Brent L. Showalter

Department of Bioengineering,
University of Pennsylvania,
424 Stemmler, 36th and Hamilton Walk,
Philadelphia, PA 19104

Dawn M. Elliott

Department of Biomedical Engineering,
University of Delaware,
161 Colburn,
150 Academy Street,
Newark, DE 19716

Weiliam Chen

Endomedix, Inc.,
Enterprise Development Center,
New Jersey Institute of Technology,
211 Warren Street,
Newark, NJ 07103

Neil R. Malhotra¹

Department of Neurosurgery,
University of Pennsylvania,
3 Silverstein Pavilion,
3400 Spruce Street,
Philadelphia, PA 19104
e-mail: Neil.malhotra@uphs.upenn.edu

Evaluation of an In Situ Gelable and Injectable Hydrogel Treatment to Preserve Human Disc Mechanical Function Undergoing Physiologic Cyclic Loading Followed by Hydrated Recovery

*Despite the prevalence of disc degeneration and its contributions to low back problems, many current treatments are palliative only and ultimately fail. To address this, nucleus pulposus replacements are under development. Previous work on an injectable hydrogel nucleus pulposus replacement composed of *n*-carboxyethyl chitosan, oxidized dextran, and teleostean has shown that it has properties similar to native nucleus pulposus, can restore compressive range of motion in ovine discs, is biocompatible, and promotes cell proliferation. The objective of this study was to determine if the hydrogel implant will be contained and if it will restore mechanics in human discs undergoing physiologic cyclic compressive loading. Fourteen human lumbar spine segments were tested using physiologic cyclic compressive loading while intact, following nucleotomy, and again following treatment of injecting either phosphate buffered saline (PBS) (sham, $n = 7$) or hydrogel (implant, $n = 7$). In each compressive test, mechanical parameters were measured immediately before and after 10,000 cycles of compressive loading and following a period of hydrated recovery. The hydrogel implant was not ejected from the disc during 10,000 cycles of physiological compression testing and appeared undamaged when discs were bisected following all mechanical tests. For sham samples, creep during cyclic loading increased (+15%) from creep during nucleotomy testing, while for implant samples creep strain decreased (−3%) toward normal. There was no difference in compressive modulus or compressive strains between implant and sham samples. These findings demonstrate that the implant interdigitates with the nucleus pulposus, preventing its expulsion during 10,000 cycles of compressive loading and preserves disc creep within human L5–S1 discs. This and previous studies provide a solid foundation for continuing to evaluate the efficacy of the hydrogel implant. [DOI: 10.1115/1.4030530]*

Keywords: intervertebral disc, nucleus augmentation, cyclic loading, compression mechanics, lumbar spine, human

Introduction

The intervertebral disc performs the mechanical roles of supporting loads, permitting motion, and dissipating energy in the spine. The disc is composed of an annulus fibrosus, which surrounds the gelatinous nucleus pulposus. The nucleus both directly supports applied loads and places the collagen fibers of the annulus in tension, resulting in a joint capable of withstanding large forces while permitting extensive spinal flexibility. Disc degeneration is a multifaceted process that manifests early in the nucleus pulposus and subsequently extends to other disc components [1]. Degeneration is strongly implicated as a cause of low back pain and results in frequent doctor visits and over $\$100 \times 10^9$ in annual costs within the United States [2,3].

Despite the prevalence of disc degeneration and its contributions to low back problems, many current treatments are palliative

only and ultimately fail [3–5]. The current gold standard for surgical treatment is fusion, in which the disc is removed and the adjacent vertebral bodies are fused [5]. While often boasting initial successful outcomes, such as reducing Oswestry Disability Index, the procedure also inherently restricts natural movement and accelerates the degenerative process in adjacent disc levels [6,7]. More recently, total disc replacements have sought to treat low back problems while retaining motion [8]. However, current replacements do not match native nondegenerative mechanical function, are subject to wear and failure, and resection is associated with risk of significant complications [9,10]. The pervasiveness of disc degeneration coupled with the failures and complications associated with the current treatments indicates a pressing need for improved treatments that permit the disc to perform its primarily mechanical functions.

