ON A NEW MICROINDENTATION METHOD FOR TESTING COMPOSITE SOFT TISSUES: THE IMPORTANCE OF RESIDUAL STRESS

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INTRODUCTION

The use of indentation as a way of probing the regional stiffness of both cells and tissues has become increasingly common over the last two decades, with the advent of the atomic force microscope (AFM) and other methods for applying very small forces [1,2,3]. Because constitutive relations estimated from stiffness data alone generally are not unique, we present a new method for determining material properties from indentation measurements. To illustrate the technique, we examine two linear elastic models that approximate specific biological structures. The first model is an axially loaded beam on an elastic foundation (e.g. stress fiber), while the second is a radially loaded axisymmetric plate on a foundation (e.g. a cell membrane). Then, a finite element (FE) model is used to study the nonlinear behavior and to determine properties of the embryonic chick heart. The behavior of these models depends strongly on residual stress. The results from our combined experimental, FE, and theoretical approach suggest that, in addition to the indenter force-displacement relation ubiquitously obtained in microindentation experiments, the deformation contour of the tissue surface can be used to estimate the residual stress and elastic properties in a large class of composite biological tissues that can be modeled approximately as either a plate or a beam on an elastic foundation.

MODELS

Theoretical Models

The governing differential equation for small deflection of a thin plate on an elastic foundation (Fig. 1(b)) is as follows:

$$D\nabla^4 w - T\nabla^2 w + Kw = p \qquad (1)$$

where D is the flexural rigidity, K is the foundation stiffness, T is the in-plane force, p is the surface pressure, and w is the transverse displacement. The 1D form of Eq. (1) governs the behavior of a beam on an elastic foundation (Fig. 1(a)). Equation (1) can be solved analytically or decomposed into a system of first-order differential equations with the appropriate boundary conditions in each case. The system was solved numerically using a boundary value problem solver in MATLAB (Mathworks, Inc.), bvp4c, which is based on a finite difference algorithm.

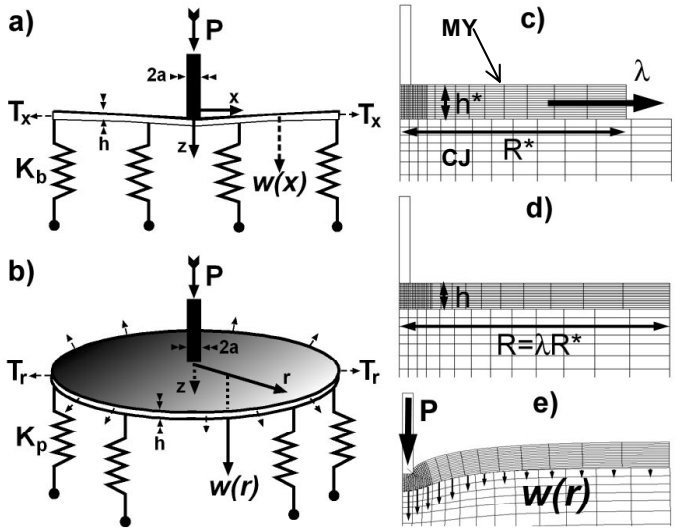

Figure 1 Schematic of linear models for a) beam, b) plate, and nonlinear FE model c) pre-stretched, d) stretched, and e) after indenter force is applied.

FE Model

To understand the effects of finite deformation and material nonlinearity we created an FE model of indentation. The geometry for the model is an axisymmetric cylinder, consisting of two layers

representing a circular plate and a foundation (Fig. 1(c)). A rigid indenter applied a force, P, at the center of the plate (Fig. 1(e)). Both layers were modeled as incompressible materials with a strain-energy density function

$$W = \frac{A}{B}\left[\exp(B(I_1 - 3)) - 1\right] \qquad (2)$$

where A and B are material parameters, and I_1 is the first strain invariant. To simulate residual stress in the plate, we prescribed a uniform radial stretch, λ, at the edge (Fig. 1(d)).

