

Modeling of Oxygen Supply Profiles in Stirred-Tank Aggregated Stem Cells Cultivation Process

Vytautas Galvanauskas, Vykantas Grincas, Rimvydas Simutis

Abstract—This paper investigates a possible practical solution for reasonable oxygen supply during the pluripotent stem cells expansion processes, where the stem cells propagate as aggregates in stirred-suspension bioreactors. Low glucose and low oxygen concentrations are preferred for efficient proliferation of pluripotent stem cells. However, strong oxygen limitation, especially inside of cell aggregates, can lead to cell starvation and death. In this research, the oxygen concentration profile inside of stem cell aggregates in a stem cell expansion process was predicted using a modified oxygen diffusion model. This profile can be realized during the stem cells cultivation process by manipulating the oxygen concentration in inlet gas or inlet gas flow. The proposed approach is relatively simple and may be attractive for installation in a real pluripotent stem cell expansion processes.

Keywords—Aggregated stem cells, dissolved oxygen profiles, modeling, stirred-tank, 3D expansion.

I. INTRODUCTION

STEM cells, including both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), are becoming an indispensable tool for various biomedical applications, including drug discovery, disease modeling, and tissue engineering [1]-[3]. Human pluripotent stem cells have vast proliferation capacity and differentiation abilities to form various types of cells. Consequently, there is an urgent need to produce a relevant quantity of pluripotent stem cells for biomedical applications (e.g., 10^8 – 10^{10} cells per batch [4]). The development of scalable and robust bioprocesses for the cultivation of these cells is the key factor to their commercial success in regenerative medicine. The existing pluripotent stem cells cultivation techniques in stirred-suspension bioreactors include cell expansion and cell differentiation processes. In this paper, the authors concentrate only on the analysis and investigation of the cell expansion process. Analysis of various stem cells cultivation techniques shows that cultivation of pluripotent stem cells, as aggregates in stirred-suspension bioreactors (3D), is one of the best alternatives for the stem cells expansion processes [5], [6]. Cultivation of stem cells as cell aggregates enables good process scalability, controllability, and monitoring options and reduces facility size requirements as compared to two-dimensional (2D) cultivation technologies [5]. By implementing advanced control algorithms for supply of

nutrients, oxygen, and growth factors, it is possible to control stem cell growth as aggregates in stirred-suspension bioreactors and consequently to produce large number of stem cells without significant differentiation of cells. For successful realization of such technology, it is very important to monitor carefully the transfer of medium components to and within the cell aggregates during the cultivation. Concentration gradients in the cell cluster, especially the concentration of dissolved oxygen, can influence significantly the proliferation potential of cells [7]. High glucose and high oxygen concentrations (OXPHOS, oxidative phosphorylation and Normoxia environment) may lead to enhanced differentiation of PSCs. Low glucose and low oxygen concentrations (Glycolysis and Hypoxia environment) are preferred for efficient proliferation of PSCs. Nevertheless, cells may be exposed to starvation and death if severe oxygen limitation conditions in the cell aggregates occur. Therefore, the oxygen consumption rate and oxygen tension in culture medium and inside of cell aggregates should be monitored on-line to design appropriate oxygen supply control loops. The research focuses on the determination of feasible solution for oxygen supply during PSCs expansion in stirred-suspension bioreactors. The paper is organized as follows with the oxygen diffusion model is presented in Section II. The model of cell aggregates growth is introduced in Section III, and in Section IV, the simulation results of oxygen concentration in cell aggregates and oxygen supply flows during the cultivation process are discussed. Finally, the conclusions are drawn in Section V.