Nucleus pulposus replacements designed to treat early stages of degeneration while maintaining or restoring disc mechanics are under development [11–14]. In this study, we will be evaluating an in situ gelable, triple-interpenetrating-network hydrogel nucleus replacement. The gel is composed of *n*-carboxyethyl

¹Corresponding author.

Manuscript received July 21, 2014; final manuscript received April 27, 2015; published online June 16, 2015. Assoc. Editor: Sean S. Kohles.

chitosan, oxidized dextran, and teleostean and is well suited as a nucleus replacement in part because after mixing it solidifies rapidly at body temperature and demonstrates increased resistance to biodegradation relative to single-network hydrogels [15]. It has also recently been demonstrated that the gel maintains cell viability, promotes proliferation, and has mechanical properties similar to native human nucleus pulposus [16]. A similar hydrogel restored compressive range of motion in single-cycle tests of injured ovine discs [17]. However, it remains unknown if this hydrogel implant will be contained in more extensive mechanical tests and if it will restore mechanics in human discs.

Mechanical evaluation of nucleus replacements often focuses on the intrinsic mechanical properties of the gel and its immediate effects on disc mechanical response [18–22]. More extensive mechanical testing is completed with the aim of testing failure properties [23,24]. While not commonly used, moderate cyclic loading of discs followed by unloaded hydrated recovery is a valuable testing modality because it is more rigorous than single-cycle tests and captures elements of the time-dependent mechanical response critical for daily function [25,26]. Within the disc, interplay between osmotic pressure and external loads causes 20–25% of the disc's water content to be expressed and re-imbibed daily (Sivan et al. [27]). Reduced hydration decreases disc height, increases stiffness, and increases range of motion, with properties returning to baseline after rehydration (Adams et al., [28]; Wilke et al., [29]). Cyclic loading followed by hydrated recovery in human discs mimics this naturally occurring diurnal response [26]. In a previous study that used cyclic loading to evaluate a hydrogel implant in injured porcine discs, the implant demonstrated recovery of angular stiffness and disc height [30]. However, that study did not evaluate the effects of hydrated, unloaded recovery and thus could not evaluate the time-dependent mechanical response. Also, human discs have greater degeneration and lower water content than animal discs, which may affect an implant's ability to improve disc function. The objective of this study was to determine if the hydrogel implant will be contained and if it will restore mechanics in human discs undergoing physiologic cyclic compressive loading with unloaded hydrated recovery.

Methods

Fourteen human lumbar spine segments were tested using physiologic cyclic compressive loading while intact, following nucleotomy, and again following treatment of injecting either PBS (sham, $n = 7$) or hydrogel (implant, $n = 7$).

Sample Preparation. Fourteen human lumbar spine segments with a mean donor age of 49.5 yr (22–75 yr) were obtained from institutionally approved sources. These spines were imaged in a 3 T magnetic resonance (MR) scanner to determine degeneration grade and T_2 relaxation times [31–33]. T_2 relaxation times of the intact samples were used to quantify the initial degenerative state of the sample, but were not used to track degenerative changes throughout the experiment. Disc cross-sectional area was measured from axial MR images. After imaging, L5–S1 bone–disc–bone segments were separated from the spine and prepared for testing. The L5–S1 disc was selected because herniation, and subsequent surgery, is most common at this level [34]. The samples for this study had degeneration grades ranging from 1 to 5 and T_2 relaxation times ranging from 79 to 204, with higher relaxation times indicating healthier discs. T_2 relaxation times were not acquired for one sample. For the remaining 13 samples, T_2 relaxation times correlated with Pfirrmann grades ($r = -0.75$) and provide an objective and continuous measure of degeneration (Fig. 1). Preparation included removal of posterior elements and extraneous soft tissue, followed by placement of 1.25 mm Kirschner wires in the L5 vertebrae and sacrum. The L5–S1 discs were wedge shaped, with a 13 ± 4 deg angle between the L5 and S1 endplates, which results in applied shear loads even during simple

Fig. 1 T_2 relaxation times are strongly correlated to Pfirrmann scores for the human L5–S1 discs used in this study

compression experiments. For consistency in mechanical testing, loads were applied perpendicular to the L5 vertebrae. This was ensured by potting the motion segments in PMMA with the L5 endplate parallel to the potting fixtures using fluoroscopic guidance. Samples were stored in a freezer at -20°C when not in use.

