

Regional Structure-Function Relationships of Lumbar Cartilage Endplate

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INTRODUCTION: Low back pain is a leading cause of disability and socioeconomic burden worldwide. This condition increases in prevalence with age, where degenerative changes to the intervertebral disc (IVD) have been implicated as an etiologic factor in pain development. Cartilage endplate (CEP) tissue, comprising a thin section of hyaline cartilage on the superior and inferior interfaces of the IVD, has been previously considered to play a role in both regulating the transport of nutrients into the disc and facilitating fluid pressurization of the joint under abnormal loads. It has been observed that the collagen-fiber organization of the IVD extracellular matrix is spatially heterogeneous (featuring concentrically organized fibers in the peripheral sections of the joint and relatively disorganized fiber alignment in the center). The region-dependent CEP biochemical composition has also been acknowledged to contribute to region-dependent CEP solute diffusion properties. However, to our knowledge, structure and biomechanical function relationships which describe how variations in CEP-bone interface morphology and biochemical composition contribute to regional differences in CEP tissue biomechanical properties are still poorly understood. The objectives of this study, therefore, were to 1) spatially quantify biomechanical properties (aggregate modulus, hydraulic permeability, and swelling pressure) in human cadaveric CEP using our previously-established confined compression technique; 2) correlate regionally obtained biochemical compositions (porosity and sulphated-glycosaminoglycan or sGAG content) of CEP tissue with biomechanical data from the same specimens; and 3) assess the CEP-bone interface morphology and disc collagen fiber insertion structure in each endplate region through label-free multiphoton microscopy.

METHODS: Six fresh frozen human cadaver lumbar spines (motion segments ranging from L1 to L5 vertebrae) were procured from a local tissue bank (We Are Sharing Hope SC) under institutional approval³. Segments were opened in the midplane of the IVD disc with a sterile surgical scalpel, and cylinder-shaped specimens were removed from segment halves in the central, lateral, anterior, and posterior regions (n=8/region) using a 5mm diameter corneal trephine. A sledge microtome (Leica SM2400) was used to trim the superior and inferior sample surfaces flat, removing bone first and then disc tissue, such that each sample had an approximate thickness of 0.7mm (consistent with histological observations). Confined compression was performed on the resultant specimens using a dynamic mechanical analyzer (TA Instruments Q800-DMA), following a previously established technique. This included a stress relaxation preconditioning step and two-hour creep displacement test. CEP swelling pressure was measured directly from preconditioning, while the creep displacement of the CEP allowed for simultaneous measurement of aggregate modulus and hydraulic permeability following a biphasic curve fitting approach. Porosity was also assessed using established techniques based on Archimedes principle, using a density determination kit (Sartorius YDK01) and analytical balance. After lyophilizing the tissues for a period of one week, the sGAG content of each specimen was assessed using a BlyscanTM assay kit. Fiber structures of the CEP were examined by label-free multiphoton microscopy of four additional sagittal cut specimens taken from each CEP region, where an Olympus FV 1200 microscope was used to image a 3 x 1 mm area of each interface. The collagen second harmonic generation (SHG) signal (845nm excitation, 420-460nm range of detection) and collagen auto-fluorescence (740nm excitation, 420-460nm and 575-630nm ranges of detection) were imaged in two steps for a combined three channels. Quantitatively, a linear mixed effects model accounting for within-donor covariation was used to determine regional variations in the cadaver tissue properties. Additionally, Pearson correlation tests were performed to understand relationships between the measured biomechanical and biochemical properties across all regions. Statistical findings were considered significant at the $\alpha < 0.05$ level.

RESULTS: Figure 1 demonstrates that aggregate modulus is higher in the peripheral endplate compared with central endplate, where the highest modulus was observed in the anterior CEP. Although no other significant variations were detected, there appears to be larger permeability, porosity, and sGAG content in the central CEP relative to peripheral endplate. Figure 2 shows that both aggregate modulus and hydraulic permeability are significantly correlated with porosity, where permeability was shown to be positively correlated, and aggregate modulus negatively correlated. Multiphoton microscopy of the CEP regions (Figure 3) suggests that disc collagen fibers anchor deep into the endplate forming a transitional layer with the CEP. These images also illustrate that peripheral endplate fibers have a more striated and angled insertion pattern when compared with central endplate fibers which have no clear directionality.

DISCUSSION: These findings demonstrate that biomechanical properties, in addition to previously studied solute diffusion properties, are regionally dependent in the endplate, where higher aggregate modulus in the peripheral regions show possible spatial fortification of the CEP against compressive bending loads. The organized insertion of transitional fiber bundles into peripheral endplate regions, in particular the anterior endplate, support this understanding. The correlations between the CEP porosity and both the aggregate modulus and hydraulic permeability suggest that the porosity is a dominant indicator of the CEP viscoelastic properties.

SIGNIFICANCE/CLINICAL RELEVANCE: This work helps to draw attention to the endplate tissue as a composite biological material, with heterogeneously mapped biomechanical properties, composition, and structure. This can benefit baseline understandings of the IVD interface biomechanics, as well as guide future studies examining degenerative or trauma related mechanisms of interfacial damage.

ACKNOWLEDGEMENTS: The authors would like to thank NIH/NIGMS COBRE: South Carolina Translational Research Improving Musculoskeletal Health (SC TRIMH; P20GM121342) and the Cervical Spine Research Society for supporting this project.

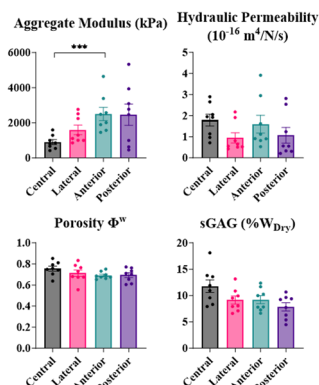


Figure 1. Regional variations in endplate biomechanical properties and biochemical compositions (error bars reported as mean \pm SE).

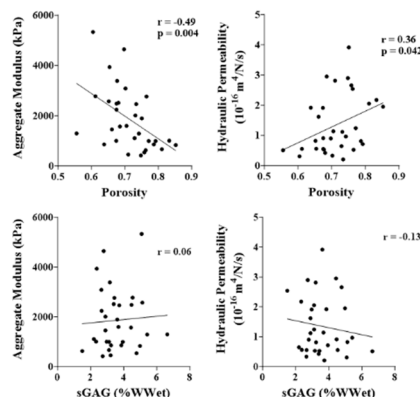


Figure 2. Correlations between endplate biomechanical properties and biochemical compositions.

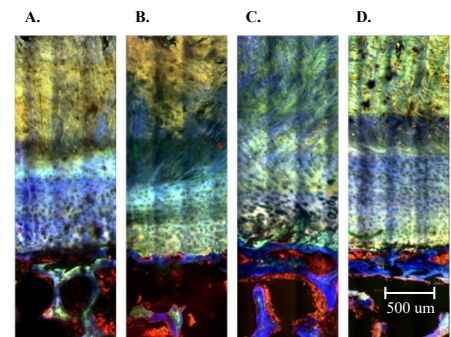


Figure 3. Multiphoton microscopy of the IVD interface from different endplate regions: (A) central, (B) lateral, (C) anterior, (D) posterior.