Use of Image Data in Kinetic Model Development for the Design of Mesenchymal Stem Cell Cultivation Processes

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Abstract

This work presents the use of image data in kinetic model development for designing mesenchymal stem cell (MSC) cultivation processes. To incorporate the initial spatial distribution of seeded cells, seeding bias in static MSC cultivation was investigated with phase contrast microscopic image acquisition on Day 1. Subsequently, a parameter was newly defined from the image analysis result calculating the standard deviation of cell number fraction among numerical 64 tiles on a squared culture space. Finally, the parameter was integrated with our previous kinetic model to consider spatial growth limitation. The model was then applied to simulate static MSC cultivation processes. Based on ordinary differential equations (ODE), Monte-Carlo simulations were conducted to find cell harvesting time when cultivation ensured given specifications for cell number and confluence degree. Feasible range of harvesting time was illustrated subject to seeding heterogeneity and density. The presented image-based ODE model could help incorporate spatial heterogeneity into the MSC cultivation process design.

**Keywords**: Cell therapy, stem cell, spatial heterogeneity, hybrid model, image analysis

* 1. Introduction

There is an increased demand for mesenchymal stem cells (MSCs) for cell therapy applications due to self-renewability, immunosuppression, and the lack of ethical concerns (Levy et al., 2020). MSCs have been studied for a wide range of therapy including acute graft vs. host diseases (Najima and Ohashi, 2017). In anticipation of the demand growth of MSCs, quality cell manufacturing by process design has been required (Lipsitz et al., 2016). To this end, ordinary differential equations (ODEs) have been developed to describe cell growth dynamics in static MSC cultivation, e.g., Jossen et al. (2020) and Hirono et al. (2022). ODEs benefit from fast simulation compared to spatially high-resolution models, which demand long calculation time. Besides, ODEs can be smoothly integrated with stochastic technology including the Monte-Carlo method to predict process variations. For example, Hirono et al. (2022) conducted an ODE-based stochastic simulation incorporating system dynamics and variabilities in MSC cultivation processes.

Primary assumptions in ODEs are regarding the system as homogeneous. For example, specific cell growth rate in cultivation is typically formulated based on the Monod kinetics (Monod, 1949) as a function of average concentration of limiting substrates and bulk cell density. However, in actual static cultivation, heterogeneous initial cell distribution can happen, which potentially causes significant growth delay due to cell–cell contact inhibition (Kagawa and Kino-oka, 2016). To model such spatial growth limitation by compensating for the ODE limitation, imaging technology can be a promising tool. In the PSE field, image-based technology has been developed for process monitoring and control applications. For bioprocess development, Oh et al. (2009) developed an automated vision system to identify human embryonic stem cell differentiation in teratoma section tissues. In static MSC cultivation studies, phase contrast microscopic images were analyzed to predict osteogenic potentials (Matsuoka et al., 2013) and to detect quality decay through passaging (Takemoto et al., 2021). However, integration and application of image information for designing MSC cultivation processes are still in infancy.

In this work, we utilized image data in kinetic modeling for the design of MSC cultivation processes. To incorporate the initial spatial distribution, our previous ODE model (Hirono et al., 2022) was extended by defining a new parameter from phase contrast microscopic images. Seeding bias in static MSC cultivation was investigated with the image acquisition on Day 1. Subsequently, the observed spatial distribution was parameterized using the image analysis results. Finally, the parameter was integrated with the kinetic model to predict spatial growth limitation due to seeding heterogeneity.

