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## An Injectable Nucleus Pulposus Implant Restores Compressive Range of Motion in the Ovine Disc

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### Abstract

**Study Design**—Investigation of injectable nucleus pulposus (NP) implant.

**Objective**—To assess the ability of a recently developed injectable hydrogel implant to restore non-degenerative disc mechanics through support of NP functional mechanics.

**Summary of Background Data**—While surgical intervention for low back pain is effective for some patients, treated discs undergo altered biomechanics and adjacent levels are at increased risk for accelerated degeneration. One potential treatment as an alternative to surgery for degenerated disc includes the percutaneous delivery of agents to support NP functional mechanics. The implants are delivered in a minimally invasive fashion, potentially on an outpatient basis, and do not preclude later surgical options. One of the challenges in designing such implants include the need to match key NP mechanical behavior and mimic the role of native non-degenerate NP in spinal motion.

**Methods**—The oxidized hyaluronic acid gelatin implant material was prepared. *In vitro* mechanical testing was performed in mature ovine bone-disc-bone units in three stages: intact, discectomy, and implantation vs. sham. Tested samples were cut axially for qualitative structural observations.

**Results**—Discectomy increased axial range of motion (ROM) significantly compared to intact. Hydrogel implantation reduced ROM 17% ( $p < 0.05$ ) compared to discectomy and returned ROM to intact levels (ROM intact 0.71 mm, discectomy 0.87 mm, post-implantation 0.72 mm). While ROM for the hydrogel implant group was statistically unchanged compared to the intact disc, ROM for sham discs, which received a discectomy and no implant, was significant increase compared to intact. The compression and tension stiffness were decreased with discectomy and remained unchanged for both implant and sham groups, as expected because the annulus fibrosus was not repaired. Gross morphology images confirmed no ejection of NP implant.

**Conclusion**—An injectable implant that mimics non-degenerate NP has the potential to return motion segment ROM to normal subsequent to injury.

## Keywords

Lumbar Spine; Hydrogel; Compression; Nucleus Pulposus; Implant; Mechanics; Sheep

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## Introduction

The nucleus pulposus (NP) of the intervertebral disc is a hydrated material with a high proteoglycan content that generates a large osmotic pressure. The NP osmotic pressure functions to provide load support in compression and places the surrounding annulus fibrosus (AF) into circumferential tension.<sup>1</sup> In the setting of disc degeneration, the NP proteoglycan content decreases, reducing pressure and altering the overall disc load support, resulting in increased range of motion (ROM) in axial loading.<sup>2,3,4</sup> Similar to degeneration, in laboratory studies, when NP is removed to simulate degeneration or discectomy surgery, the ROM increases.<sup>5,6,7</sup> Restoration of ROM, perhaps via reestablishment of NP swelling pressure, is a key parameter to treatment strategies for disc disease.<sup>4,5</sup>

There is a clinical need for an injectable NP implant to restore disc mechanical function. While injectable NP implants are under investigation as motion-preserving alternatives to spine fusion, an FDA approved injectable NP implant has yet to be offered. The advantages of injectable implants include minimally invasive delivery, potentially on an outpatient basis, and injectable implants do not preclude later surgical options. Replacement of native NP with an implant, in the setting of healthy AF, may simultaneously reduce pain, slow progression of degeneration and restore non-degenerate spinal mobility.<sup>8,9,10,11,12</sup> Significant challenges to implant design include the need to match key NP mechanical behavior *ex vivo* and mimic the role of native non-degenerate NP in spinal motion.<sup>8,11,13,14</sup> Long term development and testing of nucleoplasty devices should include five assessments: 1) mechanical testing, 2) cell-materials interaction assimilation leading to material integration, 3) kinematic testing including ROM and resistance to expulsion, 4) biocompatibility and biodurability, and 5) safety testing.<sup>15</sup> Having previously completed mechanical testing<sup>16,17</sup> and cell-materials interaction assimilation leading to material integration<sup>18</sup>, this study will focus on step 3, ROM testing. In this study we specifically investigate the ability of a recently developed NP implant, consisting of oxidized hyaluronic acid (oHA) and gelatin material,<sup>16</sup> to restore disc mechanical function characteristics that are most related to the NP.