II. OXYGEN DIFFUSION MODEL

As already mentioned in the introduction, differences in substrate and dissolved oxygen concentrations can activate the self-renewal or differentiation characteristics of stem cells. For an efficient stem cells expansion process, a continuous delivery of low oxygen tension (hypoxic O_2 level, typically pO_2 of 30 $\mu\text{m Hg}$ (4–5% O_2), or 1.2 mg/L dissolved oxygen concentration), low glucose and amino acids are required in cell growth environment [7], [8]. Such a dissolved oxygen level is favorable to maintain stem cells pluripotency and self-renewal. However, oxygen tension inside of the cell aggregates should be monitored accurately to prevent cells starvation and death. For that purpose, the authors propose to employ a soft-sensor for estimation of dissolved oxygen concentration in cell aggregates and then apply these estimation results for controlling the oxygen supply in a bioreactor. In the small-scale stem cells cultivation processes, O_2 is transferred over the gas/liquid interface to the culture medium under agitation. From the medium, bulk oxygen

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fluxes to the cells through the boundary layer around each aggregate and through the pore network within each aggregate. A simplified oxygen supply schema in the stem cells cultivation process is presented in Fig. 1. During the cultivation process, oxygen supply is realized through the headspace aeration. By changing the oxygen concentration in the inlet gas C_g or gas flow, it is possible to control the oxygen concentration in the bulk medium C_M , which then influences the oxygen concentration C_r in the stem cell aggregates. To favor stem cells proliferation in the cultivation process and to avoid cell starvation/death in the centre of cell aggregates, a preferable oxygen concentration in medium should be maintained below 1.3 mg/L (hypoxic environment), and in the centre of aggregates, it should be above the some minimal level, e.g., 10 mm Hg, or 0.45 mg/L [9].

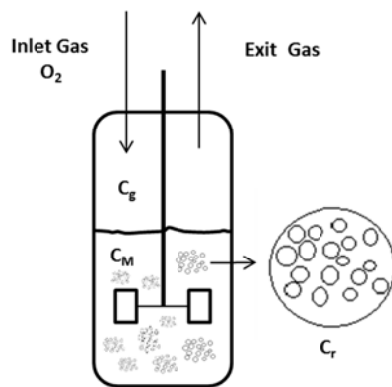


Fig. 1 Oxygen supply schema in stem cells cultivation process

For modeling of the oxygen concentration in cell aggregates, the transient reaction-diffusion models can be used [9]. By assuming that the cell aggregates are spherical, the O_2 concentration profile $c(t,r)$ in each aggregate can be presented by the following nonlinear differential equation [9]:

$$\frac{\partial c}{\partial t} = \frac{D_t^*}{r^2} \frac{\partial}{\partial t} \left(r^2 \frac{\partial c}{\partial t} \right) - V_{O_2}, \quad (1)$$

where r is aggregate radius, t is time, and D_t^* is the effective diffusion coefficient calculated using the equation:

$$D_t^* = \frac{\varepsilon D_t}{\tau}, \quad (2)$$

where D_t is the diffusion of O_2 in the cell aggregate, ε and τ are the porosity and tortuosity of the cell aggregate, respectively. The consumption of O_2 can be modeled using the well-known Michaelis-Menten (M-M) kinetics:

$$V_{O_2} = \frac{V_{max} c}{K_M + c}, \quad (3)$$

where V_{max} is the maximum O_2 consumption rate and K_M is the M-M constant. Initial and boundary conditions for (1) are given by $t = 0, c = 0; \frac{\partial c}{\partial r} = 0$ at $r = 0$ (symmetry around the aggregate centre) and $D_t^* \frac{dc}{dr} = k_l (C_m - c)$ at $r = r_0$, where r_0 is the radius of the aggregate, and k_l is the oxygen transfer

coefficient from the bulk liquid to the aggregate.

