Impact of Degeneration on Diurnal Viscoelasticity of Human Intervertebral Discs: Implications for Osmotic Kinetics

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INTRODUCTION: Osmotic cycles resulting from compression and recovery of the IVD are key mechanobiologic signals regulating cell metabolism in the IVD [1]. Although, the magnitude of an osmotic cycle is understood to be important any changes in the rate of change in osmolarity as a mechanobiologic signal are poorly understood. Given that deformation of the IVD, and corresponding changes in the tissue's fixed charge density (FCD), engender osmotic fluctuations, we aimed to understand how changes in time-dependent behaviors of the IVD with degeneration might inform the rate of osmotic cycles. Various viscoelastic models have been used to quantify degeneration associated changes in viscoelastic behaviors of IVDs and have found that time constants, which describe how long it takes for the deformation to reach steady-state, change with advancing degeneration although there is some inconsistency in reported parameter values [2–5]. It is also generally understood that the specific loading protocols used can influence the results of mechanical tests. For example, organ culture studies have demonstrated that it can take multiple days for diurnal creep/recovery behaviors to stabilize into a steady-state, which is thought to be more representative of in-vivo [6]. However, traditional mechanical testing typically uses much shorter pre-loading periods. Further, variations in the amount of time allowed for creep and recovery viscoelastic tests similarly impact the estimates of the parameters and differences in protocols and likely contribute to the variability of prior results. The aim of this study is to quantify the viscoelastic properties of human IVDs across the spectrum of disease using a simulated diurnal loading schema adopted from organ culture models with the goal of providing physiologically relevant information on the dynamics of osmotic cycles.

METHODS: Seven human lumbar spines (4 F, 3 M) were obtained from the Cooperative Human Tissue Network at The Ohio State University. One or two bone-disc-bone motion segments were isolated from each spine and potted for mechanical loading (N=12). Disc height, area and a preliminary degeneration assessment of 'Healthy' or 'Degenerated' were determined by x-ray (KUBTEC XPERT 80) after which samples were loaded for 64hrs (n=6) or 72hrs (n=6) under simulated diurnal loading consisting of 16hr of 0.6MPa compression / 8hrs of 0.2MPa compression (Fig. 1A) using an MTS 858 Bionix Test System. All loading occurred within a 37°C 0.15M NaCl bath. At the end of loading, samples were frozen at their final deformation, cut along the sagittal plane and photographs taken for Thompson degeneration grading by 3 blinded graders. The force/displacement data (Fig 1A) for each daytime creep and nighttime recovery phase from each IVD was fit to a 5-parameter rheological model (Eqn. 1, Fig 1), and stiffness parameters (S_E, S_1, S_2) and long- and short- time constants (τ_1 and τ_2) were calculated for each step utilizing a custom optimization code using the FMINCON function in MATLAB. The initial parameter guess for each sample was approximated from the raw data and the Boltzmann superposition principle was applied to account for prior displacement of each step as previously described [5]. A linear mixed model was used to assess the effect of degeneration, test duration (day 1-3) and loading phase (day vs. night) using JMP with $p<0.05$ considered significant.

