

Basic Science

Controlled immobilization-traction based on intervertebral stability is conducive to the regeneration or repair of the degenerative disc: an in vivo study on the rat coccygeal model

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ABSTRACT

BACKGROUND CONTEXT: Previous studies have shown the potential for intervertebral disc tissue regeneration is very limited. While in vivo and in vitro studies have shown that traction can restore disc height and internal pressure, in many clinical studies it was shown that axial mechanical traction for the treatment of low back pain is ineffective.

PURPOSE: The aim of this study was to identify how the disc could be distracted, how to define the state of traction, and to further examine the feasibility of regenerating or restoring the degenerative disc by means of traction.

STUDY DESIGN: A macro- and microlevel structural analysis of degenerative discs of rat tail before and after controlled immobilization-traction.

METHODS: In this study, 49 6-month-old male Sprague-Dawley rats were randomly assigned to one of seven groups. Group A was the sham control group in which caudal vertebrae were instrumented with K-wires only. In Group B (model group), caudal vertebrae were immobilized using a custom-made external device to fix four caudal vertebrae (Co7–Co10) and Co8–Co9 underwent 4 weeks of compression to induce moderate disc degeneration. In Group C, vertebrae Co8–Co9 underwent 4 weeks of compression to induce moderate disc degeneration, followed by removal of the external apparatus. Rats in the other four groups (Groups D–G), Co8–Co9 underwent 4 weeks of compression to induce moderate disc degeneration followed by 2 weeks, 4 weeks, 6 weeks, and 8 weeks of distraction, respectively. Caudal vertebrae were harvested and disc height, T2 signal intensity of the discs, disc morphology, total glycosaminoglycan content of the nucleus pulposus and the structure of the Co8–Co9 end plate were evaluated.

RESULTS: After 4 weeks of compression, the intervertebral height and T2 signal intensity of Co8–Co9 vertebrae of rats in Groups B to G were significantly reduced compared with Group A (sham group, all $p < .0001$). Histological scores of rats in Group B averaged 10.14 and the total

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Competing Interests: None.

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glycosaminoglycan (GAG) of nucleus pulposus averaged 238.21 μg GAG/ng DNA. The bony end plate structure showed significant changes in comparison with the control Group. After 2 weeks to 8 weeks of traction, the disc space and T2 signal intensity of Co8–Co9 vertebrae in Group E were significantly recovered compared to that of rats in Group B ($p < .0001$), and the intervertebral height of the Co8–Co9 in Group D, Group F, and Group G when compared with Group B ($p < .0001$). Meanwhile, the T2 signal intensity of Co8–Co9 in Group D, F, and G when compared with Group B ($p < .001$). Histological scores dropped from an average of 10.14 in Group B to 5.57 in Group E, and 5.86 in Group F (all $p < .0001$). Furthermore, the total GAG content of the nucleus pulposus increased from an average of 238.21 μg GAG/ng DNA in Group B to 601.02 μg GAG/ng DNA in Group E ($p < .0001$). The number of pores of end plates in rats in Groups D and E both were significantly increased when compared to that of rats in Group B (Groups D vs Groups B, $p < .05$; Groups E vs Groups B, $p < .0001$).

CONCLUSIONS: A mechanical degenerative model was successfully established by using a custom-made device. We demonstrated that disc degeneration is a cascade of biochemical, mechanical, and structural changes mediated by cells in an abnormal mechanical environment. Not all levels of disc degeneration can be regenerated or repaired. Regeneration or recovery of disc degeneration requires specific conditions. Based on the immobilization-traction mode, the cascade cycle of disc degeneration is interrupted. Traction of 2 to 6 weeks is a sensitive period for regeneration of the degenerative disc. Moreover, the duration and extent of the traction loading must be moderately controllable, and beyond the limits that can lead to significant degeneration. These data may help improve our understanding of the pathogenesis of clinical disc degeneration and how to optimize the use of traction devices for possible regeneration. © 2018 Elsevier Inc. All rights reserved.

Keywords: Biomechanics; Immobilization-traction; Intervertebral disc degeneration; Rat model; Regeneration; Remodeling.

Introduction

Current treatment strategies for low back pain from degenerative discs, including conservative treatments and surgeries, do not treat the underlying causes of degeneration. The current gold standard for surgical intervention includes spinal fusion [1]. This type of surgery not only sacrifices the function of the operative segment, but also changes the normal biomechanical environment of the spinal functional unit. Recently, artificial disc replacement has been used. However, these implants do not recapitulate the mechanical function of the native joint, are subject to wear and failure, and the resection procedure is complex [2]. In addition, regenerative disc therapies by injecting stem cells or other bioactive components have been performed [3,4]. Despite the potential for future success, these studies bring several new challenges, and it is difficult to accurately determine the timing and frequency of treatment. Therefore, selecting stem cells as a regenerative disc strategy requires rigorous evaluation [5]. To date, only a dynamic stabilization system can be used as a mechanical way to regenerate the disc [6]. In previous studies, it was demonstrated that disc height and intervertebral disc (IVD) pressure can be recovered by traction. The process of reproducing disc regeneration was confirmed by histological assessment and imaging studies [7,8]. However, several clinical studies reported that traction did not have a significant effect on patients with lower back pain [9–11].

The mechanism and pathogeny of IVD degeneration are complicated, and there is still some controversy about whether to repair or delay the degeneration of the disc by traction. We believe that the ability to repair or regenerate the

IVD fundamentally depends on the stage of IVD degeneration [12]. IVD degeneration proceeds until disc rupture occurs, which is considered a risky period. When the IVD environment deviates from its physiological setting, biological remodeling will cause the organizational structure to change, resulting in changes in properties of the material [13].

We hypothesized that the mechanical environment of the disc can be restored by timely and controlled immobilization-traction to effectively regenerate or repair changes of moderate disc degeneration. The aim of this study was to identify how the disc could be distracted and how to define the state of traction and to further examine regeneration or recovery of the degenerative disc by traction. Therefore, we established a mechanical degeneration model of tail vertebrae in rats to investigate reconstruction of the disc through timely and controllable traction stimulation of the degenerative disc. We studied macroscopic and microscopic structural changes of the degenerative disc as well as the changes in disc recovery over the time. Moreover, we examined effectiveness of recovery from the different stages of disc degeneration by different time traction to provide a theoretical and experimental basis for the repair or delay of IVD degeneration.