In the real stem cultivation processes, the concentration of dissolved oxygen concentration in medium C_M changes very slowly, as compared to the diffusive transport phenomena. Consequently, for practical application, (1) can be simplified and transformed into the steady-state equation describing the distribution of oxygen in cell aggregate, subject to diffusive transport and nonlinear oxygen uptake. Based on this assumption, a nondimensional governing equation and boundary conditions [10], [11] can be applied to create a simplified model of oxygen distribution in stem cells aggregate,

$$\frac{d^2 c}{dR^2} + \frac{2}{R} \frac{dc}{dR} - \frac{VC}{K+C} = 0, \quad (4)$$

subject to $\frac{dc}{dR} = 0$ at $R = 0$, and $\frac{dc}{dR} = H(1 - C)$ at $R = 1$. Here $R, C, V,$ and K are nondimensional variables and parameters:

$$R = \frac{r}{r_0}, C = \frac{c}{C_M}, K = \frac{K_M}{C_M}, V = \frac{V_{max} r_0^2}{D_t^* C_M}, H = \frac{k_l r_0}{D_t^*}. \quad (5)$$

The boundary condition at $R = 0$ ensures that the oxygen distribution is symmetric at the centre of the cell aggregate, and the boundary condition at $R = 1$ specifies a flux of oxygen at the outer surface of the aggregate. In [12] a modified, the Taylor method was proposed to obtain a handy and easily computable approximate solution of the nonlinear differential equation (4). The solution is presented in (6)-(8):

$$C(R) = G + \frac{1}{6} \frac{VG}{K+G} R^2, \quad (6)$$

$$G = \frac{1}{12H} (U + (-6K - V + 6)H - 2V), \quad (7)$$

$$U = \{ (H+2)^2 V^2 + 12H(K-1)(H+2)V + 36H^2(K+1)^2 \}^{0.5}. \quad (8)$$

Equations (6)-(8) can now be used to estimate dissolved oxygen concentration in various regions of the cell aggregate. Comparison of three different solutions of nondimensional equations (6)-(8) with the corresponding parameter values are presented in Fig. 2.

In this simulation study, the parameters of oxygen diffusion model were chosen based on [9] where transient reaction-diffusion model was used to simulate dissolved oxygen concentration profiles in human embryonic stem cells aggregates. The selected values of the model parameters are:

$$V_{max} = 1.16 \times 10^{-8} \frac{cm^2}{s}, K_M = 3.23 \times 10^{-8} \frac{mol}{cm^3},$$

$$D_t^* = 1.53 \times 10^{-5} \frac{cm^2}{s}, k_l = 2.0 \times 10^{-2} \frac{cm}{s}.$$

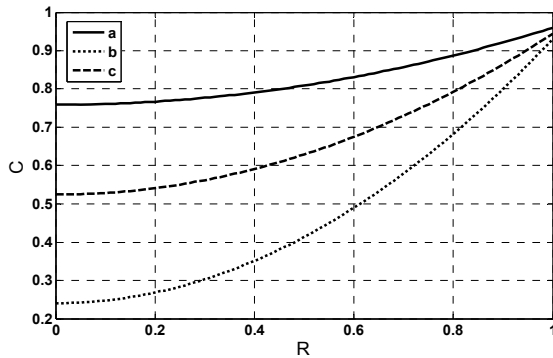


Fig. 2 Comparison of dissolved oxygen concentrations in cell aggregate for various parameter sets: (a) $V=2$ $K=0.5$ $H=10$, (b) $V=5$ $K=0.05$ $H=20$ c) $V=3$ $K=0.1$ $H=15$

To apply the diffusion model for the real stem cells cultivation process, additional tuning of model parameters is necessary. For this purpose, experimental data of oxygen consumption and dissolved O_2 concentration in the medium, along with the information about average cell aggregates size during the cultivation, are required. New innovative measurement methods, currently developed for oxygen concentration measurements inside of cell aggregates [13], could provide additional important information for a proper parameter identification procedure.

