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Volumetric Stress-Strain Analysis of Optohydrodynamically Suspended Biological Cells

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Abstract

Ongoing investigations are exploring the biomechanical properties of isolated and suspended biological cells in pursuit of understanding single-cell mechanobiology. An optical tweezer with minimal applied laser power has positioned biologic cells at the geometric center of a microfluidic cross-junction, creating a novel optohydrodynamic trap. The resulting fluid flow environment facilitates unique multiaxial loading of single cells with site-specific normal and shear stresses resulting in a physical albeit extensional state. A recent two-dimensional analysis has explored the cytoskeletal strain response due to these fluid-induced stresses [Wilson and Kohles, 2010, "Two-Dimensional Modeling of Nanomechanical Stresses-Strains in Healthy and Diseased Single-Cells During Microfluidic Manipulation," *J Nanotechnol Eng Med*, 1(2), p. 021005]. Results described a microfluidic environment having controlled nanometer and piconewton resolution. In this present study, computational fluid dynamics combined with multiphysics modeling has further characterized the applied fluid stress environment and the solid cellular strain response in three dimensions to accompany experimental cell stimulation. A volumetric stress-strain analysis was applied to representative living cell biomechanical data. The presented normal and shear stress surface maps will guide future microfluidic experiments as well as provide a framework for characterizing cytoskeletal structure influencing the stress to strain response.

1 Introduction

In an approach to studying single-cell biomechanics, isolated cellular measurements require methods capable of suspending an individual cell for repeated and dynamic manipulation of the entire membrane surface. Current available technologies include dielectrophoretic (DEP) traps creating a capturing force by acting on cell polarization induced in an oscillating electrical field [1], acoustic tweezers (AT) applying an ultrasonic standing wave to create a

pressure node attracting particles or cells [2], and hydrodynamic tweezers (HT) suspending single cells with fluid-induced forces [3]. Advancements in laser technology have facilitated another means of manipulating isolated single cells, the optical tweezers or traps (OT) [4]. Distinct loads can be applied experimentally to single cells in culture to quantify cellular, membrane, and cytoskeletal biomechanics. This type of intracellular “holding” can apply forces in extracellular microenvironments on the order of 100 pN with resolution smaller than 1 pN ($1 \text{ pN} = 10^{-12} \text{ N}$) [5,6]. All of these trapping methods apply conditions outside the physiologic range for cells, making their influence on cell behavior debatable and a topic of continued investigation.

Direct assessment of cellular stress and strain through noncontacting techniques have utilized a rheoscope that examined red blood cell properties by measuring blood viscosity as a function of cell deformation and aggregation [7,8]. Through creative OT design and available optical physics technologies, novel testing of cells with and without physical contact has become available. In an optical channel, hydrodynamic stresses caused elongation in red blood cells positioned within a focused beam [9]. A cell can also be held directly [10] or with attached beads [11] and stretched by optically generated forces.

Recent in vitro and in vivo experiments have attempted to define injurious loading regimes generally caused by large applied stresses and the resulting high magnitude strains. Experimental work on bovine articular cartilage suggests that injurious mechanical compression can stimulate cellular apoptosis as well as a range of biomechanical and biochemical alterations to the extracellular matrix [12]. Chondrocyte programmed cell death may even occur at stresses lower than those required to stimulate cartilage matrix degradation and biomechanical changes. Further, it has been observed that injurious loading may be the main cause of a decrease in matrix integrity and mechanical properties [13] as well as an increased degradation concomitant with decreased synthesis of extracellular matrix (ECM) molecules [14]. Therefore, logically, it can be concluded that chondrocyte apoptosis may be one of the earliest responses to tissue injury that leads to subsequent damage and/or degradation of the cartilage matrix.

With innovations in microscale fabrication, microfluidic devices create opportunities to study dynamic mechanical behavior of individual cells under controlled conditions. These approaches foster experimental and mathematical platforms for relating mechanical perturbation to biological response (mechanotransduction) as well as defining biometrics for disease assay [15–17]. Through fluid mechanics and optical physics, flow-based mechanical test sequences of shear and normal stresses provide unique microenvironments when coupled with single-cell suspension techniques. Here, we describe the combined platform available through optical and hydrodynamic trapping as advancement in single-cell biomechanical testing with an original mathematical development of the resulting stresses and strains induced in representative cells.

