Intervertebral Disc Dynamics and Degeneration

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ABSTRACT
Degenerative disc disease is a serious health problem worldwide, whose etiological basis—mechanical stimulus, biochemical changes, or natural aging—is poorly understood. While the disc’s known function is that of dynamic shock absorber, its dynamic response has not been fully characterized throughout the disc health spectrum (from healthy to degenerate). Therefore, this study aimed to correlate the radiographic ‘clinical’ appearance of disc degeneration with its in vitro dynamic response. Post mortem macaque tissues were utilized in a correlation study of the lumbar spine. Fifty-four functional spinal units (T10-11, T12-L1, and L6-7) from eighteen animals were excited with cyclic loading (5 to 80-Hz) and a dynamic impulse. From these, the natural frequency, stiffness, and energy absorption of each disc was correlated with its level of degeneration. No differences in mechanical properties were found between the spinal levels blocking by degeneration grade. The natural frequency of the intervertebral disc increased with degeneration grade from 51 Hz to 70 Hz. Intervertebral disc stiffness exhibited an increase between the healthy and severely degenerated tissues while the energy absorption decreased over the health spectrum. Degeneration was found to significantly affect disc dynamics which may aid in identifying the etiology of this disease.

INTRODUCTION
Back pain is the most common cause of activity limitation in individuals younger than 45 years and is the second leading cause of disability in the United States [1]. Chronic low back pain is the most prevalent musculoskeletal impairment in the U.S. afflicting between 15% and 56% of the population [1-4]. In the U.S., it is estimated that health care costs associated with low back pain alone will exceed $80 billion annually [4].

Twenty-three intervertebral discs separate the vertebrae of the human spinal column and make up approximately 20-to-30% of spinal height [5]. These intervertebral discs have been shown to change morphologically, biochemically, and biomechanically with advancing age [6-15]. These changes are directly implicated as the origin of low back pain, but the initiating and perpetuating factors of degenerative disc disease are unknown. Therefore, in spite of the pain, disability, activity limitation, and economic loss associated with disc degeneration, the etiology is still not understood so intervention and mitigation cannot effectively proceed [10, 16, 17].

The intervertebral disc is heterogeneous connective tissue acting as a hydrostatic load bearing structure within the spine [5, 18]. It contains a central gelatinous nucleus pulposus surrounded by fibrous lamellae of the annulus fibrosus circumferentially and by cartilaginous end plates superiorly and inferiorly. This avascular connective tissue contains few cells and is composed of a complex extracellular matrix which is regionally distinct.

The annulus fibrosus is composed of fibrous collagen matrix with a circumferential lamellar orientation possessing collagen types I, II, III, V, VI, IX, and XI [19-21]. These collagens are conjoined by aldehyde cross-links through hydroxylysine residues [22]. The human intervertebral disc contains the highest concentration of these crosslinkages and they support the structural organization of the annular ring of the disc [22]. This ring of connective tissue retains the central nucleus pulposus which is composed of collagen types II, VI, IX, and XI and numerous proteoglycans: aggrecan, versican, decorin, biglycan, fibromodulin, lumican, and perlecan, with aggrecan being the most abundant [5]. The nucleus pulposus of the disc contains a higher proteoglycan content than any other structure in the human body [23]. These proteoglycans of the nucleus pulposus imbibe water to provide compressive (hydrostatic) force resistance while the annulus fibrosus supports the radial forces from nuclear bulge and tensile forces placed on the disc.

These tissues are bound superiorly and inferiorly by vertebral endplates. The endplate is a bilayer of hyaline cartilage and compact bone which acts as a fluid flow path from within the disc to the vertebral body.
substantial volume of fluid (20 – 25%) is expressed from the disc during normal daily activities, but this has not been quantified nor precisely monitored for its flow path [24, 25]. The porosity of the endplate has been examined and found to have a higher permeability in immature bovine specimens compared with mature tissues [26]. Together, these tissues provide dynamic shock absorption, force attenuation, and grant motion to the torso through an integrated molecular, cellular, and systemic biochemical and biomechanical control mechanism.

