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STRATEGIES FOR AUTOMATED EXTRACTION OF YOUNG'S MODULI OF SOFT MATERIALS FROM AFM FORCE CURVES: APPLICATION TO CARTILAGE

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INTRODUCTION

Articular cartilage is a highly inhomogeneous soft tissue with composition, structure, and mechanical properties that vary with depth and region. Its primary constituents (water, collagen II fibrils, and aggrecan proteoglycans) define its functional role as a load-bearing structure. An understanding of local structure-properties relationships in cartilage can help in identifying the link between mechanical stress and biochemical processes responsible for pathological conditions such as osteoarthritis. Although constitutive laws have been developed to describe the bulk behavior of cartilage [1, 2], there remains a lack of appropriate models to describe the mechanical and thermodynamic properties of inhomogeneous, gel-like systems. Researchers have thus relied on osmotic techniques [3] and the atomic force microscope (AFM) [4] to derive contributions of the collagen network or aggrecan macromolecules to cartilage mechanics. Our goal is to map the osmotic and mechanical properties of cartilage using a tissue osmometer [5] and the AFM.

Due in large part to its versatility, the AFM has become prevalent as a tool for characterizing biological and biomimetic materials. Based on the small tip size and controlling of tip-sample interactions, it is a powerful technique for imaging surface topography with sub-nanometer resolution. Unlike electron microscopes, imaged samples can be immersed in liquid, allowing biological specimens to be maintained at or near their native conditions. The AFM is well suited for measuring the local elasticity of small, inhomogeneous samples. For example, it has been used to study the changes in the elastic modulus of cartilage along the articular surface [6].

Despite the advantages of the AFM, tip geometry and data processing issues hinder the consistency of elasticity measurements on soft biological tissues. Although improvements to experimental techniques and to the Hertzian model of contact can correct for finite sample thickness and different tip geometries [7], difficulties still arise in determining the contact point from the force curves. Here, we describe a strategy for optimizing and automating the data fitting procedure and present examples of the approach as applied to the testing of tissue-engineered cartilage.

MATERIALS AND METHODS

Preparation and Evaluation of Tissue Engineered Cartilage

Tissue engineered cartilage was grown from chick embryo sternal chondrocytes seeded in poly(vinyl alcohol) hydrogel scaffolds [5]. After a five-week culture period, the cartilaginous tissue was removed and affixed to microscope slides. The slides were immersed in PBS solution until mechanical testing.

Elasticity measurements were performed using a commercial AFM (Bioscope with Nanoscope IIIA controller, Veeco Metrology, Santa Barbara, CA) with two types of cantilever tips: sharp pyramidal with a 35° half-angle and spherical with a 9.6 μm diameter.

Curve Fitting

Mathematica (Wolframs Research, Champaign, IL) and MATLAB (Mathworks, Natick, MA) code were developed to automate the fitting procedure. Results from both programs were compared to validate consistency. In the processing of AFM force curves, tip geometry was accounted for by selecting the appropriate mathematical model. The classical Hertz theory of contact mechanics relating the force to the indentation depth of a spherical indenter is given by

$$F = 4ER^{1/2}\delta^{3/2} / 3(1-\nu^2), \tag{1}$$

where F is the normal force applied by the indenting rigid sphere of radius R; E and ν are Young's modulus and Poisson's ratio of the sample, respectively; and δ is the resulting indentation depth of the sample. Bilodeau's approximate solution to the pyramidal indenter problem [8] was used for the sharp tips. The cartilage was assumed to be incompressible.

Each curve was first preprocessed to remove overtly extraneous data (e.g., occasionally, the probe adheres to the sample and upon retraction, does not detach from the sample; this causes negative deflection, which is seen as a concave inflection near the beginning of the curve). The model was then fitted to the truncated curve by searching for the probable contact point from among the discrete (z, d) pairs in the dataset, where z is the displacement of the fixed end of the cantilever and d is the measured tip deflection. Goodness of fit was evaluated by calculating the mean-square-error (MSE). Nonlinear least-squares routines were utilized and the Golden-Section method was implemented in the search.

