

# Trans-endplate diffusion across the spectrum of human disc degeneration

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**INTRODUCTION:** The intervertebral discs are the largest avascular structures in the body and depend primarily on diffusion via the vertebral endplates to receive nutrients and expel waste products.<sup>1</sup> Due to the avascularity of the intervertebral discs, it has been suggested that reduced disc nutrition is a significant contributor to the degenerative process.<sup>1</sup> Studies have shown that the reduction of disc nutrients can occur due to the calcification of the endplate that impairs diffusion to the disc.<sup>2</sup> However, alterations in trans-endplate transport across the spectrum of spinal degeneration and the relative contributions of pathology in the bony and cartilage endplate remain poorly understood. In this study, human cadaveric endplate samples were used to assess and correlate trans-endplate diffusion with the structure, composition, and mechanical function of the bony and cartilage endplate to determine factors affecting trans-endplate transport across the spectrum of disc degeneration.

**METHODS:** Four lumbar spines (1 male, 3 female, 50-70 yo) were obtained from human cadavers (Science Care). T2-weighted MRIs were obtained for disc Pfirrmann grading, and T2 mapping was used to quantify nucleus pulposus (NP) T2 relaxation times.<sup>3</sup> Spinal motion segments (n=20) were dissected. From each disc, tissue samples of nucleus pulposus and annulus fibrosus were obtained from each motion segment and underwent biochemical assays including DMMB to quantify GAG concentration, PicoGreen to quantify DNA, and Hydroxyproline for collagen quantification. From these segments, two cylindrical cores (n=18) with a diameter of 10 mm and an average thickness of 2.50 mm were obtained that included endplate-cartilage interface with trabecular bone. One core was used for passive diffusion experiments (Figure 1A) using a custom diffusion chamber. The upstream chamber was loaded with 1.1 mg/mL of sodium fluorescein (MW = 367.27), and triplicates of the downstream chamber were collected every hour for 6 hours. Fluorescence was read via a microplate reader, and the concentration of the downstream chamber calculated based on a fluorescein standard curve. Total diffusion was quantified by calculating the area under the curve (AUC). Endplate cores were then fixed and  $\mu$ CT scanned with a resolution of 7.40  $\mu$ m to evaluate bony endplate morphometry and cartilage thickness following repeated  $\mu$ CT after staining the cores overnight with Lugol's solution.

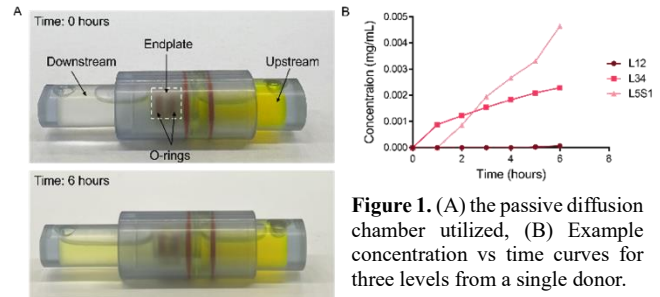
**RESULTS:** Diffusion experiments demonstrated significant variability in trans-endplate diffusion across donors and spinal levels within the same donor (Figure 1B). Correlations between NP T2 and diffusion revealed a bimodal relationship between diffusion and disc health. When discs were stratified further by Pfirrmann Grade, there was a significant positive linear correlation between NP T2 and diffusion for Pfirrmann Grade 2 discs, however, there was a trend towards increasing diffusion with decreasing NP T2 relaxation time in Pfirrmann Grade 3 discs (Figure 2A). Comparison of NP GAG content between samples with low (AUC < 5) and high (AUC > 5) diffusion demonstrated that NP GAG content trended lower in samples with high diffusion (Figure 2B). 3D  $\mu$ CT reconstructions demonstrated substantial variability in bony endplate porosity across levels even from the same donor, which could affect passive diffusion (Figure 2C). However, no significant correlation was found between endplate bone volume fraction (BV/TV) and passive diffusion (Figure 2D). Cartilage endplate thickness measured from Lugol's enhanced  $\mu$ CT (Figure 2E) was found to significantly inversely correlate with passive diffusion, demonstrating that as cartilage endplate thickness increases, passive diffusion decreases (Figure 2F).