2 Methods

2.1 Optohydrodynamic Trapping

Single cell manipulation is now available by combining optical trap and microfluidics technologies. A novel instrument was recently developed that integrates two laser-based techniques for manipulating and characterizing the mechanical environment adjacent to cellular and biomolecular structures [18,19].

Briefly, the optical tweezers or trap component of the device applies an infrared laser ($\lambda = 1064 \text{ nm}$) to suspend micrometer-sized objects with nanometer position control and constraining forces on the order of piconewton. For translucent objects larger in dimension

than the wavelength of the trapping laser (such as individual biological cells), a ray optics approach indicates that the rays of light are refracted as they pass into and out of the trapped cell. This change in direction and the associated momentum changes impart an equal and opposite force on the “trapped” object. Additionally, micrometer-particle image velocimetry can be incorporated with two frequency-doubled lasers ($\lambda = 532$ nm) aligned through the same optical path as the OT for full-field flow velocity characterization around the trapped cell.

As an enhancement to the optical trapping capabilities, a microfluidic chip configured with a silicone mold was prepared in the form of a cross-junction channel (Fig. 1). This geometry created a hydrodynamic trapping environment with extensional flow and a stagnation point at the geometric center. A cell may be positioned at the centroid with optical trapping, thus creating an optohydrodynamic trap. In this environment, the cell experiences a total drag force equal to zero, confirmed by integrating the stress tensor as defined by the normal (form drag) and shear (friction drag) stresses [19]. This reflects the stability or saddle-point nature of the cross-junction’s geometric center. Chips may be fabricated using a standard soft lithography approach using polydimethylsiloxane (PDMS). PDMS is bonded to a cover slip, after having punched ~ 0.9 mm diameter holes for microport access. Here, input and output open reservoirs (syringes) are placed at different heights, generating a gravity-driven flow. The output syringe height is held constant while the input syringe height is controlled as a means to vary the flow rate and in turn the applied stresses. Input and output syringe position can be reversed in order to drive the fluid with a lower “on chip” pressure.

2.2 Applied Extensional Flow Stress Analysis

In the following mathematical development, bolded uppercase letters indicate tensors while bolded lowercase letters indicate vectors. Here, the three-dimensional stress state is characterized as applied to the surface of a nonrotating spheroid cell of radius “a,” optically suspended in the linear extensional flow field within the hydrodynamic trap. For a nonrotating sphere suspended in a general linear flow with viscosity μ , the fluid velocity field vector $\mathbf{u}$ is [20]

$$\mathbf{u} = \mathbf{E} \cdot \mathbf{x} \left(1 - \frac{a^5}{r^5} \right) + \frac{1}{2} (\boldsymbol{\omega} \times \mathbf{x}) \left(1 - \frac{a^3}{r^3} \right) - \mathbf{x} (\mathbf{x} \cdot \mathbf{E} \cdot \mathbf{x}) \left(\frac{5a^3}{2r^5} - \frac{5a^5}{2r^7} \right) \quad (1)$$

including the corresponding pressure distribution

$$p = p_\infty - 5\mu (\mathbf{x} \cdot \mathbf{E} \cdot \mathbf{x}) \left(\frac{a^3}{r^5} \right) \quad (2)$$

For the specific linear case of planar extensional flow (where the vorticity vector is defined as $\boldsymbol{\omega} = \mathbf{0}$), the strain rate tensor $\mathbf{E}$ is reduced to the shear rate magnitude $\dot{\gamma}$ as components along the matrix diagonal:

$$\mathbf{E} = \frac{U}{a} (\mathbf{ii} - \mathbf{jj}) = \begin{bmatrix} \dot{\gamma} & 0 & 0 \\ 0 & -\dot{\gamma} & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad (3)$$