The probable solution (i.e., contact point and E) was next used as initial values in refinements of the fit. To minimize the influence of noise in the data, the estimated contact points in these subsequent fits were permitted to deviate from the original curve within a specified tolerance based on the range of cantilever deflections. Lack of significant improvement in the MSE was indication that the true contact point was not captured by the original dataset. In this circumstance, a final search was performed outside the range of the data.

RESULTS AND DISCUSSION

Approach curves were analyzed for each data set. The original and fitted curves for several representative measurements (soft, intermediate, and stiff) using the pyramidal tip are shown in Fig. 1. Also shown are the results for an example using the spherical tip. Values in parentheses are the root-mean-square error of the fit as a percentage of the range of cantilever deflections.

Data processing is a necessary step to remove contributions from tip-surface interactions and the strain-softening that is occasionally observed. Strain hardening effects can be excluded in a like manner. Inclusion of such effects skews the results because models based on Hertzian mechanics describe only linearly elastic behavior.

For substrates of intermediate and high stiffness, an upturn in the force curve at the onset of tip engagement is easily distinguished. The vertical ramp size of the cantilever can therefore be adjusted to bracket the contact point. This feature is usually absent in the testing of very soft materials. The fitting scheme described above allows for the possibility that the true contact point lies outside the ramp size. The curve corresponding to the soft sample in Fig. 1 indicates that large errors are incurred by confining the search to points within the dataset. The errors in the fits of all datasets are less than 2% of the total range of cantilever deflections. In each case, the automated fitting routine was capable of identifying the contact point that resulted in an optimal fit.

We have shown that the data fitting strategies described here are capable of reliably addressing many of the difficulties encountered in the analysis of force curves for compliant materials. The tissue-engineered cartilage used in this study exhibited a large range of mechanical properties as demonstrated by the distinct types of force curves in Fig. 1. The robustness of this approach makes it possible to automate the processing of divergent populations of datasets and facilitates the use of the AFM in data collection for statistical studies.

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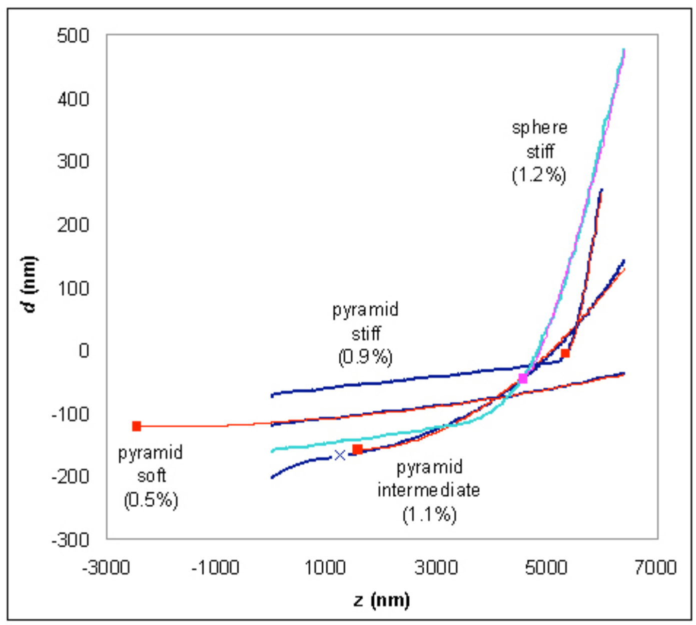

Figure 1. Plots of original (light and dark blue) and fitted (red and magenta) data. Solid squares indicate optimized contact points. "X" marks an inflection point and values in parentheses are the root-mean-square error of each fit as a percentage of the range of cantilever deflections.