The Use of a Novel Injectable Hydrogel Nucleus Pulposus Replacement in Restoring the Mechanical Properties of Cyclically Fatigued Porcine Intervertebral Discs

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Repeated flexion and extension of an intervertebral disc has been shown to affect the angular stiffness of spinal motion segments and is a barometer of the mechanical integrity of the disc. A degenerated disc that loses height causes higher levels of stress on the annulus and facet joints which may increase its level of degeneration; restoring disc height may therefore help to slow this degenerative cascade. Previous research has indicated that nucleus implants have the potential to improve the mechanical characteristics of a disc and an implant that is custom-fit to the intervertebral disc yields the best results with respect to decreasing annular degeneration. Two groups of porcine spinal motion segments were exposed to repeated flexion and extension. One group was then injected with a novel hydrogel while the other group was used as a control. Both groups were then exposed to another round of cyclic flexion and extension to examine the effect that the hydrogel had on restoring the original mechanics to the motion segments. Angular stiffness was restored to the group which received the hydrogel injection in addition to a significant improvement in specimen height. No significant changes were seen in the group which did not receive an injection. It would appear that use of the novel injectable hydrogel is able to restore angular stiffness to cyclically fatigued spinal motion segments. It is also important to note that continued repetition of the event causing specimen fatigue after performing hydrogel injection will result in an eventual return to the same fatigued state. [DOI: 10.1115/1.4024285]

Keywords: intervertebral disc, nucleus pulposus, herniation, hydrogel, angular stiffness

1 Introduction

Repetitive flexion and extension motions have been shown to cause changes in the mechanical properties of a spinal motion segment [1]. More specifically, as the disc loses height through its response to fatigue loading, angular stiffness increases [1]. From an injury perspective, these repetitive motions and associated stiffness have also been shown to produce intervertebral disc herniations [1–3]. Height loss appears to be an important variable associated with the characteristic degenerative cascade that follows injury [4], motivating efforts to prevent height loss or restore disc height [5]. One possible means of providing restoration of mechanical function which has recently come forward is the use of injectable biocompatible hydrogels implanted within the intervertebral disc [6] as a means of nucleus pulposus replacement. This material is able to be injected into the disc as a liquid at room temperature, conforming to a disc's unique geometry and then forming into a gel at body temperature. The nature of the material suggests a procedure which would result in less patient trauma given the use of only a needle puncture to deliver the hydrogel into the intervertebral disc. One problematic challenge has been to contain the implanted gel and to prevent implant migration [7].

Intervertebral disc height loss as a result of injury, degeneration, or fatigue instigates several changes. The inner margins of the annulus begin to bulge inward [8], resulting in higher levels of stress on the annulus [9]. This increased stress on the annulus can possibly result in an accelerated delamination of the collagen fibers [2]. Thus, it would be beneficial to reduce this annular bulging and restore intervertebral disc height. Data from Meakin et al. shows that replacement of the nucleus pulposus does indeed prevent this inward bulging [10]. In addition, flattened discs redirect compressive load to the facet joints, which are thought to result in secondary facet change [11].

One approach to restoring disc height has been to inject hydrogels mimicking native nucleus pulposus. Due to issues with the extrusion of implant material through damaged collagen in the annulus [7], the success of artificial nucleus material depends on the ability to form a supportive scaffold and to seal any endplate fractures [12]. Bertagnoli et al. performed mechanical testing on preformed hydrogel implants with a reinforced mesh layer [13]. They found that the implant resisted radial bulging under axial compression and resisted compressive fatigue testing, along with extrusion under bending failure tests [13]. While reinforcement layers may be warranted to prevent extrusion in a preformed implant that must be implanted through an incision, it may be less of a factor when the implant can be delivered through a less-invasive needle puncture instead. Furthermore, model data from Dahl et al. suggests that nucleus pulposus replacements that conform to the unique geometry of each disc result in decreased degeneration of the annulus [14]. This evidence would support the

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position that the disc cannot simply be 'wedged' up by an implant, but that restoring a nondegenerated stress distribution [15] to the intervertebral disc requires a material that can conform to its unique geometry.

