

## 論文の内容の要旨

論文題目    Multiscale design of cryopreservation processes  
for human induced pluripotent stem cells (ヒトiPS  
細胞向け凍結保存プロセスのマルチスケール設計)

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Regenerative medicine is a future technology that aims to heal or restore human tissues and organs damaged by age, disease, or trauma, back to the original condition. It has the potential to cure diseases for which no effective treatment is currently available, and to solve the shortage in organ transplant donors. Human induced pluripotent stem (hiPS) cells are considered one of the most promising sources of regenerative medicine products because of their various advantages compared with the conventional sources, and the demand for hiPS cells is increasing.

The cryopreservation process is required to transport hiPS cells to the next process and store them over a long period of time, although hiPS cells are known to be sensitive to damage caused by cryopreservation. Experimental studies have explored the cryopreservation process. However, it is still difficult to design the cryopreservation process that can handle an enormous number of hiPS cells.

The cryopreservation process of hiPS cells presents challenges and issues in each scale. In the molecule scale, dimethyl sulfoxide (DMSO) is still used as a cryoprotective agent (CPA), although DMSO is toxic to hiPS cells. In the unit operation scale, important operation conditions directly related to quality and productivity have not been examined comprehensively. In the society scale, the process design has not considered the impact on society, e.g., budget deficits. Therefore, the objective of this research is to present a multiscale design of cryopreservation processes for hiPS cells. The aim is to enable the overall optimal design of the cryopreservation process. To achieve

this aim, the issues in each scale are first addressed, and the achievements in each scale are then stitched together to enable the multiscale design.

Chapter 2 presents a mechanistic model for the slow freezing process design that integrated heat transfer, mass transfer, and crystallization models. These models described the radial and temporal temperature profiles in a container, the volume change of a cell through transmembrane water transport, and intracellular ice formation during slow freezing, respectively. The integration enabled calculation of the maxima of the cell volume change and intracellular ice crystal volume, and the required freezing time, as a function of cooling rate, vial diameter, cryoprotective agent, and vial material. By this way, I could provide, for the first time, the intracontainer variation of cell quality, a critical factor towards industrial manufacturing of hiPS cells. Three design cases were explored where the optimal vial size was obtained given the cell demand for a specific freezer.

Chapter 3 presents a hybrid-model-based assessment of temperature profiles of hiPS cells in slow freezing. The basis of this chapter was the mechanistic model on the single-cell, which described heat transfer, dehydration, and intercellular ice formation. I extended the model to cover the cell survival rate through statistical modeling. To obtain the necessary parameter values, freeze/thaw experiments were performed using hiPS cells. Given the temperature profile of freezing, the hybrid single-cell model was then used to calculate the cell survival rate and the required freezing time as quality and productivity objectives, respectively. The model was applied to assess 16,206 temperature profiles. The simulation results suggested fast, slow, and fast cooling in the dehydration, nucleation-promoting, and further cooling zones, respectively. The work can be the first step toward establishing a design space of slow freezing of hiPS cells with an awareness of productivity and quality. Finally, in the experimental investigation, a multiobjective optimal temperature profile for slow freezing of hiPS cells was discovered, which is a good example of computer-aided process improvements.

Chapter 4 presents a multilayered model-based approach to scale-up slow and force convection-based freezing of hiPS cells. The model enabled the calculation of spatial and time-dependent survival rate of cells within a freezer given a temperature profile and inlet coolant velocity. The model was first demonstrated on a single vial problem where it was found that at faster cooling rates, the coolant velocity affected the cell survival rate. In the

second case study, the freezing of 235 cryovials was studied with 18 different operational conditions. The model illustrated that when non-constant cooling rates were applied, special heterogeneity in the cell survival rates occurred. As such, the study indicated the benefit of such a multilayered approach which can provide high resolution results towards model-based scale-up and commercialization of hiPS cell production.

Chapter 5 presents an integrated design of the fill-freeze-thaw processes for hiPS cells using hybrid models. The freezing process was represented by a white-box single-cell model, which describes heat transfer, dehydration, and intercellular ice formation during freezing. I newly developed hybrid models for the filling and thawing processes. Reactive oxygen species accumulation during filling and thawing was defined as the quality indicator, and was described as a black-box model, for which experimental results provided the necessary parameter values. The time duration for filling and thawing was defined as the productivity indicator based on physical models. The overall fill-freeze-thaw model calculates the quality and productivity indicators given the process specifications, such as vial diameter and thawing temperature. In the case study for thawing, vial diameter largely affected the productivity. For the overall fill-freeze-thaw process, the optimal thawing temperature and vial diameter for productivity changed significantly, depending on the acceptable quality specifications and cell demand.

Chapter 6 presents a computational screening of 40 compounds as CPAs using quantum chemistry and molecular dynamics (MD) simulations. A set of models were developed to calculate the solvation free energy and the partition coefficient of a compound by quantum chemistry simulation. The models also covered the root mean square deviation (RMSD) of a phospholipid bilayer which composes a cell membrane by MD simulation. The solvation free energy, partition coefficient, and RMSD were defined as the indicators of the osmoregulatory ability, affinity with a cell membrane, and ability to spread a cell membrane, respectively. The quantum chemistry simulation elucidated that the six compounds of trimethylglycine, formamide, urea, thiourea, diethylene glycol, and dulcitol were better than DMSO in either or both of the physical properties considered. This finding is based on the inherent physical property and is thus case-independent. Further characterization with the MD simulation suggested that formamide, thiourea, and urea should be the first candidates to investigate, although the result was valid only in the simulated condition. This work serves

as the first step of multi-faceted computational evaluation of multiple compounds in the search for an effective CPA compound after DMSO.

Chapter 7 presents a model-based cost-effectiveness analysis in the manufacture of allogeneic hiPS cells in Japan. An agent-based model was developed that can quantify the DALYs of each patient and the total cost in the manufacture of allogeneic hiPS cells. The DALYs were applied as the effectiveness indicator, while the total cost was used as the cost indicator. Given the disease, the number of annually treated patients, the treatment mode, and the cell survival rate in the cryopreservation, the model can evaluate these two indicators. Two case studies were presented. Focusing on the treatment mode, when the number of treated patients is small, the treatment mode needs to be discussed carefully. Focusing on the supply chain network, the type of the supply chain network should be chosen depending on the tolerance for cryopreservation of the cells. Finally, a map was obtained that could be used for estimating the cost-effectiveness of allogeneic hiPS cell therapy from the manufacturing perspective.

Chapter 8 presents a multiscale design of cryopreservation processes for hiPS cells. First, the objective function in the society scale is defined, and the compound used as CPA is then chosen. Subsequently, based on the physical properties of the compound, the determination of the operation conditions in the unit operation scale is performed. Finally, the evaluation of the defined objective function is conducted. The above procedure is continued until it can be judged that the process is optimized. The practical usage example of the multiscale design is as follows. The objective function in the society scale is defined as the cost-effectiveness, and the computational screening of CPA candidate compounds is then performed (Chapter 6). Subsequently, based on the obtained membrane permeability coefficient, the operation conditions in the unit operation scale are determined (Chapters 2 through 5). Finally, the evaluation of the cost-effectiveness is conducted by using the cell survival rate and the manufacturing time (Chapter 7).

The proposed multiscale design enables the optimal process design considering from molecules to society, and also leads to establish cryopreservation processes that can handle an enormous number of hiPS cells. In addition, the proposed multiscale design could perform the optimization of cryopreservation processes for hiPS cells before the establishment of the process, which would enable industrial manufacturing of hiPS cells.