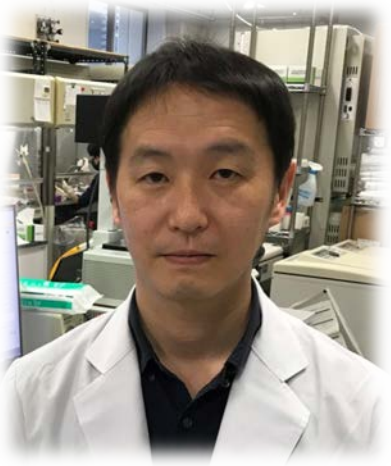
Interview with Dr. Satoshi Okamoto on the production of iPS cell-derived mini-livers

Prof. Taniguchi [Special Contract Professor, Yokohama City University, Professor, Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo] and his research group have induced differentiation of three types of cells (hepatocytes, vascular endothelial cells, and mesenchymal cells) from human iPS cells, and have succeeded in producing human iPS cell-derived mini-livers (liver organoids) (Ref.1, 2). Ajinomoto has successfully developed and commercialized StemFit® For Differentiation, an animal-origin free supplement for differentiation, in collaboration with the Department of Regenerative Medicine, Yokohama City University, Graduate School of Medicine. This product is also being used in a project at Yokohama City University for the production of iPSC-derived mini-livers (Fig. 1). Ajinomoto interviewed Dr. Okamoto, who is in charge of this project, and asked him about his cutting-edge research on the production of iPSC-derived mini-livers and his efforts towards clinical research and clinical trials. (February 8, 2021)



Satoshi Okamoto, Ph.D.
Department of Regenerative Medicine,
Graduate School of Medicine,
Yokohama City University

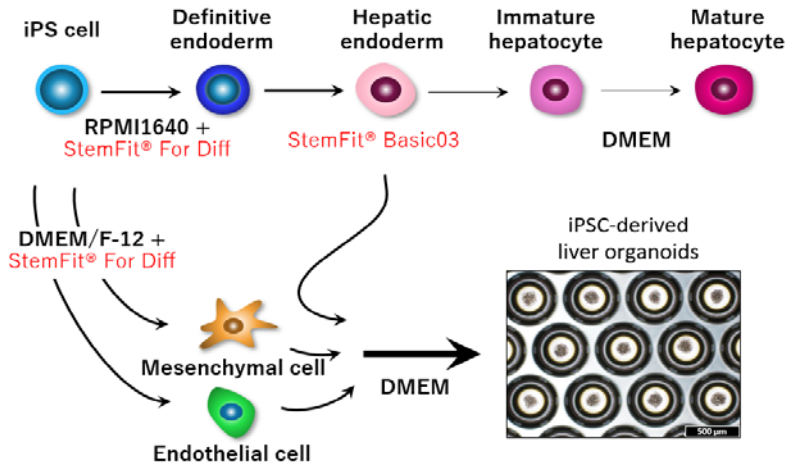


Fig.1 Liver organoid and development of clinical medium

StemFit® For Differentiation

1. Overview of the research and future clinical trials

In our study, three types of cells, including liver progenitor cells (liver endoderm cells, vascular endothelial cells, and mesenchymal cells), were induced to differentiate from human iPS cells. These cells were then mixed and seeded in a special culture plate with engraved microwells to produce small liver organoids with a diameter of about 130 μm.

Currently, we are conducting research to administer this liver organoid to patients with liver diseases as a treatment. We are actively preparing for two clinical trials; one for urea cycle disorders, and the other for liver cirrhosis. In the development of a therapeutic method for the former, the cell manufacturing process and quality evaluation method of liver organoids have been determined, and the safety and efficacy evaluation using animal models has also been confirmed. Currently, the study plan is under review by the Certified Special Committee for Regenerative Medicine. For the treatment of liver cirrhosis, we have already confirmed the basic cell manufacturing process and efficacy evaluation, and are now in the development stage in order to proceed with clinical trials.

In the case of urea cycle disorder, cells are currently administered to the liver through blood vessels. When single cells are administered in this manner, most of the administered cells stay in the liver, but a small number of cells can migrate to organs other than the liver, which presents a safety issue. In contrast, we believe that transplanting liver organoids will be a very useful method from the standpoint of safety, since migration to other organs can hardly be detected when transplanting liver organoids.

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2. Important and difficult points in cell production for clinical use

In the early stages of R&D, we were developing at a research grade and small scale, but by using StemFit® AK03N for iPS cell culture and StemFit® For Differentiation for differentiation, we were able to adopt animal-origin free reagents, which made the culture protocol very stable (Ref.3). On the other hand, when we scaled up from a small scale to a large scale in anticipation of cell production, the culture became unstable and we could not obtain stable results. It took a lot of time and effort to figure out what was causing the instability. Ultimately, we found that the cell detachment and cell seeding processes were the most important factirs that ensure stability in the cell culture. We have since standardized the cell detachment method using enzymes, the number of pipetting steps, the cell counting method, the coating method of culture dishes, and the diffusion method after cell seeding in detail, and have developed protocols and conducted training so that everyone in charge of cell production is now able to perform the same culture operations. In the culture of liver organoids using a special culture dish, organoids were often scattered to other wells when medium was added to the dish during medium change. This caused the organoids to fuse with the organoids in other wells, resulting in a larger than specified size, which was an issue from the perspective of safety after implantation. To solve this problem, we also focused on establishing a technique for medium exchange that would prevent organoids from splashing into other wells. Specifically, we are trying to control the flow rate of medium exchange so that it is slow and constant.

