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A Noninvasive Approach to Determine Viscoelastic Properties of an Individual Adherent Cell under Fluid Flow

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Abstract

Mechanical properties of cells play an important role in their interaction with the extracellular matrix as well as the mechanotransduction process. Several *in vitro* techniques have been developed to determine the mechanical properties of cells, but none of them can measure the viscoelastic properties of an individual adherent cell in fluid flow non-invasively. In this study, techniques of fluid-structure interaction (FSI) finite element method and quasi-3-dimensional (quasi-3D) cell microscopy were innovatively applied to the frequently used flow chamber experiment, where an adherent cell was subjected to fluid flow. A new non-invasive approach, with cells at close to physiological conditions, was established to determine the viscoelastic properties of individual cells. The results showed an instantaneous modulus of osteocytes of 0.49 ± 0.11 kPa, an equilibrium modulus of 0.31 ± 0.044 kPa, and an apparent viscosity coefficient of 4.07 ± 1.23 kPa·s. This new quantitative approach not only provides an excellent means to measure cell mechanical properties, but also may help to elucidate the mechanotransduction mechanisms for a variety of cells under fluid flow stimulation.

Keywords

cell mechanical property; viscoelastic; fluid-structure interaction; finite element method; osteocyte

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Conflict of Interest Statement

None.

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Introduction

One of the most common loading mechanism on cells such as osteocytes in bone is interstitial fluid shear resulting from tissue matrix deformation (Weinbaum et al., 1994). The fluid drag introduces strain inside the cell body, which may trigger the activation and transduction of biochemical signals, such as intracellular calcium signaling (Lu et al., 2012). In order to understand the spatial and temporal distributions of the stress/strain in a cell under fluid flow, knowledge of the constitutive material behavior and mechanical properties of the cell is necessary.

Several *in vitro* techniques have been developed to determine the viscoelastic mechanical properties of a variety of cells, such as micropipette aspiration, atomic force microscopy (AFM), magnetic tweezers, optical traps, and cytoindentation (Lim et al., 2006). However, these techniques are not ideal for determining the mechanical behaviors of cells under fluid flow, which is an important physiological loading mechanism on osteocytes.

In our previous studies, the 3D cell geometry of an osteocyte under fluid flow was recorded by a quasi-3D microscopy (Baik et al., 2010). The current study combines quasi-3D microscopy and a fluid-structure interaction (FSI) finite element method to simulate the viscoelastic creep behavior of osteocytes under steady flow. The objective of this study was to determine the viscoelastic properties of cells under fluid flow using this new approach.

Methods

Cell Culture and Shear Flow Experiments

MLO-Y4 osteocytic cells, a gift from Dr. Lynda Bonewald at the University of Missouri-Kansas City (Bonewald and Kato, 2002), are grown in α -modified Eagle's medium (α -MEM), with 5% fetal bovine serum and 5% calf serum. During experiments, cells were stained with Alexa Fluor 594 wheat germ agglutinin plasma membrane dye. A square glass micro-tube (width: 700 μm , height: 550 μm) was used as flow chamber. A laser cutter (Universal Laser Systems, Scottsdale, AZ) was used to cut glass slides (No. 1 thickness; Fisher Scientific, Waltham, MA) into thin micro-slides to fit into the flow chamber. Cells were plated on the micro-slides, which were coated with fibronectin (Huo et al., 2010) for 20 min. A syringe pump (New Era Pump Systems, Wantagh, NY) and gas-tight syringe (Hamilton, Reno, NV) were used to generate a steady laminar flow in the glass tube with a wall shear stress of 10 dynes/cm^2 .

Fluorescent images of individual osteocytes are captured by two objectives simultaneously at 12 Hz along the loading history, using the custom-built quasi-3D microscope as described in our previous study (Baik et al., 2010). Each image of the plasma membrane over time is further processed by means of a digital image correlation technique to measure the displacement of the cell contour. The accuracy of the deformation of the cell acquired by the quasi-3D microscopy system was verified in our previous work, with the precision for displacement ± 0.2 pixel and strain ± 0.00287 (%), respectively (Baik et al., 2010; Qiu et al., 2012).