Using spherical coordinates for the general position vector $\mathbf{x}$ (Fig. 1) the equation components are

$$\mathbf{E} \cdot \mathbf{x} = \frac{Ur}{a} \sin \phi (\sin \phi \cos 2\theta \mathbf{e}_r + \cos \phi \cos 2\theta \mathbf{e}_\phi - \sin 2\theta \mathbf{e}_\theta) \quad (4)$$

and

$$\mathbf{x} \cdot \mathbf{E} \cdot \mathbf{x} = \frac{Ur^2}{a} \sin^2 \phi \cos 2\theta \quad (5)$$

Substituting these expressions into the generalized flow field gives the individual velocity components:

$$\mathbf{u} = U \left[\frac{r}{a} - \frac{5}{2} \left(\frac{a}{r} \right)^2 + \frac{3}{2} \left(\frac{a}{r} \right)^4 \right] \sin^2 \phi \cos 2\theta \mathbf{e}_r + U \left[\frac{r}{a} - \left(\frac{a}{r} \right)^4 \right] \sin \phi \cos \phi \cos 2\theta \mathbf{e}_\phi + U \left[\frac{r}{a} - \left(\frac{a}{r} \right)^4 \right] \sin \phi \cos 2\theta \mathbf{e}_\theta \quad (6)$$

as well as the pressure distribution not previously included in a two-dimensional stress analysis [19,21]:

$$p = p_\infty - \frac{5\mu U}{a} \left(\frac{a}{r} \right)^3 \sin^2 \phi \cos 2\theta \quad (7)$$

These expressions now describe the fluidic environment surrounding the isolated cell. Translating the velocity gradients into applied stresses, the constitutive equation for an incompressible, Newtonian fluid states that [22]

$$\mathbf{T} = -p\mathbf{I} + 2\mu\mathbf{E} \quad (8)$$

where $\mathbf{T}$ is the stress tensor and $\mathbf{I}$ is the identity matrix associated with the local isotropic (hydrostatic) pressure distribution p . $\mathbf{E}$ can be further represented by the flow velocity gradient tensor and its transpose,

$$\mathbf{E} = \frac{1}{2} [\nabla \mathbf{u} + \nabla \mathbf{u}^T] \quad (9)$$

Again, breaking out the polar components of the volumetric stress state produces the following:

$$\begin{aligned} \mathbf{T}_{rr} &= -p_\infty + \frac{\mu U}{a} \left[2 + 15 \left(\frac{a}{r} \right)^3 - 12 \left(\frac{a}{r} \right)^5 \right] \sin^2 \phi \cos 2\theta \mathbf{e}_r \\ \mathbf{T}_{r\phi} &= -\frac{\mu U}{a} \left[2 - 5 \left(\frac{a}{r} \right)^3 + 8 \left(\frac{a}{r} \right)^5 \right] \sin \phi \cos \phi \cos 2\theta \mathbf{e}_\phi \\ \mathbf{T}_{r\theta} &= -\frac{\mu U}{a} \left[2 - 5 \left(\frac{a}{r} \right)^3 + 8 \left(\frac{a}{r} \right)^5 \right] \sin \phi \sin 2\theta \mathbf{e}_\theta \end{aligned} \quad (10)$$

Evaluating the stress tensor at the cellular surface, $r=a$, gives the following volumetric stress state on the boundary:

$$\mathbf{t}_r = \mathbf{T}_r = \sigma_{rr} \mathbf{e}_r + \tau_{r\phi} \mathbf{e}_\phi + \tau_{r\theta} \mathbf{e}_\theta \quad (11)$$

where

$$\begin{aligned}\sigma_{rr} &= -p_{\infty} + \frac{5\mu U}{a} \sin^2 \phi \cos 2\theta \\ \tau_{r\phi} &= \frac{5\mu U}{a} \sin \phi \cos \phi \cos 2\theta \\ \tau_{r\theta} &= -\frac{5\mu U}{a} \sin \phi \sin 2\theta\end{aligned}\quad (12)$$

A three-dimensional representation of the stresses was then developed for presentation (MATLAB, Mathworks, Inc., Natick, MA).

