to first thank my family for the life-long support they have given me. I don't know where I would be if my parents had not taken the risk of a lifetime to pack everything in a few suitcases and make the ocean-crossing trip to come to the United States almost 15 years ago. That gave me more opportunities than I could ever ask for. They pushed me as far as the stars reach and always knew that I could get even further if I just put my mind to it. On that note I cannot forget my sister, Cynthia, for being nothing but a ray of sunshine when things were tough. Little girls provide some of the best therapy, especially when thesis writing has you down. I owe the deepest thanks to both my parents and my sister. Thank you.

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If there is anyone I left out that helped me along the way, thank you!

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INTRODUCTION

The current methodology for relieving lower back pain is to give patients short term pain relief, but most often lower back problems are recurring, so a long term solution needs to be found. In most cases of recurring pain, there is damage to an intervertebral disc that is pretty severe and that is causing all the pain in the area. Some surgeons have gone as far as removing all disc tissue, with no regard to how degraded it was, and placed an artificial disc in its place. Such a surgery is highly invasive and success rates have been pretty low. A better way to treat the problem would be to catch the condition early on when there is not as much damage to the disc tissue, and simply replace the inner core of the disc, known as the nucleus pulposus. For the past few decades doctors and scientists have been making different devices and formulations of materials such as hydrogels in an effort to use them as replacements for the nucleus pulposus.

Development of these new materials for replacement of the nucleus pulposus has progressed quite well, but the testing aspect has had some issues along the way. The gel systems have undergone some static and dynamic tests but

there has not been much effort put into the testing of the gel systems when subjected to physiological loads in a physiologically accurate model of a degenerative intervertebral disc. If a material is identified as suitable for the human body and has mechanical characteristics that are similar to that of the native tissue, simply implanting it into the body does not guarantee that it will not fail just like the native tissue did. This study looked at the extrudability of a simple gel system from a degenerative intervertebral disc device under compression and then compared those results with viscoelastic results obtained through dynamic rheological testing. The goals of the research were to use a simple gel system to identify how the extrutabilty of the basic polymer gel changed as its basic characteristics were altered, like weight percent, then compare those to the rheological properties of the gel, tie it all together to how the native tissue reacts under the same conditions all while most importantly check for experimental reproducibility. The experimental reproducibility and relationship to measurable rheological properties are the key components in this study making it possible to test more complex gel systems being identified as possible replacements for nucleus pulposus material in the degenerative disc device created.

BACKGROUND

Vertebral Column

The adult vertebral column consists of 24 vertebrae, the sacrum, and the coccyx. These vertebrae provide the support column which bears the weight of the head, neck, and trunk and also transfers that weight below to the lower limbs. The vertebrae also help protect the very fragile spinal cord from being damaged and keep the body in upright positions. The vertebral column is divided into five regions: cervical, thoracic, lumbar, sacral, and coccygeal region (Van De Graaff 2002). There are seven cervical vertebrae which constitute the neck and extend into the trunk. There are twelve thoracic vertebrae which form the superior portion of the back. Then five lumbar vertebrae form the inferior portion of the back. L_5, the most inferior of the lumbar vertebrae articulates with the sacrum, which then articulates with the coccyx. The vertebrae found in the lumbar region of the spine are the largest of all the other vertebrae.

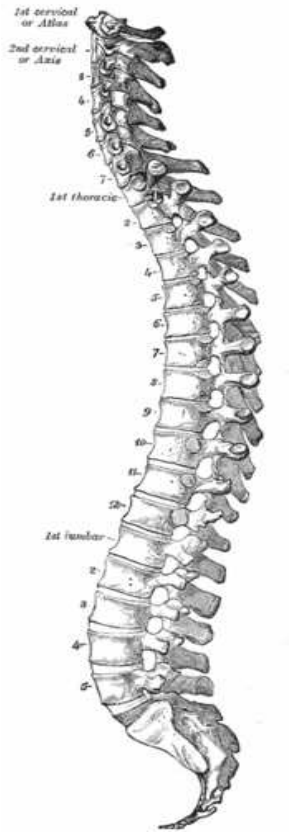

Figure 1: Lateral view of the vertebral column (Gray 1918).

The anterior part of each vertebra is a thick block called the vertebral body. The vertebral body is kidney shaped with a flat top and bottom and has slightly concave anterior and lateral surfaces. Projecting from the back of the vertebral body are the pedicles, which are small pillars of bone. From each pedicle, a sheet of bone called the lamina projects toward the midline. The two laminae fuse with one another at the midline and they form into a roof-top shape when viewed from above. Posteriorly from the junction of the laminae is a narrow bone called the spinous process. Laterally, from the junction of the pedicle and the lamina, extends a flat bar of bone called the transverse process. The vertebral body serves as the

weight bearer of the vertebra. It is not a solid block of bone, but just a shell of cortical bone surrounding a cancellous cavity which supports longitudinally applied loads.

Intervertebral Discs

Anatomy

The vertebral bodies do a great job of supporting weight, but on their own they cannot move together very well. In between the vertebral bodies, there exists a layer of strong, but deformable soft tissue called the intervertebral disc. There are two basic components that make up the intervertebral discs: the central nucleus pulposus and the surrounding annulus fibrosus. There is no clear boundary between the nucleus and annulus within the disc even though the nucleus is very distinct at the center and the annulus is very distinct on the outer edges (Bogduk 1997). The outer parts of the nucleus merge with the innermost parts of the annulus to create a continuum within the disc. The top and bottom of the intervertebral disc is covered by a layer of cartilage called the vertebral end-plate which separates the disc from the vertebral bodies.

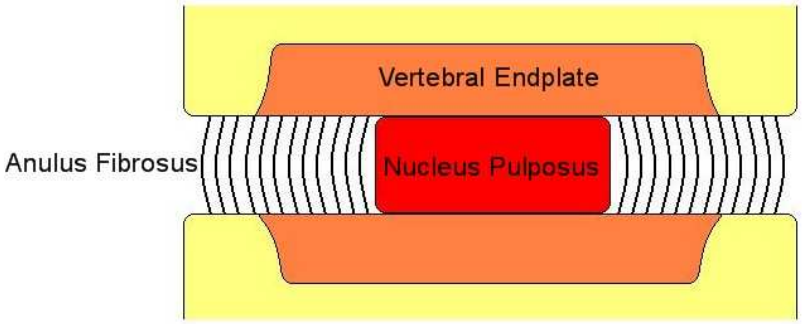

Figure 2: Cross section diagram of intervertebral disc with vertebral end plates.

In a typical healthy adult, the nucleus pulposus is a semi-fluid mass of mucoid material (Bogduk 1997). From a histological standpoint, the nucleus pulposus consists of some cartilage cells and some irregularly placed collagen fibers, scattered in a semi-fluid ground substance. The fluid nature allows the nucleus to be deformed when pressure is applied. Biomechanically this means that if the nucleus is subjected to compression, it will try to deform and transmit the pressure in all directions.

The nucleus pulposus consists of approximately 70-90% water which can range based on age (Naylor 1971; Beard and Stevens 1980). The next major component in the microstructure of the nucleus are proteoglycans, which make up about 65% of the dry weight (Beard and Stevens 1980). Proteoglycans form complex, three-dimensional molecules, which have the property of attracting and retaining water. The volume enclosed by a proteoglycan into which it retains water is know as its domain (Bogduk 1997). The water inside the nucleus pulposus is contained within the domains of the proteoglycans. Throughout the proteoglycans,

thin fibrils of type II collagen can be found which serve to hold together proteoglycan aggregates (Bogduk 1997). The mixture of proteoglycans, their aggregates, and collagen fibers is known as the matrix of the nucleus pulposus. The collagen in the nucleus constitutes about 15-20% of its dry weight (Beard and Stevens 1980). The remainder of the nucleus is made up of elastic fibers and non-collagenous proteins. Implanted within the proteoglycan medium are cartilage cells, or chondrocytes. They are responsible for the synthesis of proteoglycans and collagen for the nucleus pulposus (Urban and Maroudas 1980). As stated above, the nucleus pulposus is mainly filled with type II collagen. Type II collagen is more elastic in nature and is generally found in tissues that are usually subjected to pressure. The annulus fibrosus on the other hand is typically made up of both type I and II collagen because the annulus is involved in tension and pressure type processes (Bogduk 1997).

The collagen found in the annulus fibrosus typically makes up about 50-60% of its dry weight (Beard and Stevens 1980). These collagen fibers are arranged in a very structured pattern. The fibers are arranged in between 10 and 20 sheets that are called lamellae (Taylor 1990). The lamellae are arranged in concentric rings which surround the nucleus pulposus. Towards the center of the disc, and in the anterior and lateral portions of the annulus the lamellae are thicker as opposed to the posterior portion of the annulus where the lamellae are packed tighter and therefore are thinner (Bogduk 1997). The collagen fibers within each lamella are parallel to one another and are oriented at 30° from the horizontal

traveling from the vertebral body below to the vertebral body above. The 30° orientation alternates relative to the longitudinal axis of the spine as you go from the outer portion of the annulus towards the nucleus as shown in fig. 3. Not every lamella forms a complete ring around the nucleus. In any one of the four quadrants of the annulus, up to 40% of the lamellae are incomplete (Marchand and Ahmed 1990).

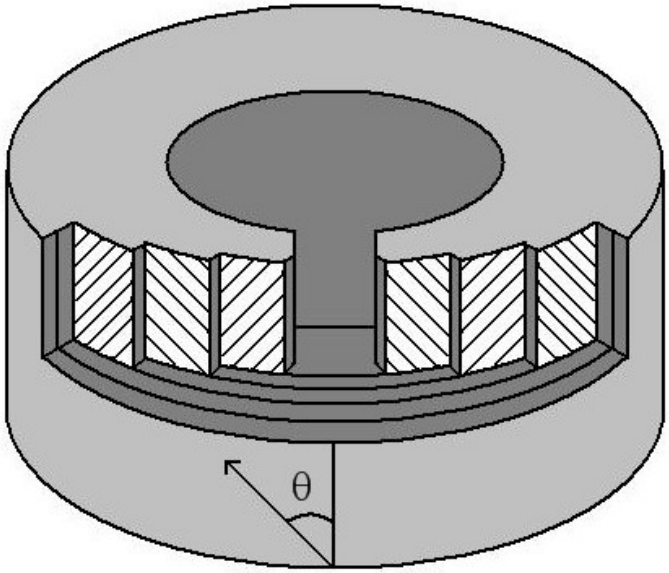

Figure 3: Alternating collagen fibers of the annulus fibrosus.

Like in the nucleus pulposus, the main component of the annulus fibrosus is water, making up 60-70% of its weight (Beard and Stevens 1980). Proteoglycans then make up 20% of the dry weight of the annulus (Beard and Stevens 1980). A proteoglycan gel fills the spaces between collagen fibers and binds the fibers and the lamellae together (Bogduk 1997). This binding prevents them from buckling. The concentration of water and proteoglycans increases as

you move from the outer to the inner annulus, and the concentration of collage decreases as you move from the outer to the inner annulus (Best *et al.* 1994). In between the collagen fibers and lamellae few chondrocytes and many fibroblasts are found which are responsible for the synthesis of the collagen and proteoglycan gel which the annulus consists of (Best *et al.* 1994). The fibroblasts are located towards the outer region of the annulus, while the chondrocytes are usually found in the inner region of the annulus towards the nucleus pulposus which has a much higher concentration of chondrocytes and virtually no fibroblasts.

The nucleus pulposus and the annulus fibrosus are similar structures from certain biochemical standpoints. Both are made up of water, proteoglycans, and collagen; however, what makes them different are the relative concentrations of those components and the particular type of collage that exists in the structure. The annulus fibrosus consists of proteoglycans and a large amount of water, but it is thickened by a high concentration of inelastic type I collagen in the outer annulus. As you approach the nucleus pulposus, type I collagen concentration decreases and type II collagen concentration increases because the nucleus mainly consists of hydrophilic proteoglycans and water with some type II collagen (Mow and Hayes 1997).

The vertebral end-plates are layers of cartilage that are around 0.6-1.0 mm thick which are attached to the vertebral body enclosed by the ring apophysis (Bogduk 1997). The end-plates of each disc cover the nucleus pulposus completely, but do not cover the entire annulus fibrosus. The end-plate consists of both hyaline

cartilage and fibrocartilage, with hyaline cartilage occurring towards the vertebral body and in young discs and fibrocartilage being found predominantly towards the nucleus pulposus and in older discs. The chemical structure is very similar to that of the disc itself. It mainly consists of water, proteoglycans, and collagen fibers that have a similar concentration profile as you move from the outer edge towards the center just like the disc itself. The tissue that is closer to the bone has more collagen, and the tissue that is nearer the nucleus pulposus has more proteoglycans and water (Bogduk 1997). This helps pass small molecules through the process of diffusion since a fairly uniform medium exists.

