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EFFECT OF HYDRATION ON INTERVERTEBRAL DISC RECOVERY

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INTRODUCTION

Intervertebral disc mechanics are largely dependent on its structure and biochemical composition. The disc consists of a collagen-rich annulus fibrosus (AF), which surrounds a gel-like core, the nucleus pulposus (NP). The high water content of these subcomponents (NP: 80-85%, AF: 70-80%) allows the disc to resist large compressive loads while maintaining spinal flexibility and mobility [1,2]. External loads induce fluid flow out of the disc while negatively charged proteoglycans attract water during bed-rest recovery. Previous studies, including work from our own laboratory, have used osmotic loading to evaluate the effect of tissue hydration (i.e. water absorption) on mechanical function. Recently, we demonstrated that an increase in disc hydration decreases disc joint compressive stiffness [3]. This previous study only considered disc mechanics once a steady-state condition was achieved; therefore, the time-dependent response to achieve a steady-state condition under osmotic loading is not well understood.

Water re-absorption into the disc increases internal pressure; however, water flow into the disc is not trivial, due to porosity differences in the NP, AF, and endplate [4]. The first objective of this study was to analyze the time-dependent response of disc components (i.e., NP, AF and whole disc) under free-swelling conditions. Swelling response of the NP and AF were evaluated as tissue samples and under in situ conditions to account for differences in tissue swelling behavior and boundary conditions. The second objective of this study was to evaluate time-dependent recovery behavior under osmotic loading.

METHODS

Skeletally mature bovine spine sections were acquired from the local abattoir (n = 14 spines, age = ~18 months). Bone-disc-bone segments were prepared by removing the surrounding muscles and posterior elements for Studies 1 and 2 (n = 31). In Study 1, discs were dissected from the superior and inferior vertebral bodies using a scalpel to assess swelling behavior of the whole disc (n=10), NP (n=6) and AF (n=6). Whole disc or tissue samples were immersed in phosphate buffered saline (PBS) solution (0.15 M or 1.5 M PBS) until equilibrium swelling was attained, which was defined as a change in wet weight of less than 0.02 g/hr. The swelling ratio was calculated as the differences between pre- and post-swollen wet weights, normalized by the pre-swollen wet weight. A stretched exponential function was used to describe the swelling ratio as a function of time (Eq. 1), with model parameters to describe equilibrium swelling ratio (Q_∞), time constant (τ), and stretch parameter (β) where $0 < \beta \leq 1$. The stretch parameter, β, describes the rate of change of tissue-swelling with respect to an exponential curve, where the response is faster than an exponential description for $t < \tau$ and slower than exponential curve for $t > \tau$ [5]. A Student's t-test was performed to determine differences in swelling behavior of the 0.15 M and 1.5 M PBS groups.

$$Q(t) = Q_\infty \left(1 - e^{-(t/\tau)^\beta}\right) \qquad (1)$$

In Study 2, unloaded recovery of bone-disc-bone motion segments was assessed with respect to osmotic loading. Motion segments were potted in bone cement to ensure parallel loading and hydrated in 0.15 M PBS solution prior to mechanical testing. A 300 N creep load was applied and held for two hours. At the end of the creep portion of the test, the water bath was replaced with fresh saline (0.015 M, 0.15 M, 0.75 M and 1.5 M PBS). Then, motion segments were allowed to recover at a nominal load (20 N) to ensure the platen was in contact with the sample during recovery (12 hours). Each sample was re-hydrated between tests and tested in a random order. Force and displacement were measured. The displacement during recovery was curve fit to a 5-parameter rheological model (lsqcurvefit function, Matlab Inc.) shown in Eq. 2 [6].

$$d = \mathrm{L}\left[\frac{1}{S_1}\left(1 - e^{-(t/\tau_1)}\right) + \frac{1}{S_2}\left(1 - e^{-(t/\tau_2)}\right) + \frac{1}{S_E}\right] \quad (2)$$

The model is composed of two Voigt solids with a spring in series and allows for the characterization of the fast response (S_1 and τ_1), the slow response (S_2 and τ_2), and the elastic response (S_E). To simplify the model, the elastic response (S_E) was set as the instantaneous displacement during recovery. The model also provided parameters for equilibrium distance (d_{eq}) and equilibrium time (t_{eq}). To validate the equilibrium time predicted by the model, additional samples were loaded under the same conditions and allowed to recover for 48 hours (n = 5). A one-way ANOVA with Tukey post-hoc test was used to determine the effect of bath osmolality on recovery properties. The 0.15 M PBS group served as the control. Significance was assumed at $p \leq 0.05$.

RESULTS

The stretched exponential function described the swelling ratio well for all groups (Fig. 1; $R^2 > 0.98$). Equilibrium swelling ratio of whole discs hydrated in 0.15 M PBS was 18% greater than the 1.5 M PBS group (Fig. 2 – '*'; $p < 0.015$). There was no difference in the time constant and the stretch parameter (Fig. 2; $p > 0.07$). Tissue swelling of the NP and AF separately (0.15 M PBS only) resulted in a significant difference in swelling ratio and the stretch parameter, as expected (Fig. 2 – '^'; $p < 0.02$).

