Development of automatic cell culture system for cell therapy and regenerative medicine

— Robotized system for high quality cell product preparation —

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We carried out R&D in order to dramatically facilitate cell culture for clinical use, the difficulty of which had been a major hurdle in adapting basic research to clinical applications of cell therapy and regenerative medicine. The world's first robotized cell culture system (MDX) was developed by Kawasaki Heavy Industries, Ltd., and the systems were installed in Shinshu University and AIST. Based on the technologies of the MDX system, we developed a novel cell culture system R-CPX (Robotized-Cell Processing eXpert system) which can produce high quality medical cell products. This system does not need to be placed in a CPC (cell processing center), which is expensive to construct and difficult to manage. We aimed to realize rapid progress of various cell therapies and production of medical cell products of global standard quality.

Keywords : Tissue engineering, cell engineering, auto culture system

1 Introduction

1.1 Why is it necessary to develop an automatic cell culture device now?

As low birth rate and aging progress, we have reached the limit of symptomatic therapy using alternative materials such as artifacts and drug administration that were done conventionally against adult-onset diseases such as cancer, diabetes, and dementia, and the development of new medical technology is expected. To fulfill this expectation, it is necessary to apply the diverse elemental technologies and research results of the fast-evolving medical fields to drug discovery, as well as the development of the analysis tools, diagnostic technology and medical equipment that support such discovery. It is important for the private companies and clinical research institutes to unite and engage in R&D to achieve quick and practical application.

In place of conventional medicine, regenerative medicine and genetic/cell therapy are expected to be the next-generation medicine. They are medical fields whose objective is to utilize the cell functions such as growth and differentiation. The patient or donor cells are harvested, grown or differentiated *in vitro* or maneuvered by gene transfer, and then transplanted to the diseased area of a patient to treat a certain disease. This field is currently expanding rapidly and many good results are being accumulated. In Japan, skin grafting using autologous cultured epidermis tissues has been put into practical use (has been commercialized).

Although the operation of cells harvested from the body is a technological development at the basic research level where the growth and differentiation of the cells are controlled, translational research (TR) from basic research is necessary for clinical application and commercialization.[1] The cultured tissues obtained from (autologous) cells harvested from the patient or (homologous) cells harvested from the donor must be handled as products, and it is necessary to guarantee their safety, and to present the efficacy of these products to the physicians and patients who are the customers. In the process of cell culture, the succession of cells in the processes of harvesting and growing, and the cell processing such as induction of differentiation are necessary. Presently, such complex cell culture operations are done by skilled technicians at the cell processing center (CPC) that are specifically set up in the hospital or company (Fig. 1A).

For the cell culture operation at CPC, the maintenance of a sterile environment is necessary since the cells and tissues cannot be sterilized. Also in the cell culture process, cross-contamination (contamination by mixing with cells of other people) and human error are not tolerated. From such a

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perspective, the therapy follows the Medical Practitioners Act in Japan, while CPC and cells and tissues used for therapy must satisfy the Good Manufacturing Practice (GMP) that is the rule for quality control, process control, and facility for the manufacture of drugs. The three principles of GMP that must be satisfied by the CPC are: ① prevention of contamination and deterioration of quality, ② minimization of human error, and ③ system to guarantee high quality.[1][2] Therefore, (1) aseptic control, biohazard countermeasure, and cross-contamination prevention, (2) mix-up prevention, and (3) documentation and recording of the operation status are essential. Currently, many CPCs are in operation in Japan, and R&D for regenerative medicine is being done, but there are limits to the medical system that uses the conventional CPC due to the maintenance cost necessary to satisfy the aforementioned high level requirements, as well as the demand to respond to various new medical technologies. Therefore, the development of improved systems and devices that are in compliance with GMP are awaited, keeping in mind the commercialization of regenerative medicine and cell therapy. For items ② and ③ of the three principles, an automatic culture system that replaces the manual operation is important, and for ①, the development is under way for an isolator system where the sterile environment of CPC that is expensive to maintain can be achieved in a small device and the operation can be done through the glove box (Fig. 1A). The culture by manual operation using the isolator is expected to become more prevalent in the future.

In this R&D, to advance the automatic culture system, the culture system is incorporated inside the isolator, with the objective of developing a robotized cell processing expert system (R-CPX) that can be used for various usages and does not require a CPC. With the development of this system, the next generation R-CPX that does not require a CPC is created to fulfill the demand of the small to medium businesses. The specialized device can be provided at low cost to satisfy the demand of medium to large companies in the diffusion phase of regenerative medicine. This is expected to help spread low-cost and safe regenerative medicine.

