EFFECT OF OSMOTIC SWELLING IN SOFT TISSUES IS DEPENDENT ON COLLAGEN FIBER ORIENTATION

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INTRODUCTION

Residual stresses are commonly reported in soft tissues, including the arterial wall and the intervertebral disc, and may act to ensure uniform stress distribution within complex fiber-reinforced tissues [1]. Extensive research has shown the important role of collagen fiber composition and architecture on tissue mechanics [2-6]. Collagen fibers are embedded within an extracellular matrix that consists of proteoglycans, water, and fibril-associated collagens that connect collagen fibers, and the role of the extracellular matrix on tissue mechanics is not well understood.

Negatively charged glycosaminoglycans (GAG) act to attract water molecules into soft tissues, resulting in osmotic swelling. Recent studies have shown that osmotic swelling stiffens articular cartilage by engaging the collagen fibers in tension prior to compression testing [7]. In contrast, osmotic swelling in the intervertebral disc caused a decrease in joint stiffness [8]. Moreover, a localized increase in GAG composition in the arterial wall altered stress distribution throughout the wall thickness, which may be a mechanism for thoracic aortic aneurysms [9]. Sophisticated computational models, such as biphasic swelling and triphasic mixture model theory, have been developed to incorporate tissue swelling in soft tissue simulations [10].

Our previous work showed that tissue shrinking or elongation of rectangular specimens under osmotic swelling conditions was dependent on fiber orientation (with respect to the long axis of the specimen; Fig. 1) [11]. We are particularly interested in the mechanical behavior of the annulus fibrosus (AF) from the intervertebral disc. The AF is a complex fiber-reinforced tissue with collagen fibers oriented at ±45° with respect to the spinal axis in the inner AF and gradually increases to ±60° in the outer AF. Taken together, the mechanical response of fiber-reinforced soft tissues includes contributions from osmotic pressure, elastic deformation of extracellular matrix, and collagen fiber stretch [12]. While it is thought that the extracellular matrix does not play a significant role in tissue tension behavior [13], it may be important for stress distribution between AF lamellae layers. Therefore, the objective of this study was to evaluate the combined effect of osmotic swelling, fiber orientation, and lamellae structure on tissue mechanics. To do this, we evaluated rectangular test specimens used for uniaxial tension testing and an AF ring structure.

METHODS

Rectangular test specimen models were developed with one, two, or three fibrous layers. Similarly, the AF ring model was developed with one, two, or three lamellae rings. Rectangular models were developed and meshed in Preview (FEBio Package) and had initial dimensions of 2 mm X 10 mm X 0.2 mm for width, length, and layer thickness, respectively [14]. Meshes for AF ring models were generated using a custom algorithm to process published disc geometry data [15]. For all models, each layer had a uniform fiber distribution, and the fiber angles alternated between adjacent layers.

Material coefficients were chosen from our previous model for the AF [11], where the extracellular matrix was described as compressible hyperelastic material: Holmes-Mow (density ρ = 1 g/cm³, Young’s Modulus E = 0.0649 MPa, Poisson’s ratio ν = 0.24, and an exponential stiffening coefficient β = 0.95). The initial solid volume fraction was 0.3. Diffusivities of Na⁺ and Cl⁻ ions were 0.00199 mm²/s and their solubilities were 1 [11]. Fibers were described using an exponential-linear function [14].
For rectangular models, one end was fixed and the other end was free to deform and rotate. The swelling was simulated by increasing the negative fixed charge density from 0 to -100 mmol/L, while the surrounding environment was held fixed to represent 0.15 M PBS. Six cases were simulated, including no fibers (extrafibrillar matrix only) and fiber orientations of 0°, ±30°, ±45°, ±60°, and ±90°. For AF ring models, two cases were considered: one is no fiber case and the other was a consistent fiber orientation (±30° with respect to the transverse plane). The no fiber cases act as negative controls to understand the role of collagen fibers in combination with tissue swelling. Volume ratio was calculated as the volume in the deformed condition divided in the volume in reference (Ref.) configuration. Normalized length (Norm. Length) was calculated as the length after swelling normalized by the length in reference configuration.

RESULTS
For all ‘no fiber’ cases (both rectangular and AF ring models), osmotic loading resulted in a uniform 42% increase in tissue volume (Fig. 2A & 3A). For single layer tissues, the inclusion of fibers decreased the uniform swelling (36% increase in volume with respect to the reference configuration; Fig. 2A). Furthermore, bulk fiber orientation rotated perpendicular to the direction of the collagen fibers (Fig. 2A). Similar to our previous work, osmotic swelling increased tissue elongation in the 0° case (1.17 normalized length), while tissue elongation along for the 0°<θ<90° cases followed a sigmoidal behavior ($\varepsilon_{xy} = -0.89 \times \sin(-20)$; Fig. 2C).

Fig. 2: (A) Volume change due to tissue swelling for one-layer rectangular models. (B) Normalized length vs fiber orientation angle. (C) xy strain vs fiber orientation angle.

Adding a second layer to rectangular specimens did not alter the tissue-swelling ratio when fibers were oriented perpendicular or parallel to long axis of the tissue specimen (Fig. 2A vs. 3A – 0° and 90° models). In contrast, a cross-ply fiber orientation reduced volume swelling (30% volume increase versus 36%) and caused tissue rotation with a twist angle, $\alpha$ (Fig. 3A). The pitch, P, was defined as $P = l/\alpha \times 360°$, where $l$ is the current tissue length. The pitch reached its minimum value when fibers were oriented between ±40° and ±45° (Fig. 3B). Three-layer rectangular models basically repeated observation from our previous study, where tissue length decreased when fiber orientation was between ±45° and ±90° (Fig. 1).

Finally, to understand tissue swelling under in situ boundary conditions, we modeled AF rings. The AF ring model experienced a 30% increase in volume with swelling. There was a decrease in the lateral dimension (long axis of the disc) and an increase in the anterior-posterior direction (short axis of the disc; Fig. 4).

DISCUSSION
In this study, we investigated the deformation of fiber-reinforced soft tissue caused by the combined effect of swelling and fiber orientation. Collagen fibers are stiff under tension and buckles under compression, while GAGs absorb water molecules, resulting in anisotropic tissue swelling. The resistance from stiff collagen fibers combined with anisotropic swelling results in a residual stress state under equilibrium conditions.

While our objective here was to understand AF mechanics with tissue swelling, the fiber architecture of the single fiber control models (0° and 90°) is similar to tendons and ligaments. Interestingly, a single group of fibers resulted in more tissue elongation along the length of the fibers, which would result in higher pre-stress of the collagen fibers (Fig. 2A – ‘no fiber’ model versus 0° model). However, the volume ratio was lower in model simulations with fibers, suggesting that fibers limited matrix expansion in the transverse direction.

Alternating the fiber orientation between lamellae, as observed in native AF tissues, resulted in a smaller increase in a volume ratio. However, the tissues continued to experience a change in length (Fig. 1 and 3A), suggesting fiber re-alignment during swelling. Previous studies have noted aligned fiber realignment during tensile loading, with changes in fiber realignment behavior during degeneration [15].

Taken together, tissue swelling alters stresses transferred to collagen fibers in adjacent lamellae. Understanding how this transfer of stresses alters disc joint mechanics is not trivial; however, the response from the AF ring model suggests that AF fiber architecture with swelling acts to create a more circular structure (Fig. 4). In the healthy native disc, this response is counter balanced by the geometry and intradiscal pressure from the nucleus pulposus.

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REFERENCE