Hydrodynamic Impact to the Cell Stress during Single Cell Recovery

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Abstract: Cell overstressed in single-cell recovery process leads to cell mortality during the isolation process. A glass capillaries micropipette can be useful as a tool for picking up cell based on the positive displacement picking up method. However, when a negative pressure applies to the micropipette glass for picking a cell, it produces hydrodynamic pressure to the cell medium. This pressure condition, which proportional to the stress of the cell during the recovery process. In this work, we present a numerical analysis of shear stress on cell cytoplasm. Parameters such as micropipette diameter size, micropipette tip distance to the target cell, and negative pressure impact to the cell are analyzed. As a result, shear stress of cell cytoplasm increased by a short distance of cell to the micropipette tip during the initial picking up process. However, during the cell flowing inside the micropipette, the shear stress produced to the cell has no difference to the micropipette diameter. Therefore, this study could provide a benefit understanding of cell stress phenomena for many types of cells in single-cell recovery application by proper selection of micropipette diameter, suction pressure and the minimal cell distance to during the recovery process.

Keywords: Cell stress; Hydrodynamic impact; Micropipette manipulation; Shear stress; Single cell recovery.

1. THE IMPORTANCE OF SINGLE CELL ANALYSIS AND TECHNIQUES TO SEPARATION AND ISOLATION

The single-cell analysis provides specific information about its functionality, served as a useful biomarker of health condition, and responds to perturbations induced by an external or internal mechanism of a biological system [1]. Furthermore, the large numbers of cells analysis provide an average measurement or analysis results which summarized all biological information of the single-cell state [2].

There are three techniques of cells separation, such as passive, active, and a combination of passive and active techniques [3]. In the passive separation techniques, these methods manipulate hydrodynamic forces, inertial forces, cellular adhesion, and immobilization in separating and sorting bulk liquid sample [4-5]. Active separation techniques, on the other hand, requires external force such as acoustics, magnets, electrophoresis, etc. for separating the cell from the bulk sample. After cells are being separate, single-cell isolation is required for further cell down-stream analysis. To isolate this single-cell, it requires manual or automated handling using micropipette technique [6].

The glass capillary micropipette uses to recover the single target cell in the separation platforms such as microfluidic chip, petri dish, and a well in a controlled environment. After cells are layouts, the micropipette is used to aspirate and also deposit the target single cell [7]. Because of cell fragility, some of the requirements of single-cell recovery, such as hydrodynamic aspiration effects corresponding to cell shear-stress, which influenced the cell viability during the process [8-9]. Cell viability rates are highly relevant such in rare cell isolation application as well as a non-rare cell for single-cell analysis [10]. Furthermore, the viability of a cell is highly crucial to many biological applications such as single-cell genomics and proteomics. Hence, by understanding the impact e.g., hydrodynamic pressure, suitable pipetting distance, and micropipette size, which might be a benefit in the single-cell isolation process. Figure 1 shows the illustration of single-cell recovery using micropipette technique.

2. ANALYSIS THEORY IN SINGLE CELL RECOVERY

The study of single-cell recovery using robotic micromanipulation has been claimed to have a high success rate of more than 80%. Furthermore, it also provides less processing time (from micropipette positioning, the picking up process, cell transportation and dispensing time on dedicated location) with less than 35 seconds per cell [9-10]. The estimation of micropipette tip position from substrate culture well or petri dish during aspiration is set between 5 to 10 µm for vertical whole-
cell aspiration. The distance between micropipette tip to targeted cell and substrate is essential to prevent the collision. The impact could damage the micropipette or even worst damaging the cell. The accuracy of the single-cell isolation system highly depends on the hardware setup, such as the optical system and imaging system, and the precision of robotic micromanipulation system and vision processing algorithm [11-15].

The cell viability success-rate highly depends not only to the cell environment such as culture medium, temperature, and toxicity condition. Furthermore, the process of single-cell recovery also contributes to the success rate. As an example, the distance between the cell and micropipette tip during the picking up process potentially damaging the cell. A high negative pressure applies typically to the partial-cell aspiration technique, whereas low pressure is more suitable for whole-cell aspiration [16]. However, in some cell sorting system, if the pressure applied is very high and fast, based on Hagen-Poiseuille flow relationships,

\[ \tau = \frac{4Q\eta}{\pi R^3} \]

where \( \tau \) is the wall shear stress, \( Q \) is the flow rate, \( \eta \) is the liquid dynamic viscosity, and \( R \) is the radius of the micropipette, the maximum shear stress occurs at the wall of the micropipette. A smaller diameter of the micropipette, increase shear stress to the cell and the faster cell passing thru the micropipette, the more potential to be damaged. The hydrodynamic impact on cell analysis is analyzed in dynamic fluid laminar flow condition in the tube (micropipette). In laminar flow condition, the relationship between fluid flow-rate and pressure determine from Poiseuille,

