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Study of Nonlinear Viscoelastic Mechanical Properties of Degenerated Intervertebral Discs

Estudio de las Propiedades Mecánicas Viscoelásticas No Lineales de Discos Intervertebrales Degenerados

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SUMMARY: Biomechanical factors are important factors in inducing intervertebral disc degeneration, in this paper, the nonlinear viscoelastic mechanical properties of degenerated intervertebral discs were analyzed experimentally. Firstly, the loading and unloading curves of intervertebral discs before and after degeneration at different strain rates were compared to analyze the changes of their apparent viscoelastic mechanical properties; The internal stress/strain distribution of the disc before and after degeneration was then tested by combining digital image technology and fiber grating technology. The results show that the intervertebral disc is strain-rate-dependent whether before or after degeneration; The modulus of elasticity and peak stress of the degenerated disc are significantly reduced, with the modulus of elasticity dropping to 50 % of the normal value and the peak stress decreasing by about 55 %; Degeneration will not change the distribution of the overall internal displacement of the intervertebral disc, but has a greater impact on the superficial and middle AF; The stress in the center of the nucleus pulposus decreases, and the stress in the outer AF increases after degeneration. Degeneration has a great impact on the nonlinear viscoelastic mechanical properties of intervertebral disc, which has reference value for the mechanism, treatment and prevention of clinical degenerative diseases.

KEY WORDS: Degeneration; Nonlinear viscoelasticity; Strain-rate-dependent; Loading-unloading; Internal stress and strain.

INTRODUCTION

Low back pain (LBP) is a very common clinical disease, which is usually caused by intervertebral disc degeneration (Bonnaire *et al*., 2021). This disease has affected people's work and life for a long time. However, the mechanism of the occurrence and development of intervertebral disc degeneration is still unclear. As mentioned in previous studies, both mechanics and biology are considered to be important factors in inducing intervertebral disc degeneration (Xiao *et al*., 2020), and they are interrelated and amplify each other, promoting tissue progression toward pathologization and eventually leading to disc failure. Some studies believe that intervertebral disc degeneration is due to excessive mechanical load, while others believe a disorder of the behavior of the intervertebral disc cells. Studying the relationship between the mechanical properties of degenerated discs can help us further understand the mechanism of intervertebral disc degeneration.

The intervertebral disc has significant viscoelastic and rate-dependent properties, which are strongly related to its specific structure (Emanuel *et al*., 2018). The intervertebral disc is mainly composed of high moisture content gelatinous nucleus pulposus (NP) and annulus fibrosus (AF) (Li *et al*., 2020). Many previous studies have shown that the viscoelastic mechanism includes three-dimensional fluid flow through the nucleus pulposus, annulus fibrosus and endplates, as well as inherent solid-phase viscoelasticity (Schroeder *et al*., 2008). Existing studies have analyzed the loading response of intervertebral discs under different loads, while the unloading response was ignored. Since both loading and unloading are key behaviors of the circadian cycle in vivo, a better understanding of the viscoelastic unloading recovery response is crucial. It has been shown that degeneration has the least impact on the outer AF of the intervertebral disc, and the interlaminar and intralaminar

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arrangement and continuity of the fiber bundles may affect the regional behavior and viscoelasticity of the lamella (Pham *et al*., 2018). Other viscoelastic tissues, including cartilage, have been shown to change in mechanical response to different applied strain rates (Han *et al*., 2020), but few are known about the response of the intervertebral disc to different strain rates. In addition, a few studies have tested the viscoelastic properties of normal lumbar intervertebral discs, but the effect of degeneration has not been analyzed.

Intervertebral disc degeneration is a complex multi-factor process, which is caused by genetic, mechanical stress, cellular senescence, and nutritional changes caused by blood vessel supply (Zhao *et al*., 2019). How the mechanical properties of the intervertebral disc will change after degeneration has been a matter of concern. (Bashkuev *et al*., 2020). Based on the finite element model of human L4-L5 spinal segments, probability generated 1000 individual segments, covering all degrees of degradation, which are subjected to combined compression and buckling / extension loads. Experiments have shown that early mechanical changes related to intervertebral disc degeneration have the greatest impact on facet joint load. The pressure in the intervertebral disc is essential for maintaining the biomechanical behavior of the intervertebral disc. The pressure in the intervertebral disc stretches the annulus fibrosus and supports the endplate, which is the main determinant of the height of the intervertebral disc and the axial compression stiffness. At present, the effect of degeneration on the changes of macroscopic mechanical properties has been studied, but little is known about the stress and strain of the annulus fibrosus tissue level, and the mechanism of intervertebral disc stress distribution is still unclear.