The ROM is the mechanical behavior most related to the NP function in axial range of motion.<sup>3,4,5,17</sup> In order to demonstrate restoration to normal ROM, a model system with a healthy disc is needed, precluding the use of human cadaver samples that are typically older and quite degenerated. The sheep disc is a suitable model of the human disc with respect to geometry and axial mechanics<sup>20,21,22,23</sup> and has previously been utilized for *in vitro*<sup>22,23,24,25,26</sup> and *in vivo*<sup>24,27,28,29,30,31,32</sup> studies of discectomy. One advantage of the sheep is that it is a suitable model for future *in vivo* evaluation of the NP implant if *in vitro* results warrant additional studies.

The objectives of this study were to 1) evaluate the ovine disc as an *in vitro* model of discectomy for NP implant testing, and 2) to determine if an injectable hydrogel could functionally replace the NP by re-establishing native mechanics, primarily ROM. In the current study, ROM was specifically evaluated as a first step in order to characterize the material in restoring the disc mechanics. It is hypothesized that discectomy will result in increased ROM and hypermobility and that injection of an oHA-gelatin hydrogel NP implant will return ROM to normal levels.

## Materials and Methods

### Study design

The experimental group consisted of three conditions: intact, discectomy, NP implant (n=10, Figure 1). Axial mechanical properties were measured in each of these conditions for all discs. The control group consisted of the same three conditions and underwent the same protocol in its entirety, but without the addition of an NP implant for the third condition (n=10, Figure 1). Gross morphological evaluation was performed at the end of the test for both the experimental group (NP treated discs) and control group (discectomy but no NP implant), and separate intact discs were also included for comparison of morphological structure.

### Specimen preparation

Lumbar spines were harvested from twenty skeletally mature sheep previously obtained for a non-spine animal study. All musculature and soft tissue were dissected and facets and transverse processes removed to eliminate any facet and transverse process contributions on disc mechanics. Bone-disc-bone units, were prepared by making parallel cuts through the vertebral bodies above and below the disc at lumbar spine level L4-L5. Samples were potted in polymethyl-methacrylate bone cement. Kirschner wires were placed through the bone cement and vertebral body to increase pull out strength. The potted samples were wrapped in saline soaked gauze throughout preparation to prevent dehydration. Prior to each mechanical testing step, the sample was thawed and hydrated for 18 hours in a 4°C refrigerated PBS bath to establish a uniform initial hydration condition.<sup>33</sup>

### Mechanical testing

Mechanical tests were performed in PBS bath on an Instron 8874 servohydraulic test frame (Instron, Canton, MA). The displacement resolution of the Instron 8874 is 0.004 mm. Mechanical testing protocol consisted of 20 sinusoidal cycles from -300 N compression to +300 N tension at 1 Hz, as previously described.<sup>20</sup> The maximum cyclic compressive load of -300 N was selected to represent approximately 1.5 times human body weight scaled for differences in cross sectional area of the human and ovine intervertebral discs.<sup>20,21,22,23</sup> Therefore, this load represents a moderately high stress within the physiological range of the human. This test protocol was repeated three times per sample (intact, discectomy, NP implant/sham).

The 20<sup>th</sup> test cycle was selected for data analysis, with the first 19 cycles of loading serving as preconditioning to establish a repeatable hydration level and force-displacement hysteresis response.<sup>5,34</sup> Axial range of motion (ROM) was directly measured as the total peak-to-peak displacement. The compression stiffness was calculated from a linear regression of load-displacement between -200 and -300 N and the tension stiffness similarly calculated between +200 and +300 N.

### Discectomy

A standard discectomy approach was performed. The sample was thawed then the right posterior AF was incised with an 11-blade (2.5 mm incision). Micro-rongeur was inserted into the nuclear cavity and loose NP material was removed. Microcurette was used to release additional nuclear material and micropituitary rongeur was used to resect remaining loose NP. The amount of nuclear material removed ( $0.024 \pm 0.003$  g, approximately 35% of the total NP mass) was determined by immediately weighing the sample. The sample was then rehydrated, mechanical testing performed as above, and refrozen.