Materials and methods

Animal models and experimental groups

In this study, 49 6-month-old male Sprague-Dawley rats (mean \pm standard deviation, weight 450 \pm 15 g) were used. Animal experiments were approved by the Institutional Animal Care Committee of the Laboratory Animal at

Table 1
Summary of study design.

Group	Instrumented level (Co8–Co9)	No. of animals
A (sham)	Instrumented with K-wires only	7
B (model)	compression -4 week	7
C	compression -4 week (remove devices)	7
D	compression -4 week/ traction -2 week	7
E	compression -4 week/ traction -4 week	7
F	compression -4 week/ traction -6 week	7
G	compression -4 week/ traction -8 week	7

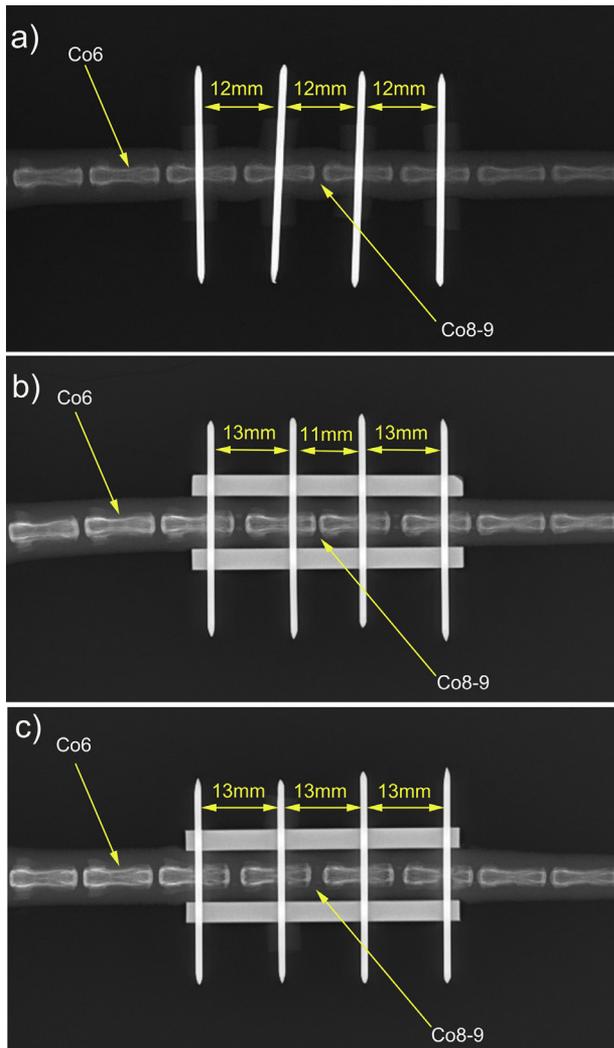


Fig. 1. Research design. (a) The caudal vertebrae were instrumented with K-wires only, the hole spacing of Co7–Co8, Co8–Co9, and Co9–Co10 were 12 mm, and served as controls. (b) The caudal vertebrae were immobilized using a custom-made external device to fix four caudal vertebrae (Co7–Co10), and Co8–Co9 underwent 4 weeks of compression. The hole spacing of Co8–Co9 was 11 mm, and the hole spacing of Co7–Co 8 and Co9–Co10 was 13 mm. (c) The Co8–Co9 underwent 4 weeks of compression, followed by 2 weeks, 4 weeks, 6 weeks, and 8 weeks of traction (mechanical test showed traction of 22 N). In the traction groups (Groups D–G): Co8–Co9 vertebrae were first compressed, and then underwent traction (22 N), the hole spacing of Co7– Co8, Co8–Co9, and Co9–Co10 vertebrae was 13 mm.

School of Medicine, Soochow University (Suzhou, China). Rats were randomly assigned to one of seven groups (Table 1). Group A (sham, caudal vertebrae were instrumented with K-wires only, Fig. 1a) served as controls. Group B (model, caudal vertebrae were immobilized using a custom-made external device to fix four caudal vertebrae (Co7–Co10), and Co8–Co9 vertebrae underwent 4 weeks of compression to induce disc degeneration, Fig. 1b). Group C (Co8–Co9 vertebrae underwent 4 weeks of compression to induce disc degeneration, followed by removal of the external apparatus (Fig. 2j). In the remaining four groups (Groups D–G, Co8–Co9 vertebrae underwent 4 weeks of compression to induce disc degeneration, followed by 2 weeks, 4 weeks, 6 weeks, and 8 weeks of traction (22 N attached and loaded traction device, Fig. 1c).

Intervertebral disc height measurement and magnetic resonance imaging analysis

After each time node completes the experiment, rats were evaluated by X-ray and magnetic resonance imaging (MRI) analysis. Radiographs were obtained under anesthesia by isoflurane (RWD Life Science Co., Shenzhen, China) using a digital, self-contained cabinet x-ray machine (SHIMADZU, RAD SPEED M, Japan, exposure time: 10 seconds, 26 kV). The disc space was determined by radiography [14]. As shown in Figs. 2 and 3, the IVD specimens of rat caudal vertebrae were scanned in a 1.5T MRI scanner (GE HDE, scanning sequence: FRFSE-XL, slice thickness: 1.4 mm), and scored by three radiologists and three spinal surgeons using the Pfirrmann system [15].

Measurement of glycosaminoglycans

Samples of NP underwent papain digestion at 65°C for 2 hours. Glycosaminoglycan (GAG) content was calculated with chondroitin-4 sulfate as the standard and based on the dimethylmethylene blue method [16]. To standardize GAG values, the DNA concentration of each sample was measured using PicoGreen analysis [17].