The establishment of degenerative disc model is helpful to understand the mechanism of disc disease. Protease injection into intervertebral disc is also a relatively fast method to simulate intervertebral disc degeneration, trypsin can induce proteolytic digestion or remove glycosaminoglycan chains to cause intervertebral disc degeneration. The decomposition of proteoglycans can lead to the loss of intervertebral disc height and changes in biomechanical stability (Alini *et al*., 2008). Roberts *et al*. (2008) cultured bovine tail intervertebral disc in different protease solutions for 3 weeks and found by microscopic observation that various regions of intervertebral disc treated with papain and trypsin had different degrees of damage, especially in the nucleus pulposus. Therefore, this paper chooses the method of making animal degeneration model with trypsin solution, and then launches experimental research.

In this paper, the nonlinear viscoelastic properties of degenerative intervertebral disc will be evaluated by quasi-static experiment, and the internal displacement / stress distribution of intervertebral disc will be measured at the tissue level. The research work of this paper can help us further understand the mechanism of intervertebral disc degeneration.

MATERIAL AND METHOD

Sample preparation. Ten fresh oxtails (24-36 months old) slaughtered within 2-4 hours were obtained from local slaughterhouse. Six intervertebral disc segments were isolated, the cartilage endplates and upper and lower vertebral bodies were preserved, and excess muscle and soft tissue were removed. The samples were wrapped with gauze soaked in normal saline to maintain the cellular activity of the intervertebral disc. The experimental samples were divided into three groups to test the stress / strain curve and internal displacement / stress distribution of intervertebral disc at different strain rates before and after degeneration. Finally, one was used for histological studies to verify the degeneration grade. The basic information statistics of the intervertebral disc samples are shown in Table I.

Table I. Statistical table of basic information of intervertebral disc samples.

Segments	Height(mm)	Area(mm^2)
No.1	7.68	594
No.2	7.12	523
No.3	6.80	475
No.4	6.28	345
No.5	5.60	314
No.6	5.49	295

Experimental apparatus and testing. The experiment was conducted at room temperature (26 °C). The WDW-10 microcomputer-controlled electronic universal testing machine was used for the quasi-static loading test (Fig. 1). The strain rates were adjusted to 0.0005/s, 0.005/s, and 0.05/s for quasi-static vertical compression loading of the disc segments, and the samples were then unloaded at the same strain rate. After the experiment, appropriate amount of 0.9 % NaCl solution and 0.25 % trypsin solution were injected into different intervertebral disc segments, and placed in a 37 ºC incubator for 4 hours. Repeat the previous experimental operation when the degeneration model is completed.

The internal displacement was measured by optimized DIC technology (Liu *et al*., 2020), the intervertebral disc was cut along the sagittal plane, and the

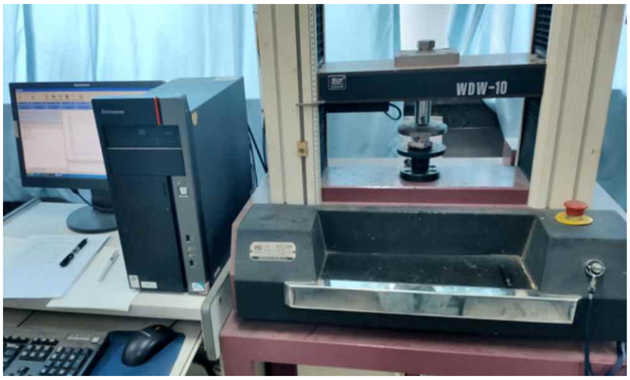

Fig. 1. The experimental equipment.

iron oxide nanoparticles were evenly coated on the incision surface as a marker point to record the deformation of different areas of the intervertebral disc. A pair of marked points a1 (x1, y1) and a2 (x2, y2) with similar x value were selected, and the axial displacement distribution will be reflected by comparing the y value between this pair of points before and after loading. A pair of marked points a3 (x3, y3) and a4 (x4, y4) with similar y value were selected, and the radial displacement distribution will be reflected by comparing the x value between this pair of points before and after loading.