* 1. Model development
		1. MSC cultivation

Figure 1 shows the experimental setup for in vitro static cultivation of bone marrow MSCs at passage 6 (19TL281098, Lonza Group Ltd., Basel, Switzerland) with MSCGM (Lonza Group Ltd) culture medium. A restricted culture area (17 × 17 mm2) was designed at the center of polydimethylsiloxane in each well of 6-well plates (353046, FALCON, NY, USA). MSCs were initially seeded in a cloning ring (20140514, AGC Inc., Tokyo, Japan) to produce seeding bias by changing hold time, $t\_{r}$, specifically 0 (numerically), 15, and 60 min. In the case of $t\_{r}=$ 0, the colony ring was not used. The cultivation was started with seeding densities, $X\_{seed}$, of 1000, 2000, and 3000 cells cm–2 under 37°C, 5% CO2 in the incubator until Day 8. Partial medium change was conducted on Days 2, 4, and 6 when half of the working medium was replaced with fresh medium. Duplicate samples were prepared for each culture condition, resulting in a total of 18 samples.

* + 1. Image acquisition

Phase contrast microscopic images were automatically acquired every 6 h during the cultivation using BioStation CT (Nikon Corporation, Tokyo, Japan) at 4× magnification (8 × 8 tiling per well, covering 15.3 × 15.3 mm2; 1000 pixels2/image). The number of evaluation time points was 32 from 6 h to 192 h after removing the ring. All images were quantified by original Python code implementing the image processing pipeline based on Matsuoka et al. (2013) and Sasaki et al. (2014). Using the image analysis results, cell numbers at each tile were measured. Specifically, the analysis result on Day 1 was used for the following parameterization.

* + 1. Parameterization



**Figure 1. Use of image data in kinetic model development for MSC cultivation.**

The cell number at each tile, $N\_{i}$ ($i=$ 1, 2, ..., 64), was counted using the images acquired from the cultivation on Day 1. Dividing $N\_{i}$ by the total cell number on Day 1, the cell number fraction at each tile was obtained. Subsequently, calculating the standard deviation for the fraction among the 64 samples, seeding heterogeneity was quantified as a new parameter, $ε$ (see Eq. (1)).

* + 1. Integrated kinetic model

To incorporate the image-driven $ε$ into our previous kinetic model (Hirono et al., 2022), spatial growth limitation was formulated as a function of $ε$, which modified the Monod equation (see Eq. (2)). The overall integrated kinetic model was developed as follows:

|  |  |
| --- | --- |
| $$ε=\left\{\frac{1}{64}\sum\_{i}^{64}\left(\frac{N\_{i}}{\sum\_{j}^{64}N\_{j}}-\frac{1}{64}\right)^{2}\right\}^{\frac{1}{2}}$$ | (1) |
| $$μ=μ\_{max}\left(1-εK\_{S}\right)\left(1-\frac{X}{X\_{max}}\right)$$ | (2) |
| $$\frac{dX}{dt}=μX \left(t\geq t\_{lag}\right)$$ | (3) |
| $$t\_{lag}=β∙ln\left(\frac{αX\_{seed}}{X^{\*}}\right)+γ$$ | (4) |

where$ε$ is seeding heterogeneity indicator; $N\_{i}$ is cell number at each tile on Day 1; $μ$ is specific cell growth rate; $μ\_{max}$ is maximum specific cell growth rate; $K\_{S}$ is a fitting parameter; $X$ is cell density;$X\_{max}$ is maximum cell density;$t$ is cultivation time; $t\_{lag}$ is lag time needed to start cell growth; $β$ and $γ$ are fitting parameters; $X^{\*}$ is unit cell density ($=$ 1 cells cm–2); $α$ is adhesion ratio on Day 1.

* 1. Model application

The developed model was applied to find a feasible range of cell harvesting time, $t\_{h}$, in MSC cultivation processes. First, parameter estimation was performed to calibrate the model. Second, dynamic and stochastic simulation was conducted with parameters sampled from discrete distributions composed of the experimental observation. Third, from the simulation outputs, the feasible $t\_{h}$ was calculated to satisfy given specifications for cell number and confluence. The feasible range was finally visualized subject to seeding heterogeneity and density.

Figure 2 illustrates the parameter estimation results. As an error indicator, the normalized root-mean-square error (NRMSE) was used. The average of NRMSE for cell number among the 32 measuring time points was minimized, resulting in $μ\_{max}$ and $K\_{S}$ of 24.7 and 3.03 $×$ 10–2, respectively. The developed model showed better fitness with the observation than the model ignoring seeding heterogeneity, especially in uneven cases.

