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Novel Design of Manufacturing Bioreactor and Facility of Cell-Based Health Care Products for Regenerative Medicine

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Abstract

The processing systems for cell and tissue cultures suitable for therapeutic application are promising devices to save labor tasks, space, and contamination risk. Similar to the isolator system in the aseptic processing of healthcare products based on pharmaceutical regulation, the installation of a decontamination apparatus into the processing system will realize a further reduction in manufacturing costs based on the proposed siting criterion in an ISO Class 8 clean area without any increment of contamination risk. Furthermore, flexible modular platform (fMP) technology will realize the flexible combination of isolator modules, the practical design for cell sheet assembling was successfully performed. These proposals of the siting criterion and manufacturing system for the isolator and fMP technologies are expected to enhance process versatility accompanied by safety, security and cost-saving.

Keywords: bioreactor, manufacturing facility, flexible modular platform, automation, cell processing facility, cell sheet.

7.1 Introduction

In the last decade, cell and tissue therapies have encompassed a broad, rapidly growing field of medicine that involves the manipulation and administration of cells for the treatment of disease. Especially, the advances in tissue engineering

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have offered promising strategies for reconstructing and repairing defective tissues in vivo (1-2), enabling damaged tissue to be replaced with cultured tissues that meet the needs of the individual patients. A number of companies manufacturing cultured tissues have been established. The manufacture of cultured tissues is still burdened by instability owing to the qualitative fluctuation of cell sources as raw materials and the risk of biological contamination.

Innovative techniques of cell and tissue processing have been developed for therapeutic applications. The subculturing for cell expansion is a core process. In manufacturing, strict management against contamination and human error are compelled due to un-sterilable products and the complexity of culture techniques, respectively. In addition, the development of a processing system is considered to lead to safety, security and cost-saving for cell and tissue cultures. However, the criterion of facility design to date has not been clear. This article describes a novel strategy for bioreactor and facility designs.

7.2 Bioreactor Design for Cell Processing

Bioreactors are a core element to produce high-value materials in biological processes using mammary cells which can be employed for many purposes on various scales of operation in pharmaceutical production, cell therapies and tissue engineering (3). These range from simple, small-scale systems for basic research to sophisticated production-scale systems, which are in use for commercial manufacture. The development of industrial-scale bioreactors was initiated in the mid-1950s to meet the demand for mass production of vaccines. The cell culture bioreactors employed stirred tanks containing micro-carriers with adherent cells, which were, in principle, an adaptation of homogeneous culture systems used for microbial culture to meet the requirements of mechanically sensitive animal cells. The fundamental idea was to overcome the major limitations of cell cultivations that caused slow cell growth and low attainable cell densities by providing an environment that allowed the cells to continuously produce the products of interest at high levels.

In recent years, a new trend has emerged, that of tissue engineering. In contrast to traditional approaches of bioreactor design for mass production, the manufacturing features inherent for cell-based health care products leads to the requirement for small-scale design of patient-oriented bioreactors for clinical use. The automation platform becomes the core technology to realize the 3S (safety, security and cost-saving of manufacturing). Especially, the installation of a processing system for cell and tissue cultures leads

to: 1) process automation of machinery operations, 2) maintenance of the closed aseptic environment to reduce contamination risks, 3) mimicry of the biological environment with chemical and mechanical stimuli, and 4) information utilization of culture monitoring. These functional progresses provide some solutions to the features inherent to cell and tissue processing, being contributable not only to the process control including the saving of labor and process stability, but also to the quality control including the evaluation of cell and tissue potentials.

In our previous study, the automation for expansion process including the operations of seeding, medium change, passage as well as observation were developed (4, 5), and proposed the intelligent culture system accompanied by automated operations (liquid transfer and cell passage) to perform serial cultures of human skeletal muscle myoblasts, as shown in Figure 7.1(6). An automated culture system that could manage two serial cultures by monitoring the confluence degree was constructed. The automated operation with the intelligent determination of the time for passage was successfully performed without serious loss of growth activity, compared with manual operation using conventional flasks. This intelligent culture system can be applied to cultures of other adherent cells and will lead to the qualitative stability of products in the practical manufacturing of cells available for transplantation.

