An optical-manipulation technique for cells in physiological flows

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Received: 19 June 2009 / Accepted: 4 September 2009 /
Published online: 29 September 2009
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Abstract We have developed a technique to manipulate human red blood cells (RBCs) in hydrodynamic flows. This method applies optical tweezers to trap and move microbead-attached RBCs in a liquid medium at various speeds, while it significantly minimizes laser heating and photon-induced stress for normal operation with laser-trapped cells. Computational fluid dynamics is applied to simulate flow-induced shear stress over the cell membrane and to correlate quantitatively the forces with the cell deformations. RBCs can be manipulated under physiological conditions by this approach, which may open an avenue to design principles for the next generation of cell sorting and delivery.

Keywords Optical tweezers · Cell–flow interaction · Red blood cell · Cell sorting · Flow–structure interaction

1 Introduction

The development of techniques for manipulating cells in physiological flows is of paramount importance for advancement in cell delivery and cell sorting. Optical tweezers have been widely used for manipulating biological cells and characterizing biophysical and biomechanical properties [1–4] because of their distinct advantages such as non-contact forces for cell manipulation, force resolution accurate to a pico-Newton resolution, and

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applicability to liquid medium environments [5]. However, there are a few intractable problems associated with the conventional laser trapping applied for manipulating living cells. One is the well-recognized heating damage, since laser trapping normally induces a temperature increase of 2°C to 4°C in cells [6, 7]. Also, the photons of the laser source bombarding the cell membrane normally generate unwanted stress and deformation to the cell. We have observed significant shrinkage in liposome volume when laser trapping is applied (unpublished data). In order to minimize these complexities, attaching the cells with a silica microbead will provide an alternative route for cell manipulation. In this case, the laser beam will trap the microbead, instead of the cell, and hence the laser heating can be minimized. Since the bead size is much smaller compared with the cell size, about 10% to 15% in diameter, the bead-induced flow will not be significant for mathematical modeling such as computational fluid dynamics (CFD) simulations. Detailed analyses for the flow-induced shear stress over the cell membrane have been performed by using commercial CFD software (COMSOL Multiphysics version 3.3). How the cell deforms under various physiological flow conditions has correspondingly been quantified. Such calculations provide a new approach which is an alternative to the previous techniques of trapping force calibration, e.g., escape force method and trapping stiffness method [8–10] for accurately determining the laser trapping force. In this paper, we have demonstrated the feasibility of the technique in manipulating human red blood cells (RBCs) attached with silica microbeads in various physiological flow conditions. In principle, the technique can potentially be applied to therapeutic delivery of various cells such as stem cells.

2 Experimental

2.1 Materials

Five milliliters of blood sample from a healthy donor were collected by venipuncture into a 10-ml Falcon tube which contains heparin. The fresh sample was centrifuged at the speed of $700\times g$ for 15 min. After collection, the supernatant consisting of plasma was removed from the tube. Human red blood cells were rinsed with $1\times$ PBS (pH 7.4) three times. Hypotonic buffer (10 mM potassium phosphate, pH 7.4, 75 mM NaCl, 155 mOsm/kg) was used to generate osmotic pressure for inflating the RBCs to spherical or nearly spherical shapes.

Silica microbeads, 2 μm in diameter, were purchased from Bangs Laboratories (Fishers, IN). The beads were rinsed three times in purified water before addition to the RBC suspension. The added amount corresponded to approximately one bead per RBC. The suspension was then maintained at 4°C for 1 h, allowing spontaneous and nonspecific adhesion of microbeads to the RBC membrane [11]. Finally, around $\sim 50,000$ microbead-attached RBCs per microliter were obtained after dilution. All observations and measurements were made at room temperature ($\sim 25^\circ\text{C}$). The bead-attached RBCs have been subjected to forces up to more than 100 pN [12]; the bead adhesion is quite stable in this study, as the maximum force is below 20 pN.

2.2 Optical tweezers system

Commercially available optical tweezers (Cell Robotic, Inc, USA) which were driven by a Windows XP based software were used in this present experimental work. The source of

laser is Nd:YAG at a wavelength of 1,064 nm pumped by a 1.5-W diode. The laser was then reflected through dichroic mirrors and focused by an inverted microscope (Nikon optical microsystem) before it reached the objects. The RBCs in $1 \times$ PBS were loaded to a Lab-Tek chamber (Nunc Inc., Naperville, IL) which contained the PBS liquid medium. The chamber was set up on an inverted microscope platform which was driven by a motorized stage at a velocity ranging from 0 to 250 $\mu\text{m/s}$. As a result, fluid flow of a constant velocity was created owing to the relative motion between the chamber and the trapped cell.