The purpose of this study was to evaluate, as a proof of concept, the efficacy of a novel injectable hydrogel that conforms to the geometry of the intervertebral disc in restoring the original mechanical properties to a spinal motion segment exposed to repetitive cycles of flexion and extension. It was hypothesized that the use of the novel injectable hydrogel would restore the angular stiffness of fatigued spinal motion segments compared to control motion segments put through the same fatigue protocol.

2 Materials and Methods

2.1 Tissue Sample Preparation. Ten porcine cervical spines (mean age: 6 months, weight: 80 kg) were utilized in this investigation, all meeting Galante's grade one criteria of a normal disc [16]. Functional spinal motion segments consisting of C5 and C6 or C3 and C4 vertebral bodies and the intervening discs were potted into stainless steel cups. The specimens were divided into two groups each consisting of four C5/C6 segments and one C3/C4 segment. A screw at each endplate along with 18 gauge wire looped bilaterally around the lamina and anterior processes secured the specimens in the cups. Filling the cups with nonexothermic dental stone (Denstone®, Miles, South Bend, IN) further secured the specimens. Wrapping saline-soaked cloth and plastic wrap around the specimens maintained hydration.

2.2 Biomechanical Testing Apparatus. All specimens were tested using a dynamic servohydraulic load testing system (Model 8511, Instron Canada, Burlington, Ontario, Canada). The bottom cup translated freely on a surface of ball bearings while flexion and extension moments were created by an electric brushless servo-motor (model BNR3018D, Cleveland Machine Controls, Billerica, MA) and a planetary gear head (model 34PL0400, Applied Motion Products, Watsonville, CA). The system was controlled using a customized software interface.

The hydrogel utilized here was an injectable liquid at room temperature but a semisolid gel at body temperature. Thus, specimens had to be warmed to body temperature using a custom-built heating apparatus (see Fig. 1). The apparatus consisted of a water bath passing heated water through silicone tubing. The silicone tubing was affixed to a portion of thermal blanket of a size which would allow it to be wrapped around the cups without restricting movement of the specimen or physically touching it. Velcro® attached to the borders of the thermal blanket and the base of the stainless steel cups allowed for the apparatus to be wrapped around the specimen and to remain in place. A micro-thermometer was periodically placed at the periphery of the anterior of the disc to measure the temperature of the specimen.

In order to facilitate the injection of the hydrogel into the disc without having it flow back around the needle puncture, all specimens were put into a small amount of traction using a custom-built apparatus that provided an equal amount of traction (5 kg) to each specimen (see Fig. 2). This apparatus consisted of a flat surface supported by four legs; bolts were inserted through the top surface, and the potted specimen was secured to the bolts underneath. A 5 kg weight was suspended from the bottom of the potted specimen, separating the two vertebral bodies and increasing the disc space to facilitate hydrogel injection.

2.3 Hydrogel Properties. The hydrogel used was a composition consisting of thermally-responsive branched copolymers of poly(N-isopropylacrylamide) (PNIPAAm) and poly(ethylene glycol) (PEG) [6]. The copolymer was prepared by free radical polymerization of NIPAAm monomer in the presence of PEG (4600 g/mol) dimethacrylate, in a molar ratio of 700 to 1, as previously described. [6]. Below its lower critical solution temperature

Fig. 1 (*a*) Heating jacket used to bring the specimens to sufficient temperature for the hydrogel to change from a liquid to a gel state. Rubber tubing was attached circumferentially and had heated water cycled through it. (*b*) The jacket itself was then wrapped around the metal cups used to secure the specimens. This ensured that the heating jacket was not in physical contact with the specimen and did not interfere with the flexion or extension motions of the segment while still providing heat. (*c*) Entire heating jacket configuration secured around the specimen while being cyclically fatigued.

