Complete mechanical characterization of soft media using nonspherical rods

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Hydrogels have been used as substrates for studying the cellular processes by many researchers. The stiffness of such gels was also characterized previously. However, in most of the cases, these soft Poisson's ratio was assumed incompressible and Poisson's ratio is assumed to be one-half. This may not be true in many cases, and might alter the calculation of the stiffness of the gels. In this study, we present equations for the complete characterization of soft media, i.e., calculation of Young's modulus, shear modulus, and Poisson's ratio. The method involves the individual measurement of either the displacement or rotation of cylindrical rods embedded in the soft media, under the application of an external force or torque. Equations involving shear modulus and Poisson's ratio for rotation of the rod and Young's modulus and Poisson's ratio for the displacement of the rod are independently derived. In addition, the displacement and rotation of the rods embedded in an elastic medium, under the application of either a force or a torque, respectively, were also calculated using finite element analysis. These values compared well with the displacements and rotations obtained using closed form equations. © 2009 American Institute of Physics. [doi:10.1063/1.3211313]

I. INTRODUCTION

Soft media such as hydrogels are used as substrates for cell locomotion,^{1,2} proliferation,^{3,4} adhesion,⁵ and differentiation.⁶ The mechanical properties of these substrates play an important role in the growth of the cells. It has been previously demonstrated that spinal cord neurons extend more primary dendrites and shorter axons on stiffer gels.⁷ For the range of the stiffness of tens of pascals to a few kilopascals, neurite branching number was found to be significantly higher and astroglia are less adherent to the softer gels.⁸ Apart from neurons, other cell types were also shown to be sensitive to the substrate compliance.^{9–12} Thus, characterizing the mechanical properties of these substrates becomes very critical.

The elastic moduli of soft media were previously determined by many researchers using the theories of rubber elasticity and viscoelasticity. The medium was assumed to be a linear, elastic, isotropic, incompressible material, and Poisson's ratio was assumed to be equal to one-half. Some of the methods employed were compression,^{13–15} macroscopic indentation,^{1,15} atomic force microscopy,^{16,17} and manipulation of spherical beads.^{18,19} Spherical magnetic beads were previously utilized by our group^{19,20} to obtain the bulk elastic modulus of soft hydrogels. However, a complete characterization of the hydrogel would involve calculating two of the three unknown quantities: Young's modulus, shear modulus, and Poisson's ratio of the material. In this study we derive analytical expressions for Young's modulus (E), shear modulus (G), and Poisson's ratio (ν) of a soft, homogenous, iso-

tropic, linear elastic medium by using nonspherical inclusions in the form of cylindrical rods assuming the no-slip boundary conditions.

Poisson's ratio of a gel network system plays an important role in many phenomena, for example, the bending of polyelectrolyte gels in electric field,²¹ gel surface pattern formation,²² network swelling kinetics,^{23,24} and gel volume phase transitions.²⁵ If measurements are performed on a time scale that is sufficiently long to allow fluid flow in the medium, nonvolume preserving distortions of the medium can be expected. In such cases the medium can exhibit Poisson's ratio, which is substantially different from 0.5 as observed by many researchers.²⁶ Li *et al.*²⁷ proposed a method for measuring Poisson's ratio of polymer gels using different swelling behaviors of free and constrained gels. Poisson's ratio of polyacrylamide gels was found to be sensitive to the amount of polymerization initiator and varied between 0.3 and 0.36. Micropipette aspiration technique was used by Boudou *et al.*,²⁸ to measure the range of Poisson's ratio values for polyacrylamide gels with concentrations in the range of $0.02\% \leq [\text{Bis}] \leq 0.2\%$ and $3\% \leq [\text{Acry}] \leq 10\%$. A mean Poisson's ratio of 0.48 ± 0.012 was obtained for these hydrogels. Young's moduli and Poisson's ratios of hydrogels were also characterized at the micron scale using tensile tests at varying degrees of swelling.²⁹ Poisson's ratio of the hydrogels was found to vary between 0.42 and 0.45 when the pH changed from 2.8 to 12. But most of these techniques were destructive in nature and cannot be used in the presence of cells.