## Implant/sham

Following discectomy and mechanical testing, the sample was thawed and the experimental group was injected with a hydrogel implant previously designed and tested for this purpose,<sup>17</sup> while the control group underwent the same procedures without an NP implant. The implant consisted of an oxidized hyaluronic acid (oHA) and gelatin material. One gram of hyaluronan ( $M_w$   $1.5 \times 10^6$  Engelhard, Inc.) was dissolved in 80 ml of water in a shaded flask and sodium periodate, dissolved in 20 ml water (pH 5.4), was added dropwise. After incubating at ambient temperature, 10 ml of ethylene glycol was added to terminate the reaction followed by stirring for one hour. The product was dialyzed for 3 days and lyophilized to obtain oxidized hyaluronic acid oHA (yield: 50-67%). Gelatin (Bloom 300, Type A,  $M_w$  100,000) solution of 20% (w/v) concentration (in pH 9.4, 0.1 M borax) was prepared. The oHA and gelatin solutions were mixed in the weight ratio of 7:3 stirred for 1 min at 37°C.

For the experimental group, the implant was injected directly into the NP cavity through the annular opening created during discectomy with a blunt 21-gauge needle. The NP cavity was filled with the liquid formulation of the implant ( $0.26 \pm 0.09$  ml) by syringe. For the control group, the 21-gauge needle was inserted but no material was injected. For all discs, the AF was not repaired or filled with material. Samples were maintained at 37°C, body temperature, for one hour to permit gel formation and then frozen until rehydration and mechanical testing, as described above.

## Statistical analysis

The data are presented as mean values  $\pm$  standard deviation. A single factor repeated measures ANOVA (levels: intact, discectomy, implant/sham) with post-hoc Bonferroni test was applied separately to the experimental and control groups. Dependent variables were ROM, compression stiffness, and tension stiffness. To confirm that the two groups were only different with respect to the final treatment condition, a two-way ANOVA with post-hoc Bonferroni test was performed between the experimental and control groups. Significance was set at  $p < 0.05$ .

## Results

### Discectomy

Discectomy significantly altered mechanical function compared to intact samples. The ROM significantly increased for both the experimental and control groups (Figure 2); for the experimental group the ROM increased 18% from 0.71 mm intact to 0.87 mm following discectomy. The ROM of sham and experimental groups were not statistically different ( $p > 0.05$ ). The compression stiffness was not significantly altered following discectomy (Figure 3) and for the experimental group was 1517 N/mm intact and 1344 N/mm following discectomy. The tension stiffness significantly decreased for both the experimental and control groups (Figure 4); for the experimental group the tension stiffness decreased 24% from 718 N/mm intact to 545 N/mm following discectomy. The intact and discectomy conditions were not significantly different between the experimental and control groups for ROM, compression stiffness, or tension stiffness ( $p > 0.05$ ).

### Treatment with a nucleus pulposus implant

No NP or implant material was exuded from the disc during mechanical testing. For the experimental group, the ROM following NP implantation was 0.72 mm, 17% lower than following discectomy ( $p < 0.05$ ) and not significantly different from the intact condition (Figure 2). Both the compression stiffness and tension stiffness following NP implant treatment were significantly lower than intact condition and not significantly different from

the discectomy condition (Figure 3 and 4). In contrast, for the sham control, with no implant, the ROM was 37% larger than the intact condition ( $p < 0.05$ ) and not significantly different from the discectomy condition (Figure 2). The sham control compression and tension stiffness were not different from the discectomy condition in the absence of an NP implant (Figure 3 and 4).

### Structural Observations

Gross morphology of the intact ovine disc shows concentric AF layers and a hydrated NP (Figure 5A). The experimental discs (Figure 5C) illustrated the successful filling of the NP cavity with implant. No implant material was ejected from the NP cavity. The control disc, where discectomy was performed and no implant placed, shows empty cavity/distribution of remaining NP material/any separation of AF lamella (Figure 5B). The site of annulotomy from discectomy can clearly be seen in both the treated and sham control discs (arrow, Figure 5B and 5C).

### Discussion

The central findings of this study are two-fold. First, discectomy performed *in vitro* on the ovine disc results in a defined, statistically significant, decline in disc mechanical function that remains consistent following repeated testing. Secondly, an injectable oHA-gelatin NP implant can restore a key factor associated with NP degeneration: increased ROM. This study both demonstrates a successful study platform for assessing injectable NP implants and an effective implant prototype for normalizing NP mechanics. The implant designed to mimic native NP was able to return post-discectomy discs to intact range of motion (Figure 2). However, as expected, the compression and tension stiffness did not return upon the implant delivery due to the annular opening created during annulotomy, which was not treated (Figure 3 and 4).

