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ELASTICITY OF CARTILAGE MEASURED BY LARGE STRAIN, NON-HERTZIAN AFM NANOINDENTATION

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INTRODUCTION

The raster scanning and nanoindentation capabilities of the AFM are exploited in force spectroscopy, a valuable quantitative technique for mapping the elastic properties of materials down to nanometer resolution. When the probe is of well-defined geometry and the indentation behavior can be represented by established contact mechanics models, the force maps can be transformed to elastic modulus maps to facilitate comparison. This is especially important in biological specimens, where sample-to-sample variability is usually high.

At relatively small strains, gels and other materials that exhibit rubber elasticity can be assumed to be linear elastic and the indentation mechanics modeled using the generalized Hertzian force-indentation (F vs. δ) relationship [1-2]:

$$F = \lambda\delta^{\beta} \tag{1}$$

where λ and the constant β are dependent on the geometry of the indenter. The elastic properties of the sample (Young's modulus E and Poisson's ratio ν) are contained in λ. For the large compressive deformation of a rubber sphere by rigid flat plates, Tatara has derived contact mechanics relationships based on the Mooney-Rivlin law [3]. These relationships appear to be pertinent, with some modification, to the case of a rigid sphere deforming an elastic half space.

Even when direct F-δ relationships exist, careful treatment of AFM data must be practiced due to the need to infer values of force and indentation from measurements of the position (z) and deflection (d) of the cantilever [1]. The complication is compounded when applying the Tatara model because it requires a numerical solution to the governing equations. Moreover, the indirect measurement of F and δ introduces an additional variable in the form of the contact point coordinates (z_0, d_0). We applied an approximate nonlinear model based on the assumption that the dependence of the contact radius on indentation depth remains unchanged at large strains. This single equation model was used to extract Young's moduli from the large strain indentation of tissue-engineered and native cartilage specimens.

MATERIALS AND METHODS

Engineered cartilage constructs were grown from chicken embryo sternal chondrocytes seeded in poly(vinyl alcohol) hydrogel scaffolds [4]. The cartilaginous tissue was separated from the scaffold after culture periods of five and 25 days, and fixed with formaldehyde. Mouse cartilage samples were harvested from the femoral heads of a one-day old homozygous mutant. Longitudinal sections of 60 μm thickness were used for all measurements.

AFM probing was performed in contact mode using a commercial instrument (Bioscope I with Nanoscope IV controller, Veeco Metrology, Santa Barbara, CA). General purpose silicon nitride probes (Veeco) with 10 μm diameter polystyrene beads attached to the tips were used.

The cantilever deflection-position datasets corresponding to the extension stroke of each indentation were fit with a modified Hertz equation based on the rubber elasticity stress-strain relationship

$$\sigma = C(\Lambda - \Lambda^2) \tag{2}$$

where σ is the normal stress, C is an elastic constant equal to the shear modulus in rubber elasticity theory, and Λ is the extension ratio (related to the normal strain ε by 1-ε). The indentation stress and strain were defined as $F/(\pi R\delta)$ and $(\delta/R)^{1/2}$, respectively, where $a = (R\delta)^{1/2}$ is the Hertz contact radius and R is the radius of the sphere. For ideal Hertzian behavior, σ/ε is a constant and proportional to

Young's modulus E_0. Combining Eq. (2) and the indentation stress and strain definitions, the following force-indentation relationship is obtained:

$$F = \frac{\lambda_H}{3}\left(\frac{\delta^{5/2} - 3R^{1/2}\delta^2 + 3R\delta^{3/2}}{\delta - 2R^{1/2}\delta^{1/2} + R}\right) \quad (3)$$

Here, λ_H corresponds to the value of λ in Eq. (1) for a spherical (Hertz) indenter and is equal to $4E_0R^{1/2}/3(1-\nu^2)$, where E_0 is Young's modulus of the sample at zero strain and ν is Poisson's ratio.

RESULTS AND DISCUSSION

When the AFM dataset is free of high levels of noise, especially in the vicinity of the contact point, it can be truncated to eliminate points that exceed a predetermined, linear strain threshold. This is illustrated in the example shown in Fig. 1, where Young's modulus evaluated using Eq. (1) with a strain threshold of approximately 15% agrees well with the result using Eq. (3). If Eq. (1) is used to fit the entire dataset, the resulting fit is poor as indicated by the large mean-square-error (MSE; see plot of errors in inset of Fig. 1). The extracted modulus is also high in comparison to those from the other fits.

