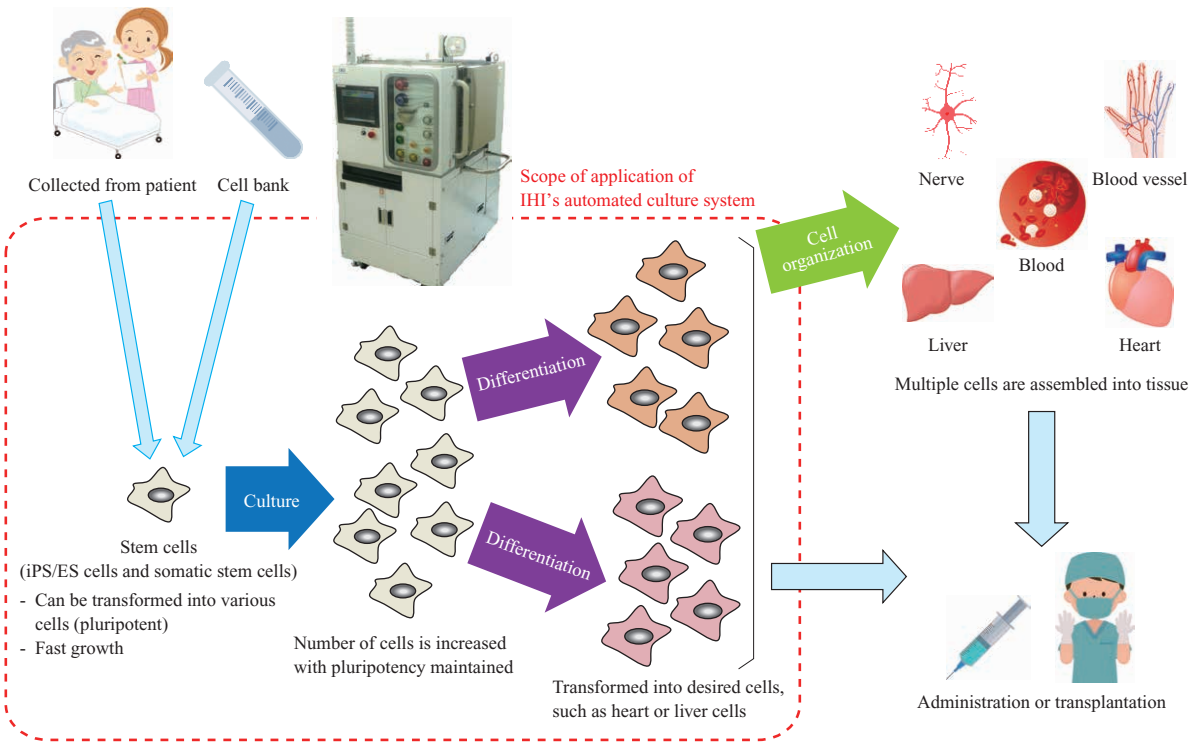
Hoping to Save More People from Serious Diseases as Soon as Possible

IHI's automated cell culture technology potentially contributable for regenerative medicine

Utilizing stem cells, such as iPS cells, in regenerative medicine requires the production of a large number of cells. IHI has developed an original automated cell culture system based on the plant engineering and fluid technologies that it has accumulated over many years. It is exploring worldwide needs in order to aim for acquiring the opportunities to apply the system to regenerative medicine.



