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The Numerical Estimation of Mass Transfer Coefficient of **Oxygen in the Large-Scale Suspension Culture of iPS Cells**

R Isobe¹, Y Kanemaru¹, A Sekimoto¹, Y Okano¹* and M Kino-Oka²

¹ Department of Materials Engineering Science, Osaka University, 1-3, Machikaneyama, Toyonaka, Osaka 560-8531, Japan ² Department of Biotechnology, Osaka University, 1-2, Yamadaoka, Suita, Osaka 565-0971, Japan e-mail: okano@cheng.es.osaka-u.ac.jp

Abstract. In order to practically apply induced pluripotent stem (iPS) cells to regenerative medicine, a large amount of undifferentiated iPS cells should be produced by using an automated/scaled-up suspension culture system. However, in large-scale culture, oxygen supply to iPS cells away from the gas-liquid interface can be insufficient. In this numerical study, the oxygen supply performance is quantitatively evaluated by estimating the volumetric mass transfer coefficient of oxygen in the suspension culture of iPS cells. And, focusing on shaking culture, where shear stress that causes death or differentiation of iPS cells is relatively reduced, we compare two different shaking methods: one direction rotation (ODR) and periodic alternate rotation (PAR). The validity of the volumetric mass transfer coefficient calculation is confirmed by comparison with the experiment. The PAR method is superior to the ODR method in terms of oxygen supply because of higher turbulence intensities, but it is much less energy efficient than the ODR method. In the ODR method, the cell size is thought to be non-uniform since iPS cells are aggregated due to cell sedimentation. On the other hand, in the PAR method, cell sedimentation is suppressed by controlling the Froude number (Fr).

1. Introduction

Induced pluripotent stem (iPS) cells [1, 2] are produced by introducing specific inducers into somatic cells such as human skin and culturing them. They can proliferate without limit and differentiate into cells of various tissues and organs. Although embryonic stem (ES) cells [3] have been extensively studied as the cells with similar abilities to iPS cells, it is pointed out that ES cells have ethical problems regarding the destruction of fertilized eggs and that the possibility of rejection by immune function is high because cells from others are transplanted. On the other hand, since the iPS cells are cultivated from somatic cells of oneself, there are no ethical problems and the rejection response is unlikely to occur.

As for the practical application to organ transplantation, the basic techniques to create a liver bud using human iPS cells and to produce the liver primordium with a vascular network from the liver bud have been reported, and the production method of a large amount of high-quality iPS cells has been established [4-6]. However, the produced liver consists of about 10^8 cells, which does not satisfy the required amount of 10^9 cells for regenerating the real-scale organ, such as the heart or liver of adults [7]. Therefore, it is essential to develop a scaled-up iPS cell culture method that replaces conventional static culture.

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The requirements for the new culture method of the iPS cells are that the cells can be produced in large quantities without requiring any manual operation and that the cells remain undifferentiated. Then, it is believed that an automated/scaled-up suspension culture is appropriate for the production of high-quality iPS cells keeping them undifferentiated [8-10]. However, in suspension culture, it has been reported that the shear stress acting on the iPS cells by agitation causes them death or differentiation during the cultivation process [11]. Furthermore, the cell size becomes inhomogeneous due to the accumulation of cells at the bottom of the vessel.

Among some agitation methods, the shaking method has recently been investigated because the shear stress acting on the cells is relatively small in a shaking bioreactor [12]. When the culture performance for the shaking culture of iPS cells is investigated, a large amount of time and cost are spent in the experiment because of a large number of operating parameters such as rotation speed and shaking radius. Then, a numerical simulation is useful because the phenomenon in the bioreactor can be evaluated quantitatively. The cultivation performance, such as the shear stress acting on the cells and cells distribution in the stirring tank and the orbital shaking tank, has been examined in the past numerical study [12]. However, the volume of the culture medium is a small as about 100 mL in that simulation, and further scale-up is necessary in case of considering a practical application to regenerative medicine. In the present simulation, the volume of the culture medium is 10 L, which is 100 times that of the previous simulation. It is expected that the flow will change from laminar to turbulent flow as the volume of the culture medium is required to analyze the smallest vortices in Kolmogorov scale is enormous, and the computation cost is too high. Therefore, in the design of the culture apparatus, a turbulence model in which the flow is averaged and analyzed macroscopically becomes useful.

