Scheduling Complexity of an Automated Cell Manufacturing Process

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Abstract. Osteoarthritis is currently the most prevalent chronic joint disease and is expected to increase continually due to aging population and obesity epidemic. Many methods exist to treat this disease but the most promising one is cell-based therapy which uses chondrocyte cells. Chondrocyte cells are cultivated in an automated cell manufacturing system (ACMS). However, ACMS suffers from long idle time caused by incubation times required to cultivate cells in different samples in different incubators. But before optimization can be used to find an optimal schedule, the complexity of the ACMS scheduling problem is established. Parallel operation of the ACMS was able to process a maximum of 26 samples in a year, while sequential operation yielded only 14. Moreover, if we produce more sample in a year the number of different schedules will increase. Then, the problem has more complicated.

1. Introduction

Cell-based therapy or regenerative medicine is capable of restoring the function of damaged cells, tissues, and organs using patients’ own cells [1]. The outcome of this treatment relies heavily on the number and quality of the cells used in the therapy. For future, the applications of cells are used in a tissue construction to transplant tissue to patient A. Jain and R. Bansal reported [2], or a cell banking is an alternative David T. Harris reported [3]. Other treatment methods based on disease-modifying drugs such as non-pharmacological, pharmacological, and surgical approaches are not effective when applied too late [4]. Conventional manufacturing of cells involves manual operation which requires highly skilled technicians and is very common at the laboratory scale [5]. However, manual operation is labour-intensive, time-consuming, prone to human errors, and unable to meet commercial needs of patients. In addition, cell processing and culturing in laboratories must be carried out under aseptic condition to avoid bacterial contamination, which is difficult for technicians to control. Therefore, an automated cell manufacturing system (ACMS), as shown in figure 1, is employed to overcome problems associated with manual operation. The ACMS consists of two robot arms housed in an isolator to carry out tasks normally done by humans. The isolator is connected to two incubators to handle the processing of two different samples simultaneously. The aseptic environment inside the system is assured by performing periodic decontamination to prevent bacterial contamination and cross-contamination between samples.

With the above-mentioned benefits, our research group has used the ACMS (CellPROi, Shibuya Cooperation, Japan) for large-scale production of human autologous chondrocytes to treat cartilage defects. The manufacturing process of chondrocyte cells involves several steps whose sequence must follow the standard operating protocol (SOP) as shown in figure 2. The procedure is divided into three main parts, namely cell expansion (cell seeding and subculture), medium replacement, and cell
collection (for subsequent applications). In “Cell Seeding”, cells initially isolated from human articular cartilage tissue (passage 0) are plated onto two 100 mm tissue culture petri dishes before transferring them into the connected incubator. The culture medium is replaced every 3 days using “Medium Replacement 1” mode. Once the cells reach 80% confluency or 80% of the culture area is covered by a monolayer of cells, “Subculture 1” is activated to expand the number of cells by transferring all the cells to two new 150 mm culture petri dishes, resulting in the cells at passage 1 with a split ratio of 1:1. “Medium Replacement 2” mode is used until the cells reach 80% confluency before starting “Subculture 2” to yield the cells at passage 2 at a split ratio of 1:3. These steps are repeated for “Medium Replacement 3” and “Subculture 3”. At the end of the SOP, the chondrocytes at passage 4 in eighteen 150 mm culture dishes are collected for further use [6].

![Image of CellPROi machine]

**Figure 1.** The automated CellPROi machine

Although the automated cell culture has several advantages, as previously mentioned, it suffers from long waiting time between each processing step, leading to inefficient utilization of the system and opportunity loss. Consequently, an important problem that arises is to find an optimal cell manufacturing schedule to maximize the number of samples while minimizing the operating cost of the two incubators in a given year, given that there are clearly many feasible schedules. At this point, waste treatment, product quality testing, and transportation of cell products are not taken into account. Optimization to find an optimal schedule is beyond the scope of this study. Instead, this work explores and addresses the complexity of the ACMS scheduling problem which shows that subsequent optimization is needed to maximize the efficiency of the system.