Metabolism

Inside the intervertebral disc, old proteoglycans and collagen are routinely removed and replaced by new ones. These activities necessitate the cells to be metabolically active. The cells need oxygen, glucose, and other substrates for the processes that are being carried out. However, the intervertebral discs are deficient in a real blood supply, being the largest avascular structure in the human body (Mow and Hayes 1997). The cells rely on nutrients coming to them through diffusion. The diffusion comes from the two nearest available systems of vessels, those in the outer annulus fibrosus and the capillary plexuses underneath the end-plates. Cells in the center of disc lie as far as 8 mm from the closest blood supply (Ferguson *et al.* 2004). If there is a deficiency in the supply of nutrients, disc degeneration can occur at much quick rates (Ferguson *et al.* 2004). In order to get to the nucleus pulposus, nutrients like oxygen, glucose, and other molecules have

to diffuse across the matrix of the vertebral end-plate or the annulus fibrosus. Then nutrients must also filter through the proteoglycans matrix to get to the nucleus (Bader 2006). That is why the rate of diffusion depends on the concentration gradient of the substance in question, the resistance to diffusion by the annulus or vertebral end-plates, and the resistance to diffusion by the matrix inside the nucleus. The annulus fibrosus is moderately permeable to most substance, but only a small central portion of the end-plates is permeable (Maroudas 1988). Because of surface area differences, it appears that the relative amount of nutrients coming from the annulus and the end-plates is about the same (Maroudas 1988). Previous studies have shown that diffusion alone satisfies the nutritional needs of the disc for small molecules like oxygen and glucose (Urban *et al.* 1982; Katz *et al.* 1986). More recently, it has been proposed that fluid flow into and within the disc augments the transport of larger molecules (Urban *et al.* 1982; Ferguson *et al*. 2004). The disc is under compression for about 16 hours each day from daily activities and since the disc is poroelastic, fluid flow is coupled to the deformation (Ferguson *et al.* 2004). About 10-20% of the total disc volume is cycled over the course of a single 24 hour period due to the daily fluid loss due to loading which is restored during rest at night (Ferguson *et al*. 2004).

Functions of the Disc

The intervertebral disc has two main functions: weight bearing and accommodating movement between vertebrae. The nucleus pulposus and annulus fibrosus play equally pivotal parts in sustaining any weight that is being held. The

annulus acts on its own and in also in conjunction with the nucleus. Even though the annulus is made up of almost 70% water, it has densely packed collagen fibers which make it fairly stiff. The collagen lamellae make the annulus bulky. When the lamellae are healthy, the annulus can resist buckling and can uphold weight passively. The inner portion of the annulus is usually not as stiff as the outer portion (Best *et al.* 1994). Some studies have shown that under smaller and briefer loads, an intervertebral disc that has had its nucleus extracted can hold the same axial load as one that is intact (Best *et al.* 1994). Over time, a disc without a nucleus would deform greatly. The lamellae would buckle under a sustained load and its water content would decrease. The height of the disc would decrease further and more deformation would occur if pressure was sustained. It is for this reason that the nucleus pulposus is so valuable to the intervertebral disc. The nucleus is believed to provide internal fluid pressurization in response to compressive loads (Iatridis *et al.* 1997). When a weight is applied to the nucleus, it will tend to reduce in height as it expands radially. The expansion in the radial direction places a pressure on the annulus that tends to make the lamellae stretch outwards, but the tensile properties of the lamellae resist the stretch (Bogduk 1997). In healthy discs, for any load that is applied, equilibrium is achieved where the radial pressure exerted by the nucleus is balanced by the tension in the annulus (Bogduk 1997). The annulus is so thick and strong that if a 40 kg load is applied to a healthy adult disc, there is only a 1 mm vertical compression and 0.5 mm of radial compression (Bogduk 1997).The nucleus also exerts its pressure in the

vertical direction. The pressure that is exerted on the vertebral end-plates is how the load is transmitted from one vertebra to the next which lowers the load that the annulus has to hold. This helps preserve the state of the nucleus and the annulus.

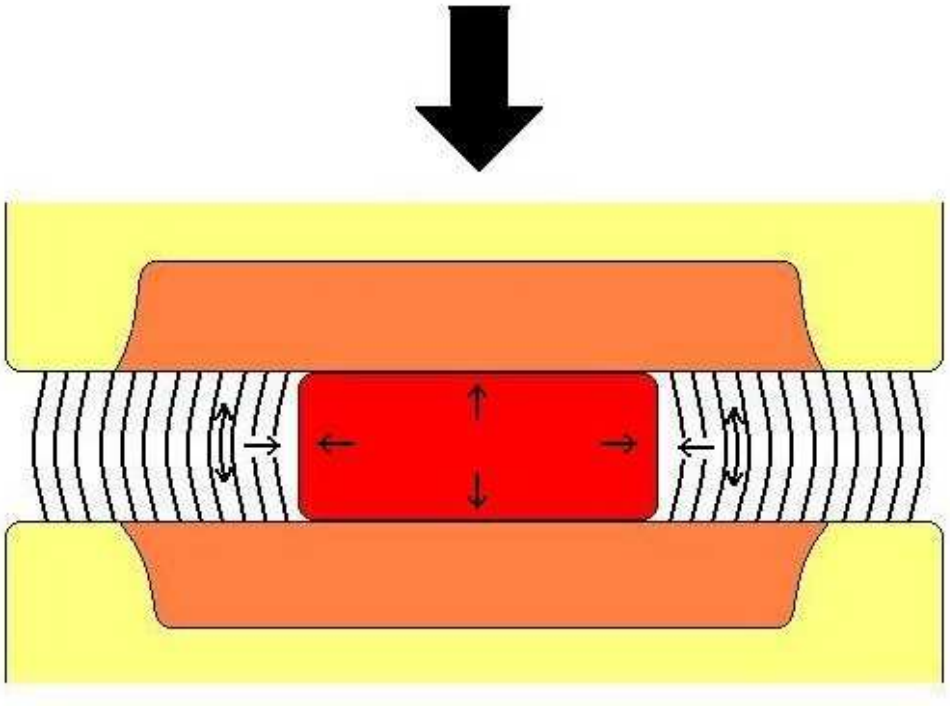

Figure 4: Vertical load distribution on the intervertebral disc.

The pressure of the nucleus pulposus was measured *in vitro* with pressure transducers and it ranged from 0.1 MPa to 0.3 MPa without being loaded externally and from 1-3 MPa under varying loading conditions (Iatridis *et al.* 1996). Measurements *in vivo* showed that nucleus pulposus pressures are dependant on loading conditions and posture, which is largest when sitting and smallest when recumbent (Iatridis *et al.* 1996). Most studies have identified the nucleus pulposus as an inviscid, incompressible fluid because it exhibits fluid-like behavior under static axial loading conditions. Others identified the nucleus

pulposus as a poroelastic solid with the following material properties: Young's modulus = 4.5-1500 kPa; Poisson's ratio=0.1 – 0.45 (Argoubi and Shirazi-Adl 1996). Neither of these classifications provides a complete depiction of the nucleus pulposus. The nucleus pulposus is now classified as a viscoelastic material due to the work done by Iatridis et. al. in a study where its behavior was examined under torsional shear (Iatridis *et al.* 1996). Differences between viscoelastic materials are much more noticeable in shear experiments than more conventional loadings like axial compression (Bader 2006). Fluids are commonly defined as materials that move and deform continuously as long as shear stress is applied or that no stress is needed to maintain a change of shape. For solids, any deformations that change their shape would require a continued application of stress (Iatridis *et al.* 1996). This distinction between fluid and solid is even more difficult for biological tissues because most of them have both components depending on the rates of loading. Iatridis et. al. subjected nucleus samples to dynamic oscillatory shear and transient stress relaxation experiments and found that under the dynamic loading the complex shear moduli and phase shift angles were more closely related to those of biological solids (Iatridis *et al.* 1996). When transient loading experiment were run, the nucleus pulposus tissue behaved more like a biological fluid having the shear stress relaxation near zero (Iatridis *et al.* 1996). This experiment showed such different behavior by the nucleus under different loading modes that is has to be classified as a viscoelastic material and not as an inviscid, incompressible fluid or as a poroelastic solid.

The second main function of the intervertebral disc is to aid in movement. If unrestricted by any posterior elements of the vertebrae, two vertebral bodies joined by an intervertebral disc can move in any direction (Bogduk 1997). Deformation of the disc allows for all the movements to take place, but the disc also confers varying degrees of stability to the inter-body joint during movements.

Disc Degeneration

One of the most drastic changes in the spine occurs in the nucleus pulposus. Changes in the biochemistry are most dramatic from the time of infancy to about the age of 10 (Bogduk 1997). These changes seem to be triggered by the regression in infancy of the inadequate blood supply to the disc (Bogduk 1997). The rate of synthesis of proteoglycans decreases and the concentration of proteoglycans in the nucleus also decreases as the disc ages (Johnstone and Bayliss 1995). In a typical adult nucleus pulposus, proteoglycans make up about 65% of the dry weight, but by the time the adult reaches age 60, proteoglycans constitute only about 30% of the dry weight (Johnstone and Bayliss 1995). The proteoglycans that do remain are much smaller in size and weight. Another major change in the nucleus is the increase in its collagen content (Buckwalter 1995). The fibril diameter of collagen in the nucleus increases in such a manner that the type II collagen of the nucleus pulposus starts to resemble the type I collagen of the annulus (Buckwalter 1995). This leads to having less distinction between collagen of the nucleus and that of the annulus. The water content in the nucleus also decreases with age. At birth it is estimated that the water content is about 88%,

which decreases to about 65-72% by the age of 75 (Buckwalter 1995). The majority of that decrease occurs during childhood and adolescence and not from adulthood to old-age (Bogduk 1997). Regardless of the amount of decrease, the intervertebral discs become drier with age and they also become more fibrous and less resilient. This makes the disc stiffer and their decreased water-binding power makes them less able to recover from creep deformation (Bogduk 1997). Yet another change that comes with aging is that the number of viable cells in the nucleus pulposus decreases, and the proportion of cells that show necrosis changes from 2% to 80% as you go from infancy to old age (Buckwalter 1995). As the nucleus dries out, it is less able to exert pressure and less able to transmit weight so a larger portion of the load is handled by the annulus. The annulus then having to hold greater weight undergoes changes to reflect the increasing strains it bears. The collagen lamellae of the annulus become thicker and increasingly fibrillated as the disc ages (Roughley 2004). Cracks and cavities are then able to develop and many enlarge to become clefts and fissures (Roughley 2004). The changes that occur in the structure of the disc as aging occurs may be caused by lots of different factors. The rate at which changes take place can be increased if there is unfavorable loading. At this point it is not clear if the catabolic events that initiate degeneration are the same ones involved in the normal aging process (Roughley 2004). If the events are the same, then aging and degeneration in the disc could be classified as the same process and individuals would show variation only in the rate that the process would occur. Iatridis et. al. performed a study to determine the

viscoelastic shear behaviors of the nucleus pulposus due to aging and degeneration and found that it was difficult to separate the effects of aging and degeneration (Iatridis *et al.* 1997). It was shown that there was as increase in the dynamic shear modulus and a decrease in tan δ, where δ is the phase shift angle, which indicate a transition from more fluid-like behavior in a younger nucleus to more solid-like behavior present with aging and degeneration (Iatridis *et al.* 1997). The continual catabolism taking place in the disc and the failure to replace degraded collagen eventually result in a functionally weakened tissue. Although an individual may have disc degeneration, that individual may or may not have back pain as a result. It has not been established as to why some individuals experience pain while others do not. It seems as though the morphological changes that take place do not necessarily cause symptoms in the individual. In the extreme case where herniation (i.e. disc prolapse) occurs, only 30% of individuals actually report pain (Bader 2006). There are many forms of back pain all of which could be from different causes. Lumbar spinal pain is perceived as radiating from an imaginary region below the T12 spinous process and above the S1 spinous process (Hardy 1993). The pain could be present in other locations as well. Radicular pain is pain that occurs as a result of irritation of a spinal nerve or its roots. A formal survey in the United States found that no more than 12% of patients with back pain had any clinical evidence of disc herniation (Deyo and Tsui-Wu 1987).

Disc herniation is the single most common cause of radicular pain, and there has been increasing evidence that this condition causes pain by mechanisms

other than simple compression (Hardy 1993). On myelography, individuals can show root compression by disc herniation but have no symptoms (Hardy 1993). Patients who were previously symptomatic can still show root compression even after symptoms were resolved (Hardy 1993). It is believed that inflammation is the actual cause.

Disc herniation can occur anywhere in the spine, but for the purposes of this study, only herniation in the lumbar region will be addressed. The lumbar region is where the majority of cases occur. Disc herniation can be defined as a condition where a tear in the annulus fibrosus allows the nucleus pulposus material to bulge out. It is normally a further development of a disc protrusion where the annulus is bulging out, but not torn due to increased disc pressure and degeneration. The main complaint for herniation is leg pain greater than lower back pain. Pain from a herniated disc is usually continuous, not pulsating like that which can be caused by muscle spasms (Deyo and Tsui-Wu 1987). Usually, symptoms are experienced only on one side of the body, but if the herniation is very large and applies pressure to the spinal cord of cauda equina, symptoms could occur bilaterally (Deyo and Tsui-Wu 1987).

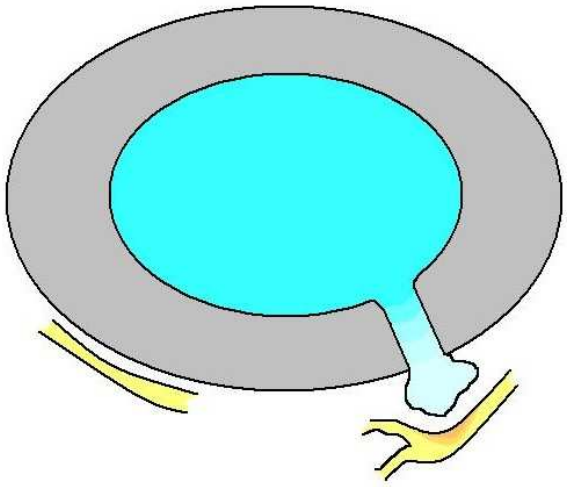

Figure 5: Diagram showing nucleus pulposus material extruded through the annulus fibrosus (herniation).