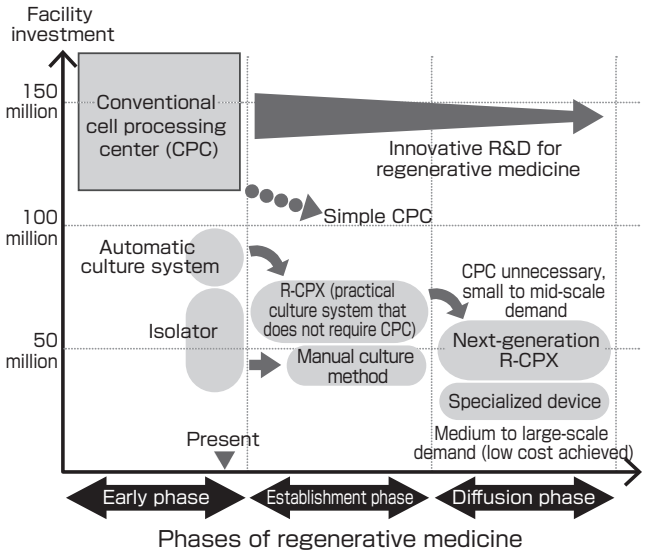


Fig. 1A Roadmap of the cell preparation system

1.2 Objective, execution, and outline of the R&D

In this R&D, the goal was to dramatically simplify the preparation of clinical cells that is one of the major barriers in the translational research from basic research to clinical application of regenerative medicine and cell therapy technologies. Figure 1B shows the technological items and their configuration of this project. Kawasaki Heavy Industries, Ltd. (hereinafter will be called Kawasaki), Shinshu University (SU), and the National Institute of Advanced Industrial Science and Technology (AIST) have already created the Medical Device Project X (MDX, Figs. 2 and 3), a cell culture robot system, for the first time in the world. The system installed at SU was called Evaluation Unit 1 and the system at AIST was called Evaluation Unit 2. SU has abundant experience and know-how in cell culture technology for regenerative medicine in CPC, while AIST has the knowledge of cell culture used for drug discovery and development. Kawasaki's cell culture robot was designed under the concept of replacing the movement of a skilled technician conducting manual cell culture with the movement of a robot (mainly a robot arm). The culture techniques of SU and AIST were incorporated in Evaluation Units 1 and 2, and tests were done for verification. The research was conducted with the goal of developing the culture system R-CPX based on the results obtained.

Regenerative medicine and cell therapy are highly novel therapies for which aggressive R&D is being done presently, and the quality standard and required functions are changing

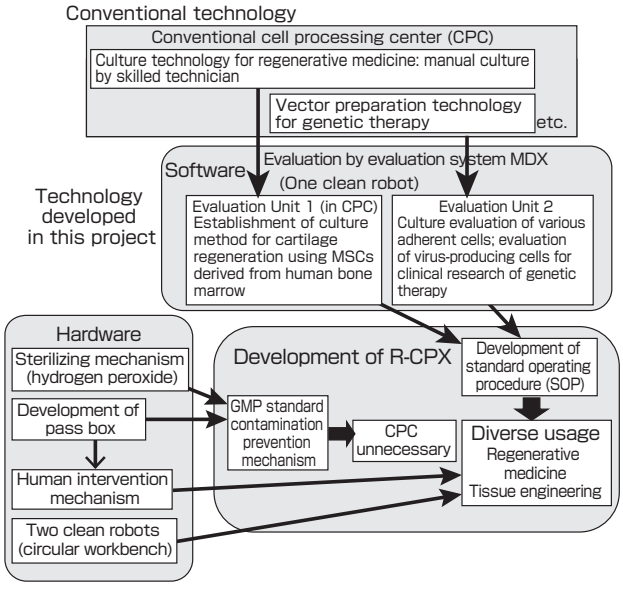


Fig. 1B Technological items in this project and the configuration diagram

daily. Also, since the method can be applied to wide-ranging diseases, the cells and tissues used as raw materials as well as the final products (cells) that are being prepared are varied. Therefore, it is insufficient to merely transplant to a robot system the cell culture technique that was already established for manual operation in one of the protocols. Therefore, development was done by selecting two different, specific projects that contained representative procedures of diverse operations, and concentrated on the improvement of these procedures. The regenerative medicine and cell therapy projects selected were "Establishment of culture method for cartilage regeneration using human mesenchymal stem cells from bone marrow" (Evaluation Unit 1) and "Evaluation of virus-producing cells for clinical genetic therapy" (Evaluation Unit 2). Since the researchers of these projects were highly experienced, the standard operating procedures (SOP) were established for the manual operation in the existing CPC for the cell reagents used in clinical trials. The actual reagents were prepared according to the SOP and the quality of the product was verified. Concurrently with these researches, the following main technical items were incorporated, to construct the R-CPX hardware that smoothly operates independently without using the CPC:

(i) Sterilizing mechanism for the interior of the R-CPX by hydrogen peroxide,
(ii) Incorporation of a human intervention mechanism by developing the pass box, without robotizing the entire process to enable diverse usages, and
(iii) Achievement of efficiency by two clean robots, which could not be achieved with one clean robot.

Particularly for the robotization process, verification tests were conducted using the existing MDX, and the aim was to automate most of the manual operation in the future.

According to the above objectives, the following five research items were set for the R&D:

① Develop the R-CPX that meets the GMP standard
② Establish and evaluate the regenerative medicine using R-CPX
③ Investigate the vector production method for genetic therapy using R-CPX
④ Develop the SOP
⑤ Evaluate the culture by evaluation equipment

Of the above items, ①, ④, and ⑤ that are directly related to the device development will be explained in detail.

2 Development of R-CPX that meets the GMP standard

2.1 Basic concept of the R-CPX design

The contamination prevention function and human intervention mechanism that were needed for achieving R-CPX were developed, and based on these the total configuration of the R-CPX was developed. For the contamination prevention, the ventilation function where the interior could be maintained at positive/negative pressure to be adaptable to P2 was verified using a prototype and its performance was confirmed. For the sterilization function, sterilization by hydrogen peroxide steam was employed after comparative study. Various tests were conducted to check the sterilizing performance, and basic data needed for the R-CPX design was obtained. For the human intervention mechanism, interlock mechanism was designed, as it had to be cooperative with the robot. Also, the fitting mechanism for gloves was designed to prevent decrease of operability when the interior of the device was at negative pressure, and this was verified in the prototype.

