

Effects of Degeneration on the Biphasic Material Properties of Human Nucleus Pulposus in Confined Compression

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Study Design. The biphasic compressive material properties of normal and degenerate human nucleus pulposus tissue were measured in confined compression.

Objectives. The objective of this study was to determine the effects of degeneration and age on the mechanical properties of human nucleus pulposus.

Summary of Background Data. The nucleus pulposus exhibits swelling behavior in proportion to proteoglycan content. In shear, the nucleus exhibits both fluid-like and solid-like properties, suggesting a biphasic nature. To date, biphasic compressive properties of human nucleus pulposus have not been reported.

Methods. Human nucleus pulposus samples were tested in confined compression. Isometric swelling stress and effective aggregate modulus were measured. Linear biphasic theory was used to determine the permeability of the tissue. Mechanical behavior was correlated with proteoglycan and water content.

Results. Degeneration produced significant decreases in swelling stress ($P_{sw} = 0.138 \pm 0.029$ MPa nondegenerate, $P_{sw} = 0.037 \pm 0.038$ MPa degenerate) and effective aggregate modulus ($H_A^{eff} = 1.01 \pm 0.43$ MPa nondegenerate, $H_A^{eff} = 0.44 \pm 0.19$ MPa degenerate). Both properties were inversely correlated with proteoglycan content. Permeability increased with degeneration ($k_a = 0.9 \pm 0.43 \times 10^{-15}$ m⁴/N-s nondegenerate, $k_a = 1.4 \pm 0.58 \times 10^{-15}$ m⁴/N-s degenerate).

Conclusions. Swelling is the primary load-bearing mechanism in both nondegenerate and degenerate nucleus pulposus. Knowledge of the biphasic material properties of the nucleus pulposus will aid the development of new treatment strategies for disc degeneration aimed at restoring mechanical function of the intervertebral disc.

Key words: intervertebral disc, biomechanics, nucleus pulposus, confined compression, proteoglycan, stress relaxation, swelling, pressure. **Spine 2005;30:E724–E729**

Intervertebral disc degeneration consists of a complex interaction of biologic, chemical, and mechanical changes within the disc. A loss of aggrecan, the primary proteoglycan of the nucleus pulposus, is one of the earliest known degenerative changes that occur.^{1,2} This loss of proteogly-

can significantly decreases the swelling pressure of the nucleus pulposus, leading to altered disc mechanics.^{3,4} New strategies for treatment of intervertebral disc degeneration, including tissue engineered nucleus pulposus constructs^{5–8} and artificial disc replacements,^{9,10} aim to restore mechanical function to the disc. Thus, knowledge of the material properties of nondegenerate and degenerate human nucleus pulposus is integral to the success of these treatments.

Composition and mechanical function of the nucleus pulposus are closely related. The nucleus pulposus is composed of a highly hydrated, disorganized matrix of collagen and proteoglycan. Fixed charge density associated with proteoglycan in the nucleus is responsible for the generation of an osmotic gradient, which drives water into the nucleus pulposus. This swelling behavior has been previously investigated and is linearly correlated with proteoglycan content.^{3,4} However, there are relatively little data available concerning the load-deformation behavior of the nucleus pulposus. In shear, the nucleus exhibits the properties of both solid and fluid material phases,^{11–13} suggesting that the nucleus may be best characterized as a biphasic material. Measurement of the compressive modulus in the nucleus pulposus has been difficult to achieve, however, because of the complexities involved in handling and testing this highly hydrophilic tissue. While there are limited data available describing the compressive behavior of nucleus pulposus,^{14,14a} the compressive behavior of the annulus fibrosus has been previously investigated in confined compression,^{15–20} and these experimental techniques are well established.

The biphasic compressive properties of the nucleus are critical to the development of new finite-element models of the disc. Previously, the nucleus pulposus has been described as an incompressible fluid^{21–23}; however, more recent models describe the nucleus as a poroelastic material.^{24–26} As there have been no direct studies of nucleus pulposus permeability, these models have relied on assumptions of permeability from other cartilaginous tissues such as annulus fibrosus and cartilage. The structure and composition of these tissues differ significantly from that of the nucleus pulposus. Thus, there is a need for direct experimental measures of nucleus pulposus modulus and permeability.