Walpole^{30,31} derived equations relating the force and the translation vectors or the couple and the rotation vectors for ellipsoidal inclusions in an elastic, isotropic, and homogeneous medium. Eshelby^{32–34} also carried out significant work

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FIG. 1. Cylindrical rods embedded in an elastic medium. An axial force would displace the rod in the direction of the force, while a torque applied about the center of the rod would tend to rotate the rods in the medium.

in the field of inclusions in an elastic medium. However, direct relations between force, displacement, torque, angle, elastic modulus, Poisson's ratio, and shear modulus were not found in literature.

Hydrogels usually exhibit tunable properties, meaning that the mechanical properties can be changed on the fly by increasing or decreasing the temperature or by adding or removing a crosslinker, changing the pH of the solution, etc., for example, the stiffness of the DNA crosslinked hydrogels can be increased by adding crosslinks and decreased by adding removal strands.³⁵ Thus, there is a need for a nondestructive technique to completely characterize the properties of the material. The equations presented in this paper can be used in conjunction with the four magnet setup developed by our group³⁶ to achieve this goal. In brief, the method involves displacing or rotating microneedles embedded in soft hydrogels using a microscope-based four magnet manipulator. The displacement and the rotation angles of the microneedle are measured by a time-lapse camera attached to the microscope. The force and the torque applied by the magnet on the needles are calculated by precalibrating the magnetic fields in the space in which the sample is positioned. Knowing the force-displacement and torque-rotation data, we determine G , E , and ν for the hydrogel.

II. MATHEMATICAL FORMULATION AND DISCUSSION

Consider a cylindrical rod in a homogenous, isotropic, linear elastic medium. No-slip boundary conditions are assumed and medium finite size effects are ignored; that is, the size of the rod embedded in the medium is taken to be very small compared to the distance between the rod and the gel surface.

In this study we consider two particular cases as shown in Fig. 1.

- (1) *Translation of the rod.* In this case the rod is placed along the z -axis and a constant force is applied along the z -axis. This will make the rod to displace along the z -axis.
- (2) *Rotation of the rod.* In this case the rod is placed along the z -axis and the torque is applied about the y -axis, which causes the rod to rotate about the y -axis. The torque is obtained by applying opposite forces on either side of the rod along the x -axis.

A. Case 1: z -axis translation of cylindrical rod

We start with the field equation for the displacement vector in a linear elastic medium and obtain the Galerkin solution³⁷ to the strain equations. The equilibrium equation in terms of the displacements is given by

$$\lambda \frac{\partial e}{\partial x} + 2\mu \left(\frac{\partial e_{xx}}{\partial x} \frac{\partial e_{xy}}{\partial y} + \frac{\partial e_{xz}}{\partial z} \right) + p_x = 0, \quad (1)$$

where λ and μ are Lamé's constants given by

$$\lambda = \frac{Ev}{(1+\nu)(1-2\nu)}, \quad (2)$$

$$\mu = \frac{E}{2(1+\nu)}. \quad (3)$$

In the absence of a body force $p_x=0$, Eq. (1) reduces to the following field equation for the displacement vector for a linear elastic medium, upon using Eqs. (2) and (3):

$$\nabla(\nabla \cdot \mathbf{u}) + (1-2\nu)\nabla^2 \mathbf{u} = 0. \quad (4)$$

Since the force is acting along the z -axis, the displacement is also along the z -axis and can be represented as

$$\mathbf{u} = u(r)\mathbf{e}_z. \quad (5)$$

Hence

$$\nabla \cdot \mathbf{u} = 0, \quad (6)$$

and Eq. (4) yields

$$(\nabla^2 \mathbf{u})_r = (\nabla^2 \mathbf{u})_\phi = 0 \quad (7)$$

and

$$(\nabla^2 \mathbf{u})_z = \nabla^2 u(r). \quad (8)$$

Thus Eq. (4) reduces to

$$\nabla^2 u(r) = 0, \quad (9)$$

which further reduces to

$$\frac{\partial}{\partial r} \left(r \frac{\partial u(r)}{\partial r} \right) = 0. \quad (10)$$

