A large animal model that recapitulates the spectrum of human intervertebral disc degeneration


Objective: The objective of this study was to establish a large animal model that recapitulates the spectrum of intervertebral disc degeneration that occurs in humans and which is suitable for pre-clinical evaluation of a wide range of experimental therapeutics.

Design: Degeneration was induced in the lumbar intervertebral discs of large frame goats by either intradiscal injection of chondroitinase ABC (ChABC) over a range of dosages (0.1U, 1U or 5U) or subtotal nucleotomy. Radiographs were used to assess disc height changes over 12 weeks. Degenerative changes to the discs and endplates were assessed via magnetic resonance imaging (MRI), semi-quantitative histological grading, microcomputed tomography (μCT), and measurement of disc biomechanical properties.

Results: Degenerative changes were observed for all interventions that ranged from mild (0.1U ChABC) to moderate (1U ChABC and nucleotomy) to severe (5U ChABC). All groups showed progressive reductions in disc height over 12 weeks. Histological scores were significantly increased in the 1U and 5U ChABC groups. Reductions in T2 and T1 relaxation times, and increased Pfirrmann grade were observed on MRI. Resorption and remodeling of the cortical boney endplate adjacent to ChABC-injected discs also occurred. Spine segment range of motion (ROM) was greater and compressive modulus was lower in 1U ChABC and nucleotomy discs compared to intact.

Conclusions: A large animal model of disc degeneration was established that recapitulates the spectrum of structural, compositional and biomechanical features of human disc degeneration. This model may serve as a robust platform for evaluating the efficacy of therapeutics targeted towards varying degrees of disc degeneration.

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responsive to conservative treatment. There is therefore a substantial unmet clinical need for new treatment options for disc degeneration and low back pain.

Disc degeneration is a progressive cascade of cellular, biochemical, structural, and biomechanical changes that manifests as a spectrum from mild to severe. It is thought that the earliest degenerative changes typically occur in the central nucleus pulposus (NP), and include loss of proteoglycan and water content, which compromises the ability of this disc compartment to swell and effectively engage the surrounding annulus fibrosus (AF) [15-18]. Because of the lack of therapeutics targeting mild- to moderate-stage degeneration, and the shortcomings of surgical solutions for late-stage degeneration, there exists considerable research interest in developing new, biological regenerative treatment strategies. These treatment strategies must aim to provide both pain relief and long-term regeneration of disc structure and mechanical function, and may include, for example, stem cell or growth factor injections into the disc, hydrogels for NP replacement or augmentation, and tissue-engineered total disc replacements [19-21].

For these therapies to be successful, they must be tailored to the degenerative state of the disc in need of treatment. As such, preclinical animal models as an essential step for translating emerging therapies to humans, should effectively recapitulate the spectrum of degeneration, including reproducible structural, compositional and biomechanical changes [10]. Animal models of disc degeneration range in scale from small species, such as the mouse, rat, and rabbit, to large species, including the sheep, goat, pig, and dog [10-14]. The benefits of large animal models include similarities in morphology to the human lumbar disc as well as sufficient disc height to mimic the challenging nutritional environment present in human discs [15]. Furthermore, in the sheep and goat, NP proteoglycan content and whole-disc mechanics are similar to human [66,77]. Certain goat breeds, specifically, are an attractive model due to greater disc height when compared to similar sized species such as pigs and sheep, and the absence adult notochordal cells [18].