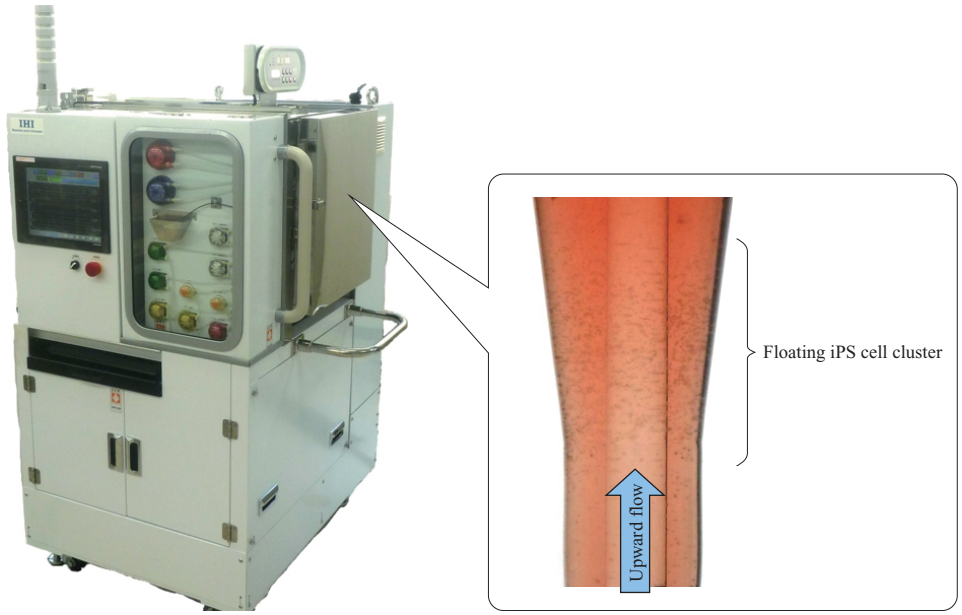
Regenerative medicine process using stem cells (example)

Current situation and challenges of regenerative medicine research

Induced pluripotent stem cells (iPS cells), which attracted significant attention when Shinya Yamanaka, Professor of Kyoto University, received the Nobel Prize, are able to form various tissues and organs (pluripotency), and it is expected that they will be used in medical treatments for the regeneration of organs damaged by disease and other causes.

In a recent clinical study, retinal pigment epithelial cells were produced from iPS cells and transplanted into patients with age-related macular degeneration, which is a serious eye disease.

At the same time, there are studies in progress that use somatic stem cells, which have limited pluripotency but can be collected directly from humans, and that use embryo-stem cells (ES cells: pluripotent cells made from fertilized eggs).



Automated cell culture system (prototype) and culture tank in which iPS cells are being cultured

In general, these stem cells are cultured manually using Petri dishes. Culturing cells with Petri dishes requires repeated processes for subculture: replacing the culture medium (culture solution) periodically, removing the cells that have grown all over the Petri dish, dividing and transplanting them into multiple Petri dishes with new medium.

However, stem cells are sensitive to external forces and, depending on the skill of the operator who performs the culture work, may die or transform into types of cells that were not intended.

In addition, a huge number of cells are required to regenerate a large organ such as the heart or liver. Several thousands to several tens of thousands of Petri dishes would be required to culture such a huge number of cells, and it is unrealistic for all of these dishes to be handled by skilled operators. There is an ongoing attempt to replace these skilled operators with robots, but a large number of robots would be required to handle a large number of Petri dishes.

For these reasons, a challenge with regard to promoting widespread use of regenerative medicine is the establishment of a method of producing a large number of cells of stable quality.

Features of IHI's automated cell culture technology

To overcome this challenge, IHI is developing an automated cell suspension culture system that allows cells to float in a culture medium so that they are not exposed to external forces. Other automated cell suspension culture systems have already been marketed, but IHI's has the two following major features under development:

(1) It is possible to replace the culture medium while culturing.

(2) It is possible to control the size of cell clusters, technically referred to as spheroids.

Through these features, IHI's automated cell culture system aims to produce a large number of cells of stable quality at low cost without the need for skilled operators. Details of each feature are as follows:

