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Indentation analysis of biphasic viscoelastic hydrogels

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ABSTRACT

Mechanical properties of soft biological materials are dependent on the responses of the two phases of which they are comprised: the solid matrix and interstitial fluid. Indentation techniques are commonly used to measure properties of such materials, but comparisons between different experimental, and analytical techniques can be difficult. Most models relating load, and time during spherical indentation are based on Hertzian contact theory, but the exact limitation of this theory for soft materials are unclear. Here, we examine the response of gelatin hydrogels to shear and indentation loading to quantify combined effects of the solid, and fluid phases. The instantaneous behavior of the hydrogels is different for each test geometry, and loading rate, but the relaxed response, measured by the relaxed modulus, is the same for all tests, within 17%. Additionally, indentation depths from 15% to 25% of the radius of the spherical indenter are found to minimize error in the estimate of relaxed modulus.

1. Introduction

Quantitative measurements of mechanical properties of soft materials, such as tissues, are important in understanding the material response to loads, and deformations. For example, elasticity imaging relies on differences in the elastic stiffness of healthy, and diseased tissues to produce contrast for tumor detection and diagnosis (Greenleaf et al., 2003). In addition to the elastic properties, time-varying viscoelastic properties can also be useful in imaging creep tests where an applied load is held and the material is imaged over time (Greenleaf et al., 2003). Mechanical properties are also important in tissue engineering, and cell cultures where cells are known to sense and respond to the material with which they are in contact. The viability of cells in culture is greatly influenced by the effective stiffness of their extra cellular matrix (ECM) (Augst et al., 2006). A quantitative understanding of the mechanical properties of the ECM would help in understanding the cellular response of the mechanical environment.

The complexity of biological systems makes quantitative mechanical testing of such systems difficult. Inhomogeneities, irregular geometries, and difficulty in the isolation/extraction of tissue samples are just a few of the factors that affect mechanical measurements on these materials. Simplified systems, such as hydrogels, that mimic some of the mechanical properties of biological systems are useful to study basic material behavior. Tissue engineering and cell culture studies rely on the use of scaffold materials, often hydrogels (Dubruel et al., 2007; Fischback et al., 2007), on which cells are grown. Hydrogels are often used in bioimaging studies (Hall et al., 1997; Khaled et al., 2006; Han et al., 2003) as phantoms before more complicated systems, like tissue samples with tumors, are examined.

Indentation techniques are widely applied in the characterization of biological materials, and have received considerable attention over the last several years (Chen et al., 2007; Darling et al., 2006; Darling et al., 2007; Mahaffy et al., 2000; Mattice et al., 2006; Mooney et al., 2006; Krouskop et al., 1998; Wellman et al., 1999; Samani et al., 2007). Although

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the experiment is simple, effects of the thickness of biological samples, loading and boundary conditions need to be isolated when geometry-independent properties are sought. In the analysis of indentation data of biological materials, an incompressible, elastic material model using Hertzian contact theory is often assumed (Dimitriadis et al., 2002; Hayes et al., 1972). As a result of this assumption, a single parameter is found to describe the behavior, the Young's modulus, E (though sometimes, μ, the shear modulus is used). For spherical indentation of a semi-infinite elastic medium, Hertz calculated the contact pressure at the surface of the medium by approximating the spherical contact surface as a paraboloid (Hertz, 1881). The approximation is valid for small indentation depths compared to the radius of the spherical indenter. At larger depths the increasing difference between the contact area of a spherical versus parabolic indenter for the same indentation depth results in an increasing bias in the estimation of the elastic modulus from the experimental load-displacement data. An acceptable limit on the depth of indentation for Hertzian theory to be valid is not well understood for poroviscoelastic materials. It is envisaged that the validity of the semi-infinite assumption depends on the radius of the indenter, and thickness and width (in-plane dimensions) of the medium.