EXPERIMENTAL METHODS

For illustration, the FE model was used to analyze indentation data from stage 12 embryonic chick hearts. At this stage the heart is bi-layered, consisting of an outer, two-cell thick myocardium (MY), the "plate", that envelops a much thicker extracellular matrix compartment known as cardiac jelly (CJ), the "foundation". Fertile white Leghorn chicken eggs were incubated blunt end up at $38.5^{o}C$ for two days. The intact heart was dissected from the embryo by cutting its connections at the caudal and cranial ends. To isolate the cardiac jelly (CJ), the hearts were incubated in a Ca++, Mg++-free 10mM EDTA solution for 2 hours. This treatment was followed by incubation (2h) in a 1mM solution of deoxycholic acid dissolved in distilled water. To enable tracking of the surface contours during indentation, we placed polystyrene microspheres (Polysciences, Inc.) on the myocardium.

INDENTATION PROCEDURE AND MODEL FITTING

We have developed a microindentation device, the "heart poker", that provides local force-displacement data, and allows us to visualize the tissue deformation (Fig. 3(a,b)). We measure the deformation of the surface of the heart with spline fitting techniques.

A simulated annealing optimization algorithm was used to find material properties of both layers. Experimental forces were prescribed input to the FE model, and the least-squares difference between the experimental and model displacements along the tissue surface was minimized. We determined material properties for the foundation by using the isolated CJ data. After the CJ properties were known, we added the MY to the model and repeated the optimization using data from the intact heart experiments. For the MY we included the stretch ratio, λ, as an additional parameter.

RESULTS AND DISCUSSION

Linear Models

For both the beam and plate models an increase in D/K causes the normalized displacement contour, $\Gamma = w/w_{\max}$, to spread out from the indenter (Fig. 2(a,b)). Increasing tension causes Γ to spread out for the beam model (Fig. 2(c)), but Γ becomes more localized in the plate model (Fig. 2(d)). Hence, residual stress strongly affects the contour shape.

Experimental Results

We found that to fit both the apparent stiffness and contour data for the intact heart, residual stress had to be included. Otherwise, the contours from the model did not fit the experimentally measured

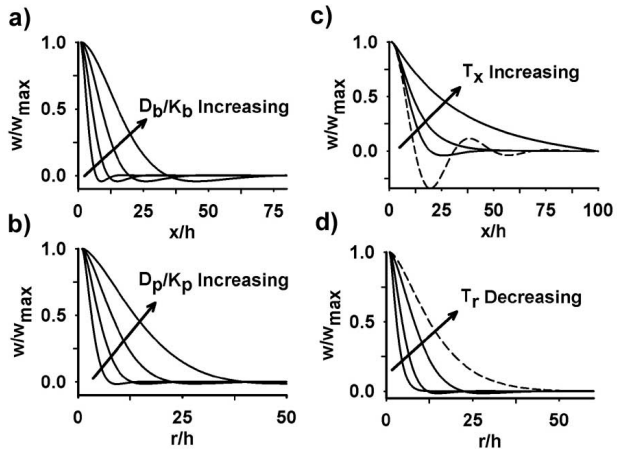

Figure 2 Effect of stiffness and tension on displacement contours for beam (a,c) and plate (b,d). Dashed lines indicate compressive forces.

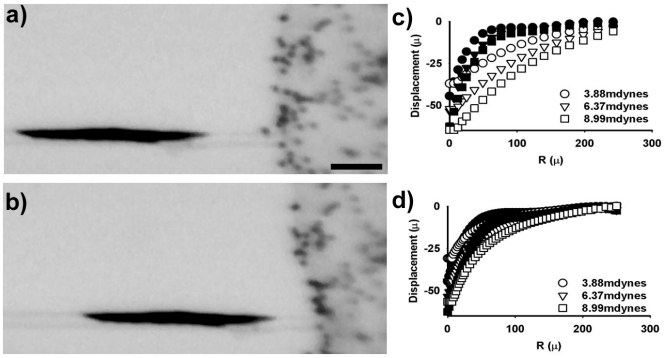

Figure 3 Contour before (a) and during (b) a poke. Fits to Eq. (2) for MY are shown for e) $A = 8000, B = 15, \lambda = 1.0$ **and f)** $A = 500, B = 2.5, \lambda = 1.20$**. The open symbols are model data, while the closed symbols are experimental data. Scale bar = 50 μm.**

contours (Fig. 3(c)), even if the force-displacement data matched (data not shown). Figure 3(d) shows how the model can fit the data when the MY is stretched by 20% before being indented.

In summary, from the cellular to macroscopic level this new microindentation method should prove useful in testing various tissues that have residual stress.

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