### Discectomy

Discectomy (or nucleotomy) in animal models and human cadaver studies demonstrates changes similar to those that occur in early human disc degeneration,<sup>4,35,36,37,38,39</sup> supporting the use of this *in vitro* model system. Human cadaveric studies demonstrate that discectomy alters disc mechanics via decreased pressure, decreased disc height, increased deformation and flexibility, and increased AF bulging.<sup>4,35,36,37,38</sup> Diminishing the amount of NP results in stepwise reduction of swelling and redistribution to support disc mechanics.<sup>5,35,36</sup> The observed increase in ROM with discectomy (Figure 2) substantiates the notion that ROM relies heavily on the quantity of functional NP.<sup>19</sup> In the sham control group, ROM appears to continually increase from its initial discectomy ( $p > 0.05$  compared to discectomy). It is hypothesized that further cyclic loading in the discectomy group with no implant would lead to increased ROM from fatigue damage.

While NP plays a central role in ROM, compression and tension stiffness rely on the AF function.<sup>40,41</sup> This has been clearly shown by using a trans-endplate nucleotomy to isolate the NP function without annular disruption.<sup>5</sup> With trans-endplate nucleotomy the ROM is increased, while the compression and tension stiffness are unaltered,<sup>5</sup> thus delineating the roles of NP and AF in functional mechanics. In this present study, the ROM increased with discectomy, consistent with the trans-endplate nucleotomy. Therefore, it is concluded that decreased compression and tension stiffness is due to annulotomy to access the NP. This decline in traits related to AF function was expected in this model because the annular opening created during discectomy was not repaired or treated. In addition, decreased compression and tension stiffness from annulotomy also may be due to significantly smaller

ovine disc area ( $\sim 500 \text{ mm}^2$ ) compared to human discs ( $\sim 1900 \text{ mm}^2$ ). This effect will be improved if human cadaveric model is used.

### Treatment with nucleus pulposus implant

In the setting of degeneration, or discectomy, load transfer changes occur via a loss of NP pressure, which results in increased ROM and subsequent AF bulging, which may initiate and propagate AF tears.<sup>2,3,4,9,35,41,42</sup> Restoration of ROM is a key factor for treatment strategies designed for painful disc degeneration.<sup>4,5</sup> Since much of disc degeneration begins in the NP, and subsequently results in altered ROM, a critical goal of implant development is the ability to restore ROM. ROM, subsequent to discectomy, returned to normal after treatment with the NP implant, while ROM in discs without an implant remained 33% greater than intact values (Figure 2). Return to normal ROM subsequent to implantation demonstrates that an injectable implant can serve as a proxy for healthy NP in this crucial mechanical role. The current study reported no NP implant herniation or extrusion upon implantation via qualitative analysis. However, future studies will address potential for herniation more rigorously. In addition, future studies will also investigate how the implant functions when subjected to high number of axial tension-compression cycles via fatigue testing.

While many treatment paradigms for disc disease exist, it is increasingly apparent that less invasive, less destructive, motion preserving alternatives are needed. Many spine arthroplasty technologies are currently being tested with the intent of relieving pain and maintaining motion while preserving the health of adjacent segments.<sup>43</sup> Arthroplasty options include total disc replacement (TDR) and subtotal disc replacement or nucleoplasty.<sup>13,44</sup> TDR, while permitting some motion preservation, is destructive to the native joint and requires highly invasive implantation techniques. Nucleoplasty via injectable implant has the advantages of being less invasive in deployment and does not preclude future surgical alternatives, including TDR or spinal fusion.<sup>15</sup> The advantage of injectable implants include, inter-digitation with native NP and potential to recreate the natural NP function with uniform stress distribution and shock absorption capability.<sup>15</sup> Contrary to injectable implants, solid mechanical implants are not able to maintain even stress distribution and lack of the ability to absorb shock.<sup>15</sup> A further division between the types of NP implants is delivery approach. Prefabricated mechanical implants necessitate surgical approaches associated with some joint destruction. With motion preservation and minimal tissue destruction as a focus, injectable implants will restore short term and long term function of the disc.<sup>14,44,45,46</sup>

