Poroelasticity is the dominant energy dissipation mechanism in cartilage at the nano-scale
**INTRODUCTION:** Recent studies of micro- and nano-scale mechanics of cartilage and chondrocyte pericellular matrix have begun to relate macromolecular features to its mechanical response [1,2]. AFM-based indentation has revealed rate-dependent stiffness at the micro-scale [1]. While multi-scale elastic behavior has been studied, and poro- viscoelastic properties have been extensively documented at the tissue-level [3], time-dependent behavior and energy dissipation mechanisms of cartilage matrix at the nano-scale are not well understood. Here, we used AFM-based dynamic compression in conjunction with poroelastic finite element modeling to study the frequency-dependent behavior of cartilage using nano-scale oscillatory displacement amplitudes. We introduce the characteristic frequency $f_{\text{peak}}$ at which the maximum energy dissipation occurs as an important parameter to characterize matrix time-dependent behavior. Use of micron-sized AFM probe tips with nano-scale oscillatory displacements over a 3-decade frequency range enabled clear identification of this characteristic frequency $f_{\text{peak}}$. The length-scale dependence of poroelastic behavior combined with judicious choice of probe tip geometry revealed flow-dependent and flow-independent behavior during matrix displacement amplitudes on the order of macromolecular dimensions and intermolecular pore-sizes.

**METHODS Sample Preparation:** Middle zone cartilage disks (9 mm diameter × 0.5 mm thick) were harvested from the femoropatellar condyle. The length-scale dependence of poroelastic mechanics was evidenced by a micro-scale pre-indentation and hold, and subsequent 15 nm amplitudes sinusoidal compression at 0.3 – 130 Hz (Fig. 1). The amplitude of the sinusoidal displacement, $\delta_0$, was set much larger than the radius of the indenter ($R = 0.1 \mu m$) and transversely isotropic models $E_2 = 0.46 \text{ MPa}$, $G_{12} = 0.37 \text{ MPa}$ (values taken from literature [5,6]) were used to simulate the dynamic behavior of the cartilage. The spherical indenter was prescribed using a displacement history shown in the experiment of Fig. 1b. The indenter and the substrate surface were assumed to be rigid and impervious, and all other surfaces were assumed to be free draining. The dimension of the sample was set much larger than the radius of the indenter ($R = 0.1 \mu m$) to simulate an infinite domain for the width and depth of the sample. Both isotropic ($E = 0.1 \text{ MPa}$, $\nu = 0.1$) and transversely isotropic models $E = 5.8 \text{ MPa}$, $\nu = 0.46 \text{ MPa}$, $G_{12} = 0.37 \text{ MPa}$ (values taken from literature [5,6]) were used to simulate the dynamic behavior of the cartilage. The dynamic indentation modulus is defined as $E^* = f_{\text{peak}}/[2\delta (R\delta_0)^{1/2}]$ [7] (parameters defined in Fig. 1) and $f_{\text{peak}}$ is the measured sinusoidal force amplitude. The angle $\phi$ is the measured phase lag between the force and applied displacement.

**RESULTS:** $E^*$ was found to asymptote at low frequencies at 0.09 MPa and 0.13 MPa for $\delta_0 = 2.4$ and $\delta_0 = 3.3 \mu m$, respectively. $E^*$ increased with frequency and began to level off near the frequency at which the phase lag was maximum. Upon further increases in frequency, $E^*$ plateaued at 0.6 MPa and 0.8 MPa for $\delta_0 = 2.4$ and $\delta_0 = 3.3 \mu m$, respectively. The phase lag between the force and displacement reached a maximum at $f = 29.2$ Hz for $\delta_0 = 2.4$ and $\delta_0 = 3.3 \mu m$, respectively (Fig. 2b). Both the isotropic and transversely isotropic models predicted the same patterns for $E^*$ and $\phi$ vs. frequency as those observed experimentally. However, in the isotropic model the value of maximum phase lag was $\phi_{\text{max}} = 8^\circ$, while in the transversely isotropic model the maximum phase lag varied with the choice of elastic constants. Both models predicted a shift in peak frequency of $\phi$ proportional to the inverse square of the contact distance, i.e., $f_{\text{peak}} \propto 1/d^2$. This prediction is in close agreement with the experimental peak frequencies of $f = 40.2$ to $29.2$ Hz, corresponding to the contact distances of $d = 7.9$ and $d = 9.3 \mu m$, respectively.

**DISCUSSION:** The measured phase lag (related to energy dissipation) during sinusoidal loading of cartilage over a 3-decade wide frequency range suggests that poroelastic dissipation is the dominant loss mechanism even at displacement amplitudes ~15 nm (e.g., compared to intrinsic matrix viscoelastic effects). This conclusion is supported by the observed shift in the phase lag peak frequency, $f_{\text{peak}}$, caused by the change in indentation depth, $\delta_0$ and the associated change in contact distance, $d$ (Fig. 2), consistent with the trends predicted by the linear poroelastic prediction: $f_{\text{peak}} \propto 1/d^2$ (Fig. 3). Comparing the isotropic and transversely isotropic poroelasticity models (Fig. 3), it was found that the transversely isotropic model better predicted the range of changes in both the magnitude and phase lag of the dynamic modulus, as has been observed at the tissue scale [6,8]. Ongoing studies focus on a range of probe tip diameters to further explore the robustness of the approach, and parametric analysis using the theoretical model.

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**Fig. 1** (a) The geometry of the AFM-based dynamic compression experiment. (b) The applied displacement and resulting force profiles included a micro-scale pre-indentation and hold, and subsequent 15 nm amplitude sinusoidal compression at frequencies 0.3 – 130 Hz.

**Fig. 2** Cartilage dynamic indentation modulus $|E^*|$ (a) and phase lag $\phi$ (b) at two pre-indentation depths $\delta_0 = 2.4$ and $3.3 \mu m$ (mean±SD, n=10).

**Fig. 3** Theoretically predicted dynamic indentation modulus (a) and phase lag (b) based on isotropic and transversely isotropic models.


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