Integrating Eq. (10) yields

$$u(r) = A \ln(r) + B. \quad (11)$$

Considering the rod as a rigid body, $u(R)$ equals the displacement of rod, on the surface of the rod. Also, the displacement field vanishes at a distance $L/2$ from the rod, where L is the length of the rod. Thus applying the boundary conditions $u(R) = \delta$ and $u(L/2) = 0$ we obtain

$$A = \frac{\delta}{\ln(L/2R)} \quad \text{and} \quad B = -A \ln(L/2). \quad (12)$$

Substituting the values of A and B in Eq. (11) yields

$$u(r) = \delta \frac{\ln(L/2r)}{\ln(L/2R)}. \quad (13)$$

The shear stress tensor component of τ_{zr} is given by

$$\tau_{zr} = \frac{E}{2(1+\nu)} \left(\frac{\partial u_r}{\partial z} + \frac{\partial u_z}{\partial r} \right). \quad (14)$$

Simplifying Eq. (14) we obtain

$$\tau_{zr} = \frac{E\delta}{2(1+\nu)r \ln(L/2R)}. \quad (15)$$

The total force along a cylinder of length L and radius R is obtained by integrating the shear stress over the length of the rod. Thus,

$$F = \int_0^L dz \int_0^{2\pi} R \tau_{zr}(R) d\theta. \quad (16)$$

Solving the integral gives rise to

$$F = \frac{2\pi E\delta L}{2(1+\nu)\ln(L/2R)}.$$

Hence

$$E = \frac{2(1+\nu)\ln(L/2R)}{2\pi L} \frac{F}{\delta}. \quad (17)$$

B. Case 2: Rotation of cylindrical rod

For obtaining the relationship between the shear modulus and the torque-rotation data, we start with the two-dimensional Kelvin solution also called as the fundamental solution.

$$u_{ij} = \frac{1}{8\pi G} \{ (3-4\nu) \ln r \delta_{ij} + r_{,i} r_{,j} \}. \quad (18)$$

Consider the cylindrical rod to be along the z -axis, and force acting along the x -axis.

Hence $\mathbf{F} = F_x \mathbf{e}_x$.

Substituting in Eq. (18) we obtain

$$u_i = \frac{F_x}{8\pi G} \{ (3-4\nu) \ln r \delta_{ix} + r_{,i} r_{,x} \}. \quad (19)$$

Taking $r = \sqrt{(x^2 + y^2)}$ we obtain

$$r_{,x} = \frac{x}{\sqrt{(x^2 + y^2)}} \quad \text{and} \quad r_{,y} = \frac{y}{\sqrt{(x^2 + y^2)}}. \quad (20)$$

The displacement in the direction of the applied force is obtained from Eq. (19) as

$$u_x = \frac{F_x}{8\pi G(1-\nu)} \{ (3-4\nu) \ln r \delta_{xx} + r_{,x} r_{,x} \}, \quad (21)$$

$$u_x = \frac{F_x}{8\pi G(1-\nu)} \left\{ (3-4\nu) \ln r + \frac{x^2}{x^2 + y^2} \right\}. \quad (22)$$

This is the displacement of any point on a plane whose normal is along the axis of the rod. In order to obtain the average displacement of all the points in this plane, we average the displacement obtained in Eq. (22) as

$$\langle u_x \rangle_R = \frac{1}{2\pi R} \int_0^{2\pi} u_x R d\theta, \quad (23)$$

where $\langle u_x \rangle_R$ represents the average displacement. Expressing the displacement in the cylindrical coordinates we obtain

$$u_x = \frac{F_x}{8\pi G(1-\nu)} \{ (3-4\nu) \ln r + \cos^2 \theta \}. \quad (24)$$