From the results of Evaluation Unit 1, and Unit 2 that was developed for drug discovery and installed at AIST, the total configuration of R-CPX was considered, the specifications were determined, and the design diagram was completed. Prototypes were used for items that required verification and confirmation of performance, such as pipette insertion tests, improvement of operability of the culture instruments, and attachment/removal of the pipetter. The configuration was kept simple to ensure the sterilization performance by hydrogen peroxide steam and to improve the ease of maintenance. The environmental recognition technology by

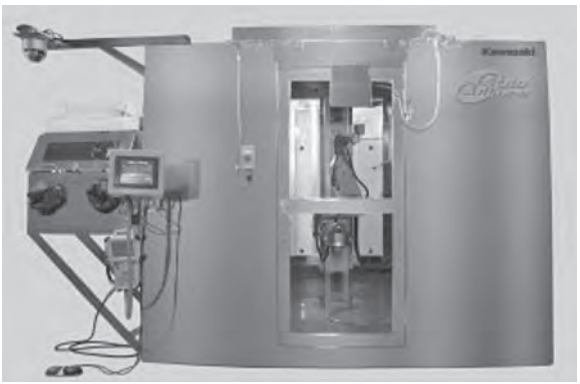


Fig. 2 MDX Evaluation Unit 1 (installed in Shinshu University) (for regenerative medicine)

Fig. 3 MDX Evaluation Unit 2 (installed in AIST) (for drug discovery)

image processing was also investigated.

The detailed design of R-CPX was done, and the prototype was completed after reviewing the generation mechanism for hydrogen peroxide steam. The sterilization of the interior and pass box by hydrogen peroxide, and the culture operation using two clean robots that were not present in Evaluation Units 1 and 2 were created (Fig. 4), and a performance test was conducted for confirmation. For Unit 1, the issues in the hardware that arose during the culture tests at SU were improved, and after revising the automatic culture SOP, the culture evaluation was conducted concurrently with manual culture using the same donors and the same period of time.

2.2 Development of the contamination prevention method that meets the GMP standard

For contamination prevention, the following four functions were required:

(a) Interior of the device is not contaminated from the exterior
(b) Interior of the device is not contaminated when loading/unloading from the exterior
(c) Cross-contamination does not occur when handling different samples within the device
(d) Exterior is not contaminated from the interior of the device, in handling the cultures for genetic therapy

To realize these four functions, the R-CPX was equipped with a ventilation function where the interior could be maintained at either negative or positive pressure, as well as the sterilization function of the device including the pass box. For the sterilization function, all the areas with the possibility of contamination must be sterilized. In the R-CPX, hydrogen peroxide steam sterilization was employed since there was little damage to the interior equipment and the sterilization time was relatively short. The hydrogen peroxide steam sterilization is employed for the sterilization of the isolator used in pharmaceutical and medical research, and sufficient sterilization performance could be expected if appropriate conditions were set.

In the hydrogen peroxide steam sterilization, the surface that comes into contact with the hydrogen peroxide steam is sterilized. Therefore, the issue was whether the steam reached the target surface sufficiently. Since there was a concern that the steam may not sufficiently reach the interior of complex structures or narrow spaces, the structure was kept simple. Since the presence of gaps and screws could not be avoided, sterility of those parts was checked. After the sterilization process, the degree of sterility was tested using the bio-indicator, and the results indicated high sterilization capacity.

One of the characteristics of R-CPX that was not present in the previous devices (Evaluation Units 1 and 2) is the human intervention mechanism, and this was accomplished using the glove box (Fig. 5). The gloves replaced part of the culture operation done by the robot. Therefore, among the motion range of the robot engaging in culture operation, the locations where human intervention by use of gloves occurred were allotted to the areas that could be reached by the robot, and the areas that could be accessed by the person facing the front side of the device. The material for the gloves was limited and the operability was poor because they were exposed to hydrogen peroxide steam and there would be pressure difference (approximately 150 Pa to −50 Pa) with the external environment. Particularly, when the interior of the device was kept at negative pressure, the gloves expanded and would not fit the operator's hands. Therefore, we devised a way to have the gloves fit closely to the operator's hands and thereby improving the operability. The configuration is as seen in Fig. 6. The airtight ring was placed at the upper arm area of the operator, air was pumped into the ring for sealing, and then air was drawn from the space beyond the airtight ring to fit the gloves closely to the operator's hands.

2.3 Basic configuration of R-CPX

The automation mechanism was considered by reducing the number of places that may be contaminated, and by simplifying and making the mechanism as close to hand culture as possible, in addition to the contamination

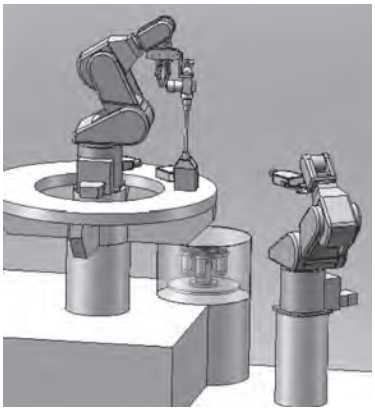


Fig. 4 Arrangement of the robot and rotating workbench

Fig. 5 Gloves that can be treated by hydrogen peroxide steam sterilization

prevention and human intervention mechanism that were considered above. The operability of Evaluation Unit 1 installed at SU and Unit 2 at AIST were compared, and the following basic configurations were determined.