Mechanical Testing. The mechanical test consisted of six steps (Fig. 2): (1) overnight hydration, also called initial hydration, (2) measure mechanical parameters (*initial*), (3) 10,000 cycles of compressive loading, (4) measure mechanical parameters (*cyclic*), (5) overnight hydration, also called unloaded recovery, and (6) measure mechanical parameters (*recovery*) [26].

During the first step, overnight hydration, samples were submerged in a PBS bath containing protease inhibitors (phenylmethylsulfonyl fluoride, *N*-ethylmaleimide bioxta, and benzamidinium hydrochloride from Sigma-Aldrich, St. Louis, MO) at 4°C for 16–24 hr. The remainder of the test was completed in a PBS bath held at room temperature ($\sim 20^\circ\text{C}$). Second, compression stiffness and range of motion were measured by applying 60 cycles between 0.48 MPa (741 ± 112 N) in compression and 375 N in tension cycles at 0.2 Hz, which is similar to previous studies [35]. The compressive stress of 0.48 MPa corresponds to one body weight or to low-intensity activities such as sitting upright without support or relaxed standing [29,36,37]. The tensile load of 375 N was applied to all samples instead of applying a uniform stress to prevent sample pull-out from the PMMA for discs with large cross-sectional areas while ensuring sufficient load to describe the mechanical parameters of interest. The third step was to apply 10,000 cycles at 2 Hz of compressive loads between 0.12 and 0.96 MPa (1482 ± 224 N), which are loads similar to moderate physical activities such as jogging or lifting a 10 kg weight [29,37]. The number of cycles and cycle frequency were selected for comparison with earlier studies and because these loads are representative of moderate physical labor [38,39]. Fourth, mechanics were measured immediately after cyclic loading in the same manner as the second step. For the fifth and sixth steps, discs were rehydrated overnight followed by measurement of compressive mechanical parameters. All testing was completed on an ElectroPuls E3000 test system (Instron, Norwood, MA). The three time points in which mechanical parameters were measured (steps 2, 4, and 6) are referred to in this work as initial, cyclic, and recovery.

Nucleotomy. Nucleotomy was performed by first hydrating the discs in PBS for 8 hrs. Next, a #11 scalpel blade was used to create a cruciate incision of approximately 4×4 mm width extending through the depth of the posterolateral annulus. Access to the nucleus pulposus was confirmed using a blunt probe prior to proceeding to nucleotomy. Afterward, either 2 or 4 mm pituitary rongeurs were used to remove as much nuclear material as

Fig. 2 The mechanical testing procedure altered disc hydration. Discs are fully hydrated in a PBS bath containing protease inhibitors (steps 1 and 5), and have reduced hydration after cyclic loading (step 3). Mechanical parameters are measured after each change in hydration level (steps 2, 4, and 6).

possible through the single incision, which was 1.70 ± 0.36 g. This is approximately 1.7 mL and 50% of nucleus pulposus volume and slightly less than the 2.1 ± 0.9 g was removed during a clinical discectomy [40–43]. Following nucleotomy, the samples were frozen until further testing. The results for these nucleotomy tests have already been published [26].

Treatment. Samples were separated into sham and implant groups based on blocked randomization of intact initial compressive strain. Prior to injection, discs were hydrated in PBS at 37°C . Both sham and implant groups received an injection to fill the void created by nucleotomy (0.5 ± 0.02 mL). The nucleus pulposus remaining in the disc following during nucleotomy expanded during rehydration, and as a result, less material was injected than was removed. Sham samples ($n = 7$) received an injection of the PBS, while the implant group ($n = 7$) received an injection of the hydrogel implant. The hydrogel implant was created by mixing aqueous solutions of 20% teleostean, 3% *N*-carboxyethyl chitosan, and 7.5% oxidized dextran were at a ratio of 1:1:2 [15]. Toluidine blue was mixed in the aqueous solution of teleostean before mixing the hydrogel components to permit visualization of the implant. After injection, both sham and implant groups were placed in a PBS bath held at 37°C to allow the implant to cure. It is likely that the PBS from the sham injection and the bath were interchanged during this period. However, the implant mixture cured sufficiently in few minutes prior to placement in the PBS bath that the gel was not observed to be dispersed in the bath during the curing period. Afterward, the samples were tested as described above.