3 Modeling and simulations

The red blood cells experienced shear stress in the chamber, and their cell shape underwent a great change. The change in cell shape, in turn, had an influence on the fluid flow. The fluid–structure interaction (FSI) was solved by a commercial software package, Comsol Multiphysics (version 3.3). Lagrange multipliers were used to simulate the forces exerted on the flow-deformed structure, while velocity constraints were applied to the fluid at the fluid–structure boundary, to ensure that the fluid at that location was moving at the same velocity as the structure undergoing deformation. Structural deformation and fluid dynamics are determined simultaneously. An Arbitrary Lagrange Euler mesh was used to allow FSI simulations to be performed.

The key challenge of the simulation is to describe the deformation of red cell membranes with a thickness of 5–10 nm, i.e., 1,000 times smaller than the average cell size of 8 μm . The human red blood cell membrane comprises the phospholipid bilayer, the underlying spectrin network, and transmembrane proteins. The cell membrane structure is commonly modeled as an incompressible effective continuum. The conventional method for cell membrane simulation is to take the whole cell as a shell model [13]. However, usually the 3-D shell model will take a lot of computer resources. In this paper, an alternative technique was developed for this simulation challenge.

The fluid flow was described by the Navier–Stokes equations with a continuity equation, solving for the velocity field $\mathbf{u} = (u, v)$ and the pressure p in the spatial (deformed) moving coordinate system:

$$\rho \frac{\partial \mathbf{u}}{\partial t} - \nabla \times (-p\mathbf{I} + \eta (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)) + \rho ((\mathbf{u} - \mathbf{u}_m) \times \nabla) \mathbf{u} = \mathbf{F} \quad (1)$$

$$\nabla \times \mathbf{u} = 0 \quad (2)$$

where ρ is the fluid density and η is the fluid viscosity, $\mathbf{I}$ is the unit diagonal matrix, and $\mathbf{F}$ is the volume force affecting the fluid. We assume no gravitation or other volume forces affecting the fluid, so $\mathbf{F} = 0$. The coordinate system velocity is $\mathbf{u}_m = (u_m, v_m)$.

At the entrance, the inlet velocity was assumed to be identical with the stage movement velocity, and the flow had fully developed to be of laminar characteristics. At the outlet boundary condition, it was assumed that $p = 0$ for the flow simulation. On the undeformed solid surface, no-slip conditions were imposed, while on the deformed interface, the velocities equaled the structure deformation rate, i.e., $u_0 = u_t$ and $v_0 = v_t$.

The structure deformations were solved by using an axial symmetry plane stress-strain model. The principle of virtual work, which means the sum of virtual work from internal strains is equal to work contributed from external loads, was used to formulate the problem.

This formulation in this paper was applied to both the solid (internal cell) and the boundary (cell membrane).

For the internal cell:

$$dW = \int_A r \left(-\varepsilon_r^{\text{test}} \sigma_r - \varepsilon_\phi^{\text{test}} \sigma_\phi - \varepsilon_z^{\text{test}} \sigma_z - 2\varepsilon_{rz}^{\text{test}} \tau_{rz} + r \times uor^{\text{test}} F_r + S_z^{\text{test}} F_z \right) dA \\ + \int_s r \left(r \times uor^{\text{test}} F_r + S_z^{\text{test}} F_z \right) ds + \left(r \times uor^{\text{test}} F_r + S_z^{\text{test}} F_z \right) / 2\pi \quad (3)$$

where W is the total stored energy, r and z are the radial and axial coordinates, A is the cross-sectional area, ε is the global strain ($\varepsilon_r = \frac{\partial S_r}{\partial r}$, $\varepsilon_\phi = \frac{S_r}{r} = uor$, $\varepsilon_z = \frac{\partial S_z}{\partial z}$, $\varepsilon_{rz} = (\frac{\partial S_r}{\partial z} + \frac{\partial S_z}{\partial r})$), and σ and τ are stress tensors. F is the external load. S_r and S_z are the deformation rates in the r and z directions. The superscript “test” denotes a function used for the weak form of the partial differential equations. The cell membrane with a thin thickness was described as a boundary of the whole cell. The virtual work for the cell membrane was described as:

$$dW = \int_L \left(-\varepsilon_n^{\text{test}} \sigma_n A + \mathbf{S}^{\text{test}} \mathbf{F}_L \right) dL \quad (4)$$

where ε_n is the local strain rate, A is the cross-sectional area. $\mathbf{S}$ is the displacement and $\mathbf{F}_L$ is the force on the boundary L .