Another problem is that oxygen is not sufficiently supplied to all of the cells in the large-scale automated cultivation. On the other hand, it has been reported that the growth rate of iPS cells is improved in the culture at low oxygen concentration [13]. Thus, quantitative assessment of oxygen transfer rates is considered to be critical in the design of iPS cells suspension culture systems. The oxygen transfer rate is calculated from the product of the volumetric mass transfer coefficient and the difference between the bulk concentration and the saturation concentration in the culture medium. In this study, we introduce a model for calculating the volumetric mass transfer coefficient of oxygen in the shaking culture of iPS cells.

We focus on the shaking method in which a cylindrical vessel rotates on a circular orbit, and consider two different shaking methods; the One-Direction Rotation (ODR) method which is widely used and investigated, and the Periodic Alternate Rotation (PAR) method which is expected to keep the iPS cells floating in the vessel. In this study, the volumetric mass transfer coefficient ($k_L a$) of oxygen is calculated using the numerical simulation since the oxygen supply performance is an important evaluation parameter in the suspension culture of iPS cells. In terms of energy efficiency, power consumption should be considered when operating a suspension culture apparatus. Furthermore, the uniform distribution of the cell colonies is required for the high quality of the undifferentiated iPS cells. Therefore, the power consumption (P) and the sedimentation ratio (R_B) of iPS-cell colonies are examined quantitatively and compared in each shaking method.

2. Numerical methods

2.1. Numerical model and assumptions

The numerical model is shown in Figure 1. The movement of the cylindrical vessel follows a circular path horizontally without self-rotation. The shaken tank is shown in Figure 1 (a). The inner diameter (d) of the vessel is 390 mm, the tank height (H) is 450 mm and the tank volume (V) is 50 L. The culture medium is water, whose height (h) is 84 mm and volume (V_L) is 10 L. The circular orbit of the shaken tank is shown in Figure 1 (b), where R_s is the shaking radius and ω is the angular velocity.



Figure 1. The numerical model in the present simulation. (a) The shaken tank, where left half is the computational mesh. (b) The circular orbit of the shaken tank.

The iPS-cell colony, which consists of 10^4 cells, is modeled as solid spherical particles whose shape and size remained constant during the simulation. The discrete element method (DEM) coupled with computational fluid dynamics (CFD) is applied to trace the particles in the culture fluid [14, 15].

The following assumptions are applied in the simulation:

- The culture fluid is Newtonian and incompressible.
- The temperature and the physical properties of the system remain constant.
- Biological, biochemical and chemical reactions don't occur.
- The collision of particles and the particle action on the fluid is neglected due to the very low particle concentration, then one-way coupling is applied.

2.2. The governing equations

2.2.1. Fluid phase. Since the flow of the culture fluid is predicted to reach turbulent, a large number of computational grids are required. Therefore, it is necessary to use the turbulence model to reduce the computation cost and to predict the effects of turbulence. In this simulation, the realizable k- ε turbulence model [16] is applied for the fluid phase (air and water) analysis. The governing equations are the Reynolds-averaged continuity equations, the Reynolds-averaged Navier-Stokes equations and the transport equations for k and ε :

$$\nabla \cdot \boldsymbol{v}_{\mathrm{f}} = 0 \tag{1}$$

$$\frac{\partial}{\partial t}\rho_{\rm f}\boldsymbol{v}_{\rm f} + \nabla \cdot (\rho_{\rm f}\boldsymbol{v}_{\rm f}\boldsymbol{v}_{\rm f}) = -\nabla p + \nabla \cdot \left((\mu_{\rm f} + \mu_{\rm t})\nabla \boldsymbol{v}_{\rm f}\right) + \rho_{\rm f}\boldsymbol{g} - \rho_{\rm f}\boldsymbol{a}_{\rm c} + \sigma\kappa\boldsymbol{n}_{\rm s}\delta$$
(2)