Data Analysis. Disc area was measured from MR images by outlining the disc in a midaxial slice using the image processing software OSIRIX (Pixmeo, Switzerland). Average disc height was calculated from a lateral fluoroscopic image. Using MATLAB (MathWorks, Natick, MA), the disc space was manually outlined and the disc height calculated by dividing the outlined area by the disc midaxial anteroposterior width [44].

The maximum and minimum displacements of a single loading cycle decrease during the 10,000 cycles of compressive loading. Creep was defined as the change in maximum position from cycle 50 to 10,000 of the cyclic loading, after which creep strain was calculated by dividing the creep by initial disc height.

Mechanical parameters were measured on the 50th cycle of the mechanical measurement steps of the testing procedure (steps 2, 4, and 6) using a previously described custom MATLAB program [26,35]. Compressive range of motion was defined as the displacement between 0 MPa and 0.48 MPa of applied load and compressive stiffness was defined as the slope of the line fit through the data between 0.38 and 0.48 MPa in compression (Fig. 3). Compression strain was calculated by dividing the compression range of motion by disc height. Similarly, compression apparent modulus was calculated from stiffness by multiplying by disc height and then dividing by disc area. Intact disc height and area were used for this normalization.

Statistics. The effects of treatment (sham and implant) and loading history (initial, cyclic, and recovery) on the mechanical parameters (compression modulus and compression strain) were

analyzed using a two-way analysis of variance (ANOVA) with repeated measures for loading history. Significant treatments and loading histories were analyzed with a Sidak's multiple comparison test.

Degeneration effects on cyclic loading for implant and sham groups were examined using linear correlations between T_2 relaxation time of the intact samples and the percent change of parameters between the initial and cyclic time points of the treated samples. Similarly, degeneration effects on the changes caused by sham or implant treatment were analyzed by performing linear correlations between intact T_2 relaxation time and the percent change between nucleotomy and treatment parameter values at their respective loading history state (initial, cyclic, or recovery). To include the sample with no measured T_2 relaxation time in the degeneration analysis, its T_2 relaxation time was estimated based on its Pfirrmann grade and the regression line calculated from the other 13 samples.

The effect of treatment on creep strain was analyzed using a Student's *t*-test. Because data were taken at multiple time points for each sample, all statistical tests except for the direct comparison between sham and implant samples used repeated measured or paired statistics; that is, the statistical tests evaluated if the mean change per sample between time points was nonzero. Significance for all tests was set at $p < 0.05$.

Results

No visible tissue degradation was observed at the end of mechanical testing and no nucleus pulposus material was ejected from the sham discs. More importantly, the hydrogel implant was not ejected from the disc during testing and was undamaged when discs were bisected following all mechanical tests (Fig. 4). As seen in the figure, implant material interdigitated with the surrounding disc tissue. However, no physical bonds were formed between the nucleus pulposus and the implant, and minimal force could separate the gel and disc tissue. Implant retention was previously reported for three of these seven discs [16]. All numerical results in the remainder of this document are presented as mean $\pm$ standard deviation. Intact disc area was 1543 ± 233 mm² and disc height was 8.6 ± 2.4 mm. Samples were separated by blocked randomization based on the compression strain of the intact disc at the initial time point. Intact initial compression strain for the sham group was 0.12 ± 0.05 and for the implant group it was 0.11 ± 0.05 , which was not statistically different ($p = 0.74$). All mechanical data presented are normalized by disc geometry as described in the Methods section.

