

Repetitive Pure Shear Loading Leads to Permanent Fetal Articular Cartilage Mechanical Changes due to Collagen Fiber Densification and Fiber Alignment

Kangxin Wang, Jill M. Middendorf

Department of Mechanical Engineering, Johns Hopkins University

DISCLOSURES: Wang (N), Middendorf (N)

INTRODUCTION: Collagen fiber orientation, proteoglycan content, and depth-dependent mechanics are essential for cartilage's smooth, durable, low-friction surface. Thus, to repair damaged and arthritic cartilage, researchers have attempted to recreate the mature cartilage structure with varying degrees of success. Understanding the development of the mature cartilage structure from immature cartilage may provide insights. In mature cartilage, mechanical properties vary with depth due to the heterogeneous distribution of collagen fibers and proteoglycans. Collagen fibers in the superficial region are highly aligned, while proteoglycans are more prevalent in the middle zone [1]. Proteoglycans are evenly distributed in immature cartilage, and collagen fibers are randomly oriented. Previous studies investigated cartilage structure and composition in discrete development stages, finding that through development, cartilage starts to create a highly aligned collagen fiber structure in the superficial region with low proteoglycan content, while maintaining the random collagen fiber orientation and high proteoglycan content in the middle region [2]. Previous studies have shown that collagen fibers tend to align parallel to the loading direction at large tensile strain [3], and that repetitive tensile loading can also alter fiber orientation in hydrogel with cells embedded [4]. However, these previous studies have not examined shear loading, a common loading mode in daily joint activity. These previous studies also did not investigate how collagen and proteoglycan concentrations contribute to surface region fiber development, fiber alignment, and densification. Our objective is to determine whether repetitive shear loading can modify the fiber architecture and mechanical properties of immature cartilage, promoting a transition toward a mature cartilage-like structure, and understanding how proteoglycan content influences this behavior.

METHODS: Femoral condyle samples (N = 10) were obtained from fetal bovine joints in the late third trimester. Cylindrical explants (5mm diameter * 3mm height) were prepared from each condyle. Five samples were treated with trypsin for 3 hours to remove proteoglycans, as this treatment fully depletes proteoglycans [5]. The remaining five were left intact. All explants were stained with 14 µg/mL D-TAF for 20 minutes, rinsed with PBS, and bisected into half-cylindrical shapes to create a flat imaging surface. Each sample was mounted between two plates in a Tissue Deformation Imaging Stage (TDIS), with both the articular surface and bone side glued to prevent motion [6]. Both groups were subjected to 1 Hz oscillatory shear loading at a peak shear strain of 3% for 8 hours, followed by a 12-hour stress relaxation period. Mechanical behavior was recorded at 0h, 4h, 8h, and after relaxation using an LSM 700 confocal microscope with a 10x objective. To assess shear modulus changes attributable to the solid matrix, a quasistatic oscillatory strain rate of 0.0125 Hz with the same 3% amplitude was applied. Samples were immersed in PBS with protease inhibitors throughout to maintain hydration. Displacement along the depth was tracked to generate shear stress-strain curves. Our analysis focused on the surface region (0-200 µm), where fiber reorientation and damage are most likely. Key mechanical parameters, such as toe modulus, linear modulus, effective shear modulus, and energy dissipation, were calculated for comparison across loading times and treatments. Picrosirius Red (PSR) staining combined with Fast Fourier Transform (FFT) analysis was used to examine fiber orientation.

RESULTS: Stress-strain curves (Fig. 1: A-B) from the surface region showed clear changes in shape and nonlinearity between 0 and 8 hours in both trypsin-treated and untreated groups. Toe modulus, linear modulus, and effective shear modulus (Fig. 1: C-H) decreased with loading time and did not recover after relaxation, indicating permanent changes. In the intact group, the largest decrease in toe modulus occurred between 4 and 8 hours, while in the trypsin-treated group, it happened within the first 4 hours; other modulus changes were similar between groups. Energy dissipation (Fig. 1: I-J) decreased only in the trypsin-treated group. PSR and FFT analyses (Fig. 2) revealed higher collagen fiber orientation energy after 8 hours in both groups, with greater increases when proteoglycans were removed.

