Improvement of Injection Accuracy in the Media Changing Process for Cell Processing

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Abstract

In regenerative medicine, manual cell processing is labor-intensive and costly and needs to become more efficient. Recently, automatic cell culture systems equipped with vertically articulated robots have been developed. However, automating all cell processing tasks complicates these systems. This study aims to develop a simple and rational cell processing system by combining robot and human tasks. In a previous study, we improved the efficiency of discarding and injecting tasks using a robot in a media changing process. In this study, we further investigated the injection method and developed and evaluated two types of algorithms that are both accurate and can handle variable target volumes. One algorithm predicts the time to stop injecting based on the flow rate and corrects the stop timing based on the y-intercept of the injection volume and the target volume. The other algorithm uses machine learning to predict the time to stop injecting based on the flow rate, y-intercept, and target volume. Our experimental results revealed the feasibility of both algorithms. With these algorithms, a robot can perform the injecting task more efficiently than a human.

Introduction

In recent years, cell transplantation has been used to repair damaged tissues. For example, skeletal myoblasts, mesenchymal stem cells, myocardial stem cells, embryonic stem cells, and induced pluripotent stem cells have been used to repair myocardial tissue damaged by ischemic heart diseases such as angina pectoris and myocardial infarction. Under these circumstances, a cell structure formed using a scaffold and a cell sheet, where cells form in a sheet shape, has been developed [1, 2].

Conventionally, these cells have been manufactured manually by an operator with specialized knowledge in a clean room called a cell processing center. The cost of manufacturing cells is high, and there is still room for improvement in its efficiency. In manual operation, there are individual differences in terms of operation time and contamination risk. Therefore, automatic cell culture apparatuses that use a robot arm to perform tasks related to cell culture have been developed [3-10]. Moreover, techniques related to cell culture automation have also been proposed [11-14]. Although automated injection has been promoted in fields other than regenerative medicine, accurately controlling the injection volume is difficult because teaching playback control is adopted [15, 16].

Our investigation revealed that most of the existing automatic cell culture apparatuses use single-arm or dual-arm robots, which can be used unattended [17]. However, the automation of all cell processing tasks complicates the system; the developed apparatuses lack flexibility (e.g., versatility and expandability), and the cost of the apparatuses is high. Because automatic cell culture apparatuses have been mainly developed by companies, such as machine manufacturers, they tend to be large-scale systems that automate all tasks or systems that use advanced technologies, such as robot arms.

The regenerative medicine products approved for manufacture and sale at this time have been approved by culture methods. Thus, a cell culture-assisting apparatus that collaborates with humans in a safety

cabinet is considered easier to introduce than a fully automated apparatus. This study aims to develop a simple and rational cell processing system through the combination of the tasks performed by a robot and those performed by a human. In a previous study, we proposed improving the efficiency of discarding and injecting tasks using a robot in a media changing process [18, 19]. Such tasks have a high risk of liquid dripping and rely on the operator's experience.

In this study, we report the results of further investigation of the injection method and the development and evaluation of two types of algorithms that are both accurate and can handle variable target volumes. One algorithm predicts the time to stop injecting based on the flow rate and corrects the stop timing from the y-intercept of the injection volume and the target volume. The other algorithm uses machine learning (ML) to predict the time to stop injecting based on the flow rate, y-intercept, and target volume. ML has been used for prediction and factor analysis in optical communications [20], and infectious disease epidemiology [21], and its effectiveness has been confirmed. However, there is no study on the application of ML to the task of injecting a liquid. The application of ML to the injecting task will reduce the necessity for manual data analysis, as in the previous study. Furthermore, if the learning model is simple, the amount of data required for learning should be minimal, allowing for highly accurate predictions. We conducted the preliminary experiments to calculate the parameters of the prediction equation for each algorithm. Afterward, we conducted evaluation experiments using the prediction equation to confirm the effectiveness of each algorithm. Our experimental results reveal that both algorithms are feasible and which algorithm is more suitable depending on the situation.

Methods

Injection algorithm using ΔT and X

The method for improving the efficiency of the injecting task is shown in the previous study [19]. Figure 1 shows the injecting task performed by a robot in this study. The angle of the injection bottle changed from 45° to 70° to reduce the culture media left in the injection bottle. The robot performs the injecting task only by rotating around the tool center point (TCP), the injecting spout of the injection bottle. To reduce the risk of liquid dripping, a protruding inspiration port on the lid of the injection bottle is changed to a non-protruding inspiration hole.

