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A HYPERELASTIC CONTACT MODEL FOR THE INDENTATION OF CHONDROCYTES AND CARTILAGE EXTRACELLULAR MATRIX

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INTRODUCTION

The use of micro and nanoindentation to map the elasticity of cartilage chondrocytes and extracellular matrix has been driven by the desire to understand the changes in microstructure and micromechanical properties of the tissue associated with development and with diseases such as osteoarthritis. Advances in atomic force microscopy (AFM) have facilitated application of the technique to soft tissues, allowing the concurrent imaging and mechanical probing of minute sample regions at high resolution. Examples of the capabilities of the AFM in studying cartilage biomechanics include its use in investigating changes in the elastic properties of the articular surface during development [1], comparing the dynamic elastic response at the microscale and the nanoscale [2], and measuring the elastic moduli of different zones [3].

The Hertz spherical contact equation and its underlying assumption of linear elasticity are employed by most researchers (e.g., see refs. [1,3]) when analyzing AFM indentation data. Although the equation works well for the majority of published data, it requires that indentations be restricted to the linear elastic regime. In certain soft biological materials, however, the linear limit may not be resolvable by the instrument. Overestimation of the elastic modulus is a normal consequence of using the Hertz equation to fit data in the strain-stiffening regime. Hence, contact equations based on hyperelastic models that have proven to be appropriate for modeling the large-strain deformation of soft tissues are desirable. We had previously developed and experimentally validated a force-indentation equation based on the Mooney-Rivlin formalism [4]. In this work, we extend that approach to derive an equation using the model of Fung [5]. The equation is utilized to determine the elastic moduli of chondrocytes and cartilage extracellular matrix. Preliminary results from a finite element validation study are also presented.

MATERIALS AND METHODS

Sixty-micrometer thick cartilage layers were transversely sectioned from the femoral head of one-day old wild-type mice using a microtome. Samples were fixed in 3% formaldehyde, rinsed thoroughly in PBS, and frozen in embedding medium prior to sectioning. Slices were immediately transferred to glass slides, where the embedding medium was allowed to dry and bond the tissue samples to the glass surface. The samples were then rinsed several times with a physiological buffer solution and equilibrated to room temperature. AFM imaging and microindentation were performed with the samples submerged in the buffer.

General-purpose silicon nitride tips with 5 μm polystyrene beads attached were used for the AFM measurements, conducted using a commercial instrument (Bioscope I with Nanoscope IV controller, Veeco). The spring constants of the cantilevers were measured by the thermal tune method while bead diameters were measured from images acquired during the attachment process. A raster scanning approach ("force-volume") was applied to automatically perform indentations over an area of ~ 30×30 μm, at a resolution of 32×32 (1024 indentations). Code written in Matlab was used to automatically process each dataset and extract values of Young's modulus. Height images were used to determine whether each measurement location corresponded to the extracellular matrix or to the cells.

In deriving the hyperelastic contact equations, we assumed that the contact radius varied with the indentation depth in a Hertzian manner [4]. To verify the validity of this assumption, nonlinear, contact finite element analysis (FEA) was performed in ABAQUS. The model consisted of a rigid, 10 μm sphere indenting a Mooney-Rivlin material (initial Young's modulus = 30 kPa, first material constant = 10x second material constant) to a depth of 5 μm in 250 ms.

RESULTS AND DISCUSSION

Following the approach described elsewhere [4], the Fung and Mooney-Rivlin force (F) – indentation (δ) equations are

$$F = C\pi R^{1/2}\left(\frac{\delta^{5/2} - 3R^{1/2}\delta^{2} + 3R\delta^{3/2}}{\delta - 2R^{1/2}\delta^{1/2} + R}\right) \exp\left[b\left(\frac{\delta^{3/2} - 3R^{1/2}\delta}{-R\delta^{1/2} - R^{3/2}}\right)\right];\ C = \frac{8E_0}{9\pi(1-\nu^2)} \quad (1)$$

