Osmotic Swelling Alters Tensile Mechanics in Fiber-Reinforced Tissues

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INTRODUCTION: Fiber-reinforced tissues of the musculoskeletal system, including tendons, ligaments, menisci, and the annulus fibrosus of the intervertebral disc, consist of collagen fibers embedded within an extrafibrillar matrix. The intervertebral disc is a fibrocartilaginous material consisting of a soft nucleus pulposus surrounded by the fiber-reinforced annulus fibrosus (AF). The AF has a lamellar structure with collagen fibers oriented at ±45° in the inner AF to ±60° in outer AF (with respect to vertical/spinal axis) [1]. These fibers are embedded in an extrafibrillar matrix that consists of proteoglycans (~3.0 %/ wet weight & water content ~70% in the AF), which contribute to the swelling behavior of the nucleus pulposus [2]. It is well known that the nucleus pulposus has an excellent capacity to imbibe water and swell, which increases the intradiscal pressure *in situ,* supporting the disc under large compressive loads [3]. However, the effect of swelling on the mechanical behavior of the AF is not well understood.

Therefore, it is likely that osmotic loading greatly affects the mechanical response of AF. Recent work by Han et al. demonstrated that hyperosmotic loading (2 M PBS) increased the apparent stiffness of the outer AF [4]. This observation contradicts observations of swelling on the mechanical response of articular cartilage, which has a significantly lower collagen composition and decreased collagen alignment [5]. To better understand the effect of the osmotic loading on tensile mechanics of fiber-reinforced tissues, we developed a finite element model (FEM) describing multi-lamella tissue. The model used a triphasic material description to simulate swelling [6]. In this study, we varied the fiber angle orientation to represent collagen fiber orientations of a variety of tissues; however, the material properties used were based on experimental data for the AF.

METHODS: *Geometry and mesh*: AF tissue geometry was defined with dimensions of 10 mm x 2 mm x 0.6 mm for length, width, and lamellae thickness, respectively. Three welded lamellae were included in the model to simulate the alternating fiber angle orientation in the annulus fibrosus (used for the ±30º, ±45º, and ±60º models). The model was developed using FEBio modeling suite (PreView 1.19.0; hexahedral elements; 33915 nodes and 30600 elements). *Constitutive model and material parameters*: A triphasic material description was used to describe tissue swelling. The AF extrafibrillar matrix solid was modeled as a compressible hyperelastic material using the Holmes-Mow model, which consists of parameters for material density (ρ = 1 g/cm^3), Young's modulus (E = 0.0649 *MPa*), Poisson's ratio (ν = 0.24), and an exponential stiffening coefficient (β = 0.95) [7]. The fixed charge density was defined as -120 *mmol/L*. Fibers were described using an exponential-linear function, as previously described [8]. The strain-dependent Holmes-Mow model was used, where parameters defined permeability (k_0 = 0.0064 mm^4/Ns), the exponential strain-dependence coefficient (M = 4.8), and an exponential term (α = 2). The solid volume fraction was 0.3 [2]. The osmotic coefficient under different initial osmotic environments was approximated using linear interpolation based on the data reported in [9]. Finally, diffusivity for Na^+ and Cl^- ions was set to 0.00199 mm^2/s. We assumed that all pores were accessible for solute diffusion from the saline solution (solubility κ = 1).
Loading Conditions: Tissues were loaded in a two-step process. First, the tissue was allowed to swell until a steady state condition was achieved. During swelling, we simulated the effect of hyperosmotic loading (0.15 M, 0.75 M and 1.5 M PBS), where the 0.15 M PBS group served as the control. Swelling was followed by uniaxial tension to 20% stretch at different loading rates (steady state, 1%/s, and 4%/s). Stretch was defined to be current length normalized by original length, and stress was calculated as the exerted force divided by the original cross section area.