\[ \Delta P = \frac{8\eta LQ}{\pi R^4} \]

\[ Q = \frac{\pi R^4(P - P_0)}{8\eta L} \]

where \( P \) is pressure, and \( L \) is the length of the micropipette tube. This equation generally presents the flow rate, \( Q \) is profoundly affected by the radius of the tube. Hence, we analyze the effect of stresses on the cell using several radii of the micropipette. Numerical simulations have been performed using COMSOL Multiphysics® software (version 4.2. COMSOL, Inc., Burlington, MA, USA) using Navier-Stokes solver [17]. The incompressible formulation of the continuity represents the conservation of mass

\[ \rho \nabla \cdot u = 0 \]

and the conservation of momentums equations

\[ p \frac{\partial u}{\partial t} + \rho u \cdot \nabla u = -\nabla p + \nabla \cdot \left( \mu (\nabla u + \nabla u^T) - \frac{2}{3} \mu \nabla \cdot u I \right) + F \]

where \( u \) is the fluid velocity, \( \rho \) is the fluid density, \( p \) is the fluid pressure, \( F \) is instantaneous force, \( I \) is the identity tensor and \( \mu \) is the dynamic fluid viscosity. The acting surface force on the single-cell surface arises from a variety of pressure forces and viscous forces in the fluid flow. From the Navier-Stokes equation, the surface force could be defined as

\[ p \left( \frac{\partial u}{\partial t} + u \nabla u \right) = -\nabla p + u \nabla^2 + \rho g \]

where pressure gradient on the right represents the pressure force, and velocity multiply the viscosity on the lefthalf represents the viscous force. \( g \) represents body acceleration acting on the continuum.

In the analysis of the single-cell model, the structural, mechanical module is used to determine the shear force to the cell and deformation of the sphere model cell type. The force acting to the sphere model determine using surface forces which act directly on the surface of the fluid element, result from the fluid-dynamics couple in the mechanic’s model of a single cell. The time-dependent analysis implements Newton’s second law as,

\[ \rho \frac{\partial^2 u^2}{\partial t^2} = F v + \nabla \cdot \sigma \]

where \( Fv \) is body force per volume, \( \sigma \) is stress, \( \rho \) is the density of the material and \( u \) is velocity. Thus the stress such as force load, tensor-stress and cell surface i.e. cell cytoplasm stress of modeled material could be determined.
The stress of cell in micropipette tube in suction is (f) because the pressure is (g) shown the example of the simulation result where (i) is when the cell distance is 20 μm, (ii) is 0 μm, (iii) is cell stress increased by increasing both the diameter of micropipette and negative suction pressure as in Figure 2(a). For distance 0 μm, the stresses are higher for 30 μm diameter compared to others. The ratio between 30 μm diameter and 40 μm diameter is smaller compared to 40 μm to 50 μm diameter respectively is about 1.2. This condition happens due to hydrodynamic force load is proportional to the area which holds the Continuity equation.

The pressure on the cell surface (cytoplasm) in suction (i) is when the cell distance is 20 μm and pressure suction is applied in the range of 10-50 Pa with an interval of 10 Pa. Then, micropipette distance is set to 20 μm to 30 μm, the stresses are higher for 30 μm distance was about 2.5, while the ratio for the micropipette diameter 40 μm and 50 μm for 0 μm and 20 μm respectively is about 1.2. This condition happens due to hydrodynamic force load is proportional to the velocity of the fluid as described earlier in the Bernoulli equations.

The pressure influenced by hydrodynamic force load is proportional to the velocity of the fluid as described earlier in the Bernoulli equations. The stress of cell in micropipette tube in suction is (f) because the pressure is (g) shown the example of the simulation result where (i) is when the cell distance is 20 μm from the micropipette tip, and (ii) is 0 μm, (iii) show when the cell stress in micropipette tube during suction and (iv) the shear-stress (tensor) of cell in micropipette tube during suction.

**Table 1. Material properties and conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value / Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Elastic Modulus</td>
<td>$E$</td>
<td>1000 Pa</td>
</tr>
<tr>
<td>Cell Poisson-s Ratio</td>
<td>$\nu$</td>
<td>0.5</td>
</tr>
<tr>
<td>Cell Density</td>
<td>$\rho_c$</td>
<td>1012 kg/m$^3$</td>
</tr>
<tr>
<td>Fluid Density</td>
<td>$\rho$</td>
<td>1000 kg/m$^3$</td>
</tr>
<tr>
<td>Fluid Dynamic Viscosity</td>
<td>$\mu$</td>
<td>$8.90 \times 10^{-4}$ Pa.s (25°C)</td>
</tr>
<tr>
<td>Micropipette Diameter</td>
<td>$M_d$</td>
<td>range(30; 10 (step size): 50) μm</td>
</tr>
<tr>
<td>Micropipette Distance</td>
<td>$M_r$</td>
<td>0 and 20 μm</td>
</tr>
<tr>
<td>Recovery Pressure</td>
<td>$P_r$</td>
<td>range(0;10;50) Pa</td>
</tr>
<tr>
<td>Fluid Depth</td>
<td>$F_d$</td>
<td>1000 μm</td>
</tr>
<tr>
<td>Wall</td>
<td></td>
<td>Non-slip condition</td>
</tr>
<tr>
<td>Fluid flow</td>
<td></td>
<td>incompressible, non-turbulence</td>
</tr>
</tbody>
</table>