(a) The use of automated machine with complicated structure was minimized. The main bodies were two clean robots, where one mainly engaged in transportation (a transport robot) and the other mainly engaged in culture operation (an operation robot).

(b) The one-touch cap was used so the capper would not be used to open/close the screw cap.

(c) For the pipetter, the method of joining the syringe pump and tube was not employed, and an independent pipette similar to the one used by an operator was used. The pipette was exchanged for each operation, and it would be removed and sterilized after each series of culture operation.

(d) Since the multiple use of sensors made the structure complex, the processed image from the TV camera was used as the sensor for identification in areas that could be replaced by remote visual inspection.

(e) Hip flask that is often used in hand culture operation was used as the culture container.

(f) The doors of the incubator and refrigerator were opened/closed by a robot rather than by cylinder.

(g) The workbench for the culture operation was arranged in a circle with the robot in the center, and the rotation of the workbench was controlled. The movement of the rotary workbench reduced the range of culture operation to be done by the pipette attached to the robot.

(h) The centrifuge tubes were used as common containers for the medium or reagent, and the empty containers were used as liquid waste containers.

(i) The hand mechanisms were installed on the robot, and the robot operated the pipette attached to it and also held the container.

(j) Storages other than the incubator (such as room-temperature and refrigerated storages) were concave without much depth, considering the ease of sterilization.

The R-CPX system was constructed based on these basic configurations. The overall diagram and the main components of the completed device are shown in Figs. 7 and 8.

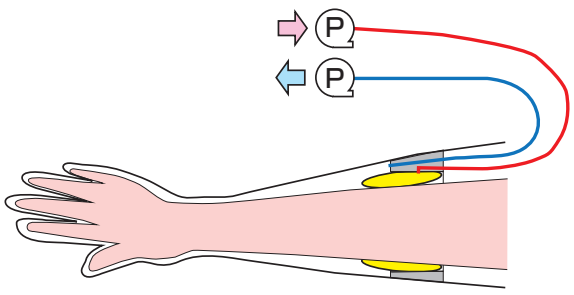


Fig. 6 Glove fitting mechanism

3 Development of standard operation procedure (SOP)

In the case of culture by manual operation, SOP serves as a standard manual, and if an operator with a certain degree of skill reads the manual and does the operation, he/she should be able to obtain the same results. When this is replaced by the automatic culture, it is composed of four parts.

(a) Operations that are needed by both manual and automatic culture operation: Preparation of culture, seeding on collagen, etc.

(b) Operations not needed in manual but necessary in automatic culture: Packing of disposable items, etc.

(c) Operations of the automatic culture device: Loading of disposable items, unloading of test samples, etc.

(d) Motion of the automatic culture device

The most important item here is (d). The motion of the automatic culture device will not be entirely the same as the movement of a human operator. For example, in changing the media immediately after the primary culture, the blood components from the bone marrow will remain, and the dish must be shaken before the medium change to allow the blood components to rise into the supernatant as much as possible before disposal. The operator visibly checks the amount of shaking, but the robot is incapable of doing this. The manner of shaking must be determined beforehand. Therefore, the strength and frequency of the shaking motion were experimentally compared and determined. In Fig. 9 (left),

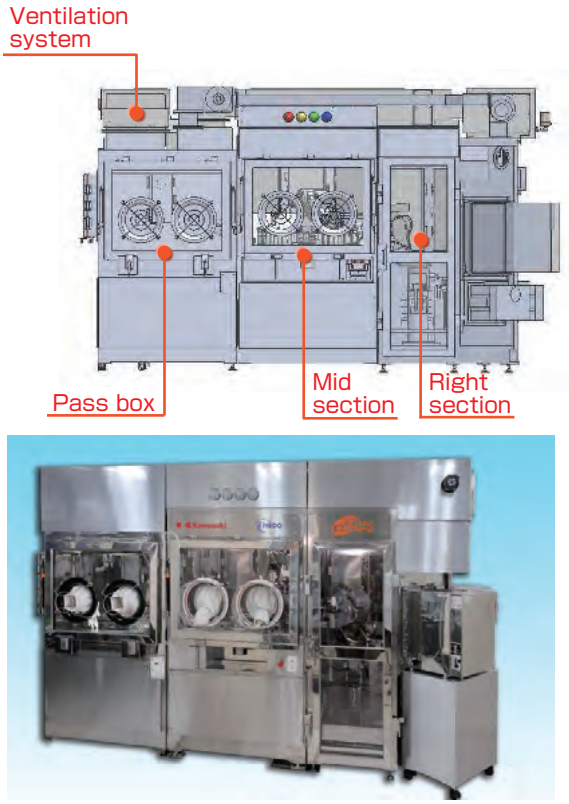


Fig. 7 Overall diagram of the R-CPX (top) and photograph (bottom)

the dish after the medium change at initial shaking condition had a reddish color, indicating that there were residual blood cells. As a result of correcting the shaking condition, the red color could no longer be visibly seen in the dishes after medium change. As shown in Fig. 9 (center, right), the blood components were hardly seen in the microscopic observation.

