Original Article

Numerical investigation of particle dispersion in the preprocessing stage for a static cell cultivation

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In order to produce high-quality cells in a static culture, the initial placement of the cells is one of the most important factors. Dense distribution of the cells increases the risk of cell death. Thus, the cells need to be uniformly distributed during the preprocessing of a static culture. This process depends on the operator’s experience and has not been standardized. In this study, we have performed numerical simulations to investigate the efficiency of cell dispersion by using OpenFOAM. The numerical domain is a square-shaped dish. Two shaking methods, one-direction and multi-direction reciprocal shaking, were considered and calculations were conducted under five oscillation frequencies. The cell colony was assumed as a solid spherical particle. The initial particles were densely positioned at the center. The numerical result showed that the multi-direction reciprocal shaking was more effective to disperse the particles than the one-direction reciprocal shaking. In addition, at a low frequency, almost all particles sank to the bottom and hardly dispersed. These results indicate that strong fluctuations can lift particles from the bottom and that frequent changes in the flow direction make for more even distribution.

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1. Introduction

Induced Pluripotent Stem (iPS) cells have the ability to differentiate into another tissue cell [1, 2]. Compared with Embryonic Stem (ES) cells [3] that have similar characteristics, iPS cells do not have ethical and immunological problems because iPS cells can be produced from the patient’s somatic cells. They are expected to contribute to regenerative medicine and the development of new drugs. However, culturing iPS cells needs delicate techniques and this process depends on the operator’s experience. In addition, a large number of cells cannot be cultured by one operation. An automated process for massive production of cells is required, which is a common demand in cell cultivation in general. In our previous study, we focused on the suspension culture and investigated numerically the shear stress acting on particles which were the model of cell colonies of 1000 of iPS cells [4].

In a static culture, the initial placement of the cells is one of the most important factors to produce high-quality cells. Cells have to be positioned uniformly in a culture dish because the dense distribution of cells increases the risk of cell death. To obtain an optimal shaking method, we have to quantify the particle distribution and find out exactly what happens in a culture dish. However, there are few reports that investigate the cell seeding process by using numerical simulation. In this study, we have performed numerical simulations to investigate the cell dispersion in a bioreactor with three horizontal reciprocating movements.

2. Numerical methodologies

The iPS-cell colonies are modelled as rigid spherical particles whose shape remains unchanged for the duration of the simulation. The discrete element method (DEM) coupled with computational fluid dynamics (CFD) is applied to investigate the dynamics of the particles in the culture fluid.

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The following assumptions are applied in the simulations:

- The culture fluid is Newtonian and incompressible.
- The temperature and the physical properties of the system remain constant and homogeneous during the process.
- The collision between particles is neglected since the number of particles is small enough.
- Biological, biochemical or chemical reactions do not occur during the simulation.
- The particle behaviour does not affect the fluid motion due to the very low cell concentration. A one-way coupling is assumed in the present simulations.

The modelled square dish has a length of 74 mm on each side (d) and its height (H) is 37 mm. The culture fluid phase is water and the height (h) is 3 mm as in Fig. 1(a).

2.1. Numerical model and governing equations

2.1.1. Fluid phase

The governing equations for the fluid phase (air and water) are the continuity and the Navier–Stokes equations:

$$\nabla \cdot \mathbf{v}_t = 0 \tag{1}$$

$$\frac{\partial \rho_t \mathbf{v}_t}{\partial t} + \nabla \cdot (\rho_t \mathbf{v}_t \mathbf{v}_t) = -\nabla p + \nabla \cdot (\mu_t \nabla \mathbf{v}_t) + \rho_t \mathbf{a} + \sigma \mathbf{n} \delta \tag{2}$$

where $t$ is time, $\rho_t$ is the density, $\mathbf{v}_t$ is the velocity vector, $\mu_t$ is the viscosity of the fluid, $p$ is the pressure, $\mathbf{a}$ is the centrifugal acceleration, $\sigma$ is the surface tension of the water, $k$ is the curvature of the air–water interface, $\delta$ is the delta function and $\mathbf{n}$ is a unit vector normal to the liquid surface. The density ($\rho_t$) of water and air is 996 and 1.2 kg/m$^3$, respectively. The kinematic viscosity ($v = \mu_t/\rho_t$) is $1.1 \times 10^{-6}$ m$^2$/s for water and $1.5 \times 10^{-5}$ m$^2$/s for the air.