(LCST) around 33 °C, PNIPAAm forms a miscible solution with water. Above the LCST, it becomes hydrophobic, so that the polymer and water separate, forming a compact gel. Therefore, aqueous solutions of PNIPAAm can solidify *in situ* without the use of toxic monomers or crosslinkers.

2.4 Testing Protocol. Specimens were placed in the Instron testing machine for compression/bending once they reached a temperature between 33 °C and 37 °C; this temperature was maintained throughout testing since 33 °C was the minimum temperature the hydrogel was required to be at to remain in its gel state. Temperature readings were taken from the anterior periphery of the disc; inserting the micro-thermometer into the disc would have resulted in an unacceptable level of damage, altering the mechanics and integrity of the disc. Pilot work revealed that if the specimens were slowly heated over a time interval of at least one hour, the temperature at the disc's periphery was the same as the temperature inside the disc.

Specimens were preloaded to 300 N of axial compression for 15 min in order to counter any post-mortem swelling which may have occurred [1,9]. Specimens were then loaded to 1500 N of axial compression and were subjected to a passive test establishing the linear region of angular displacement versus axial torque described by Panjabi et al. [17]. Two groups of specimens were

Fig. 2 Custom-built apparatus used to provide traction to specimens in order to achieve sufficient endplate separation facilitating hydrogel injection. Specimens were suspended while a 5 kg weight was hung to the carriage holding the bottom disc in place. A sleeve with tubing running heated water on its periphery provided heat to the specimens without interfering with flexion or extension motions.

created by random assignment. Group 1 consisted of specimens exposed to 8000 cycles of flexion and extension, injected with the hydrogel, and then exposed to another 8000 cycles of flexion and extension. Group 2 served as a control group and consisted of specimens exposed to 8000 cycles of flexion and extension, traction, needle puncture, and then exposed to another 8000 cycles.

2.5 Hydrogel Injection Protocol. Hydrogel was injected into the specimens using an 18-gauge needle and syringe only if the disc periphery was at a temperature between 33 °C and 37 °C. Given small differences in volume within the intervertebral discs of the specimens, hydrogel was injected into the disc until the plunger on the syringe would not depress any further and the disc could not contain any more, ensuring consistency between specimens. The amount of hydrogel injected into each specimen was relatively similar, remaining within a range of 1.2 and 1.4 ml. The needle was left in the specimen for the remainder of the traction period, acting as a stopper and preventing the backflow of hydrogel out of the puncture. After a change to its gel state, there was no backflow of hydrogel out of the puncture hole made by the needle due to its change in viscosity and the needle was removed. All specimens were placed under this traction protocol along with the needle remaining in the disc for 30 min. Group 2 was subjected to the same protocol (including traction and needle puncture), but without the hydrogel injection step.

Prior to being placed back into the testing apparatus, the temperature at the periphery of the anterior portion of the disc was recorded and ensured to be between 33 °C and 37 °C. Specimens were then placed back into the servohydraulic testing machine and subjected to an additional 8000 cycles of flexion and extension. After this second set of flexion and extension cycles, the temperature at the periphery of the anterior portion of the disc was recorded again. If the temperature was recorded to be below 33 °C, the data for that specimen would not be included in the analysis; no specimens experienced this occurrence.

2.6 Data Analysis. Angular stiffness (measured in Nm/deg) was calculated for specimens in 10% incremental time points of the trial to generate a time-history representing the mechanical degradation of each specimen as the number of cycles they were exposed to accumulated. Over time, angular stiffness increased as

the specimen lost height and the loading machine was required under position control to provide higher levels of torque to bring the specimens to the same flexion and extension angles.

Specimen height loss was measured from the relative position of the ram on the Instron. The hydraulic ram is capable of measuring minute changes in vertical displacement accurate to ±0.5% of its travel during a testing trial. Over time the ram dropped due to the creep response of the specimen itself and this was measured from a relative starting position of no height loss to the final vertical displacement (relative to the start of testing) of the specimen at the end of testing.

