Analysis of Stem Cell Culture Performance in a Microcarrier Bioreactor System

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ABSTRACT

An analysis of the effects of the turbulent shear stress on cell culture in a stirred tank microcarrier bioreactor system using a synergistic combination of computational fluid dynamic (CFD)-based simulations and experiments is presented. A 3D computational model of Corning's bench-scale spinner flask was built using a state-of-the-art computational fluid dynamics (CFD) software, Flow3D (www.flow3d.com). The effects of parameters such as the impeller speed, culture medium fluid properties and particle size on the steady-state shear stress acting on the cell-laden microcarrier particles in the bioreactor are studied using CFD analysis. This is used to predict the precise shear conditions experienced by cells and identify optimum operating conditions that prevent turbulent shear damage of the cells. In addition, the effect of shear on the pluripotency of hPSCs is studied by determining the percentage of cells carrying the pluripotency markers *Oct4*, *Sox2* and *Nanog* using flow cytometry and quantitative PCR.

Keywords: Human pluripotent stem cells (hPSCs), Computational Fluid Dynamics (CFD), Turbulent shear stress, cell culture, Kolmogorov length scale, pluripotency.

1 INTRODUCTION

Human pluripotent stem cells (hPSCs) are capable of differentiating into all somatic cell types and therefore hold great potential for future clinical application. To induce the differentiation of hPSCs, biochemical and biophysical stimuli plays a central role in stem cell fate decision. In addition, biomechanical forces have also been shown to affect differentiation propensity of mouse embryonic stem cells (mESCs) into lineages such as endothelial, haematopoietic and cardiac cells [1, 2].

Often, laminar flow environment was employed to investigate shear-stress which was easy to control experimentally [3]. However, large-scale expansion and differentiation of stem cells typically utilized stirred-suspension bioreactor where shear stress effect may be different from laminar flow environment. For example, shear stress in stirred suspension bioreactors was found to improve mESC pluripotency [4] while in 2D laminar environment it primed mESCs to differentiate[5]. Two potential reasons can explain this discrepancy: First, shear stress in bioreactor system, as a 3D environment was not as homogeneous as 2D environment, and second stem cells in stirred-suspension were subjected to diffusion limitation of oxygen and nutrients [6] as cell aggregates.

To overcome these two limitations, we utilized computation fluid dynamics (CFD) to investigate shear-stress effect on hPSC phenotype and compared it to experimental results using a microcarrier bioreactor system. Microcarrier system provide culture environment closer to monolayer, largely reducing the diffusion limitation into cell aggregates However, agitation in bioreactors caused due to stirring induces flow stresses which are capable of inducing damage to microcarriers and indeed to stem cells[7]. In addition, cells attached to microcarriers cannot easily change position or rotate in response to shear forces in the fluid. This, coupled with the lack of a protective cell wall, make animal cells on microcarriers especially susceptible to shear damage [8]. Although excessive fluid shear can be detrimental, moderate fluid shear may prove to be desirable as it can lead to increased cell permeability [9] and increased secretion of extracellular proteins [10]. CFD modeling is therefore essential to predict the flow and the precise shear conditions experienced by cells in the stirred vessels employed for cell cultures. The regions experiencing the stress that could cause cell damage can be readily identified from the analysis of spatial distributions of shear stress and turbulent length scales obtained from the simulation. It has been reported that cell damage can be avoided if the particle size is smaller than the size of the smallest eddy as characterized by the Kolmogorov length scale for turbulence [8]. Thus, by carefully controlling the operating conditions of the reactor and microcarrier particle size, we could potentially adjust shear stress hPSCs were exposed to based on competing considerations of shear induced damage and agglomeration. On the other hand, CFD method can not only provide shear stress field in more detail but also predict microcarrier trajectory as a moving object.

2 THEORY AND MODELING

Turbulent fluctuations in stirred vessels are associated with high Reynolds number and are characterized by a cascade of eddies of various length scales. When a stirred vessel is agitated with a rotating impeller, the Reynolds number for the bulk flow is given by[8]

$$N_{RE} = \frac{ND_i^2}{\nu} \quad (1)$$

Where, ν is the kinematic viscosity (m^2/s), N is the agitation rate (rpm), D_i is the impeller diameter and. For complete off-bottom suspension of microcarriers, virtually all stirred vessels must be agitated in the turbulent regime[11]. In the turbulent flow field of a microcarrier culture, short term hydrodynamic forces arise through the motion of turbulent eddies. In conjunction with the transfer of energy from large to small eddies, there exists a spectrum of eddy sizes down to the viscous dissipation regime. The intensity of shear depends on the size of eddies that exist in the bioreactor relative to the microcarrier particles. If the particles are small relative to the eddies, they tend to be captured or entrained in the eddies as shown below in fig 1[8] .

