INTRODUCTION: Articular cartilage plays an essential role in joint health by providing articular surface lubrication and load transmission across articular surfaces. The health and integrity of the cartilage’s extracellular matrix (ECM) is maintained by chondrocytes, the cells within articular cartilage, which are responsible for synthesizing structural macromolecules. The well-documented viscoelastic behavior of cartilage, such as hysteresis under cyclic loading, may provide insight into the functional demands of the tissue and cells. For instance, it has been shown that the strain rate-dependent viscoelastic response of cartilage can substantially reduce periodic alterations of tissue deformations during cyclic compressions [1]. However, neither theoretical nor experimental studies have fully addressed the viscoelastic responses of chondrocyte deformations in situ under dynamic mechanical loading [2-4]. Specifically, experimental studies using isolated chondrocytes embedded in agarose gel [5] have shown that the viscoelastic response of chondrocytes is significantly different from that of articular cartilage, highlighting the need for further investigation of the viscoelastic properties of chondrocytes in situ [6].

METHODS: Sample preparations: Articular cartilage and its subchondral bone were extracted from the femoral groove of skeletally mature swine on the day of slaughtering. Cylindrical cores (diameter = 6.4 mm) were extracted from the harvested osteochondral blocks (N = 4). Fluorescein conjugated dextran (Alexa, excitation: 488nm) was suspended in DMEM (Dulbecco’s Modified Eagle’s Medium, Gibco, OR, USA) at a concentration of 6.7 mg/ml (2.2 mM). The osteochondral cores were incubated in the dextran solution for 4-10 hours at 4°C prior to mechanical testing and confocal imaging.

Single stress relaxation test: A single static compressive load (10% nominal tissue strain; 20 min stress relaxation time) was applied to the surface of the osteochondral blocks using a custom-designed indentation system at a speed of 49.5 µm/s [6] (Fig. 1A). Confocal image sections were recorded before loading; at 2 and 17 minutes of relaxation; and at 30 s and 1.3, 5, 7, and 10 min after removal of the load.

Three cyclic compression test: Then, a series of three compressive load ramps (10% strain, 3%/s held for 2 min each) were applied to the articular surface (Fig. 1B). Confocal scans were taken before loading, during each loading phase, during each unloading phase, and following loading as outlined in the single stress relaxation test above (Fig. 1B).

Dynamic Cyclic Compression Test: The final test consisted of a half-sinusoidal compressive load (13% strain) applied for 100 cycles at 0.35 Hz (Fig. 1C). Confocal scans were taken before loading and after loading as explained for the tests above. Total tissue strain, local tissue strain (denoted as ECM), and compressive cell strain were analysed to quantify the viscoelastic responses of each component.

Data analysis: Tissue, ECM, and cell strains at different experimental time points were compared to the unloaded state using paired t-tests (SYSTAT 12.0). The level of significance was set at α = 0.05. Results are presented as means ± standard deviations.

RESULTS: Single stress relaxation test: After 17 minutes of static loading, local ECM strains were -59 ± 4% and average compressive cell strains were -38 ± 8%. The cartilage recovered to its original shape and chondrocytes to their original height 5 minutes after load removal (p = 0.03 and p < 0.001, respectively; Fig. 1A).

Three cycle compression test: Local ECM strains and compressive cell strains remained similar across the three loading cycles (p > 0.25 and p > 0.5, respectively). Tissue and cells never fully recovered during the 2 minute unloading phase (p = 0.02 and 0.04, respectively; Fig. 1B). In the 10 minute recovery period after loading, the cartilage tissue was almost fully recovered (-0.6 ± 0.3%). Similarly, local tissue strain recovered fully within 7-10 min (p = 0.027), and compressive cell strain was fully recovered within 5-7 min (p = 0.008, Fig. 1B).

Figure 1: A: Single stress relaxation test B: Three cyclic compression tests C: Dynamic cyclic compression test (P < 0.05; * tissue, * ECM, + cell).

DISCUSSION: The results of our study are in contrast with findings from isolated chondrocytes embedded in agarose gel, which recovered immediately and nearly elastically following mechanical loading [2]. The extracellular matrix also showed a highly viscoelastic response following a single static compression load. Therefore, we speculate that the viscoelastic response of chondrocytes in situ is associated with the viscoelastic behaviour of the extracellular matrix environment. Our results are consistent with a previous study in the intact mouse tibia-femoral joint [5], where it was found that chondrocytes deform rapidly upon loading but take minutes to recover shape and volume upon unloading. This result suggests that cell deformations recover very little (if at all) in the unloading phase of cyclic cartilage compression, thereby minimizing shape changes and volume fluxes of chondrocytes during cyclic loading conditions which are common in everyday movements, such as in the loading of lower limb joints during locomotion. This result also implies that if cell deformations are related to signaling pathways, such signaling would primarily occur at the beginning of cyclic loading and might be quiescent for subsequent cycles.

SIGNIFICANCE: The findings of our study are important for gaining an understanding of the basic mechanics of articular cartilage and chondrocytes in vivo and during physiological loading conditions, and how these mechanics might relate to cell signaling and adaptive/degenerative responses of joints. Our results might also provide new insights into how chondrocytes may be optimally stimulated in an engineered cartilage in order to mimic in vivo conditions.