The cell membrane was subjected to a load, $\mathbf{F}_T$, contributed from the fluid flow, which is given by

$$\mathbf{F}_T = -\mathbf{n} \times \left(-p\mathbf{I} + \eta \left(\nabla \mathbf{u} + (\nabla \mathbf{u})^T \right) \right) \quad (5)$$

where $\mathbf{n}$ is the normal vector to the boundary, and other symbols are the same as those in Eq. 1. This load represents a sum of pressure and viscous forces.

The input parameters were: liquid density of 1,000 kg/m³; viscosity of 0.001 kg/ms; Young modulus for RBC membrane of 2,000 Pa; Young modulus for RBC internal structure of 2.0 Pa; Poisson’s ratio for both RBC membrane and RBC interior of 0.49, i.e., nearly incompressible [4].

4 Results and discussion

Figure 1 shows that one RBC with attached microsphere was fixed in the optical trap, and the RBC was subjected to a shear force due to the fact that the stage, where the RBC/liquid-containing chamber was mounted, was moving in a circular path. The focused optical trap is indicated by the white cross in Fig. 1, clearly demonstrating that the optical force was exerted on the microsphere rather than the RBC. The RBC was subjected to a steady shear force, and the cell experienced a smaller deformation. The observation confirms that the method can avoid directly striking optical photons onto cells which could induce a temperature increase or produce stress on cells.

The effect of different shear forces due to different stage moving velocities ranging from 0 to 100 $\mu\text{m/s}$ on a RBC is shown in Fig. 2. It can be seen that when the RBC was trapped due to the optical tweezers, the RBC was not aligned with the direction of the liquid movement. When the RBC experienced shear force, it started to align in the direction of liquid flow, and its shape started to change.

Fig. 1 The laser beam was focused on the microbead rather than the RBC when the microbead-attached RBC moved in the liquid. Using this technique, the RBC can avoid direct contact with the laser light, which can heat and exert stress on cells

Analyzing the images in Fig. 2 with MATLAB's image processing toolbox (Mathworks, USA), the edges of RBC and bead were detected, as shown in Fig. 3. The boundaries of cell and bead were determined by comparison with original images. In Fig. 3b and schematic drawing Fig. 3c, L_1 is the distance between the edge of the bead and the edge of the cell membrane. L_2 is the diameter of the bead, which is a fixed value. In this context, $L_1 - L_2/2$

Fig. 2 Cell deformation under various flow speeds

Fig. 3 An image (a) at 70 $\mu\text{m/s}$ was processed by the MATLAB toolbox to generate the edges of cell membrane and bead in b. A schematic drawing of the image b is shown in c. L_1 is the distance between the edge of the bead and the edge of the cell membrane. L_2 is the diameter of the bead, which is a fixed value. $L_1 - L_2/2$ is the maximum dimension of the cell in the horizontal direction. The extent of cell deformation was defined as $(L_1 - L_2/2)/L_0$. L_0 is the diameter of the cell at 0 $\mu\text{m/s}$

is the maximum dimension of the cell along horizontal direction. The extent of the cell deformation was defined as $(L_1 - L_2/2)/L_0$. L_0 is the diameter of the cell at 0 $\mu\text{m/s}$. The extent of cell deformation via the flow rate is shown in Fig. 4. It can be seen that, as the flow rate increases, the cell deformation increases accordingly. However, at flow rates ranging from 70 to 100 $\mu\text{m/s}$, cell deformation is not very significant. When increasing the moving velocity above 100 $\mu\text{m/s}$, the cell escaped from the stage moving direction. Based on the equation

$$F = 6\pi\eta ru, \quad (6)$$

Fig. 4 The extent of cell deformation as a function of flow rate. The extent of cell deformation was extracted from Fig. 2 by using the MATLAB imaging processing toolbox. The *small squares* represent the experimental data and data points; *large squares* show simulation results. Error bars represent $\pm 30\%$

Fig. 5 Boundary conditions for incompressible flow and RBC deformation simulation

where η is the liquid viscosity, r is the RBC radius, and u is the escape velocity, the force can be calculated as 7.54 pN. At this low force, the membrane remains in the small deformation range. Hénon et al. [11] used a double trap to fix a RBC with two silica beads in diametrical position and then measured the cell deformation under different forces by changing the applied power. It was found that the cell deformation was fairly minor at 7 pN, consistent with the experimental observations described in the present paper.