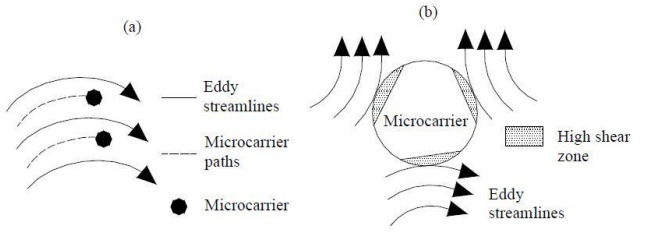

Fig 1: Eddy-microcarrier interactions. (a) Eddy size larger than microcarrier particles. (b) Eddy-size smaller than microcarrier particle

A single eddy that cannot fully engulf the particle will act on part of its surface and cause the particle to rotate in the fluid as shown in fig 1b; this will result in a relatively small level of shear at the surface of the bead. However, much higher shear stress result when several eddies with opposing rotation interact with the particle simultaneously. It has been reported that cell damage can be prevented if the particle size is smaller than the size of the smallest eddy as characterized by the Kolmogorov length scale for turbulence[11]. It is calculated from the equation shown below:

$$\lambda = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4} \quad (2)$$

Where, λ is the Kolmogorov length scale that is the characteristic dimension of the smallest eddies and is usually in the range of 30-100 μm , ν is the kinematic viscosity of the fluid, and ε is the local rate of turbulent energy dissipation per unit mass of liquid. At steady state the rate of energy dissipated by turbulence is equal to the power supplied by the impeller.

Mixing power for non-aerated fluids depends on the stirrer speed, the impeller diameter and geometry, and properties of the fluid such as density and viscosity. The relationship between these variables is usually expressed in terms of dimensionless numbers such as the impeller Reynolds number N_{RE} and the power number N_p. N_p is defined as:

$$N_p = \frac{P}{\rho N_i^3 D_i^5} \quad (3)$$

Where, N_p is the dimensionless power number, N_i is the agitation rate (rpm), D_i is the impeller diameter (m) and ρ is the medium density (kg/m^3). The above equation express the strong dependence of power consumption on the impeller diameter and the stirrer speed. The dimensionless power number can be estimated through the Nagata (1975) correlation [12]:

$$N_p = \frac{P}{N^3 D^5 \rho} = \frac{K_1}{N_{RE}} + K_2 \left(\frac{10^3 + 1.2N_{RE}^{0.66}}{10^3 + 3.2N_{RE}^{0.66}}\right)^{K_4} \quad (4)$$

$$K_1 = 14 + \left(\frac{W}{D_T}\right)\left[670\left(\frac{D_i}{D_T} - 0.6\right)^2 + 185\right] \quad (5)$$

$$K_2 = 10^{K_3} \quad (6)$$

$$K_3 = 1.3 - 4\left(\frac{W}{D_T} - 0.5\right)^2 - 1.14\left(\frac{D_i}{D_T}\right) \quad (7)$$

$$K_4 = 1.1 + 4\left(\frac{W}{D_T}\right) - 2.5\left(\frac{D_i}{D_T} - 0.5\right)^2 - 7\left(\frac{W}{D_T}\right)^4 \quad (8)$$

Where, N_{RE} is the dimensionless Reynolds's number, W is the impeller width (m), D_T is the bioreactor diameter (m) and D_i is the impeller diameter (m). The impeller Reynolds number, used to describe flow regimes, is a ratio of inertial forces to viscous forces and is defined as shown in equation-1. For these calculations, the following media properties determined at 37 ^{0}C were used: density (ρ) =990.8 kg/m^3, kinematic viscosity (υ) =9.14×10^{-7} m^2/s and viscosity (μ) =9.05×10^{-4} Pa/s.

