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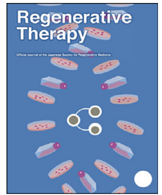
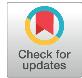


Original Article

In-process evaluation of culture errors using morphology-based image analysis



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ABSTRACT

Introduction: Advancing industrial-scale manufacture of cells as therapeutic products is an example of the wide applications of regenerative medicine. However, one bottleneck in establishing stable and efficient cell manufacture is quality control. Owing to the lack of effective in-process measurement technology, analyzing the time-consuming and complex cell culture process that essentially determines cellular quality is difficult and only performed by manual microscopic observation. Our group has been applying advanced image-processing and machine-learning modeling techniques to construct prediction models that support quality evaluations during cell culture. In this study, as a model of errors during the cell culture process, intentional errors were compared to the standard culture and analyzed based only on the time-course morphological information of the cells.
Methods: Twenty-one lots of human mesenchymal stem cells (MSCs), including both bone-marrow-derived MSCs and adipose-derived MSCs, were cultured under 5 conditions (one standard and 4 types of intentional errors, such as clear failure of handlings and machinery malfunctions). Using time-course microscopic images, cell morphological profiles were quantitatively measured and utilized for visualization and prediction modeling. For visualization, modified principal component analysis (PCA) was used. For prediction modeling, linear regression analysis and the MT method were applied.
Results: By modified PCA visualization, the differences in cellular lots and culture conditions were illustrated as traits on a morphological transition line plot and found to be effective descriptors for discriminating the deviated samples in a real-time manner. In prediction modeling, both the cell growth rate and error condition discrimination showed high accuracy (>80%), which required only 2 days of culture. Moreover, we demonstrated the applicability of different concepts of machine learning using the MT method, which is effective for manufacture processes that mostly collect standard data but not a large amount of failure data.
Conclusions: Morphological information that can be quantitatively acquired during cell culture has great potential as an in-process measurement tool for quality control in cell manufacturing processes.
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1. Introduction

Human-derived mesenchymal stem cells (MSCs) are among the most promising cell sources for clinical applications of cell therapies in regenerative medicine. MSCs, which can be harvested relatively easily from patients, are widely studied somatic stem cells, and have been successfully used in clinical applications, leading to the introduction of commercial cellular products in the market [1–6].

Industrial-scale manufacture technologies for producing MSCs are required to widely distribute established cell therapies [7–11]. However, one of the most difficult tasks in cell manufacture is controlling cell quality [7–9]. Numerous patient-derived variations exist in MSCs; these variations can trigger unexpected alterations in cell quality during their manufacture.

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In contrast to other industrial product manufacturing processes, technological difficulties affect cell quality control in cell manufacturing processes. One of the most important issues is the lack of effective in-process measurement methods for monitoring the transition of cellular states during the long and complex cell culture processes. Therefore, the detailed culture process is not completely understood, and little information is available for understanding the "on-going" status of cultured cells. This can impact cell manufacturing facilities in two ways. The first is the possibility of failure of a large culture after a long and costly culture process. Because partial testing by sampling may not indicate the status of the total cellular population, the lack of an in-process measurement for monitoring the entire sample can result in unexpected quality alterations at the end of culture; particularly, the impact of an unexpected yield of cells following stable manufacture is costly. The other is the difficulty in providing feedback for troubleshooting of the process. Understanding the essential points of the process based on feedback information from in-process monitoring data is one of the most common and effective approaches to improving manufacturing processes. However, such data-driven process improvement is currently difficult for MSC culture.

Monitoring of cellular morphology has long been the most practical and effective in-process evaluation technique for cell culture. Determining irregularities in cellular morphology is an important quality criterion in cell culture and clinically applied cell culture protocols. However, cell morphology is typically evaluated in a non-quantitative manner; therefore, special training and skill is required for cell culture experts to stably control the cell culture. Although such expert manual skills are essential, there is an increasing need to mechanize the process using advancing technologies. Image processing and analysis by computational technology have attracted attention, and an increasing number of reports has described the successful application of such technology for cellular evaluations [1,12–18]. Our group proposed using computational analysis techniques to quantitatively utilize information obtained from phase-contrast microscopic images as "morphology-based prediction techniques" for non-invasive cell quality evaluation in regenerative medicine [19–24]. We suggested using a combination of advanced imaging instruments, image-processing, and the multiple parametric machine-learning technique based on morphological parameters to construct models for predicting the experimentally defined quality data using only images.