Current Treatments

Non-Surgical vs. Surgical Treatment

Although it may seem contrary to common sense, the severity of pain from a herniated disc does not always correlate to the amount of physical damage to the disc. Additionally, less serious back problems may cause more pain than a herniated disc. The severity of pain is not a determining factor for identifying a herniated disc. The care of a patient with a herniated disc is not standardized and most times it is individualized for each patient. The treatment options for a lumbar herniated disc will largely depend on the length of time the patient has had his or her symptoms and the severity of the back pain. Patients are generally advised to start with 6 to 12 weeks of conservative treatment (Foster 2007). There is a wide range of conservative treatment options for patients to try for treatment of a

herniated disc. The primary goals of treatment are to provide relief of pain and to allow return to normal functioning level (Foster 2007). The most common conservative treatment options are:

- Rest, followed by slow mobilization
- Pain medications
- Chiropractic/osteopathic manipulations
- Physical therapy
- Epidural steroid injections

The recommended amount of conservative treatment for the herniated disk is individualized for each patient. For those patients who are not in severe pain and can function well, a longer period of conservative treatment is reasonable (e.g. 12 weeks) (Foster 2007). For those patients with severe pain that is not responsive to conservative treatment, more radical treatment needs to be administered.

If conservative treatment does not provide pain relief after 12 weeks it is reasonable for the patient to consider surgery. The goal of surgery is again to alleviate the pain faster. In recent years, the morbidity of surgery for a lumbar herniated disc has decreased and overall results have improved making surgery a more reasonable option to healing faster (Foster 2007). The most common surgical treatment options include:

- Microdiscectomy (the most common procedure)
- Fusion
- Lumbar laminectomy

- Chymopapain injections
- Arthroscopic lumbar discectomy
- Microendoscopic surgery

A lumbar microdiscectomy (also called a lumbar micro-decompression) is considered the gold standard and is the most common surgery to alleviate pain from a lumbar herniated disk (Foster 2007).

Discectomy

Discectomy (also called open discectomy) is the surgical removal of herniated disc material that presses on a nerve root or the spinal cord (Ullrich 1999). Before the disc material is removed, some of the bone from the affected vertebra may be removed using a laminectomy to give the surgeon a better view of the area. Microdiscectomy uses a special microscope to view the disc and nerves. This procedure is considered more effective because the magnified view makes it possible for the surgeon to remove the herniated disc material through a smaller incision and therefore cause less damage to the surrounding tissue (Ullrich 1999). The success rate for a microdiscectomy is approximately 90%, although 5-10% of patients develop a recurrent disc herniation at some point in the future (Ullrich 1999; Atlas 2001). A recurrent disc herniation may occur right after the surgery or years later, although most occur within 3 months after surgery (Atlas 2001). Recurrent herniated discs are not thought to be directly related to a patient's activity, and more to do with the fact that within some disc spaces there are multiple fragments of disc that can come out at later times (Ullrich 1999). Through

a posterior microdiscectomy only about 5 to 7 % of the disc space can be removed and most of the disc space cannot be visualized (Ullrich 1999). Also, the hole in the disc space where the herniation occurs (annulotomy) usually never closes because the disc itself does not have a blood supply, so the area does not heal or scar over and there is no real way to surgically repair the annulus (Ullrich 1999). As with any surgery, there are risks and complications including:

- Dural tear (cerebrospinal fluid leak). This occurs in 1% to 2% of these surgeries, does not change the results of surgery, but post-operatively the patient may be asked to lay recumbent for one to two days to allow the leak to seal (Ullrich 1999).
- Nerve root damage
- Bowel/bladder incontinence
- Bleeding
- Infection

For patients with multiple herniated disc recurrences, a spine fusion surgery is usually recommended. Spine fusion surgery involves placing small morsels of bone either in the front of the spine (in the disc space) and/or along the back of the spine (in the posterolateral gutter) so that the bone grows together and fuses that section of the spine (Ullrich 1999). It is designed to eliminate motion in that segment therefore decreasing or eliminating the pain associated by the motion. The spine is not actually fused at the time of the surgery, but instead, the surgery

creates the conditions for the spine to fuse over a 6 month period following the surgery.

Removing the entire disc space and fusing the level is the most common way to absolutely assure that no further disc herniations can occur. If the posterior facet joint is not compromised and other criteria are met, an artificial disc replacement can be considered. Although there has been some success, the implantation of a total artificial disc is very invasive and can require many revisions, which are also invasive. These devices also require the removal of all remaining tissue, regardless of how degenerated the actual tissue is. A different approach to the problem which is less invasive is the replacement of just the nucleus pulposus.

Nucleus Pulposus Replacement

Nucleus pulposus replacement is less invasive than total disc replacement and it maintains the healthy tissue of the intervertebral disc right where it is. It is not necessary to remove all of the surrounding tissue in order to put in a replacement for the nucleus pulposus. In the recent past, researchers have made the claim to use hydrogels as the implant material of choice in place of the nucleus pulposus (Di Martino *et al*. 2005; Boyd and Carter 2006). Hydrogels are a network of polymer chains that are water-insoluble, and are sometimes found as a colloidal gel (Di Martino *et al.* 2005). They are superabsorbent; they have the capability to retain large quantities of water. Hydrogels also possess a degree of flexibility similar to natural tissue, due to their significant water content (Di Martino *et al.*

2005). They are commonly used as scaffolds in tissue engineering where they contain human cells in order to repair tissue. Besides being able to hold large quantities of water, another reason why hydrogels are a great candidate for nucleus pulposus replacement is their high permeability to low molecular weight solutes like oxygen and glucose. This is very critical to maintaining the health of the rest of the intervertebral disc. Hydrogels have also been shown to have similar mechanical properties as the native tissue (Bader 2006). Poly(vinyl alcohol) (PVA) hydrogels have been identified as the most favorable for implantation because of their excellent biocompatibility, high elasticity, low toxicity, and swelling capability (Bader 2006). Another area of research has been in hydrogels that are injectable and photopolymerizable. The traditional approach of cutting someone open and placing the implant in the degenerated disc can be avoided by using an injectable material. Hydrogels are prepared, injected into the void space, and then they are photopolymerized *in vivo* in order for the gel to solidify.

Current devices

There are currently no available nucleus pulposus replacements for implantation available in the United States. Hydrogels are the obvious choice for an implantable material but nothing has been released commercially yet. There are quite a few products in the development and testing phases but all the published results about them are vague and lacking much detail. The Prosthetic Disc Nucleus (PDN®) (Raymedica, Inc., Bloomington, MN) is probably the furthest in the race to be approved in the United States. It is currently available for use outside the US

and Canada and has had success rates up to 90% in recent trials (Klara and Ray 2002). It is made of a hydrogel pellet that is encased in a poly(ethylene) jacket and it is supposed to mimic the nucleus pulposus in strength but no actual results have been published to show this (Bader 2006). Even though published results reveal high success rates, this device has a few disadvantages like an improper fit into the cavity which could lead to more extrusion (Bader 2006). There are a few other devices that are undergoing clinical trials in Europe like the NeuDisc, Newcleus, Aquarelle, DASCOR™, and BioDisc™ all of which are a hydrogel formulation, but not necessarily the same material. They each have their own advantages and disadvantages but none of them stand out as devices that will replace the native tissue without any problems. Much more product development and testing needs to be conducted before an implant device could be ready for commercial use in the United States.

MATERIALS & METHODS

Model of a Degenerative Intervertebral Disc

In order to ensure that materials proposed for implantation can hold up to normal physiological loads, a model of a degenerative intervertebral disc was created. This device was created out of a Plexiglas™ material and had a hollowed center representative of where the nucleus pulposus would be found.

The device was made out of two 12.7 mm thick Plexiglas™ blocks each 63.5 mm by 75 mm that are bolted together so there is no gap in between them. A thru hole was drilled completely through the top block and 3 mm into the bottom block at a diameter of 25 mm. The hole was placed at a distance of 15 mm from the edge of the block. The top block also has a small channel cut out on the bottom side of it running from the edge into the drilled hole. The channel has a width of 8 mm and a depth of 1.5 mm. The dimensions of this device were created as such to mimic those of an actual human intervertebral disc. The hole diameter was set to be similar to that of the nucleus pulposus of a human. The channel length was set to equal the diameter of the annulus without the nucleus pulposus. It was set to mimic the distance that the nucleus pulposus would have to travel through the defect when herniation occurs.

An assembly is shown in fig. 6 as drawn in Solid Works of the two blocks. The main features of the testing device can be seen in this wire-frame assembly drawing. For more detail, a section cut-out of the testing device is shown in fig. 7. A longitudinal cut was made so that the hole and channel can be seen in more detail.

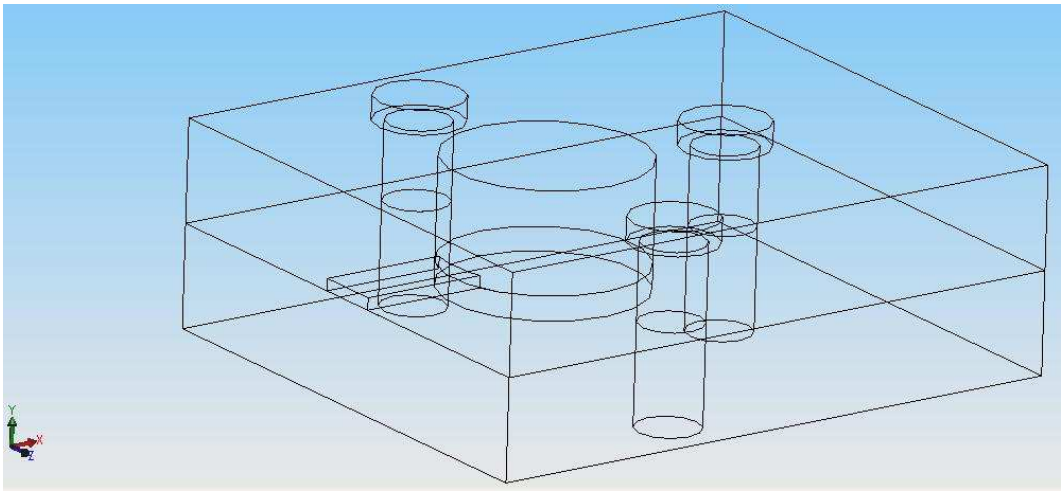

Figure 6: Solid Works assembly wire-frame drawing.

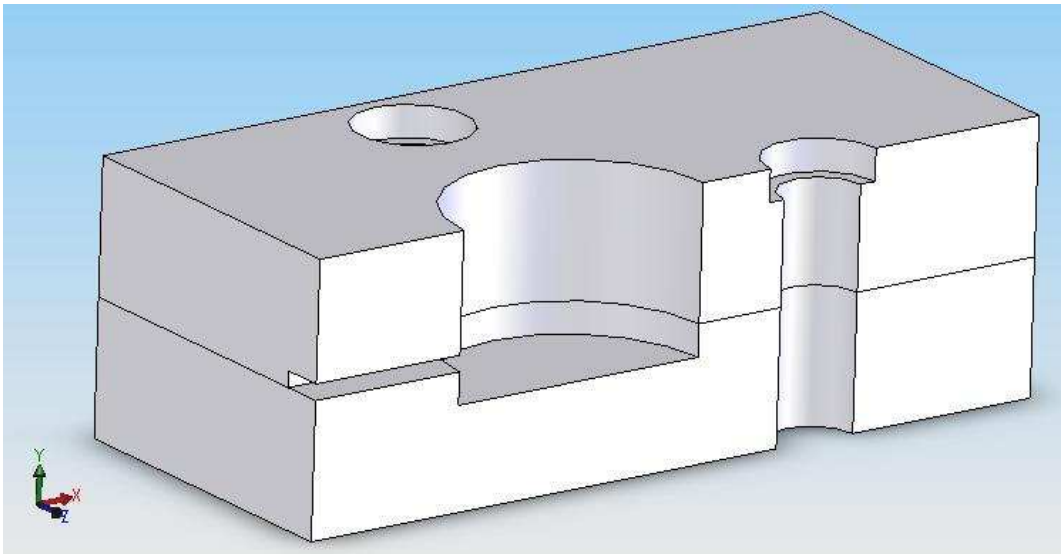

Figure 7: Solid Works section drawing.

Preparation of Agarose Gel

Classification of the extrudability of a gel material in the degenerative disc device created had to be done with lots of repeatability. A gel material for this use needed to be fairly inexpensive and easy to make. Agarose was chosen as the gel to be tested in the apparatus. Agarose is purified from agar which is a gelatinous substance mostly used as a culture medium for biological work. Chemically, it is a polymer made up of subunits of the sugar galactose. It is obtained from the cell walls of some species of red algae or seaweed.