The objective of the present study was to directly measure biphasic material properties of nondegenerate and degenerate human nucleus pulposus in a confined compression experiment. It was hypothesized that 1) the isometric swelling stress of degenerate nucleus tissue would be lower than nondegenerate tissue due to decreased pro-

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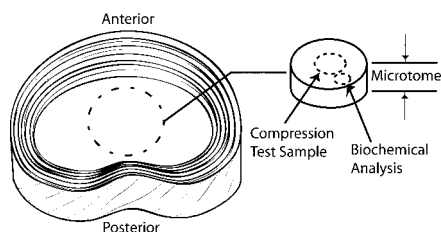


Figure 1. Schematic representation of the locations from which nucleus pulposus test samples were removed for mechanical and biochemical testing.

teoglycan content, 2) the compressive stiffness (effective aggregate modulus, H_A^{eff}) would increase, and 3) permeability would increase. Biochemical content was measured in order to perform correlations between tissue composition and mechanical function.

Materials and Methods

Ten human lumbar spines (age range, 19–80 years; mean, 57.5 years) were obtained from IRB approved tissue sources (National Disease Research Interchange, Philadelphia, PA and International Institute for the Advancement of Medicine, Jessup, PA). Two lumbar intervertebral discs were removed from each spine *via* sharp dissection at the superior and inferior endplates. Using a modified Thompson scale,²⁷ discs were assigned a degenerative grade (1–5 scale; Grade 1, nondegenerate; to Grade 5, degenerate) based on observations of gross morphology made by three independent orthopedic surgeons. The center of the nucleus pulposus was removed (Figures 1, 2) and placed onto the freezing stage (Model BFS-30; Physitemp, Clifton, NJ) of a sledge microtome (Model SM2400; Leica, Nussloch, Germany). Samples were sectioned to uniform thickness and thickness measurements were taken at four distinct locations across the sample using a micro laser sensor (LM10 Laser, Aromat, New Providence, NJ). Average thickness was 2.40 ± 0.18 mm ($n = 19$). Samples were frozen at -80 C until testing.

A circular punch was used to prepare 4.37-mm-diameter cylindrical test samples from the sectioned nucleus pulposus tissue. Test samples were placed into the confining chamber (Figure 3) of a custom-built load and displacement-controlled compression testing device.²⁸ The device consisted of a linear stepper motor (Model 18512; Spectrums Physics Oriel, Stratford, CT) connected to an LVDT in order to record displacement data (Model PR812-200, Macro Sensors, Pennsauken, NJ). Load reaction force was continuously measured using a uniaxial load cell (Model 31; Honeywell Sensotec, Columbus, OH).

A porous platen (50% porosity, 45–53 μm pore size) was lowered at 10 $\mu\text{m}/\text{sec}$ until a contact load of 0.045 N was reached. The chamber was then filled with room temperature 0.15 mol/L phosphate-buffered saline, and the sample was held in this position for 5 minutes. Compressive load was observed

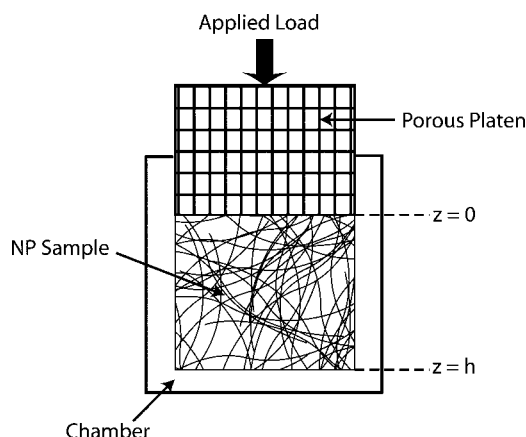


Figure 3. Schematic of confined compression chamber.

to increase during this time, indicating that the sample had expanded slightly to fit against the walls of the confining chamber and platen. An isometric swelling test was performed by applying a small 1% compressive strain followed by a 3-hour hold period. Following the swelling experiment, a 5% compressive strain increment (ramp rate, 0.25 $\mu\text{m}/\text{sec}$) was applied and relaxation was then recorded for 2 hours. In pilot studies, the isometric swelling phase was found to reach greater than 95% of equilibrium swelling pressure within 3 hours. Similarly, in pilot stress-relaxation tests, samples were found to reach greater than 95% of equilibrium relaxation within 2 hours.