Histological analysis

At termination of the experiment, animals were euthanized by an excess of isoflurane (isoflurane, RWD Life Science co. Shenzhen, China). The caudal vertebrae were harvested (Rats in Group A and B were euthanized immediately after using K-wire and compression for 4 weeks, respectively, whereas rats in Group C were killed after removing the compression device and after 8 weeks of free feeding and activity) for further analysis. The target discs (Co8–Co9) were harvested, fixed in 10% buffered formalin (Shanghai Yuanye Bio-Technology Co. Ltd, Shanghai, China) for 24 hours, and decalcified in 10% ethylenediaminetetraacetic acid (Biosharp, Hefei, China) for 30 days. Subsequently, discs were paraffin-embedded (Leica, Richmond, VA, USA), and sectioned into 5 μ m-thick sections

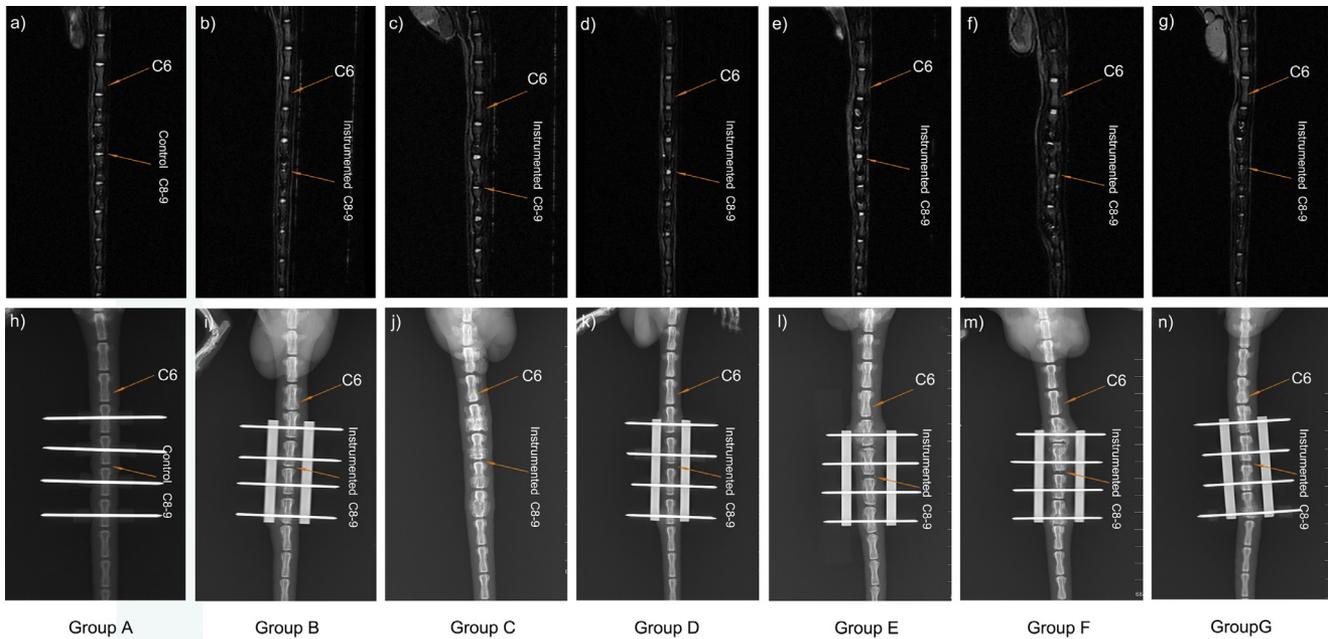


Fig. 2. MRI scans and radiographs. (1) Figure a to g (scanning sequence: FRFSE-XL, slice thickness: 1.4 mm) uses magnetic waves to create pictures to determine nucleus pulposus size and hydration status according to T2 signal intensity. (2) Figures h to n Radiographs were obtained under anesthesia using a digital, self-contained cabinet x-ray machine (exposure time: 10 seconds, 26 kV). After 4 weeks of compression in Group B to Group G, the intervertebral space and T2 signal intensity of Co8–Co9 in each experimental group was significantly decreased when compared to that in Group A. After 2 weeks to 8 weeks of traction in Group D to Group G, Co8–Co9 disc space was significantly improved when compared with that in Group B (Figures k–n), and T2 signal intensity was significantly reestablished (Figures d–f). The disc height and T2 signal intensity was significantly reduced in Group C (Figures c and j).

using a histotome (Leica, Heidelberg, Germany). For histological analysis, sections were stained with hematoxylin/eosin (Beijing BiotoppedScience & Technology Co. Ltd, Beijing, China). Staining was evaluated using a binocular microscope (XSP-2CA, Shanghai, China), as shown in Fig. 4. Histological evaluation was based on a grading system that was developed by Han et al. [18] (Fig. 5a).

Evaluation of bony end plates by scanning electron microscopy

Rats were euthanized by an excess of isoflurane, and the coccygeal vertebrae (Co8–Co9) were removed. To ensure the integrity of the vertebral end plate, the surrounding tissues were removed, including the surrounding ligaments, intervertebral discs, spinal cord, and nerve roots. The Co8–Co9 vertebrae were placed into preprepared test tubes, 20 ml of a mixed enzyme solution containing type I collagenase and type II collagenase (1%) was added. Tubes were placed in a 37°C incubator, and the enzyme solution was replaced every 2 days for 6 consecutive days. Then, soft tissue and cartilage on the surface of vertebral specimens were removed, and the integrity of the bony end plate was preserved. Vertebral specimens were repeatedly washed with saline. Next, vertebral specimens were dehydrated using a gradient of 70%, 80%, and 90% alcohol, after which the specimens were placed in a cool, ventilated area and dried for 24 hours. After this treatment, bony end plates were separated from the vertebral body (some bony end plates had been completely separated from

the vertebral body). Then, the cranial end plate of the Co8–Co9 intervertebral space was treated by metal spraying and observed by SEM (FEI Quanta 250, USA).

Statistical analysis

Data management and statistical analysis were performed using Excel 2016 (Microsoft, Corp., Redmond, WA, USA) and SPSS 24.0 (IBM SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation (SD). Significant differences between study groups were obtained by using a one-way analysis of variance with a Tukey's multiple comparisons test to analyze the influence of loads (compression and traction) and time. Statistical significance was set at $p \leq .05$.