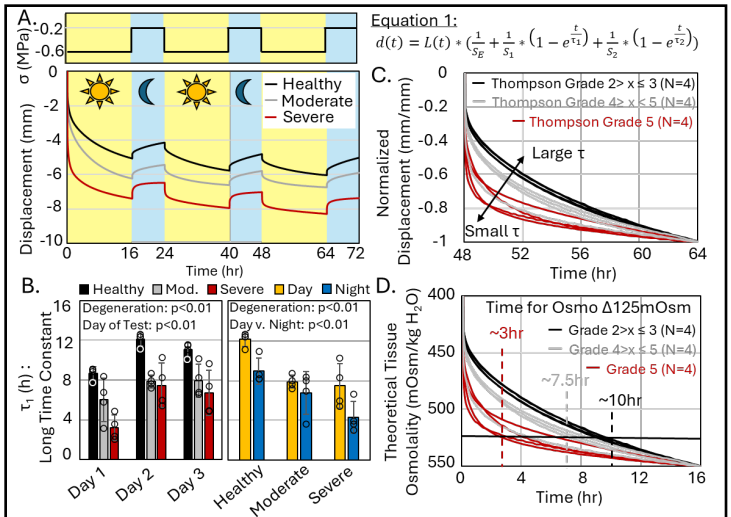

Figure 1 – Degenerated IVDs deform quicker than healthy IVDs: **A.** Representative diurnal displacements of a healthy and degenerated motion segment loaded over 3 days under 16hr of 0.6MPa (Daytime) and 8hrs of 0.2 MPa (Nighttime) compressive stress. **B.** Change in long time constant (τ_1) (left) of daytime creep phases over testing duration with advancing degeneration and (right) comparing daytime creep and nighttime recovery τ_1 from day 2 at each disease state. Results demonstrate degenerated IVDs creep and recover much quicker than healthy IVDs and that nighttime recovery if faster than daytime creep **C.** Normalized displacements from 48 to 64hrs for all IVDs (N=3/mild, N=3/moderate, N=3/severe) demonstrating that degenerated IVDs reach equilibrium much faster (i.e., smaller time constant). **D.** Theoretical schematic demonstrating differences in daily kinetics of an osmotic cycle with degeneration assuming the magnitude of the daily cycle is the same across degeneration.

RESULTS: Thompson degeneration scoring grouped IVDs into three groups (N=4/grp); Healthy (Grade <3), Moderate degeneration (Grade 4 to <5) and Severe (Grade = 5) Qualitatively, comparing the shapes of the diurnal deformation curves degenerated IVDs exhibited time-dependent deformations that more quickly approached a steady-state value, more closely resembling a square wave, while healthy IVDs had a much more gradual change (Fig 1A&C). Quantitatively, degenerated IVDs exhibited significantly lower long-term and short-term time constants, τ_1 and τ_2, respectively (Fig 1B&C). Degenerated IVDs also had significantly greater elastic (S_E) and long-term (S_1) stiffnesses. As expected, all parameters from the first day of loading were significantly lower than those from Days 2 and 3 (Fig. 1B). Additionally, the long-time constants from the nighttime recovery phase were significantly lower than the daytime creep on day 3 (Fig 1B).

DISCUSSION: This study aimed to quantify degeneration-associated changes in viscoelastic behaviors of the IVD to inform how the rate of osmotic cycles within the tissue may change with disease, and is underpinned by the understanding that deformation induced changes in the tissue's FCD would engender osmotic fluctuations. These results indicate that as degeneration advances, IVDs deform more quickly (shown quantitatively in lower time constants (Fig 1B) and qualitatively in Fig 1C) than healthier IVDs, a finding consistent with prior work [2,7] although with different absolute values given different loading protocols. Conceptually, if all IVDs experienced an osmotic cycle of the same magnitude throughout the day changing from 400 to 550 mOsm/kg H_2O, which likely is not the case, degenerated IVDs would change by 125mOsm in ~3hrs while in healthy IVDs this would take ~10hrs. This time dependent behavior has implications in cell culture applications, where many studies apply an instantaneous change in osmotic environment via solution changes, which our results suggest may mimic a more degenerated environment. The finding that IVDs have shorter time constants overnight is consistent with prior work demonstrating direction-dependent resistance to fluid flow [8] and consistent with the natural requirement that IVDs recover the height lost during 16hrs of the day in a shorter time-period overnight (8hrs). The significant effect of test duration highlights that it takes time for the IVD to reach a "dynamic steady state," emphasizing that comparing parameter values from the test starting at time = 0, while helpful to make relative comparisons, may not fully be representative of what occurs physiologically.

SIGNIFICANCE/CLINICAL RELEVANCE: Overall, these results demonstrate that degeneration has a significant effect on the viscoelastic properties of the IVD under simulated physiological diurnal loading. These results have implications for cell culture studies and suggest the rate of change in osmolarity may be another mechanism of how mechanobiology within the IVD changes with disease.

REFERENCES: [1] Zelenski +2015, [2] Le +1995, [3] Van der Veen +2013, [4] Pollintine +2010 [5] O'Connell +2011, [6] Emanuel +2015, [7] Kazarian +1975, [8] Ayotte +2001 ACKNOWLEDGEMENTS: This work was funded by NSF CAREER 2143779.