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The region-dependent biomechanical and biochemical properties of bovine cartilaginous endplate

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ABSTRACT

Regional biomechanical and biochemical properties of bovine cartilaginous endplate (CEP) and its role in disc mechanics and nutrition were determined. The equilibrium aggregate modulus and hydraulic permeability between the central and lateral regions were examined by confined compression testing. Biochemical assays were conducted to quantify the amount of water, collagen, and glycosaminoglycan (GAG). The equilibrium aggregate modulus of the CEP in the central region (0.23 ± 0.15 MPa) was significantly lower than for the lateral region (0.83 ± 0.26 MPa). No significant regional difference was found for the permeability of the CEP (central region: $0.13 \pm 0.07 \times 10^{-15}$ m^4/N s and lateral region: $0.09 \pm 0.03 \times 10^{-15}$ m^4/N s). CEPs were an average of 75.6% water by wet weight, 41.1% collagen, and 20.4% GAG by dry weight in the central region, as well as an average of 70.2% water by wet weight, 73.8% collagen, and 11.7% GAG by dry weight in the lateral region. Regional differences observed for the equilibrium aggregate modulus were likely due to the regional variation in biochemical composition. The lateral bovine endplate is much stiffer and may share a greater portion of the load. Compared with the nucleus pulposus (NP) and annulus fibrosus (AF), a smaller hydraulic permeability was found for the CEP in both the central and lateral regions, which could be due to its lower water content and higher collagen content. Our results suggest that the CEP may block rapid fluid exchange and solute convection, allow pressurization of the interstitial fluid, and play a significant role in nutrient supply in response to loading.

1. Introduction

The degenerative changes in the intervertebral disc (IVD) and disc herniation have been implicated as possible primary etiologic factors for low back pain (LBP) (Deyo, 1986; Luoma et al., 2000; Morinaga et al., 1996; Videman and Nurminen, 2004). A thin layer of hyaline cartilage endplate (CEP), which surrounds the cranial and caudal surfaces of the central regions of the disc, is critical for disc health by helping to resist disc herniation or tears (Harada and Nakahara, 1989; Lama et al., 2014; Rajasekaran et al., 2013). Abnormal loading may cause CEP damage and internal disc disruption, which then may initiate disc degeneration or the herniation processes (Adams and Hutton, 1982; Callaghan and McGill, 2001; Veres et al., 2010). CEP damage was also found to be strongly correlated with the onset of innervation in the bony endplate layer (interface between vertebral body and CEP) (Fields et al., 2014a). Mechanical and chemical stimuli may further sensitize the nerves under this pathological condition leading to the main cause of LBP (Cox, 1990; Koike et al., 2003; Lotz and Ulrich, 2006). Therefore, damage to the CEP may play an irreplaceable role in the progression of disc degeneration and LBP.

Due to the avascular nature of the disc, the nutrients that disc cells require for maintaining disc health are supplied by blood vessels at the margins of the disc. Two possible pathways for nutrient transport into the IVD include through the CEP as well as through the perianular region of the disc (Nachemson et al., 1970). Most *in vivo* studies (using animal models) and *in vitro* studies suggest that the endplate route is the main pathway for exchange of fluid and solutes between the nucleus pulposus (NP) [and inner annulus fibrosis (AF)] and surrounding blood vessels (Holm et al., 1981; Maroudas et al., 1975; Nachemson et al., 1970; Ogata and Whiteside,

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1981; Urban et al., 1982). As a result of calcification, the water content/porosity of the CEP as well as its transport properties (lower hydraulic permeability and solute diffusivity) would be dramatically decreased (Gu and Yao, 2003; Gu et al., 2004; Roberts et al., 1993). The transport of nutrient solutes and metabolites such as glucose/oxygen inflow and lactate outflow may be hindered to a greater extent in a disc with a calcified CEP, than in a disc with a normal CEP (Roberts et al., 1996; Wu et al., 2013). By contrast, a degenerated or damaged CEP may have an inverse effect due to the loss of proteoglycan or small lesions in its extracellular matrix (ECM) (Johnstone and Bayliss, 1995; Rajasekaran et al., 2004; Urban and McMullin, 1988). It could "open up" the channels and accelerate the inflow of cytokines or enzymes, which have deleterious effects on the behavior of the disc cells (Koike et al., 2003; Roberts et al., 1996). Therefore, knowledge of the mechanical and transport properties of the CEP is crucial for understanding the mechanisms of disc mechanics, nutrition, and degeneration.

A previous study suggests that the average equilibrium tensile modulus of normal human CEP is similar to that of femoral articular cartilage (AC) in adults (Fields et al., 2014b). The compressive modulus of the baboon CEP is also found to be within the same range as that in bovine and human AC, while the hydraulic permeability of the baboon CEP is two orders of magnitude higher than that of human AC (Setton et al., 1993). By contrast, the permeability coefficient of human CEP is found to be about 1/3 and 1/10 of that in human AF and cartilage (Maroudas et al., 1975). Compared with NP and AF tissue, previous studies also indicated that CEP has a unique 3D morphology, inhomogeneous biochemical composition, and regional dependent solute diffusion rate (Fields et al., 2014b; Rajasekaran et al., 2004, 2008, 2010; Roberts et al., 1989, 1996). Therefore, we hypothesized that the biphasic viscoelastic properties of the CEP may also be regional dependent. Due to the scarcity of normal human tissue as well as previous studies have shown that the bovine is an appropriate animal model to study human IVD biomechanics and biology, healthy bovine CEP was chosen for this study (Demers et al., 2004; Oshima et al., 1993). Specifically, we will determine the compressive aggregate modulus, swelling pressure, and hydraulic permeability of the bovine cartilage endplate in the central and lateral regions, and further characterize its related biochemical composition.