A variety of approaches to induce disc degeneration have been utilized in both large and small animal models, including chemonucleolysis (to enzymatically induce NP degradation), nuclear aspiration, annular injury, or altered mechanical loading. Annular injury models (laceration or needle puncture) have been characterized in mice, rats, rabbits, sheep and goats, with the extent of degeneration dependent on the size of the injury relative to the size of the disc [15,19-21]. Chemonucleolysis, most commonly achieved via intradiscal delivery of chondroitinase ABC (ChABC) or chymopapain, has been utilized in an effort to recapitulate the hallmark loss of proteoglycans that occurs in human disc degeneration [15,22]. ChABC specifically degrades the chondroitin and dermatan sulfate side chains of proteoglycans, and has yielded mild to moderate degeneration in rats, rabbits, sheep, goats, and dogs, although spontaneous regeneration has been observed in smaller species [18,23-27]. Chymopapain is a proteolytic enzyme that cleaves the non-collagenous protein connections of proteoglycans, and induces degeneration in dogs that is typically more severe than that achieved via ChABC [24,28]. A spectrum of degeneration has also been achieved via chymopapain in vitro organ culture models [29]. Previous models of intradiscal ChABC injection in large animals, such as the goat, sheep, and dog, are primarily characterized by mild degenerative changes until the evaluation of the various species [18,24,25]. Therefore, a spectrum of degeneration, which has utility for the evaluation of a variety of pre-clinical regenerative therapeutics, has yet to be achieved in a large animal model via these methods. Thus, the purpose of this study was to establish an inducible large animal model that reproducibly recapitulates the spectrum of intervertebral disc degeneration found in human patients.

Methods

Animals and surgical procedures

The study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Nine large frame castrated male goats (Thomas D. Morris Inc., Reisterstown, MD), approximately 3 years-of-age, were utilized in this study. Except for surgical procedures and radiographs, animals were group housed in a barn, and evaluated daily by a veterinarian for signs of pain, behavior changes or gait abnormalities for the duration of the study. All animals underwent a surgical procedure to induce degeneration of the lumbar intervertebral discs, as per the study design in Fig. 1(A). Animals were anesthetized via intravenous injection of ketamine (11–33 mg/kg) and midazolam (0.5–1.5 mg/kg), and then intubated and maintained on an isoflurane–oxygen mixture throughout the surgical procedure. Using standard aseptic technique, the lumbar intervertebral discs were exposed via a open, left lateral retroperitoneal, transposacral approach. The disc spaces were identified and counted using lateral fluoroscopy, and a titanium Kirschner wire was placed in the L1 or L2 vertebral body as a fiducial marker to enable identification of vertebral levels on radiographs [Fig. 1(B) and (C)]. As indicated in Fig. 1(A), the L1–L2, L2–L3, L3–L4 and L4–L5 discs were randomized to receive either subtotal nucleotomy (i.e., a nuclectomy) (n = 10), sham injection (n = 5), or injection of 0.1U (n = 5), 1U (n = 10) or 5U (n = 5) of ChABC (Ambosio, Cambridge, MA). The T12–L1 and L5–L6 discs served as intact controls (n = 10), for a total of six experimental groups. Each dose of ChABC was resuspended in 200 µL of vehicle (sterile PBS containing 0.1% BSA) for injection, and delivered to the NP using a 5-mm, 22G spinal needle. Sham injection consisted of 200 µL of vehicle only. Subtotal nucleotomy was performed using a cruciate annular incision followed by 2 mm pituitary rongeurs to remove NP (0.43 ± 0.17 g). The surgical incision was then closed in layers, and the animals were hand-recovered by veterinary staff until ambulatory, upon which they were returned to standard housing. Peri-operatively, animals were administered transeptal fentanyl (2.5 mcg/kg/hr) and intravenous flunixin meglumine (Banamine, 1.1 mg/kg) for analgesia. Florfenicol (40 mg/kg) was administered for antimicrobial prophylaxis.

Radiographs and magnetic resonance imaging (MRI)

