Computer-aided optimization of   
cooling temperature profiles in slow freezing for   
human induced pluripotent stem cells

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Abstract

Human induced pluripotent stem (hiPS) cells are one of the most promising sources of regenerative medical products. After successful clinical studies of hiPS cells, the demand for these cells is increasing. Thus, establishing the freezing processes of these cells for storage and transportation is necessary. Here, we present a computer-aided optimization of multiobjective optimal temperature profiles in slow freezing for hiPS cells. This study was based on a model that calculates cell survival rates after thawing, and the model was extended to evaluate cell potentials until 24 h after seeding. To estimate the necessary parameter values for this extension, freezing experiments were performed using constant cooling rates. The model was applied to assess 8,705 temperature profiles by quality and productivity indicators, and a promising profile was obtained.

**Keywords**: Hybrid modeling, Industrialization, Numerical simulation, Optimization, Regenerative medicine

* 1. Introduction

Human induced pluripotent stem (hiPS) cells are one of the most promising resources for the future industrialization of regenerative medicine. Recently, several clinical studies involving hiPS cells were performed (e.g., Parkinson’s disease (Morizane, 2019)), and industrial-scale production of hiPS cells is becoming possible. Thus, establishing slow freezing processes for their preservation and transportation is urgently needed (Carpenter and Rao, 2015).

Several cryobiological studies have investigated the slow freezing of cells. Cell damage mechanisms during slow freezing were explored in Chinese hamster cells by Mazur and colleagues (Mazur et al., 1972). The authors also generated a cell dehydration assessment model and suggested that the extent of cell dehydration and intracellular ice formation was affected by cooling rates. Subsequently, several studies improved on these cell dehydration and intracellular ice nucleation models (Anderson et al., 2019; Traversari and Cincotti, 2021).

Several studies focused on slow freezing processes for hiPS cells. Ntai et al. (2018) applied new CPAs to hiPS cells. Li et al. (2020) measured temperature profile effects on intracellular ice crystals. Most recently, a mechanistic model that can estimate cell damage as a function of process conditions was published previously by the research group of the authors (Hayashi et al., 2020). In addition, a hybrid model that evaluates the cell survival rate after thawing was also published by the same group (Hayashi et al., 2021). Based on the simulation results in this study, it was suggested that a three-zone temperature profile could contribute to quality and productivity improvement. However, a rigorous investigation of the temperature profile is yet to be presented.

Here, we present a computer-aided exploration of multiobjective optimal temperature profiles in slow freezing for hiPS cells. This study was based on a numerical simulation model that evaluated the cell survival rate after thawing, depending on the given conditions (Hayashi et al., 2021). Furthermore, we extended the model using statistical modeling to calculate cell potentials until 24 h after seeding. To estimate required parameter values for extension, freezing experiments were conducted using constant cooling rates. We evaluated 8,705 temperature profiles using the model, and a promising candidate profile was obtained.

* 1. Experimental Methods

Figure 1 represents an overview of the experimental methods. First, the cells were separated using centrifugation for 3.0 min at 180 × g. After removing the supernatant, the cells were resuspended in commercially available cryopreservation solution with 10% dimethyl sulfoxide (STEM-CELLBANKER GMP grade; ZENOGEN PHARMA Co., Ltd, Fukushima, Japan) containing 10 μM Y-27632. Cell counting was performed using an automated cell counting instrument (TC20; Bio-Rad Laboratories, Inc., Hercules, CA, USA) based on the trypan blue exclusion method. Then, the cell suspension was filled into cryotubes at a volume of 0.50 mL each. The cryotubes were placed on an aluminum tube holder in a programmable freezer (Program Deep Freezer FZ-2000; STREX Inc., Osaka, Japan) and maintained for 15 min at 277 K, and then cooled to 193 K using the predetermined cooling temperature profile. During cooling, the temperature of the holder was measured using a temperature sensor built in the freezer, and that of the cell suspension was measured using a platinum resistance thermometer inserted in a cryotube placed at the center position of the holder. After cooling, the cryotubes were stored in the vapor phase of a liquid nitrogen tank for at least 24 h.

To evaluate the cell quality, the cell survival rate after thawing, [−], was defined using the following equation:

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**Figure 1.** Overview of freezing experiments using hiPS cells

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where [−] is the number of live cells before freezing and [–] is the number of live cells after thawing. The attachment efficiency at 24 h after seeding, [−], was defined as follows:

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where [−] is the number of adherent cells on the culture plate at 24 h after seeding. The cell potential until 24 h after seeding, [−], was obtained using the following equation:

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* 1. Numerical simulation model

Figure 2 shows an overview of the numerical simulation model. The inputs were defined as the determined cooling rate of the freezer, [K min−1], and the temperature at which the freezer temperature was changed (changing point hereafter), [K]. The outputs were defined as the required freezing time, [min], and the cell potential, , which are the quality and productivity indicators, respectively. The physical part of the model overview consists of the models for heat transfer, mass transfer, and crystallization, which calculates the cell volume change and ice crystal volume. The statistical part connects these two parameters with the cell potential until 24 h after seeding. The overall model can evaluate the quality and productivity indicators given the process conditions.