2.3 Stress Versus Strain Response

Quantifying the mechanical state through three-dimensional stress versus strain relationships, the six deviatoric stresses were combined as an effective stress value, σ_{eff} [23]:

$$\sigma_{\text{eff}} = \frac{1}{\sqrt{2}} [(\sigma_x - \sigma_y)^2 + (\sigma_y - \sigma_z)^2 + (\sigma_z - \sigma_x)^2 + 6(\tau_{xy}^2 + \tau_{yz}^2 + \tau_{zx}^2)]^{1/2} \quad (13)$$

Volumetric strain, e , was defined to encompass the full-field deformation response of the cell to the multiaxial fluidic loads based on the strain invariants, I_i , which coincide with the Cartesian axes as determined from a Mohr's circle analysis [19,24]:

$$e = I_1 + I_2 + I_3 = \varepsilon_x + \varepsilon_y + \varepsilon_z + \varepsilon_x \varepsilon_y + \varepsilon_y \varepsilon_z + \varepsilon_z \varepsilon_x + \varepsilon_x \varepsilon_y \varepsilon_z \quad (14)$$

Since the experimental approach is a planar measurement technique, the off-axis strain value, ε_z , can be determined through a transposition of the planar loading scenario, again, through a Mohr's circle analysis [25]:

$$\varepsilon_z = -\frac{\nu}{1-\nu}(\varepsilon_x + \varepsilon_y) \quad (15)$$

The applied deviatoric stress state was related to volumetric strain and each of the individual principal strains. In addition, the droplet deformation strain, $\varepsilon_{\text{drop}}$, a ratio incorporating the major ($A=2a$) and minor ($B=2b$ and $C=2c$) axes, was applied:

$$\varepsilon_{\text{drop}} = \frac{A-B}{A+B} \quad (16)$$

Under suspension and hydrostatic loading, the cell's volume can be defined as a sphere, $V=3/4\pi abc$, with radii $a=b=c$. However, in the extensional loading state, the cell deforms into either an oblate spheroid ($a=b<c$), a prolate spheroid ($a=b>c$), or a scalene ellipsoid ($a\neq b\neq c$). It makes intuitive sense that the resulting shape would replicate a scalene ellipsoid due to different maximum stresses applied in the three orthogonal axes. Ongoing experiments and modeling will attempt to clarify this multiaxial biomechanical response.

2.4 Multiaxial Single-Cell Biomechanics

As previously reported in the development of an optohydrodynamic trap, living nonadhered, suspended osteoblasts, chondrocytes, fibroblasts, and myoblasts have been isolated and mechanically manipulated [6,19,26]. Briefly, primary cultures of chondrogenic and

osteogenic tissues were generated directly from rat long bones. The muscle cells were the mouse-derived myoblast C2C12 cell line (ATCC, CRL-1772; Manassas, VA). Cells were cultured in tissue culture flasks using α -minimum essential medium (MEM) (Cellgro, Herndon, VA) supplemented with 10% fetal bovine serum (GIBCO, Gaithersburg, MD), 2% glutamine, 1–2% penicillin-streptomycin (Cellgro), and 2% 1 M hydroxyethylpiperazine ethanesulfonic acid (HEPES) under standard culture conditions (37°C, 5% CO₂, and pH=7) [27]. Cells directly harvested from cartilage tissue consisted mainly of chondrocytes (rounded cells) and chondroblasts (attached). Osteogenic cultures exhibited firmly attached osteoblasts with a typical epithelial morphology. Myoblasts were firmly attached elongated cells that retained the ability to form myotubes when exposed to tissue culture medium with reduced serum content (2%). Harvested cells were diluted (10,000 cells/ml) in a solution containing 50 ml of artificial cerebrospinal fluid, 1 ml of HypoThermosol (FRS) (BioLife Solutions, Bothell, WA), and 1 μ m of ethylenediamine tetra-acetic acid (EDTA) to avoid clustering. Cells were tested at room temperature for all experiments (~20.5°C). The flow media consisted of a physiological buffer (127 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 0.5 mM Na₂HPO₄, 2 mM CaCl₂, 5 mM NaHCO₃, 10 mM glucose, 10 mM HEPES, 0.1% bovine serum albumin (BSA) adjusted to pH =7.4) resulting in a media viscosity of ~1 mPa s.