With aging, biologic and biomechanical changes in the intervertebral disc reduce its ability to effectively transmit forces and retain its structure. These changes have been characterized as degeneration of the disc and lead to painful low back symptoms in certain cases. The etiology of disc degeneration has not been conclusively identified; there is evidence for biochemical changes altering morphology and affecting biomechanics as well as indications for disc mechanical injury inducing biochemical adaptations and modifying morphology.

The altered biomechanical response of degenerate intervertebral disc tissues have been measured in several studies [7-9, 27-36]. These works found that disc stiffness increases with degeneration while the energy it absorbs decreases. Unfortunately, these studies were primarily quasi-static, belying the function of the healthy lumbar disc as a dynamic shock absorber. A few studies have examined the dynamics of the lumbar spine, but have not fully related these data to the health or degree of degeneration of the intervertebral discs studied [37, 38].

In an effort to examine these integrated morphological, biomechanical, and biochemical factors of disc degeneration, in vivo animal models have been utilized. Animal models can clarify pathomechanisms and aid in the development of therapeutic strategies. While most in vivo animal models for disc degeneration require mechanical or chemical induction [9, 39-51], the macaque monkey has naturally degenerating intervertebral discs [52, 53]. Macaques have been used extensively as models of various human conditions due to their phylogenetic proximity and similarities in behavior and anatomy to humans. Macaques are quadrupeds but spend much of their time sitting with their spine in a vertical orientation, especially when caged. Macaque spines are similar to those of humans, except that macaques have 7 lumbar vertebra. Macaques, in general, age approximately 3-3.5 times the rate of humans [54-56], so a 20 year old macaque is equivalent in age to a 60-70 year old human. A naturally occurring disc degeneration animal model would facilitate our understanding of the initiation and progression of disc degeneration as well as attempts to regulate, ameliorate, or eliminate it.

Since the intervertebral disc’s primary function is that of dynamic shock absorber, we aimed to investigate this role as a function of the degeneration process. We therefore set out to examine the dynamic response of the macaque intervertebral disc with respect to its degenerative radiographic appearance.

**METHODS**

A factorial study design was used to examine the relationship between the radiographic appearance of disc degeneration and its biomechanical consequences. The evidence of disc degeneration and the genetic proximity of macaque monkeys to humans make them an attractive model for examining degenerative disc disease. Radiographic features associated with disc degeneration were measured from radiographs taken in anesthetized macaque monkeys. Post-mortem, the disc biomechanical properties of these same monkeys were measured in vitro using a dynamic testing protocol. The syntheses of these data describe the relationship between radiographic and biomechanical characteristics of disc degeneration and provide evidence for the macaque monkey as a naturally degenerating disc model.

**EXPERIMENTAL ANIMALS.** Eighteen (18) macaque monkeys (*Macaca fascicularis*) born in captivity and singly caged until their demise were investigated. At death, the five female and thirteen male macaques were 22.3 ± 0.9 years. Prior to the current study, these animals were used to examine the long-term effects of mercury exposure. This study identified negligible changes in the animals over their lifetime and, thus, the macaques presented as an elderly population without prior history of biomechanical compromise to the lumbar spine.

**RADIOGRAPHIC ASSESSMENT.** Lateral and antero-posterior radiographs were taken of each animal’s spine less than 18 months prior to their death. These radiographs were used to score the degree of disc degeneration for each thoracolumbar spinal level of each animal. Disc degeneration was quantitatively assessed by its degree of osteophytosis and disc space narrowing [52]. Each level of the thoracic and lumbar spine was scored for disc space narrowing defined as the change in disc height relative to the adjacent intervertebral spaces and osteophytosis defined by the presence of osteophytes on each anterior vertebral margin. This scoring was accomplished by a single observer using an atlas method (grade 0-3) prior to any biomechanical analysis and blind to the biomechanist [52, 57, 58].
**Tissue Preparation.** Post-mortem, each macaque specimen was dissected free of musculature and isolated functional spinal units were removed for testing (T10-11, T12-L1, and L6-7). The posterior elements were then carefully removed maintaining only a vertebral body-disc-vertebral body preparation. The anterior and posterior longitudinal ligaments remained intact along with the intervertebral disc. Each superior and inferior vertebral body was prepared for rigid fixation to the testing apparatus by passing wires through the vertebral body and then embedding these wires along with 1/3 of the body height into poly-methylmethacrylate (FIGURE 1). After preparation, each of the 54 specimens was wrapped in a towel, bathed in 0.9% saline, double-bagged, and frozen at -20ºC until testing [59].