If the level of noise in the data is high or the linear regime comprises a small number of data points, the Hertzian small strain analysis using Eq. (1) becomes unreliable. This is demonstrated in Fig. 2, where a representative dataset from the indentation of mouse cartilage is shown. The value of Young's modulus thus obtained is significantly less than the values obtained from the large strain analyses. It is obvious that the small subset of the dataset corresponding to small strain is greatly influenced by noise. The neo-Hookean equation again provides a superior fit when compared to Eq. (1) and appears to correctly identify the contact point.

Use of the AFM in measuring the elastic properties of biological materials such as cartilage has become a common and accepted technique. Due to the lack of an accessible and easily implemented nonlinear contact model, classical Hertzian mechanics as represented by Eq. (1) are used even in cases where nonlinear behavior is apparent. Equation (3) is a direct force-indentation relationship that we have found to be superior to the Hertzian models for cases of large indentation strains. We successfully applied this approximate model to extract Young's moduli of synthetic poly(vinyl alcohol) gels known to exhibit rubber elastic behavior (data not shown). Here, we have shown that the neo-Hookean model can also be applied to the large strain indentation of biological gels such as cartilage.

ACKNOWLEDGMENT

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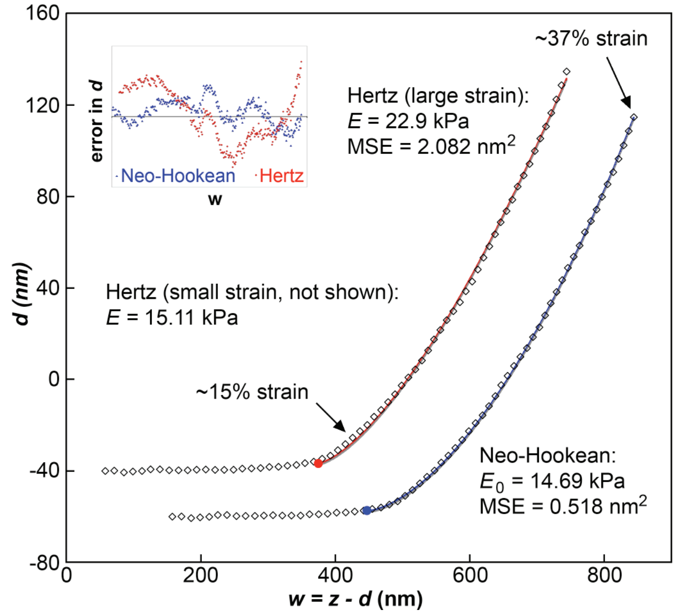

Figure 1. Extension data for engineered cartilage probed using a 10 μm diameter sphere. Every seventh data point is plotted. Fits using the Hertz and neo-Hookean equations are compared. Contact points are indicated by the filled circles. The force is equal to $k(d - d_0)$, where k is the spring constant of the cantilever, and indentation is $(z - d) - (z_0 - d_0)$. Inset shows plots of errors.

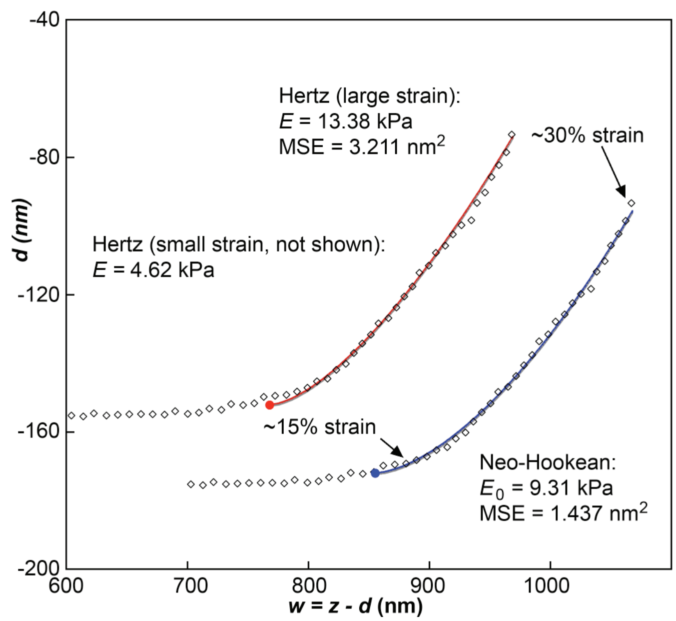

Figure 2. Extension data for mouse cartilage probes using a 10 μm diameter sphere. Every fifth data point is plotted.