Isometric swelling pressure, P_{sw} , was measured as the final equilibrium stress reached at the end of the 3-hour swelling experiment. Data from the stress relaxation experiment were analyzed using the linearly elastic, isotropic biphasic theory.²⁹ Effective aggregate modulus (H_A^{eff}) was calculated directly from the relaxation data, and relaxation curves were fit to a forward finite-difference approximation of the biphasic theory modified from Soltz and Ateshian³⁰ in order to determine the apparent permeability (k_a) of the tissue.

In comparing the biphasic and triphasic theory, Ateshian *et al* define the biphasic aggregate modulus, H_A^{eff} , as an “effective” modulus that is the sum of the solid confined modulus, H_A , and a term Π , the negative of the rate of change of osmotic pressure with dilatation: $H_A^{\text{eff}} = H_A + \Pi$.³¹ Under small strain conditions, shear modulus, μ , is not influenced by Π and can therefore be measured directly. For an isotropic material, the solid matrix aggregate modulus, H_A , is related to shear modulus according to: $H_A = 2\mu\nu/(1 - 2\nu) + 2\mu$ in which μ is the shear modulus and ν the Poisson’s ratio. The confined modulus, H_A , was computed using shear modulus data from the literature (nondegenerate shear modulus $\mu = 5.8$ kPa, degenerate shear modulus $\mu = 22.5$ kPa),¹² assuming a Poisson’s ratio of 0.2. Poisson’s ratio has not been measured in nucleus pulposus; therefore, this value was selected based on articular

Figure 2. Preparation of human nucleus pulposus tissue for confined compression experiment. A cylindrical plug was removed from the center of the nucleus pulposus and microtomed to uniform thickness.



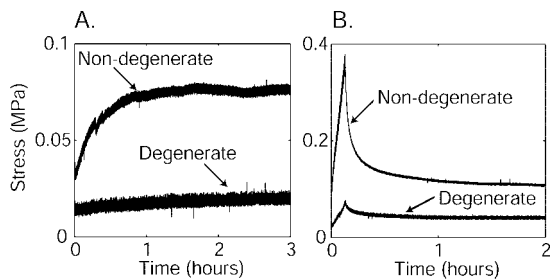


Figure 4. Representative isometric swelling (left) and stress-relaxation (right) results for nondegenerate and degenerate human nucleus pulposus. Nondegenerate tissue exhibited higher swelling, peak, and equilibrium stress than degenerate tissue.

cartilage data.³² The contribution of Π as a percentage of the effective aggregate modulus, H_A^{eff} , was computed.

Adjacent nucleus pulposus tissue (Figure 1) was used to determine water content and sulfated-glycosaminoglycan (s-GAG) content as follows: After recording wet weight, tissue samples were incubated at 65 C for 48 hours to obtain dry pellets. Sample wet and dry weights were measured in triplicate. Percentage water content was determined as $\%H_2O = (\text{wet weight} - \text{dry weight})/\text{wet weight}$. Dried samples were digested with proteinase K in order to solubilize s-GAG. A 50- μL aliquot was used to determine s-GAG content using 1,9-dimethylmethylene blue in a microplate reader assay.³³

A total of 19 samples, from 10 human lumbar spines, were mechanically tested and analyzed for biochemical composition (one disc was too severely degenerate to prepare a test sample). For statistical analysis, samples were separated into nondegenerate (grade <2.5 , $n = 8$) and degenerate (grade >2.5 , $n = 11$) groups based on their gross morphologic degenerative grade. Student's t test was performed on the mechanical and biochemical parameters to test for the effect of degeneration. A correlation matrix was created using linear regression analysis to assess the influence of biochemical composition (water, s-GAG) and degeneration (age, degenerative grade) on mechanical behavior (P_{sw} , H_A^{eff} , and k_a). All statistical analyses were performed using SYSTAT software (Systat Software Inc., Point Richmond, CA) with significance set at $P \leq 0.05$.