Based on the above basic concept, the SOP was developed for the automatic culture for cartilage regeneration and automatic culture for genetic therapy.

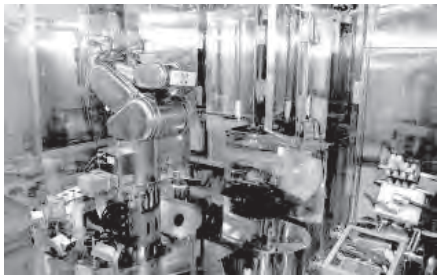
The SOP for culture by manual operation has the following issues: methods are different by different operators even at the same facility; records are not kept accurately; transfer to other operators is difficult; and the quality of the product is not stable. Assuming the development of SOP for automatic culture, the SOP for manual culture was reviewed, and the points that were ambiguous or lacking in scientific evidence were investigated and clarified. Moreover, the thinking for the transfer of the SOP from manual culture to automatic culture was organized. Through such studies, the ways to achieve the SOP for manual and automatic cultures became clear.

4 Culture evaluation by evaluation equipment

The cultured subjects of R-CPX include not only the mesenchymal stem cells (MSCs) for regenerative medicine, but also various cells such as the virus-producing cells for genetic therapy and other clinical research. The evaluation of MSC culture was done in Evaluation Unit 1. Adherent cells other than MSCs were evaluated using Evaluation Unit 2.

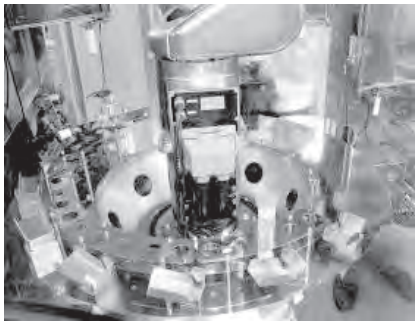
4.1 Outline of Evaluation Unit 1 installed at Shinshu University

Evaluation Unit 1 was installed in the CPC of Shinshu University Hospital for the purpose of culturing MSCs for regenerative medicine in clinical research. For use in clinical



(1) Interior of the mid section and the operation robot

(2) Interior of the right section and the transport robot



(3) Rotating workbench at the center of mid section

(4) Pass box

Fig. 8 Photographs of the main sections of R-CPX

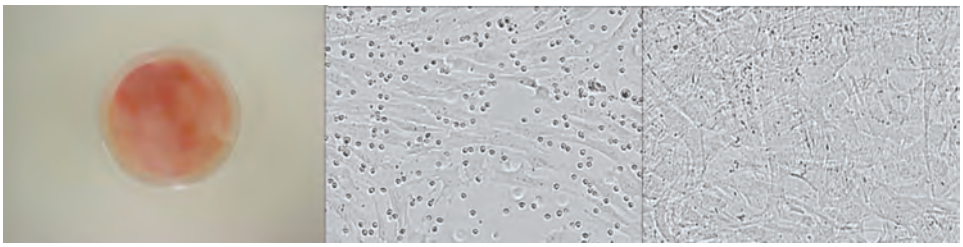


Fig. 9 Dish immediately after the primary culture (reddish color also seen after medium change) (left). Microscope photograph comparison of the effect of improving the shaking method (middle: before improvement, right: after improvement).

research, it was necessary to obtain approval based on the "Guidelines on clinical research using human stem cells" of the Ministry of Health, Labour and Welfare, and we conducted culture evaluation at the level applicable to clinical research.

Unit 1 has the following characteristics:

(a) The culture is started from bone marrow, the amount of MSC necessary for cartilage regeneration is cultured, and then it is obtained as cell suspension.
(b) The device is placed in the managed area with cleanliness level 100,000, and the interior of the device is maintained as sterilized space of cleanliness level 100.
(c) The interior contamination is prevented by using a glove box when loading/unloading the disposable items or cells, and by using sterilized packages.
(d) Primary culture, medium change, successive culture, cell recovery, and cell observation can be done automatically within the device. The main body of culture operation is done by a clean robot. Also, samples for testing can be made.
(e) The culture operation procedure is managed by a computer. Not only can scheduling be done freely, but also all movements are logged.
(f) There is a storage space within the device, and disposable items are stored in the room-temperature storage, reagents in the refrigerator. Complete automatic culture operation is possible.
(g) There is a remote monitoring function that enables monitoring the same information as the terminal in the CPC, and the image inside the device can also be viewed.

4.2 Outline of the cell culture method and evaluation method by Evaluation Unit 1

Concurrently with the dry run (culture in which the cultured cells are not transplanted to the donor) of manual culture, the culture of the bone marrow cells of the same donor was conducted in the dry run of the automatic culture. The culture medium was the same as the one used in the manual dry run, and was prepared from the donor's autologous serum. For evaluation, the sterility test and quality tests were done for endotoxin and mycoplasma during succession and cell recovery. Cell count was conducted and surface antigens were analyzed, to calculate the percentage of cells considered to be MSCs within the obtained cells. The standards for cell reagents, converted to 9 ml of bone marrow from the donor, were a total number of cultured cells of 10^7 or more within 3 weeks of culture period, and cells with 90 % or higher MSC marker positive recovered at 80 % or higher purity.