The acceleration is $\mathbf{a} = d\mathbf{u}/dt = d^2\mathbf{r}/dt^2$, where $\mathbf{u}$ is the velocity vector of the trajectory of the vessel and $\mathbf{r}$ is the position of the vessel centre. The last term in Eq. (2) represents a surface tension called Continuum Surface Force (CSF) model [5]. A volume of fluid (VOF) method is used to represent the free surface of the gas–liquid interface in a reciprocal shaking dish [6,7].

The shaking trajectory is modelled as a cosine curve and the position of the square dish is set as

$$\mathbf{r} = (x, y, z) = n|R_0\cos(\omega t)| \tag{3}$$

where $n$ represents the shaking direction as illustrated in Fig. 1(b). $T_p = 2\pi/\omega$ is the time period of one cycle of shaking, and eight cycles are considered. At each period, $(m-1)/T_p < t < m(T_p)$, the direction of shaking is $n = (1, 0, 0)$ for $1 \leq m \leq 8$ in the single-reciprocal shaking (SRS) method,

$$n_i = \begin{cases} (1, 0, 0) & \text{for } m = 1, 3, 5, 7 \\ (0, 1, 0) & \text{for } m = 2, 3, 6, 8 \end{cases}$$

for the multi-reciprocal shaking method (MRS1), which alternates the shaking direction every period, and

$$n_i = \begin{cases} (1, 0, 0) & \text{for } m = 1, 2, 5, 6 \\ (0, 1, 0) & \text{for } m = 3, 4, 7, 8 \end{cases} \tag{5}$$

After the eight cycle ($t/T_p > 8$), the process is stopped and the final particle distribution is measured at $t = 14$ s.

2.1.2. Solid phase

Discrete element method (DEM) [8,9] was used to calculate the movement of the particle, which is governed by Newton’s second law of motion as:

$$m_p \frac{\partial \mathbf{v}_p}{\partial t} = \mathbf{F}_D + \mathbf{F}_C - m_p \frac{\partial \mathbf{U}}{\partial t} \tag{6}$$

where $m_p = \pi d_p^3/6$ is the mass of the particle, and $d_p$ is the particle diameter, and $\mathbf{v}_p$ is the velocity vector of the particle.

$$\mathbf{F}_D = C_D A_p \frac{\rho_t}{\rho} \frac{|\mathbf{v}_t - \mathbf{v}_p|^2}{2} \frac{\mathbf{v}_t - \mathbf{v}_p}{|\mathbf{v}_t - \mathbf{v}_p|} \tag{7}$$

is the force from the liquid phase, $A_p = \pi d_p^2/4$ is the projected area of a particle, and

$$\mathbf{F}_C = m_p \mathbf{g} \left(1 - \frac{\rho_t}{\rho} \right) \tag{8}$$

is the net gravitational force acting on a particle considering the buoyancy force that stems from the density difference between fluid and the particle. The empirical drag coefficient

$$C_D = \begin{cases} \frac{24}{Re} \left(1 + \frac{1}{6} Re^{2/3}\right) & Re \leq 1000 \\ 0.424 & Re \geq 1000 \end{cases} \tag{9}$$

is applied, where $Re = |\mathbf{v}_t - \mathbf{v}_p|d_p/\nu$ is the particle Reynolds number based on the relative velocity, $|\mathbf{v}_t - \mathbf{v}_p|$, of particles to the fluid. In this study, the particle diameter is $d = 0.3$ mm, the specific gravity of the particles is set as 1.08, and the total number of particles is 990. Initially, the particles are densely distributed at the center of the dish, and the fluid is stationary.

Fig. 1. (a) Configuration of the reciprocal shaking method. (b) The top view.
2.2. The nondimensional control parameters

By choosing the reference velocity as \( U_{\text{ref}} = Ra_u \) and the reference length as the dimension of the square dish \( L_{\text{ref}} = d \), the nondimensional equation for \( \frac{v}{C_3} = v_f / U_{\text{ref}} \) is obtained as:

\[
\frac{\partial \mathbf{v}}{\partial t} + \nabla \cdot \mathbf{v} \mathbf{v} = -\nabla p + \frac{1}{Re_u} \nabla^2 \mathbf{v} + 2 \frac{Fr_d}{Re} \mathbf{e}_z - \frac{\mathbf{a}^*}{Dr} + \frac{1}{We} \mathbf{n} s,
\]

(10)

where \( \mathbf{e}_z \) is the unit vector in the vertical direction, \( \mathbf{a}^* \) is the nondimensional acceleration by the apparent forcing:

\[
\mathbf{a}^* = -\mathbf{n} \cos(\omega t)
\]

(11)

The nondimensional control parameters for the system are the shaking Reynolds number for water \( (Re_u = Ra_u d / \nu) \), the Froude number \( (Fr = Ra_u^2 / \sqrt{g}) \) which represents the ratio of the inertial centrifugal force and gravitational body force, the ratio of the diameters of the trajectory of the shaking method and the square vessel \( (Dr = R_s / d = 0.46) \), and the Weber number \( (We = \rho_f R_s^2 u^2 d / \sigma) \). The effect of surface tension is weak in our model and also in a large-scale orbital shaking vessel.