This study was performed in a non-human *in vitro* model that permits collection of healthy baseline mechanical data in the same animal model intended for future *in vivo* pre-clinical testing. There are limitations in the use of sheep, which has a small disc height compared to the human that resulted in large discectomy-related changes to the AF. However, the use of a sham control group and repeated testing on the same sample enabled separation of implant-related treatment effects and discectomy-related damage. Moreover, there is a key advantage to use of the same model for *in vitro* and *in vivo* phases by providing baseline mechanical effects prior to biological remodeling *in vivo*. Another limitation is that the damping properties of the disc were not considered. However, viscoelastic effects are highly dependent on the relative size and fluid flow pathways, which cannot be appropriately determined in the ovine model. Future studies using cadaveric human discs will investigate these properties.

The conclusion of this work is that an injectable implant will return ROM, a crucial parameter of disc mechanical function, to baseline. While the current study established a number of preliminary requirements for the current material to be developed for an NP

implant, future work will comprehensively address material characterization, biocompatibility assessment, and safety evaluation. For the millions of patients each year not responding to conservative therapy and also not meeting criteria for surgical intervention there is currently no proven treatment modality. An NP injectable implant may be a new treatment for disc disease to reach these patients.

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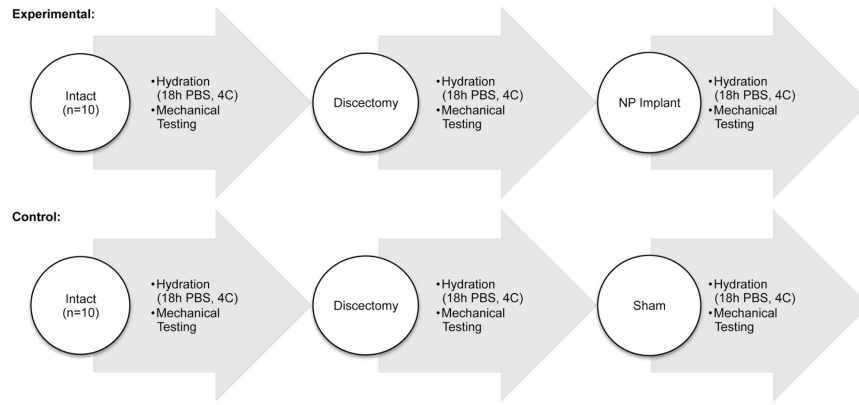
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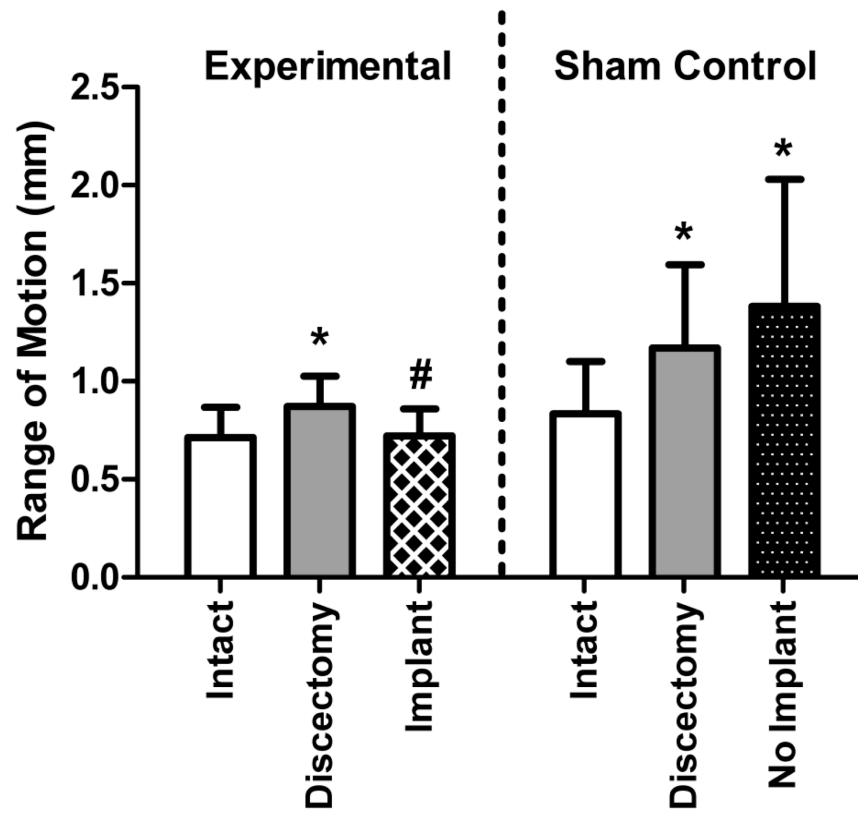
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### Key Points