III. GROWTH OF CELL AGGREGATES

Predicting and controlling the stem cell aggregates size distribution is a very important task in the development of efficient stirred-tank cells expansion processes. Various approaches can be used to model the growth of aggregates in the cultivation medium, beginning from the well-known Population Balance Equations (PBE) models [14], Gompertz equation [15], linear and nonlinear regression models, or more complicated hybrid models. The critical issue, when selecting the right model structure, is the availability of experimental data required for identification of model parameters. In most cases, experimental data are limited. Consequently, simple models for process modeling are preferred. In this simulation study, for reasons of simplicity, only the average cell aggregate size is modeled. This simplification has substantial advantage because the estimated average cell aggregate size can then be easily involved in various process control schemas. The growth rate of average aggregate Agg during the cells expansion process is modeled using the Gompertz equation [15], [16]:

$$\frac{dAgg}{dt} = \alpha Agg \ln\left(\frac{Agg_{max}}{Agg}\right) \quad (9)$$

with the following analytical solution;

$$Agg(t) = Agg_{max} \exp\left(\ln\left(\frac{Agg(0)}{Agg_{max}}\right) \exp(-\alpha t)\right), \quad (10)$$

where $Agg(0)$ and Agg_{max} are initial and the maximal attainable aggregate size, and α is a constant characteristic of the cell growth. In this simulation study, model parameter

values are taken from [9]: $Agg_{max}=1600 \mu\text{m}$, and $\alpha=5.72 \times 10^{-3} \text{h}^{-1}$. To use this model in a real stem cells cultivation process, additional tuning of model parameters will be necessary. Predicted values of the cell aggregate size in a typical stem cells cultivation process are shown in Fig. 3. These predicted average aggregate size values will be used for estimation of the rational oxygen concentration profile, which should be realized during the stem cell cultivation process.

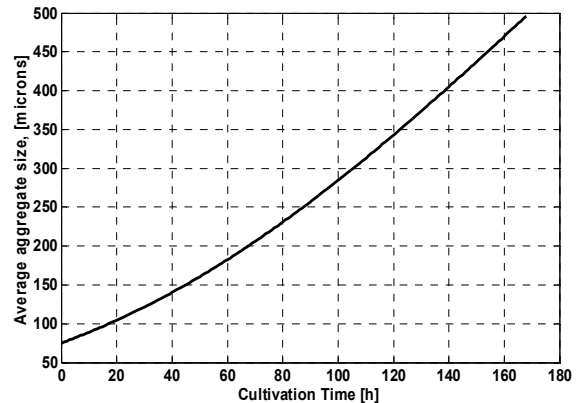


Fig. 3 Predicted values of stem cell aggregate size in typical stem cultivation process

IV. SIMULATION RESULTS

The proposed oxygen diffusion model was implemented as a soft-sensor to estimate the dissolved oxygen concentration inside of the cell aggregates. The concentration of dissolved oxygen decreases as the radius of cell aggregate increases and thus it can achieve level, which leads to cell starvation and death. Fig. 4 presents the simulation results of dissolved oxygen concentration inside of cell aggregates when dissolved oxygen concentration in cultivation medium is $C_M=2.5 \text{ mg/L}$ (40% of saturation, headspace aeration with air). In this case, a part of cell aggregates with radius $r=350 \mu\text{m}$ and $r=450 \mu\text{m}$ is exposed to strong oxygen limitation ($c < 0.45 \text{ mg/L}$). To avoid such a situation, the oxygen concentration in cultivation medium C_M should be increased.

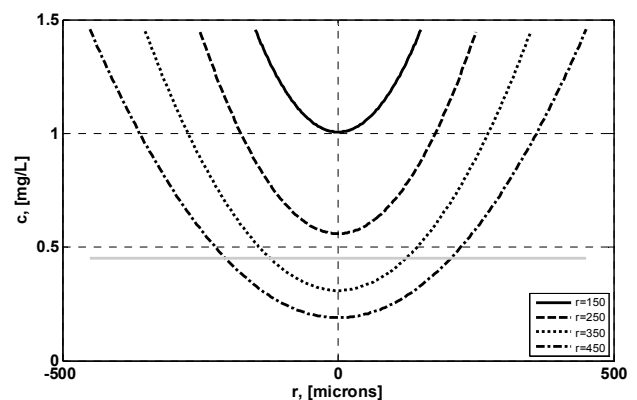


Fig. 4 Simulation results of dissolved oxygen concentration inside of the various-radius cell aggregates ($C_M= 3 \text{ mg/L}$). Solid gray line marks border ($c < 0.45 \text{ mg/L}$) where strong oxygen limitation inside of the cell aggregates occurs

