Assessing Differences between Interlamellar Properties of the Annulus Fibrosus from Degenerative Disc Disease and Non-Diseased Young Donor Patients

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INTRODUCTION: Degenerative disc disease (degen) is accompanied by mechanical and biochemical changes in the intervertebral discs (IVD) [1]. The IVDs in these patients often exhibit severely degenerated annulus fibrosus (AF) tissue at the time of surgery performed to alleviate significant pain and mobility issues. The lamellae that form the AF are interconnected through the inter-lamellar matrix (ILM) [2], consisting of mainly collagen type VI [3], proteoglycans [4], and elastin [5] to help prevent delamination. The ILM contains the interlamellar cross-bridges that connect the lamellae radially in 3-dimensions [6]. Weakening of the ILM and less interlamellar cross bridges could contribute to delamination between the lamellae, reducing their ability to resist loads. We hypothesized that surgical tissue from degen patients will have decreased AF interlamellar mechanical properties compared to the samples from young donor ("normal") patients. The objective of this study was to quantify the differences in interlamellar mechanical properties from surgical and normal fresh tissue.

METHODS: Fresh human AF tissue from patients undergoing surgery for degenerated discs and "normal" samples from organ donors through the Southern Alberta Tissue Donation program were collected (Ethics ID: REB18-1308, Conjoint Health Research Ethics Board, University of Calgary). Fresh tissue with minimum dimensions of 15 mm X 3 mm X 2 mm (circumferential length X tissue width/disc height X radial length) were obtained from surgery. A peel test was performed to measure the interlamellar properties of the AF as per Gregory et al. [7]. An incision was made in the circumferential direction in the middle of the sample to create two annular tabs for mounting and the peel length was standardized to 8-9 mm. After mounting, the tissue was exposed to 100% water vapor and allowed to reach equilibrium for 10 minutes. The tissue then underwent 20 cycles of preconditioning at 0.5 Hz, followed by stress-relaxation for 5 minutes. The tissue was peeled at 0.5 mm/s until complete separation [7]. A force-displacement curve was analyzed using a custom Python script to calculate the properties. One-tailed Student t-tests based on Shapiro-Wilk normality and F-test were performed to assess the differences between the degen and "normal" samples. The Type I error was set to $\alpha = 0.05$. Depending on the interlamellar property, 0-2 samples were removed based on Tukey's outlier criteria (k = 1.5).

RESULTS: Detailed patient demographic information is presented in Table 1. Normal and degen samples had a mean Peel Stiffness of 0.27 ± 0.07 N/mm^2 and 0.19 ± 0.08 N/mm^2, respectively ($p < 0.01$) (Figure 1). Mean Peel Strength for the Normal and degen samples was 2.39 ± 0.93 N/mm and 1.61 ± 0.76 N/mm ($p < 0.01$) (Figure 1). The mean Peel Toughness of Normal samples was 34.34 ± 15.04 J/m whereas it was 22.59 ± 13.64 J/m for degen samples ($p < 0.05$) (Figure 1). No statistically significant differences were observed in the Peel Region Length and Standard Deviation of the Peel Stress. The Peel Region Length for Normal and degen samples was 4.42 ± 2.65 mm and 3.57 ± 2.81 mm ($p = 0.15$). The Standard Deviation of the Peel Stress for Normal and degen samples was 0.065 ± 0.030 N/mm and 0.045 ± 0.035 N/mm ($p = 0.08$).

DISCUSSION: Increased Peel Stiffness and Peel Toughness for Normal samples showed that greater force and energy were required to peel the lamellae apart. The increased Peel Strength indicated greater adhesion between the lamellae for "Normal" samples compared to the degen samples. Although no significant differences were observed for Peel Region Length and Standard Deviation of the Peel Stress, increasing trends were observed for Normal samples based on Hedge's g effect size measurements. Some degen samples exhibited properties similar properties to the Normal samples, suggesting that the ILM in degen condition exists on a spectrum. Gregory et al. measured Peel Stiffness and Strength in frozen human samples with mild degeneration [7] and in rabbit annular disc puncture models to induce disc degeneration [8]. Although our reported values were similar in order of magnitude, we tested fresh samples only which may be the reason behind increased Peel Strength values in our study. Our study also had some limitations. Samples obtained from surgery had spatial heterogeneity of degeneration that was not apparent upon visual inspection. This led to selecting homogeneous portions for mechanical testing and the sample's entire interlamellar integrity was not assessed which may introduce some bias. This study provided quantitative evidence that the mechanical integrity of ILM may be decreased in surgical patients with the degen condition compared to the non-degen/"normal" patients.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrated that decreased interlamellar adhesion observed in the samples from human surgical patients may contribute to weakening of the AF and the IVD. With an expanded cohort of degen and spine deformity patients, the results will provide fuller insights into the contributions of the mechanical integrity of ILM in development and progression of these spine conditions.

REFERENCES: [1] Adams M, et al. *Spine*. (2006). 31(18):2151-61, [2] Schollum M, et al. *J. Anat*. (2009). 214(6): 805–816, [3] Melrose J, et al. *Eur Spine J*. (2008). 17(2):314-24, [4] Adams M, et al. *Eur Spine J*. (1993) 2(4):203-8, [5] Yu J, et al. *Spine*. (2015). 40(15):1149-57, [6] Han S, et al. *J. Orthop. Res*. (2015). 33(3):304-11, [7] Gregory D, et al. *Eur Spine J*. (2012). 21:1716-23, [8] Gregory D, et al. *Spine J*. (2014), 14(7):1265-71

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IMAGES AND TABLES:

	Normal	degen
Sample Size	13	18
Number of Patients	7	16
Age (years)	35 ± 9	46 ± 10
Sex (Male/Female)	(9/4)	(10/8)
Level (L4-L5/L5-S1)	(7/6)	(6/12)

Table 1: Basic demographics information of the patients included in the Normal and degen groups. The age is calculated based on the number of patients whereas sex distribution and level distribution are calculated based on the number of samples.

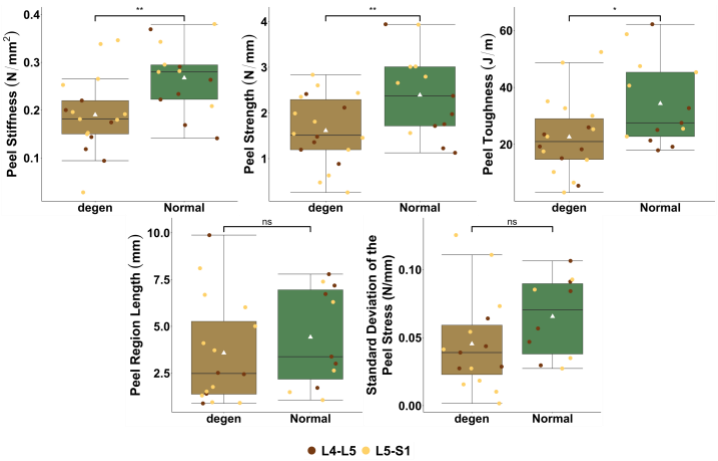

Figure 1: Comparison of the interlamellar mechanical properties between degen and Normal samples. Both L4-L5 and L5-S1 levels, and males and females are combined. The horizontal line in the middle of the boxplot represents the median and the white triangle represents the mean of the group. ** represents $p \leq 0.01$, * represents $p \leq 0.05$, and ns represents $p \geq 0.05$.