**DISCUSSION:** Our results suggest that trans-endplate diffusion is not altered in a linear fashion across the spectrum of disc degeneration, as both healthy (high NP T2) and degenerative (low NP T2) discs exhibited high trans-endplate diffusion – a trend also observed in prior human MRI studies of diffusion into the disc.<sup>4</sup> A limitation of the current study is that our sample set contained primarily moderately degenerative discs, and therefore we are currently expanding our sample set to include more healthy and severely degenerative discs to more rigorously quantify the spectrum of disease. Our data also suggests that cartilage endplate thickness is the main structural factor affecting solute transport under passive diffusion. Prior studies have demonstrated the effect of cartilage endplate composition on diffusion, which is currently being investigated in our ongoing work, in addition to cartilage endplate mechanical properties.<sup>5</sup> Interestingly, only weak correlations between diffusion and bony endplate density were observed, in contrast to our prior work in a rabbit disc degeneration model.<sup>6</sup> It is possible that the bony endplate may have a greater impact on disc nutrition during convective transport. It has shown that dynamic loading induced convective flow can augment transport into the disc, and future work will focus on understanding the endplate structure-function properties conducive to enhanced transport under convective flow.<sup>7</sup>

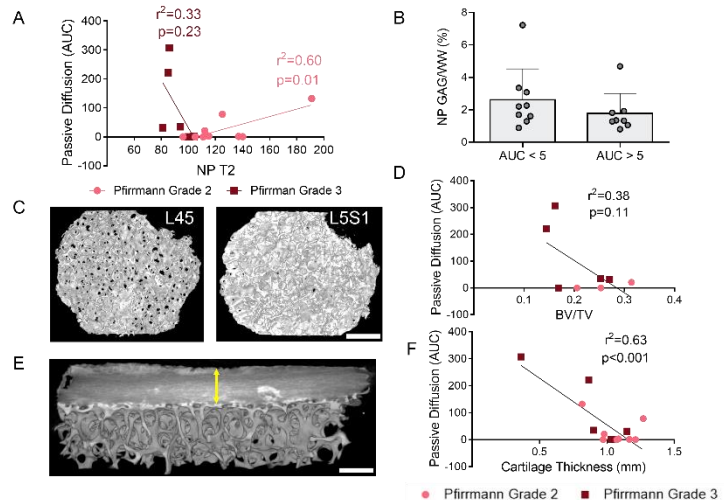
**SIGNIFICANCE:** The results from this study further our understanding of the pathomechanisms of disc degeneration, which can ultimately contribute to improved diagnostics and treatments for this debilitating condition.

**REFERENCES:** [1] Maroudas+ *J. Anat.*, 1975 [2] Benneker+ *Spine*, 1976 [3] Ashinsky+ *J. Anat.*, 2020 [4] Rajasekaran+ *Spine*, 2004 [5] Dolor+ *J. Plos One*, 2019 [6] Ashinsky+ *ASBMR*, 2020 [7] Sampson+ *J. of Biomechanics*, 2019

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**Figure 1.** (A) the passive diffusion chamber utilized, (B) Example concentration vs time curves for three levels from a single donor.



**Figure 2.** (A) Passive Diffusion quantified by area under the curve (AUC) vs. NP T2, (B) Nucleus Pulposus GAG Content compared to AUC, (C) Images of  $\mu$ CT that show porosity of samples among the same donor, (D) Passive Diffusion (AUC) not correlated with BV/TV, (E) Image of an endplate cross-section showing stained cartilage endplate (indicated by the arrow) with Lugol's Solution, (F) Passive Diffusion (AUC) correlated with cartilage thickness. Scale = 0.5 mm.