Substituting Eq. (24) in Eq. (23) and simplifying yields

$$\langle u_x \rangle_R = \frac{1}{2\pi R} \frac{F_x}{8\pi G(1-\nu)} \int_0^{2\pi} \{ (3-4\nu) \ln r + \cos^2 \theta \} R d\theta \quad (25)$$

or, carrying out the integration,

$$\langle u_x \rangle_R = \frac{F_x}{8\pi G(1-\nu)} \left\{ (3-4\nu) \ln r + \frac{1}{2} \right\}. \quad (26)$$

Now, considering the three-dimensional case of the rod, the average displacement $\langle u_x \rangle_R$ depends on the distance of the plane from the center of the rod. Consider $r = L/2R$ where L is the length of the rod and R is the radius of the rod. Also since $L \gg R$, we can ignore the constant of $\frac{1}{2}$. Thus Eq. (26) can be written in the form

$$\langle u_x \rangle_R = \frac{F_x}{8\pi G(1-\nu)} \left\{ (3-4\nu) \ln \left(\frac{L}{2R} \right) \right\}, \quad (27)$$

which yields

$$F_x = \frac{8\pi G(1-\nu)}{(3-4\nu)\ln(L/2R)} \langle u_x \rangle_R. \quad (28)$$

The torque acting on the rod can now be obtained by using

$$\Gamma = \int_{-L/2}^{L/2} F_x z dz = \frac{8\pi G(1-\nu)}{(3-4\nu)\ln(L/2R)} \int_{-L/2}^{L/2} z \langle u_x \rangle_R dz. \quad (29)$$

The displacement $\langle u_x \rangle_R$ can be expressed in terms of the angle of rotation of the rod (θ) as

$$\langle u_x \rangle_R = z\theta. \quad (30)$$

Substituting Eq. (30) into Eq. (29) yields

$$\Gamma = \int_{-L/2}^{L/2} F_x z dz = \frac{8\pi G(1-\nu)}{(3-4\nu)\ln(L/2R)} \int_{-L/2}^{L/2} z^2 \theta dz. \quad (31)$$

Simplifying Eq. (31) and carrying out the integration gives

$$\Gamma = \frac{2\pi G(1-\nu)}{3(3-4\nu)\ln(L/2R)} \theta L^3. \quad (32)$$

Hence,

$$G = \frac{3(3-4\nu)}{2\pi(1-\nu)} \frac{\Gamma \ln(L/2R)}{\theta L^3}. \quad (33)$$

In Eq. (17), F is the force applied on the rod and δ is the independently measured displacement. Similarly, in Eq. (33), Γ is the torque applied on the rod while θ is the indepen-

FIG. 2. (Color online) Comparison of the displacement of the rod using finite element analysis and the closed form equations, when an axial force is applied on a cylindrical rod embedded in an elastic medium. Poisson's ratio is varied from 0.36 to 0.499 and the displacement is calculated in each simulation.

dently measured rotation angle of the rod. In addition to Eqs. (17) and (33), for a linearly elastic and isotropic material, E , G , and ν are related by

$$E = 2G(1 + \nu). \quad (34)$$

Thus we have three equations using which the three unknowns G , E , and ν can be determined.

It can be seen from Eq. (17) that the displacement is directly proportional to the force applied on the rod and also depends on the length and radius of the rod. Similarly the rotation angle is directly proportional to the applied torque but is dependent on the cube of the length, which means that a small change in the length of the rod would greatly affect the rotation angle.