The fiber Bragg grating (FBG) technology was used as the experimental method of internal stress measurement, the principle is that when the FBG is illuminated, it will reflect a single narrow spectral wavelength centered on the Bragg wavelength. FBG undergoes certain deformation when subjected to pressure, resulting in a shift of Bragg wavelength. By calculating the wavelength shift, the magnitude of the internal stress can be obtained. The optical fiber coated with a protective layer was passed through the intervertebral disc at different locations with a syringe needle respectively, so that the sensor partially rested inside the disc and was compressively loaded with a digital push-pull gauge pressure tester (Fig. 2), while the FBG wavelength shift was recorded with a spectral analyzer. Figure 2 shows the different locations of the discs where the fiber was inserted (¨outer AF ≠ inner AF Æ nucleus pulposus).

Statistical analysis. A one-way analysis of variance (ANOVA) with repeated measures was performed to detect the differences among the experimentally measured values of the strain as well as the displacement in the compression tests. Statistical significance was accepted for $P<0.05$. Data points in the figures represent mean values, whereas error bars indicate the standard errors above and below corresponding mean values.

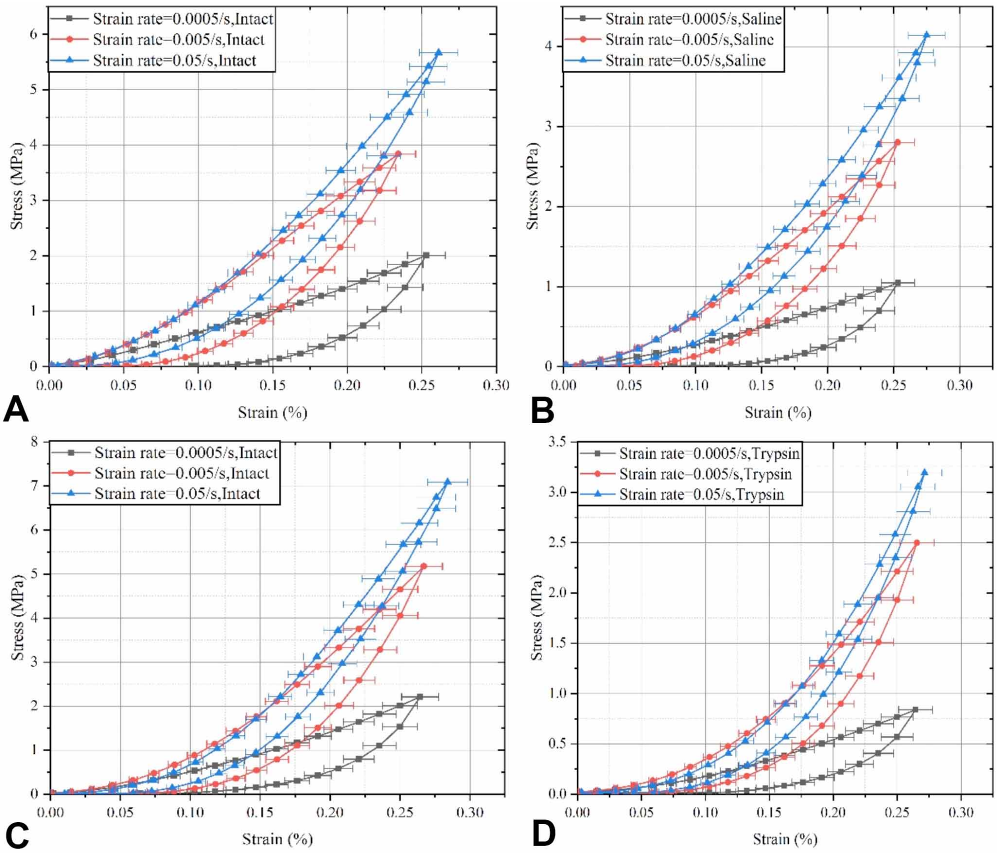

Fig. 2. The digital push pull force tester and the different locations of the discs where the fiber was inserted.