$$\frac{\partial}{\partial t}\rho_{\rm f}k + \nabla \cdot (\rho_{\rm f}k\boldsymbol{\nu}_{\rm f}) = \nabla \cdot \left\{ \left(\mu_{\rm f} + \frac{\mu_{\rm t}}{\sigma_{\rm k}} \right) \nabla k \right\} + P_{\rm k} - \rho_{\rm f}\varepsilon$$
(3)

$$\frac{\partial}{\partial t}\rho_{\rm f}\varepsilon + \nabla \cdot (\rho_{\rm f}\varepsilon\boldsymbol{\nu}_{\rm f}) = \nabla \cdot \left\{ \left(\mu_{\rm f} + \frac{\mu_{\rm t}}{\sigma_{\varepsilon}}\right) \nabla \varepsilon \right\} + c_1 \frac{\varepsilon}{k} P_{\rm k} - \rho_{\rm f} c_2 \frac{\varepsilon^2}{k + \sqrt{\nu_{\rm f}\varepsilon}} \tag{4}$$

where t is time, $\boldsymbol{v}_{\rm f}$ is the velocity vector, $\rho_{\rm f}$ is the density, p is the pressure, $\mu_{\rm f}$ is the viscosity, $\mu_{\rm t}$ is the eddy viscosity, g is the gravity acceleration, $\boldsymbol{a}_{\rm c}$ is the centrifugal acceleration, σ is the surface tension

of air-water, κ is the curvature of the air-water interface, δ is the delta function, \mathbf{n}_s is an unit vector normal to the liquid surface, k is the turbulent kinetic energy, P_k is the generation of the turbulent kinetic energy, ε is the dissipation rate of turbulent energy, c_1 is a model coefficient, v_f is the kinematic viscosity, and σ_k , σ_{ε} and c_2 are model constants.

The centrifugal acceleration is $a_c = dU/dt = d^2 r/dt^2$, where U is the velocity of the trajectory of the vessel and r is the position of the vessel center, which is expressed as:

for the ODR method,

$$\boldsymbol{r} = \begin{bmatrix} \boldsymbol{x} \\ \boldsymbol{y} \\ \boldsymbol{z} \end{bmatrix} = \begin{bmatrix} \boldsymbol{R}_{s} \cos \omega t \\ -\boldsymbol{R}_{s} \sin \omega t \\ \boldsymbol{0} \end{bmatrix}$$
(5)

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and for the PAR method, which alternates the rotation direction each one period ($T_{\theta} = 2\pi/\omega$).

$$\boldsymbol{r} = \begin{bmatrix} \boldsymbol{x} \\ \boldsymbol{y} \\ \boldsymbol{z} \end{bmatrix} = \begin{bmatrix} -R_{\rm s} \cos\left(\pi \cos\frac{\omega}{2}t\right) \\ -R_{\rm s} \sin\left(\pi \cos\frac{\omega}{2}t\right) \\ 0 \end{bmatrix}$$
(6)

The last term in equation (2) indicates a surface tension which is calculated using Continuum Surface Force (CSF) model [17]. In the VOF method, the governing equation is the transport equations for α :

$$\frac{\partial \alpha}{\partial t} + \nabla \cdot (\alpha \boldsymbol{v}_{\rm f}) + \nabla \cdot \left((1 - \alpha) \alpha \boldsymbol{v}_{\rm r} \right) = 0 \tag{7}$$

where α is the volume fraction of the liquid phase and v_r is the relative velocity of the liquid phase to the gas phase. The phase relative velocity v_r is considered as compressive velocity v_c , which is expressed as:

$$\boldsymbol{v}_{\rm r} = \boldsymbol{v}_{\rm c} = \boldsymbol{n}_{\rm s} \min \left[c_{\alpha} |\boldsymbol{v}_{\rm f}|, \, \max(|\boldsymbol{v}_{\rm f}|) \right] \tag{8}$$

where c_{α} is a constant of the interface compression strength. In this simulation, c_{α} is set to 1. When using the CSF model, $n_s \delta$ and κ is approximated as:

$$\boldsymbol{n}_{\rm s}\delta = \nabla \alpha \qquad \text{and} \qquad \kappa = \nabla \cdot \left(\frac{\nabla \alpha}{|\nabla \alpha|}\right).$$
 (9)

2.2.2. Solid phase. The particle is traced by solving Newton's second law of the motion using the Lagrangian method:

$$m_{\rm p}\frac{\mathrm{d}\boldsymbol{\nu}_{\rm p}}{\mathrm{d}t} = \boldsymbol{F}_{\rm D} + \boldsymbol{F}_{\rm g} - m_{\rm p}\frac{\mathrm{d}\boldsymbol{U}}{\mathrm{d}t}$$
(10)

where $m_p = \pi \rho_p d_p^3/6$ is the mass of a particle, ρ_p is the density of the particle, d_p is the particle diameter, and v_p is the velocity of a particle. In equation(10), F_D is the drag force from the fluid phases:

$$\boldsymbol{F}_{\mathrm{D}} = C_{\mathrm{D}} A_{\mathrm{p}} \frac{\rho_{\mathrm{f}}}{2} |\boldsymbol{v}_{\mathrm{f}} - \boldsymbol{v}_{\mathrm{p}}| (\boldsymbol{v}_{\mathrm{f}} - \boldsymbol{v}_{\mathrm{p}})$$
(11)

where $A_p = \pi d_p^2 / 4$ is the surface area of a particle. C_D is the drag coefficient:

$$C_{\rm D} = \begin{cases} \frac{24}{Re_{\rm p}} \left(1 + \frac{1}{6} Re_{\rm p}^{2/3} \right) & \left(Re_{\rm p} < 1000 \right) \\ 0.424 & \left(Re_{\rm p} \ge 1000 \right) \end{cases}$$
(12)

where $Re_p = |v_f - v_p| d_p / v_f$ is the particle Reynolds number. In equation (10), F_g is the gravity force considering the buoyancy force that stems from the density difference between the fluids and the particle.

$$\boldsymbol{F}_{\rm g} = m_{\rm p} \boldsymbol{g} \left(1 - \frac{\rho_{\rm f}}{\rho_{\rm p}} \right) \tag{13}$$

2.3. Operating condition and physical properties

The density of water and air is 996 and 1.2 kg/m³, respectively. The kinematic viscosity of water and air is 8.0×10^{-7} and 1.6×10^{-5} m²/s, respectively. In this simulation, ρ_f and ν_f is defined by using the volume fraction of liquid phase α :

$$\rho_{\rm f} = \alpha \rho_1 + (1 - \alpha) \rho_2 \tag{14}$$

$$\nu_{\rm f} = \alpha \nu_1 + (1 - \alpha) \nu_2 \tag{15}$$

where subscript 1 represents liquid phase, and subscript 2 represents gas phase. The surface tension of air-water is 0.071 kg/s². The specific gravity of the particle to the liquid phase is 1.08 and the particle diameter d_p is 0.3 mm. The total number of particles is 10,660 and their initial configuration is uniformly distributed. The fluid is initially stationary. The simulation is performed for 60 seconds, which is sufficient for the average evaluation, and the average value after 30 seconds is used for the evaluation.