Results

Animals

All 49 rats lived until completion of the study and tolerated application of the external custom-made device. No infection or other complications were observed. The device that was used for fixation of the coccygeal vertebrae weighed roughly 5.0 g and was well-tolerated by the rats, as evidenced by their ability to lift and easily move their tails with the devices attached. Although the segments within the apparatus were largely immobilized, rats could move and control their tails both proximal and distal to the

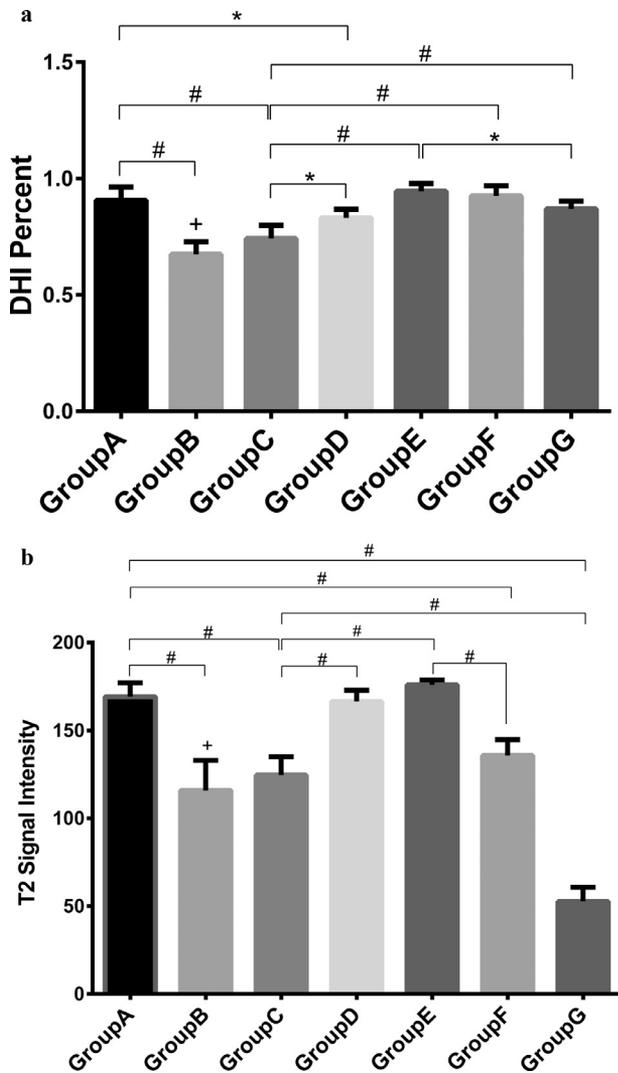


Fig. 3. A to B. Disc Height Index and T2 signal intensity. (A) Disc Height Index (DHI). (#) indicates significant differences compared to discs in other groups ($p < .0001$). (*) indicates significant differences between groups ($p < .05$). (+) indicates significant differences between discs in other groups, except for Group C ($p < .05$). (B) T2 signal intensity. (#) indicates significant differences between discs from other groups ($p < .0001$). (+) indicates significant differences between discs from other groups, except for Group C ($p < .05$).

device. None of the animals showed significant changes in weight, food intake, and sleep habits.

Invertebral disc height and T2 signal intensity

The continuous traction of the degenerative disc can effectively restore disc height and induce disc rehydration, which closely relates with time. After 4 weeks of compression, the intervertebral space and T2 signal intensity of Co8–Co9 vertebrae of rats in Groups B to G were significantly decreased when compared to that of rats in Group A (Fig. 2a, h, b, and i). Moreover, according to the Pfirrmann grading system criteria, disc degeneration was classified as grade III to IV. After 2 weeks to 8 weeks of continuous traction, the Co8–Co9

intervertebral space of rats in Group D to Group G was significantly improved compared with that of rats in Group B ($p < .05$, Fig. 3a). In addition, the T2 signal intensity was significantly reestablished ($p < .05$, Fig. 3b), and the disc recovery was classified as grade I to II (Group E, Table 2). The recovery of disc height of Co8–Co9 vertebrae of rats in both Group D and Group F were weaker compared to that of Group E (Group D vs Group E, $p < .05$ and Group E vs Group F, $p > .05$, Fig. 3a). The recovery of the T2 signal intensity of Co8–Co9 vertebrae of rats in both Group D and Group F were weaker when compared to that of rats in Group E (Group D vs Group E, $p > .05$, and Group E vs Group F, $p < .05$, Fig. 3b). Furthermore, the restoration of disc space and the T2 signal intensity of Co8–Co9 vertebrae of rats in Group G both were worse when compared to that of rats in Group E ($p < .05$, Fig. 3a and b). The disc height and T2 signal intensity were not significantly different between Group C and Group B ($p > .05$, Fig. 3a and b). However, in rats in Group C, both disc height and T2 signal intensity were persistently reduced.

Histological findings and glycosaminoglycan assay

After 4 weeks of compression, histological analysis of the loaded discs of rats in Groups B showed typical qualitative morphologic changes when compared with rats in the Group A (control group, Fig. 4a, h, and o vs b, i, and p), in which the NP comprised relatively few cells and less proteoglycans. The AF showed less organized fibrocartilage lamellae, and the collagen fibers formed a wavy arrangement. After 2 weeks to 8 weeks of traction of previously loaded discs (Groups D–G), discs of rats in Group D to Group F showed signs of tissue regeneration. Signs of tissue regeneration were not observed in discs of rats in Group G. Instead, significant scar tissue formation was observed. The NP of Co8–Co9 vertebrae of rats in Group D to Group F showed that fibrous tissue decreased whereas the volume increased, which was accompanied with restoration of the disc height. Disappearance of tissue shrinkage was obvious in rats in Group E compared with Group B. NP cells were less separated by a matrix of proteoglycans and included more round-shape cells. Disorganization of the AF became more organized with continuous traction. Moreover, after continuous traction, the disorganized layered structure of the degraded AF turned into a representative physiological “fish-bone” structure. In addition, the hyperplasia of cartilage tissue in the AF decreased with traction. By extending the traction time, these changes became more obvious with the disappearance of clefts or fissures in the AF and reduced herniation of disc materials or osteophyte formation as shown in Fig. 4 (e, l, and s). However, by increasing the duration of traction in rats in Group G, the above-mentioned changes of the disc degeneration were further deteriorated when compared to that of rats in Group B (Fig. 4g, n, and u). The histological score of the discs of rats in Groups B and G were significantly higher when compared to the control group (Group A, $p < .0001$, Fig. 5a). In addition, histological scores