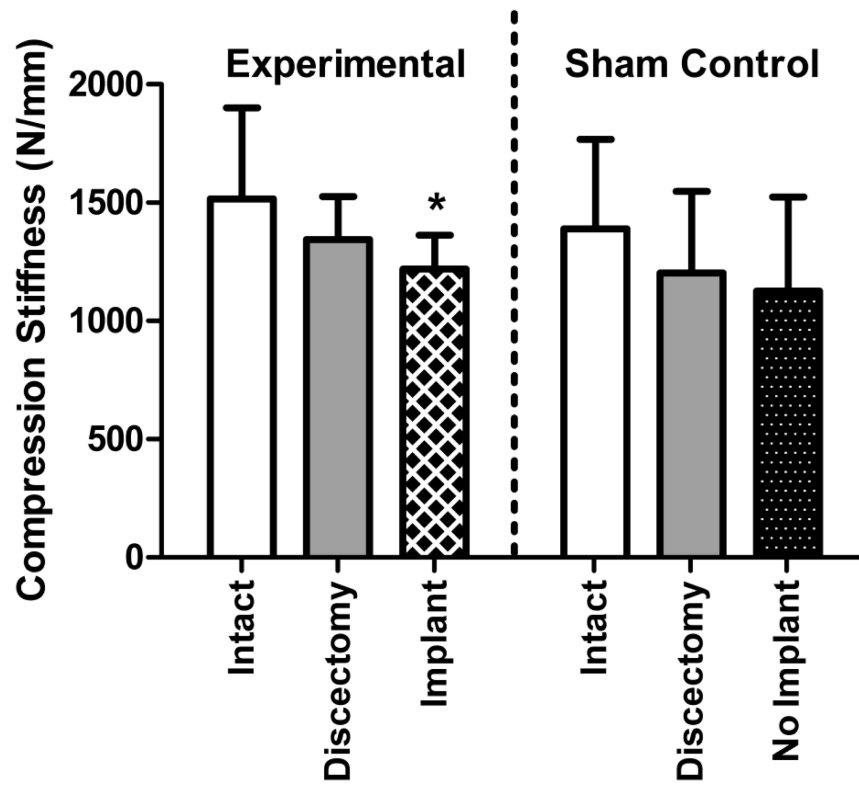
- A significant increase in ROM resulting from discectomy supports the notion that ROM depends on the quantity of functional NP.
- Return to normal ROM following an NP implant suggests that the injectable implant can serve as a proxy for healthy NP in this crucial mechanical role.
- Decline in properties related to AF function (tension and compression stiffness) is expected in this model because the annular opening created during discectomy was not repaired.
- The conclusion of this work is that an injectable implant will return ROM to normal, a crucial parameter of NP mechanical function.