3D Fluid-Structure Interaction Finite Element Modeling

Two orthogonal images of the plasma membrane of an osteocyte obtained simultaneously with quasi-3D microscopy are shown in Fig. 1A and Fig. 1B. Cell contours, shown as white lines, were plotted by spline curve-fitting in SolidWorks (Dassault Systèmes, Concord, MA), as shown in Fig. 1C, which was lofted into a 3D structure in SolidWorks (Fig. 1D). The 3D cell shape was then imported into ADINA (ADINA R&D, Watertown, MA) as para-solid geometric data and meshed using tetrahedral elements (Fig. 1E). Meshes at the

interface of cell and fluid were refined to capture small deformations (Fig. 1G&F). The cell-flow interaction FE model described an osteocyte cell in a relatively large flow chamber (Fig. 1F). Fully developed pipe flow of culture medium was obtained by applying a parabolic velocity profile as the inlet flow condition in the fluid domain of the FE model. The interaction between the cell and fluid was staged in the ADINA FSI module (Bathe, 2009).

The cell was modeled as a deformable viscoelastic material with isotropic and homogeneous properties (Haider and Guilak, 2000; Park et al., 2010), also known as the standard linear solid (SLS) model (Lim et al., 2006), with equilibrium modulus k_1 , elastic modulus k_2 , and viscosity η (Fig. 1I).

Strategy to Determine Viscoelasticity Parameters

Equilibrium modulus k_1 was determined by an iterative optimizing algorithm on the equilibrium configuration at the moment t_1 . The objective function Φ_t is defined as the error between predicted and measured cell shapes at moment t , as shown in (1),

$$\Phi_t(k_1, k_2) = \sum_{s=1}^n \left\| \vec{P}_{t,s}^{EXP} - \vec{P}_{t,s}^{FEA}(k_1, k_2) \right\|^2 + \sum_{b=1}^m \left\| \vec{P}_{t,b}^{EXP} - \vec{P}_{t,b}^{FEA}(k_1, k_2) \right\|^2 \quad (1)$$

where s and b are the indices of sampling points on the contours of the cell from side view and bottom view with the total number of n and m respectively; $\vec{P}$ is the position of the sampling points, with the superscripts of 'EXP' and 'FEA' representing the results from experimental and FEA models, respectively.

Elastic modulus k_2 was also obtained through the similar optimization of the instantaneous configuration with the fixed k_1 when cell reached its initial deformation after flow was turned on.

Cell viscosity coefficient η was determined by fitting the whole cell displacement curve of the creep response using an exponential function. The whole cell displacement is defined as the norm of the average displacement of all the sampling points on side-view cell contour, as shown in (2).

$$U(t) = \left[\left(\frac{\sum_{i=1}^N U_{xi}(t)}{N} \right)^2 + \left(\frac{\sum_{i=1}^N U_{yi}(t)}{N} \right)^2 \right]^{1/2} \quad (2)$$

The calculation flow chart of the three parameters of cell viscoelasticity is shown in Fig. 2.

RESULTS

Cell Deformation under Fluid Flow

The whole cell displacement obtained from the experiment is shown in Fig. 3. The instantaneous and equilibrium responses occurring at t_0 and t_1 are labeled appropriately.

The 3D strain pattern of E_{xx} and E_{xz} in cell body at the instantaneous and equilibrium states is shown in Fig. 4. These two components of strain are most significant compared to others.

Calculation of Viscoelastic Parameter

The convergence procedure of the objective function to optimize the viscoelastic parameter k_1 features an asymmetric parabola, and is based on the cell configurations at t_1 , as shown in Fig. 5A. Taking a sample cell, its side-view contour from FEA and experiments are well matched when k_1 is optimized (Fig. 5B). The convergence process of k_2 at instantaneous moment t_0 is shown in Fig. 5C, which is also a concave curve. The configurations of side-view cell at t_0 are shown in Fig. 5D. The rationality of the optimization for k_1 is based on the fact that the value of objective function in the equilibrium state is only determined by k_1 (Fig. 5E). The creep behavior, which is between moment t_0 and t_1 (Fig. 3), is fitted by an exponential function (Fig. 5F).