RESULTS: Tissue swelling decreased with hyperosmolar (volume ratio = 1.45 for the 0.15 M PBS vs 1.1 for 1.5 PBS). The increase in tissue volume resulted in tissue elongation or shrinking, which was dependent on the fiber orientation (e.g., Fig.1A – tissue shrinking for ±60º model). Tissue shrinkage during swelling was observed for models with fiber orientations greater than 45º and less than 90º (Fig. 1B). During tension holding osmotic loading unchanged, tissue stiffness increased with loading rate (Fig.1C). Under the same loading rate, the toe-region apparent modulus had a 45% decrease in hyperosmotic loading (1.5 M PBS case compared with 0.15 M PBS case) (Fig1.C&D), while the linear-region modulus (~16.2 MPa) showed little change.

DISCUSSION: Fiber-reinforced tissues with glycosaminoglycans, which act to attract water molecules, swell under osmotic loading conditions [2, 4]. The results from this study demonstrated that the tissue-swelling response was greatly dependent on the fiber orientation. Including swelling-related properties resulted in rate-dependent increase in tissue stiffness (Fig. 1C), as observed in experimental results [10]. Hyperosmotic loading decreased the toe-region apparent modules, which agreed with swelling result for articular cartilage [5]. However, this trend is different from that reported in [4]; the difference may be due to stress being calculated with respect to the swollen cross-sectional area rather than the cross sectional area in the reference configuration, as was done in [4]. Importantly, collagen fiber orientation significantly altered tissue pre-stress during the swelling phase prior to tensile loading. Residual stresses have been widely reported in cardiovascular tissues and in the annulus fibrosus [11], and are thought to be important for maintaining homeostasis. The findings from the FEM simulation will be used to guide experimental work that can be used to validate the findings reported here.

SIGNIFICANCE: We developed a swelling-based FEM model of fiber-reinforced tissues that demonstrated the effect of fiber angle orientation on tissue swelling behavior and the effect of swelling on tensile mechanics.

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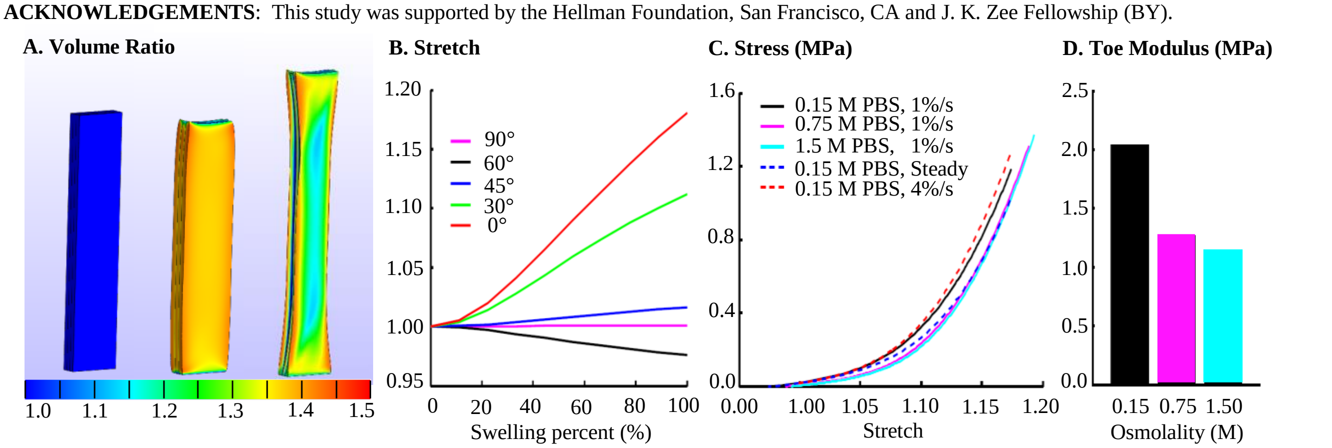

Fig.1 (A) Tissue (±60° fiber) volume change during 1 PBS swelling and 1.2 stretch tension. (B) Tissue shrinking and elongation during swelling. (C). Tissue tensile behavior under different osmolality and loading rate. (D) Toe-region tensile modulus of tissue (±60° fiber) after swelling in different osmolality PBS.