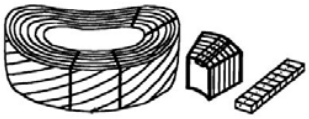
Role of Biomolecules on Cross-Ply Mechanics of the Annulus Fibrosus

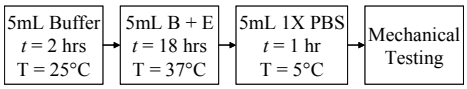
[1]Isaacs, JL; [2]Vresilovic, E; [3]Marcolongo, M
[1]Drexel University, Department of Mechanical Engineering, [2]Pennsylvania State University, Department of Orthopedic Surgery [3]Drexel University, Department of Materials Science and Engineering

Statement of Purpose: Intervertebral discs (IVDs) stabilize and maintain alignment of the spine, allow movement between vertebrae and are responsible for load distribution and energy absorption under loading. IVDs are composed of three distinct parts: cartilaginous endplate, annulus fibrosus (AF), and nucleus pulposus (NP). The AF is composed of 25 unidirectional collagen fiber lamellae. Consecutive lamellae run in opposite directions with collagen fibers at an angle of 60° to the long axis of the spine. Collagen fibers may also bind the lamellae together as elastin fibers pass radially from one lamella to the next [1]. Elastic and collagenous elements are embedded in a hydrated glycosaminoglycan matrix. At lamellar interfaces, the arrangement of elastic fibers forms discrete connections between collagen bundles in consecutive lamellae. There is also evidence that elastic fibers form 'cross-bridges' to connect collagen bundles in non-consecutive lamellae [2]. Understanding how each of these components relate to the mechanical characteristics of individual lamellae is important in creating a successfully engineered AF. To understand the mechanical attributions of these macromolecules we have previously [3] developed a single lamellar model under different conditions to test annulus lamellar biomechanics in plane to the collagen and elastin fiber directions using a micro-mechanical test protocol. In this work, using the same technique, we investigate the degenerative mechanics that occur inter-lamellar (perpendicular to the plane containing the collagen fibers across the layers).

Methods: Whole IVDs were removed at the endplates using a scalpel. Discs were hemi-sected mid-sagittally and inter-lamellar (150 μm thick) human lumbar annulus fibrosus (AF) samples were cut using a Leica 3050S cryostat with a tungsten carbide blade. The samples were placed into four groups and digested using the following protocol.



Group	pH	Enzyme	Buffer
CON	7.4	–	1x PBS
PG	8.0	Chondroitinase ABC (0.125U/5mL)	0.02% BSA, 50mM TRIS, 60mM Sodium Acetate
COL	7.4	Collagenase (10U/5mL)	50mM TES, 2mM $CaCl_2$
ELA	8.5	Elastase (10U/5mL)	0.1M TRIS



Samples were pre-strained to ε = 0.05 then with an average strain rate of 0.02 mm/sec were tested until failure. Micromechanical stress-strain curves were compiled and concurrent video streams captured morphological damage mechanics. Engineering stress (force divided by undeformed cross-sectional area at equilibrium hydration) vs. strain (the instantaneous length divided by the starting length) was plotted for each specimen. Initial modulus (tangent to the toe region of the response), ultimate modulus (tangent to the linear region of the response) and failure strain were calculated from the raw data.

Results:

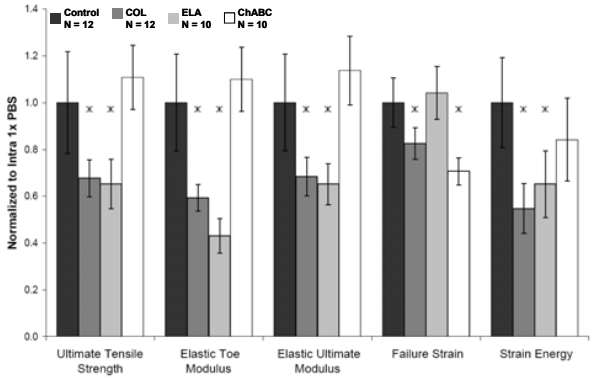


Figure 1: Mechanical properties of enzymatically-treated cross-ply slices of the AF. *p<0.05 from 1X PBS.

Conclusions: The collagenase-treated group reduced the ultimate tensile strength by on average 70% from the non-degraded group (1X PBS), indicating the importance of collagen to the tensile strength of the annulus fibrosus. The Elastase-treated groups resulted in a reduction of all properties except failure strain. This suggests that elastic fibers function to guide and restrain the deformation of the collagen matrix and that on their removal, the collagenous elements may play an even more dominant role in the tissue mechanics. A limitation is that AF mechanical properties in the radial orientation have been shown to be radially heterogeneous [4].The fact that radial position was not strictly controlled may have therefore contributed to the variances. The properties described here provide insights into the inter-lamellar mechanical behavior and how the separate molecular components that interact with each other. This may be important in diagnosis, prevention and repair of debilitating IVD disorders and manufacturing of tissue-engineered AF.

References: [1] Yu, J; CP Winlove; S Roberts; JP Urban. J Anat. 2002; 201:465-475. [2] Yu, J; U Tirlapur; J Fairbank; P Handford; S Roberts; CP Winlove; Z Cui; JP Urban. J Anat. 210:460–471, 2007. [3] Isaacs, JL; E Vresilovic; M Marcolongo. ORS Poster 2010. [4] Fujita, Y; NA Duncan; JC Lotz. J Orth Res. 15:814–819, 1997.

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