Figure 3 depicts dynamic and stochastic simulation results. The simulation was conducted until Day 10 with 50 vol% medium changes on Days 2, 4, 6, and 8. As an input variable, $ε$ was investigated among three values corresponding to the mean calculated from Base Case, Uneven Case 1, and Uneven Case 2, respectively. Besides, $X\_{seed}$ was varied from 1000 to 3000 cells cm–2 based on the experimental conditions with an interval of 500 cells cm–2. Regarding uncertain parameters, $α$ and $X\_{max}$ were randomly sampled from independent discrete distributions consisting of their experimental observations. The stochastic simulation with the sampling was conducted 10000 times to capture the resulting variations in the cultivation.



**Figure 3. Dynamic and stochastic simulation results (**$X\_{seed}=$ **2000 cells cm–2).**



**Figure 2. Parameter estimation results (**$X\_{seed}=$ **2000 cells cm–2).**

Using the simulation outputs, a feasible range of $t\_{h}$ was obtained subject to $X\_{seed}$ and $ε$ such that MSC cultivation ensured given specifications. The feasible range, $FR$, was mathematically defined as follows:

|  |  |
| --- | --- |
| $$FR=\left\{f\left(x,θ,t\_{h}\right)\in A\right\}$$ | (5) |
| $$A=\left\{5.0×10^{4}\leq SX∧\frac{X}{X\_{max}}<0.80\right\}$$ | (6) |

where$f$ is the developed model; $x$ is a set of input variables (i.e., $ε$ and $X\_{seed}$); $θ$is a set of uncertain parameters (i.e., $α$ and $X\_{max}$); $A$ is quality specifications; $S$ is surface area. Here, $A$ was based on the reason that cell quantity was directly related to medical needs, while confluence level would decrease stem cell quality (Jossen et al., 2020).

 Figure 4 visualizes the calculated $FR$ depending on both $ε$ and $X\_{seed}$ as a box-whisker plot with the whiskers within 1.5 times the interquartile range. Given $ε$ of 3.12 $×$ 10–2 and $X\_{seed}$ of 2000 cell cm–2, for example, Days 4–7 were feasible, while harvesting before Day 4 or after Day 7 was unfeasible. Areas under and over the feasible range were out of the specifications due to insufficient cell number and exceeded confluence level, respectively. Interestingly, wider feasible ranges were obtained in higher $ε$ (i.e., uneven seeding) because of lower growth rate (see Eq. (2)) even though long-term cultivation was needed. Regardless of $ε$, higher $X\_{seed}$ achieved the feasible range earlier due to larger initial cell density and shorter lag time (see Eqs. (3)–(4)).Figure 4 can serve as a basis for quantitative design of MSC cultivation processes given seeding heterogeneity and input cell resources. The default *scipy.integrate.solve\_ivp* was used in Python 3.9 for solving the ODEs. The total CPU time for the feasible range calculation was ca. 10 min using an Intel Xeon Gold 6142 CPU @ 2.60 GHz with 128 GB RAM.

* 1. Conclusions and outlook

This work presented the use of image data in kinetic model incorporating the effects of initial spatial distribution into the design of MSC cultivation processes. Utilizing image analysis result with phase contrast microscopy, a new parameter was defined representing seeding heterogeneity and integrated with our previous kinetic model. As a model application, a feasible range of harvesting time was calculated subject to seeding heterogeneity and density. Future work would include more experimental and numerical investigations to achieve practical probability distributions of seeding heterogeneity towards robust process conditions for MSC cultivation. Recently, computer-aided studies on cell and gene therapy have gathered attentions in the PSE field (Hayashi et al., 2023; Triantafyllou et al., 2023). This work would contribute further model-based investigation in the relevant area.



**Figure 4. Calculated feasible range of cell harvesting time.**

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