The experimental observation was further confirmed by the numerical simulation. Figure 5 shows the boundary conditions which were used for incompressible fluid flow and RBC deformation. Because the cell membrane was deformed during the fluid flow and this deformation affected the fluid flow, the flow and deformation were simulated simultaneously. Figure 6a and b show the cell deformation at liquid velocities of 50 and 100 $\mu\text{m/s}$, respectively. The maximum deformation was found to be 0.51 μm when cells were subjected to fluid shear force at a flow velocity of 100 $\mu\text{m/s}$, while the deformation was 0.27 μm at a flow velocity of 50 $\mu\text{m/s}$. The deformation against the flow velocity was found to be nearly linear within this flow speed range. However, the maximum deformation is quite small compared to the diameter of 8 μm . The simulated data points are also shown in Fig. 4. The simulation of cell deformation is a bit larger than experimental data. However,

Fig. 6 Numerical simulations of RBC deformation (unit: μm). **a** Liquid velocity at $50 \mu\text{m/s}$; **b** liquid velocity at $100 \mu\text{m/s}$

because the cell deformation is minor, there are errors in the MATLAB processing of the original images. Combining systematic errors from experimental setup with errors from MATLAB processing, the simulation results are in agreement with experimental data. To integrate the force on the membrane along the liquid movement direction, the force was calculated to be 7.24 pN , which is nearly the same value as that obtained from the escape velocity. When the liquid velocity reduced to $50 \mu\text{m/s}$, the maximum deformation value was $0.27 \mu\text{m}$, and the force on the membrane was integrated to be around 3.6 pN . The force via the flow rates are shown in Fig. 7. It can be seen that the simulation results are in good agreement with the values obtained from the Stokes equation.

Fig. 7 Comparison of force exerted on the cell during flow. Squares are from the simulation results, and triangles from the Stokes equation

5 Summary

A technique for optical tweezers was developed to avoid direct contact between the optical photons and biological cells. A single microbead was attached to a RBC, and the optical force from the optical tweezers was exerted on the microbead rather than the RBCs. The experiment can be used to characterize cell deformation during cell movement in blood flow, and the information obtained will allow better understanding of laser-trapped deformation under hydrodynamic force and also improve the design of cell delivery systems.

Acknowledgements The work is partly supported by the project funding (BB/D014786/1), which is co-funded by BBSRC and EPSRC (Life Science Interface Programme). Part of the work was done when KKL was at Keele University.

References

1. Titushkin, I., Cho, M.: Distinct membrane mechanical properties of human mesenchymal stem cells determined using laser optical tweezers. *Biophys. J.* **90**, 2582–2591 (2006)
2. Zhang, H., Liu, K.K.: Optical tweezers for single cells. *J. R. Soc. Interface* **5**, 671–690 (2008)
3. Hormeno, S., Arias-Gonzalez, J.R.: Exploring mechanochemical processes in the cell with optical tweezers. *Biol. Cell* **98**, 679–695 (2006)
4. Li, C., Liu, Y.P., Liu, K.K., Lai, A.C.K.: The deformation of an erythrocyte under the radiation pressure by optical stretch. *J. Biomech. Eng. ASME* **128**, 830–836 (2006)
5. Grier, D.G.A.: Revolution in optical manipulation. *Nature* **424**, 810–816 (2003)
6. Liu, Y., Cheng, D.K., Sonek, J.G., Berns, M.W., Chapman, C.F., Tromberg, B.J.: Evidence for localized cell heating induced by infrared optical tweezers. *Biophys. J.* **68**, 2137–2144 (1995)
7. Liu, Y., Sonek, G.J., Berns, M.W., Tromberg, B.J.: Physiological monitoring of optically trapped cells: Assessing the effects of confinement by 1064-nm laser tweezers using microfluorometry. *Biophys. J.* **71**, 2158–2167 (1996)
8. Neuman, K.C., Block, S.M.: Optical trapping. *Rev. Sci. Instrum.* **75**, 2787–2809 (2004)
9. Sheetz, M.P.: *Laser Tweezers in Cell Biology*. Academic Press, New York (1998)
10. Svoboda, K., Block, S.M.: Force and velocity measured for single kinesin molecules. *Cell* **77**, 773–784 (1994)
11. Hénon, S., Lenormand, G., Richert, A., Gallet, F.: A new determination of the shear modulus of the human erythrocyte membrane using optical tweezers. *Biophys. J.* **76**, 1145–1151 (1999)
12. Lim, C.T., Dao, M., Suresh, S., Sow, C.H., Chew, K.T.: Large deformation of living cells using laser traps. *Acta Mater.* **52**, 1837–1845 (2004)
13. Parker, K.H., Winlove, C.P.: The deformation of spherical vesicles with permeable, constant-area membranes: application to red blood cell. *Biophys. J.* **77**, 3096–3107 (1999)