Depth-dependent self-stiffening, energy dissipation and poroelastic properties of normal human cartilage via broad-spectrum dynamic nanoindentation

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Introduction: Energy dissipation and self-stiffening are important features of dynamic loading of cartilage at loading rates encountered in running, jumping and traumatic impact joint injury. The frequency (time) scales of such loads ranges from ~1 Hz (1 s) to ~1000 Hz (1 ms) [1,2]. These features are attributed to the complex molecular structure of cartilage that varies significantly through different zonal areas. While depth-dependent molecular composition and mechanical properties such as equilibrium modulus have been studied extensively in cartilage [3-5], rate-dependent mechanical properties are not well understood as a function of cartilage depth. Here, we employed a previously developed AFM-based method for dynamic nanomechanical analysis [6,7] to quantify important depth-dependent poroelastic features of normal young-adult human cartilage, including frequency-dependent stiffness, energy dissipation, and hydraulic permeability. We found that the dynamic stiffness increased with frequency at all depths and that this self-stiffening effect, and the associated energy dissipation, increased with depth in a manner similar to the known increase in aggrecan content with depth.

Methods: Cartilage Sample Preparation: Full-thickness human cartilage disks (3 mm diameter × ~2 mm thick) were harvested from lateral and medial sides on the tibial plateau (Collins grade 0) of a 19-year-old male donor obtained postmortem from the Gift of Hope Organ and Tissue Donor Network (Elmhurst, IL). Procedures were approved by the Office of Research Affairs at Rush–Presbyterian–St. Luke’s Medical Center and the Committee on Use of Humans as Experimental Subjects at MIT. A 700 µm-wide (2mm deep) strip of cartilage was cut through each disk to expose a flat surface with complete zonal variation (Fig 1a). On each strip two rows of indentations were performed (+ signs in Fig 1a). The indentation sites started from near the edge of the intact superficial surface and moved toward the deep zone with an interval of 100 µm for the first 6 sites and 200 µm for the rest (11 total sites). The plugs were maintained and tested in PBS. Wide-frequency AFM dynamic testing: AFM colloidal tips 25 µm in diameter and cantilevers with a nominal spring constant k = 30 N/m were used. The applied displacement profile consisted of a 2µm pre-indentation and force relaxation, followed by sinusoidal displacements over a frequency range f = 1-1000 Hz (Fig. 1b). Data Analysis: A discrete Fourier transform (DFT) was used to obtain the fundamental frequency component of the z-piezo and deflection signals, from which δ and F_m (Fig. 1b) were calculated at each frequency, f. The dynamic stiffness was calculated as the ratio of F_m to δ, normalized by the tip radius and pre-indentation δ_0 (for details see [6]). Statistics: The data (Fig. 3) are based on the two rows on each of 3 available plugs (two medial, one lateral). To test independence of peak frequency, f_p, on Fig. 3d, linear regression was performed on the normalized peak frequency vs. depth.

Results: Nanoindentation revealed that the magnitude of the dynamic modulus of cartilage increased monotonically with frequency (Fig 2a), consistent with previous results [3]. To our knowledge, however, this is the first report showing the dramatic increase with depth of the dynamic modulus of normal young adult human cartilage at any frequency, measured over a 3-decade frequency range relevant to various human loading activities (Fig. 2). The low frequency modulus E_L (i.e., the quasi-static or equilibrium modulus) was ~0.04 MPa in superficial zone, and increased by a factor of 5 at the middle/deep zone (Fig. 3a). The ratio of E_f/E_L (where E_f is the magnitude of the dynamic modulus at the frequency of the peak in phase angle, f_p) represents the self-stiffening behavior of cartilage (i.e., the tissue presents a higher effective dynamic modulus at higher loading rates/frequencies). E_f/E_L increased substantially with depth from ~2 at the superficial zone to ~3.5 at middle/deep zone (Fig. 3b). Energy dissipation (over a cycle of sinusoidal dynamic displacement) is proportional to the tangent of the peak phase angle θ_p, which increased from 22° in the superficial zone to 36° in deep zone (Figs. 2b, 3c). Finally, the peak frequency f_p, which is proportional to the product of equilibrium modulus E_L and the hydraulic permeability, k, from poroelastic theory, was statistically invariant with depth (Fig. 3d), (slope of linear regression = -0.08 with (-0.3,0.1) 95% CI).

Discussion: We quantified the frequency-dependent behavior of normal human tibial plateau cartilage as a function of depth as measured via dynamic nanoindentation. The equilibrium modulus increased significantly from superficial zone to the middle/deep zones, consistent with data in literature [3]. We showed that the self-stiffening and energy dissipation behavior of cartilage, two properties that are especially important at high loading rates, also increases with depth. These observations are all attributable to the known increase in aggrecan-GAG concentration with depth, since increased GAG concentration would confer higher equilibrium modulus [3] and higher resistance to fluid flow, even at the nanoscale [6]. The peak frequency f_p (i.e., the inverse of the characteristic poroelastic relaxation time), remained constant through the depth, which suggests that cartilage relaxes at the same rate at different zonal areas. Since this peak frequency is proportional to product of the equilibrium modulus and the hydraulic permeability, and the equilibrium modulus increases with depth (Fig. 3a), these results strongly suggest that the hydraulic permeability decreases with depth, again consistent with the known increase in aggrecan content with depth.

Significance: Quantification of the variation of dynamic nanomechanics of cartilage vs. depth over a wide frequency range common in daily activities provides the basis to connect the depth-dependent molecular composition and structure to the the depth-dependent function of cartilage. Such knowledge is important in understanding degradation, repair and tissue engineering of cartilage.

Fig. 1 (a) Indentation sites across different zonal areas (b) The applied displacement and resulting force profiles included a 2 μm pre-indentation and hold, and subsequent 10 nm amplitude sinusoidal indentation at sequential frequencies 1 – 1000 Hz.

Fig. 2 Typical wide spectrum magnitude (a) and phase (b) of the dynamic modulus of cartilage at different zonal areas (see Fig 1(a) for location).

Fig. 3 (a) Equilibrium modulus, $E_L$; (b) dynamic self-stiffening ratio, $E_p/E_L$ (ratio of dynamic modulus magnitude at frequency $f_p$ to the equilibrium modulus); (c) peak phase angle $\phi_p$ related to energy dissipation; (d) characteristic frequency $f_p$ (product of $E_L$ and hydraulic permeability, $k$) for $n = 6$ (mean ± SE).