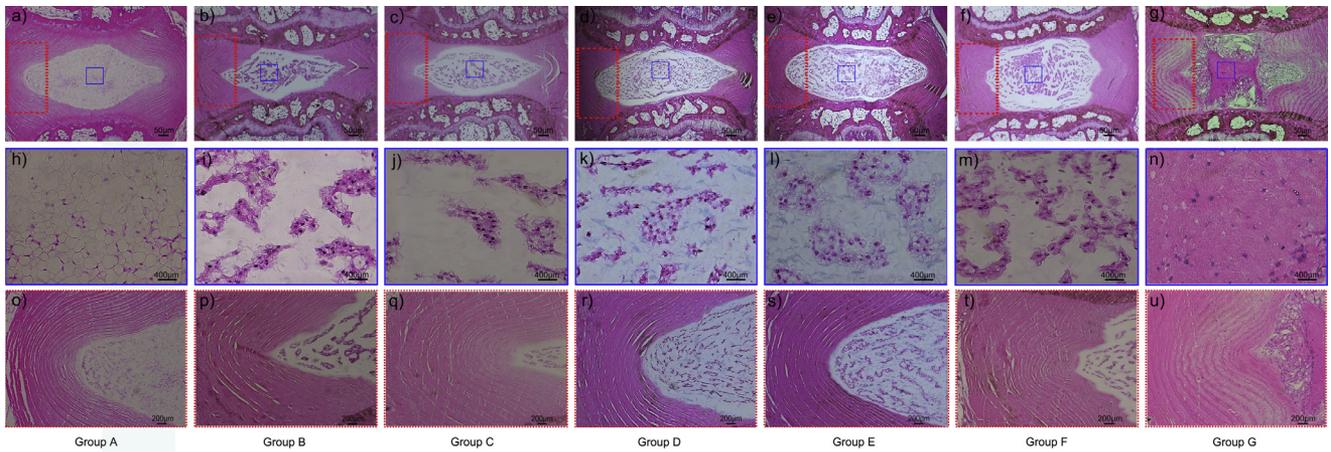


Fig. 4. Hematoxylin/eosin stain. Figure a to g, intervertebral disc (IVD) at magnification 50×. Figures h to n, IVD at a higher magnification (400×), and Figures o to u, IVD at magnification 200×. Figures h to n in the middle row are enlarged images of the blue solid line box of the top row (a–g). Figures o to u on the bottom row are enlarged images of the red dotted rectangle in top row (a–g).

were not significantly different between Group B and Group C ($p > .05$). However, the histological scores of rats in Group C were higher when compared to that of rats in Group D–F ($p < .0001$, Fig. 5a). In addition, the histological scores of rats in Group E and Group F were not significantly different when compared to that of rats in the control group ($p > .05$). The total GAG content of the NP in rats in Group B decreased significantly when compared to that of rats in the control group ($p < .0001$, Fig. 5b). Compared with each traction group (groups D–G), the total amount of NP in rats in group B was significantly reduced ($p < .05$, Fig. 5b), which

corresponded to the total GAG content of the NP in Group E, which significantly increased when compared with that of rats in groups C, D, F, and G ($p < .0001$, Fig. 5b). However, the total GAG content of the NP was not significantly different between Group B and Group C ($p > .05$).

Evaluation of bony end plates

The bony structure of the end plate-IVD interface of unloaded discs contains a large number of irregular pores (“concave lens” shape) (Fig. 6a, h, and o). After 4 weeks