A one-way repeated measures analysis of variance was performed to test for differences between groups of specimens (pre-intervention, post-intervention, control group, experimental group) and progression through the trial. The Greenhouse-Geisser correction factor was used to ensure that sphericity of data was not violated. A Bonferroni *post hoc* was used to correct for family-wise comparisons. Two independent samples of t-tests were performed to evaluate the differences between the specimen height at the end of the first testing cycle and the beginning of the second testing cycle. This was used to evaluate the efficacy of the hydrogel intervention in restoring height to the segment. All statistical analysis was performed using SPSS software (IBM, Somers NY).

3 Results

Figures 3 and 4 outline the average time-history of specimens in the experimental and control groups, respectively. Hydrogel injection was able to return the mechanical profile to a fatigued specimen in a way that matched the natural progression of a pristine specimen brought through the same fatigue protocol. Specifically, a statistical analysis revealed an interaction between group and progression through the trial. A Bonferroni *post hoc* test revealed no significant differences in the "pre" condition between the control or experimental groups ($p = 1.00$); the same pairwise comparison found significant group differences in the post-intervention condition between the experimental and control group ($p = 0.005$), significant differences between the control "pre" and 'post' conditions ($p = 0.041$), and no significant differences between the experimental group 'pre' and 'post' conditions ($p = 1.00$).

The average specimen height loss after the first trial of cyclic flexion and extension for the experimental and control group was 3.1 mm and 4.7 mm, respectively. The average specimen height loss after the intervention stage was 0.92 mm and 4.16 mm for the experimental and control group, respectively; data for the specimen height loss is presented in Table 1. There was a significant difference between the specimen height in the experimental group at the end of the first testing cycle and the beginning of the second testing cycle ($p = 0.002$), indicating that the hydrogel injection was able to increase the specimen height. No significant difference was found for the control group.

Pairwise comparisons between the percentage increments through the trial revealed statistically significant differences between the four groups (pre-intervention experimental and control, and post-intervention experimental and control) up until the specimens reached 80% progression through the trial of 8000 cycles. This indicated that while the hydrogel restores the original mechanical profile of a fatigued specimen, if it is put under further stress, it will eventually lose mechanical integrity, although the mechanism remains elusive.

Both the heating and traction apparatus were found to be highly effective in achieving the desired effects of bringing specimens to body temperature along with maintaining them at this level and providing the necessary disc space increase required to inject the hydrogel.

4 Discussion

The hypothesis that a hydrogel injection would be able to restore the original angular stiffness of a cyclically fatigued spinal
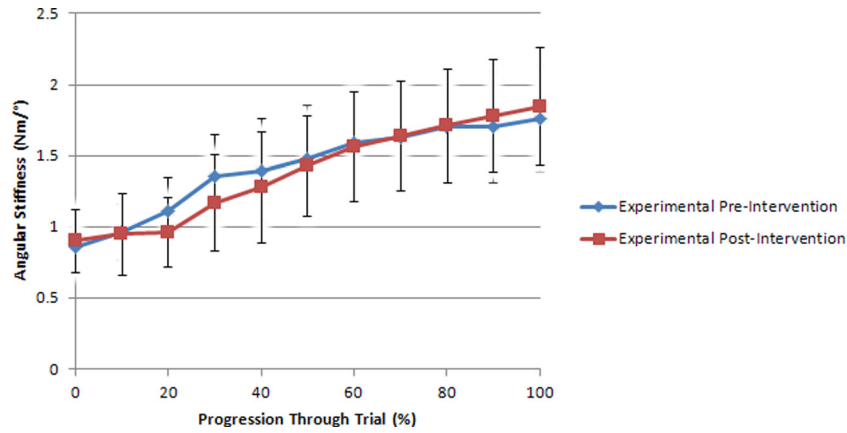
Fig. 3 Angular stiffness values before and after the hydrogel injection for the experimental group of specimens reveal successful restoration of their mechanical behavior. Even after going through 8000 cycles of repetitive flexion and extension the fatigued specimens still closely follow the same pathway as a fresh specimen. Statistical tests revealed the pre- and post-conditions for the experimental group to not be significantly different from each other ($p = 1.00$).
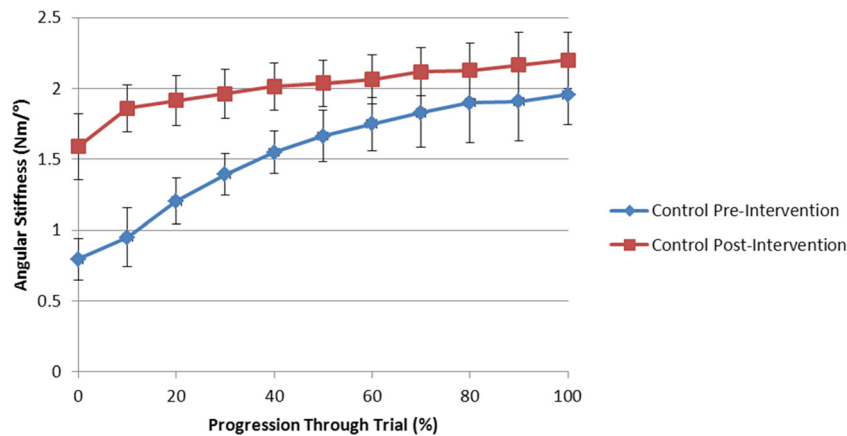