2. Materials and methods

2.1. Mechanical characterization

Bovine (2–3 years old; male) cartilaginous endplates were harvested at both the superior and inferior surfaces between C2–3 and C3–4 from bovine tails obtained from a local slaughterhouse within 4 h of death. First, the discs were opened through the median plane with a scalpel, then using an 8 mm corneal trephine, cylindrical disc tissue plugs including the bone were obtained from both the central and lateral regions of the bovine disc (Fig. 1A and B). The plugs were immediately wrapped in a plastic membrane and gauze soaked in a normal saline solution with protease inhibitors and stored at −80 °C for less than one week.

Before mechanical testing, the disc plugs were thawed at room temperature for 10 min and care was taken when separating the disc tissue from the bone under a dissecting microscope. A sledge microtome was used to carefully remove the NP or AF tissue (relatively more transparent than the CEP tissue) from the CEP to prepare disc shaped samples with an average height of 0.6 mm. The thickness of the CEP has been found to be approximately 0.6 mm in our histological study as well as in the literature (Roberts et al., 1989). The sample was then punched by a 5 mm

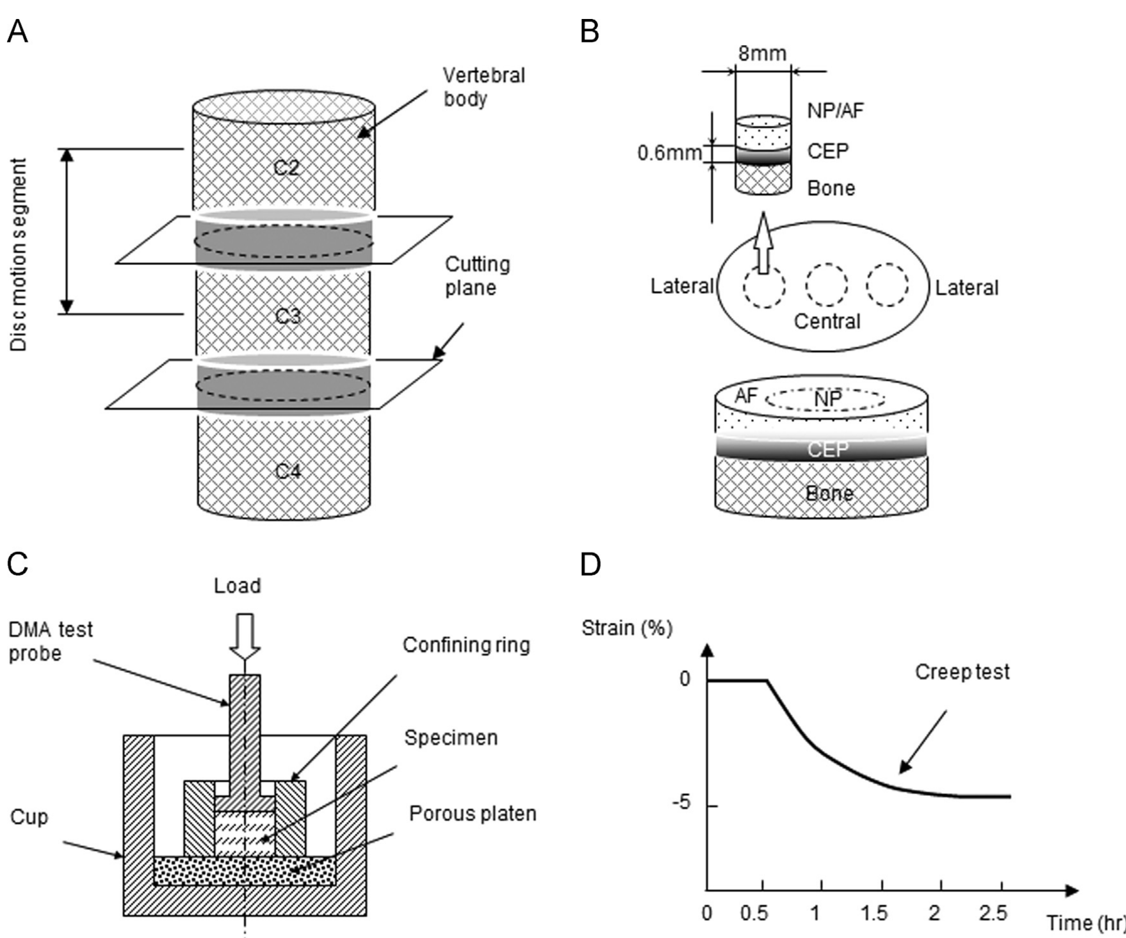

Fig. 1. (A) Disc motion segments in the bovine tail used in this study. (B) Schematic of specimen preparation. The region and size of test specimens are shown. (C) Schematic of the confined compression test chamber. (D) Schematic of the mechanical testing protocol.