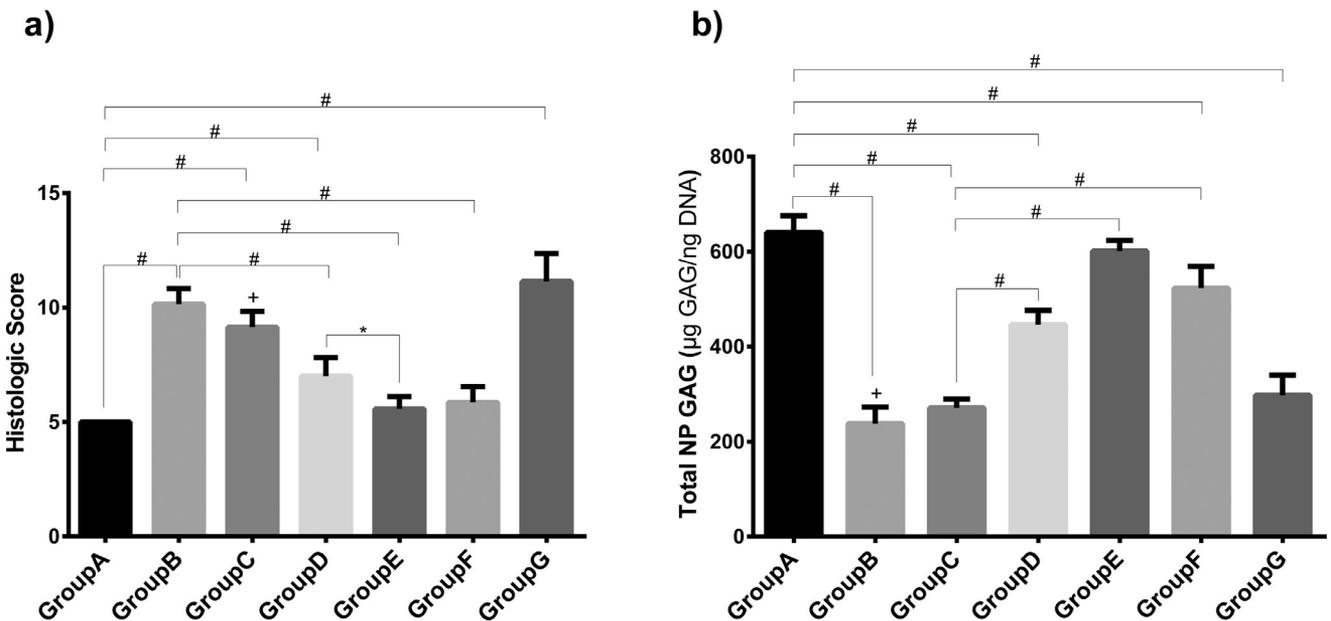


Fig. 5. Histological analysis and glycosaminoglycan assay. (a) Histologic analysis. (#) indicates significant differences between groups ($p < .0001$). (*) indicates significant differences between groups ($p < .05$). (+) indicates significant differences between groups, except for Group B ($p < .05$). (b) Glycosaminoglycan (GAG) assay. (#) indicates significant differences between groups ($p < .0001$). (+) indicates significant differences between groups, except for Group C ($p < .05$).

Table 2
Summary of pre- and post-traction of each experimental group

Group	Pfirrmann grade (I–V)							No. of animals
	Pretraction		Post-traction					
A(control)	I(7)		-					7
B(model)	III(5)	IV(2)	-					7
C	III(4)	IV(3)	I(0)	II(0)	III(1)	IV(6)	V(0)	7
D	III(5)	IV(2)	I(1)	II(4)	III(2)	IV(0)	V(0)	7
E	III(5)	IV(2)	I(3)	II(4)	III(0)	IV(0)	V(0)	7
F	III(6)	IV(1)	I(1)	II(3)	III(3)	IV(0)	V(0)	7
G	III(3)	IV(4)	I(0)	II(0)	III(1)	IV(4)	V(2)	7

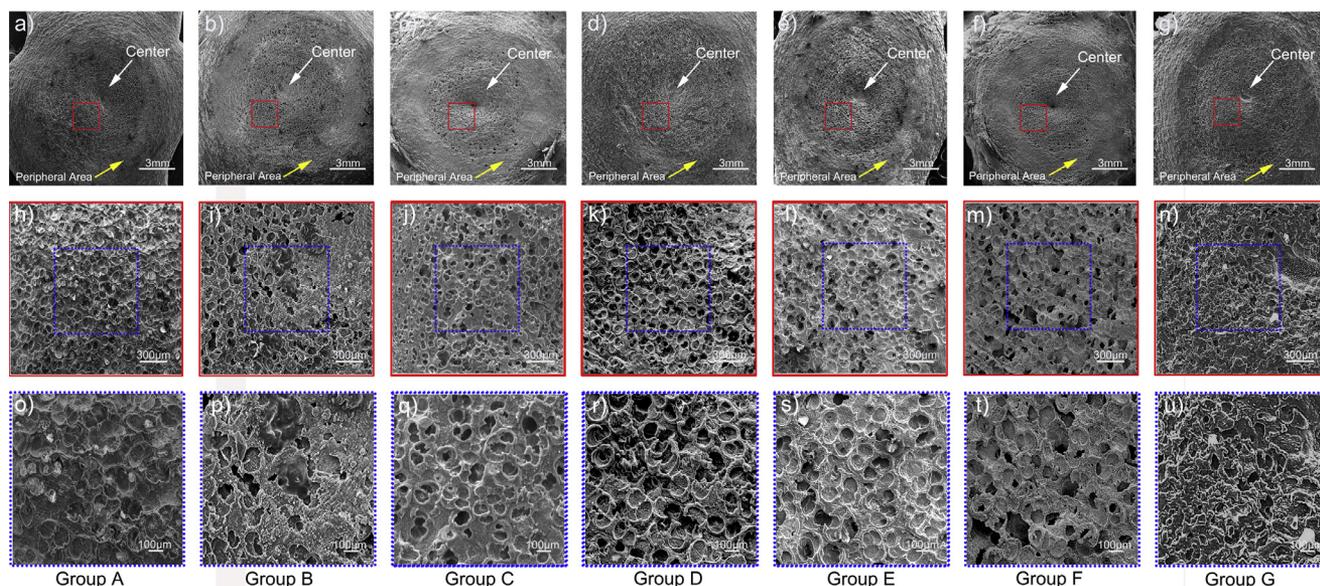


Fig. 6. Evaluation of bony end plates by scanning electron microscopy. (1) Figures a to g, bony end plates at magnification 100 \times . Figures h to n, intervertebral disc (IVD) at a higher magnification (500 \times), and Figures o to u, IVD at magnification 1000 \times . (2) Figures h to n in the middle row represent an enlarged image of the red box of the top row (a–g). Figures o to u on the bottom row are enlarged images of the blue dotted rectangle in middle row (h–n). The area indicated by the white arrow is the central area of the bony end plate. The area indicated by the yellow arrow is the peripheral area of the bony end plate.

of compression, the bony end plate structure showed significant changes in comparison with the control group (Fig. 6b, i, and p), including rough end plate surfaces that were progressively ablative and increasingly irregular, with increased calcification and osteophyte formation. In addition, the central area of the end plate was more obvious than the surrounding area, and a significant reduction in the number of pores ($p < .0001$, Fig. 7). After 2 weeks to 6 weeks of traction, the pore structure showed significant recovery-related changes in comparison with rats in Group B (Fig. 6r and s). Moreover, the number of pores of end plates in rats in groups D–F were significantly increased when compared to that of rats in Group B ($p < .0001$, Fig. 7). By increasing the traction time, the bony end plate structure of rats in groups G was increasingly deteriorated, and the number of pores was further reduced, which positively correlated with time (Group B vs Group G, $p < .0001$, Fig. 6n and u). Compared with rats in Group B, the end plate structure of rats in Group C was

further calcified or ossified, and the number of pores was further reduced ($p > .05$, Fig. 6j and q).