2.4. Nondimensional parameters

In order to investigate the nondimensional parameters governing the flow, the Navier-Stokes equation is transformed into dimensionless form. In this simulation, the velocity of the vessel is chosen as the reference velocity, and the inner diameter of the vessel is chosen as the reference length. The nondimensional equation is

$$\frac{\partial \boldsymbol{v}^*}{\partial t^*} + \nabla^* \cdot \boldsymbol{v}^* \boldsymbol{v}^* = -\nabla^* p^* + \frac{1}{Re} \nabla^{*2} \boldsymbol{v}^* + \frac{1}{Fr^2} \frac{2}{Dr} \boldsymbol{e}_z - \frac{2}{Dr} \boldsymbol{a}_c^* + \frac{1}{We} \kappa^* \boldsymbol{n}_s \delta^*$$
(16)

where $\boldsymbol{v}^* = \boldsymbol{v}_f / U_{ref}$ is the nondimensional velocity, $U_{ref} = R_s \omega$ is the reference velocity, and \boldsymbol{e}_z is an unit vector in the vertical direction.

In equation (16), there are four nondimensional parameters. They are the Reynolds number ($Re = R_s\omega d/\nu$), the Froude number ($Fr = R_s\omega/(R_sg)^{1/2}$) which represents the ratio of the centrifugal force to the gravity force, the diameter ratio ($Dr = 2R_s/d$) and the Weber number ($We = \rho_f R_s^2 \omega^2 d/\sigma$) which represents the ratio of the inertial force to the surface tension. The contributions of viscosity and surface tension terms are smaller than those of inertial term because the Re and We are high order in the present simulation. Thus, we control Fr and Dr when we compare the ODR and PAR method.

The nondimensional acceleration (a^*) by the centrifugal force is expressed as:

$$\boldsymbol{a}_{c}^{*} = \begin{pmatrix} -\cos\omega t\\ \sin\omega t\\ 0 \end{pmatrix} \qquad (ODR) \tag{17}$$

and

$$\boldsymbol{a}_{c}^{*} = \begin{pmatrix} -\frac{1}{4}(-\pi^{2} + \theta^{2})\cos\theta - \frac{1}{4}\theta\sin\theta\\ -\frac{1}{4}(-\pi^{2} + \theta^{2})\sin\theta + \frac{1}{4}\theta\cos\theta\\ 0 \end{pmatrix}$$
(PAR) (18)

where $\theta = \pi \cos(\omega t/2)$, $|\mathbf{a}_c^*| = 1$ for the ODR method and $|\mathbf{a}_c^*(t)| = ((-\pi^2 + \theta^2)^2 + \theta^2)^{1/2}/4$ for the PAR method. In order to directly compare results of the ODR and PAR method, the effective Froude number for each method, which is the ratio of the 3rd term to the 4th term on the right side of equation (16), is defined as:

$$Fr^* = Fr\left((1/T_{\theta})\int_0^{T_{\theta}} |\boldsymbol{a}_c^*| \, \mathrm{d}t\right)^{-1/2} = Fr$$
 (ODR) (19)

$$Fr^* = Fr\left((1/T_{\theta})\int_0^{T_{\theta}} |\boldsymbol{a}_c^*(t)| \,\mathrm{d}t\right)^{-1/2} = FrA_{\theta}^{-1/2} \qquad (PAR)$$
(20)

where T_{θ} is the rotation period and $A_{\theta} = 1.48$ is the compensation coefficient for the PAR method. Fr^* is set to the same level in each shaking method to equalize the external force applied to the fluid.

2.5. Evaluation of oxygen supply, power consumption and particle distribution

2.5.1. Volumetric mass transfer coefficients of oxygen. The volumetric mass transfer coefficient $(k_L a)$ of oxygen is simulated to evaluate oxygen supply quantitatively. The volumetric mass transfer coefficient can be separated into two parts: the specific interfacial area (a) and the mass transfer coefficient (k_L) . a is calculated as:

$$a = \frac{A}{V_{\rm L}} \tag{21}$$

where A is the interfacial area and V_L is the liquid volume. k_L is estimated by applying the eddy cell model [18], which assumes that the small-scale turbulent motion affects the mass transfer rate and is expressed as:

$$k_{\rm L} = K \sqrt{D} \left(\frac{\bar{\varepsilon}}{\nu}\right)^{1/4} \tag{22}$$

where K = 0.4 is the model constant, $D = 2.4 \times 10^{-9}$ m²/s is the diffusion coefficient of oxygen in water, $\bar{\varepsilon}$ is the average dissipation of turbulent energy in the liquid phase and ν is the kinematic viscosity of water.