Figure 2 shows the transition of the injection volume after the start of the injection end motion. The injection algorithm predicts the injection volume ($X\%$) to be injected in a fixed period (ΔT). If the injection is continued without stopping, the injection volume during ΔT is assumed to be 100%. The injection end motion is triggered when the injection algorithm predicts that X will reach the target volume. ΔT and X depend on the injecting angle and the angular velocity of the injecting task, the structure of the lid of the injection bottle, and the control cycle of the robot. Therefore, it is necessary to determine ΔT and X in advance by analyzing data obtained when the robot performs the injecting task.

In the previous study [19], a slope from 40 to 60 g was adopted, considering that the injection volume was fixed at 75 g and that the volume closer to the injection end motion could be more accurately predicted.

However, the injection volume should be changed. The range slope, excluding 20 g at the start and end of the injection volume, was to be used since the flow rate is stable within the range, excluding the start and end of the injection.

Correction by the y-intercept

Although the conventional algorithm can achieve the same accuracy as the operator, it was found that the injection volume varied depending on the number of injections. The injection volume when the injection is repeated multiple times is shown in Fig. 3. Specifically, the remaining volume in the injection bottle decreases as the number of injections, and the following phenomenon occurs.

1. The y-intercept decreases (the x-intercept increases) because of the decrease in the volume injected during the injection start motion.
2. The final volume decreases because of the decrease in the volume injected during the injection end motion.

Therefore, the difference in (a) is used to compensate for the effect of (b). Figure 4 shows the system overview. First, ΔT and X are calculated using load cell data when the injection experiment is repeated. The maximum volume of the culture media in the injection bottle is 500 g. Because the injection bottle is tilted 70° for injection, it is assumed that the remaining approximately 50 g cannot be injected. If the injection end motion is started when the injection volume exceeds 68 g, approximately 75 g is injected. Thus, it is possible to inject six times in a row. The six times of this experiment was repeated six sets. The average value was adopted; ΔT was determined to be 1.28 s, and X was determined to be 75.0%.

Afterward, the injection algorithm using the obtained ΔT and X was applied to the original data. Because the data were obtained when the injection end motion was started after the injection volume exceeded 68 g, it was possible to calculate a theoretical value for how much of a target volume should be set. An additional experiment with a target volume of 75 g can be conducted using the obtained ΔT and X to determine the difference between the theoretical and measured values; however, this was not conducted in this study. Figure 5 shows the correlation between the y-intercept of the injection volume and the difference between the theoretical and measured values. The smaller the y-intercept, the smaller the measured value, whereas the theoretical value remains constant. A linear approximation equation can be obtained because there is a linear correlation between the y-intercept and injection error. By calculating the volume of increase or decrease from the y-intercept and shifting the stop timing, it is possible to approach the theoretical value. The equation for calculating the correction volume C_1 using the y-intercept b_y is as follows.

$$C_1 = -0.0847(b_y - 15.5478) \quad (1)$$

A correction using the x-intercept is also possible. Figure 6 shows the correlation between the x-intercept of the injection volume and the difference between the theoretical and measured values. Each correction

was applied to the original data to calculate the root mean square (RMS) of the difference between the theoretical and measured values. The result was 0.261 when the y-intercept was used and 0.262 when the x-intercept was used. Although both can be used, this study uses the y-intercept. By employing the correction by the y-intercept, it is possible to respond to changes in the injection volume caused by the number of injections.

Correction by the target volume

In Fig. 3, if the target volume is different, even if the y-intercept is the same, the effect of (b) is different because the remaining volume at the start of the injection end motion is different. The correction by the y-intercept is for a target volume of 75 g. It includes the correction for the effect of (b). However, if the target volume is not 75 g, the remaining volume at the start of the injection end motion increases or decreases compared to the case where the target volume is 75 g. In other words, the effect of (b) changes and the correction of only (a) is insufficient. Therefore, the correction method for the case where the target volume is not 75 g is considered.

Figure 7 shows the correlation between the remaining volume at the start of the injection end motion and the injection volume after the injection end motion. The smaller the remaining volume, the smaller the injection volume. A linear approximation equation can be obtained because there is a linear correlation between the remaining volume and injection volume. Then, the change in injection volume per unit remaining volume can be calculated. Because the difference between 75g and the target volume is the difference in the remaining volume, the equation for calculating the correction volume C_2 using the target volume V_t is as follows.

$$C_2 = -0.0017(V_t - 75.0) \quad (2)$$

Using the correction by the target volume, the injection algorithm using ΔT and X can be applied to target volumes other than 75 g.

Flowchart of the injection algorithm

A flowchart of the injection algorithm using ΔT and X with two types of corrections is shown in Fig. 8. In the conventional method, the injection end motion is started when the injection volume exceeds the stopping volume V_e . However, it turned out that an injection error occurred depending on the number of injections and target volume. Therefore, the correction volumes C_1 and C_2 are added to V_e . Although not employed in this study, another method for adding correction volumes C_1 and C_2 to the target volume V_t is shown in Figure 9.