To assess longitudinal changes in disc height with degeneration, lateral plain radiographs of the lumbar spine were obtained in the standing and fully weight-bearing position pre-operatively, post-operatively and at 1, 2, 4, 6, 8, 10, and 12 weeks post-operatively. Disc height index (DHI) was quantified by a blinded assessor using a custom MATLAB program [Mathworks, Natick, USA] [30]. Twelve weeks post-operatively, animals were euthanized by an overdose of pentobarbital solution according to American Veterinary Medical Association guidelines [31], and the lumbar spines harvested. The lumbar spines were imaged using a 3T clinical MRI scanner (Siemens Magnetom TrioTim, Munich, Germany) with a voxel size of 0.6 mm × 0.6 mm × 5 mm. T2-weighted mid-sagittal images were obtained for Pfirrmann grading. Series for T2 (Echo Time = 13 * i, i = 1, 2, 3) and T1 (Spin Lock Time = 12 * i, i = 1, 2, 3) mapping were also obtained, and the T2 and T1 relaxation times were quantified in a manually segmented circular region of interest in the NP using ImageJ (NIH, Bethesda, USA) as previously described [32]. Following MRI, each lumbar spine was divided into vertebra-disc-vertebra motion segments, vacuum sealed, and stored frozen at −20°C. Five motion segments from each experimental group were utilized serially for microcomputed tomography (µCT) and histologic analyses. Additional segments...
from the intact control, nucleotomy, and 1U ChABC groups underwent biomechanical testing.

μCT

Motion segments were imaged en bloc at an isotropic 20.5 μm resolution (VivaCT 75, Scanco, Bruttisellen, Switzerland) to investigate alterations to the vertebral endplate with degeneration. The cortical bony endplate and the adjacent trabecular bone between the cortical endplate and growth plate were manually segmented to create volumes of interest (VOI), spanning the whole vertebral endplate area. These cortical and trabecular VOIs were analyzed for the cranial and caudal endplate for each motion segment (n = 10 endplates per group) to obtain cortical and trabecular bone volume fraction (BV/TV).

Histological grading

After μCT imaging, lumbar spinal motion segments were fixed in 10% neutral buffered formalin, decalcified (Formical 2000; Decal Chemical Corporation, Tallman, USA), and processed into paraffin. Mid-sagittal sections were double-stained with either Alcian blue and picrosirius red (to visualize glycosaminoglycan and collagen distribution) or hematoxylin and eosin (for cell number and morphology). Sections were graded by three blinded observers using a modification of a grading scheme proposed by Masuda et al.21 Details on the grading system are provided in Supplemental Table 1. Using a custom MATLAB program33, slides were graded using a visual analog scale from 0 (normal) to 100 (most degenerate) in five categories; organization of the AF, NP matrix density, demarcation of the AF/NP border, NP cellularity, and cartilage endplate (EP) structure.

Biomechanical testing

Five samples from each of the intact control, nucleotomy, and 1U ChABC groups underwent biomechanical testing following MRI analysis. The dorsal bony elements of each motion segment were removed, and the specimens were allowed to equilibrate overnight at 4°C in PBS. The cranial and caudal vertebral bodies of each motion segment were then potted in a low-melting-temperature indium casting alloy (McMaster-Carr, Princeton, NJ) and mechanical properties determined using an electromechanical testing system (Instron 5948, Instron, Norwood, MA). To determine axial displacement, two ink marks were placed on the vertebral bodies adjacent to the disc and were tracked optically using a digital camera (A3800, Basler, Exton, PA)20. Specimens were subjected to 20 cycles of tension/compression at 0.5 Hz from −230N to +115N, followed by 1 h of creep at −230N (~0.48 MPa, 1× body weight)16. Mechanical testing was conducted in a PBS bath at room temperature.

The twentieth cycle of tension—compression was fit to a sigmoid function using custom MATLAB software20. The boundaries of the neutral zone (NZ) were defined by the maximum and minimum second derivatives of the sigmoid function, allowing for the calculation of NZ ROM and NZ stiffness. Compressive range of motion (ROM) was defined as the displacement between the inflection point of the sigmoid curve and the maximal displacement in compression. Compressive stiffness was defined as the slope of the linear region of the force—displacement curve. Total ROM was defined as the displacement between maximal tensile and compressive loads. Stiffness was normalized to disc height and disc area as measured on sagittal and axial MRIs15. Creep behavior was fit to a five-parameter viscoelastic constitutive model, as previously described34, to quantify the early (S1) and late (S2) damping stiffness and associated time constants (τ1 and τ2). Creep displacement was measured via optical tracking and normalized to disc height as quantified by MRI.