RESULTS

Quasi-static experiments. The study of the loading-unloading curves of the intervertebral disc under compressive loading can help us to further determine its structural properties. Figure 3 shows the loading-unloading curves of intervertebral disc segments before and after degeneration at different strain rates. The results show that the intervertebral discs exhibit nonlinear stress–strain characteristics at different loading rates ($P < 0.05$). The stiffness changed slowly at lower strain rates and increased when the strain rate increased.

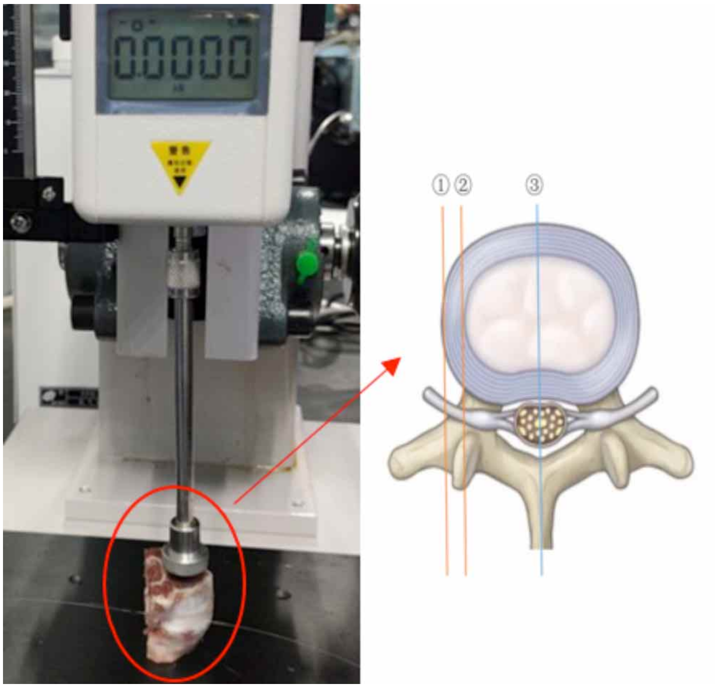

Fig. 3. Loading–unloading curves of the intervertebral disc at different strain rates. (a) Intact intervertebral disc; (b) Saline-injected intervertebral disc. (c) Intact intervertebral disc; (d) Trypsin-injected intervertebral disc.

The modulus of elasticity of the linear section and the variation of the peak stress were calculated based on the stress-strain curve shown in Figure 3, and as shown in Table II. The results show that before and after the disc degeneration, the elastic modulus increases with the increase of the strain rate, which is significantly dependent on the strain rate. The modulus of elasticity of the degenerated disc is reduced to 50 % of the normal value and the peak stress is also reduced by about 55 %.

Distribution of axial displacement inside the intervertebral disc. Figure 4 shows the internal axial displacement distribution of intervertebral disc before and after degeneration under compressive load ($P < 0.05$). The results showed that the overall displacement distribution of the intervertebral disc did not change after the degeneration. Overall, the displacement of superficial AF is the largest and that of deep AF is the smallest. The overall internal displacement of the disc increases after degeneration, especially in the superficial and middle AF.

Distribution of radial displacement inside the intervertebral disc. Figure 5 shows the internal radial displacement distribution of intervertebral disc before and after degeneration under compressive load ($P < 0.05$). The results showed that the overall displacement distribution inside the degenerated intervertebral disc is basically consistent with that in the normal disc. Overall, the displacement of the inner AF is the largest and the displacement of the outer AF is the smallest. The displacement of middle AF increased significantly after intervertebral disc degeneration.

Table II. Mechanical properties of saline-injected intervertebral discs and trypsin-injected intervertebral disc.

Segments	Processing method	Strain rate	Elastic modulus (MPa)	Change ratio of peak stress
No.1	Intact	0.0005	7.947	
		0.005	19.310	
		0.05	27.062	
No.1	Saline	0.0005	8.805	-48 %
		0.005	20.006	-27 %
		0.05	28.670	-26.9 %
No.2	Intact	0.0005	9.654	
		0.005	28.487	
		0.05	40.758	
No.2	Trypsin	0.0005	4.048	-62.1 %
		0.005	14.840	-51.7 %
		0.05	22.104	-54.9 %