Effect of Cyclic Loading Followed by Hydrated Recovery. Cyclic loading increased the compression modulus by $8.7 \pm 2.8\%$ from 6.7 ± 2.7 MPa for the sham group and by $5.5 \pm 4.1\%$ from 7.8 ± 2.8 MPa for the implant group (Fig. 5(a)). Similarly, cyclic loading increased compressive strain by $14.4 \pm 13.4\%$ from 0.17 ± 0.08 for the sham group and by $13.7 \pm 9.7\%$ from 0.15 ± 0.09 for the implant group (Fig. 5(b)). The differences in initial values and percent increases between the sham and implant samples were not significant. Changes in compression modulus completely recovered following a period of unloaded hydrated recovery (Fig. 5(a)). Compression strain partially recovered, with

Fig. 3 Mechanical parameters are found using a trilinear fit of force–displacement curve from 50th cycle of the mechanical measurement steps (steps 2, 4, and 6 of the testing procedure). The compression loading curve (diamonds) is separated from the data. Compression range of motion is the displacement between 0 and 0.48 MPa on the compression loading curve. Compression stiffness is the slope of the line fit through 0.38 and 0.48 MPa. Compression stiffness and range of motion are then normalized by intact disc area and height.

compression strain remaining $7.3 \pm 7.9\%$ higher than initial for sham samples and $7.8 \pm 7.5\%$ higher for implant (Fig. 5(b)). These trends were similar to those seen for samples while they were intact and following nucleotomy [26].

Effect of Sham and Implant Treatment. Nucleotomy previously caused a 43% increase in creep strain from the intact value of 0.12 ± 0.08 mm/mm (Fig. 6(a)) [26]. For sham samples, creep strain further increased by 15% from nucleotomy, while for implant samples, creep strain decreased by 3% (Fig. 6(b)). The difference in creep strain between implant and sham groups was statistically significant. Nucleotomy decreased compression modulus 19% from initial intact value of 8.9 ± 2.8 MPa and increased compression strain 38% from 0.12 ± 0.04 mm/mm [26]. Compression modulus was not affected by either sham or implant treatment ($p > 0.2$) (Fig. 7(a)). For sham samples, compression strain was $4.6 \pm 7.6\%$ and $7.3 \pm 7.6\%$ higher than nucleotomy values at the cyclic and recovery time points (Fig. 7(b)). For implant samples, compression strain was $4.3 \pm 8.9\%$ and $5.0 \pm 8.9\%$ higher than nucleotomy values at the cyclic and recovery time points (Fig. 7(b)). The difference in compressive strain between sham and implant samples was not significant ($p > 0.9$).

Effect of Degeneration. For implant samples, intact T_2 relaxation time was correlated with the amount of change in compression modulus caused by cyclic loading (Fig. 8). There was a larger effect of the implant in more degenerated discs (e.g., those with a lower T_2 relaxation time), with a slope of -0.076% change/ms and an intercept of 15.7%. This correlation was not significant for the intact and nucleotomy controls of the implant group ($p > 0.4$, Fig. 8). There were no other significant correlations between T_2 relaxation time and mechanical parameters for the sham or groups ($p > 0.25$).

Discussion

The hydrogel implant presented in this work successfully remained intact and contained in human discs under physiologic

cyclic compressive loads and had a positive impact on the mechanical response of the disc. Implant expulsion has been a significant problem with nucleus pulposus replacements for both human and animal studies, with expulsion rates as high as 33% [45,46]. Some research groups have addressed this problem by developing annular repair techniques such as sutures or cyanoacrylate glue, but the long-term efficacy of such repairs has not been established [23,47]. In this study, the hydrogel remained intact throughout the 10,000 cycles of compressive physiologic loading without repairing the large cruciate incision created during the nucleotomy procedure, which is due in part to the interdigitation of the gel with the disc (Fig. 4). This successful result merits further implant retention testing in more rigorous loading modalities such as bending and torsion.