For the purposes of this experiment, chemical grade agar powder (DIFCO Laboratories product #214530, Detroit, MI) was used to make each gel. The weight percent of agarose was varied from 0.3 % to 1.5 %. To make each different weight percent, the corresponding amount of powdered agarose was poured into a 250 ml flask containing 75 ml of deionized water. For example, to make a 1.5 wt % solution, 1.125 g of powder would be used since:

0.015 g/ml * 75 ml = 1.125 g

The solution was then mixed thoroughly and heated in a microwave for 2 minutes or until the liquid started boiling over. The new mixture was poured into small Petri dishes and left to cool. Once the mixture had reached room temperature, the Petri dish was covered and placed in a refrigerator for at least 24 hours.

Compression Testing

In order to determine the extrudability of a gel sample, compression tests had to be conducted on the gels placed inside the degenerative disc device created. Before any tests could be run, the gel samples were removed from the refrigerator where they were set and placed in the room where the testing would take place at least 12 hours in advance in order for them to acclimate to the testing environment. Gel samples were then cut out of the mold with a metal punch with the exact diameter of the prototype disc device so there would be a close fit. The gel disc was then placed in the hole of the Plexiglas™ test block and placed in the INSTRON testing machine (INSTRON Model #5567, Instron Corporation, Norwood, MA) as shown in fig. 8.

Figure 8: INSTRON used in compression testing.

The attached computer was turned on and *Merlin* software in compression mode was selected. The test control mode was compressive extension at 1 mm/min without a preload and data capture was set at an interval of 100 ms. A cylindrical plunger was created specifically for the testing device so that none of the material would be extruded around the plunger, but only out the channel when it reached a certain load. The load was balanced and each test was conducted. Each cylindrical specimen was loaded in compression until failure, where failure was defined as the point when the specimen was extruded through the channel. Data was recorded and then analyzed using Microsoft Excel. Graphs of load versus extension, stress versus strain, and Young's Modulus were created from the raw data.

Rheological Testing

To determine the rheological properties of the agarose, its behavior had to be analyzed using a more dynamic test. The mechanical properties of the gel were evaluated under dynamic torsional shear. A Bohlin controlled stress Rheometer, as shown in fig. 9, (Bohlin Rheometer CS, Malvern Instruments, Inc., Southborough, MA) was used for the dynamic tests.

Figure 9: Picture of Bohlin Rheometer used for testing.

Twenty-five millimeter parallel plates were used in the test setup which can be seen in fig. 10. Both the parallel plates were covered with a thin piece of sandpaper to prevent slip. A small compressive strain was also applied to maintain contact and also prevent slip. Under these test conditions, a sinusoidal angular displacement of θ was applied to each gel and dynamic frequency sweep experiments were conducted.

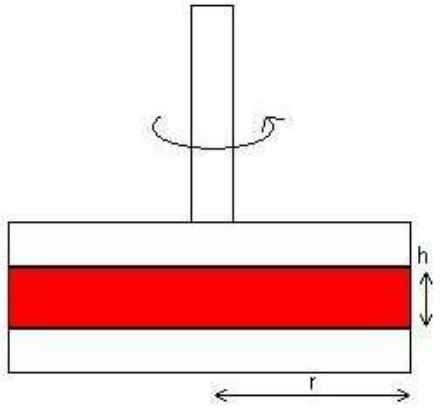

Figure 10: Parallel plate geometry.

A shear strain amplitude of $\gamma = 0.05$ rad was set over the range of $0.01 \leq f \leq 10$ Hz. The shear stress (σ) was computed from the applied torque (T) through the equation:

$$\sigma = \frac{T \times r}{J}$$

where r is the sample radius and J is the polar moment of inertia and is calculated by $J = \frac{\pi \times r^2}{2}$. The shear strain was computed using the equation:

$$\gamma = \frac{\theta \times r}{h}$$

where h is the thickness of the sample and can be seen in fig. 10. The test methodology followed a strict protocol as indicated by ASTM Standards (ASTM 2005). The experiments were run and the elastic (storage) and viscous (loss) moduli, G' and G" respectively, were resolved as functions of frequency (f). The elastic modulus is associated with the amount of energy stored and released during each periodic deformation. The viscous modulus is associated with the amount of energy dissipated in the form of heat per cycle of deformation per unit volume. Both G' and G" can be plotted against frequency to show trends, but it is more convenient to show the magnitude of the complex shear modulus, |G*|, and the tangent of the phase shift angle (δ) for analysis purposes. The two values are computed by the following equations:

$$|G^*| = \sqrt{G'^2 + G''^2}$$

$$Tan\delta = \frac{G''}{G'}$$

These two variables reveal quite a bit of information about the sample being tested. The complex shear modulus is a gauge of stiffness when you have dynamic conditions. The phase shift angle is an indicator of internal dissipation. The phase shift angle has the capability to tell you if a fluid is more like a solid or a liquid. When the stress is in phase with the strain the phase shift angle is zero, and the material being tested is said to be a purely elastic solid. When the stress is completely out of phase with the strain the phase angle is 90º, and the material being tested is said to be a viscous fluid, which can be seen in fig. 11.

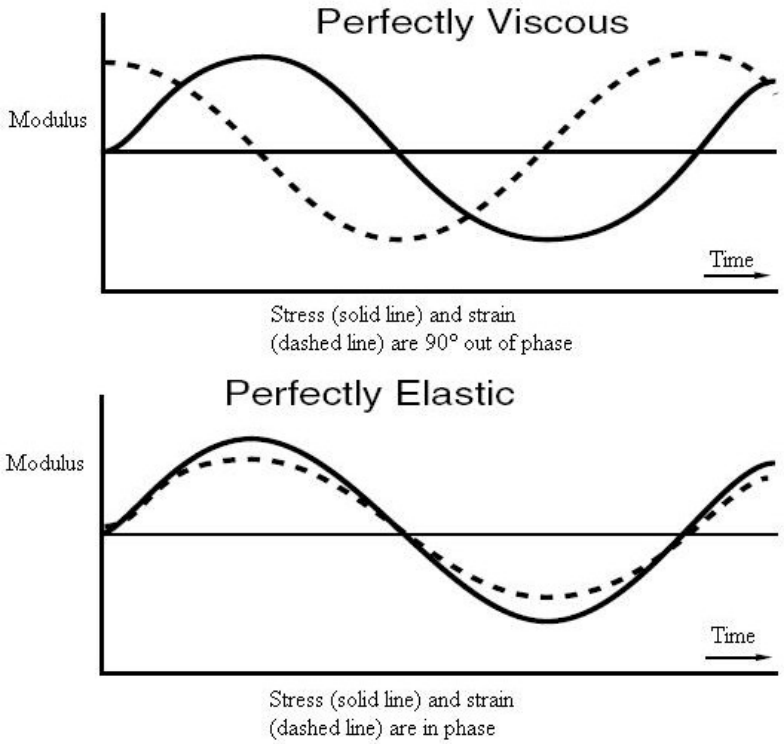

Figure 11: Viscous and Elastic Responses to applied strain.

Phase angles in between is termed viscoelastic but as one gets closer to the extremes the material gets closer to the ideal solid or fluid depending on which side of the spectrum it is.

In order to determine what shear strain amplitude to run the experiments at, a strain sweep dynamic test was conducted. The frequency was kept constant at 0.1Hz and the shear strain amplitude was varied in the range of $0 \leq \gamma \leq 0.5$. The experiment was conducted and the elastic and viscous moduli, G' and G" respectively, were determined as functions of the shear strain, γ. The data was plotted and analyzed to see where elastic modulus deviated from the linear viscoelastic region. On a plot of elastic modulus versus strain, the moduli values initially form a flat and linear region with small increases in strain, but as the strain gets much larger, the moduli start to deviate from the linear zone and that point is said to be when the material is no longer in the linear viscoelastic region and can be related to the yield strain. It is critical that testing take place in the linear viscoelastic region so that the constitutive equations for simple shear can be used when the data is analyzed.

Statistical Analysis

Statistical analysis of the rheological and mechanical data was performed using one-way ANOVA. All values reported are averages with the standard deviation also being shown in the plots.

RESULTS

Rheological Characterization

The first tests that were conducted were the strain sweep experiments. Figure 12 below shows the elastic (storage) modulus plotted against the shear strain that was imposed onto each sample gel at a frequency of 0.1 Hz. The linear viscoelastic region can be seen for very small strains where the elastic modulus curve is fairly flat. For the 0.75 weight percent gel, this region extends to about a strain of 0.1. For the 1.0 weight percent gel, this region extends to only a strain of 0.05. Lastly, for the 1.5 weight percent gel, the linear region is even narrower and only extends to a strain of 0.035. As the strain is increased, the moduli start to deviate from the linear region, which indicates the material is yielding. The shear strain where the deviation began seems to be slightly different for each weight percent tested. Another visible trend is that as the weight percent is increased, the modulus at a given strain also is increased.

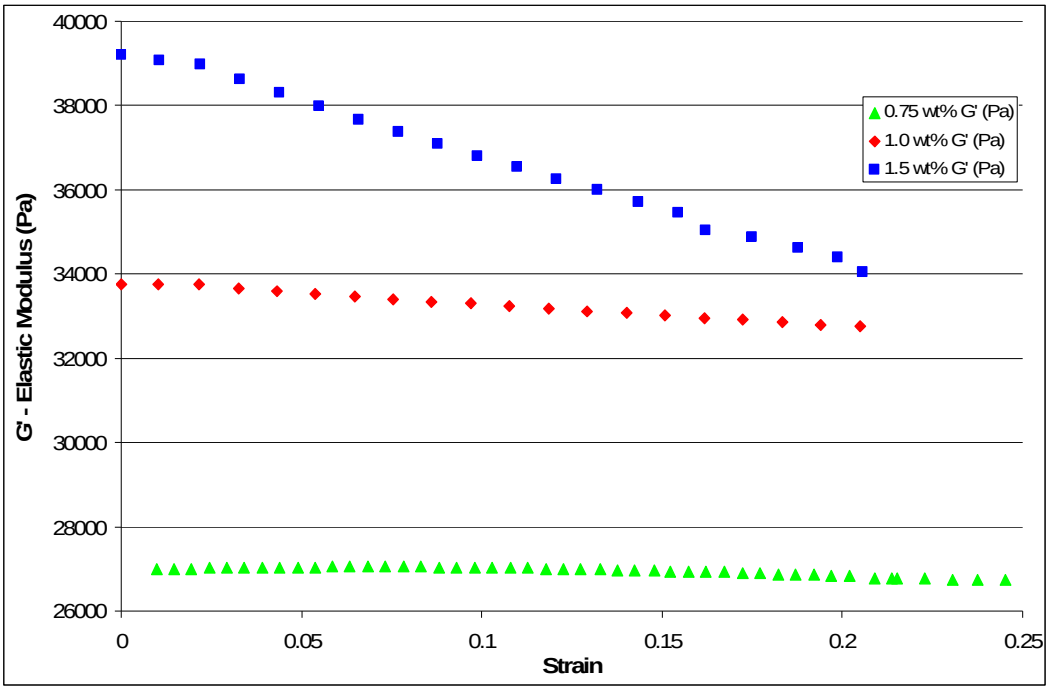

Figure 12: Strain amplitude sweep performed at 0.1 Hz for different weight percents of agarose gel showing G' – Elastic Modulus values.

In order to effectively characterize the viscoelastic properties of each gel, frequency sweeps had to be conducted with a shear strain amplitude that was in the linear viscoelastic region. Using the strain sweep data shown in fig. 10, it was decided that a shear strain of 0.05 would satisfy all the different weight percents. Next, frequency sweep experiments were conducted at the shear strain amplitude of $\gamma = 0.05$ and in the frequency range of $0.01 \leq f \leq 10$ Hz. The viscoelastic data of complex modulus is plotted below in fig. 13. Here the elastic and viscous moduli are not plotted directly, but instead the complex shear modulus and the tangent of the phase angle (δ), shown in fig. 14, are displayed in order to compact the data for analysis. $|G^*|$ increased with increasing weight percent at smaller frequencies like

0.01 Hz. For higher frequencies, |G*| seemed to increase at a constant rate and showed no dependence on weight percent. |G*| is a little bit deceptive here because the contribution from the loss modulus is not represented in the plot since its values are fairly constant over the range of frequencies. The plot is more just an indicator of the storage modulus since it is doing the majority of the changing over the frequency range. Tan δ was greater for a larger weight percent at frequencies less than 0.1 Hz. As the frequency was increased, tan δ approached zero. Since the phase angles were all less than 45°, corresponding to 0< tan δ<1, the material showed more solid like behavior. Phase angles greater than 45° are indicative of fluid-like behavior.