Results

All specimens exhibited increasing swelling stress (P_{sw}) during the isometric swelling experiment, although nondegenerate tissue exhibited far greater swelling than degenerate tissue (Figure 4A). Swelling pressure and effective aggregate modulus were significantly lower in degenerate tissue (nondegenerate $P_{\text{sw}} = 0.138 \pm 0.029$ MPa, degenerate $P_{\text{sw}} = 0.037 \pm 0.038$ MPa; nondegenerate $H_A^{\text{eff}} = 1.01 \pm .43$ MPa, degenerate $H_A^{\text{eff}} = 0.44 \pm 0.19$ MPa) (Table 1). The solid aggregate modulus, esti-

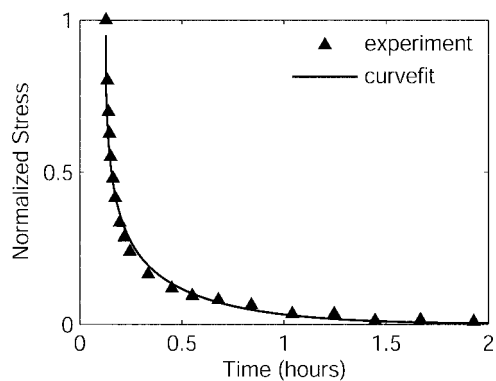


Figure 5. Biphasic relaxation curve fit (dashed) for a representative nondegenerate sample.

mated from shear data in the literature, increased with degeneration (nondegenerate $H_A = 0.016$ MPa, degenerate $H_A = 0.027$ MPa). The effective aggregate modulus was dominated by the term Π in both nondegenerate and degenerate tissue (nondegenerate 98%, degenerate 86%).

The stress-relaxation response was characterized by a large peak stress that was significantly greater in nondegenerate tissue (nondegenerate peak stress = 0.70 ± 0.15 MPa, degenerate peak stress = 0.26 ± 0.22 MPa) (Figure 4B). Relaxation data were fit well with the linear biphasic solution; average coefficient of determination for curve fitting was $R^2 = 0.95$. A representative dataset and corresponding curve-fit are shown (Figure 5). Permeability was found to increase significantly with degeneration (nondegenerate $k_a = 0.9 \pm 0.43 \times 10^{-15}$ $\text{m}^4/\text{N}\cdot\text{s}$, degenerate $k_a = 1.4 \pm 0.58 \times 10^{-15}$ $\text{m}^4/\text{N}\cdot\text{s}$).

s-GAG content significantly decreased with degeneration (nondegenerate s-GAG 439.6 ± 85.0 $\mu\text{g}/\text{mg}$ dry weight; degenerate s-GAG 148.3 ± 111.7 $\mu\text{g}/\text{mg}$ dry weight). Water content did not change significantly with degeneration, although there was a decreasing trend (Table 1).

Correlations between mechanical properties, biochemical composition, and degenerative grade revealed that the best predictor of mechanical function was age, followed by s-GAG content and degenerative grade (Figure 6). Swelling pressure and effective aggregate modulus were negatively correlated with age and degenerative grade (P_{sw} and age $R = -0.82$, P_{sw} and Grade $R = -0.67$; H_A^{eff} and age $R = -0.55$ and H_A^{eff} and Grade $R = -0.48$), and positively correlated with s-GAG content (P_{sw} and s-GAG $R = 0.65$, H_A^{eff} and s-GAG $R = 0.51$). Water content was not significantly correlated with any of

Table 1. Summary of Mechanical and Biochemical Data

	P_{sw} (MPa)	H_A^{eff} (MPa)	k_a ($\times 10^{-15}$ $\text{m}^4/\text{N}\cdot\text{s}$)	Water Content (%)	s-GAG/Dry Weight ($\mu\text{g}/\text{mg}$)
Nondegenerate ($n = 8$)	0.138* (0.029)	1.01* (0.43)	0.900* (0.425)	79.9† (4.7)	439.6* (85.0)
Degenerate ($n = 11$)	0.037* (0.038)	0.44* (0.19)	1.447* (0.581)	74.9† (5.9)	148.3* (111.7)
Overall	0.079 (0.061)	0.68 (0.42)	1.216 (0.58)	77.0 (5.8)	270.9 (177.8)

Data are mean (SD).