The implant successfully preserved the disc's creep response, while creep strain for the sham group continued to increase the strain for the implant group remained constant (Fig. 6). Increased creep strain indicates lower disc height, which in turn alters spinal motion and potentially affects low back pain [48]. The increased strain for the sham samples indicates that the mechanical test was damaging the tissue. The damage is probably due in part to the depressurization of the nucleus pulposus, which forces the annulus to carry more of the load. This finding is consistent with previous nucleotomy experiments [17]. The creep response for implant samples did not return to intact values. However, this was anticipated given that the implant did not restore the disc pressure lost in the injury procedure. The implant's ability to preserve the creep response is a positive outcome for this mechanically based treatment.

The significant correlation between degeneration and the effect of cyclic loading on the implant samples highlights the decrease in annular function with degeneration. Although the regression lines for the intact and nucleotomy tests of the same samples are similar to that of the implant treatment, the variability inherent in biological samples prevented the trends from being statistically significant ($p > 0.4$). When that biological tissue was replaced with a consistent, synthetic material, the variability seemed to decrease and correlations became significant. More degenerate discs (those with lower T_2 relaxation times) experienced greater changes in modulus as a result of cyclic loading (Fig. 8). As changes in cyclic loading are largely due to redistribution or expulsion of fluid within the disc [26], greater changes in cyclic loading properties suggest that the annulus fibrosus of the degenerate samples is more permeable and less structurally coherent than those nondegenerate discs. The correlation also demonstrates that the hydrogel implant does not compensate for existing degenerative changes in the annulus. This is an expected result and indicates that a nucleus replacement would be most beneficial for discs that had undergone degenerative changes in the nucleus pulposus, but prior to the more severe changes subsequently occur in the annulus.

The implant did not alter the stiffening effects, and subsequent recovery, caused by cyclic loading followed by unloaded recovery (Fig. 5). Following nucleotomy, cyclic loading caused a 7% increase in compressive modulus and a 15% increase in compressive range [26]. In this work, neither the implant nor sham samples altered the cyclic loading effect. Although this process is an important aspect of the disc's in vivo diurnal cycle, the effect of a nuclear implant on those compressive properties has not been previously been studied. As a result, it is not known whether other nuclear replacement treatments currently under development preserve cyclic loading effects. As noted above, these responses may be governed more by the annulus fibrosus. It is also possible that the testing frequency of 0.2 Hz did not capture changes in compressive properties that would be more evident at different frequency. In part, this is because the cyclic loading effects are likely due to fluid distribution within the disc, and the rate at which redistribution occurs has not been studied extensively. Also, one of the primary mechanical functions of the intervertebral disc is to absorb energy, and this damping capacity would be affected by

Fig. 4 Bisected human L5-S1 disc after complete mechanical test, including 10,000 cycles of compressive loading. The hydrogel implant stayed intact within the disc, although some of the toluidine blue used to dye the gel leached into the surrounding disc tissue. The enlarged region shows that the implant (dark region in lower left corner of inset) filled in the irregular contours of the remaining nucleus pulposus (light region of inset).

Fig. 5 Cyclic loading caused an increase in compression modulus (a) and compression strain (b) for both sham and implant samples. These increases were recovered following a period of overnight hydration, indicating that the changes are due to changes in hydration distribution within the intervertebral disc. Recoverable increases in compression modulus and strain are consistent with trends in intact and nucleotomy samples. Data presented as mean + standard error (* indicates $p < 0.05$).

testing frequency. In future studies, it would be interesting to evaluate how the mechanical response of the intact, nucleotomy, and treated discs in various frequencies.

Maintaining disc mechanical properties represents a significant improvement over current surgical procedures, which do not maintain or restore native disc mechanical behavior [6,7,10]. In addition, in situ curable nucleus pulposus implants, such as this hydrogel, mimic native nucleus function by creating more uniform stress distributions within the disc than other surgical implants [49]. By mimicking native function, the implants may prevent additional damage to the annulus fibrosus. Also, these injectable implants are less invasive than conventional surgeries. This is of particular importance given that repair and regeneration within the disc are limited because of its avascular nature and low

Fig. 6 Nucleotomy increased creep strain (a). Following treatment, strain continued to increase for sham samples, but decreased for implant samples (b). Data presented as mean + standard error (* indicates $p < 0.05$).