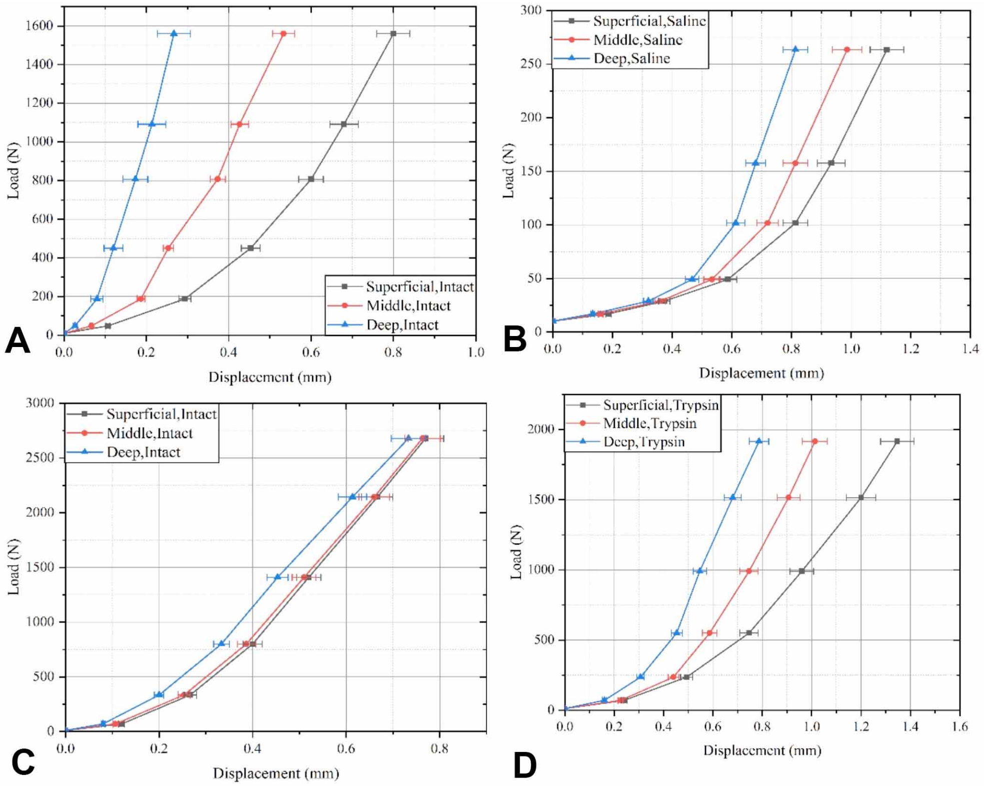

Fig. 4 Axial displacement distribution of the disc. (a) Intact intervertebral disc; (b) Saline-injected intervertebral disc; (c)Intact intervertebral disc;(d) Trypsin-injected intervertebral disc.