Injection algorithm using machine learning

As an alternative to the injection algorithm using ΔT and X with two types of corrections (called Algorithm A), the injection algorithm using ML (called Algorithm B) is considered.

Algorithm B uses multiple linear regression to predict the time to stop injecting based on the flow rate, the y-intercept of the injection volume, and the target value. When two features are fixed, there is a linear relationship between the remaining and predicted values. Therefore, linear regression is considered to be effective. Because the features include the y-intercept and target value, the correction of Algorithm A is included. The prediction equation by the linear regression model is shown in Eq. (3), and each feature value of the linear regression model is shown in Fig. 10. The slope x_1 , y-intercept x_2 , and target value x_3 are feature values; the weights w_1 , w_2 , w_3 , and the offset b are parameters of the learned model; and the stopping volume V_{ea} is a predicted value from the model. The model is developed using scikit-learn [22], a Python ML library.

$$V_{ea} = w_1x_1 + w_2x_2 + w_3x_3 + b \quad (3)$$

First, preliminary experiments were repeated to collect training data, and a linear regression model was developed from the collected training data. For each target volume, six sets of injection experiments were conducted. The target volume was set between 50 and 150 g at 10-g intervals. The injection end motion began at 7 g less than the target volume to keep the final injection volume close to the target volume. 500 g of culture media was repeatedly injected. However, the remaining volume should not be less than 50 g. Each parameter of Algorithm B was calculated using the training data. Algorithm A can also be expressed in Eq. (3). The values of each parameter for both algorithms are shown in Table 1. V_{ea} in Fig. 8 can also be calculated using Algorithm B.

Results And Discussion

Evaluation experiment

Evaluation experiments were conducted to verify the effectiveness of each algorithm. For each target volume, two sets of evaluation experiments were conducted. The target volume was set between 50 and 150 g at 10-g intervals. 500 g of culture media was repeatedly injected. However, the remaining volume should not be less than 50 g. Table 1 shows the parameters used in the experiment.

Table 2, Table 3, and Fig. 11 show the evaluation experiment results. According to our previous study [19], the standard deviation (3σ) when the operator performed the injecting task was 1.5 g, and the standard deviation (3σ) when the robot performed the injecting task using the conventional injection algorithm without correction was 1.18 g. On the other hand, both Algorithms A and B showed a better standard deviation (3σ) than the conventional injection algorithm without correction for all target volumes. Therefore, both algorithms can be used, and which one depends on the situation. For example, if data for a certain target volume can only be collected and data analysis can be performed manually, Algorithm A is suitable. In contrast, if data for various target volumes can be collected and the data analysis cannot be performed manually, Algorithm B is suitable.

Discussion on data used to calculate the parameters of Algorithm A

Parameters calculated using the experimental data when the target volume was 75 g were used in the evaluation experiment of Algorithm A. The case to be considered is when the parameters are calculated using data from another target volume. Simulations are performed using preliminary ML experimental data to verify injection accuracy.

For comparison, the parameters were calculated using the experimental data when the target volume was 50 g or 150 g. Calculating a theoretical value for how much of a target volume should be set was possible with these parameters. The difference between the theoretical and measured values was shown in Fig. 12. Although the results slightly differed depending on the data used to calculate the parameters, the difference was within ± 1.5 g for all target volumes.

Discussion on data used to construct the model for Algorithm B

Parameters calculated using all data with target volumes from 50 g to 150 g were used in the evaluation experiment of Algorithm B. The case to be considered is when the parameters are calculated using different training data. Simulations are performed using the preliminary ML experimental data to confirm injection accuracy.

For comparison, the parameters were calculated using the training data of four patterns. Each pattern has a different range and amount of training data. Calculating a theoretical value for how much of a target volume should be set was possible with these parameters. The difference between the theoretical and measured values was shown in Fig. 13. As shown in Fig. 13(a), Algorithm B achieved poor performance when experimental data with only a target volume of 75 g were used. For all other patterns, the difference was within ± 1.0 g for all target volumes.

Conclusions

Most of the existing automatic cell culture apparatuses are fully automated and lack flexibility. We aimed to develop a cell culture-assisting apparatus that collaborates with humans. In a previous study, we proposed an injection algorithm using a robot to improve the efficiency of the injecting task in the media changing process. In this study, we further investigated the injection method and developed two injection algorithms that are both accurate and can be applied to various target volumes. Evaluation experiments were conducted, and the following results were obtained.

1. The injection accuracy was improved by adding a correction using the y-intercept of the injection volume and the target volume to the conventional injection algorithm.
2. A linear regression model was developed by ML using the flow rate, y-intercept, and target value as training data. The injection accuracy using the linear regression model was equivalent to that of the injection algorithm with the correction.
3. Both injection algorithms were applicable to target volumes ranging from 50 to 150 g.