3 Results

Representative experimental flow fields within the optohydrodynamic trap confirmed the mathematical descriptions leading to the full-field stress calculations (Fig. 2). Previous validation indicated a high correlation between experimental and analytic descriptions of flow velocity in the xy -plane ($|R|>0.90$) [19]. The volumetric pressures can now be mapped throughout the region of the geometric center as a means to explore local stresses as well as those directly applied to the cellular membrane (Fig. 3). The overall resulting dynamic stress distribution is now described in three dimensions where peak polar coordinate stresses are aligned with the Cartesian coordinates for normal stresses and at 45 deg offsets for shear stresses (Fig. 4). In the actual fluidics, hydrostatic pressure p_{∞} would be additive to the normal stress values, dominating the pressurized environment.

The experimental application of the optohydrodynamic trap to a representative living cell created a maximum midplane shear rate of $\dot{\gamma}=42.5$ strain/s at the geometric center of the trap (as determined without cell perturbation) due to a 100 mm height differential between input and output fluid reservoirs. This resulted in maximum characteristic flow velocities ($U=\dot{\gamma} a$) of 359 μ m/s and 465 μ m/s applied to the deformed cell at the membrane boundaries with the minor and major axes, respectively (Fig. 5). Incremental effective stress values calculated from the maximum stress components at advancing pressure head-heights were plotted versus the responding volumetric strains indicating nonlinear and inconsistent representative stress-strain relationships within the manipulated myoblast.

4 Discussion

Advancements in technologies for assessing biomechanics at the cellular level have led to ongoing discoveries in the relationship between mechanics and biology (mechanotransduction). The aim of the described work was to fully characterize the applied local stresses and the resulting volumetric strains established within a microfluidic environment while providing a more complete knowledge of individual cellular biomechanics. Outcomes from this effort have included the introduction of an original set of equations fully describing the state of stress applied by the optohydrodynamic trap and the formation of a database archiving cellular biomechanical properties.

Although the optohydrodynamic trap provides a novel approach to cell biomechanics, significant limitations were identified in the previously introduced laser-based instrument [18]. These limitations include the following:

- *Low applied stress levels:* The described optical trap and microfluidic devices are currently only capable of applying megapascal stresses ($\times 10^{-3}$ Pa) to cells with typical kilopascal stiffness ($\times 10^3$ Pa). Current cell biomechanics studies apply stresses on the order of a pascal [28] and living joints experience healthy stresses on the order of megapascal ($\times 10^6$ Pa). As presented here in a myoblast (Fig. 5), cells tested at low stress levels may demonstrate incompressible behavior. This phenomenon has also been demonstrated in osteoblastlike cells, suspended in media under hydrostatic pressures to up to 7 MPa [29].
- *Lack of cell incubation:* Environmentally sensitive cells need to be kept alive over lengthy time periods before, during, and after testing. A thermofluidic heat transfer analysis was conducted in the design of microscope stage cell incubator [30]. Preliminary experiments identified a strategy for heating within the microfluidic device itself. Thin-film resistive heaters provided a suitable heating solution establishing a temperature distribution from 36.9°C to 37.4°C from the channel centerline to the outer surface, respectively. Manufacture of prototype on-chip resistors is ongoing through physical vapor deposition using an electron-beam evaporator.
- *Nonadhered cell testing:* Cells are typically adhered to surfaces in living physiologic environments. This interaction encourages normal cellular communication and healthy biologic activity. While the suspended cell testing approach provides full-surface access to individual cells resulting in unique volumetric data, it is not physiologic. Ongoing efforts are exploring microfluidic configurations with biocompatible wall-surface characteristics (roughness, chemistry, etc.) as a means to enhance the biologic relevance of optohydrodynamic trapping.
- *Toxic nanoparticles:* The current blend of nanoparticles applied in past applications of particle image velocimetry was not biocompatible and eventually contributed to cellular death. Unfortunately, current attempts to “clean” the antibacterial ingredients have failed. Cell-nanoparticle interactions have been successfully applied as a means to explore the mechanical deformability of living cytoplasm [31] and will be adapted to this approach.
- *Laser heating:* Direct trapping of cells with the infrared laser leads to localized heating and cell death. Low laser power is viable for short periods and is a benefit when optically and hydrodynamically trapping living cells (<30 mW required). As water is the main constituent of biological cells (~70%) and has minimal absorption properties, laser-induced heating may be relatively mild for laser wavelengths of 200–1100 nm. It was previously observed that for a laser operating at 1064 nm, laser-induced heating of 1.2°C was produced for every 100 mW of applied laser power entering a trapped ovarian cell [32].