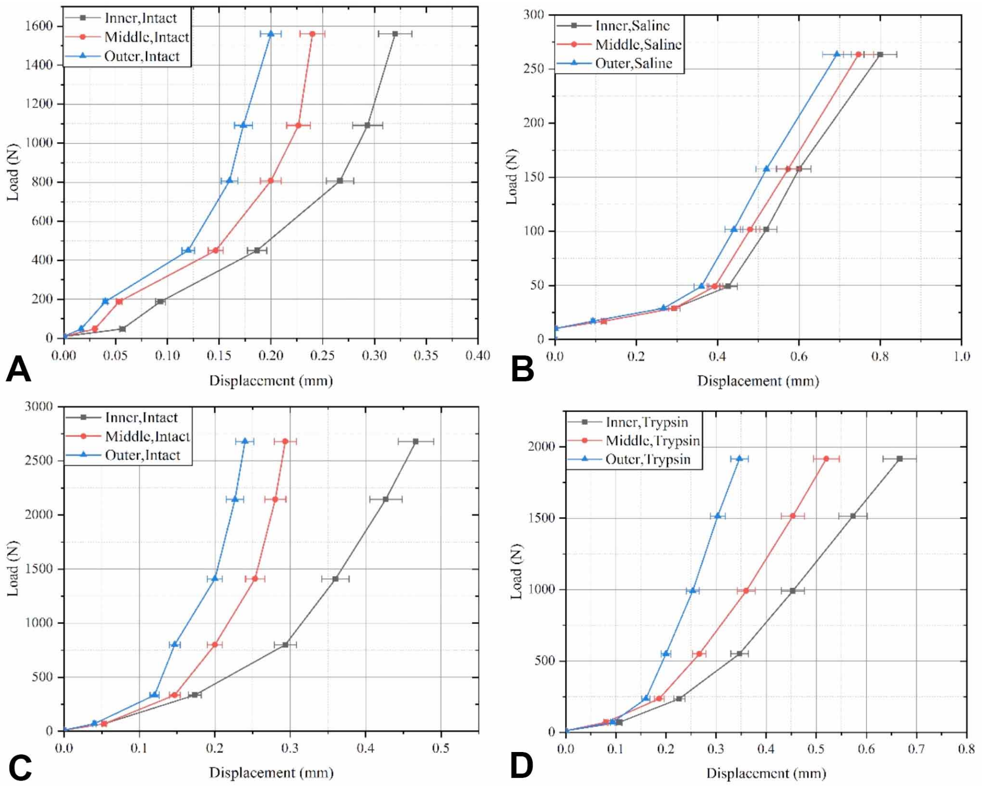

Fig. 5 Radial displacement distribution of the disc. (a) Intact intervertebral disc; (b) Saline-injected intervertebral disc; (c) Intact intervertebral disc; (d) Trypsin-injected intervertebral disc.

The internal stress distribution of the intervertebral disc. Figure 6 shows the internal stress distribution of the disc before and after degeneration ($P<0.05$). The results show that all parts of the intervertebral disc under vertical compression are under pressure, but the distribution is uneven. Overall, whether before or after degeneration, the compressive stress in the center of nucleus pulposus is the largest and the compressive stress in the outer AF is the smallest. After intervertebral disc degeneration, the central stress of nucleus pulposus decreased, the stress of outer AF increased, and the stress of inner AF basically did not change.

Morphological changes of the intervertebral disc. Figure 7 shows the staining of tissue sections of intervertebral discs under different treatment methods. It can be seen that the tissue morphology of intervertebral discs treated with trypsin has changed significantly.

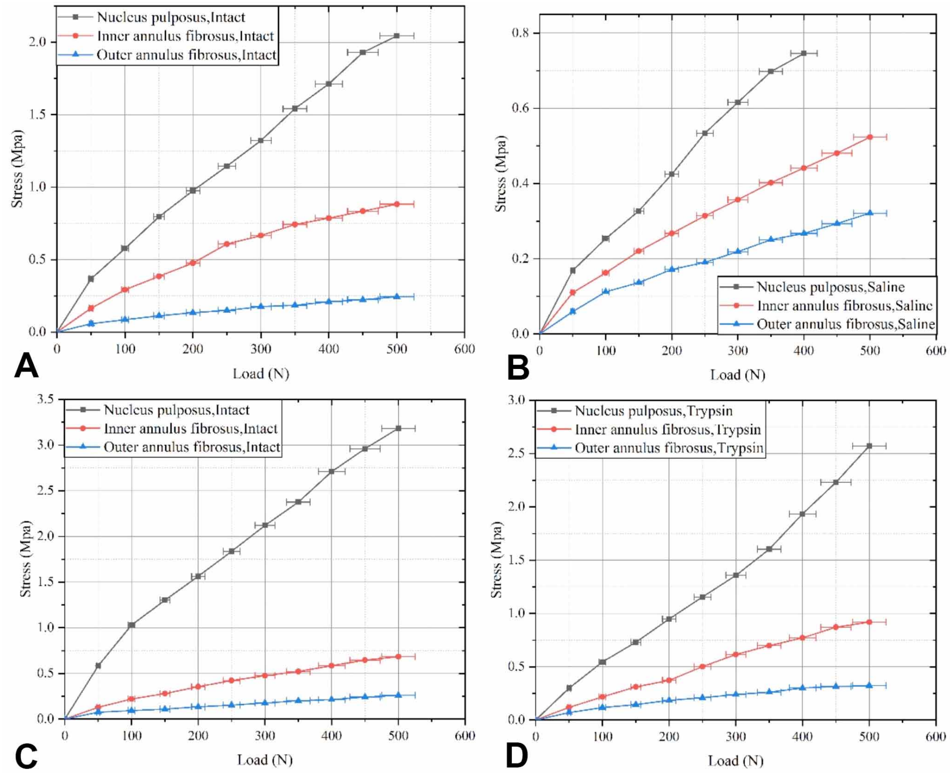

Fig. 6. The internal stress distribution of the disc. (a) Intact intervertebral disc; (b) Saline-injected intervertebral disc; (c) Intact intervertebral disc; (d) Trypsin-injected intervertebral disc.

