Cyclic Loading Decreases Stiffness and Causes Surface Damage in Articular Cartilage

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INTRODUCTION: Articular cartilage is a connective tissue that provides a low-friction, wear-resistant surface in synovial joints [1]. Cartilage is comprised of a type II collagen network surrounded by a hydrated proteoglycan (PG) matrix [2]. Osteoarthritis (OA) is the breakdown of articular cartilage. Knee OA affects more than 20 million people in the US alone, making it an important societal burden [3]. Obesity and heavy manual labor as well as advancing age increase the risk of OA, suggesting that this disease is mechanically mediated [4]. To evaluate the link between mechanical loading and damage that results in OA, previous studies have investigated cartilage degradation following the creation of an initial crack and observing the propagation via cyclic loading [5, 6] or have cyclically loaded cartilage without an initial crack [7-11]. These studies have found that repetitive cyclic loading can propagate an initial crack or create new surface damage [5-6,8]. Mechanical changes following cyclic loading include decreased tensile modulus prior to any evidence of surface damage [10], decreased effective stiffness [11], reduced relaxation time constant, and reduced peak and equilibrium forces [7]. However, the effect of cyclic loading on properties that reflect the integrity of the cartilage matrix, including linear modulus, energy dissipation, and thickness, is not fully known. The aim of this study was to characterize the response of articular cartilage to repetitive cyclic loading. The hypothesis was that an increased number of cycles would decrease the linear modulus, energy dissipation, and thickness; and would increase surface damage.

METHODS: Full-thickness, 4-mm diameter osteochondral cores were removed from porcine patellae (5-6 months old, 2 animals, gender unknown and assumed random) using a scalpel and biopsy punch. Samples were kept hydrated in phosphate-buffered saline (PBS) with protease inhibiters (PI) throughout sample preparation and mechanical testing. Using a microtome, subchondral bone was trimmed to a thin layer (0.58 ± 0.07 mm). In order to more accurately mimic an *in vivo* environment while testing *in vitro*, samples were loaded in cartilage-on-cartilage contact. All mechanical testing was conducted using a tabletop test machine (TA Electroforce 3230-AT Series III) and custom fixtures. Subchondral bone was attached to the fixtures using a small amount of cyanoacrylate. Cartilage thickness was determined optically using a Phantom V1211 camera (9.149 ± 0.002 μm/pix).

Cyclic loading was conducted to induce damage. Following a 45 N tare load, samples were loaded between -6.0 ± 0.2 MPa and -10.3 ± 0.2 MPa 1st Piola-Kirchhoff stress; stress magnitudes were chosen from previously reported joint contact stress [12]. Samples were loaded to 10,000 or 50,000 cycles (n = 6 samples per condition). Following cyclic loading, samples were unloaded and left in PBS/PI for 2 hours to reach an equilibrium unloaded thickness. Mechanical properties were assessed before and after cyclic loading. Following a 0.5 N tare load, samples were loaded to 10% strain at 1 Hz for 10 cycles. During all mechanical testing, force and displacement data were collected to calculate stress and strain. Data were analyzed using custom MATLAB code. Averages of the last 5 cycles of the 10 cycle compression tests were used to calculate results for each sample, since the mechanical testing was repeatable over these cycles. From each cycle, linear modulus was determined by fitting the loading portion of the stress-strain curve to a line, and energy dissipated per cycle was calculated from the area between loading and unloading stress-strain curves using trapezoidal numerical integration.

Surface damage was assessed by staining samples with India ink both before and after cyclic loading, then imaging the articular surface using a Nikon D300 camera (7.933 ± 0.005 μm/pix) (Fig. 1). Surface damage was quantified by taking the average intensity of the pre- and post-test images using MATLAB. Prior to calculating the intensity, red visible from the subchondral bone was removed by applying the inverse color with the Adobe Photoshop photo filter feature. Filter parameters were held constant across all images.

