Mechanical Structure-Function Relations in Neonatal Bovine Articular Cartilage Under Shear

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Introduction: Recent developments in confocal elastography have enabled high-resolution depth-dependent measurements of articular cartilage (AC) shear properties [1-4]. Early studies employing this technique discovered a region of tissue near the articular surface that is 10-100 times more compliant than the bulk [1-5]. Dynamical measurements also show this same region dissipates \(\sim90\%\) of the energy absorbed during shear [5,6], suggesting that the articular surface plays a key role in protecting the underlying tissue from injurious strains. Though the mechanical data has depth-dependent trends that closely parallel the arcade-like organization of collagen fibers, no systematic quantitative studies have been performed to explore this connection. Here, we perform the first set of high-resolution same-sample measurements comparing structural, compositional, and shear mechanical data. We show that a fiber-reinforced interpretation of the collagen network is inconsistent with experimental facts, and that the shear mechanics is largely independent of fiber organization at small strains. Instead, we find the shear modulus strongly correlates with cartilage matrix density leading to the unexpected finding that a modest variation in matrix density \(\sim50\%)\) leads to large variations in the shear modulus \(\sim10,000\%\). We interpret these results in terms of a mathematical model that illustrates how a mechanical percolation phase transition drives this behavior [7].

Methods: Eight cylindrical explants were harvested either from the patellofemoral groove (PFG) or tibial plateau (TP) of 1-3 day old neonatal bovine [5]. Each sample was halved with one portion designated for mechanical testing, and the other for biochemical composition analysis. Mechanical testing was performed using confocal elastography, a technique that combines a rheometer with a high-speed confocal microscope to produce high-resolution \(\sim10\ um\) depth-dependent measurements of the complex shear modulus \(G^*(z)\). A combination of quantitative polarized light microscopy (QPLM) and Fourier transform infrared imaging (FTIR-I) was also used to measure with similarly high spatial resolution the collagen fiber alignment, orientation, and density, as well as the proteoglycan density [8-10]. Same-sample depth-dependent measurements were directly compared to determine correlations between the structural and mechanical data, which was quantified with linear regression analysis. To explore empirical structure-function relations in a conceptual manner, we developed a mathematical model of AC using Fortran 90 and Matlab that approximates the collagen fibers as an ordered network of filaments with randomly broken segments [11], embedded in an elastic gel representing the proteoglycans. The model uses experimental inputs such as the depth-dependent collagen density to simulate the network’s shear modulus as a function of depth. For a given set of input parameters, simulations were repeated for a network of \(~10^5\) nodes, and averaged over 10 realizations yielding a statistical view of the mechanical properties. This process was repeated for a range of input parameters to identify robust predictions of the model.

Results: Consistent with previous work, mechanical testing showed that the magnitude of the complex shear modulus \(|G^*(z)|\) has a compliant region near the articular surface localized to the first 200 um of tissue. The relative magnitude compared to the bulk-averaged shear modulus varies by a factor of 10 to 100, depending on the anatomic location from where the sample was harvested. Measurements of collagen fiber alignment and orientation were consistent with the well-known arcade model of collagen organization. Comparing depth-dependent alignment (Fig. 1(A)) and orientation (Fig. 1(B)) to the depth-dependent shear modulus revealed minimal correlation between data sets \((R = 0.28 \text{ and } 0.20, \text{ respectively})\). This suggests collagen fiber organization, in spite of the remarkable similarities between the depth-dependent shear modulus and the depth-dependent alignment of collagen, is not the primary source of variations in \(|G^*(z)|\).

FTIR-I measurements of the collagen (Fig. 1(C)) and proteoglycan (Fig. 1(D)) concentration were also compared to \(|G^*(z)|\) and found to have a strong correlation \((R = 0.79 \text{ and } 0.82, \text{ respectively})\). These results indicate that matrix density is the primary driver of the shear mechanics at low strains; however, it raises the question as to the microscopic mechanism underlying this relation.

Numerical simulations of the ordered network of filaments with randomly broken segments showed that a mechanical phase transition occurs as the probability of having a missing filament segment (bond between network nodes) increases. Specifically, for a fully connected percolating network, the shear modulus is high and varies little when a few bonds are removed. As the probability of a missing bond increases, the shear modulus becomes more sensitive to the presence of a few discrete sites that connect larger well-connected clusters. Beyond a critical probability, the network is no longer able to bear or transmit...
mechanical stresses and the shear modulus goes to 0. Near this transition from finite modulus to zero modulus, the network’s mechanical strength becomes increasingly sensitive to the number of missing bonds. The qualitative nature of this behavior was robust to a wide variation in the model parameters, including values consistent with experiments.

**Discussion:** While the classic arcade model of collagen fiber organization suggests a source for the depth-dependent variations in the shear mechanics, same-sample measurements show that cartilage matrix density is the primary driver of $|G^*(z)|$ in neonatal bovine AC at small strains. Findings such as these enhance our understanding the microscopic structure-function relations at play in AC, and in turn, provide useful biomechanical targets for tissue engineered constructs. Finally, we note that our mathematical model, which naturally generates large variations in $|G^*(z)|$ from small variations in the matrix density, is distinct from typical continuum and finite-element approaches more commonly found in studies of cartilage mechanics. We hypothesize that future explorations of this class of mathematical models will lead to a more unified picture of shear and compressive properties, bridging the current gap between these theoretical descriptions.

**Significance:** Same-sample mechanical and structural measurements reveal that matrix density, and not collagen organization, drives the depth-dependent shear mechanics of articular cartilage. This unexpected result can be explained in terms of a mechanical phase transition wherein large variations in the shear modulus (~10,000%) naturally arise from modest variations in the matrix density (~50%).

**Acknowledgments:** The authors would like to thank P. Carubia, L. Bartell, K. Novakofski, M. Delco, D. Griffin, L. Fortier, E. Donnelly, N. Pleshko, the Cohen lab and Bonassar labs for useful discussions. J.L.S. was supported by the National Science Foundation through a Graduate Research Fellowship. This work made use of the Cornell Center for Materials Research Facilities supported by the NSF under aware number DMR-1120296.

Fig. 1 The magnitude of complex depth-dependent shear modulus $|G^*(z)|$ was measured for 8 samples (PFG in red; TP in blue; each sample corresponds to a unique shade) and plotted against structural and compositional quantities. (A) The complex phase is plotted as a function of $\langle P \rangle$ (a.u.). (B) The complex phase is plotted as a function of $\langle \phi \rangle$ (deg). (C) The magnitude of the complex shear modulus is plotted as a function of $V_c$. (D) The magnitude of the complex shear modulus is plotted as a function of $V_a$. 