![FIGURE 1](image)

**FIGURE 1.** Wiring and embedding of test segments. (A) Vertebra wired through the inferior and superior vertebral bodies. (B) Wired segment embedded inferiorly in PMMA. (C) Radiograph of segment fully prepared for testing.

**Experimental Procedure.** The experimental testing included an impulse test and cyclic test utilizing an apparatus similar to Kasra et al.[37] but modified to accommodate a monkey lumbar spine. After thawing, each specimen was hydrated in a solution of ionic concentration similar to cerebrospinal fluid under a static preload of 45 N [60]. Each specimen was then loaded into a high-rate MTS (Model 318.10S, MTS, Eden Prairie, MN) with an integrated linear variable differential transformer coupled to the ram superiorly. This MTS has a maximum velocity of 12 m/sec and maintains PIDF feedback closed-loop control for stroke rates up to 3 m/sec. A uniaxial load cell (Model 41, Sensotech, Columbus, OH) was mounted superior to the specimen and a six-axis load cell (Model 4526, Denton, Inc. Rochester Hills, MI) was mounted inferiorly. The load and displacement across the specimen were recorded using a custom virtual instrument in LabVIEW v.7 (National Instruments Inc., Austin, TX) on a personal computer.

Load controlled preconditioning was applied to each specimen to 100 N for 50 cycles at 1 Hz. Following preconditioning, an impulse was applied to the specimen using a haversine input waveform up to 350 N over a 50 msec duration to mimic a dynamic event. This loading and unloading curve was captured at 10 kHz and was used to identify the dynamic stiffness and energy absorption of the disc. Stiffness was calculated from the load-displacement plot on the loading cycle of the impulse by fitting the data from 20% to 80% of peak force with a linear regression ($r^2>0.90$). The area between the loading and unloading curves – the hysteresis energy – was computed as the difference of the trapezoidal integration of each curve.

Following the impulse test, each specimen was exposed to cyclic sinusoidal loading in displacement control to a displacement corresponding to 350 N. The cyclic loading profile included 10 cycles at each frequency, sweeping from 5-85 Hz (5-20 Hz in 5 Hz increments, 25-65 Hz in 2.5 Hz increments, and 65-85 Hz in 5 Hz increments). These data were captured at 10 kHz and were used to identify the natural frequency of the intervertebral disc.
The natural frequency or resonance of each intervertebral disc was determined by the phase shift between the input and output load data. A phase shift of 90º on the bode plot demarcated the natural frequency of the disc.

**DATA ANALYSIS.** The factors of (i) dynamic stiffness, (ii) energy absorption, and (iii) natural frequency were compared by radiographic degeneration grade (osteophytosis and disc space narrowing). Initially, analysis of variance tests were performed to examine if differences between the spinal levels exist. A Pearson's correlation analysis was then performed on each biomechanical metric as a function of radiographic degeneration grade. Subsequently, a two-way analysis of variance (ANOVA) was performed to examine the significance of these biomechanical factors in describing the degree of disc degeneration. Post-hoc Tukey HSD contrasts with Bonferroni correction were performed to compare between the grades of degeneration. Significance was established at an alpha level of 0.05.

**RESULTS**

No differences were found between the spinal levels (T10-11, T12-L1, and L6-7) for each mechanical property (ANOVA blocking by degeneration grade, p>0.3361). Therefore, subsequent analyses included data grouped by degeneration grade with indifference to spinal level.

Dynamic stiffness across the intervertebral disc was found to exhibit a direct correlation with radiographic degeneration grade for both osteophytosis and disc space narrowing (p<0.0001). Further, with regard to dynamic stiffness, at least one radiographic degeneration grade was found to be statistically different from the others for both osteophytosis (p=0.036) and disc space narrowing (p=0.0001) (FIGURE 2). Higher mean stiffness values were measured at each advancing disc degeneration grade such that the most severely degenerated disc stiffness was approximately 61% higher than the lowest grade.