Fig. 4 Angular stiffness values before and after the sham intervention protocol for the control group reveal statistically significant differences between the pre- and post-conditions. This indicates that the mechanical profile was not restored to the specimen after the control intervention ($p = 0.041$). Controls went through the same traction protocol as experimental specimens and were unloaded for the same amount of time, but they did not receive a hydrogel injection.

Table 1 Specimen height changes after fatigue protocol as a result of intervention

	Post-fatigue (mm)	Post-intervention (mm)
Experimental	3.1 (0.7)[a]	0.92 (0.78)[a]
Control	4.7 (1.07)	4.16 (1.04)

Note: Presented as mean and (standard deviation).
[a]Indicates significance between groups.

specimen was supported in this study. This indicates that it has the potential to be a viable candidate for nucleus pulposus replacement. The unique properties of this particular hydrogel allow it to initially flow and conform to any unique shape inside the intervertebral disc, which has been shown to result in decreased levels of degeneration [14]. What is certainly noteworthy is that while the hydrogel was able to restore the mechanical profile of a spinal segment, continual repetitive flexion and extension under load returned the specimen to its degraded profile. It remains unknown whether the gel degraded or there was a mechanical change in the components of the disc. Results from this study are from an

ex vivo mechanical test which cannot be directly related to the response of this material *in vivo*. If this response were to be observed *in vivo*, it would highlight the need for patients to exercise conservative movement patterns that do not replicate the mechanism of injury and prevent a return of the mechanics of the spine to those of a fatigued state. The authors stress that this cannot be directly concluded from the current work.

Certainly one of the important keys in the ability to restore the original angular stiffness to a fatigued segment resides in the ability of the hydrogel to restore height to the specimen. Future investigations will need to examine the mechanical properties of the disc in more detail; namely, the pressurized environment and stress distribution within the intervertebral disc and the efficacy of the hydrogel in mimicking a pristine disc in that respect. A significant advantage of using this novel copolymer in biomaterial applications is that solidification occurs *in situ*. This property allows it to be injected into a disc through a small needle puncture at room temperature and to conform to the unique shape of the disc.