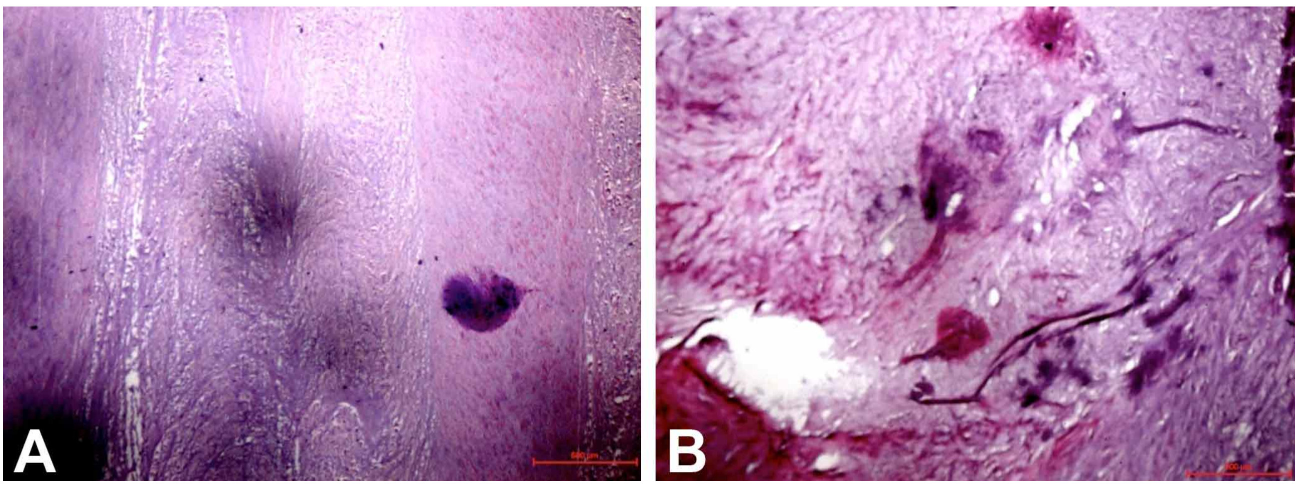

Fig. 7. Staining of tissue sections of intervertebral discs under different injection methods. (a) Saline-injected intervertebral disc; (b) Trypsin-injected intervertebral disc.

DISCUSSION

The purpose of this study is to experimentally study the nonlinear viscoelastic mechanical properties of degenerated intervertebral discs. The internal displacement was observed and analyzed by optimized digital image correlation techniques. At the same time, with the help of fiber grating technology, the changes in the internal stress of the intervertebral disc were measured, and the observed phenomena were analyzed and predicted.

In this study, we experimentally tested the mechanical properties of the intervertebral disc before and after degeneration at different strain rates, and found that there was strain-rate-dependence, which is a manifestation of viscoelasticity. Tavakoli & Costi (2018) made slices of sheep lumbar vertebrae, and measured the viscoelasticity and failure characteristics of the interlaminar matrix (ILM) in both tensile and shear directions for the first time. They found that during the dynamic loading process, the strain rate-dependent response of ILM had higher capacity absorption capacity at low strain rates in the two loading directions, which confirmed the viscoelasticity of the intervertebral disc. It was found that the stiffness changed slowly at low strain rates, and the stiffness and Young's modulus increased gradually with increasing strain rates, which is largely due to the pore elasticity of the intervertebral disc (DiSilvestro *et al*., 2001). When the intervertebral disc is degenerated, the elastic modulus is significantly reduced (Campana *et al*., 2011) used magnetic resonance imaging technology to evaluate the relationship between the viscoelasticity of lumbar intervertebral discs and the degree of degeneration. They classified the elastic modulus and viscosity according to the degree of degeneration and found that for mildly degenerated intervertebral discs, the elastic modulus varies from 9MPa to 19.7Mpa, which is in agreement with our experimental results.