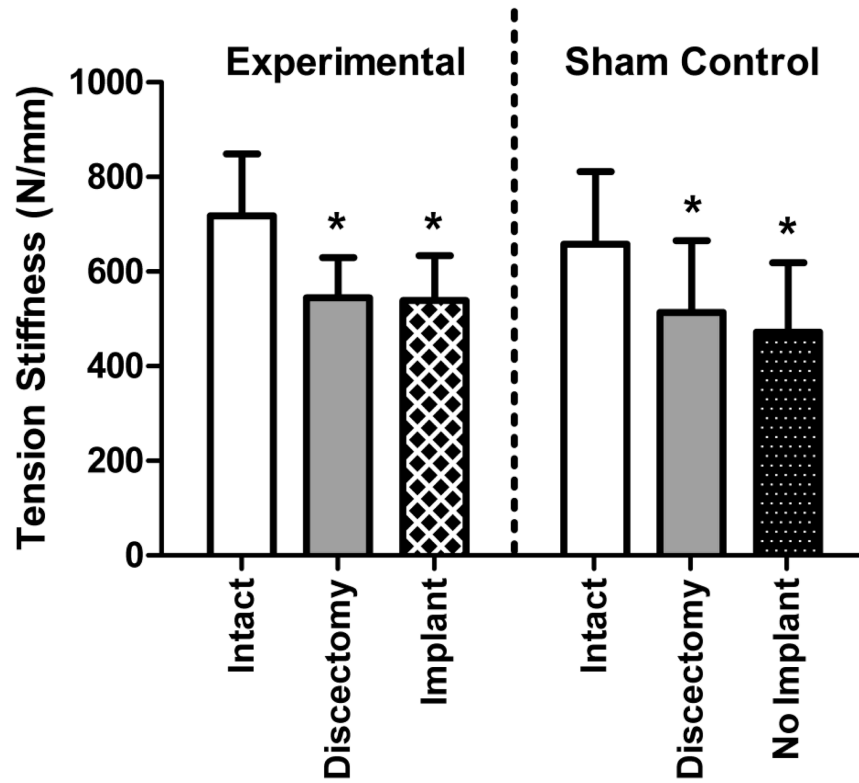
**Figure 1.** Experimental Design. Mechanical tests were performed in three conditions for each disc: intact, post-discectomy, and following implant treatment (n=10). Another set of samples underwent the same procedure, but without implant after discectomy (n=10).



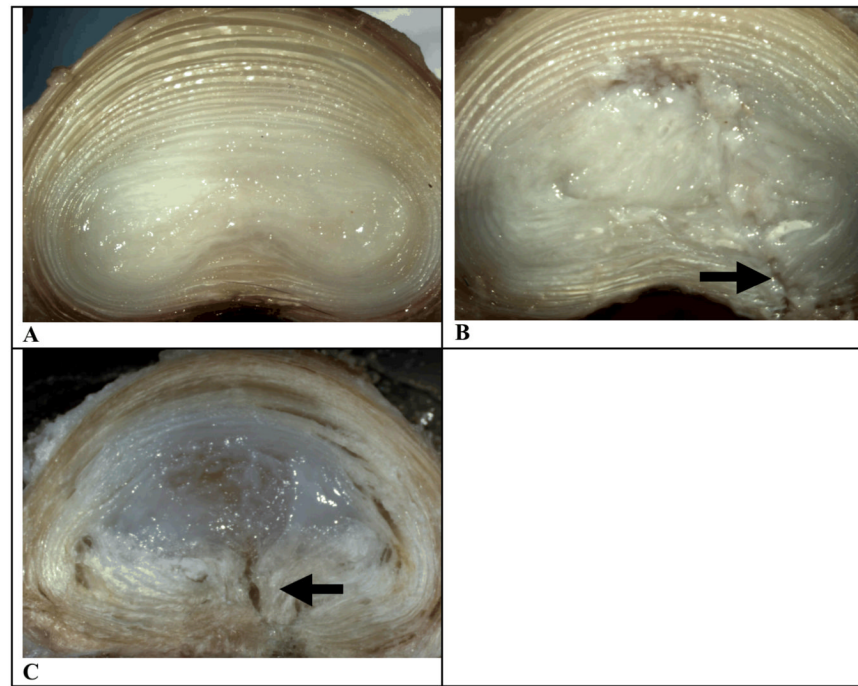
**Figure 2.** Range of Motion (ROM). Discectomy alone significantly increased ROM for both groups. ROM following NP implant treatment significantly decreased, back to its intact state. \*  $p < 0.05$ .



**Figure 3.** Compression Stiffness. Compressive stiffness was not significantly altered following discectomy for both groups. Compression stiffness following NP implant treatment was significantly lower than intact control, but not significantly different than the discectomy condition. \*  $p < 0.05$ .



**Figure 4.** Tension Stiffness. Tension stiffness was significantly decreased following discectomy for both groups. Tension stiffness following NP implant treatment was significantly lower than intact control, but not significantly different than the discectomy condition. \*  $p < 0.05$ .



**Figure 5.** Representative images of axially sectioned discs. **A:** Gross morphology of a healthy, intact ovine disc. **B:** Gross morphology of an ovine disc treated with discectomy. **C:** Gross morphology of an ovine disc treated with discectomy, followed by NP implant injection.