This study was limited in its quantification of specimen height loss. The values used were measured from the position of the ram on the Instron, which makes no distinction between fluid loss in the disc and endplate and trabecular bone deformation, all of

which cause a segment to lose height. Nevertheless, the differences in height loss between the control and experimental groups are clear and show a high fidelity of height restoration using a purely disc-based intervention. The differences in the height changes between the two groups after the initial fatigue protocol appear to be notably different. While there were differences, this did not appear to affect the stiffness properties between the control and experimental specimens for the initial fatigue protocol. It is possible that control group specimens, on average, had slightly taller discs than experimental specimens and thus were able to creep further. We submit that this difference in specimen height is not practically significant since it does not affect the mechanical properties of the specimen with respect to angular stiffness.

A further limitation of this study is in its use of an animal model. Previous research shows, however, that the porcine cervical spine is a suitable analog to the human lumbar spine with respect to geometry, anatomy [18], and function [2] in discerning injury mechanisms. The pig neck, being a quadruped, undergoes large compressive forces from the constant extensor muscle activity needed to support this posture. Further the pig "roots" for food requiring powerful neck extension moments, explaining the similar requirements of the human lumbar and the porcine cervical spine. There are some differences between human and porcine spine specimens such as the large anterior processes and smaller pars interarticularis on porcine spines [18]. Interestingly, our surgeons are usually unaware of whether the disc and vertebral body is human or porcine until told, or they see the small anterior processes. In addition, the average endplate area of a porcine cervical spine is smaller ($500~mm^2$) while human endplate areas are usually larger (generally about $1000~mm^2$) [18].

The authors are also aware of the somewhat unrealistic conditions with which this investigation was conducted. A small amount of traction was required in order to achieve sufficient endplate separation to inject the hydrogel; any eventual surgical procedure involving this injection method would most likely need to employ a method to deliver traction to the spine to achieve the same result. Furthermore, while the control group was exposed to traction which increased the vertical spacing of the disc, this was a temporary intervention that quickly dissipated once compressive load was added. Use of a saline injection in the control group would have provided a better outlook on the true efficacy of the hydrogel itself in restoring the mechanical profile to a fatigued spinal segment over a prolonged period. It was not experimentally feasible, however, to inject saline into an injured disc without having it rapidly extrude through the needle puncture site and fissures in the annulus within the first cycles of flexion and extension under a given compressive load, especially when they have already been fatigued. Andersson and Schultz have injected saline into cadaveric specimens in the past, yet used a light compressive load and still had issues with fluid retention [19]. Nevertheless, the current experiment was successful in achieving the purpose and characterizing the mechanical response of a fatigued spinal motion segment after hydrogel injection.

The results of this study indicate that it is possible to restore angular stiffness to a cyclically fatigued spinal segment using a novel minimally invasive injectable hydrogel. Although this study suggests the feasibility of a hydrogel nucleus replacement, future research examining the mechanical benefits of this hydrogel on the progression of the degenerative cascade may be warranted. This hydrogel has the potential to improve upon current surgeries which seek to repair a damaged disc. Furthermore, this type of hydrogel has the potential to cause less trauma to a patient in addition to providing an implant that is "custom fit" to the nucleus geometry of every potential candidate. Future work should be directed towards maintaining the hydrogel properties during prolonged fatigue loading. Its use as a viable surgical alternative in more practical and diverse surgical scenarios remains to be seen.

References

[1] Callaghan, J. P., and McGill, S. M., 2001, "Intervertebral Disc Herniation: Studies on a Porcine Model Exposed to Highly Repetitive Flexion/Extension Motion With Compressive Force," Clin. Biomech. (Bristol, Avon), **16**(1), pp. 28–37.

[2] Tampier, C., Drake, J. D., Callaghan, J. P., and McGill, S. M., 2007, "Progressive Disc Herniation: An Investigation of the Mechanism Using Radiologic, Histochemical, and Microscopic Dissection Techniques on a Porcine Model," Spine, **32**(25), pp. 2869–2874.

[3] Yates, J. P., Giangregorio, L., and McGill, S. M., 2010, "The Influence of Intervertebral Disc Shape on the Pathway of Posterior/Posterolateral Partial Herniation," Spine, **35**(7), pp. 734–739.