The overall displacement distribution inside the degenerated intervertebral disc is basically consistent with that in the normal disc. Overall, the axial displacement of superficial AF is greater than that of deep AF. This is consistent with the findings of Liu *et al*. (2020), who studied the effect of fatigue loading on the internal displacement of the disc by applying the optimized digital image correlation technique and found that the axial displacement of the superficial AF was the greatest before fatigue loading. Meakin *et al*. (2000) measured the displacement of the internal marker points of the nucleus pulposus discectomy with the interactive image analysis software, and concluded that when the nucleus pulposus discectomy is subjected to compressive load, the outer edge of the fibrous ring deforms outward, which is consistent with the phenomenon we observed. When the disc is severely degenerated, the nucleus pulposus loses its original mechanical properties. We also found that the internal axial displacement and radial displacement of the disc increased after degeneration. Tsantrizos *et al*. (2005) found a similar law when evaluating the degeneration relationship function between nucleus pulposus migration and intradiscal strain: Degeneration significantly increased the radial tensile and compressive strain of the disc during all loading periods. Some scholars also believe that the reason the degenerated intervertebral disc exhibits higher axial compressive load and radial tensile strain, may be due to the fact that decompressed NP apply more applied loads directly to the AF (O'Connell *et al*., 2011).

We found that the compressive stress in the center of the nucleus pulposus was greater than that in the inner and outer AF both before and after disc degeneration, which may be strongly related to the structure and mechanical function of the nucleus pulposus. Newell *et al*. (2019) investigated the high-velocity compression response of the disc before and after nucleus pulposus decompression, and found that nucleus pulposus pressure did not affect the load transfer as well as energy absorption under impact loading. After the degeneration of the intervertebral disc, the water in the nucleus pulposus decreases and the internal pressure decreases, which significantly affects the force of the annulus fibrosus. Yang & O'Connell (2019) evaluated the development of residual stress after the entire intervertebral disc degeneration and found that moderate to severe degeneration reduced the swelling capacity of the NP by 40 %, the swelling of the AF and cartilage endplates by 25 %, and that these changes in tissue swelling led to a reduction in NP pressure, which is consistent with the results we observed. After disc degeneration, there is a significant change in residual stress and fiber stretch in AF, with radial forces converting from compression to tension, which may increase the risk of AF tearing or delamination. In this study, we observed an increase in the stress of the outer fibrous ring of the degenerated intervertebral disc, which is in good agreement with the results of Park *et al*. (2013). They established a degenerative L4-L5 lumbar finite element model and studied the changes of intradiscal pressure. It was found that the intradiscal pressure of adjacent anterior cruciate ligament increased by 31 % and 8 % respectively with the progress of flexion and scoliosis degeneration.

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RESUMEN: Los factores biomecánicos son importantes en la inducción de la degeneración del disco intervertebral. En este estudio se analizaron experimentalmente las propiedades mecánicas viscoelásticas no lineales de los discos intervertebrales degenerados. En primer lugar se compararon las curvas de carga y descarga de los discos intervertebrales, antes y después de la degeneración, a diferentes velocidades de deformación para analizar los cambios aparentes de sus propiedades mecánicas viscoelásticas. La distribución interna de tensión/deformación del disco antes y después de la degeneración se probó luego combinando tecnología de imagen digital y tecnología de rejilla de fibra. Los resultados mostraron que el disco intervertebral depende de la velocidad de deformación antes o después de la degeneración; El módulo de elasticidad y la tensión máxima del disco degenerado se reducen significativamente, cayendo el módulo de elasticidad al 50 % del valor normal y la tensión máxima disminuyendo en aproximadamente un 55 %; La degeneración no cambiará la distribución del desplazamiento interno general del disco intervertebral, pero tiene un mayor impacto en la FA superficial y media; El estrés en el centro del núcleo pulposo disminuye y el estrés en el FA externo aumenta después de la degeneración. La degeneración tiene un gran impacto en las propiedades mecánicas viscoelásticas no lineales del disco intervertebral, que tiene valor de referencia para el mecanismo, tratamiento y prevención de enfermedades clínicas degenerativas.

PALABRAS CLAVE: Degeneración; Viscoelasticidad no lineal; Dependencia de la velocidad de deformación; Carga descarga; Esfuerzo y estrés interno.

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