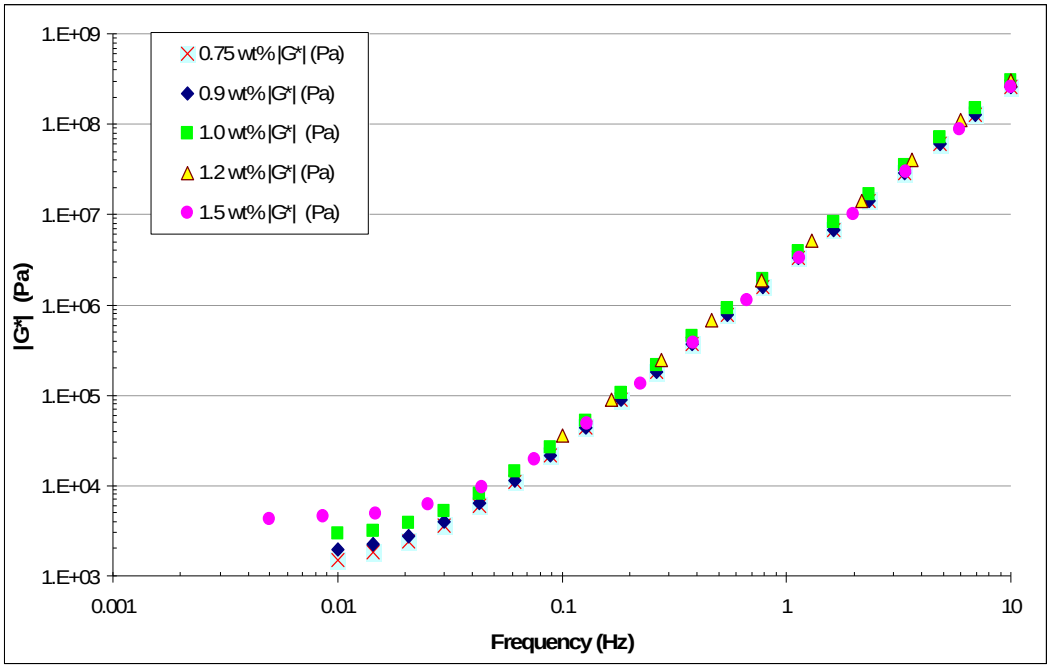

Figure 13: Frequency sweep performed at γ = 0.05 for different weight percents of agarose gel showing |G*| – Complex Modulus values.

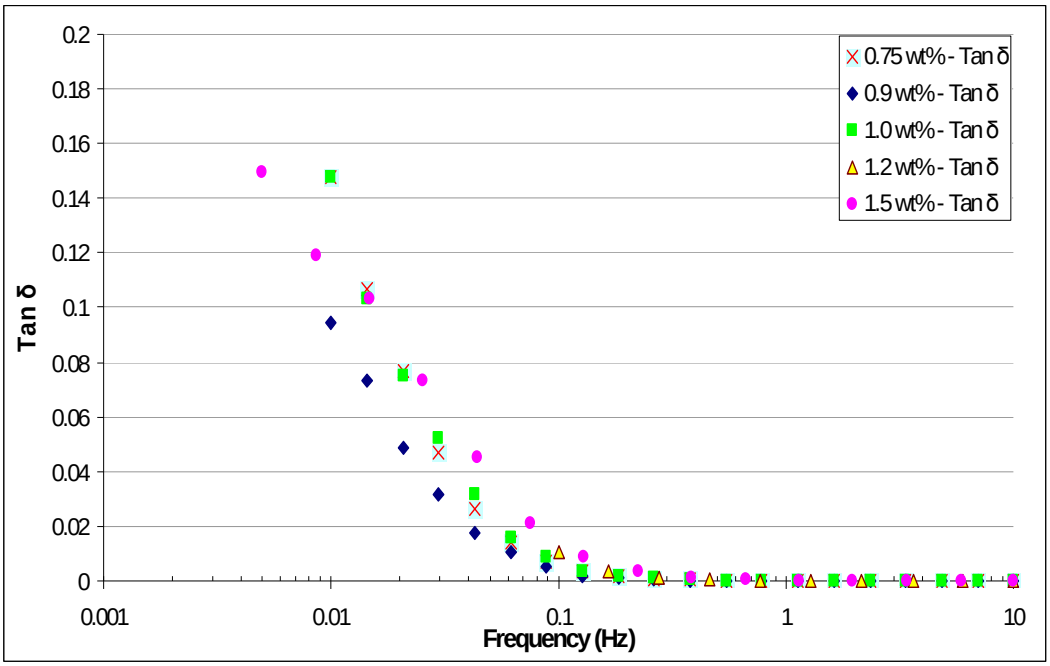

Figure 134: Frequency sweep performed at $\gamma = 0.05$ for different weight percents of agarose gel showing tan δ values.

In order to gain a better understanding of the elastic and viscous behavior acting individually in each gel under dynamic conditions, the frequency sweep plots are shown in fig. 15. Once again for a given frequency, as you increase the weight percent of agar, the modulus increases. Here it can be seen how G' and G", the elastic and viscous moduli respectively, actually act as frequency is increased. One explanation for why the elastic moduli increase as the frequency increases without a difference between weight percents is that in the higher frequency range the sample size of the molecule is much smaller. When you increase frequency, you are looking more at the backbone of the molecule, which is stiff, making the elastic moduli much higher regardless of the weight percent. Figure 16 shows an expanded view of just the viscous moduli plotted against frequency. As frequency

is increased, the moduli values remain fairly constant indicating that there is not much viscous change in the material being tested and since the phase angles indicate that the material behaves more like a solid it seems logical that the viscous moduli would remain fairly constant throughout the experiment. The loss content is constant.

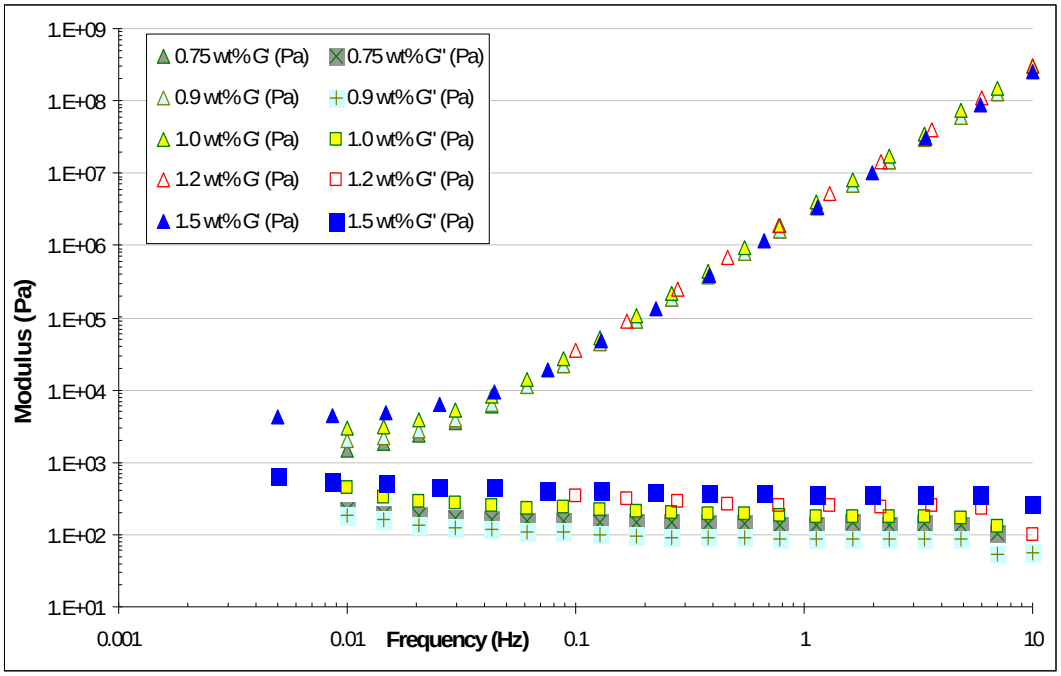

Figure 15: Frequency sweep performed at $\gamma = 0.05$ for different weight percents of agarose gel showing Elastic and Viscous Modulus values.

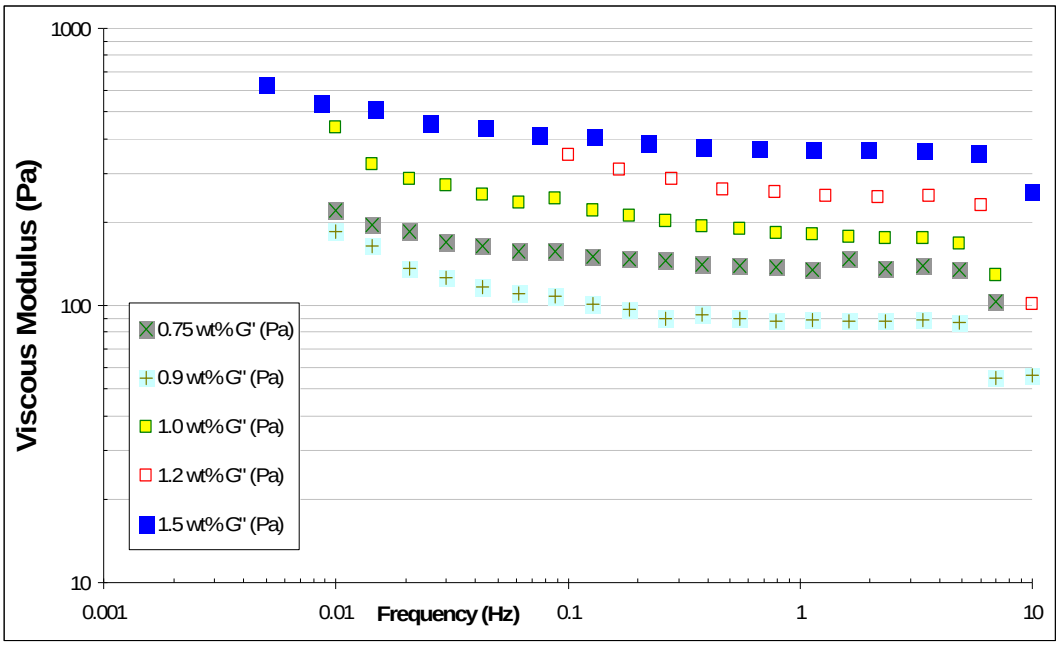

Figure 16: Frequency sweep performed at $\gamma = 0.05$ for different weight percents of agarose gel showing Viscous Modulus values.

After initial testing, it became evident that more tests needed to be performed at much lower frequencies than were originally designed. This was necessary because from about 0.1 Hz, the elastic modulus data converged and increased at a constant rate for all the samples so one weight percent could not be distinguished from any other. A frequency range of $0.001 \leq f \leq 0.1$ Hz was set at $\gamma = 0.05$ and data was collected. The viscoelastic data can be seen below in fig. 17. Here, the differences between the weight percents can be seen more clearly. Increasing the weight percent at any given frequency in the range of $0.001 \leq f \leq 0.1$ Hz yields to a larger elastic and viscous modulus. It can also be seen that up to about a frequency of 0.02 Hz, the elastic moduli are fairly linear, which was not the case at higher frequencies. Figure 17 looks very similar to the

rubbery plateau region of a linear viscoelastic flow regime where both the moduli start out increasing from the viscous region, then flatten out in the rubbery region, and finally increase going into the transition phase at higher frequencies. The tangent δ of the lower frequency range is shown in fig. 18. The values seemed to converge to zero with increasing frequency and were between 0 and 1 indicating solid-like behavior.

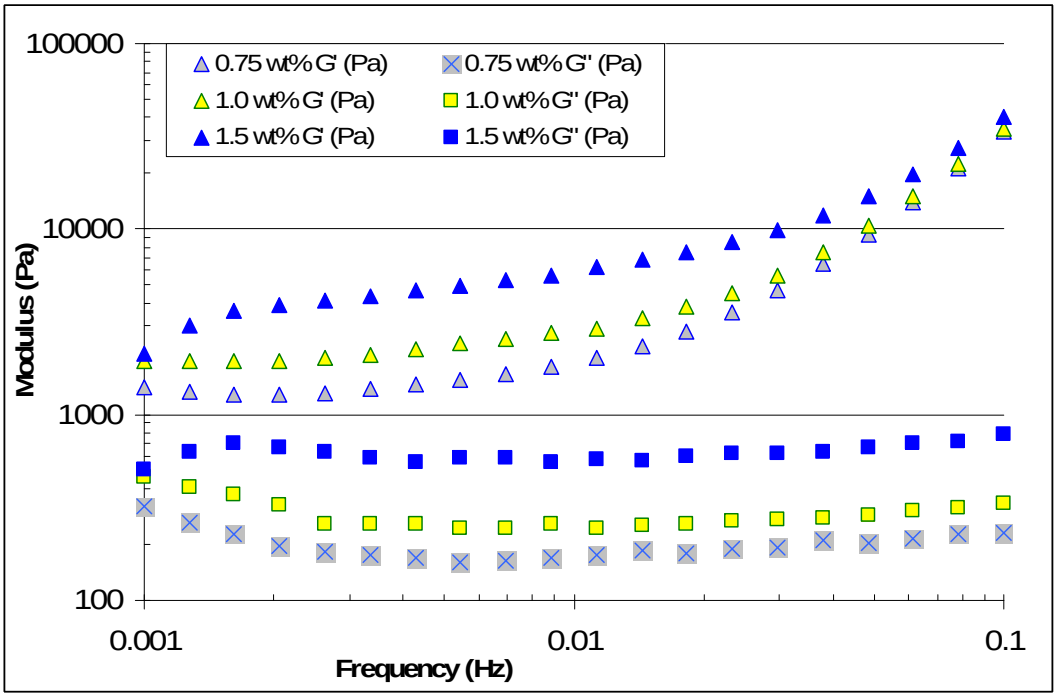

Figure 17: Small frequencies sweep performed at $\gamma = 0.05$ for different weight percents of agarose gel showing Elastic and Viscous Modulus values.