Viscoelastic Properties of Osteocytes

Deformation data from five cells were analyzed using our self-developed approach, and results are listed in Table 1. Three viscoelastic parameters of the MLO-Y4 osteocytic cells were acquired in mean $\pm$ SE form as $k_1=0.31\pm0.044$ kPa, $k_2=0.18\pm0.083$ kPa, $\eta=4.07\pm1.23$ kPa $\cdot$ S. All of the R^2 values of the exponential fitting for viscosity calculation were around 0.95.

Discussion

In this study, techniques of FSI finite element method and quasi-3D cell microscopy were employed in a classic cell-fluid shear experiment. A new approach was established to determine the viscoelastic properties of individual cells. The instantaneous modulus of osteocytes measured in the initial adhesive state was fairly close to the reported value obtained by AFM (Bacabac et al., 2008).

In comparison with other traditional techniques to measure the cell mechanical properties, the measuring approach presented in this study is non-local, non-invasive, and maintains close to physiological conditions of the cell. The shear force was exerted naturally to the whole cell body by the extracellular fluid flow, a process similar to the shearing action from the physiological fluid on the osteocytes buried in lacuna of bone mineral. These techniques allow for the study of the mechanical properties of cells, detailed strain/stress distribution in cells, and other specific dynamic biochemical processes at the same time induced by extracellular fluid shear flow.

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Fig. 1. 3D Fluid-Structure interaction FE model. (A–D) The process of 3D cell shape reconstruction. (E, F) The meshed model of cell and the fluid surrounding it. (G) The discrete elements with gradient size at the interface between cell and fluid. (H) The loading profile of inlet flow. (I) Standard Linear Solid model. Scale bar is 5 μm .

Fig. 2.

Flow chart to obtain the three viscoelastic parameters of the cells. The section of light gray shows the process to optimize k_1 or k_2 , and light blue section represents the process to obtain viscosity coefficient η .

Fig. 3. Whole cell displacement throughout the entire deformation history. The loading of steady shear flow is triggered at instantaneous moment t_0 and turned off at equilibrium time t_1 .

Fig. 4.
The 3D strain patterns of cell body at the instantaneous and equilibrium states. (A, B) Strain E_{xx} . (C, D) Strain E_{xz} . One half of the cell body is shown here.

Fig. 5.

The procedure of the noninvasive approach to determine cell mechanical properties. (A) The convergent process of k_1 at t_1 . (B) The configurations of side-view contour from FEA and experiment at t_1 with the optimal k_1 . (C) The convergent process of k_2 at t_0 . (D) The configurations of side-view cell at t_0 with the optimal k_2 . (E) The value of objective function almost remains constant when k_1 is kept as a constant while k_2 and η both vary over a wide range. (F) Fitting curve of the creep response with an exponential function, $R^2 > 0.95$.

Table 1

Intermediate and measured variables during the determination of viscoelastic parameters of osteocytes

	Cell #1	Cell #2	Cell #3	Cell #4	Cell #5	Mean±SE
<u>Optimal value (kPa)</u>	0.42	0.20	0.21	0.37	0.33	0.31±0.044
<u>Min. obj. (μm²)</u>	2.546	12.34	10.25	15.63	10.41	10.24±2.15
<u>Iteration Numbers</u>	12	9	10	9	9	9.8±0.58
<u>low boundary (kPa)</u>	0.22	0.15	0.15	0.15	0.15	null
<u>upper boundary (kPa)</u>	0.62	0.35	0.45	0.45	0.45	null
<u>Optimal value (kPa)</u>	0.20	0.10	0.013	0.49	0.10	0.18±0.083
<u>Min. obj. (μm²)</u>	3.207	7.257	8.351	7.442	2.801	5.8±1.16
<u>Iteration Numbers</u>	8	9	11	8	17	10.6±1.69
<u>low boundary (kPa)</u>	0.05	0.025	0.005	0.05	0.025	null
<u>upper boundary (kPa)</u>	0.332	0.25	0.15	0.55	0.25	null
<u>fitting Value (s)</u>	59.35	53.43	26.14	20.76	43.26	40.59±7.50
<u>R2</u>	0.95	0.94	0.96	0.93	0.95	null
<u>η (kPa·s)</u>	8.04	4.25	0.318	4.39	3.37	4.07±1.23
<u>k₁+k₂(kPa)</u>	0.62	0.3	0.223	0.86	0.43	0.49±0.11

Last column presented as Mean±SE, "Min. obj." represents the minimum value of objective function.