Differences between nondegenerate and degenerate groups: * $P < 0.05$ (significance); † $P < 0.1$ (trend).

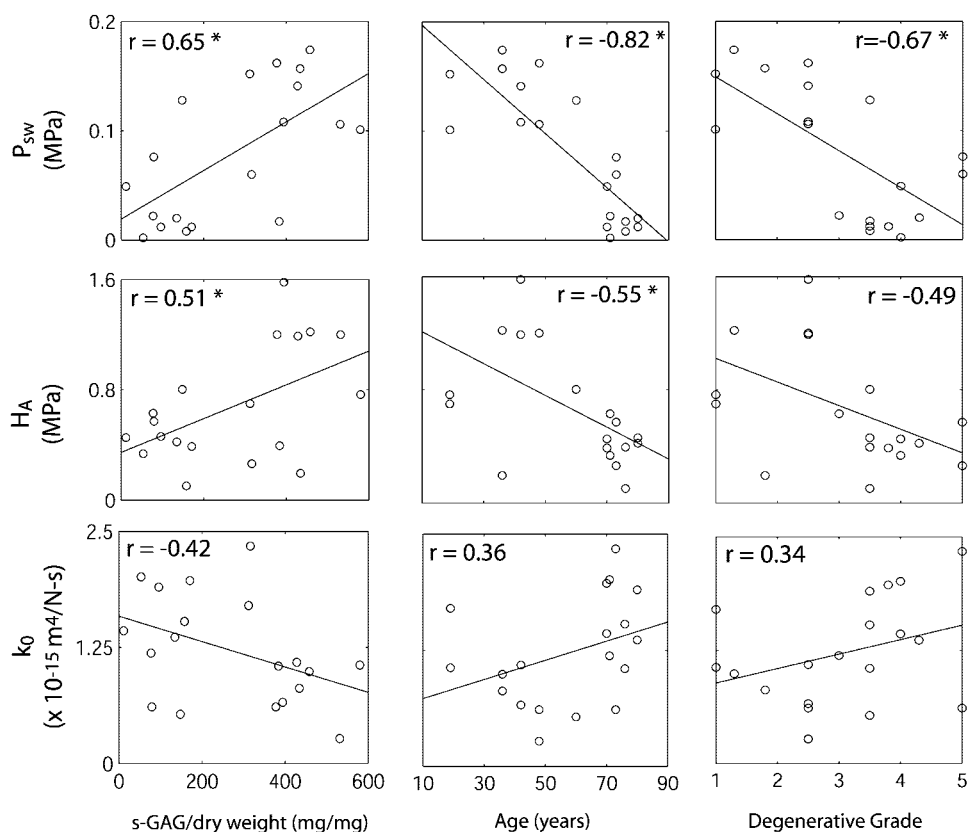


Figure 6. Correlation between biphasic material parameters and s-GAG content, age, and degenerative grade. The strongest predictor of mechanical function was age, followed by s-GAG content and degenerative grade.

the measured mechanical parameters, although there was a negative correlation between water content and morphologic grade ($R = -0.69$) and a weak correlation between water content and age ($R = -0.50$). The s-GAG content showed a strongly negative correlation with both age ($R = -0.75$) and degenerative grade ($R = -0.69$).

Discussion

Confined compression experiments of nondegenerate and degenerate human nucleus pulposus were performed to determine the effects of degeneration on the biphasic compressive properties of the nucleus pulposus. The experiment consisted of an isometric swelling test followed by a stress-relaxation experiment, from which isometric swelling pressure, effective aggregate modulus, and hydraulic permeability of the tissue were determined. Swelling pressure and effective aggregate modulus were found to decrease with degeneration. In contrast, permeability increased with degeneration. Swelling pressure and modulus were positively correlated with proteoglycan content.