Our cell culture and processing facility is a unique device that consists of multiple isolators connected by an automated conveyor belt system (Fig. 2). The device allows for the exchange of cells, media, consumables, and other items necessary for culture between each isolator. The isolator is equipped not only with a place for culture operations, but also with a unit for aseptic storage of large quantities of materials necessary for culture, making it the ideal facility for mass production of cells.

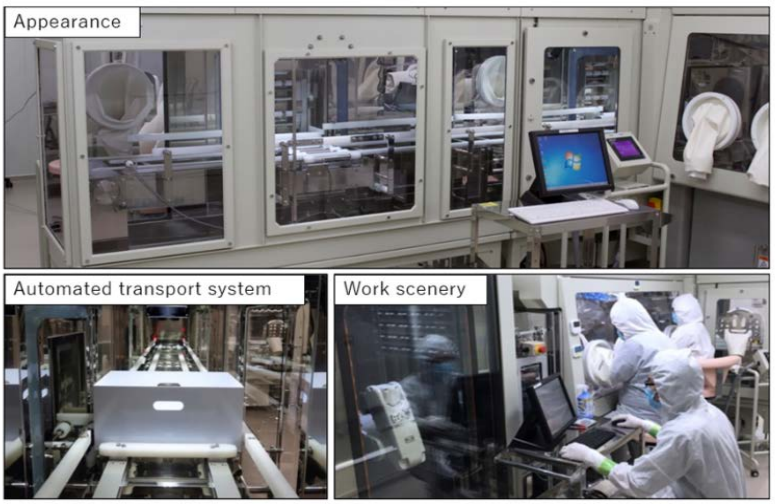

Fig.2 Cell processing facility

3. Important and difficult points in terms of laws and regulations for regenerative medicine

From the early stages of our research and development, we have been involved in the selection of cell culture media and reagents for clinical grade, and have also developed a clinical differentiation supplement (StemFit® For Differentiation) in collaboration with Ajinomoto. Since our research team did not have any experts on regulatory compliance, we have been considering the implementation of pre-clinical studies and regulatory compliance with the opinions of PMDA and outside experts. As for growth factors, many animal-origin free reagents were available from various manufacturers, but most of the differentiation supplemants (serum-substitute reagents such as FBS, KSR, B27, etc.) contained human and animal-origin components at that time. The joint development of StemFit® For Differentiation, an animal-origin free differentiation supplement, with Ajinomoto has solved the safety issue and was a very important initiative.

4. Quality control of cell products

Previous studies have shown that as the number of iPS cell passages increases, the percentage of undifferentiated iPS cells remaining in differentiated cells increases, so the number of iPS cell passages need to be kept to a minimum. Even at the research stage, we have established a working cell bank (WCB) and use the WCB every time we induce differentiation and start differentiation at a certain number of passages. StemFit® medium is very useful in that it allows us to construct WCBs with a short number of passages and to start differentiation induction because it can efficiently grow iPS cells. Through previous studies, we have been optimizing our protocol to achieve stable cell production. In our laboratory, we have a fixed day on the culture schedule for WCB thawing, cell passaging, and start of differentiation induction, which makes it easier for us to plan and carry out our work efficiently. As a result, it is easier to manage the pre-clinical study schedule for the evaluation of transplantation into animal models after the production of liver organoids. In order to control quality in the manufacturing process, we have discovered not only the expression of markers specific to differentiated cells, but also markers that confirm cell viability, and are applying these markers to cell quality test systems. The final product, liver organoids, is also under size control. In other words, we have been able to achieve stable cell production by using reagents with stable performance, such as StemFit®medium, and by establishing standardized culture techniques.

5. Points to be considered when selecting raw materials

In the early stages of research and development, we used research grade reagents, but we have been recently replacing them with reagents that comply with Japanese biological raw material standards, even in the early stages, once our protocol is close to being established. We also found that some reagent manufacturers took a lot of time to provide information on their reagents, resulting in no information being available, and those reagents had to be replaced with other reagents that could provide information. When selecting reagents, we believe it is important to adopt reagents that are clinical grade and that the reagent manufacturers are willing to provide prompt information and support for IND.

6. Feeling about using StemFit® For Differentiation

StemFit® For Differentiation allows for stable differentiation induction and is very easy to use. In our project, we induce differentiation of three types of cells from human iPS cells for the production of liver organoids, and we feel that StemFit® For Differentiation is a very versatile supplement because it can be used for any of the differentiation induction. In addition, we believe that the reason why we have been able to achieve stable culture even in scale-up is because we have been using this supplement to build a differentiation induction system from the small-scale stage, and also because StemFit® For Differentiation is a chemically-defined supplement.

7. Future prospects, issues, etc

As mentioned earlier, we plan to make steady progress in our research and development regarding the implementation of clinical trials for the treatment of urea cycle abnormalities and liver cirrhosis using iPS cell-derived liver organoids. After that, we are planning to develop a system to enable mass production of liver organoids in order to widely spread regenerative medicine using liver organoids. We have already been working on the stability of liver organoids during storage and transport. For the transportation of the final product for the treatment of urea cycle abnormalities, we have already confirmed that stable transportation for several hours is possible. We are also working on obtaining data on the stability of transportation for longer periods of time, and studying cryopreservation for mass production through industrialization.

For the treatment of liver cirrhosis, we are developing a cell product that adds another function to the liver organoids used for urea cycle abnormalities by further modifying them. We are also conducting research to improve the function of organoids by changing the cells that make up the organoids. We are planning to conduct extensive research and development on liver organoids with improved functions, with the goal of applying them to many more liver diseases.

Reference

1) Nature 2013 Jul 25;499(7459):481-484
2) Cell Reports 2017 Dec 5;21(10):2661-2670
3) Scientific Reports 2020 Oct 21;10(1):17937