Statistical analyses

Statistical analyses were conducted in SYSTAT 13 (Systat Software, Inc., San Jose, CA). Differences in DHI between groups at each time point were assessed via two-way ANOVA with Tukey’s post-
hoc tests. Statistical differences in NP T2 and T1-p values, biomechanical, and μCT parameters at 12 weeks were established using one-way ANOVA with Tukey’s post-hoc tests. Histology grading outcomes were analyzed with two-way ANOVA with Tukey’s post-hoc tests, with both experimental group and blinded grader serving as independent variables. Significance was defined as $P < 0.05$ for all tests; a trend towards significance was defined as $P < 0.1$. All results are presented as mean ± standard deviation unless stated otherwise.

Results

All animals recovered from the surgical procedure without adverse events, and no complications arose during the 12 weeks study duration.

Disc height changes

Progressive loss of disc height occurred over time for both ChABC-injected discs (all doses) and in discs that underwent subtotal nucleotomy. Significant (18.7% and 25.7%) mean reductions in DHI in the 1U and 5U ChABC groups, respectively, were observed at 12 weeks compared to sham ($P = 0.006$, Fig. 2). DHI was significantly reduced for the nucleotomy group compared to sham at all post-operative time points. In the 0.1U ChABC group, DHI was only reduced significantly compared to sham at 4 weeks post-operatively ($P < 0.001$), and was not different from sham at the 12 weeks time point. DHI between control and sham injected discs did not reach significance at any time point.

MRI

T2-weighted MRI images [Fig. 3(A)] obtained 12 weeks post-operatively showed a gradient of disc degeneration across experimental groups, as well as variability within each group. Mean NP T2 and T1-p values were closest to sham and control for the 0.1U ChABC group, and progressively lower for the nucleotomy, 1U and 5U ChABC groups, respectively [Fig. 3(B) and (C)]. NP T2 and T1-p values were both significantly lower compared to sham for the nucleotomy ($P = 0.005$ and $P = 0.01$, respectively), 1U ($P < 0.001$ and $P = 0.001$, respectively), and 5U ($P < 0.001$) ChABC groups. No significant differences in NP T2 or T1-p values were detected between sham and control discs. Mean Pfirrmann grade was significantly greater for the 1U and 5U ChABC groups (means of 3.2 and 3.8, respectively, $P < 0.001$) compared both to sham and control groups (means of 1.5 and 1.4, respectively, Fig. 3(D)). Pfirrmann grade significantly correlated with both T2 ($r^2 = 0.50$, $P < 0.001$, Fig. 3(E)) and T1-p ($r^2 = 0.60$, $P < 0.001$, Fig. 3(F)).

Microcomputed tomography of bony endplates

Three-dimensional μCT reconstructions of the cortical and trabecular bone adjacent to the intervertebral disc revealed no obvious qualitative differences in bone content or morphology between control, sham, and nucleotomy groups. However, for ChABC-injected discs, localized lower bone content in the cortical bony endplate and greater trabecular bone content in adjacent secondary ossification centers was observed [Fig. 4(A)]. The extent of the cortical endplate bone changes increased with increasing ChABC dosage. Of the five samples analyzed for each group, one sample exhibited clearly localized decreased endplate bone content for the 0.1U ChABC group, three for the 1U ChABC group, and five for the 5U ChABC group. Quantitative analyses revealed significantly reduced cortical bone BV/TV for the 1U ($P = 0.02$) and 5U ($P = 0.05$) ChABC groups compared to control [Fig. 4(B)]. Significantly greater adjacent trabecular bone BV/TV was observed in the 1U ChABC group compared to control ($P = 0.04$, Fig. 4(C)). Samples with the lowest cortical BV/TV also exhibited the greatest adjacent trabecular BV/TV, regardless of the ChABC dose.