Through the five dry runs of automatic culture, we were able to achieve culture that reached the qualifying level. The marrow supernatant during the primary culture was sterility test negative (2 week culture) and endotoxin of less than 0.1 EU/ml. In the quality test for successive and cell recovery by automatic culture, sterility test negative (2 week culture), endotoxin of less than 0.1 EU/ml, and mycoplasma negative were confirmed, and pathogenic contamination was not observed in the culture process. After the improvements in the automatic culture SOP and the device, the product quality standard was demonstrated in the fifth dry run, and an MSC reagent with high quality in both purity and specificity was created. Since the quality guaranteed MSC was created, the adequacy of the automatic culture SOP was verified.

4.3 Outline of Evaluation Unit 2 installed at AIST

The Evaluation Unit 2 is an automatic cell culture device that was created for the purpose of usage in non-clinical research such as drug discovery, and has the following characteristics:

(a) Direct automation of manual culture operation: The series of procedures that were done by hand, from changing the culture medium, succession, cell recovery, and cell observation are automated.
(b) Capacity to handle various cells: The general culture operations are programmed, and the user can set various parameters such as the peeling time during succession, and quantity and discharge speed of the reagents.
(c) Culture support by image identification: It is equipped with image processing unit that enables observation of the cells inside the device and automatic recording. The cell occupancy rate can be displayed and automatically recorded.
(d) Culture scheduling function: Each culture operation can be freely scheduled.
(e) Stability and uniformity of cell quality: Stability and uniformity of performance and culture quality can be achieved through automatic culture operation.
(f) Contamination prevention: The cleanliness level inside the device is 100. Since the operations are done by a clean robot, the contamination of the cultured cells can be prevented. It is equipped with a decontamination function by automatic alcohol spray to prevent cross-contamination.
(g) Compact size: Approximate dimensions of the device are width of 3 m, depth of 1 m, and height of 2 m.

4.4 Outline of the cell culture method and evaluation method by Evaluation Unit 2

The subjects of culture by R-CPX are not only the MSCs for regenerative medicine, but also include virus-producing cells for genetic therapy and various cells used for various studies of clinical research. In Evaluation Unit 2, the target was cells other than MSCs, and the evaluations were done for a wide range of cell types as possible. The evaluation was done for the adherent cells. For virus-producing cells for genetic therapy, the cytokine-producing cells that excrete protein components into the culture supernatant, much like the virus, was used as the evaluation cells.

4.4.1 Culture evaluation of a wide range of adherent cells

Although the main objective of this system is the automatic culture of adherent cells, there are wide variations in the adhesiveness of the cell types. There were 13 types of cell lines such as HeLa and NIH3T3. Good results were obtained for the culture parameters by preliminary determining the initial cell count, frequency of succession, and dilution rate where stable hand culture was possible and by fine-tuning using Unit 2 based on the results. In the cells with weak adhesiveness such as 293 gp/mIL2, the discharge speed of the PBS and culture medium (from the pipette) was studied. Figure 10 shows the situations of cell peeling at discharge rate 3, 1, 0.3, and 0.1 ml/sec. The optimal discharge speed was 0.3~1 ml/sec. In the case of cells that were hard to peel such as PC-12, the peeling time was increased compared to the initial setting, and the number of tappings was increased to improve the peeling rate. By setting the culture parameter with consideration for the cell characteristics enabled the automatic culture of a wide range of cells.

4.4.2 Culture evaluation of the virus-producing cell assuming clinical research for genetic therapy

Since the retrovirus used in genetic therapy requires the P2 level dispersal prevention device, it is necessary to seal the device and to enclose the virus inside by maintaining negative pressure against the exterior of the device. However, in the evaluation stage, the investigation using the cell line that released secreted protein was sufficient because the operation procedures were the same as the recombinant retrovirus, and the evaluation was done for whether the virus supernatant could be actually recovered. The cell line used was NIH3T3/mIL2. This is a cell made to release mIL2 into the culture supernatant through genetic transfer. To evaluate the recovery performance of automatic culture, the culture supernatant was recovered at 34 h, 58 h, and 82 h after cell seeding, and the results were compared to the manual culture. The mIL2 concentration in the supernatant was quantified, and we were able to obtain the supernatants with the same concentration for automatic and manual cultures. From the results of the conducted evaluation tests, it was found that the automatic culture device could perform stable cell culture almost at the same level as manual culture, and the cells that required specific conditions could be cultured by setting fine-tuned culture parameters.

5 From manual culture to automatic culture

This system was developed on the concept that the operation conducted smoothly by manual operation of a skilled technician could be replaced by the movements of a robot arm. A skilled technician conducts each maneuver based on experience, under optimal condition for the cell while observing through the culture microscope. There is a SOP and the work of the machine is programmed accordingly. However, it is difficult to incorporate the unconscious operation nurtured through experience to the machine unconditionally. The cells that readily peal are injected from the pipette to the culture medium softly. The cells that do not peel readily are injected vigorously onto the medium, and sometimes tapping is done. Even for the same cell, the adhesiveness and reproducibility differ slightly according to slight conditional differences. To conduct one operation completely (peeling the cell or changing the medium without cells peeling off) regardless of the differences, it is necessary to employ conditions that do not cause problems, yet be stricter than the optimal conditions of the SOP. This is the difficulty of determining the SOP. A novice is instructed to not leave the door of the CO_2 incubator open for a long time. A skilled technician does that unconsciously. If the SOP is made disregarding the unconscious, the door may be left open too long, the CO_2 concentration inside the incubator changes and this affects the culture. First, we were unaware of this, and got worse results than manual culture, but this was improved by adjusting the timing of open/close of the door. How to reflect the unconscious operation that is not written in the operation protocols done by humans in the SOP was one of the challenges of automation.