Discussion

Mechanical degeneration of the IVD is a cascade reaction of biochemical, structural, and morphologic changes induced by changes in the mechanical environment, which further damages the function of the intervertebral disc. Moderately controllable traction can help to regenerate or repair a disc [13].

Disc height and the T2 signal intensity are effectively recovered after traction

The proteolysis of proteoglycans and the loss of polysaccharides may negatively affect IVD function and be directly involved in IVD degeneration. In addition, the loss of polysaccharides and proteoglycans further reduced the

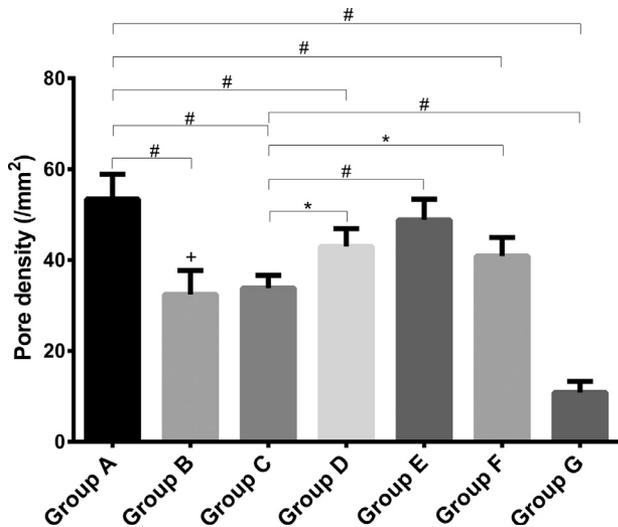


Fig. 7. Statistical analysis of pore density of bony end plates. (#) indicates significant differences between groups ($p < .0001$). (*) indicates significant differences between groups ($p < .05$). (+) indicated significant differences between groups, except for Group C ($p < .05$).

expansion of the disc, thereby causing mechanical damage [19]. Proteoglycans can withstand pressure on the spine, cause the disc to expand, and maintain the separation of vertebral bodies [19]. In our study, after 4 weeks of continuous compression, the level of disc height, and MRI T2 signal intensity decreased. Moreover, the total GAG content of the NP of rats in Group B (model group) decreased significantly when compared with the control group. These changes were consistent with findings presented in previous studies showing that proteoglycan loss occurs during the initial stage of disc degeneration [17,20]. This was likely due to the loss of proteoglycans in the degenerated discs, which reduced osmotic pressure, reduced stiffness of the disc, and increased deformation in response to a compressive load [21]. The continuous load reduced the proteoglycan content, and eliminated moisture, thereby increasing the level of proteoglycans and its expansion potential. With continuous traction and recovery of the disc height, the GAG content gradually increased and water is introduced into the IVD by osmosis, so the T2 signal gradually recovers. In our study, we confirmed that recovery of disc height and T2 signal intensity of Co8–Co9 vertebrae of rats in Group D was weaker when compared to that of rats in Group E by short-term traction (2 weeks). Moreover, we found that the recovery of the disc height and signal intensity was best compared with other experimental groups (6 week and 8 week) after 4 weeks of continuous traction. In addition, the disc height and T2 signal intensity between rats in Group B and Group C was similar, indicating that by removing the compression device the disc height reduction was irreversible with low traction. The degeneration process progressed after removing the compression device. An increased duration of traction (6 weeks to 8 weeks) resulted in a decrease of

proteoglycan permeability, which may be due to fatigue of the cells, and failed to provide the necessary energy for the biosynthesis process and converts anabolism into catabolism [22,23]. Moreover, due to the massive death of NP cells, proteoglycans cannot be synthesized, resulting in a reduction in IVD space, and T2 signal intensity.

Disc regeneration promoted after controlled traction

Degeneration of IVDs is the process that involves changes in the biomechanical structure or environment around its cells that slowly deviates from the normal tissue under the precise regulation of cells. Therefore, IVD regeneration requires a specific environment. The appearance of cell clusters and a disordered AF, accompanied with gradual disappearance of the division between the NP and the AF, is a typical feature of intervertebral disc degeneration [24]. Kroeber et al. confirmed that loads induced disk degeneration was irreversible after 28 days of unloading [25]. However, after 4 weeks of traction of previously loaded discs, the disc showed a decrease in fibrous tissue and an increase in volume accompanied with restoration of the disc height. The results of this study indicated that in the rabbit lumbar spine model, disc regeneration can be caused by axial dynamic traction [13]. Guehring et al. showed distraction leads to the rehydration of IVDs, stimulated the expression of extracellular matrix genes and increased the number of protein-expressing cells [26]. In our study, we confirmed that regeneration after 4 weeks of traction, and disc recovery were consistent with the above findings. However, disc recovery was weaker compared to that of 4 weeks of traction by the short-term traction (2 weeks). Moreover, by increasing the traction from 6 weeks to 8 weeks, the NP of the intervertebral disc was gradually replaced by proliferative granulation tissue and scar tissue, and the AF was progressive hyperplasia and hypertrophy. These results indicated that when the tissue environment deviates from its physiological setting value, biological remodeling will lead to changes in tissue structure and material properties. Significant degeneration can be induced, depending on the duration and extent of the loading. Furthermore, we hypothesize that long-term immobilization-traction (6 weeks–8 weeks) will create a unique mechanical state that can cause disc degeneration [27].