[4] Kirkaldy-Willis, W. H., Wedge, J. H., Yong-Hing, K., and Reilly, J., 1978, "Pathology and Pathogenesis of Lumbar Spondylosis and Stenosis," Spine, **3**(4), pp. 319–328.

[5] Arbit, E., and Pannullo, S., 2001, "Lumbar Stenosis: A Clinical Review," Clin. Orthop. Relat. Res., **384**, pp. 137–143.

[6] Vernengo, J., Fussell, G. W., Smith, N. G., and Lowman, A. M., 2008, "Evaluation of Novel Injectable Hydrogels for Nucleus Pulposus Replacement," J. Biomed. Mater. Res., Part B: Appl. Biomater., **84**(1), pp. 64–69.

[7] Klara, P. M., and Ray, C. D., 2002, "Artificial Nucleus Replacement: Clinical Experience," Spine, **27**(12), pp. 1374–1377.

[8] Adams, M. A., Stefanakis, M., and Dolan, P., 2010, "Healing of a Painful Intervertebral Disc Should Not Be Confused With Reversing Disc Degeneration: Implications for Physical Therapies for Discogenic Back Pain," Clin. Biomech., **25**(10), pp. 961–971.

[9] Adams, M. A., McNally, D. S., and Dolan, P., 1996, "'Stress' Distributions Inside Intervertebral Discs. The Effects of Age and Degeneration," J. Bone Jt. Surg., Br. **78**(6), pp. 965–972.

[10] Meakin, J. R., Reid, J. E., and Hukins, D. W., 2001, "Replacing the Nucleus Pulposus of the Intervertebral Disc," Clin. Biomech., **16**(7), pp. 560–565.

[11] Shim, C. S., Lee, S. H., Shin, H. D., Kang, H. S., Choi, W. C., Jung, B., Choi, G., Ahn, Y., Lee, S., and Lee, H. Y., 2007, "Charite Versus Prodisc: A Comparative Study of a Minimum 3-Year Follow-Up," Spine, **32**(9), pp. 1012–1018.

[12] Nerurkar, N. L., Elliott, D. M., and Mauck, R. L., 2010, "Mechanical Design Criteria for Intervertebral Disc Tissue Engineering," J. Biomech., **43**(6), pp. 1017–1030.

[13] Bertagnoli, R., Sabatino, C. T., Edwards, J. T., Gontarz, G. A., Prewett, A., and Parsons, J. R., 2005, "Mechanical Testing of a Novel Hydrogel Nucleus Replacement Implant," Spine J., **5**(6), pp. 672–681.

[14] Dahl, M. C., Ahrens, M., Sherman, J. E., and Martz, E. O., 2010, "The Restoration of Lumbar Intervertebral Disc Load Distribution: A Comparison of Three Nucleus Replacement Technologies," Spine, **35**(15), pp. 1445–1453.

[15] Adams, M. A., Freeman, B. J., Morrison, H. P., Nelson, I. W., and Dolan, P., 2000, "Mechanical Initiation of Intervertebral Disc Degeneration," Spine, **25**(13), pp. 1625–1636.

[16] Galante, J. O., 1967, "Tensile Properties of the Human Lumbar Annulus Fibrosus," Acta Orthop. Scand. Suppl., **100**, pp. 1–91.

[17] Panjabi, M. M., Duranceau, J. S., Oxland, T. R., and Bowen, C. E., 1989, "Multidirectional Instabilities of Traumatic Cervical Spine Injuries in a Porcine Model," Spine, **14**(10), pp. 1111–1115.

[18] Yingling, V. R., Callaghan, J. P., and McGill, S. M., 1999, "The Porcine Cervical Spine as a Model of the Human Lumbar Spine: An Anatomical, Geometric, and Functional Comparison," J. Spinal Disord., **12**(5), pp. 415–423.

[19] Andersson, G. B., and Schultz, A. B., 1979, "Effects of Fluid Injection on Mechanical Properties of Intervertebral Discs," J. Biomech., **12**(6), pp. 453–458.