2.5.2. Evaluation of power consumption. The power consumption (P) by operating the suspension culture apparatus is equal to the energy loss of the fluids, which includes the dissipation of turbulent energy and the pressure loss. The dissipation of turbulent energy is more dominant than the pressure loss and the power consumption is calculated using the dissipation rate of turbulent energy as following formula:

$$P = \int \rho_{\rm f} \varepsilon dV \tag{23}$$

where ρ_f is the density of fluids, ε is the dissipation rate of turbulent energy and V is the volume of the vessel.

2.5.3. *Evaluation of particle distribution*. The sedimentation ratio, which expresses the accumulation of particles at the bottom of the vessel, is defined as:

$$R_{\rm B} = N_{\rm B}/N_{\rm A} \tag{24}$$

where $N_{\rm B}$ is the number of particles attached on the bottom and $N_{\rm A} = 10,660$ is the total number of particles existing in the vessel.

2.6. Numerical implementation

The governing equations associated with the present simulation are implemented in the open source CFD toolbox, OpenFOAM as a VOF solver, so-called *interFoam*. In the present simulation, the governing equations are discretized using the finite volume method. The space discretization is performed using the second-order linear interpolation scheme. The interface curvature is estimated using the second-order *vanLeer* scheme [19]. The velocity and pressure fields are coupled by using the Pressure Implicit with Splitting Operator (PISO) algorithm [20]. The total number of grids in this simulation is 1,080,000. The boundary condition of the velocity at the wall is the no-slip condition. The code validation is shown in the previous study, where the computational accuracy was confirmed by comparing with Salek *et al.* [21].

3. Results and discussion

 Fr^* and Dr in each method are set to the same value to equalize the external force applied to the fluid when comparing the ODR and PAR method. The operating conditions in the present simulation are shown in table 1.

	ω [rad/s]	<i>R</i> _S [m]	Fr* [-]	Dr [-]
ODR	3.1	0.20	0.45	1.0
	4.2	0.20	0.60	1.0
PAR	2.6	0.20	0.45	1.0
	3.4	0.20	0.60	1.0

Table 1. Operating conditions for 4 cases in the present simulation.

3.1. Validation of calculation of the volumetric mass transfer coefficient

To confirm the reliability of the calculation method of the volumetric mass transfer coefficient of oxygen in the present simulation, the results in this simulation are compared with the empirical equation obtained from the experiment performed by Klöckner *et al.* [22]. The numerical domain is the same as that in Figure 1, and shaking method is the ODR method. To investigate the effects of angular velocity and shaking radius, comparisons are performed by fixing one and changing the other. Figure 2 shows the results of the validation. The values are generally consistent and the numerical errors are $3 \sim 20\%$. Therefore, it is suggested that the numerical results on the volumetric mass transfer coefficient are sufficiently reliable.

3.2. Oxygen supply performance

For quantitative evaluation of the oxygen supply performance, the volumetric mass transfer coefficient of oxygen is calculated and its magnitude is compared between the ODR and PAR method. The results



Figure 2. The comparison of the present numerical results of the volumetric mass transfer coefficient with the experimental equation by Klöckner *et al.* [22]. (a) $k_{\rm L}a$ vs. ω where $R_{\rm S} = 0.05$ m. (b) $k_{\rm L}a$ vs. $R_{\rm S}$ where $\omega = 100$ rpm.



Figure 3. The volumetric mass transfer coefficient in the cases of ODR and PAR methods.