Fig. 2. Longitudinal changes in disc height from plain radiographs. A. Disc height (measured as DHI, as a percent of pre-operative values) declined progressively over the 12 weeks study for all experimental groups, except control and sham; * $P < 0.05$ vs sham group. B. DHI measured immediately post-operatively as a percent of pre-operative values. C. DHI measured 12 weeks post-operatively as a percent of pre-operative values; bars denote significance, $P < 0.05$; ANOVA with Tukey’s post-hoc tests. D, and E, Representative radiographs of the lumbar spine for the same animal taken pre-operatively and 12 weeks post-operatively, respectively.
Biomechanical properties

Alterations in disc biomechanical properties as a consequence of 1U ChABC injection and nucleotomy were determined by tension-compression testing of spine motion segments. Average force-displacement curves [Fig. 5(A)] showed greater NZ length and total ROM for 1U ChABC and nucleotomy groups compared to controls. Significant reductions in compressive ($P = 0.03$) and NZ modulus ($P = 0.001$) were found for both nucleotomy and 1U ChABC groups compared to control [Fig. 5(B) and (D)]. NZ ROM was significantly greater for the 1U ChABC group ($P = 0.04$), while total ROM was significantly greater for both the 1U ChABC ($P = 0.001$) and nucleotomy ($P = 0.01$) groups compared to control [Fig. 5(E) and (C)]. No significant differences in creep parameters were found between groups, although representative creep curves suggested greater creep displacement in the 1U ChABC group compared to control. There was a trend ($P = 0.09$) towards lower early damping stiffness ($S_1$) in the nucleotomy and 1U ChABC groups compared to control (Supplemental Fig. 1).

Histological grading

A gradient of degenerative changes to the disc was observed histologically across experimental groups [Fig. 6]. Qualitatively, the
nucleotomy and 0.1U ChABC groups exhibited mild to moderate degenerative changes characterized by slight in-folding of the AF lamellae, mild to moderate loss of proteoglycan staining in the NP, thinning of the EP, and fibrotic changes to the NP [Fig. 7(A)]. Moderate to advanced degeneration was observed in the 1U and 5U ChABC groups, with the most severe changes occurring in the 5U ChABC group, including loss of proteoglycan in the NP, NP fibrosis, disorganization of the AF and loss of endplate structure. Endplate disruptions, characterized by apparent protrusions of NP material into the underlying bone, were observed adjacent to ChABC-injected discs for all doses [Fig. 7(B) and (C)]. No obvious degenerative changes were apparent for sham discs.

Semi-quantitative grading, where a higher score indicates a more degenerative disc, supported the above histological observations [Fig. 8]. No significant differences between blinded graders were found for the AF organization and AF/NP border categories. Significant differences between graders were found for the NP ECM ($P = 0.03, 4.0\%$ of total variation), NP cellularity ($P = 0.02, 5.7\%$ of total variation) and EP structure ($P < 0.001, 9.5\%$ of total variation) categories. Scores for the 1U and 5U ChABC groups were
significantly higher than sham and control for all grading categories ($P < 0.001$). Scores for the 0.1U ChABC group were not significantly higher than sham and control in any category, and were significantly lower than both 1U and 5U groups for AF organization ($P = 0.01$), AF/NP border ($P = 0.003$) and NP matrix ($P = 0.004$). Scores for the nucleotomy discs were significantly higher than sham and control for AF organization ($P = 0.02$), NP matrix ($P = 0.001$), NP cellularity ($P = 0.02$) and EP structure ($P = 0.001$). Scores for the 1U and 5U ChABC groups were not significantly different from each other for any grading category. Similarly, scores for the 0.1U ChABC and nucleotomy groups were also not significantly different from each other for any category. No significant differences were observed in scores between sham and control discs for any category.

### Discussion

In this study we established a large animal model of disc degeneration that presents with a spectrum of degenerative changes, from mild to severe, as demonstrated by alterations in disc height, MRI score, histologic grade, biomechanical properties, and bony endplate changes. Two mechanisms for inducing degeneration were investigated: a subtotal nucleotomy and intradiscal ChABC injection. Nucleotomy or low dose ChABC injection (0.1U) resulted in mild to moderate degeneration, 1U ChABC injection resulted in moderate to severe degeneration, and 5U ChABC injection resulted in severe degeneration after 12 weeks. The nature of these interventions may account for these findings: subtotal nucleotomy physically removes disc material, leaving some healthy NP behind, while ChABC specifically degrades glycosaminoglycans, acutely disrupting the normal function of the proteoglycan-rich NP.

