

Distinguishing Disc Aging from Disc Disease: Mechanical Integrity and Proteinase Expression in Asymptomatic, Intervertebral Disc Degeneration, and Degenerative Scoliosis Samples

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Nothing to Disclose

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Introduction

Intervertebral disc degeneration (IVDD) involves biochemical, structural, and mechanical changes that impair disc function (Vergroesen et al., 2015). Many of these changes also occur as part of normal aging, complicating the definition of “healthy” control tissue. IVD from asymptomatic individuals without back pain, often termed “normal”, frequently exhibit delamination or radiographic signs of degeneration (Brinjikji et al., 2015). Thus, it may be more accurate to describe them as asymptomatic, rather than true normal controls. This distinction underscores a critical knowledge gap: is degeneration primarily a consequence of age, while disease is defined by the presence of pain and dysfunction? Changes in the disc’s extracellular matrix have been shown to accompany growth, maturation, aging, and degeneration (Antoniou et al., 1996; Roughley, 2004), highlighting the central role of matrix remodeling in these processes. To begin addressing this, we stratified samples into young asymptomatic (“young normal”), older asymptomatic (“old normal”), and pathological groups including IVDD and degenerative scoliosis (dScoli). The aim of this study was to test whether reductions in annular biomechanics during aging are paralleled by changes in proteinase expression, and whether symptomatic pathologies display distinct or convergent patterns relative to asymptomatic aging.

Methods

Human annulus fibrosus (AF) tissue was collected from patients undergoing surgery for IVDD at L4-5 or L5-S1 (n=3, age: 43 ± 11 years, 2M/1F) and dScoli (n=3, age: 66 ± 3 years, 2M/1F). Asymptomatic samples from L4-5 and L5-S1 were obtained from organ donors, stratified into young asymptomatic (n=3, age: 27 ± 4 years, 2M/1F) and older asymptomatic (n=3, age: 57 ± 8 years, 3M). Each sample was divided, with one portion assessed biomechanically and a second piece frozen at -80°C for biochemical analysis, enabling direct correlation within matched specimens. Frozen tissues were powdered, extracted with PBS (pH 7.2), and analyzed by SDS-PAGE. Proteinase (MMP-3, MMP-1, and HTRA1) expression levels were assessed using western blotting (Mahmood and Yang, 2012) using commercially available antibodies (Abcam) that were validated for specificity to the indicated proteinases. Mechanical integrity of the AF was evaluated using peel tests (Gregory et al., 2012; Dhiman et al., 2025). Statistical analysis was performed using one-way ANOVA with Tukey’s post-hoc tests to compare peel stiffness, peel strength, and peel toughness across groups. This study was approved by the Ethics Committee.

Results

Biomechanical testing revealed significant group differences. Young asymptomatic discs had the greatest mechanical integrity, with stiffness of 0.31 ± 0.06 N/mm², significantly higher than IVDD (0.13 ± 0.03 N/mm², p = 0.036) and dScoli (0.14 ± 0.05 N/mm², p = 0.047), but not older asymptomatic discs. Peel strength also differed across groups, with young asymptomatic samples showing the highest strength (3.20 ± 0.65 N/mm), and seeing a significant decline in older asymptomatic (1.03 ± 0.11 N/mm, p = 0.005), IVDD (0.98 ± 0.02 N/mm, p = 0.005), and dScoli (0.96 ± 0.87 N/mm, p = 0.005). Peel toughness followed a similar trend, with young asymptomatic discs demonstrating the greatest toughness (48.94 ± 9.11 J/m), significantly greater than older asymptomatic (16.00 ± 1.07 J/m, p = 0.003), IVDD (13.54 ± 1.41 J/m, p = 0.002), and dScoli (14.93 ± 12.08 J/m, p = 0.003). Western blotting revealed strong MMP-3 expression in young asymptomatic discs, reduced and more variable expression in older asymptomatic and IVDD samples, and consistently low expression in dScoli. MMP-1 expression was variable within groups. HTRA1 expression was fairly consistent across groups without a clear progressive pattern.

Discussion

This preliminary analysis highlights both shared and distinct features of disc aging and pathology. Young asymptomatic discs exhibited the greatest mechanical integrity, whereas older asymptomatic and IVDD samples showed similar reductions in strength and toughness, suggesting that age-related changes alone are sufficient to compromise AF function. dScoli samples, though clinically distinct, also demonstrated mechanical deficits that overlapped with IVDD. While both symptomatic conditions show similar features of AF failure, differences between the pathologies suggest they may not converge on a single endpoint. As shown in Figure 1, MMP-3 expression was strongest in young asymptomatic discs and declined with age, remaining consistently low in dScoli. This pattern suggests a role in early matrix remodeling rather than late-stage degeneration, though differences in cell number between young and older discs may also contribute. While MMP-1 expression variability was present within groups, the presence of MMP-1 across all samples suggests it may play a more general role in AF turnover rather than serving as a differentiating marker between aging and pathology. HTRA1 was expressed across all groups without a clear progression, suggesting more complex regulation that may be context-dependent rather than age-driven. Taken together, these findings indicate that disc aging and disease cannot be defined by a single mechanical or proteinase profile but rather by the interactions between molecular and mechanical changes. Proteinases are important for normal matrix regulation and may be differentially regulated at the individual level, meaning their presence or absence cannot be directly associated with symptoms. IVDD may involve adaptive responses, which may compensate for changes in disc structure. Loss of AF integrity itself may or may not induce symptoms, depending on how secondary disc functions such as load distribution and motion are affected.

Significance/ Clinical Relevance

While limited by sample size, this study provides an early framework for distinguishing asymptomatic aging from symptomatic disease. Future work involves using paired samples for spatial localization of proteinase activity and larger stratified cohorts to clarify how age and pathology uniquely contribute to annular failure. Such studies may enable the identification of early biomarkers of disease and the development of targeted therapies.

Group	Peel Stiffness (N/mm ²)	Peel Strength (N/mm)	Peel Toughness (J/m)
Young Asymptomatic	0.31 ± 0.06	3.20 ± 0.65	48.94 ± 9.11
Older Asymptomatic	0.26 ± 0.10	1.03 ± 0.11	16.00 ± 1.07
IVDD	0.13 ± 0.03	0.98 ± 0.02	13.54 ± 1.41
dScoli	0.14 ± 0.05	0.96 ± 0.87	14.93 ± 12.08

Table 1. Biomechanical properties of annulus fibrosus tissues

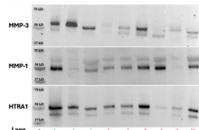


Figure 1. Western Blotting of tissue extracts

Lane	Pathology
1	MW Ladder
2	Young
3	Asymptomatic
4	Older
5	Asymptomatic
6	IVDD
7	IVDD
8	dScoli
9	dScoli
10	dScoli

Figure 1 Legend