The average power input per unit mass can be calculated from the power number correlation by solving the above algebraic equations simultaneously. The corresponding shear stress and the Kolmogorov length scale are obtained from the Flow 3D simulation. Based on the empirical correlations of cell death with the shear stress we can tune the parameters including fluid media viscosity, stirring speed and microcarrier particle size in order to obtain optimum growth of the cells. The calculations indicate that the optimal stirring rate for the growth of hPSCs in the 250 ml traditional lab scale spinner flask is 60 rpm. At spin rates above 60 rpm the turbulence generates eddies which are smaller than the size of the microcarriers leading to shear damage because of high levels of shear developed due to opposing rotation interaction on the particle surface as shown in fig. 1b

2.1 MODEL DESCRIPTION

A state-of-the-art multiphysics CFD program Flow-3D (www.flow3d.com) was used for this analysis. The Fig.3

below shows the 3D CFD model of the Corning's bench-scale stirred-flask bioreactors. In the computational model, the reactor is filled with 50 ml of cell culture media and loaded with micro carrier particles that are 150 microns in diameter in a concentration of 0.5 g/ml. The simulation takes into account fully-coupled particle/fluid interactions wherein the particles move in response to viscous drag imparted by the flow field and their motion, in turn, alters the flow. Two-equation $k-\varepsilon$ model was chosen to model turbulence. Second order monotonicity momentum advection was activated and the computational grid was refined near the bottom portion of the impeller which is in contact with the fluid in order to accurately capture the eddies. The shear stress experienced by the microcarrier is given by the expression

$$\tau = 2\mu^* |S| \tag{8}$$

Where τ is the shear stress, μ is dynamic viscosity and $|S|$ is the magnitude of the strain rate.

$$|S| = \sqrt{\frac{1}{2}\left(\frac{\partial u}{\partial x}\right)^2 + \frac{1}{2}\left(\frac{\partial v}{\partial y}\right)^2 + \frac{1}{2}\left(\frac{\partial w}{\partial z}\right)^2 + \frac{1}{4}\left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x}\right)^2 + \frac{1}{4}\left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial x}\right)^2 + \frac{1}{4}\left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y}\right)^2} \tag{9}$$

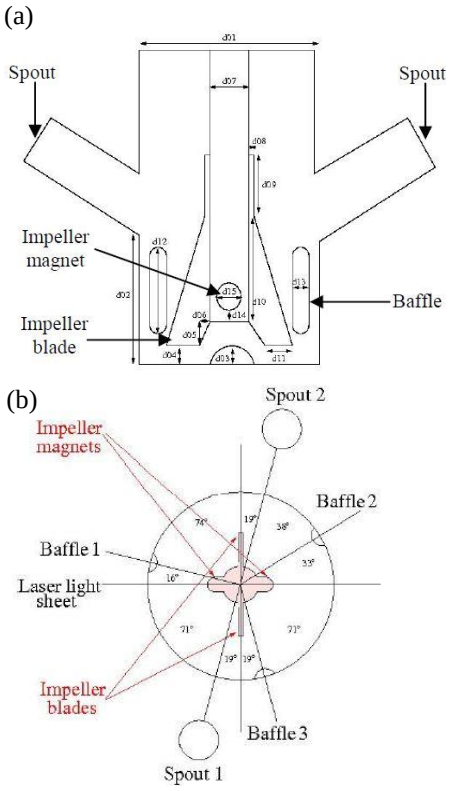

Fig.2 (a) Corning's bench-scale stirred-flask CFD model geometry setup. (b) Positioning of impeller within flask and positioning of baffles (refer to Table 1 for lengths).

Table 1

Geometry parameter	Length (mm)	Geometry parameter	Length (mm)	Geometry parameter	Length (mm)
d01	65	d06	1	d11	10
d02	52.5	d07	18	d12	38
d03	4	d08	0.5	d13	10
d04	7.5	d09	20	d14	3.5
d05	5	d10	35	d15	8

3 RESULTS AND DISCUSSION

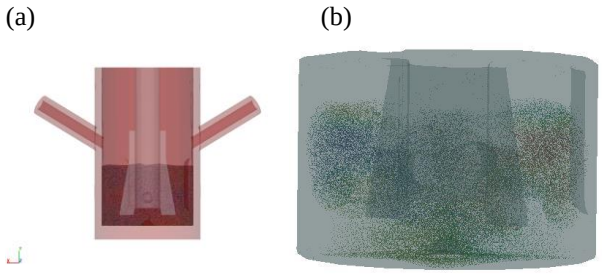

Fig. 3. (a) 3D CFD model of Corning's bench-scale stirred-flask bioreactor, (b) microcarrier particle dynamics colored by x-velocity.