Figure 4. The power consumption in the cases of ODR and PAR methods.

are shown in Figure 3. $k_L a$ for the PAR method is much larger than that for the ODR method. This is because the Reynolds number is higher in the PAR method. In other words, in the PAR method, the diffusion of oxygen in the culture medium is enhanced by the strong turbulence intensity. Further, the oxygen supply performance is also improved by increasing Fr^* . The strong centrifugal force changes the shape of the liquid surface and lead the large area of the gas-liquid interface. Therefore, high Fr^* enhances the diffusion of the oxygen.

3.3. Power consumption in the large-scale shaking culture

There are large differences in the power consumption between the ODR method and the PAR method as shown in Figure 4. The power consumption for the PAR method is required 10 to 100 times greater than that for the ODR method. This is because a heavy load is applied to the rotation of the vessel due to a rapid change in the acceleration of the flow.

3.4. Particle distribution in the vessel

The typical snapshots of the particle distribution are illustrated in Figure 5. In the ODR method, particles tend to aggregate near the center of the vessel. The flow near the bottom of the vessel is slower than the flow near the gas-liquid interface because the viscous stress has a greater effect than the centrifugal force in the bottom boundary layer. Therefore, an inward secondary flow within the bottom boundary layer is





Figure 5. Top view of the vessel at 60 seconds. The particle distribution is expressed by the red dots in the (a) ODR and (b) PAR method for $Fr^* = 0.60$.

Figure 6. The sedimentation ratio $R_{\rm B}$ as functions of time in the cases of ODR and PAR methods.



Figure 7. The average nondimensional velocity in *z*-direction ($v_{f,z}^* = v_{f,z}/R_s\omega$) at *z* = 1mm. The vessel is shaken with the PAR method for (a) $Fr^* = 0.45$ and (b) $Fr^* = 0.60$.

generated in the direction toward the center of the vessel, which causes the particles to be aggregated. On the other hand, the PAR method overcomes this problem significantly and the particles are somehow dispersed in the vessel. This is because the particle sedimentation is suppressed and the particles are not affected by an inward secondary flow within the bottom boundary layer. Figure 6 shows the time evolution of the sedimentation ratio. In the ODR method, the number of bottom particles continues to increase monotonically and almost all particles eventually sink. In comparison, in the PAR method, the bottom particles can float because of the change in the direction of rotation. Furthermore, the sedimentation of all particles is relatively suppressed at higher Fr^* since the frequent alternation of vessel rotation produces strong vertical velocity as shown in Figure 7.

4. Conclusion

The suspension culture of the iPS cells requires the development of a new shaking method that optimizes various evaluation parameters such as oxygen supply performance, energy efficiency, the shear stress acting on the iPS cells, and iPS-cell colonies aggregation. In the present study, we focus on the oxygen supply performance and have developed a model for estimating the volumetric mass transfer coefficient of oxygen in shaking culture using the numerical simulation. The value estimated by the present numerical simulation is almost in agreement with the empirical formula obtained from the experiment, indicating the validity of the present numerical model.

We have compared the two shaking methods: the ODR method which is used and investigated generally and the PAR method which changes the rotation direction each one period. Since the

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volumetric mass transfer coefficient of oxygen in the PAR method is more than twice that in the ODR method, it is shown more oxygen is supplied to the culture medium in the PAR method compared to the ODR method. However, it is pointed out that the power consumption is too large to be neglected as in the PAR method. When shaking the vessel with the PAR method, the aggregation of cell colonies near the center of the bottom of the vessel which is observed in the ODR method is reduced.

In the PAR method, there is the critical Fr^* which determine if the iPS-cell colonies float or sink between 0.45 and 0.60. It is also expected that the sedimentation of the particles can be further suppressed by increasing Fr^* . When Fr^* is further increased, the shear stress acting on the iPS cells becomes an important evaluation parameter. In the future, it is necessary to examine the effect of Fr^* on the culture performance by further simulation. In addition, since Dr determines whether vortices are formed in the vessel or not, the effects of Dr should be also investigated. Therefore, it is expected that there will be the optimum Fr^* and it is considered that the optimization of the large-scale culture of the iPS cells using the PAR method is necessary.

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