Histologically, the spectrum of degeneration achieved in this model recapitulates the range of structural and compositional alterations to the disc observed in human degeneration, including a progressive reduction in disc height, loss of proteoglycans in the NP, fibrosis of the NP matrix, and disorganization of the AF. Reductions in NP T2 and T1 r values and increases in Pfirrmann grade with increasing severity of degeneration are also consistent with the well-characterized MRI changes observed in human discs with aging and degeneration.

Interestingly, alterations to the vertebral bone adjacent to the disc were also observed in ChABC-injected discs in this study. Degeneration of the EP and remodeling of the subchondral bone in human disc degeneration has not been extensively investigated, yet increases in trabecular thickness and bone volume fraction of the vertebral endplate have been associated with disc degeneration.

Additionally, endplate pathology, including fibrovascular endplate...

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**Fig. 6. Histology.** Substantial alterations to disc structure and composition are evident in nucleotomy and ChABC-injected discs. Minimal degenerative changes are present in sham discs compared to control. Variations in disc structure and composition are also evident within experimental groups. Alcian blue (glycosaminoglycans) and picrosirius red (collagen) staining; mid-sagittal sections; scale bar = 1 mm.
Fig. 7. Histological details of disc substructures. A. Degenerative changes to disc substructures are evident for ChABC-injected and nucleotomy groups, including in the nucleus pulposus (NP; increasing fibrosis and decreased glycosaminoglycan staining), the annulus fibrosus (AF; inward folding of lamellae) and the EP (thinning). B. Magnified views of single and C. multiple endplate disruptions (arrows, eruption of NP material into adjacent vertebral bone). Alcian blue (glycosaminoglycans) and picrosirius red (collagen) staining; Scale bar = 0.1 mm for NP and EP images in panel A; scale bar = 0.5 mm for AF images in panel A. Scale bar = 0.25 mm in panels B and C. IAF = inner annulus fibrosus, OAF = outer annulus fibrosus, VB = vertebral body.

Fig. 8. Semi-quantitative histological grading of degeneration. Grading was performed independently by three blinded assessors using a visual analog scale (0 (normal)—100 (most degenerate)) to rate each disc substructure. A. AF organization. B. AF/NP border. C. Cartilage EP structure. D. NP matrix. E. NP cellularity. Differently shaded symbols indicate different graders. Bars denote significance; * indicates significantly different from nucleotomy, 1U and 5U groups (P < 0.05).
marrow and nodular type endplate defects, have been identified in human discs on histologic sections and bear similarity to those observed in this model\textsuperscript{15,16}. The endplate pathology of this model also bears resemblance to Schmorl’s nodes, which are observed concomitant with human degeneration and are associated with the occurrence of frequent back pain\textsuperscript{19}. The etiology of these endplate disruptions in humans and in this model is unclear, but may be related to an inflammatory or immune reaction to ChABC in the degenerative disc\textsuperscript{40}.

The structural alterations to the disc in the 1U ChABC and nucleotomy groups were associated with measurable changes in whole-disc mechanical function. These included lengthening of the NZ, increased total ROM, reduced compressive modulus and increased creep strain. Reduced NP glycosaminoglycan content has been previously shown to affect increases both total and NZ ROM, as well as reduced NZ stiffness\textsuperscript{41}. Additionally, increases in ROM and reductions in elastic modulus have been demonstrated in human degenerate discs compared to healthy discs\textsuperscript{12,43}. Increases in creep displacement and the early creep time constant \(\tau_c\) have also been observed with human degeneration\textsuperscript{44}. Collectively, our mechanical findings support the structural and histological findings of moderate degeneration with 1U ChABC and nucleotomy.