* + 1. Heat Transfer Model

The radial and temporal temperature profiles inside the vial were defined as follows:

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where [K] is the temperature, [s] is the time, [m2 s−1] is the thermal diffusion coefficient, and [m] is the radial distance from the vial center. The productivity indicator, , was defined using the following equation:

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**Figure 2.** Overview of the numerical simulation model.

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* + 1. Mass Transfer Model

The mass transport of water was modeled as follows:

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where [m3] is the volume, [m s−1 Pa−1] is the water permeability, [m2] is the surface area, and [Pa] is the pressure difference. The normalized maximum cell volume change, [−], was defined as follows:

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where the superscripts init and fin represent the initial and final state of freezing, respectively.

* + 1. Crystallization Model

In general, ice nucleation in a cell is categorized into two different mechanisms, homogeneous (HOM) and heterogeneous nucleation. The latter is further categorized into two types, namely, surface-catalyzed nucleation (SCN) and volume-catalyzed nucleation (VCN). The ice nucleation rate was estimated using the following equation:

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where [s−1] is the ice nucleation rate, [s−1 m−3 or s−1 m−2] is the nucleation rate per volume or area, and the subscript m represents the melting point. The total volume of intracellular ice crystals, [m3], was obtained from the following equation:

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The normalized maximum ice crystal volume, [−], was defined as follows:

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* + 1. Cell Potential Model

As the output of the model, the calculated cell potential until 24 h after seeding, [−], was defined using the following equation:

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where [−], [−], [−], and [−] were the fitting coefficients for the experimental results. The freezing experiments using constant cooling rates provided the necessary values of the cell potential model.

* 1. Results and Discussion
     1. Freezing experiments using constant cooling rates

The circles in Figure 3(a) show the cell potentials until 24 h after seeding, , that were observed in freezing experiments using constant cooling rates. The observed cell potentials were measured at the predetermined cooling rate, = 0.10, 1.0, 2.0, 3.0, 4.0, and 5.0 K min−1. Three measurements were obtained at each cooling rate ( 3). One-way analysis of variance was applied to analyze the experimental results in Figure 3. The *p* value was found to be 1.910−6. Therefore, the relevance of the cooling rate for the cell potential was confirmed. The solid line in Figure 3(a) represents the fitted curve for the experimental results using the determined fitting coefficients.

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**Figure 3.** (a) Experimental results of the observed cell potentials and their fitted curve and (b) Model-based assessment result of 8,705 temperature profiles.

* + 1. Model-based assessment of candidate temperature profiles

For model-based assessment, 8,705 candidate temperature profiles with two temperature-changing points were produced. To determine the optimal temperature profile, a multiobjective optimization problem was formulated, as shown in the following equation:

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| subject to   |  |  |  | | --- | --- | --- | |  |  |  | |  |  |  | |  |

where [−] is the joint objective function, [K min−1] is the first cooling rate, [K] is the first changing point of the freezer temperature, [K min−1] is the second cooling rate, [K] is the second changing point, and [K min−1] is the third cooling rate. The joint objective function, , was defined as follows:

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where [−] is the calculated cell potential until 24 h after seeding, [−] is the minimum calculated cell potential in the considered profiles, and [min] is the maximum required freezing time.

**Table 1.** Top 5 temperature profiles judged using the joint objective function.

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| Rank | [K min–1] | [K min–1] | [K min–1] | [K] | [K] |
| 1 | 5.0 | 1.0 | 4.0 | 228 | 213 |
| 2 | 5.0 | 1.0 | 4.0 | 233 | 213 |
| 3 | 5.0 | 1.0 | 5.0 | 228 | 213 |
| 4 | 5.0 | 1.0 | 5.0 | 233 | 213 |
| 5 | 4.0 | 1.0 | 4.0 | 228 | 213 |

The relationship between the calculated cell potential, , and the required freezing time, , for the 8,705 profiles is shown using cross marks in Figure 3(b). The red circle and green triangles in Figure 3(b) represent the optimal and top 5 profiles, respectively. Table 1 shows the top 5 temperature profiles judged using . Fast, slow, and fast cooling was effective for both short freezing time and high cell potential.

* 1. Conclusions and Outlook

This work presented a computer-aided optimization of multiobjective temperature profiles in slow freezing for hiPS cells. A numerical simulation model was developed to calculate the cell potential until 24 h after seeding using statistical modeling. The model was applied to assess 8,705 temperature profiles by quality and productivity indicators, and a promising profile was obtained. In the field of computer-aided process engineering, cell therapy related studies are becoming relevant, e.g., Triantafyllou et al. (2023). Further model-based studies in this area are encouraged.

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