The Targeted Cleavage of Proteoglycans Alters the Viscoelastic Properties of Bovine Articular Cartilage

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INTRODUCTION Osteoarthritis (OA) is a debilitating disease associated with the degeneration of articular cartilage that leads to pain and loss of mobility with significant economic costs [1,2]. OA initiates with alterations in the matrix composition including the degradation of proteoglycans and followed by gradual loss of the mechanical function of the cartilage [3]. Cartilage is a multiphasic viscoelastic material whose proper mechanical function is dependent on its matrix composition [4]. Thus alterations in the proteoglycans in cartilage may play a role in the tissue’s mechanical performance. The objective of this study is to investigate the contributions of proteoglycans on the elastic and viscoelastic behavior of bovine articular cartilage by using a novel dynamic microindentation system.

METHODS 6 Bovine tibial plateaus (3 mon. old) with intact cartilage were obtained and divided into two groups. The treatment group (n=3) was first tested mechanically to determine the native mechanical behavior prior to incubation. The samples were incubated in cathespin D, which enzymatically cleaves the glycoaminoglycans [5] at 2.5U/mL for 120 mins at 37°C and immediately assessed mechanically. Finally the samples were incubated for an additional 120 mins at the same conditions (total incubation time = 240 mins) and then mechanically evaluated. The control group was subjected to the same time course treatment but was incubated in a phosphate buffered saline (PBS) solution.

Mechanical testing was performed using a microindentation system using a 1.47 mm diameter cylindrical punch tip [6]. 3 sites/sample were indented to a depth of 150 µm at test frequencies of 1 Hz – 20 Hz. From the force-displacement data, the following elastic and viscoelastic parameters were computed: dynamic elastic modulus (geometrically adjusted peak of the force-displacement curve), tan delta (trigonometric tangent of the phase shift between load and displacement), energy dissipation (area enclosed by the force-displacement curve), anelastic deformation ratio (the ratio of the time-dependent deformation to the total deformation). After the incubations, biopsies of cartilage at the tested sites were taken to determine the tissue proteoglycan content as measured by the 1,9-dimethyl methylene blue (DMMB) assay, and collagen content as measured by a hydroxyproline assay. ANOVAs were used to determine the effects of frequency, and incubation time on the parameters of mechanical behavior and tissue composition. Fisher’s LSD tests were used for post-hoc comparisons.

RESULTS The cleavage of proteoglycans by cathepsin D resulted in the increased viscous behavior of the intact articular cartilage as shown by the increasing tan delta with incubation time and indentation frequency (Fig 1; p<0.001). Significant differences were also observed in anelastic deformation ratio (Fig 2; p<0.01) and energy dissipation (p<0.01) in a dose-dependent manner with the frequency. There were no statistically significant changes in the dynamic elastic modulus of the articular cartilage due to cathepsin D incubation (p=0.67), and the changes in matrix composition in DMMB (+8.1%; p=0.65) and collagen (-3.5%; p=0.75) were not statistically significant before and after treatment.

DISCUSSION As a viscoelastic elastic material, bovine articular cartilage exhibited frequency-dependent mechanical behavior, and this frequency-dependent response may be derived from the proteoglycans. Treatment by cathepsin D, which targets the protein backbones of proteoglycans and glycoaminoglycans [5], resulted in minor changes in the matrix composition and elastic modulus of the cartilage. However, cathepsin D resulted in significant changes in the viscoelastic properties including tan delta, energy dissipation, and anelastic deformation ratio. These results suggest that the structural integrity of the proteoglycans may be critical for resisting dynamic loads in cartilage. A novel parameter describing the viscoelasticity of cartilage, anelastic deformation ratio, shows excellent correspondence with tan δ (Fig 3; p<0.001), and may provide additional insights into the mechanical behavior of the tissue. Increased expression cathepsin D in human cartilage have been associated with OA, and it may play a role in the progression of OA [5]. Similar to early OA, the changes in the cartilage mediated by cathepsin D were undetectable in the elastic aspects of the mechanical behavior [7]. The result of this study show that changes in proteoglycan of cartilage can greatly affect its viscoelastic behavior, and parameters such as ADR can effectively characterize these changes.


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