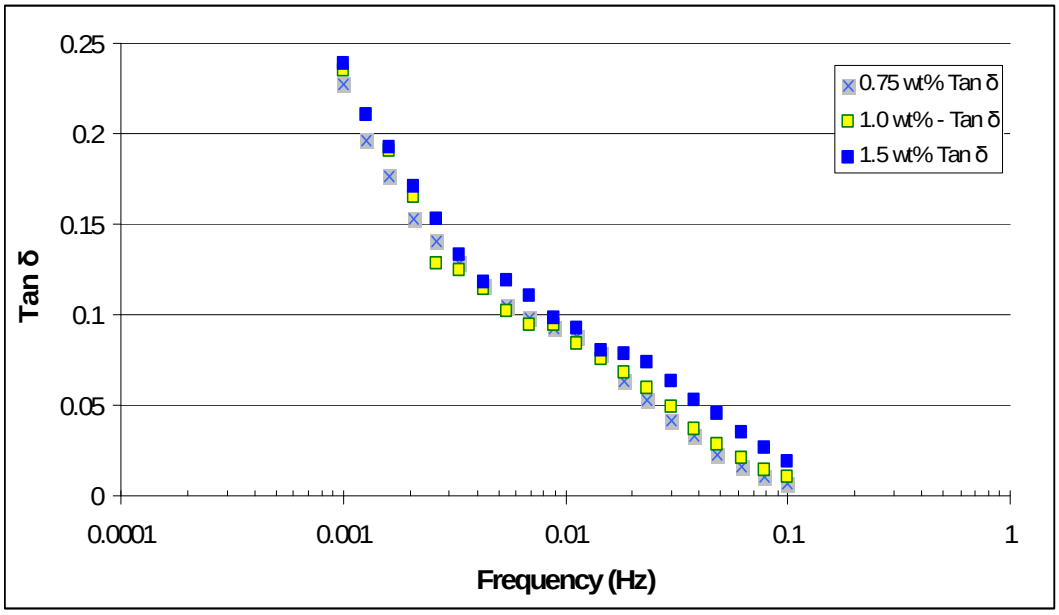

Figure 18: Small frequencies sweep performed at $\gamma = 0.05$ for different weight percents of agarose gel showing tan δ values.

Mechanical Characterization

Static mechanical testing of the agarose gel material was done with much iteration. One sample elongation versus load plot is shown in fig. 20. The plot shows how the gel is being loaded in compression inside the cavity until it reaches a certain point where the load increases very rapidly. The gel is expanding as much as it can to fill the cavity completely until it reaches the yield point. This is when extrusion through the defect occurs and the gel starts to squirt out. It can be seen on the plot as the spike that occurs around 7 mm. The gel has different behavior as it is extruded depending on the weight percent being tested. The stiffer and higher weight percent gels create a more violent event when they extrude, meaning that the gel is squirted out at once in an explosion type event. The less stiff and lower weight percent gels extrude much slower and they flow out of the defect. This can

be explained by the difference in stiffness of each material, with stiffer materials being more brittle and breaking at one final yield point, and softer materials being less brittle and breaking in a slower, more gradual fashion. When viewed from above it looks like ripples coming out of the defect. A diagram of what the gel after extrusion looks like can be seen in fig. 19. The extruded material in the defect looks as if it rippled out. The gel left in the cavity has a few fracture rings close to where the defect is. After the initial extrusion, there is less material in the cavity, but the gel is still being compressed. At this point, the gel once again starts to fill all the available space in the cavity because it has nowhere else to go. Since it has already been fractured once, it is less stiff and stable. Another yield point occurs where the gel is extruded from the defect and that can be seen closer to 8 mm on fig. 20. At this point the gel is not a solid structure any more and it is extruding out of the cavity at a constant rate. It is important to note that the material is yielding around the defect because a stress concentration exists there. Everywhere else in the cavity the material is confined to that space so it can’t go anywhere. Around the defect the gel can expand outward, and when it reaches a critical value of shear stress, it yields.

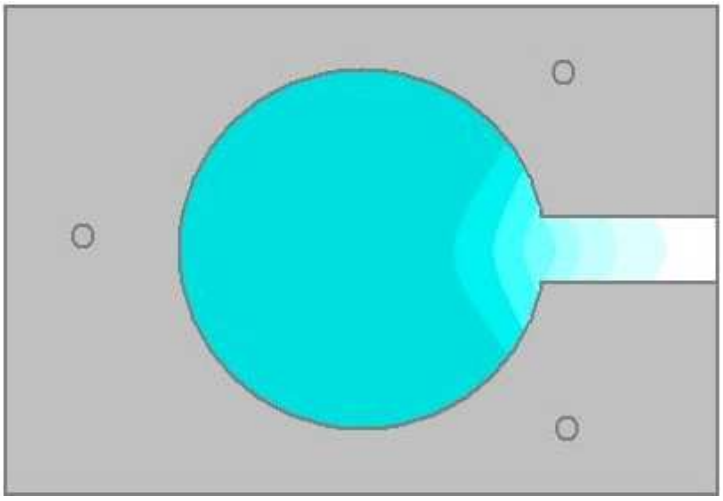

Figure 19: Top view schematic interpretation of degenerative disc device with gel after extrusion.

The elongation – load plot was then converted to a normal stress – strain plot and that is shown in fig. 21. The shape of the curve is the same as that for the elongation – load curve except the numerical values are different since the strain and stress values and the cross-sectional area of the gel sample being tested is constant throughout the test since it is confined to the diameter of the cavity. Some extra shear stress could have been included in the calculated stress due to the friction between the plunger and the walls of the cavity.

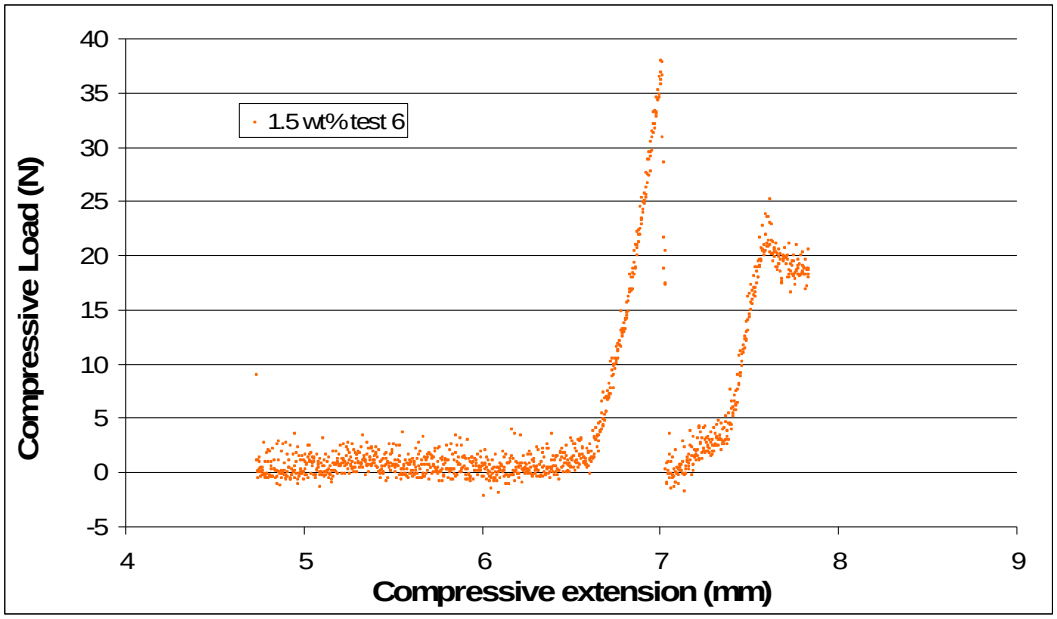

Figure 20: Typical raw compressive data plot for a 1.5 weight percent agarose gel

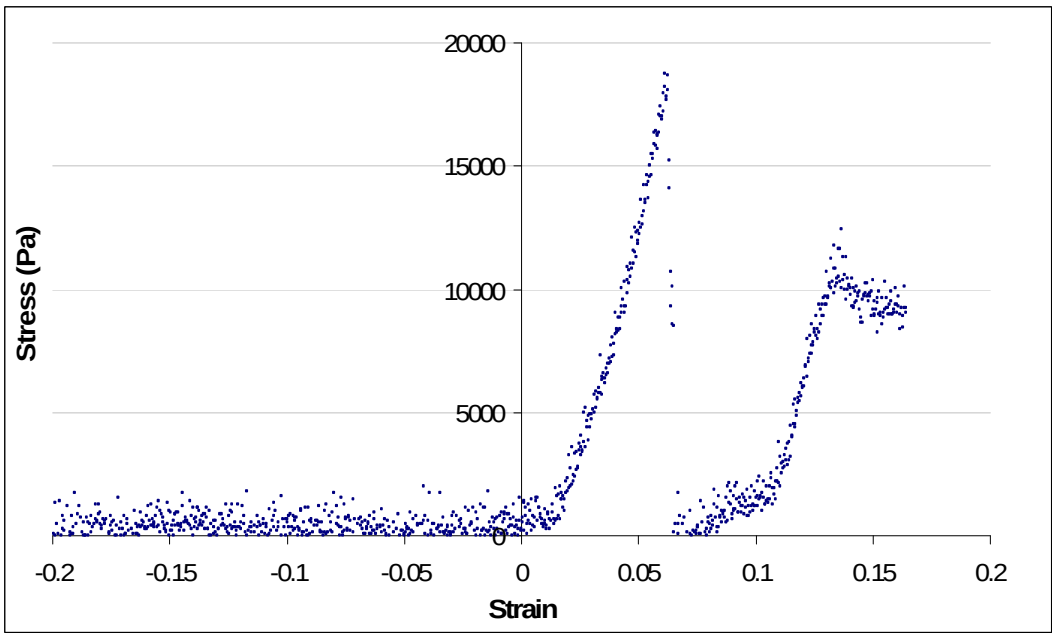

Figure 21: Typical stress - strain plot for a 1.5 weight percent agarose gel.

The normal stress and strain values were tabulated for all of the compression tests that were run and the data was plotted. The yield stress values plotted for weight percent represent the stress value when the material first yielded which occurred when the extrusion first took place in the cavity. These values are found below in fig. 22. There seems to be an increasing yield stress trend with increased weight percent of agarose gel. Higher weight percents of the agarose gel produced gels that were stiffer to the touch so it seems logical that a stiffer gel could withstand more pressure before yielding. The yield strain values versus weight percent are plotted in fig. 23. These values also show an increasing trend when weight percent is increased, but the trend is not as strong as it was with the yield stress. There is more deviation in the strain data as well. The material is yielding at strain values in the range of $0.35 \leq \gamma \leq 0.5$ for weight percents of agarose gel in the range of $0.75 \leq wt\% \leq 1.5$. The corresponding yield stress versus yield strain values are plotted together in fig. 24. This plot shows that as strain is increased at yield points, so is the stress at those corresponding points. This makes sense because if a material can withstand much more strain, it will yield at a much higher stress value. The error bars in the stress and strain directions show the variability in the data. Figure 25 shows the Young's modulus data for the compression tests. This is a measure of the initial slope in the stress – strain curve as the material begins yielding. Young's modulus is a measure of stiffness of the given material. It is also known as a measure of the elasticity in a material so the values of Young's modulus found under compression can be

compared to the elastic modulus from the dynamic tests to find a correlation. Young's modulus values also showed an increasing correlation with increasing weight percent. This indicates that the higher percent gels are stiffer and that makes sense because there is less water and more agarose molecules making up the gel.

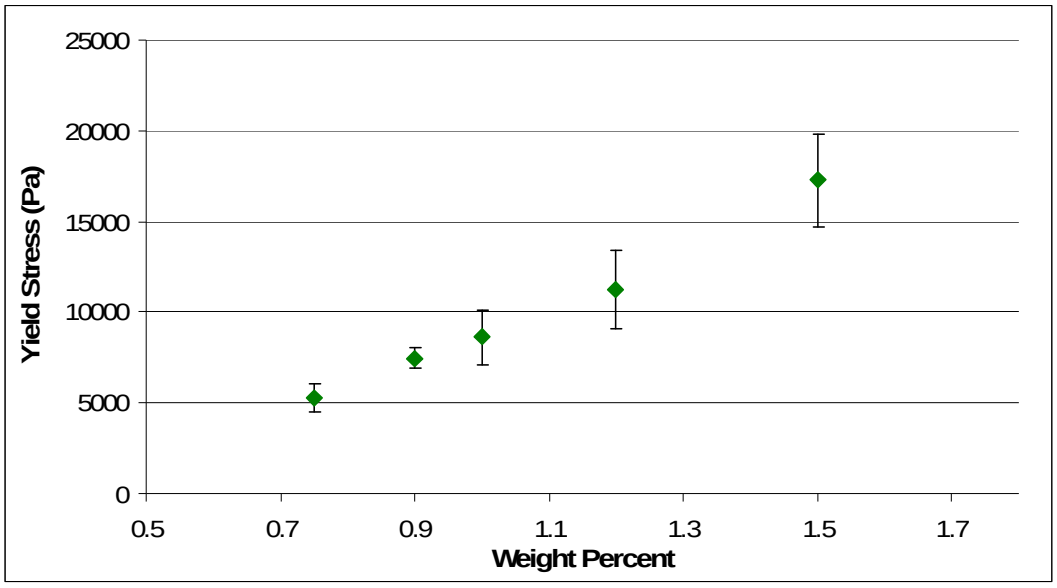

Figure 22: The stress value for each gel when the material yielded (extrusion from the cavity).