Using the proposed oxygen diffusion model, it is possible to determine the minimal dissolved oxygen concentration in the cultivation medium C_{M_min} , by which the oxygen concentration in the core of the cell aggregate is minimal (hypoxic growth conditions are aimed), and the given concentration limit is not violated (e.g., $c < 0.45$ mg/L). The corresponding algorithm for identification and implementation of the rational oxygen concentration $C_{M_min}(t)$ profile during the stem cells cultivation process can be realized in the following way:

- measure/estimate the average cell aggregate size during the cultivation;
- apply the oxygen diffusion model and search for minimal oxygen concentration in medium $C_{M_min}(t)$, which ensures the given minimal oxygen concentration (e.g., $c > 0.45$ mg/L) in the core of cell aggregate;
- manipulate the oxygen concentration in inlet gas or inlet gas flow and maintain the determined oxygen concentration profile $C_{M_min}(t)$.

The proposed algorithm was employed for the estimation of the rational $C_{M_min}(t)$ profile when the average aggregate size changes from 75 to 490 μm . Fig. 5 shows the simulation results. It presents the rational oxygen concentration profile for stem cells cultivation run when minimal oxygen concentration in the core of the cell aggregate was chosen to be $c > 0.45$ mg/L and $c > 0.9$ mg/L.

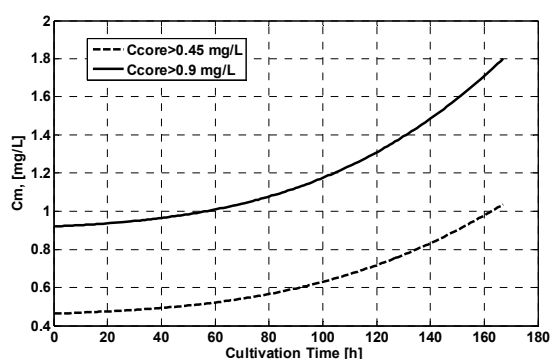


Fig. 5 Rational profiles of dissolved oxygen concentration $C_{M_min}(t)$ in stem cells cultivation process

The obtained time profile of $C_{M_min}(t)$ now can be used as a set-point variable for the dissolved oxygen controller. To apply this approach for the real stem cells expansion process, additional tuning of the parameters for oxygen diffusion and aggregate size prediction models must be carried out. By implementation of such a dissolved oxygen control algorithm, the hypoxic environment in stem cells cultivation process can be maintained, which is preferred for the cell expansion process [8], [17]. On the other hand, the minimal concentration of dissolved oxygen in the core of the cell aggregates will prevent the cells from oxygen starvation.

V. CONCLUSION

Control of the oxygen supply to the stem cell aggregates is an important factor for stem cell culture proliferation. In this work, a modified oxygen diffusion model was employed to

predict the oxygen concentration profile inside of stem cell aggregates. Using this model, the oxygen concentration profile, preferred for the stem cells expansion process (hypoxic environment), was estimated. This profile can be controlled during the cultivation process by manipulating the oxygen concentration in the inlet gas or gas flow. The proposed approach is relatively simple and may prove to be useful in development of rational oxygen supply control strategies for real stem cells expansion processes.