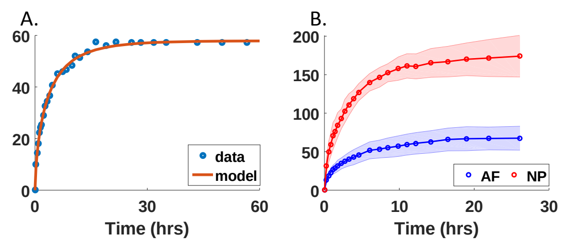

Figure 1: A) A representative AF sample showing the swelling ratio, Q (%), with model fit and B) swelling ratio for NP and AF - whole disc data not shown. Circles indicate the experimental data.

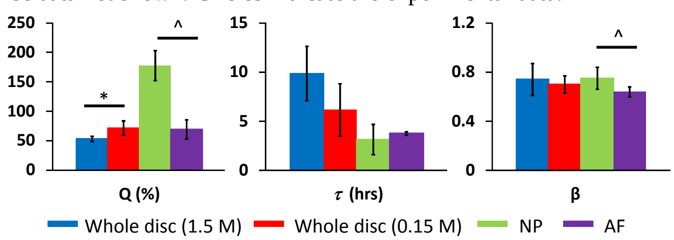

Figure 2: The parameters for the stretched exponential function.

Full recovery was not observed within 12 hours for any group, but there was a decrease in recovery with osmotic loading ($p < 0.001$; Fig. 3A). Recovery in a hyperosmotic saline ($\geq$ 1.5 M PBS) demonstrated a reverse trend in disc height after ~3 hours, such that the disc exhibited features of loading rather than recovery (Fig. 3A – purple line). The 5-parameter model fit well to the experimental data (Fig. 3B, $R^2 > 0.99$) except in 1.5 M due to the decrease in disc height during recovery. The instantaneous displacement (elastic response) during recovery was 0.52 ± 0.12 mm (pooled average across all osmotic loading conditions). The equilibrium displacement predicted by the model agreed well with the results obtained from samples tested for 48h (Fig. 3B). The fast response behavior (S_1 and τ_1) was altered with osmotic loading ($p < 0.05$), while the slow response behavior (S_2 and τ_2) was not dependent on the osmotic loading condition (Fig. 4, S_2 = 1620 ± 422 N/mm and τ_2 = 26.7 ± 8.76 min, pooled average across all osmotic loading conditions).

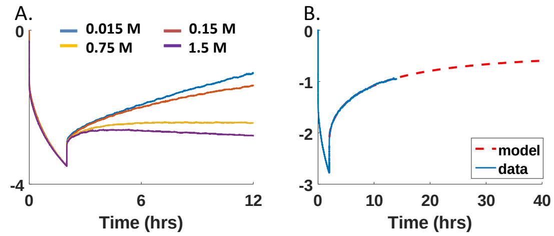

Figure 3: A) Disc height recovery for each osmotic loading group (PBS solution). B) A representative sample of data and model fit.

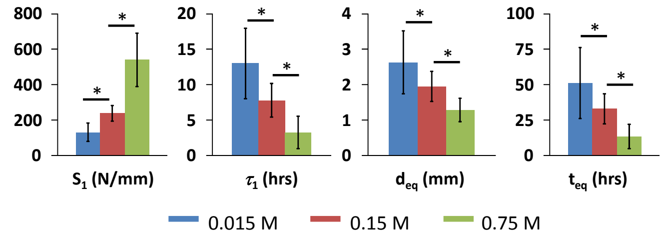

Figure 4: The results obtained from the 5-parameter model. Only statistically significant results are shown.

DISCUSSION

The disc needs to maintain adequate hydration in order to preserve flexibility, mobility, and resistance to external loads. This study evaluated the effect of osmotic loading on disc recovery behavior. In Study 1, swelling ratios of NP and AF tissue structures were evaluated separately and as a composite material (i.e. whole disc). The swelling ratio of excised discs decreased with hyperosmotic loading, which agreed well with disc joint recovery measured in Study 2. As expected, swelling behavior was greatly altered by the boundary conditions. The swelling ratio of the whole disc was closer to the AF swelling ratio, even when accounting for differences in the initial volume of each subcomponent (Fig. 2; NP volume ≅ 30% of disc volume) [7].

In hyperosmotic saline ($\geq$ 1.5 M PBS), disc height recovery did not follow the expected behavior. After approximately 3 hours of recovery, disc height recovery reversed, suggesting a shift between recovery from mechanical loading and loading from the osmotic environment. To estimate the balance between mechanical loading and osmotic loading, the displacement at 12 hours (d_t=12hrs) was compared to the elastic displacement (S_E). The findings reported here suggest that the disc is in osmotic equilibrium in a saline bath with a concentration of ~1.0 M.

The intervertebral disc loses a significant amount of water during diurnal loading. Rehydration plays a crucial role in the disc mechanics and tissue maintenance. In conclusion, changes in the disc's external osmotic environment greatly alters disc recovery following mechanical loading, which may result in long-term degenerative like changes in composition and mechanical function.

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