Recently, this technique applied to the development of chip culture system for maturation of retina pigment epithelial cells derived from human iPS cells (Figure 7.2). The chip bioreactor system for long-term culture of human retina pigment epithelial (RPE) cells consists of incubation unit and medium supplier unit. In the incubation unit, the chip as closed vessel (2.5 mm in diameter, working volume 25 ml) was set to be 37°C and 5% CO_2, where gas permeable resin (PDMS) was used for the vessel wall. Whole bottom surface of chip was observed through the culture to detect the immature

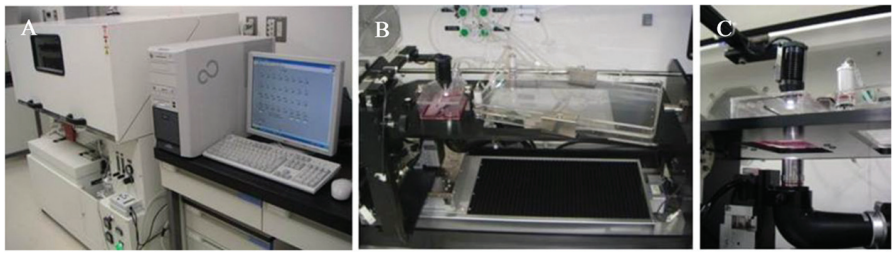

Figure 7.1 Intelligent bioreactor system for passage automation. A; overview of the system, B; culture vessel, and C; monitoring system.

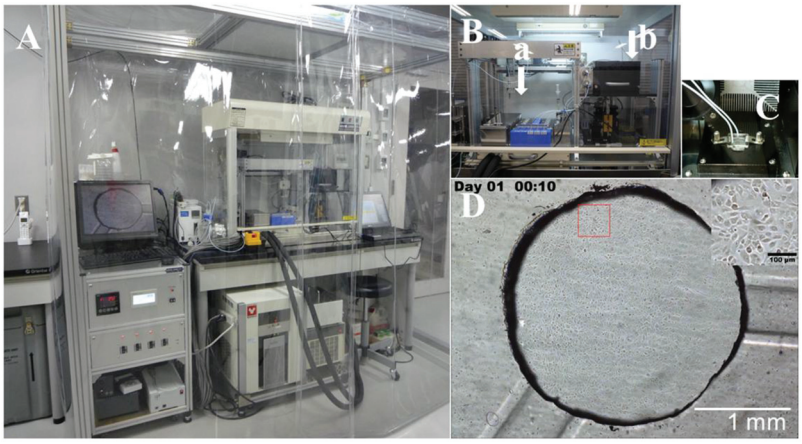

Figure 7.2 Chip bioreactor system for maturation of retina pigment epithelial cells. A; overview of the system, B; medium supplement (arrow a) unit and incubation unit (arrow b), C; chip culture vessel, and D; culture image in chip.

RPE cells. The medium was changed with fresh one every day by introducing from the medium supplier unit by syringe pump. Here, the storage solutions were stocked in the refrigerator and freezer parts, and the fresh medium was prepared on time for the medium change by warming up to 37°C and mixing. The seeding were conducted at 5.0×10^4 cells/cm^2 to be confluent state at initial, and long-term culture for 36 days is initiated for the maturing of RPE cells. The culture system will contribute to the process control as well as the quality control by detecting morphology of whole cells in their chip.

7.3 Facility Design for Cell Processing

Efforts to commercialize cell-based therapies are driving the need for capable, scalable, manufacturing technologies (7–10). It should be certificated that these therapies meet regulatory requirements and are economically valuable at the industrial scale production. In a commercial aspect, a major challenge is to translate lab-scale designs into production-scale designs of biologically functioned products that are reproducible, safe and clinically effective, as well as being economically acceptable and competitive, so that the engineering knowledge for the strategies of cell and tissue processing can be realized on the production scale (11).