$$F = C_1\pi R^{1/2}\left(\frac{\delta^{5/2} - 3R^{1/2}\delta^{2} + 3R\delta^{3/2}}{\delta - 2R^{1/2}\delta^{1/2} + R}\right) + C_2\pi R^{1/2}\left(\frac{R^{1/2}\delta^{5/2} - 3R\delta^{2} + 3R^{3/2}\delta^{3/2}}{-\delta^{3/2} + 3R^{1/2}\delta - 3R\delta^{1/2} + R^{3/2}}\right);\ C_1 + C_2 = \frac{4E_0}{9\pi(1-\nu^2)} \quad (2)$$

respectively, where the C's and b are fitting parameters, R is the radius of the spherical indenter, E_0 is the initial (or linear) Young's modulus, and ν is Poisson's ratio. Equations (1) and (2) assume that the contact radius a varies with indentation according to the Hertzian relationship

$$a = R^{1/2}\delta^{1/2} \quad (3)$$

For a Mooney-Rivlin material, results of the FEA, summarized in Fig. 1, indicate that Eq. (3) is valid for hyperelastic indentation at strains (ε^*), defined as $\varepsilon^*=a/R$, up to ~40%. It is expected that the trend is characteristic of all hyperelastic models. Since we performed all indentations up to a maximum strain of ~30%, Eq. (3) can be accurately used to describe the contact radius. At larger strains, a different relationship must be used in place of Eq. (3). It should be noted that the validity of the Mooney-Rivlin force-indentation relationship was previously verified by comparing values of E_0 to those obtained from the macroscopic compression of rubber-like gels [4].

A sample dataset from the indentation of a chondrocytes is shown in Fig. 2. It can be seen that the fit using Eq. (1) is excellent. By comparison, Eq. (2) yields a mean-square-error (MSE) of 16 nm^2 and E_0=22.7 kPa. Likewise, the Hertz model grossly overestimates Young's modulus (E_0=72.3 kPa) and results in an error of 24 nm^2. Similar results were found for regions of extracellular matrix. Averaging over ten random datasets each from the indentation of cells and matrix, we found the following properties of the neonatal mouse cartilage using Eq. (1): cells – E_0=11.81±1.76 kPa, MSE=0.125 nm^2; matrix – E_0=19.83 kPa, MSE=0.138 nm^2.

We propose Eq. (1) as a viable alternative to the Hertz equation in the micro and nanoindentation of cells and soft tissues. The FEA substantiated the assumption that the contact area remains Hertzian up to large strains (~40%). Use of this approach overcomes one of the chief shortcomings of Hertzian analysis by permitting indentation beyond the linear elastic regime.

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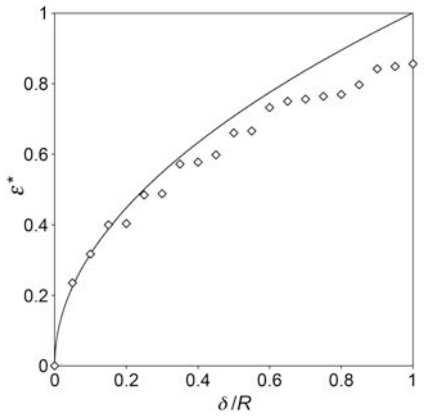

Figure 1. Contact radius vs. indentation, both normalized by the radius of the indenter. Data points are results from FEA while the curve is the theoretical Hertzian relationship.

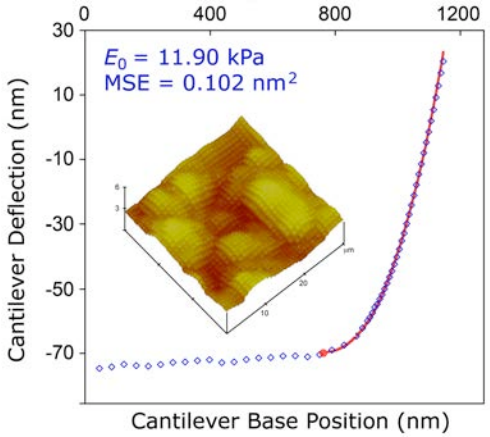

Figure 2. Sample AFM force curve from the indentation of a chondrocyte. The solid curve represents the fit using Eq. (1). Inset shows the topographical height image that allows delineation of cells and matrix. MSE: mean-square-error.