DISCUSSION: Our findings demonstrate that the mechanical modulus of the femoral condyle decreases permanently after 8 hours of oscillatory shear loading due to altered collagen fiber orientation and density for both undigested and trypsin-treated groups. Furthermore, proteoglycan content appears to provide some resistance to collagen fiber reorientation, because earlier changes were observed in the absence of proteoglycans. These changes may be due to the proteoglycan's role in attracting and retaining water, resisting mechanical loading, and maintaining cartilage structure [1]. In our study, the estimated principal strain in the superficial region for the proteoglycan-removed group was of a similar magnitude (10%) to the tensile strains previously observed to cause fiber alignment in hydrogel [4]. This shows that a threshold strain in the superficial zone may be required to cause fiber alignment.

SIGNIFICANCE/CLINICAL RELEVANCE: Repetitive shear loading mimics normal joint use during development; thus, this work shows that repetitive shear loading on immature cartilage may be essential for forming the aligned surface region necessary to ensure healthy joint function in mature tissue.

REFERENCE: [1] D. J. Responde, et al., Crit. Rev. Biomed. Eng. [2] P. Julkunen, et al., Osteoarthritis Cartilage [3] Y. Sasazaki, et al., J. Anat. [4] T.-A. N. Kelly, et al., J. Biomech. [5] P. Raj et al., ACS Sens. [6] T. Wyse Jackson et al. Sci. Adv.

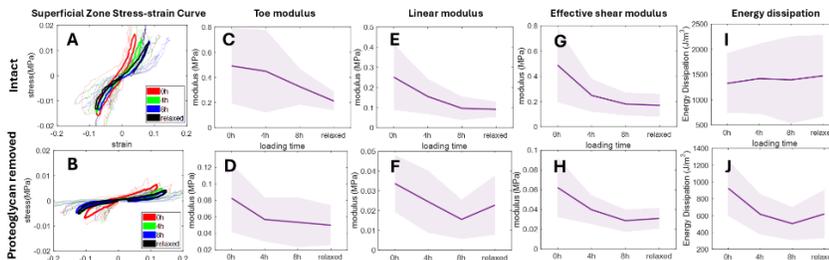


Figure 1: Changes in both the shape and degree of nonlinearity between 0 and 8 hours for both the group (A–B) with trypsin treatment and without the trypsin treatment. Proteoglycan removed group is less stiff comparing to the intact group. There is no recovery observed at the stress-strain curve after relaxation. The toe modulus (C–D), linear modulus (E–F), and effective shear modulus (G–H) decreased over the 8-hour repetitive shear loading for both the intact and the proteoglycan removed group. There is little change of energy dissipation for the intact group (I), while there is a decreasing trend for the proteoglycan removed group (J).

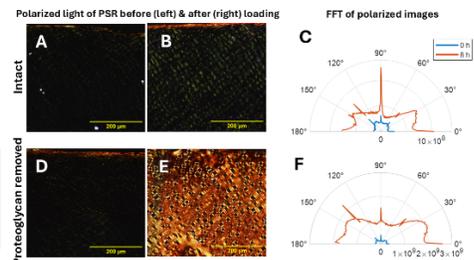
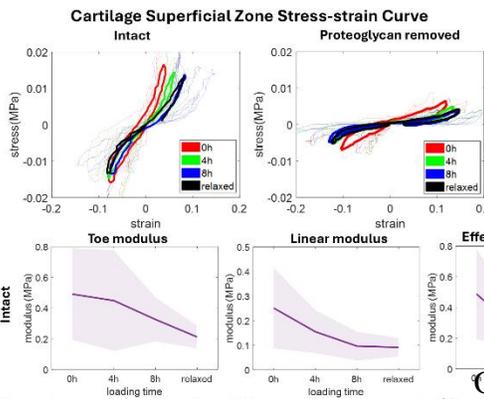


Figure 2: The Picrosirius red stain results under polarized light microscope (A – B: intact group; D – E: proteoglycan removed group) and the orientation energy distribution based on FFT analysis (C: intact group; F: proteoglycan removed group). The orientation energy was increased for both groups, while the proteoglycan-removed group had a more significant increase in orientation energy intensity.



◀ **Figure 1:** Changes in both the shape and degree of nonlinearity between 0 and 8 hours for both the group with trypsin treatment and without the trypsin treatment. Proteoglycan removed group is less stiff comparing to the intact group. There is no recover observed at the stress-strain curve after relaxation.

▼ **Figure 2:** The toe modulus, linear modulus, and effective shear modulus decreased over the 8-hour repetitive shear loading for both the intact and the proteoglycan removed group. There is little change of energy dissipation for the intact group, while there is a decreasing trend for the proteoglycan removed group.