6 Summary

We developed a practical culture system R-CPX (robotized cell processing expert system) where high quality cell reagents could be prepared without a CPC. The characteristic of the R-CPX is the flexible structure that can handle various usages, including the GMP-standard contamination prevention mechanism and operation by two clean robots.

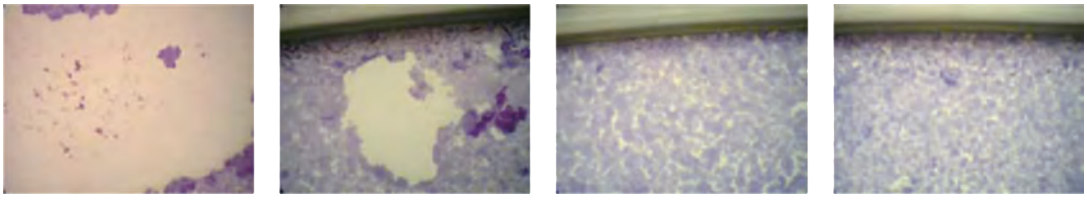


Fig. 10 Relationship between the discharge speed and cell peeling (293 gp/mIL2 cell)

7 Desired research stance and issues for the future

Through this research, we aimed for the swift achievement of diverse regenerative medicine and cell therapy, as well as the development of a device. The diseases that were the subjects of the study were the regenerative medicine of joint cartilages, for which one of the authors has abundant research experience and over 50 cases of clinical research, and the regenerative medicine of periodontal tissues including jaw bone regeneration using the MSCs, for which there are about 40 cases in Japan. If the R-CPX system can be applied to such diseases, the exploratory clinical tests for regenerative medicine and cell therapy for "refractory diseases derived from adult-onset diseases" involving cranial nerves, heart muscles, or marrow that use the same cell source, and the execution of clinical trials for the following commercialization can be facilitated dramatically.

This R&D was conducted to realize the vision of "R-CPX System Development Center" as shown in Fig. 11, to break through the adverse effect of individual research institutions conducting research separately for individual diseases.

Through the advancement of this R&D, the network with leading researchers of Japan was built. Also, collaboration with industry is essential for commercialization and practical use. In realizing regenerative medicine and cell therapy, the role of the cell preparation companies is large, and the participation of the major companies with ample resource of specialists in various fields and abundant financing is necessary. On this point, collaborations are being built along with the advancement of this R&D.

Also, it is clear that commercialization cannot be done if the cell preparation companies depended on manual culture for preparing the cells, and many companies are awaiting the development of the automatic culture device. Therefore, it is necessary to form a consortium of companies that possess various technologies that comprise the device.

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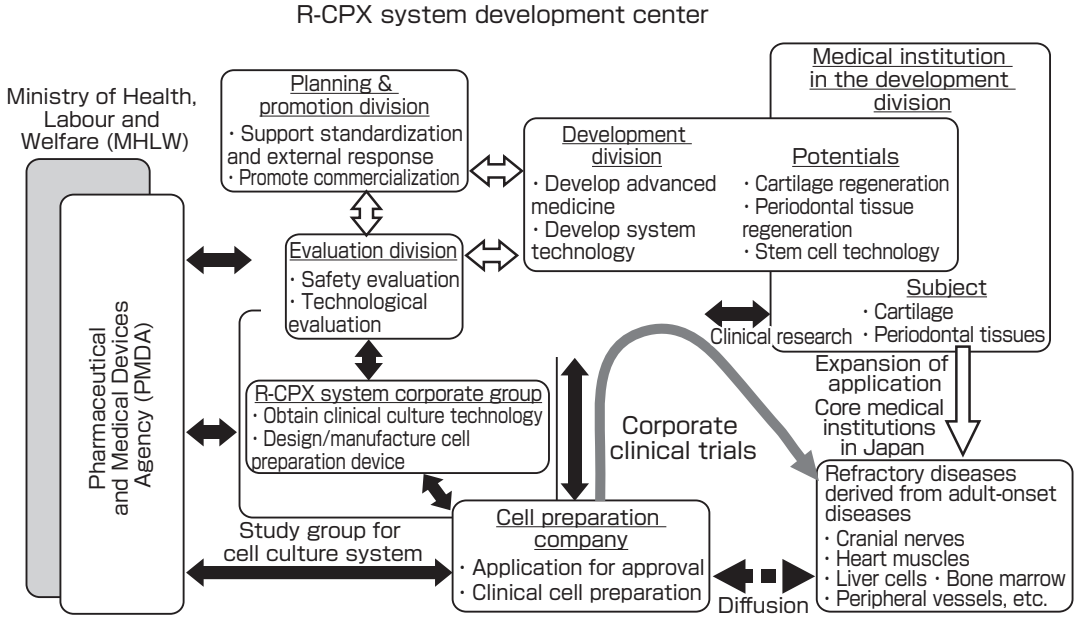