This work builds on previous goat models of disc degeneration, which have successfully achieved mild degenerative changes to the disc within a similar time scale. Previous studies of low dose intradiscal injection of ChABC (approximately 0.04U per disc) reported non-significant changes in DHI at 12 weeks, but significant alterations to histologic scores and MRI\textsuperscript{15,44}. Increases in disc ROM in lateral bending and axial rotation, and reduced stiffness in axial rotation have also been reported in this model, consistent with the alterations in mechanical properties observed in the current study\textsuperscript{45}. Increases in catabolic gene expression have also been illustrated in this model with increasing severity of degeneration\textsuperscript{46}. Mild to moderate changes to the goat disc have also been reported following surgically induced injury to the annulus, based on histological outcomes\textsuperscript{13,47,48}.

Due to the spectrum of degeneration that can be achieved at a size scale relevant to the human spine, this large animal model provides a versatile platform for the pre-clinical evaluation of experimental therapeutics. The degenerative state induced by nucleotomy or 1U ChABC injection could be used to evaluate the safety and efficacy of injectable stem cell, hydrogel or pharmacological regenerative therapies\textsuperscript{49–51}. The advanced degeneration achieved via high dose ChABC injection may be well-suited for the evaluation of tissue-engineered total disc replacements\textsuperscript{52}.

As with any animal model of human disease, there are several important limitations to the current study. In this model, degeneration of the disc was induced by acute insult, either by physical removal of NP material or chemical depletion of NP glycosaminoglycan content. While loss of proteoglycans from the NP is a hallmark of early-stage degeneration in humans, compositional changes are slow to manifest and progress, and the underlying causative factors are complex and multifactorial\textsuperscript{52,53}. All outcomes measured in this study, with the exception of DHI, were performed terminally at a single time point, 12 weeks following the initiation of degeneration, and as such, the time course of changes to the disc following nucleotomy or ChABC injection cannot be determined. While we reproducibly achieved a spectrum of degeneration via varying dosages of ChABC injection, there was variation in the degenerative response of discs within each treatment group, which may be accounted for by inter-animal variability or subtle differences in the surgical delivery of the enzyme. It is also important to note that potential disc height recovery, suggestive of an innate regenerative response or re-swelling of residual NP, was observed in the 0.1U ChABC and nucleotomy groups between 10 and 12 weeks. A similar phenomenon was previously observed in a rat ChABC degeneration model\textsuperscript{17,18}. Future studies should confirm this by monitoring DHI at additional time points beyond 12 weeks, as it may indicate that there is a critical threshold for ChABC-mediated NP glycosaminoglycan depletion that is necessary to ensure irrecoverable degenerative changes. Finally, it is unknown whether this pattern of degenerative changes gives rise to discogenic pain, an outcome that would add important clinical relevance to this model. While in the current study none of the animals exhibited clinically perceptible pain, ongoing work is aimed at quantifying animal behavioral and activity changes that may be associated with disc degeneration, in addition to examining the presence of nociceptive nerve fiber ingrowth into degenerated discs. The presence of pro-inflammatory cytokines may also contribute to discogenic pain in humans by driving tissue catabolism and promoting innervation. Increases in inflammatory factors have been identified in animal models of injury or altered mechanical loading of rat intervertebral discs\textsuperscript{43,54}. Ongoing work will determine the presence of TNF-\(\alpha\), IL-6 and other pro-inflammatory cytokines and their roles as potential triggers of degeneration and pain in this model.

In conclusion, we have developed a reproducible large animal model of intervertebral disc degeneration that presents consistently with characteristics mimicking the spectrum of human pathology, from mild to severe. It establishes a clinically relevant testing platform for a wide range of regenerative therapeutics.

**Author contributions**

SEG, NRM, TPS, AHM, GRO, EJV, DME, RLM, and US were responsible for study design and conception. Acquisition and analysis of data was performed by SEG, NRM, TPS, ZZ, JTM, and JRB. The article was drafted by SEG. All authors revised the article for important intellectual content, and all authors gave final approval of the version to be submitted.

**Conflict of interest**

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this manuscript.

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**Supplementary data**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2016.08.006.
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