Radial Variations in Composition and Swelling Properties of Bovine Caudal Discs

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Disclosures: The authors have nothing to disclose.

INTRODUCTION: A variety of animal models are used for spine research, due to the limited availability of human tissue. Bovine caudal discs have been shown to be an ideal animal analog for studying disc biomechanics because of the larger disc area and height, lower inter-specimen variability, and mechanical and biochemical properties that are comparable to healthy human lumbar discs [1-3]. Previous studies showed that mechanical and biochemical properties vary greatly throughout the human disc, although spatial resolution of the compositional analyses in these studies has been limited to a few sites (*i.e.,* nucleus pulposus (NP) versus annulus fibrosus (AF), or inner versus outer AF) [2-7]. To date, radial variation of bovine disc composition has not been characterized, despite extensive use of bovine discs in the field. Thus, the aim of this study was to measure radial variation in biochemical composition of bovine caudal discs from the center of the disc (*i.e.*, NP) to the outer AF.

METHODS: Fresh bovine tails were obtained from local abattoirs (9 spines, age ~18 months). Surrounding musculature and ligaments were removed, and tails were hydrated in 0.15 M saline for 18 hours. Discs were removed from the top two levels (C1-C3) and placed on a freezing stage microtome (-16°C) to prepare ten concentric rings of equal thickness using biopsy punches/scalpel. Each layer was trimmed to produce specimens that weighed ~90 mg. Specimen wet weight was measured, then each sample was freeze-dried for 48 hours before re-weighing to obtain the sample dry weight (DW). Tissues were digested overnight in proteinase-K and biochemical assays were performed to measure sulfated glycosaminoglycan (s-GAG), collagen, and DNA contents. Water content was calculated as the difference between the wet and dry weights divided by the wet weight.

Eight additional discs were used to evaluate radial variation in swelling properties. Tissue sections were prepared, as described above, and the wet weight of each fresh tissue section was recorded. Specimens were immersed in 10 mL of 0.15 M saline and allowed to free swell for 16 hours. The swelling ratio was calculated as the percent change in water content after free swelling. s-GAG content was measured for tissue digests and swelling solutions to calculate the percentage of s-GAGs leached into the saline bath during swelling.

RESULTS: Changes in water, s-GAG, and collagen content were continuous and smooth from center of the disc to the outer AF (Fig. 1A-C). Although DNA content was relatively constant within the NP, there was a ~3-fold increase in DNA from the inner AF to the outer AF (Fig 1D). Similar to s-GAG content, swelling ratio gradually decreased from the disc center to the outer AF (Fig. 1E), and the layer-average swelling ratio was strongly correlated with s-GAG content (Pearson's correlation, r = 0.95). There was also a gradual decrease in s-GAGs released into the surrounding solution from the center of the disc to the outer AF (*i.e.*, 242 mg/g DW in the NP to 12 mg/g DW in the outer AF, Fig. 1F). Greater s-GAG leakage was observed in the NP than the AF, such that the measured tissue s-GAG corresponded to ~50% of the total s-GAG in the NP and ~70% of the total s-GAG in the AF.

DISCUSSION: Biochemical properties in the disc varied greatly as a function of radial location. Changes in disc composition between the NP and AF were gradual and continuous, rather than a distinct separation, as observed with intradiscal pressure [8]. Although some computational studies account for differences in structural and biochemical properties of the NP and AF, heterogeneity within tissue subcomponents has often been neglected for simplicity [9-11]. The findings reported here are important for computational studies that aim to accurately describe disc mechanics under complex loading. Furthermore, the heterogeneous biochemical distribution partially explains wide variances in tissue composition reported in the literature. Interestingly, we observed a drastic increase in DNA content between the NP/AF transition zone and outer AF, agreeing with computational studies that reported restricted nutrient diffusion at the NP/AF interface [12], due to nutrient diffusion occurring either through the healthy endplate or outer AF. Our DNA results suggest that there may be more sufficient nutrient diffusion from the outer AF than the center of the endplate (*i.e*., NP or Layer 1). Swelling ratio was strongly correlated with s-GAG content, confirming the significant role of proteoglycans in tissue swelling. Consistent with observations in human discs, a greater percentage of s-GAG leaked from NP explants during swelling than from AF explants [13], likely due to differences in porosity, collagen architecture, and, possibly, difference in the size of proteoglycan molecules [13]. There is a close relationship between compressive mechanics and GAG content [2]; therefore, s-GAG leakage during hydration should be considered when analyzing mechanical behaviors with respect to degeneration, as degenerative discs will likely lose a smaller percentage of s-GAGs.

SIGNIFICANCE: Bovine caudal discs exhibit highly heterogeneous disc composition and swelling properties. Values reported here can be used to predict disc composition at any location within the disc, and to develop more sophisticated computational models that accurately consider local tissue swelling behavior and disc composition heterogeneity.

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ACKNOWLEDGEMENTS: This work was supported by NSF CAREER Grant (1751212).

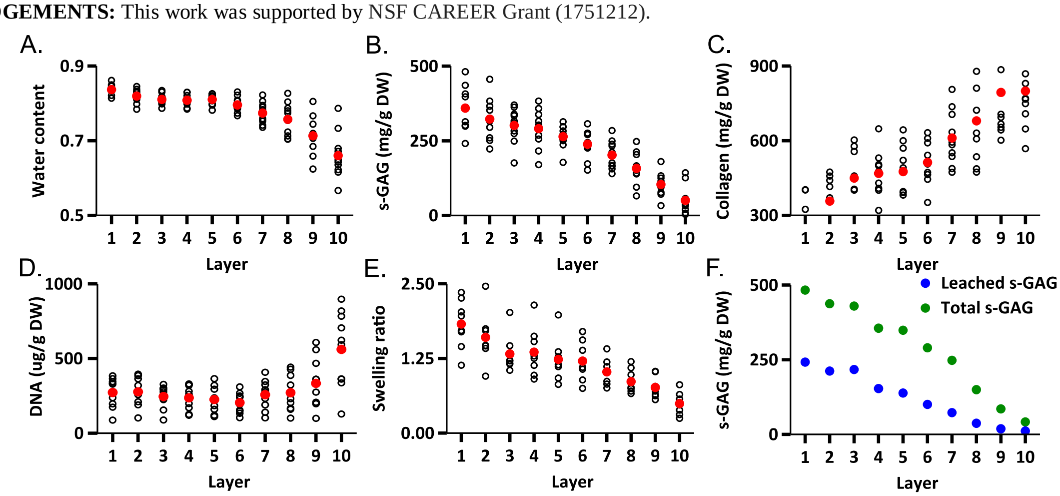

Figure 1: A-D) Biochemical composition and E) swelling ratio from the center of the disc (Layer 1) to the outer AF (Layer 10). Black circles = individual specimen data points; red circles = average data for each layer. F) Layer-average total s-GAG (green) and layer-average leached s-GAG (blue).