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Abstract of Thesis

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Title	Stability Analysis of Filling Process in Human-Induced Pluripotent Stem Cell Manufacturing （ヒト人工多能性幹細胞製造における充填プロセスの安定性解析）

Abstract of Thesis

Chapter 1: General Introduction

The filling process is an important part of the downstream processes involved in manufacturing cell-based products, including human-induced pluripotent stem cell (hiPSC) based products. Designing the filling process for hiPSC products considering the challenges of cell manufacturability requires evaluation of process instability. Since analyzing biological attributes for process stability with conventional statistical process control (SPC) is not recommended due to the limitations of parametric techniques, this study focuses on developing quality indices and a nonparametric analysis algorithm for the hiPSC filling process. The thesis aims to evaluate process instability and implement stabilization strategies for improving the filling process during scale-up. Chapter two focuses on defining quality indices for the output of the filling process designed for hiPSC products. In this chapter, three quality indices are proposed, which measure the physical and biological attributes of the output. Chapter three begins with the calibration method for the filling equipment and the development of an algorithm for analyzing process instability. The algorithm detects outliers in the quality index measurements and classifies process instability as fluctuations within a batch and variations between batches. It quantifies process instability as the difference between the standard coefficient of variation (CV) and CV for stability to get CV for instability.

Chapter 2: Defining quality indices for the hiPSC filling process

This chapter defined quality indices, namely V/V^*, D/D^*, and P/P^*, for quantifying the quality attributes of the output from a filling process and observed the trends of these indices as a result of interactions between the input material and the process parameters. The quality index V/V^* measures the fill volume in each vial from a batch. D/D^* measures the particle density within each filled vial when a suspension is used as the input material. P/P^* measures the biological attributes when a cell suspension is used as the input material for the filling process. It is derived as a product of previously defined viability indices. The yield (Y) for the process was defined as the product of the above-mentioned quality indices based on the nature of the input material to understand process stability. After observing the trends of quality indices, it was determined that the mixing operation is an essential part of the filling process to maintain output homogeneity when a suspension was used as the input material. It was also determined that the mixing operation affects the fill volume of the final vials filled in a batch. The biological quality index had higher differences between samples of the same batch and saw considerable degradation after filing. Strategies such as the filling process at 4 ° C were implemented based on previous studies to mitigate the degradation of the biological attribute.

Chapter 3: Analysis of fluctuation within batch and variation between batches

In this chapter, an analysis method that utilizes a nonparametric, unsupervised outlier detection algorithm (Isolation Forest) to quantify stability and instability in a filling process was developed. Initially, a new method for establishing the threshold of outlier classification for quality index measurements was developed. After establishing the threshold of outlier classification for each quality index, fluctuation and variation between batches for each of those quality indices were quantified and compared. The quantification was done with CV stability (δ) and CV for instability (Δ) derived after analysis with the algorithm. Initially, filling order-dependent fluctuations were analyzed for the three quality indices. V/V^* had order-dependent fluctuations for vials towards the end of each batch. Order-dependent fluctuations for D/D^* were only observed for the filling process without mixing operation. hIPSC suspension was always filled with mixing operation to avoid heterogeneity in the output that may affect the viability and proliferation assays. Five batches were analyzed

for quantifying fluctuations within batches, and ten were analyzed for determining variation between batches. Based on this assessment, the filling process stability for different types of input materials was compared. It was shown that the filling process was more stable for the physical quality indices. If not, it could be made more stable by introducing operations like mixing during the filling process. Cell potential (P/P_*) after the filling process fluctuates more than physical quality indices even after implementing some improvement strategy.

Chapter 4: General conclusion and future perspectives

Process instability was quantified after defining quality indices for the output of the filling process and analyzing fluctuations within a batch and variation between batches using an algorithm that utilizes nonparametric statistical techniques. Furthermore, process improvement strategies were implemented based on this analysis. However, further investigations must be done to mitigate the fluctuations observed in cell potential, possibly from mechanical stress due to mixing operation. This approach can be implemented while designing a filling process for hiPSC and similar cell-based products.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文では、ヒト人工多能性幹細胞（hiPSC）の細胞製造における充填工程での工程特性評価を行う際に、指標提案、工程安定性・不安定性の観点から方法論を提案している。

第 1 章では，充填工程は、hiPSC 製品を含む、細胞ベースの製品製造に関わる下流工程の重要な一部であることを示し、細胞製造性という課題を考慮して hiPSC 製品の充填工程を設計するには、工程の不安定性を評価する必要があることを示している。従来の統計的工程管理による工程の安定性に関する生物学的属性の分析は、パラメトリック手法の限界から推奨されないため、本研究では、hiPSC 充填工程の品質指標とノンパラメトリック分析アルゴリズムの開発に焦点を当て、プロセスの不安定性を評価し、スケールアップ中の充填プロセスを改善するための安定化戦略を実施することを示している。

第 2 章では、hiPSC 製品用に設計された充填プロセスの出力である物理的および生物的属性をしめす 3 つの品質指標を提案している。ここで、3 つの指標としては、各充填バイアルの充填量比率（物理的品質指標）V/V*、各充填バイアル内の粒子密度比率（物理的品質指標）D/D*としている。粒子または細胞の懸濁液を投入材料として使用する場合、混合操作は、生産物の均質性を維持するために充填工程では不可欠な操作であると判断されている。また、混合操作は、順番で充填される際、最終に充填されたバイアルの充填量が、大きく変動することについて明らかにしている。さらに、P/P*は、同じバッチのサンプル間で変動が大きく、細胞劣化もあることを示している。この生物学属性の劣化を軽減するために、先行研究に基づいて 4℃での充填工程などの戦略が提案されている。

第 3 章では、充填装置のキャリブレーション方法と、プロセスの安定性と不安定性を分けて分析するアルゴリズムを開発している。このアルゴリズムは、品質指標測定値の外れ値を検出し、工程の不安定性を標準変動係数（CV）と安定性 CV の差として定量化し、不安定性 CV を求めている。特に、定量化するために、ノンパラメトリックで教師なしの外れ値検出アルゴリズム（Isolation Forest）を利用した分析手法を開発している。品質指標測定における外れ値分類の閾値を確立するための新しい方法を提案し、各品質指標について外れ値分類の閾値を設定した後、各品質指標についてバッチ間の変動とばらつきを定量化し、比較している。その結果、V/V*に対しては、充填操作の最終のバイアルで、不安定となることや、攪拌による D/D*の安定性向上について示している。さらに、生物的指標の P/P*に対しては、物理的品質指標の変動より大きく安定性に劣ることが示されている。

第 4 章では、上記の議論を踏まえ、今後の展望について述べ、本アプローチは、hiPSC や類似の細胞製造における充填工程を設計する際に貢献できることを示している。

以上のように、本論文は、ヒト iPSC 製造の充填工程において、工程特性評価を行う際の新規なアプローチを提案しており、学問分野に重要な知見を与えている。よって本論文は博士論文として価値あるものと認める。