Thus, it was determined that the robot could perform the injecting task more efficiently than the operator. Which algorithm to use depends on the purpose. The choice can be made based on the amount and type of data collected and the effort required analyzing the data. In the future, we will incorporate the new injection algorithm into the injecting robot and optimize the task procedures in combination with the discarding robot.

Abbreviations

ML: Machine learning

TCP: Tool center point

RMS: Root mean square

Declarations

Availability of data and materials

Not applicable.

Competing interests

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Not applicable.

Authors' contributions

RN conducted the research and drafted the manuscript. KY and KS provided requirement specifications and their knowledge on cell processing and designed the lid of the injection bottle. MJ supervised the research and reviewed the manuscript. All authors read and approved the final manuscript.

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Tables

Table. 1 Parameters for the Evaluation Experiment of Algorithm A and Algorithm B.

Parameters	Algorithm A	Algorithm B
w_1	-0.9634	-0.8120
w_2	-0.0847	-0.1421
w_3	0.9983	1.0007
b	-1.1894	-3.5116

Table. 2 Experimental results of Algorithm A.

Target volume [g]	RMS of injection error [g]	Average [g]	Standard deviation (1 σ) [g]	Standard deviation (3 σ) [g]
50	0.44	50.24	0.38	1.13
60	0.27	60.18	0.21	0.62
70	0.40	70.17	0.38	1.13
80	0.37	80.18	0.34	1.01
90	0.32	90.24	0.23	0.69
100	0.25	100.13	0.23	0.70
110	0.24	110.08	0.24	0.72
120	0.23	120.15	0.18	0.54
130	0.20	129.88	0.18	0.55
140	0.27	139.86	0.25	0.75
150	0.31	149.91	0.32	0.97

Table. 3 Experimental results of Algorithm B.

Target volume [g]	RMS of injection error [g]	Average [g]	Standard deviation (1 σ) [g]	Standard deviation (3 σ) [g]
50	0.37	50.18	0.34	1.01
60	0.24	59.97	0.25	0.74
70	0.34	70.15	0.32	0.97
80	0.30	79.95	0.32	0.95
90	0.28	90.06	0.29	0.87
100	0.32	99.89	0.32	0.96
110	0.29	110.11	0.28	0.85
120	0.21	120.10	0.20	0.60
130	0.30	129.94	0.33	0.98
140	0.35	140.05	0.37	1.12
150	0.29	150.04	0.32	0.95

Figures

Figure 1

Injecting task performed by the robot. (1) The robot rotates the injection bottle and injects the culture media into a flask. (2) The robot stops at 70° and continues to inject. (3) The robot returns to the initial position and finishes the injecting task.

Figure 2

Transition of the injection volume after the start of the injection end motion. To inject the target volume V_t , the injection end motion is started when the injection volume exceeds the stop volume V_e .

Figure 3

The injection volume when the injection is repeated multiple times. The injection volume varies depending on the number of injections.

Figure 4

System overview. The injection was performed only by rotating the 6th axis of a 6-axis articulated robot.

Figure 5

Correlation between the y-intercept of the injection volume and the difference between the theoretical and measured values. The measured value decreases as the y-intercept decreases, whereas the theoretical value remains constant.

Figure 6

Correlation between the x-intercept of the injection volume and the difference between the theoretical and measured values. The measured value decreases as the x-intercept increases, whereas the theoretical value remains constant.

Figure 7

Correlation between the remaining volume in the injection bottle and the injection volume after the start of the injection end motion. The injection volume decreases as the remaining volume decreases.

Figure 8

Flowchart of the injection algorithm using ΔT and X with two types of correction. The correction volume C_1 and C_2 are added to V_e .

Figure 9

Flowchart of the injection algorithm using ΔT and X with two types of correction. The correction volume C_1 and C_2 are added to V_t .

Figure 10

Each feature value of the linear regression model. The slope x_1 , y-intercept x_2 , and target volume x_3 can be used to calculate the stop volume V_{ea} .

(a)

(b)

Figure 11

The injection error for each target volume: (a) Algorithm A; (b) Algorithm B.

Figure 12

The difference between the theoretical and measured values for each stops volume. To calculate the parameters of Algorithm A, experimental data for various target values were used: (a) The target volume is 75 g; (b) the target volume is 50 g; (c) the target volume is 150 g.

Figure 13

The difference between the theoretical and measured values for each stops volume. Machine learning was performed using a variety of training data: (a) Six sets of data with a target volume of 75 g; (b) six sets of data with target volumes from 50 to 150 g at 10-g intervals; (c) one set of data with target volumes from 50 to 150 g at 10-g intervals; (d) six sets of data with target volumes of 50 and 150 g.