2.3. Numerical scheme

The governing equations are discretized using the finite volume method and a second-order linear interpolation scheme is used for the spatial discretization. The velocity and pressure fields are coupled by using the Pressure Implicit with Splitting Operator (PISO algorithm) [10]. The interface curvature is estimated using the Van Leer scheme [11]. The total number of grid points in the simulation is 237,000. The validation was checked in a previous study [12], and the computational accuracy of the numerical codes was confirmed by comparing with Zalc et al. (2002) [13].

2.4. Dispersion rate and shear stress acting on particles

The Numerical results are quantified in 6 s after the shaking process was stopped. The number density \( (N_D) \) of the particle position is calculated in the square dish.

\[
N_D(x, y) = \frac{\text{Number of particles in a bin}}{\text{Total number of particles}} \times 100
\]

(12)

The dispersion efficiency can be evaluated by using the standard deviation \( (\sigma_D) \) of \( N_D \). Small \( \sigma_D \) indicates high dispersion rate.

The shear stress acting on particles is an important factor which affects the quality of undifferentiated iPS cells. We also examine quantitatively the shear stress \( (\tau) \) on the particles from our simulation data. Applying Stokes’ approximation for very slow flow around a sphere, the magnitude of total shear stress over the surface of a spherical particle can be estimated as,

\[
|\tau| = 2 \pi \rho_f \mu_4 |v_f - v_p|
\]

3. Results and discussion

A typical snapshot of the particle dispersed at the end of the shaking process of MRS1 is shown in Fig. 2(a) for \( Fr = 1.08 \). The particle distribution is measured at the end of the shaking process. The number density of the particles is computed as in
Fig. 2(b), where the yellow (or light grey) region represents highly aggregated particles, more than 1%. Note that the ideal equally distributed case has $N_D = 0.25\%$. The uniformity of the particle distribution can be improved by using a large $Fr$, however, attention should be paid to the damaging of the cells. Fig. 3 shows the mean shear stress $|\tau|$ on the particles during the shaking process, where it is clear that the strong shaking increases $|\tau|$ monotonically, so that we should choose as small $Fr$ as possible, keeping the distribution of particles uniform.

Fig. 4 shows the particle distribution for MRS1 method at different $Fr$. For small $Fr$, the particle sank quickly and the shaking could not disperse the particles, while a strong $Fr$ distributed the particles in the whole square dish, although some regions had a large aggregation of particles. The uniformity of the particle distribution is evaluated by the standard deviation of $N_D$ as in Fig. 5. As $Fr$ increases, the standard deviation decreases for $Fr < 1$, however, there is a saturation of $\sigma_D$ for a larger $Fr$. Since we consider the optimal shaking process where the energy consumption and the shear stress acting on the particles $|\tau|$ should be minimized, $Fr \approx 1.1$ is a better choice.

The profiles of $\sigma_D$ are similar for these three methods. The particle distributions of the three methods (SRS, MRS1, MRS2) are, however, different as in Fig. 6 and the distribution of SRS has a line band as seen in Fig. 6(a). We further quantify the anisotropy of $\sigma_D$ in the $x$ and $y$ directions as in Table 1. It is clear that the SRS method shows strong anisotropy regarding the particle distribution, while MRS1 and MRS2 exhibit homogeneous particle dispersions. Fig. 7 shows the effect of the number of shaking cycles for MRS1 method, which reveals that the standard deviation is almost independent of the number of cycles for $Fr > 1$. For $Fr < 1$, $\sigma_D$ may approach the asymptotic value as the number of cycles increases, however, the longer shaking process may affect the quality of cells.

![Fig. 4. The number density of particles, $N_D(x,y)$, for the MRS1 method.](image)
4. Conclusion

We have developed a numerical method, using CFD and DEM methods to evaluate the cell dispersion rate during the pre-processing of the static culture. There is an optimal $Fr = 1.08$, i.e., the optimal combination of shaking distance and speed. Our approach using a computational model can be also useful to find an optimal cultivation condition and significantly reduce the cost to develop a new automated culture method.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2019.04.003.

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