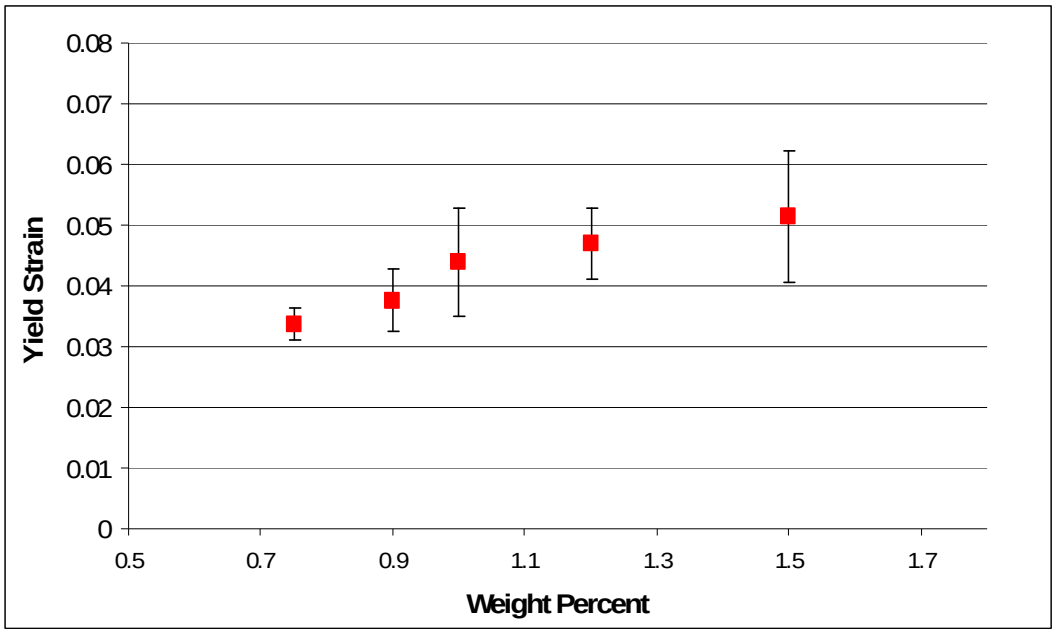

Figure 23: The strain value for each gel when the material yielded (extrusion from the cavity).

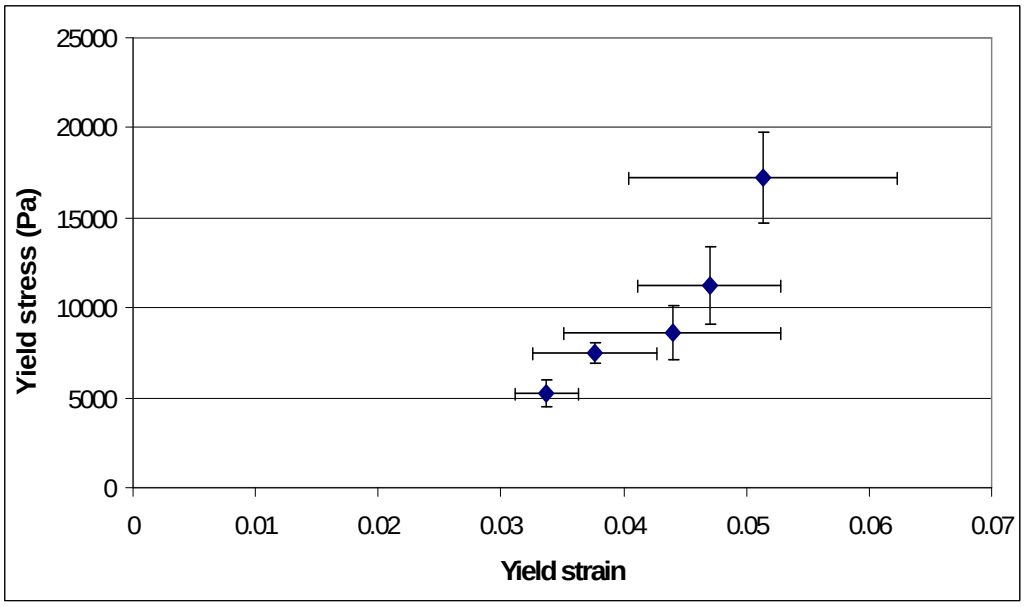

Figure 24: Yield stress versus yield strain at each weight percent of agarose gel.

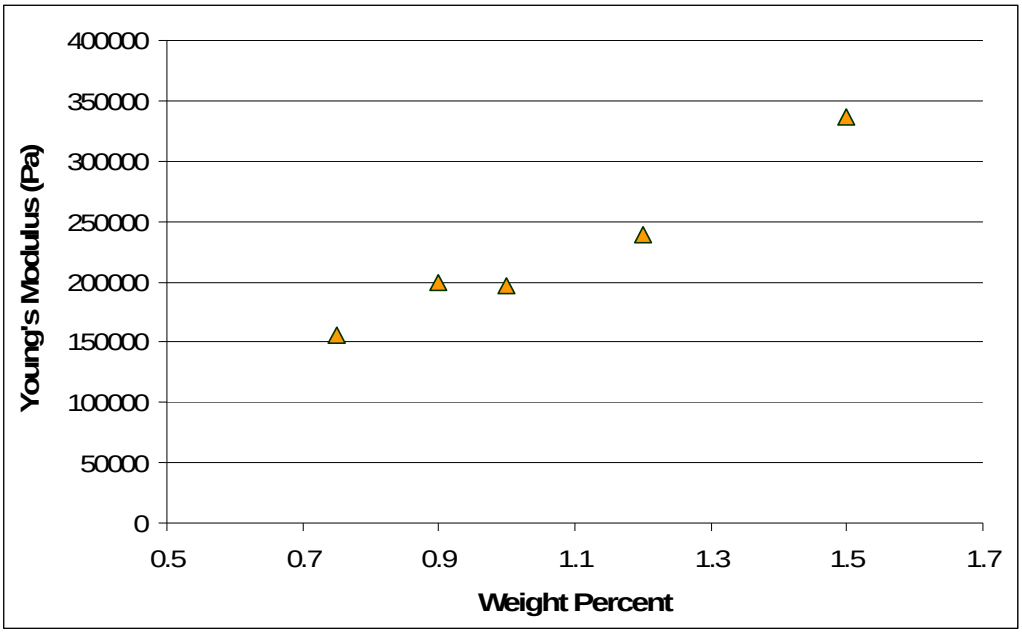

Figure 25: Young's modulus values at each weight percent of agarose gel.

All compression tests performed in the degenerative disc device constructed in this study had reproducible results for the range of weight percent of the agarose gel system tested.

DISCUSSION

Most of the recent work being done to create a nucleus pulposus replacement has focused on matching the behavior of the implant material to the actual nucleus pulposus, but there has not been any extensive testing on an implant material if subjected to physiological loads when a defect is present. This study has taken into consideration the effect of a defect for a basic gel system under compression in order to better understand the mechanical characteristics needed for an implant material. The experimental reproducibility needed to be verified before testing on more complex systems was to be carried out.

In order to classify the data found in the testing, actual nucleus pulposus data was first needed. Figures 26-29 show the frequency sweep data. Superimposed on the plot is a boundary region where the elastic, viscous, and complex moduli under dynamic testing of a human nucleus pulposus would lie. Iatridis et. al. tested the human nucleus pulposus and found the viscoelastic moduli values to be in the range shown below in fig. 26-29. Figure 26 shows the elastic modulus data and it can be seen that the agarose gel has moduli values that are much lower than the nucleus at low frequencies and moduli values that are too large at high frequencies. The reason that the elastic moduli values for the nucleus

pulposus material are found in a narrow band is that for the frequency range tested it is in the rubbery plateau region. The rubbery plateau region for the gel system tested in this study is narrower and found at lower frequencies than that of the nucleus pulposus. The viscous modulus data shown in fig. 27 shows that the agarose material is not as viscous as the human nucleus pulposus under dynamic testing. The complex modulus data reveals the same relationship that the elastic modulus data showed in relation to the agarose material. The agarose gel material is obviously not a good replacement for the nucleus because of many reasons and a few of those are shown in the plot. The gel has moduli values that are much lower than the nucleus at low frequencies, and moduli values that are too large at high frequencies. The agarose gel also behaves too much like a solid and not enough like a viscoelastic material in order to be effectively implanted into the lumbar spine. It is true that one flaw in the actual nucleus pulposus is that once the annulus is ruptured, the nuclear material easily flows out, so any material that you would want to implant would need to behave more like a solid than a fluid in order to resist extrusion, but the agarose gel is too much like a solid in this case.

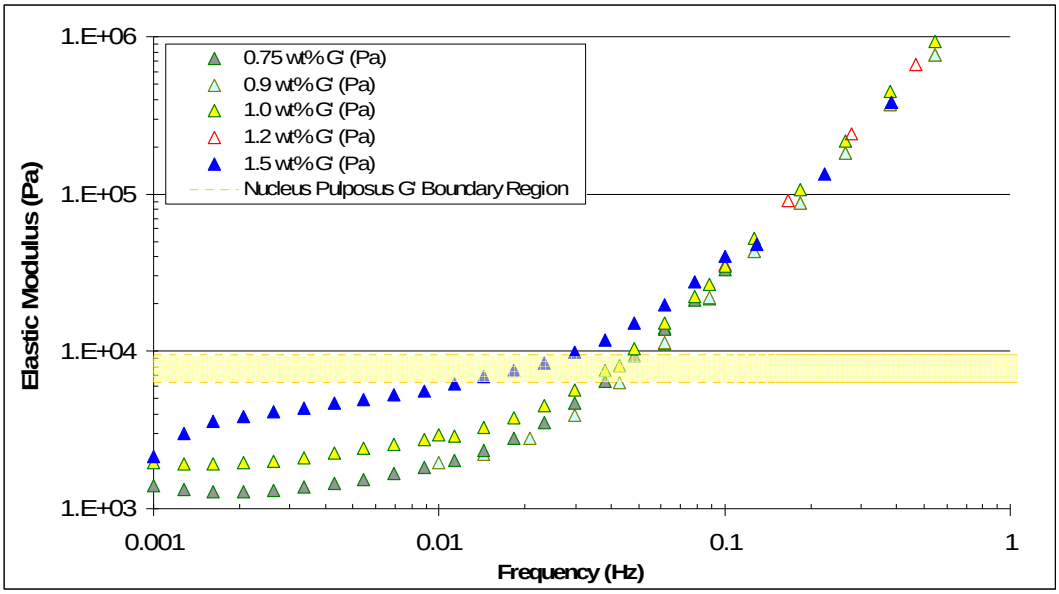

Figure 26: Frequency sweep performed at γ = 0.05 for different weight percents of agarose gel showing G' values and a boundary region of elastic moduli from the Nucleus Pulposus under the same loading mode.

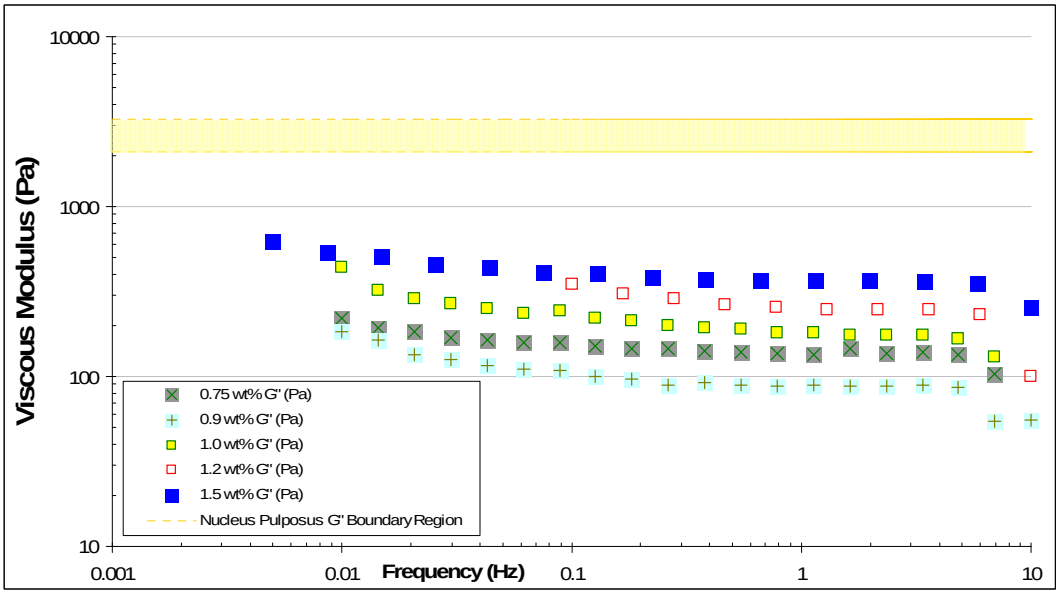

Figure 27: Frequency sweep performed at γ = 0.05 for different weight percents of agarose gel showing G" values and a boundary region of viscous moduli from the Nucleus Pulposus under the same loading mode.