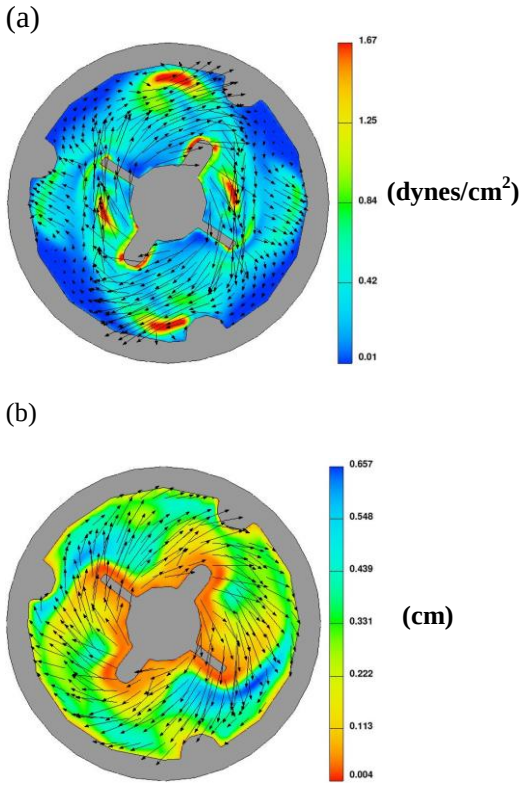

Fig. 2. (a) Shear stress distribution along with velocity vectors in a cross sectional plane of the bioreactor running at 60 rpm, (b) Kolmogorov length scale distribution at the same plane under the same conditions.

From Fig.3, we can clearly visualize the generation of shear forces in the boundary layers and near the solid objects. During operation of the Corning stirrer-flask, the minimum Kolmogorov length scale was found to be lowest when an impeller magnet passes a baffle. It is observed that the wake region created behind the impeller blades experience relatively low λ in the order of 50 µm. Because of the low pressure created in the wake the microcarrier particles will be driven towards the wake region, therefore the λ in the wakes of the impeller blades becomes the determining factor for the cell damage or the differentiation outcome.

Studies have been done on H9 hPSCs seeded as single cells on Matrigel-coated polystyrene micro-carriers. The successful cell proliferation and maintenance of pluripotency using the Corning bioreactor at 60 RPM is demonstrated in Figure 4 using Nanog, SSEA4, and Oct4 gene expressions.

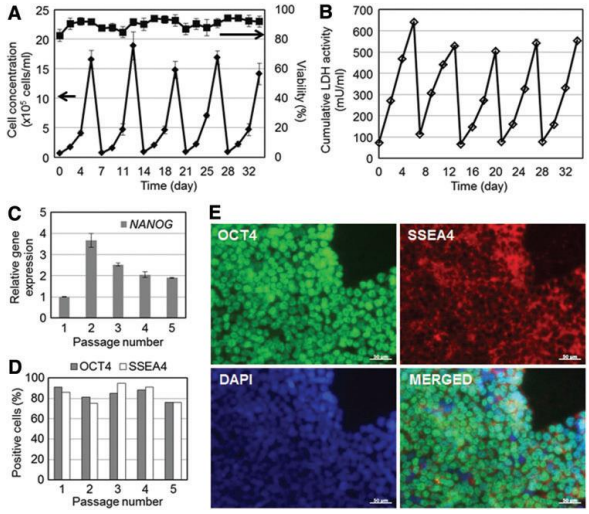

Fig. 5[13]. Growth profile and pluripotency studies on H9 hPSCs cultured on Matrigel-coated micro-carriers at 60 RPM. (A) Viability and cell growth on micro-carriers over five passages (B) Cumulative Lactate Dehydrogenase activity studies (C) Gene expression of pluripotency markers (D) Flow cytometry of the percentage of pluripotent cells on micro-carriers (E) Pluripotency observed by immunostaining.

CONCLUSION

The hydrodynamics within the cornings stirred-flask bioreactor was studied using a combination of CFD and experiments. The effects of shear stress on cell proliferation and cell viability has been studied. In addition, the maintenance of pluripotency at a particular agitation rate was presented. This work shows that CFD modeling is essential to accurately model the microscopic flow fields arising due to the microcarrier-eddy interactions and predict the precise shear conditions experienced by the microcarriers during cell culture. Furthermore, CFD modeling helps in identifying the possibilities for microcarrier exposure to regions of stress levels that could cause turbulent shear damage to the cells.

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