Although an increasing number of image-based analysis reports has reported the applicability of image-based analysis in non-invasive cell quality evaluations, few studies have focused on detecting "practical and trivial errors" that can occur during the actual cell culture process. In the actual cell manufacture process, the culture protocol is carefully determined and designed to reproduce an identical cellular status during culture. Application failure of such protocols are mostly caused by unexpected errors and are trivial. Therefore, it is more important that the performance of image-based analysis for in-process measurements be investigated under conditions where such variations (such as differences that can occur in the same medium) commonly occur rather than under conditions where the variations are too distinct (such as differences between completely different types of media). However, most culture error is considered as "immatureness" of the experiment and their actual differences from the normal state or their difficulty of detection has not been examined.

Therefore, we compared the differences caused by errors under standard culture conditions that have not been quantitatively examined and investigated the performance of "morphology-based image analysis" as an example of in-process measurement for monitoring cellular status. Practically, conditions under which intentional errors occur were designed and compared with the standard condition: Condition A, normal medium containing 2% dimethyl sulfoxide (DMSO), assuming insufficient removal of DMSO in a cryopreserved cell stock containing 10% DMSO; Condition B, damaged medium that had been repeatedly warmed to represent repeated mishandling; Condition C, normal medium culture under 0% CO_2, assuming unexpected errors in the sensors and air supply in the incubator; and Condition D, normal medium culture under 10% CO_2, also assuming incubator malfunction (Table S1). Most of these designed errors were exaggerated from the aspect of matured researchers; we consider it meaningful to quantitatively determine the detection performance of these errors. "Logically abnormal" conditions are rarely quantitatively examined and presented; therefore, it is unknown how extensively morphological features respond under such conditions. Understanding the limit of measurement data is important for designing measurement equipment, such as image-based monitoring software. Additionally, to advance cell manufacture, various factors cannot be judged based on the experience of the researcher. When manual processes are carried out using robotics, mis-operation or mis-programming in panel operation is difficult to eliminate. Thus, an automated detection process is needed. Moreover, in industrial manufacturing in which higher production requires increased human resources at a lower cost, not all operators are sensitive to errors. To advance cell manufacture, we determined the effectiveness of quantitative morphological analysis using in-process image data by comparing different conditions (Fig. 1). With visualization and prediction model construction, the intentionally designed "errors in the culture" were quantitatively compared. We found that continuous label-free quantification of morphological parameters effectively described the in-process measurements, supporting the cell manufacture processes for regenerative medicine products.

2. Materials and methods

2.1. Cells and culture

Nine lots of human bone-marrow derived MSCs were purchased from Lonza Japan, Ltd. (Tokyo, Japan) and 1 lot was purchased from Lifeline Cell Technology (Frederick, MD, USA). Nine lots of human adipose derived stem cells (ADSCs) were purchased from Lonza Japan, Ltd. and 1 lot was purchased from KURABO (Osaka, Japan). Cells were maintained in MSCGM (Lonza Japan, Ltd.) supplemented with BulletKit (Lonza Japan, Ltd.) under conditions of 37 °C and 5% CO_2 according to the companies' protocols; these were designated as the "Standard" conditions. The antibiotics penicillin (100 U/mL) and streptomycin (0.1 g/mL) were added. The medium was stored at 4 °C protected from light for the Standard condition. Four irregular conditions mimicking the "errors" that can occur in standard conditions were prepared: (Condition A: 2% DMSO) The cells were cultured at 37 °C and 5% CO_2, with a final concentration of 2% (v/v) DMSO added to the medium. This condition represented a situation in which DMSO in the cryopreservation stock (using 10% DMSO) was not sufficiently removed from the culture. (Condition B: Damaged medium) The cells were cultured at 37 °C and 5% CO_2, but the used medium was warmed for 10 cycles of (37 °C for 2 h) before use. This condition was an exaggerated condition designed to assume that the pre-warming period of the medium was conducted in error. (Condition C: 0% CO_2) The cells were cultured at 37 °C in an incubator with no CO_2 supply. This condition was designed to mimic a malfunction of both the CO_2 monitoring sensor and gas supply bulb. Because the CO_2 supply is one of the most essential conditions for maintaining the medium pH, this condition was

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