On the basis of the guidelines for aseptic processing for healthcare products, the siting criterion of the processing systems for cell and tissue cultures is discussed in perspective of manufacturing therapeutic products (12, 13). The practical managements of processing systems for cell and tissue cultures have referred to the guidance for aseptic processing of healthcare products. The guidance describe that aseptic processes are designed to minimize exposure of sterile articles to the potential contamination hazards of the manufacturing operation. Limiting the duration of exposure of sterile product elements, providing the highest possible environmental control, optimizing process flow, and designing equipment to prevent entrainment of lower quality air into the clean room are essential to achieving aseptic process for product sterility. In addition, the International Organization for Standardization (ISO) guideline for the aseptic processing of healthcare products (Part 1: General requirements, ISO 13408-1) states the set-up of an aseptic processing area (APA). To site the highly controlled area, the influx prevention of chemical and biological contamination sources is one of the critical issues. The critical processing zone in the APA is defined to be an ISO Class 5 clean area using the biological safety cabinet (or laminar flow hood) where the sterilized drug products, containers, and closures are exposed to environmental conditions that must be designed to maintain product sterility. Moreover, the critical processing zone must be set in the direct support zone (ISO Class 7 clean area) where the indirect support zone (ISO Class 8 clean area) is surrounded.

In terms of autologous cell processing for therapeutic application, the fundamental criterion of the APA has been applied to the siting of the cell processing facility (CPF) for therapeutic purpose. In this respect, the bioreactor system should be installed in the direct support zone (ISO Class 7) of CPF because some culture operations including the cell seeding and harvesting are conducted in the critical processing zone of a laminar flow hood. The costs for operation and facility maintenance for the processing in CPF are known to be very high. Therefore, it is difficult to turn a profit for the small scale production with the autologous cell processing. For the cost saving, the aseptic processing has required a promising device: 1) to minimize the space where the closed and regulated environments are maintained rather than minimizing the critical processing zone within the aseptic processing area, 2) to minimize operator's entrance to reduce contamination risk, and 3) to minimize cross-contaminations leading to catastrophic events such as the expansion of serious contaminations which is transmitted from one batch operation to another by the aerosolized route.

In the pharmaceutical manufacturing of healthcare products, the minimization of space, operator's entrance and cross-contamination raise the development of the isolator as useful alternatives to full-scale clean rooms, being described as: "A device creating a small, enclosed, controlled or clean-classified environment in which a process or activity can be placed with a high degree of assurance that effective segregation will be maintained between the closed environment, its surroundings and any personnel involved with the process or manipulation" (14). According to the ISO guideline for the aseptic processing of healthcare products (Part 6: Isolator systems, ISO 13408-6), the isolator is placed in a clean room in which the environment is controlled to give the same conditions as an ISO Class 8 clean area equivalent to an indirect supporting zone in the aseptic processing of healthcare products. An economic analysis using the parameter of lifecycle cost indicated that the total cost per lot in the infrastructures for aseptic cell processing was based on: (i) the critical processing zone with manual operations, (ii) the isolator with manual operations, and (iii) the isolator with automated operations using the robot arm. Aseptic cell processing based on the isolator system with manual operations could reduce the lifecycle cost by 43%, compared with that based on the critical processing zone (15). The installation of a robotic system to realize automated processing in the isolator was suggested to achieve a 38% reduction in cost in the production scale, although the expenses related to facility costs increased by 2% compared with that based on the critical processing zone. Even though a further estimation will be required for practical management of the aseptic processing of cells and tissues for therapeutic use, these estimations are considered to promote the broad utility of the isolator for the aseptic cell processing for not only healthcare products but also for therapeutic cells and tissues.