![Flow chart of automated cell culture]

**Figure 2.** Flow chart of automated cell culture
In general, the complexity of an optimization problem is defined by the number of decision variables and the number of constraints (collectively known as the size of the problem) and how its feasible solution space increases with the problem size. For example, the solution space can increase linearly or exponentially as the problem size increases [7]. Obviously, the latter is much more difficult to solve. In certain optimization problems, the problem solution space or the number of feasible solutions can be established precisely as a function of the number of decision variables. Unfortunately, this often is not the case, and the ACMS scheduling problem is no exception. As a result, before optimization can be carried out, this study seeks to establish the complexity of the scheduling problem.

2. Experimental

2.1. Production of human primary chondrocytes
The ACMS (CellPROi) manufactured by Shibuya Cooperation Co., Ltd. (Japan) was installed in a laboratory at the Bangkhuntien Campus of KMUTT. The machine consists of two robotic arms and two incubators, which allow to process two cell samples simultaneously. The process procedure following the SOP is shown in figure 2. The cells must be subcultured when their confluency reaches 80% to avoid contact inhibition. If the cell confluency does not reach 80%, the medium will be replaced again. A flow chart in figure 3 shows the steps involved in achieving a confluency of 80% in a cell culture.

2.2. Simulation
All data used in the calculations involving schedules come from actual experiments at the Bangkhuntien laboratory of KMUTT. First, it should be noted that the ACMS scheduling problem is multi-level. The first issue is to maximize the number of samples that can be produced in one year, given the number of incubators. After that, two sub-objective functions will be considered, which are to minimize the total production time and to minimize the operating cost. For simplification, the incubation time is assumed to be the same for all sample although cells in different samples behave differently depending on the type and the quality of cell. All samples must follow same processing sequence as shown in figure 3.

In the ACMS, a feasible schedule can be found by simple hand calculations for both sequential and parallel production. A feasible schedule is one that does not violate any constraints in the system such as sequences of process follow the standard operating protocol and all product can be processed in one year. These calculations can be programmed and automated in software such as MATLAB. Random feasible schedules or solutions are then generated for both sequential and parallel production. For each feasible schedule, the value of its objective function, e.g. the operating cost, is calculated. To establish the complexity of the scheduling problem, the total number of random schedules, \(N_R\), is varied, each of which is treated as one case. For each case, the number of schedules \(N_F\) that give different values of the
objective function is collected. The trend for $N_F$ is then observed. If $N_F$ starts to plateau quickly as $N_R$ increases, it indicates that the scheduling problem does not contain many feasible solutions.

First, the maximum number of samples that can be processed in one year via sequential and parallel production was determined by trial-and-error. This was achieved by randomly generating 10,000 feasible schedules for each case of $N_S$, the number of samples to be processed, with $N_S = 2, 3, 4, \ldots N_{\text{max}}$. When $N_{\text{max}}$ was reached in which all 10,000 schedules generated required more than one year to process, $(N_{\text{max}} - 1)$ was taken the maximum number of samples that the ACMS can process in one year.

3. Results and discussion
The scheduling of the ACMS is based on two existing incubators for the production of human primary chondrocyte cells. Because the cell cultivation must follow the SOP, the production is by nature sequential. The incubation time of each sample is the same, and we assume that the specific growth rate of each sample is the same.

3.1. Preliminary calculation
The chondrocyte cell production can be carried out either sequentially or in parallel. In sequential production, the ACMS handles one sample at a time, which allows the use of only one incubator. Decontamination is performed only once at the beginning of the sample. When the cells of sample 1 are collected, sample 2 will be processed in a sequential manner. The processing time of each sample is 37.78 hours. The maximum sample in one year was found to be 14 samples which resulted in an idle time of 8,032 hours and a production time of 8,560.97 hours. Then the operating cost, which consists of material, chemical and electricity cost, was evaluated. The operating cost is 68,550 Baht/sample. On the other hand, in parallel production, the ACMS handles two samples at a time by using two incubators. However, the SOP must still be followed which means that cross-contamination between samples must be avoided. The decontamination step must be performed every time when there is a switch between samples. This leads to a total of 18 decontaminations during one run of two samples. The processing time of the two samples is 459.57 hours. The maximum number of samples for year is found to be 26 which resulted in an idle time of 2,316.98 hours and a production time of 8,291.35 hours. The operating cost is 87,450 Baht/sample. The results of the two types of production are summarized in Table 1.