The volumetric strain analysis presented here facilitated a comparison with the common assumption applied in cell biomechanics, that of basing the mechanical properties within the framework of liquid droplet models [33]. This approach is typically applied to neutrophil biomechanics and has two main drawbacks. It treats the cytoplasm as a single phase rather than as multiphase or composite material, and it does not address the cell as a living entity having active cellular deformation and force generation. Of course, the latter issue of passive versus active mechanics is a significant hurdle in any cell biomechanical analysis and one not necessarily approached here. An active compensatory cellular strain response may be

evident as the reasoning behind the relatively constant strain values calculated during a concomitant increase in the applied stress (Fig. 5).

In this present paper, we observed representative changes in the physical properties of single cells under the influence of external stress. As explored previously, any mechanical stress, be it static or dynamic, has an influence on matrix dynamics, cellular apoptosis, and ultimately cartilage mechanical properties. Outcomes of the presented work have provided insight toward ongoing efforts addressing chondrocyte migration, apoptosis, and extracellular matrix growth kinetics [15–17]. Researchers have reported the effects of mechanical stress on cellular kinetics, providing insight toward definitive physical laws, which may eventually explain how stress-strain directly affects individual biochemical reactions within the intracellular or extracellular environment [34]. Current efforts utilize a systems biology modeling approach whereby anabolic and catabolic pathways are described, which influence tissue remodeling and matrix homeostasis. Ergo, chondrocyte behavior governs these fundamental pathways and maintains the matrix homeostasis through a harmonic relationship between cytokine and growth factor abundance.

Despite the limitations in the technical components of this research endeavor, many opportunities are available in this microscale bioengineering approach. Making cell-level knowledge relevant to tissue-level therapies will be a critical element for establishing future contributions to healthcare [35]. Thus, an emphasis on multiscale experimentation and mathematical modeling amplifies the significance, extending the reach of the single-cell approach. In this philosophy, a strong link is being made between cell engineering and tissue engineering. Cell population biomechanics and biomaterials science then become important areas of applied research when harnessing the adaptability of the living system toward repair, regeneration, and healing. Ongoing efforts are addressing these multiple-scale links by exploring interactive spatial and temporal relationships.

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Fig. 1. Schematic representations of (a) the microchannel configuration facilitating the optohydrodynamic trap and (b) the relationship between Cartesian (x - y - z) and spherical polar (r - θ - ϕ) coordinate systems [19]

Fig. 2. Planar experimental flow velocities in an isolated quadrant surrounding an optically and hydrodynamically trapped analogous cell (20.6 μm diameter polystyrene microsphere) as measured with micrometer-resolution particle image velocimetry [6]. Maximum velocity gradients are associated with maximum applied normal and shear stresses.

Fig. 3.

The fully characterized volumetric pressure field at the geometric center of the cross-junction channel (*a*) with and (*b*) without the cell perturbing the flow field (Eq. (10)). The balance in applied stresses leads to an efficient hydrodynamic trap. Axis units are locations relative to the radius of the cell (a/r).

Fig. 4.

Individual components of the volumetric stress state applied to the surface ($r=a$) of a single cell as modeled within the optohydrodynamic trap. These stresses include (a) normal stress, σ_{rr} , (b) shear stress in the xy -plane, $\tau_{r\phi}$, and (c) shear stress in the xz -plane, $\tau_{r\theta}$ (Eq. (12)). The colors of the full-field stress map indicate locations with positive (+red), negative (−blue), and zero (0 green) hydrodynamic stresses based on the prescribed coordinate system. Axis units are spatial locations relative to the radius of the cell (a/r).

Fig. 5.

Volumetric stress versus strain of an optohydrodynamically trapped myoblast. The z-direction view cell images represent the cell under no dynamic load (left) and with the maximum applied hydrodynamic load (right). Volumetric strains are equivalent to that of the fractional change in volume of the spherical cell as well as those calculated from the orthogonal principal strains. Strain due to droplet deformation theory is shown for comparison [26].