![FIGURE 2. Intervertebral Disc Dynamic Stiffness as a Function of Disc Degeneration. The radiographic grade of the disc as defined by space narrowing and osteophytosis demonstrates increasing dynamic stiffness values with advancing degeneration severity. Statistically significant differences between the grades are indicated by the same symbol (p<0.05). Samples sizes per grade are indicated on the bars.](image)

The energy absorbed by the disc was significantly correlated with the radiographic degeneration grade for osteophytosis and disc space narrowing (p<0.0001). Differences in energy absorption were also observed between degeneration grades for osteophytosis (p=0.001) and disc space narrowing (p=0.013) (FIGURE 3). Between each grade of degeneration, the mean energy absorbed by the disc decreased; the most severely degenerate discs (grade 3) absorbed only 26% the energy of the healthiest discs (grade 0).
Intervertebral disc natural frequency demonstrated a significant correlation with the degree of disc degeneration as defined by osteophytosis and disc space narrowing (p<0.004) (FIGURE 4). Statistically, ANOVA revealed at least one radiographic degeneration grade to have a distinct natural frequency from the others for both osteophytosis (p=0.048) and disc space narrowing (p=0.041). These weak statistical results are indicative of the small change in natural frequency from 51 Hz for grade 0 to 70 Hz for grade 3.

**FIGURE 4.** The Natural Frequency of the Intervertebral Disc with Respect to Disc Degeneration. The radiographic grade of the disc as defined by space narrowing and osteophytosis demonstrates a small increase in natural frequency with degeneration. Statistically significant differences between the grades are indicated by the same symbol (p<0.05). Samples sizes per grade are indicated on the bars.

**DISCUSSION**

The dynamic response of intervertebral disc tissues demonstrates a distinct relationship with advancing disc degeneration. The macaque model exhibits natural disc degeneration which can be quantified by radiographic appearance. This degeneration also affects the dynamic properties of the disc such that severely degenerated intervertebral discs behave poorly as dynamic shock absorbers.
The phylogenetic proximity and upright biomechanics of nonhuman primates make them ideal for examining degenerative disc disease [52, 53]. Although degeneration has been found in all non-human primate species that have been assessed, more is known of disc degeneration in macaque monkeys (genus *Macaca*) than any other non-human primate. Pioneering studies by de Rousseau [56, 61] showed rhesus macaques (*M. mulatta*) exhibited spinal osteophytosis and endplate degeneration. Pritzker and colleagues [62] proposed using rhesus macaques as a model of joint degeneration in humans. Others have used plain film radiographs to assess degeneration, including osteophytosis and disc narrowing, in rhesus [55, 63] and pig-tail (*M. nemestrina*) [52] macaques. The macaque model appears to have great utility for understanding the progression of disc degeneration and examining therapeutic strategies to prevent or inhibit its course.

Previous biomechanics research has identified the stress distribution across the disc, intervertebral pressures, spinal segment compressive stiffness, and spinal segment bending kinematics for various degeneration grades [27, 28, 34, 35, 37, 64-69]. These previous works found altered spinal mechanics with advancing age and degeneration, suggesting a causal relationship between disc degeneration and distorted mechanical function. To our knowledge, no previous study has isolated the vertebral body-intervertebral disc-vertebral body complex without the posterior elements in an investigation of degenerative dynamics. Unfortunately, because the degenerative process has been shown to include load transfer to the facet joints, comparisons between previous works and the current study are not equivalent.

Limitations of the current study include the history of the animals, the radiographic consistency, and the subjectivity of morphologic disc grading. The macaque monkeys utilized in these experiments were singly-caged their entire lives and, consequently, the intervertebral disc tissues may have adapted to disuse. Thus, the outcome – disc degeneration – appears the same, but the natural initiator might not be the same. Further, although the radiographs of each animal were taken in a consistent manner, the exact position was not reproducible such that variability in disc grading may be present due to radiograph quality. Finally, while our radiographic grading system has been used extensively, the absolute values of the grades may be shifted compared with other observers.

In summary, we have identified a relationship between radiographic evidence of disc degeneration and intervertebral disc dynamic response. We have a reproducible macaque model which is excellent for examining the natural initiation and progression of disc degeneration. Further examination of this model will provide more data on the relationship between the mechanical response of an intervertebral disc and its biology and morphology such that we may approach therapeutic intervention and prevention of disc degeneration.

**LITERATURE CITED**