| Parameters | Sequential production | Parallel production |
|------------|-----------------------|---------------------|
| Number of samples | 14 | 26 |
| Production time (hour) | 8,560.97 | 8,291.35 |
| Idle time (hour) | 8,032 | 2,316.98 |
| Expense (Baht) | - Materials 18,000 | 36,400 |
| | - Chemicals 43,700 | 46,500 |
| | - Utilities 6,850 | 4,550 |
| Operating cost (Baht/sample) | 68,550 | 87,450 |

Comparative calculations between sequential and parallel production show that the latter can reduce the idle time by 5,715 hours due to its higher efficiency and the ability to produce 12 more samples in a year. Even though parallel production incurs a higher operating cost than sequential production because of the need for more decontaminations, the average utility cost per sample in parallel production is cheaper because more samples are produced.
3.2. Randomization method

With two incubators, the ACMS can process up to a maximum of 26 samples per year. The simulation shows that if 27 samples are to be processed, total production time of 370.88 days is needed. Clearly, more samples to be processed means there are more possible ways or schedules to process them. To establish this relationship and the complexity of the AMCS scheduling problem, random feasible schedules are generated for each $N_S$, starting with $N_S = 2$ up to $N_S = 26$. For each $N_S$, a total number of 10,000 random feasible schedules are generated based on coding in MATLAB. The total number of different feasible schedules $N_F$ is then recorded for each $N_S$, which are then plotted in figure 4. Note that $N_F \leq 10,000$ for all cases as it should be figure 4 shows that as $N_S$ increases, $N_F$ also increases and goes up rapidly when $N_S > 10$, suggesting that when $N_S$ is large and approaches 26, the number of possible feasible schedules also increases. At $N_S = 24$, only about 20% of the random schedules generated are repeats or the same. This randomization of feasible solutions indicates that simply picking the best schedule from the 10,000 random schedules does not guarantee an optimal schedule or even a good schedule. Hence, optimization is needed which will lead to an optimal or a near-optimal schedule using a lot less computation time than the randomization method.

To further illustrate the need for optimization, the computation times required to generate 10,000 solutions in each case of $N_S$ are recorded and shown in figure 5. It is clear that when $N_S = 26$, it took a laptop computer (Intel® Core™ i7-7700HQ CPU @ 2.80GHz 2.81 GHz, 64-bit operating system, x64-bases processor) about 10 hours to complete the solution generation. With optimization, the computation time is expected to be just a fraction of that of the randomization method with almost a guarantee that the optimized solution would be superior to the best solution from randomization.

It is possible that different feasible schedules result in the same objective value. In the context of optimization, these two solutions with the same objective values are equivalent. Moreover, it is also important to note the variance in the values of the objective function for the schedules generated. figure 6 shows the distribution of feasible schedules for the case of $N_S = 26$ in terms of the frequency of feasible schedules with the same objective values and the variance of the objective values. The range of the production time is between 8,291.5 - 8,760 hours (345.47 – 365 days), while the range of the operating cost is between 86,260 - 87,450 Baht/sample. As the operating cost depends on the utility cost and the decontamination cost, different production schedules will yield different production duration and operating cost. At the maximum number of samples per year ($N_S = 26$), the relationship between the production time and operation cost from each randomly generated feasible schedule was investigated, as shown in figure 7. As expected, the operating cost is inversely proportional to the production time. A shorter production time requires the decontamination step to be performed more frequently, leading to a higher cost. This result shows that the overall production cost of each sample can be more affected by the frequency of the decontamination process. Therefore, optimization is necessary to determine a processing schedule that can provide a lower operating cost within the desirable production duration.
Figure 6. Frequency of feasible solution at $N_S = 26$

Figure 7. The relation of sub-objective function between production time and operating cost at $N_S = 26$

4. Conclusions
In this work, the scheduling problem of an ACMS with two incubators to produce chondrocyte cells was studied. Because many feasible schedules exist, ACMS scheduling is an optimization problem, whose optimal solution depends upon the objective function chosen to be minimized or maximized. In this study, the complexity of the scheduling problem was explored and quantified. First, two types of schedules are possible, namely sequential production and parallel production. By generating a large number of feasible manufacturing schedules, it was found that the maximum number of samples that could be produced in a year was 14 for sequential production and 26 for parallel production with the operating costs for each sample of 68,550 Baht and 87,450 Baht, respectively. Parallel production was more desirable, as it can produce more samples per year despite a higher production cost. Therefore, an application of optimization would be particularly beneficial to the ACMS as it can help determine an optimal production schedule that yields a large number of samples while minimizing the operating cost.

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