Relationship Between OA Cartilage Shear Mechanics and Composition is Dictated at the Articular Surface

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INTRODUCTION: Osteoarthritis (OA) is a chronic disease that affects more than 500 million individuals worldwide[1]. With aging populations, rising rates of obesity, genetics, and joint injury, the prevalence of OA is expected to continue growing globally[1]. Therefore, understanding and defining tissue-level changes between healthy and OA cartilage will be a critical step in slowing disease progression. The most abundant components in cartilage are collagen, especially type II, and aggrecan; any depletion of either or both can impair tissue mechanics. Collagen molecules form a network of fibrils with very high tensile strength, and damage to this network results in loss of tissue integrity and diminished capacity to resist shear strains. Conversely, the aggrecan network consists of highly charged sulfate molecules that generate osmotic stress, attracting water and swelling the tissue. Loss of aggrecan reduces osmotic swelling, making the tissue less capable of recovering from compression and weakening shear properties. As these networks degrade, tissue mechanics weaken, leading to tissue failure. During OA development, ECM depletion occurs gradually, beginning at the surface and progressing to deeper zones, eventually reaching the bone[2]. However, little is known about how matrix depletion affects the depth-dependent shear mechanics of OA human cartilage. We hypothesize that OA cartilage will exhibit a lower shear modulus due to ECM loss compared to healthy cartilage. This study aims to: 1) determine how changes in collagen and aggrecan composition influence the depth-dependent microscale mechanics of OA human cartilage, and 2) evaluate how effectively the grading scale predicts shear mechanics.

METHODS: *Tissue Collection:* Cartilage samples were obtained as operating room discard after approval by an IRB (both healthy and OA tissue) and patient consent. The age range for healthy cartilage (from osteochondral allograft) was 14 to 28 years, while OA samples (from total knee replacement) ranged from 69 to 73 years old. Articular cartilage plugs (6 mm diameter and 2 mm height) were harvested from the femoral condyle (n=7 donors, k=13 samples) for healthy tissue and OA (n=2, k=6), then bisected into halves. Outerbridge scoring was performed prior to tissue dissection, and OARSI grading was applied to histological slides. *Mechanical Testing:* Depth-dependent shear mechanics were tested following an established protocol and custom MATLAB code[3]. Samples were compressed to 15% of tissue thickness and subjected to 1% peak shear strain. Strain data were fitted to a sinusoidal function, and stress was calculated based on the force exerted by the stationary plate on the tissue cross-section. The shear modulus (G*) was derived by dividing the depth-dependent shear strain by a constant shear stress. G* min represents the minimum modulus, while G* plateau was determined once the modulus ceased increasing at depths greater than 1000 µm. *Composition Analysis:* FT-IR spectroscopy was utilized to study the depth-dependent composition of the samples[4]. Polarized light and second-harmonic generation imaging were conducted throughout the tissue depth. Concentrations of aggrecan and collagen were measured using dimethyl methylene blue and hydroxyproline assays, respectively. *Statistical Analysis:* Welch's t-test compared the shear G* min and plateau between healthy and OA samples, while Pearson correlation assessed relationships between grading, mechanics, and biochemical composition using R Studio ($\alpha = 0.05$).

RESULTS: The shear strain of OA samples showed higher than healthy cartilage in the first 400 µm (**Fig. 1A**). The shear modulus of OA samples was lower throughout the tissue depth compared to healthy, both of which increase as the depth goes towards the bone (**Fig. 1B**). We highlighted the first 200 µm to show where the difference of shear modulus is approximately two orders of magnitude (**Fig. 1B**). The healthy cartilage showed higher stiffness compared to OA cartilage in both the shear min (p=0.0071) and plateau (p=0.0074) (**Fig. 1C-D**). The collagen content for the OA tissue was decreased throughout the depth of the tissue compared against the healthy, while aggrecan level followed a similar pattern but the difference was not the same level as the collagen (**Fig. 1E**). Pearson correlation was performed only on the OA samples to better understand the relationship between grading scales (OARSI and Outerbridge), shear mechanics (shear min, shear and plateau), and bulk biochemical composition, and showed that shear min, collagen (volume), and aggrecan (volume) are negatively correlated with Outerbridge. However, there was a strong positive correlation with shear min and collagen (volume) (**Fig. 1F**).