Fig. 11 Scheme for achieving regenerative medicine and tissue engineering by innovative cell preparation system

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Discussions with Reviewers

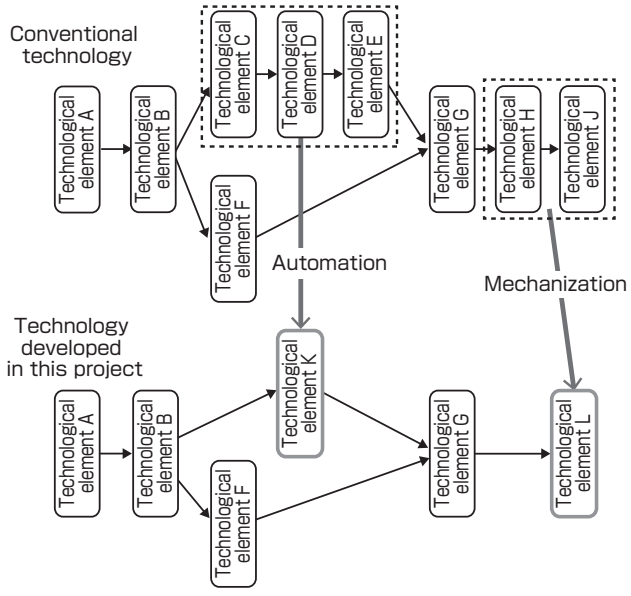
1 Motivation for development, research objective, technical elements, etc.

Question and comment (Tai Kubo, Molecular Profiling Research Center for Drug Discovery, AIST)

Please rewrite to emphasize the necessity of the automatic culture device and your motivation for its development, such as how CPC requires high level of safety, hygiene, and quality control, how expensive it is to build and maintain such a facility, as well as the requirement for solid skills and the need to satisfy the demand for "quantity" that will be necessary for the clinical application in the future. Also, as the descriptions of the experiment and results for Evaluation Unit 2 are too detailed, please narrow down the content appropriately.

Question and comment (Toshimi Shimizu, AIST)

The research objective of this project is to develop the R-CPX culture system that allows the preparation of high quality cell reagents without setting up a specialized CPC. To gain understanding of the readers who are not experts in the field, I recommend you draw a configuration diagram that allows immediate grasp of what technical items and components (procedures) there are, to what extent they are completed, how technological elements were modified to enable the automation, mechanization, and human intervention. Moreover, the large framework and detailed technologies are mixed throughout the paper, and this makes it difficult reading. Particularly, I recommend you keep to a minimum the descriptions of cell culture and evaluation methods by Unit 1, and the evaluation of adherent cell culture by Unit 2.



Answer (Toshimasa Uemura)

Since this project was an integration of the technological developments from many fields, it was difficult to maintain uniformity of the description, and perhaps it had become rather difficult for the readers to understand. First, I greatly revised "1 Introduction," "1.1 Why is it necessary to develop an automatic cell culture device now?" and "1.2 Objective, execution, and outline of the R&D." Also, based on the development roadmap for the cell preparation system, the configuration diagram of the technological items that show the correlation of the conventional and new technologies was added as Fig. 1B.

2 GMP, the rules for facility, procedure control, and quality control for drug manufacture

Question and comment (Tai Kubo)

For GMP, you need an explanation to enable the readers to understand the incredibly high hygiene and quality levels required for the approval of this machine.

Answer (Toshimasa Uemura)

I added the explanation of the GMP required particularly for CPC in chapter 1.

3 Examples of failure and trial-and-error in the development process

Question and comment (Tai Kubo)

It is in the scope of the *Synthesiology* paper for you to describe the failures, trial-and-errors, and requests for improvement you heard from the companies and clinical practices, in the process of the device development. Please include them in the paper, if any.

Answer (Toshimasa Uemura)

I added a story of hardship as chapter 5 "From manual culture to automatic culture."

4 Contribution of the multiple authors and various research institutions

Question and comment (Toshimi Shimizu)

Please introduce briefly in the paper the roles and contributions to the individual technology elements by the listed multiple authors.

Answer (Toshimasa Uemura)

There seems to be quite many authors, but there are five times more people who contributed to this project, and the authors are the central figures amongst them. In this project, Kawasaki Heavy Industries, Ltd. participated in creating the device as part of their corporate mission. For other institutions, the individuals rather than the organizations were important. The roles and missions of the organizations were not so important, but the roles and missions of the people with experiences or technologies that no one else possessed were important. I explained the chapters penned by the authors in their profiles, and I think that will help in understanding which part of the project the authors contributed.

5 Technological contribution by AIST; its role, characteristic, and superiority

Question and comment (Toshimi Shimizu)

Please explain clearly the content and characteristic of the core technology of AIST in this project. Particularly, the Evaluation Units 1 and 2 were installed at Shinshu University and AIST, respectively. Please explain the technological contribution, role, characteristic, and superiority of AIST pertaining to these units.

Answer (Toshimasa Uemura)

I think the technological contribution by AIST can be summed up by its experience in cell culture. The automatic culture system was designed with the concept of a robot, namely a robot arm, conducting the same movements carried out by a skilled technician engaging in manual operation. The necessary human movements were programmed into the Evaluation Unit 2 installed at AIST. The movements could be checked progressively, and I think this led to perfection. Although this did not involve intellectual property, this was an extremely important contribution.