Figure 1. (a) Conceptual image of single cell recovery by micropipette manipulation. (b) 2D image of proposed single cell recovery simulation.

3. MATERIAL PROPERTIES

The estimation model of single-cell material, fluid properties, pressure, micropipette diameter, distance to the target cell is shown in Table 1. The 2D model was built, with geometry, as shown in Figure 1. The model shows a micropipette with a flat tip is used in the culture dish area with targeted single-cell on the center of the micropipette diameter. The model uses a spherical type cell shape. All the parameters and properties are mostly based on properties of leukocyte [18-19].

The analysis begins with the distance of micropipette tip to the cell is 0 μm and pressure suction is applied in the range of 10-50 Pa with an interval of 10 Pa. Then, micropipette distance is set to 20 μm from the cell model surface and the analysis stress process is repeated. Form the numerical result, the stresses on the cell due to hydrodynamic effects are analyzed.

4. RESULT AND DISCUSSION

It is noted cell stress increased by increasing both the diameter of micropipette and negative suction pressure as in Figure 2(a). For distance 0 μm, the stresses are higher for 30 μm diameter compared to others. The ratio between 30 μm diameter on 0 μm distance to 30 μm diameter on 20 μm distance was about 2.5, while the ratio for the micropipette diameter 40 μm and 50 μm for 0 μm and 20 μm respectively is about 1.2. This condition happens due to hydrodynamic force load is proportional to the velocity of the fluid as described earlier in the Bernoulli equations.

The pressure on the cell surface (cytoplasm) in Figure 2(b), for both distance, is increased by negative suction pressure as well as the micropipette diameter. However, the ratio of 50 μm to 40 μm micropipette diameter is smaller compared to 40 μm to 50 μm diameter, which are about 1.4 and 2.5 respectively, for 20 μm tip to cell distance case. In the case of 0 μm tip to cell distance, the ratio is almost equal for 50 μm to 40 μm and 40 μm to 30 μm diameter, which is about 1.2. The pressure influenced by larger micropipette diameter and far distance to the cell surface.

The stress of cell in micropipette tube in Figure 2(d) shown similar stress and pressure in Figure 2(e) which not affected by the diameter. However, stress and pressure are different in a smaller diameter in Figure 2(f) because the pressure is proportional to velocity, but it is inverse-proportional to the area which holds the Continuity equation.

The cell shear-stress in Figure 2(f) is determined during cell traveling in the micropipette based on tensor-stress. Smaller diameter caused higher tensor stress to cell due to a great response to fluid velocity. This value is higher than von-misses in the tube because it is independent of the first stress invariant, and stress tensor present real stress at a point of the material. In Figure 2(g) shown the example of the simulation result where (i) is when the cell distance is 20 μm from the micropipette tip, and (ii) is 0 μm, (iii) show when the cell stress in micropipette tube during suction and (iv) the shear-stress (tensor) of cell in micropipette tube during suction.
Figure 2. (a) Cell stress during recovery with a distance of 0 and 20 µm. (b) The surface pressure of cell during recovery. (c) Cell load force during recovery. Cell surface pressure and stress during flowing in the tube (micropipette) in (d) and (e) respectively. (f) cell shear-stress (tensor) during cell movement related to hydrodynamic pressure and micropipette diameter. (g) partial simulation image of distance of cell position to the micropipette tip, (i) 20 µm and (ii) 0 µm, (iii) the surface pressure and stress in a tube and (iv) the shear-stress during cell flowing in micropipette.
5. CONCLUSION

We present a single cell stress analysis during cell recovery using a micropipette technique. As a conclusion, hydrodynamic pressure and the diameter of micropipette profoundly affecting the cell stress during the picking up process. This work provides a basic understanding of the relationship between pressure, stress, distance, cell shape, culture medium, and diameter of micropipette which would be useful for setting up a system used for a single-cell recovery application. Furthermore, the recovery process has a high risk caused by cell-damaging or dead during the process. However, limited literature, model, and analysis of real practical cell stress are remaining unclear. In particular, of rare cell recovery application as an example, this viability success is critical for downstream analysis. Thus, further investigation and evaluation of real cell in single-cell recovery become a priority.

REFERENCES