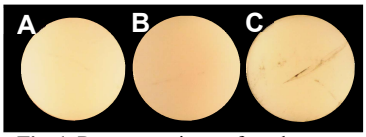

Fig. 1. Representative surface damage images before (A), after 10,000 cycles (B), and after 50,000 cycles (C).

Statistical significance was determined using two-tailed, paired t-tests to compare the effect of cyclic loading on linear moduli, energy dissipation, cartilage thickness, and articular surface intensity (indicating surface damage). Values of $p \leq 0.05$ were considered statistically significant.

RESULTS: Cyclic loading significantly decreased linear moduli, decreased energy dissipation, decreased thickness, and induced significant surface damage (Fig. 2). Linear moduli were significantly lower after 50,000 cycles than after 10,000 cycles. Both 10,000 and 50,000 cycles significantly reduced linear moduli compared to pre-test values. Energy dissipation after 50,000 cycles was significantly lower than both the pre-test value and the 10,000 cycle value. Energy dissipation was not significantly changed from pre-test after 10,000 cycles. Finally, thickness and intensity values were significantly lower after 50,000 cycles compared than after10,000 cycles. Both 10,000 and 50,000 cycles significantly reduced thickness and intensity values compared to pre-test.

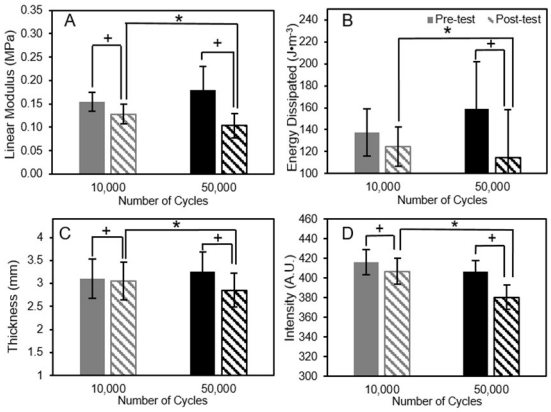

Fig. 2. Linear modulus (A), energy dissipation (B), thickness (C), and surface damage measured by intensity (D). $^{+}p \leq 0.05$ 10,000 vs. 50,000 cycles $*p \leq 0.05$ vs. pre-test.

DISCUSSION: This study demonstrates that repetitive cyclic loading results in tissue softening and surface damage. These results are consistent with previous literature, but are the first to evaluate linear modulus [5,7,9-11]. Loading for 10,000 cycles at or above physiological stress levels at the frequency of normal gait initiates surface damage in cartilage, even in the absence of an initial crack [5,6,8]. That cartilage damage can be initiated by mechanical loading alone suggests that cyclic loading can cause *in vivo* damage.

Decreased thickness suggests irreversible damage to the PG matrix, decreased modulus suggests irreversible damage to the collagen matrix, and increased surface damage likely reflects damage to both PGs and collagen. Cartilage thickness is maintained by the balance between PGs attracting fluid and collagen resisting tension. Thus, a permanent decrease in thickness suggests damage primarily to the PG matrix. At 1 Hz, cartilage behaves approximately as an incompressible solid because fluid does not have time to exude [13]. Therefore, the compressive linear modulus in this study is an indirect measure of collagen stiffness, and the observed decrease in modulus suggests damage to the collagen. Because surface damage indicates a gross disturbance to the cartilage surface, it likely reflects a combination of damage to both PGs and collagen. A previously reported decrease in specimen thickness after cyclic loading was attributed to fatigue damage [11]; the results of this study suggest that fatigue damage includes damage to both PGs and collagen.

In conclusion, this study confirmed that with increased number of cycles, linear moduli, energy dissipation, and thickness decreased, while surface damage increased. Following cyclic loading, cartilage exhibits dose-dependent irreversible damage. Future research will directly assess structural damage and will evaluate additional loading regimes.

SIGNIFICANCE: Understanding mechanisms underlying cartilage damage could lead to the development of prevention and treatment options for OA.

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