Fig. 7 Percent change from nucleotomy values for sham and implant samples. Treatment caused no change in compression modulus (a). Compression strain continued to increase for both sham and implant samples at the cyclic and recovery time points (b). Data presented as mean + standard error (+ indicates $p < 0.05$ versus nucleotomy values).

cell content [51,52]. Finally, treatment with an injectable implant does not preclude future surgical alternatives [49].

Unexpectedly, the hydrogel implant did not restore compressive strain in the human discs (Fig. 7(b)), despite restoring compressive strain when implanted in ovine discs [17]. This difference may be attributed to a combination of factors, including the degenerate human annular tissue, the annular nucleotomy injury, and the quantity of hydrogel injected. The human discs used in this study are more degenerate than the ovine discs used in the previous study in which compressive range of motion was

Fig. 8 Percent change in compression modulus between the initial and cyclic time point for the samples in the implant group. This correlation was not significant for the samples while they were intact or following nucleotomy, but was significant following injection of the hydrogel. The slope (m) and y -intercepts (b) of the regression are shown in the legend.

restored [17]. As a result, the hydrogel implant may have been less beneficial because the surrounding tissue already had degraded mechanical properties. Also, the nucleotomy incision depressurized the nucleus pulposus, which prevents the implant from functioning in the same manner as the native nucleus. While the nucleotomy injury was required as a negative control for this study, the implant could be delivered with a much smaller injury in clinical practice, thus avoiding disc depressurization. Finally, in the previous ovine study, a greater than 1:1 ratio of implant to nucleotomy volume was delivered. Here, however, there was a reduced amount of implant (0.5 mL) compared to nucleus pulposus removed (1.7 mL), a less than 1:3 implant to nucleotomy ratio. This may have prevented some of the beneficial effects of the hydrogel implant. This reduced implant amount may have occurred because samples underwent three periods of overnight hydration in PBS between the nucleotomy procedure and treatment injection. The nucleus pulposus has been shown to swell up to 200% or more in its initial volume [52]. As a result, the remaining nucleus tissue may have expanded to partially fill to nucleotomy void prior to receiving the implant. The hydration steps between nucleotomy and treatment were necessary for this study to establish the nucleotomy samples as an injured control. However, those steps are unique to this study, and in future implementation of the hydrogel, the implant will be placed immediately following nucleotomy.

While implant retention is a significant biomechanical benchmark, it is also encouraging regarding the broader goal of using an injectable hydrogel as a restorative therapy for treating disc degeneration. Bench tests in this gel and others have shown that mesenchymal stem cells (MSCs) within hydrogels can differentiate along a chondrogenic lineage, with aggrecan and collagen production [16,53]. MSCs embedded in a hydrogel maintained metabolic activity seven days after injection into porcine discs [54]. The interdigitation of the current hydrogel with the surrounding disc tissue provides interface for collagen, aggrecan, and other extracellular matrix components produced by MSCs co-injected with an implant to interact with and reinforce the remaining disc tissue. This reinforcement is an important aspect of establishing hydrogel implants as a restorative therapy for treating disc degeneration and will be the focus of future work.

Previous work on the hydrogel implant has shown that it has properties similar to native nucleus pulposus, can restore compressive range of motion in ovine discs, is biocompatible, promotes cell proliferation, and has the potential to incorporate pharmaceuticals [16,17,19]. This work expands on those findings by showing that the implant interdigitates with the surrounding disc tissue and was not expelled during 10,000 cycles of compressive loading and preserves disc creep within human L5–S1 discs. This body of work provides a solid foundation for continuing to evaluate the efficacy of the hydrogel implant to functionally regenerate the nucleus pulposus.

Acknowledgment

We would like to thank Dhara B. Amin and Ian S. MacLean for performing some of the mechanical testing. Spines were acquired from National Disease Research Interchange, Philadelphia, PA and Platinum Training, Henderson, NV. This study was funded by NIH R01AR050052, NSF Graduate Research Fellowship No. DGE-0822, and a Neurosurgery Research and Education Foundation grant.

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