The installation of isolator technology applied to the cell and tissue processing for therapeutic application would be a similar layout to that for aseptic processing of healthcare products as mentioned above. The critical issue of the isolator is to equip the pass box with a decontamination apparatus, so that the aseptic environment can be prepared by exposing it to decontamination reagents such as vaporized hydrogen peroxide using the decontamination apparatus, enabling materials such as culture vessels and containers for cells and medium to pass through the border from the ISO Class 8 clean area (equivalent to indirect supporting zone) to the ISO Class 5 clean area (equivalent to critical processing zone) without any additional buffer spaces (equivalent to the ISO Class 7 clean area of direct support zones), enabling the saving of costs for the operation and maintenance as well as space in the manufacturing of cell and tissues. As the siting criteria depend on the

respective processing, further discussion on the availability of these proposed manufacturing systems with the decontamination apparatus will be required in terms of the GMP regulatory issue, accompanied by validation data for the aseptic environment and biohazard level. The expectation shos that culture systems equipped with a decontamination apparatus takes countermeasures against biohazards and can be met for the autologous cell and tissue cultures because of specific requirements including: (i) the small-scale production for each patient, (ii) the utilization of non-sterilable materials derived from patient's cells, and (iii) independent biohazard spaces for healthy and virus-carrying (infected) patients. These specific requirements provide reasonable basis for the installation of manufacturing systems equipped with a decontamination apparatus for autologous cell processing. On the basis of the siting criterion mentioned above, the comparison of management between CPF and cell aseptic processing system (CAPS) employing isolator technology revealed that CAPS leads to reductions of the running cost as well as operational laboriousness in the small production.

7.4 Flexible Modular Platform Technology

Recently, as shown in Figure 7.3, a novel design of manufacturing facility based on a flexible Modular Platform (fMP) has been proposed to realize that the individual aseptic modules can connect and disconnect flexibly with keeping the aseptic environment in each module, leading to the compactness of aseptic processing area and quick change-over for multi-purposes and patients. This technology will make cost-saving with safety and security. To effectively implement this fMP technology an interface that can be aseptically detached and attached from one device to another is required and such a device must be able to handle diverse aseptic processing requirements. A common approach utilized in isolator based manufacturing of sterile pharmaceuticals is a transfer system using rapid transfer ports (RTP) or Double Porte de Transfert Etanche (DPTE). However, its interface limited to a circular configuration, and a more versatile aseptic transfer mechanism is sought for handling the connection between devices.

As shown in Figure 7.4, in the collaboration with Profs. Okano and Shimizu in Tokyo Women's Medical University and several companies in Japan, the isolator module system based on fMP for the cell sheet assembly has been developed, which will make automated formation of multilayered sheets and their incubation. The machinery operations were successfully performed. And this system can realize some procedures by having flexible connections

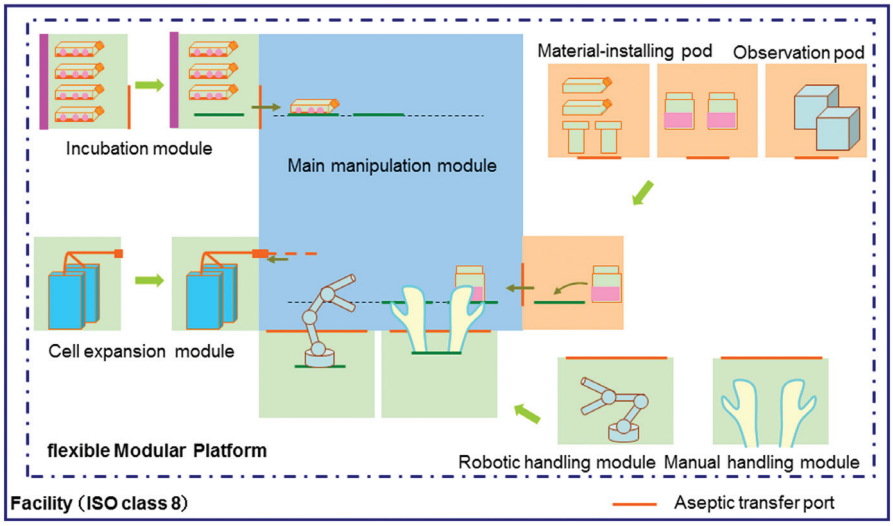

Figure 7.3 Proposal of manufacturing system based on flexible Modular Platform (fMP)

Figure 7.4 Automation system of sheet assembly based on the fmp technology

between modules under aseptic conditions by developing the interface of double door system for modules, suggesting the broad versatility for the production in other types of multilayered cell sheets.

7.5 Acknowledgments

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