The swelling stress of hydrated soft tissues, such as intervertebral disc and cartilage, is due to close packing of negatively charged proteoglycans and the osmotic pressure resulting from their associated fixed charge density. The isometrically determined swelling stress (nondegenerate $P_{sw} = 0.138 \pm 0.029$ MPa) compares favorably with values reported from osmotic free-swelling studies (0.13–0.22 MPa)^{3,4} and *in vivo* intradiscal pressure measurements (0.1 MPa, prone position).^{34,35} As expected, the swelling stress of degenerate tissue was sig-

nificantly lower than that of nondegenerate tissue. As proteoglycan content decreases with age and degeneration, the total fixed charge density of the tissue decreases, resulting in a loss of swelling pressure. In support of this mechanism, we found a linear correlation between s-GAG concentration and isometric swelling behavior, consistent with findings reported by Urban and McMullin.^{3,4}

We found the effective aggregate modulus, H_A^{eff} , of nondegenerate nucleus pulposus (1.01 MPa) to be higher than values reported in nondegenerate anulus fibrosus (0.38–0.56 MPa).¹⁶ H_A^{eff} decreased with degeneration. In contrast, the shear modulus of degenerate nucleus tissue is significantly higher than that of nondegenerate tissue.¹⁵ Thus, the observed decrease in H_A^{eff} was somewhat unexpected. This difference may be attributed to the swelling stress of the tissue. We found that Π accounts for approximately 98.5% of H_A^{eff} in nondegenerate tissue and 86% in degenerate tissue. Thus, even in degenerate nucleus pulposus, functional behavior is dominated by the effects of swelling.

The hydraulic permeability of human nucleus pulposus has not been previously reported, although some have made use of permeability estimates from cartilage and anulus fibrosus studies in finite-element models.^{25,26} The experimental value determined here (nondegenerate $k_a = 0.9 \pm 0.43 \times 10^{-15} \text{ m}^4/\text{N}\cdot\text{s}$) is somewhat higher than estimates in the literature ($0.3 \times 10^{-15} \text{ m}^4/\text{N}\cdot\text{s}$).²⁶ We found a significant increase in permeability with degeneration, most likely due to the loss of proteoglycans which bind

water. However, there was not a significant correlation between proteoglycan content and permeability.

While this study is the first to quantify the biphasic material properties of human nucleus pulposus, it is not without limitations. Although the nucleus is confined by the superior and inferior endplates and surrounded by the annulus fibrosus *in situ*, this loading condition is neither fully confined nor fully unconfined. However, the confined compression test used here is a standard well-defined technique to measure material properties of soft hydrated tissues. The values for permeability and aggregate modulus reported here are for a linear, isotropic material and are determined from a single strain level; however, permeability may exhibit strain dependence due to compaction of the solid matrix as the tissue is compressed.³⁶ Strain dependence in the annulus fibrosus has been described using a nonlinear permeability coefficient.¹⁵ In this study, we selected a slow ramp rate and small strain magnitudes in order to limit local tissue strains such that the assumptions of infinitesimal strain theory remain valid. Our calculations for Π and the solid aggregate modulus, H_A , are dependent on our assumption of Poisson's ratio, which has not been measured. However, our conclusion that the effective aggregate modulus, H_A^{eff} , is dominated by Π holds for values of Poisson's ratio within the range of 0.1 to 0.4 (nondegenerate $\Pi = 96\%$ – 98% , degenerate $\Pi = 70\%$ – 88%).

■ Conclusion

The findings of this study demonstrate that swelling remains the primary load-bearing mechanism in the nucleus pulposus, even with degeneration. Biochemical analysis confirms that nucleus pulposus function is correlated to proteoglycan content of the tissue. The confined compression biphasic material properties reported here are essential to the development of new functional tissue engineered nucleus pulposus and intervertebral disc constructs and finite-element modeling studies.

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■ Key Points

- The mechanical function of nondegenerate human nucleus pulposus is dominated by the large swelling stress of the tissue. Swelling remains the dominant load support mechanism in degenerate tissue.
- Swelling stress and effective aggregate modulus decrease significantly with degeneration; both are positively correlated with proteoglycan content. Permeability increases with degeneration.

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