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REFERENCES

- S. Yamanaka, "Induced pluripotent stem cells: Past, present, and future," *Cell Stem Cell*, vol. 10, pp. 678–684, 2012.
- K. G. Chen, B. S. Mallon, R. D. McKay, and P. G. Robey, "Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics," *Cell Stem Cell*, vol. 14, pp. 13–26, 2014.
- M. J. Jenkins and S. S. Farid, "Human pluripotent stem cell-derived products: Advances towards robust, scalable and cost-effective manufacturing strategies," *J. Biotechnol.*, vol. 10, pp. 83–95, 2015.
- Y. Wang, L. Cheng, and S. Gerecht, "Efficient and scalable expansion of human pluripotent stem cells under clinically compliant settings: A view in 2013," *Ann. Biomed. Eng.*, vol. 42, pp. 1357–1372, 2014.
- V. C. Chen and L. A. Couture, "The suspension culture of undifferentiated human pluripotent stem cells using spinner flasks," *Methods Mol. Biol.*, vol. 1283, pp. 13–21, 2015.
- A. Elanzew, A. Sommer, A. Push-Klein, O. Brüstle, and S. Haupt, "A reproducible and versatile system for the dynamic expansion of human pluripotent stem cells in suspension," *J. Biotechnol.*, vol. 10, pp. 1589–1599, 2015.
- S. Sart, S. P. Agathos, and Y. Li, "Process engineering of stem cell metabolism for large scale expansion and differentiation in bioreactors," *Biochem. Eng. J.*, vol. 84, pp. 74–82, 2014.
- T. Ezashi, P. Das, and R. M. Roberts, "Low O₂ tensions and the prevention of differentiation of hES cells," *Proc. Natl. Acad. Sci. USA*, vol. 102, pp. 4783–4788, 2005.
- J. Wu, M. R. Rostami, D. P. Cadavid Olaya, and E. S. Tzanakakis, "Oxygen Transport and Stem Cell Aggregation in Stirred-Suspension Bioreactor Cultures," *PLoS ONE*, 9:e102486, 2014.
- S. H. Lin, "Oxygen diffusion in a spherical cell with nonlinear oxygen uptake kinetics," *J. Theoret. Biol.*, vol. 60, pp. 449–457, 1976.
- M. J. Simpson and A. J. Ellery, "An analytical solution for diffusion and nonlinear uptake of oxygen in a spherical cell," *Appl. Math. Modell.*, vol. 36, pp. 3329–3334, 2012.
- H. Vazquez-Leal, M. Sandoval-Hernandez, R. Castaneda-Sheissa, U. Filobello-Nino, and A. Sarmiento-Reyes, "Modified Taylor solution of equation of oxygen diffusion in a spherical cell with Michaelis-Menten uptake kinetics," *Int. J. Appl. Math. Res.*, vol. 4, pp. 253–258, 2015.
- D. Lambrechts, M. Roeyers, G. Kerckhofs, S. J. Roberts, J. Hofkens, T. Van de Putte, H. Van Oosterwyck, and J. Schrooten, "Fluorescent oxygen sensitive microbead incorporation for measuring oxygen tension in cell aggregates," *Biomaterials*, vol. 34, pp. 922–929, 2013.
- D. E. Kehoe, D. Jing, L. T. Lock, and E. S. Tzanakakis, "Scalable Stirred-Suspension Bioreactor Culture of Human Pluripotent Stem Cells," *Tissue Eng. Part A*, vol. 16, pp. 405–421, 2010.
- G. F. Brunton and T. E. Wheldon, "The Gompertz equation and the construction of tumor growth curves," *Cell Tissue Kinet.*, vol. 13, pp. 455–460, 1980.
- A. K. Laird, "Dynamics of tumor growth," *Br. J. Cancer*, vol. 18, pp. 490–502, 1964.
- M. Serra, C. Britto, M. F. Sousa, J. Jensen, R. Tostoes, J. Clemente, R. Strehl, J. Hyllner, M. J. Carrondo, and P. M. Alves, "Improving expansion of pluripotent human embryonic stem cells in perfused bioreactor through oxygen control," *J. Biotechnol.*, vol. 148, pp. 208–215, 2010.