Stress reconstructed the structure of the bone end plate

The IVD is nonvascular, and mainly depends on diffusion of nutrients through the vertebral end plate [28]. Change in the end plate structure affects the opening of the IVD nutrition channel, and the NP will undergo degeneration with insufficient nutrient supply of the IVD [29,30]. Compression loads resulted in cartilage end plate degeneration, and bone end plate vascular channel volume decreased. Furthermore, the volume of vascular channels significantly increased after traction [31]. This further suggested that positive reconstruction of the end plate structure guarantees nutrition supply of the IVD, and delays further degeneration of the disc. Kandel

et al. demonstrated that nutritional factors and transport disorders were considered key factors in the slow recovery of human intervertebral discs [32]. The pore structure (“concave lens” shape) of the bony end plate is conducive to the transfer of nutrients and metabolites in the position of the NP [33]. The spongy structure of the bony end plate is the structural basis of nutrient transmission [34]. In our study, we confirmed that the typical structure of the bony end plate (concave lens) after 4 weeks of compression was significantly disorganized, and calcification and osteophyte formation was observed. The central area of the end plate was more affected than the surrounding area, and a significant reduction in the number of pores. Gradual recovery of the pore structure was observed after 2 weeks to 4 weeks of traction loading. However, by increasing amount of traction time, the bony end plate structure of discs of rats in Group F (6 weeks) and Group G (8 weeks) was increasingly deteriorated, which positively correlated with time. These findings further indicated that the compression load caused bone end plate degeneration, that after traction the structures were progressively restored or reversed, and closely related with time. A duration of 4 weeks of traction was the ideal time for end plate reconstruction. Conversely, by applying a longer traction time, the pore structure recovery will be worse. This was consistent with the timing of degenerative disc reversal or recovery. This further proved that changes in the end plate structure were closely related to degeneration of the IVD.

Continuous immobilization-traction effectively maintains the stability of the mechanical environment of the degenerative intervertebral disc

Disc degeneration includes the loss of water content in the NP, disorganization of the AF, gradual disappearance of the division between the NP and the AF, and end plate ossification and osteophyte formation [35–37]. These changes further lead to loss of the entire disc height, resulting in reduced spinal stability [25,37,38]. Adams and Zhao et al. reported that with increasing age and degeneration, the disc internal pressure decreased and the stress bone spur increased. This supported the contention that the pressure and shear force in the adult IVD were not balanced [39]. Studies by Adams et al. showed that degenerative IVD height decreases significantly changed the biomechanical properties of the spine motion segments, reduced the loads in the NP and AF, thereby leading to a weakened load resistance ability of the IVD and spinal instability [35,38]. Previous clinical studies have confirmed that height loss of a degenerative IVD may affect the function of articular processes [40–42]. These processes are accompanied by a narrow joint space, poor joint faces, hyperplasia of the joint, and instability of the spine [40–42]. In addition, instability can lead to accelerated disc degeneration, which can lead to pain and neurologic dysfunction [13]. In our study, disc degeneration caused by compression loads was irreversible after 8 weeks of unloading (Group C). These findings indicated that intervertebral

instability caused by disc degeneration due to compression cannot be fully repaired by itself. Moreover, we confirmed that through continuous immobilization-traction (Group D to G), the degenerative disc was well-reconstructed and regenerated (Group E), and by increasing the time of continuous traction, disc degeneration in Groups F and G gradually increased deterioration. We believe that compression induced degeneration triggers a cascade of disc degeneration. Based on the immobilization-traction mode, the cascade cycle of disc degeneration is interrupted. In addition, we believe that this is the result of the reverse stretching of the biomechanical adaptation principle because cells and its regulated micro-nano environment are sensitive to external forces or load conditions including load time, amplitude, and changes. After 4 weeks of traction, the disc structure was nearly normal, thereby indicating that the traction force and duration were relatively appropriate. In addition, 6 weeks to 8 weeks of traction to repair the degenerative discs was not sufficient, indicating that cells were tension overstimulated and that synthesis of extracellular matrix and cells trying to adapt to or repair to another more deviation from normal environment lead to excessive correction. These findings implied that the duration and extent of the traction loading must be moderately controllable, and beyond the limits that can lead to significant degeneration, and that not all degrees of disc degeneration can be regenerated or repaired. We believe that this may be one of the reasons for the poor clinical efficacy of traction in the treatment of disc degeneration and lower back pain. In previous studies, it has been demonstrated that NP and AF tension caused by the interaction between NP and AF were time-dependent, and controlled by hydration of the NP and stress relaxation of the AF. The steady state between NP pressure and AF shear forces was likely to be restored [43]. Therefore, we hypothesized that by continuous immobilization-traction of the tail vertebrae, the stability of the caudal vertebrae is maintained by restoring the disc height. In addition, stress on the intervertebral disc as well as the stress concentration were reduced, thereby preventing the pathologic process of disc degeneration. The intervertebral stability based on immobilization-traction can delay the acceleration of IVD degeneration, which may be due to the nonphysiological mechanical environment caused by intervertebral instability. This would result in degenerative discs that were well-reconstructed and regenerated, easily delayed IVD degeneration, thereby further proving that intervertebral instability caused by IVD degeneration was the cause of increased degeneration of the disc. Therefore, the stress conduction mode was changed by immobilization-traction, and was consistent with the biomechanical stability of the spine. Moreover, the duration and extent of the traction loading must be moderately controllable, and beyond the limits that can lead to significant degeneration.

Our study further showed that in the early and mid-stages of IVD degeneration (Pfirrmann grade III–IV), timely and controllable immobilization-traction is conducive to the regeneration and recovery of the disc. In this

study, Sprague-Dawley rats were used to construct a model of disc degeneration, which was both economical and reliable but does not completely recapitulate the changes that occur in human disc degeneration. However, the rat model is the currently accepted model and was easier to establish and replicate than alternatives. More importantly, it provides relevant experimental parameters of the IVD structure before and after degenerative disc traction that human IVDs cannot provide. Therefore, we believe that the observed changes in rat IVDs will help further elucidate the etiology and pathogenesis of human intervertebral disc degeneration, and provide theoretical and experimental basis for traction inducing regeneration and delaying disc degeneration as well as clinical treatment and prevention of disc degeneration and related secondary diseases.

Conclusions

In this study, a mechanical degenerative model was successfully established by using a custom-made device. It has been demonstrated that disc degeneration is a cascade of biochemical, mechanical and structural changes mediated by cells in an abnormal mechanical environment. Not all levels of disc degeneration can be regenerated or repaired. Regeneration or recovery of disc degeneration requires specific conditions. Based on the immobilization-traction mode, the cascade cycle of disc degeneration is interrupted. In addition, the duration and extent of traction loading must be moderately controllable, and beyond the limits that can lead to significant degeneration. In summary, it is necessary to improve our understanding of the pathogenesis of clinical disc degeneration and discogenic lower back pain, as well as how to optimize the use of traction devices for possible treatment.

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