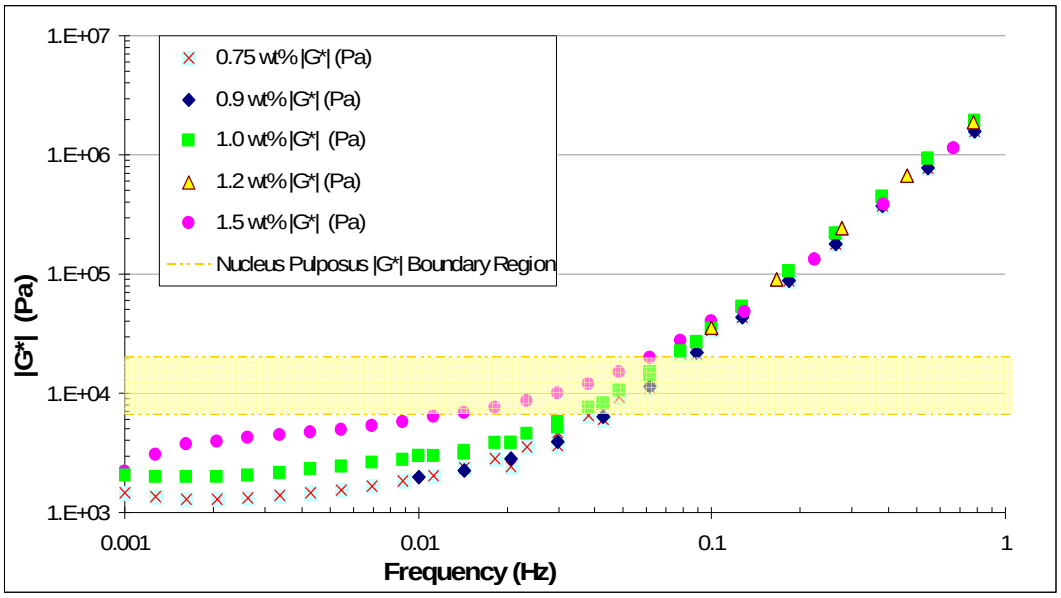

Figure 28: Frequency sweep performed at γ = 0.05 for different weight percents of agarose gel showing |G*| – Complex Modulus values and a boundary region of complex moduli from the Nucleus Pulposus under the same loading mode.

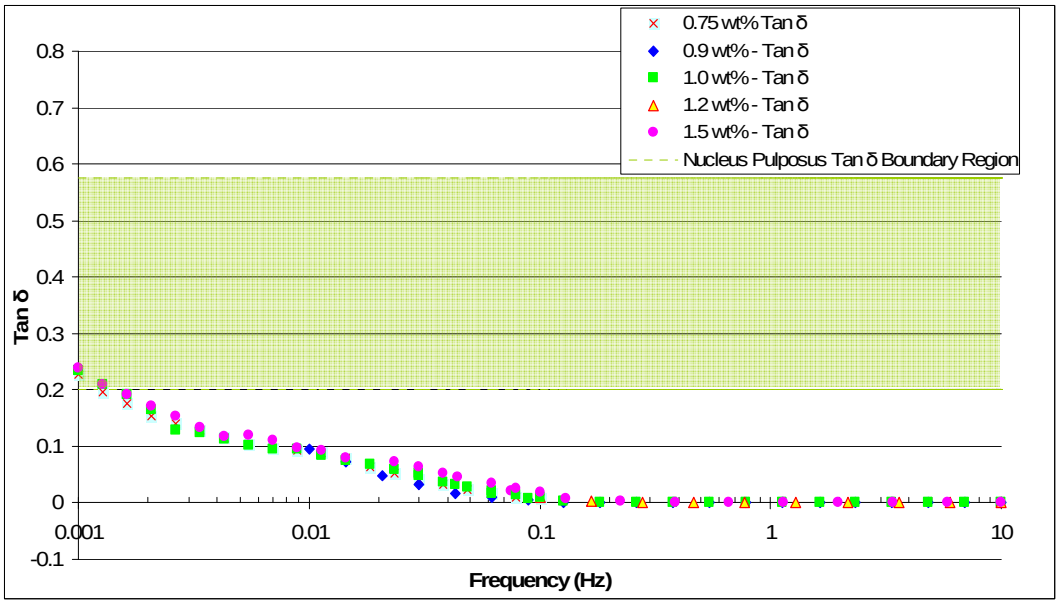

Figure 29: Frequency sweep performed at γ = 0.05 for different weight percents of agarose gel showing tan δ values and a boundary region of tan δ from the Nucleus Pulposus under the same loading mode.

The rheological testing was very important in figuring out the rheological properties of the agarose gel system and then being able to relate them to the extrudability testing done with the model of the degenerative intervertebral disc. The low frequency dynamic tests showed that if weight percent is increased the stiffness of the material was also increased since the elastic moduli were higher. This was then compared to the static compression tests and a similar result was found. As the weight percent of each agarose gel sample was increased its Young's modulus, a measure of stiffness, also increased. The values for Young's modulus varied from 150 kPa to 340 kPa for the range of weight percents of agarose gel tested. In a previous study, the static compressive modulus of human nucleus pulposus was found to be 310 kPa (Perie *et al.* 2005). The fact that the human nucleus pulposus compressive moduli falls within the range of the moduli for agarose does not indicate that agarose should be used as a replacement. It does however indicate that a multitude of different tests need to be performed on a proposed implant material because there are so many different aspects that need to be considered. Rheological tests are good benchmark for evaluating the materials in dynamic tests because physiological conditions can be mimicked. Compression tests are also very important because the spine is under compression most of the time so implant materials need to be able to withstand heavy compressive loads without failing. You can very easily make a material that is great at withstanding oscillatory stress and strain without yielding but may not withstand as much compression. You can also make a material that is very stiff and can withstand a

large compressive force without failure, but it will probably not handle oscillations quite well. There is a very delicate balance that has to be achieved when creating an implant material because the native tissue is so complex. You are also not trying to create the same exact properties as the native tissue because the native tissue failed, so the implant material would fail as well under the same conditions. The material needs to withstand a great deal of fatigue and dehydration as the human body goes through its daily cycle. So far, researchers have identified various forms of hydrogels as possible implant materials but even as promising as they look, it is not certain how they would react in the human body because there is still not enough evidence showing their behavior. The testing protocol carried out in this study with the degenerative disc apparatus proved to yield reproducible results that could be related to those measured rheological properties. More complex materials such as were discussed earlier would need to be thoroughly tested in the apparatus to gain a better understanding of how they would behave if implanted.

In summary, the simple polymer gel system used in this study, agarose gel, was tested in the degenerative disc apparatus with reproducible results. Its properties however were identified using rheological characteristics from dynamic oscillatory tests and compared to static mechanical characteristics obtained through compression tests. The disc apparatus created shows great promise in identifying how a proposed implant material would behave if placed in a degenerative disc and compressed at physiological loads.

CONCLUSIONS

The study conducted here is just one “stepping stone” in a long path that needs to be built in order to have a commercially available device that mimics the native nucleus pulposus yet does not extrude as easily as the native tissue. Many studies have been done to try to create materials suitable for implantation, but there have not been any studies that have looked at the extrudability of each material from a model degenerative intervertebral disc device. A simple degenerative disc apparatus was designed and constructed and testing on it began with a simple gel system of agarose. Weight percent of agarose gel was varied and compression tests in the degenerative disc apparatus were carried out to reveal that the stiffer gels could only be extruded at higher loads. Rheological tests also correlated that the higher weight percent gels had larger stiffness values through the high elastic (storage) moduli and low viscous (loss) moduli. It was very clear that agarose gel is not a suitable nucleus pulposus replacement material because it does not possess the same mechanical characteristics and that was not being questioned in this study. The emphasis here was placed on designing and constructing a device that could be used for testing possible materials for nucleus pulposus replacement. A test protocol was established and very reproducible

results were obtained for the simple gel system analyzed. The extrudability results were then correlated with the measured rheological properties.

FUTURE WORK

The next step in the process is to identify a more complex gel system that more closely mimics the mechanical properties of the nucleus pulposus and test it using the protocol found here with the degenerative disc apparatus. A more suitable gel system would be a hydrogel one which has shown great promise in mimicking the native tissue and even being a bit more “solid-like” in order to resist extrusion. Materials with inclusions such as rods or spheres could be tests as well.

BIBLIOGRAPHY

Argoubi, M. and A. Shirazi-Adl (1996). "Poroelastic creep response analysis of a lumbar motion segment in compression." Journal of Biomechanics **29**: 1331-1339.

ASTM (2005). Standard Test Method for Plastics: Dynamic Mechanical Properties: Melt Rheology. ASTM, ASTM International. **D 4440 - 01**.

Atlas, S. T. (2001). "Surgical and nonsurgical management of sciatica secondary to a lumbar disc herniation: Five-year outcomes from the Maine Lumbar Spine Study." Spine **26**(10): 1179-1187.

Bader, R. A. (2006). Development and Characterization of novel hydrogels for nucleus pulposus replacement. PhD Dissertation in Materials Science. Corvallis, Oregon State University**:** 109.

Beard, H. K. and R. L. Stevens (1980). Biochemical changes in the intervertebral disc. The Lumbar Spine and backache. M. I. V. Jayson. London, Pitman**:** 407-436.

Best, B. A., F. Guilak, L. A. Setton, W. Zhu, F. Saed-Nejad, A. Ratcliffe, M. Weidenbaum and V. C. Mow (1994). "Compressive mechanical properties of the human anulus fibrosus and their relationship to biochemical composition." Spine **19**: 212-221.

Bogduk, N. (1997). "Clinical Anatomy of the Lumbar Spine and Sacrum."

Boyd, L. M. and A. J. Carter (2006). "Injectable biomaterials and vertebral endplate treatment for repair and regeneration of the intervertebral disc." European Spine.

Buckwalter, J. A. (1995). "Aging and degeneration of the human intervertebral disc." Spine **20**: 1307-1314.

Deyo, R. A. and Y. J. Tsui-Wu (1987). "Descriptive epidemiology of low-back pain and its related medical care in the United States." Spine.

Di Martino, A., A. R. Vaccaro, J. Y. Lee, V. Denaro and M. R. Lim (2005). "Nucleus pulposus replacement. Basic science and indications for clinical use." Spine **30**: S16-S22.

Ferguson, S. J., K. Ito and L. Nolte (2004). "Fluid flow and convective transport of solutes within the intervertebral disc." Journal of Biomechanics **37**: 213-221.

Foster, M. R. (2007). Herniated Nucleus Pulposus, WebMD. **2007**.

Gray, H. (1918). Anatomy of the Human Body. Philadelphia, Lea & Febinger.

Hardy, R. W. (1993). Lumbar Disc Disease. New York, Raven Press.

Iatridis, J. C., L. A. Setton, M. Weidenbaum and V. C. Mow (1997). "Alterations in the mechanical behavior of the human lumbar nucleus pulposus with degeneration and aging." Journal of Orthopaedic Research **15**(2): 318-322.

Iatridis, J. C., L. A. Setton, M. Weidenbaum and V. C. Mow (1997). "The viscoelastic behavior of the non-degenerate human lumber nucleus pulposus in shear." Journal of Biomechanics **30**(10): 1005-1013.

Iatridis, J. C., M. Weidenbaum, L. A. Setton and V. C. Mow (1996). "Is the nucleus pulposus a solid or a fluid? Mechanical behaviors of the nucleus pulposus of the human intervertebral disc." Spine **21**(10): 1174-1184.

Johnstone, B. and M. T. Bayliss (1995). "The large proteoglycans of the human intervertebral disc." Spine **20**: 674-684.

Katz, M. M., A. R. Hargens and S. R. Garfin (1986). "Intervertebral disc nutrition: Diffusion versus convection." Clinical Orthopaedics and Related Research **210**: 243-245.

Klara, P. M. and C. D. Ray (2002). "Artificial Nucleus Replacement." Spine **27**: 1374-1377.

Marchand, F. and A. M. Ahmed (1990). "Investigation of the laminate structure of lumbar disc anulus fibrosus." Spine **15**: 402-410.

Maroudas, A. (1988). Nutrition and metabolism of the intervertebral disc. The biology of the intervertebral disc. P. Ghosh. Boca Raton, CRC Press. **II:** 1-37.

Mow, V. C. and W. C. Hayes (1997). Basic Orthopaedic Biomechanics. Philadelphia, Lippincott Williams & Wilkins.

Naylor, A. (1971). "Intervertebral disc prolapse and degeneration. The Biochemical and biophysical approach." Spine **1**: 108-114.

Perie, D., D. Korda and J. C. Iatridis (2005). "Confined compression experiments on bovine nucleus pulposus and annulus fibrosus: sensitivity of the experiment in the determination of compressive modulus and hydraulic permeability." Journal of Biomechanics **38**: 2164-2171.

Roughley, P. J. (2004). "Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix." Spine **29**(23): 2691-2699.

Taylor, J. R. (1990). "The development and adult structure of lumbar intervertebral discs." J Man Med **5**: 43-47.

Ullrich, P. F. M. (1999). Spine Surgery, Spine Health. **2007**.

Urban, J. and A. Maroudas (1980). "The chemistry of the intervertebral disc in relation to its physiological function." Clin Rheum Dis **6**: 51-76.

Urban, J. P., S. Holm, A. Maroudas and A. Nachemson (1982). "Nutrition of the intervertebral disc: Effect of fluid flow on solute transport." Clinical Orthopaedics and Related Research **170**: 296-302.

Van De Graaff, K. M. (2002). Human Anatomy. New York, McGraw-Hill.