Many soft materials cannot be adequately characterized by a single parameter such as a modulus due to their inherent viscoelastic nature. The correspondence principle, in which elastic parameters in an elastic solution are replaced by the analogous viscoelastic differential or integral functions, is often used to determine the theoretical viscoelastic solution. For viscoelastic indentation problems, the analysis often begins with the elastic Hertz solution (Lee and Radok, 1960; Oyen, 2005; Cheng et al., 2005; Mattice et al., 2006; Darling et al., 2006; Mahaffy et al., 2000), and thus the viscoelastic indentation solutions are subject to the same restrictions as the Hertz solution. Recently, creep (Oyen, 2005; Cheng et al., 2005), load relaxation (Cheng et al., 2005; Mattice et al., 2006), and microrheology tests (cyclic loading) (Mahaffy et al., 2000) with a spherical indenter geometry have been used to study the viscoelastic response of soft materials. These time-dependent or frequency-dependent tests provide a good insight into viscoelastic behavior, but add complexity since there could be possible environmental effects on the sample during the length of test time, and also require specialized equipment. For example, creep and load relaxation tests that last longer require environmental control for biological materials such as tissues or cell cultures. Similarly, microrheological tests that can be conducted over a wide range of frequencies, require sophisticated instrumentation, and synchronization to achieve accurate results. Hence, a simple, and quick test that probes the viscoelastic behavior of soft biological tissue would be ideal. The quasistatic indentation test can be conducted on a simple load frame, and in a short testing time. This inherent characteristic of the indentation test potentially eliminates the need for specialized equipment or environmental control. Additionally, when indentation load-displacement data is analyzed with an appropriate viscoelastic model, the time-dependent material behavior can be estimated.

Modulus values for soft tissues have been estimated in the literature using varying experimental techniques, and analyses. To illustrate this variation, Table 1 contains the estimated elastic modulus values from current literature of three types of human breast tissue; adipose tissue, normal glandular tissue, and infiltrating ductal carcinomas (IDC). The experimental method, and test variables, such as strain rate, prestrain, and frequency, are indicated. It can be seen that, even when similar experimental techniques are used, there can be differences of nearly an order of magnitude between measured modulus values. These discrepancies emphasize the difficulty in comparing the moduli estimated using different analyses, and test methods.

In this work, we estimate the relaxation modulus of gelatin hydrogels using common experimental methods for comparison between experiments, and we examine the bias in the Hertzian theory, and its effect on the estimated material modulus. Time-dependent moduli estimated from a shear stress relaxation experiment, stress relaxation, are compared with moduli estimated from two types of indentation tests, load relaxation, and quasistatic indentation. The instantaneous moduli, and relaxed moduli estimated from each type of experiment used in this study are compared to examine the short and long time effects, respectively, of the geometry of the specimen, load application, and rate of loading. The quasistatic indentation experiment is explored in detail using a standard linear solid material model to estimate a viscoelastic, time-dependent modulus. The limitations of the Hertz solution for elastic indentation are explored to seek

Table 1
Example modulus measurements (average ± standard deviation) on three types of breast tissue from different studies using various indentation techniques, and analysis. The average modulus varies greatly between studies, even when the experiments are similar (e.g. frequency = 0.1 Hz).

Type of tissue	Experiment	Experiment details	N	Elastic modulus (kPa)	Ref.
Adipose	Sinusoidal, flat punch indentation	0.1 Hz, 5% precomp.	40	18 ± 7	(Dimitriadis et al., 2002)
Adipose	Flat punch indentation	Varying rates, strain = 0.01	26	5 ± 3	(Chadwick, 2002)
Adipose	Sinusoidal, flat punch indentation with FEA	0.1 Hz, preconditioned	71	3 ± 1	(Selvadurai, 2004)
Normal glandular	Sinusoidal, flat punch indentation	0.1 Hz, 5% precomp.	31	28 ± 14	(Dimitriadis et al., 2002)
Normal glandular	Flat punch indentation	Varying rates, strain = 0.01	7	18 ± 9	(Chadwick, 2002)
Normal glandular	Sinusoidal, flat punch indentation with FEA	0.1 Hz, preconditioned	26	3 ± 1	(Selvadurai, 2004)
IDC	Sinusoidal, flat punch indentation	0.1 Hz, 5% precomp.	32	106 ± 32	(Dimitriadis et al., 2002)
IDC	Flat punch indentation	Varying rates, strain = 0.01	25	47 ± 20	(Chadwick, 2002)
Intermediate grade IDC	Sinusoidal, flat punch indentation with FEA	0.1 Hz, preconditioned	21	20 ± 4	(Selvadurai, 2004)