In order to assess the accuracy of the closed form equations, finite element analysis was performed using ANSYS[®] WORKBENCH[™] software, on a cylindrical rod of length 10 μm and diameter 1 μm embedded in a cylindrical matrix of radius 300 μm and 30 μm height. The rod is glued to the gel using contact elements, thus ensuring rigid bonding and no-slip conditions. An arbitrary value of 3000 Pa is assumed for Young's modulus of the gel. The rod is modeled using the properties of nickel, namely, Young's modulus of 210 GPa and Poisson's ratio of 0.3. An axial force of 15 nN is applied on the face of the rod, and the displacement of the rod is calculated in each case as Poisson's ratio is varied from 0.36 to 0.49. In order to compare the closed form equations with the finite element solution, it is convenient to write Eq. (17) in the form

$$\delta = \frac{2(1 + \nu)\ln(L/2R)}{2\pi L} \frac{F}{E}. \quad (35)$$

Using the same values for E , F , L , and R as used in the finite element simulations, the displacement δ is plotted against Poisson's ratio ν as shown in Fig. 2. It is evident from the graph that the closed form solutions match well with the finite element solutions, although there is an increasing disparity between the displacements obtained from the two methods with increasing Poisson's ratio. From Eq. (35) it can be observed that the displacement is directly proportional to the term $(1 + \nu)$. As a result, the displacement increases with increasing Poisson's ratio. However, in the

FIG. 3. (Color online) Comparison of angle of rotation of the rod using finite element analysis and the closed form equations, when a torque is applied on a cylindrical rod embedded in an elastic medium. Poisson's ratio is varied from 0.36 to 0.499 and the angle of rotation is calculated in each simulation.

finite element solution, as Poisson's ratio of the isotropic material approaches 0.5, the errors in measurement increase due to the presence of a term in the linear elasticity field equations containing the quantity $(1 - 2\nu)$, which approaches zero as Poisson's ratio approaches 0.5. This results in a divide by zero error when Poisson's ratio equals one-half.

In a similar way, finite element simulations were also performed for the rod in rotation when a torque is applied. Using Eq. (34), it is convenient to write Eq. (33) in the form

$$\theta = \frac{3(3 - 4\nu)(1 + \nu)}{\pi(1 - \nu)} \frac{\Gamma \ln(L/2R)}{E L^3}. \quad (36)$$

The gel and rod system are modeled using ANSYS[®] WORKBENCH[™] software, as described above with the same properties. The angle of rotation of the rod upon the application of the torque was measured as Poisson's ratio was increased from 0.36 to 0.499, and the results are compared with Eq. (36) in Fig. 3. The close match between the two methods gives us confidence in the closed form solutions derived in this paper.

The approach considered in this study is only appropriate within a limited range of deformation of the medium and is useful for determining the material properties in the linear elastic regime. Also, no-slip boundary conditions were assumed and the finite size effects were ignored. So care should be taken to make sure that the length of the rods (L) is much smaller compared to the size of the medium. Otherwise the factor of R_m/L , where R_m is the defining dimension of the elastic medium, should be taken into consideration.²⁰ If slip boundary conditions apply, the motion of the particles on the surface of the rod is restricted to the trajectories tangential to the rod's surface, and Eqs. (17) and (33) no longer hold. The treatment of these conditions is beyond the scope of this paper.

III. CONCLUSIONS

The mechanical properties of hydrogels, which act as substrates, play an important role in the growth and proliferation of many cell types. In most of the studies the hydrogels are assumed to be incompressible and Poisson's ratio is taken as 0.5. However, Poisson's ratio can differ from 0.5. Hence, a more complete characterization of the elastic prop-

erties of hydrogels requires that one experimentally obtains the value of at least two of the three quantities: Poisson's ratio, shear modulus, and elastic modulus. In this study, we were able to obtain analytic expressions for the force displacement and the torque rotation for nonspherical rods placed in a soft, homogenous, elastic, and isotropic medium. These expressions are used to obtain the elastic modulus, Poisson's ratio, and the shear modulus of soft hydrogels, in a nondestructive manner, from data on the rotation and translation of rod shaped inclusions subjected to known torques and forces. In addition, finite element analysis was performed to validate the accuracy of the closed form equations obtained.

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