DISCUSSION: This study examined depth-dependent shear mechanics in healthy and OA human cartilage. The results showed that OA cartilage experiences shear strain levels that are one order of magnitude higher than healthy tissue, with most of the strain concentrated within the first 400 µm. The shear modulus of OA samples exhibited a decrease of more than 20-fold around the surface, while the deeper zone did not match the healthy shear modulus level but still showed approximately a 5-fold decrease. Other studies have reported similar findings, with very low shear modulus around the first 100–400 µm when tissues were treated with trypsin or collagenase, although shear modulus tends to return to healthy levels at the deeper zone[5,6]. However, our data indicate that the modulus in the deeper zone does not recover, which is characteristic of total ECM breakdown. The compositional analysis supported these mechanical findings, showing that both collagen and aggrecan were present at low levels at the surface and did not increase as they do in healthy tissue. The collagen content decreased 4-fold around the first 200 µm but stayed around 3-fold throughout the rest of the tissue depth compared to healthy samples. Our data also demonstrated that the change of collagen concentration is higher than the aggrecan level throughout the depth of the tissue. Meanwhile, the correlation analysis revealed that the grading system may be more predictive of overall biochemical properties than shear mechanics.

SIGNIFICANCE: The shear mechanics of OA cartilage are governed by compositional changes, and are more sensitive to collagen than aggrecan, while most of the change occurs at the articular surface.

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REFERENCES:. [1]Leifer, V P et al. *Osteoarthritis and cartilage* 2022. [2]Man et al *Journal of medicine and life* 2014. [3]Buckley et al. *Journal of biomechanics* 2008. [4]Silverberg J et al. *JOR* 2013. [5]Wyse Jackson et al. *Science advances* 2022. [6]Griffin et al. *JOR* 2014.

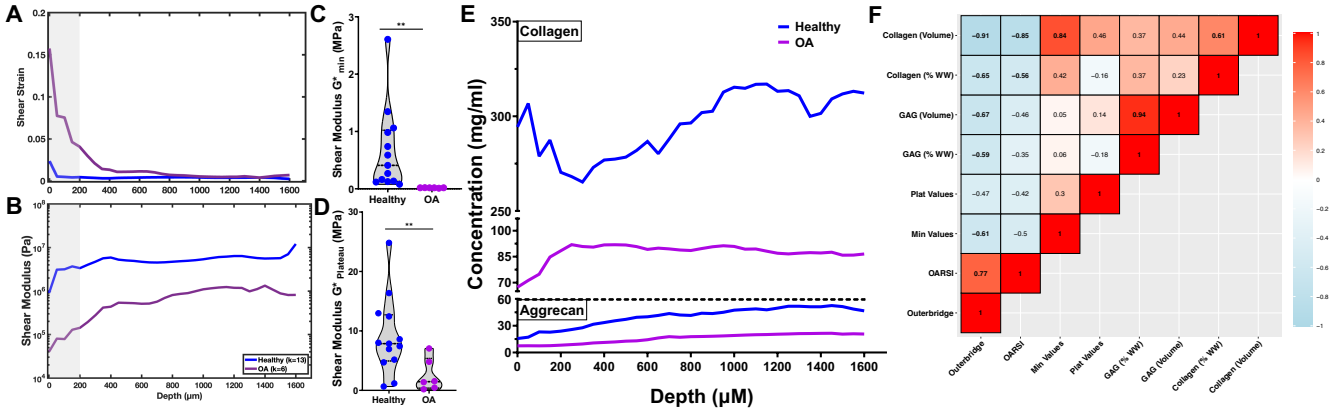

Figure 1: A: Depth-dependent shear strain of healthy and OA. **B:** Depth-dependent shear modulus for healthy and OA. **C:** The Shear modulus minimum showed OA has the lowest mechanics (p=0.0071). **D:** The G* plateau for healthy and OA (p=0.00714). **E:** Depth-dependent FT-IR composition for both collagen (top) and aggrecan (bottom) for both OA and healthy samples. **F:** Correlation plot of pairwise comparisons between grading scales, shear mechanics, and bulk biochemical composition. The data shown is geometric median with quartiles. Analyzed using Welch's t-test *($p<0.05$)*.