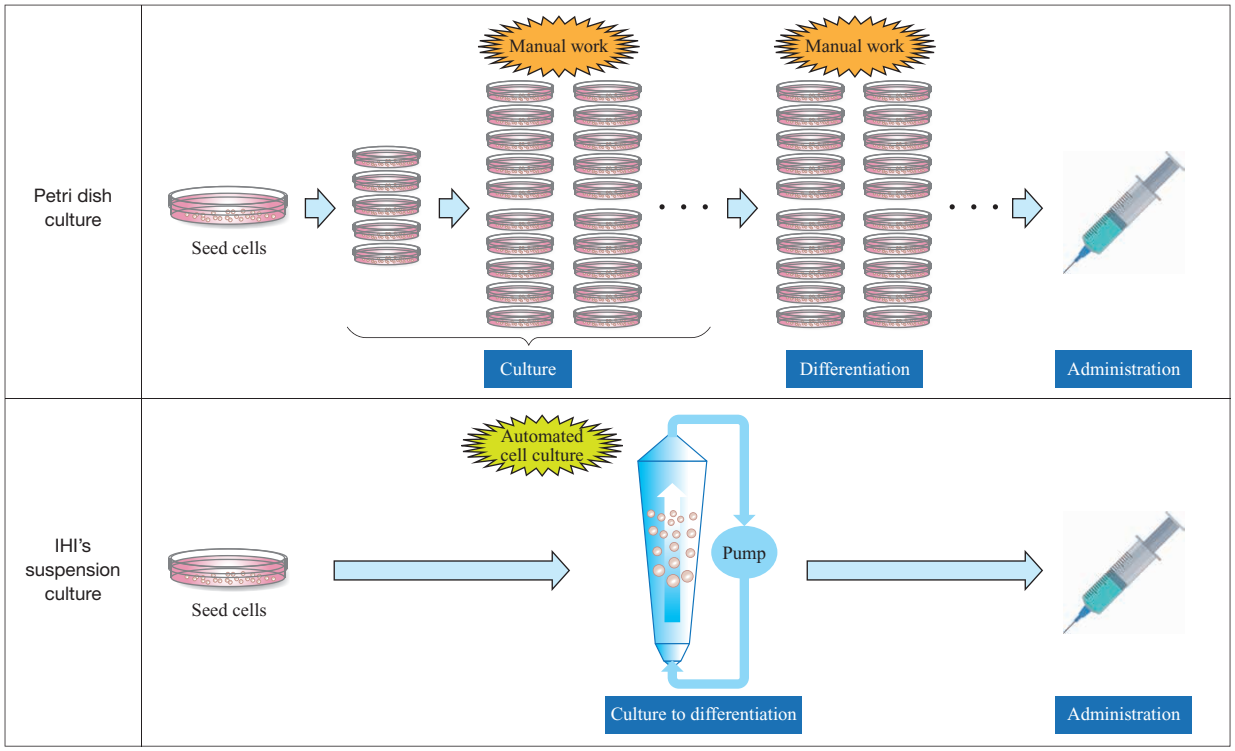
(1) Replacing the culture medium while culturing

When cultured using the suspension method, stem cells, due to their nature, generally form cell clusters several hundred micrometers in size. However, each cell cluster becomes gradually large as the cells divide and grow. In addition, during culturing, cell clusters also become larger by combining together and, as a result, cell cluster size becomes non-uniform.

Suspension culture systems generally have a stirring mechanism (e.g., rotary blades) in the flask to stir the culture medium, thereby preventing floating cell clusters from combining together. In these systems, when the culture medium is replaced, stirring is stopped to allow the cells to settle out, and only the supernatant is replaced. Hence, because the precipitated cells need to remain, unremovable part of the culture medium must remain old without replacement.

IHI's culture system uses a unique culture tank that has an inverted cone shape and optimally designed internal flow distribution. This allows the cell clusters to be kept floating in the culture tank simply by having the culture medium flow from bottom to top.

In the upper part of the culture tank, the cross-sectional area is large, and so the upward flow velocity is low. Conversely, in the lower part of the tank, the cross-sectional area is small, and so the upward flow velocity is



Comparison between conventional Petri dish culture and IHI's suspension culture

high. When cell clusters of various sizes are put in the tank, the small, light clusters are kept floating in the upper part and the large, heavy ones in the lower part, so that the upward flow velocity and the downward velocity of cells due to gravity are balanced. Hence, the cell clusters do not leak out of the culture tank and can be kept inside it without complex structure for preventing them from leaking out, such as the use of a filter.

During normal culturing, the cells are kept inside the culture tank by discharging the culture medium from the top of the tank and recirculated it from the bottom. When the culture medium is replaced, the fresh medium is introduced from the bottom of the tank while the old medium is simultaneously discharged from the top at the same rate. This allows replacement of almost all of the medium whilst continuing to culture the cells in the tank.

Using this mechanism, another type of culture medium can be supplied when the medium is replaced. For regenerative medicine, differentiation of stem cells is needed in many cases. After several differentiation steps, cells differentiate into the desired type of cells, such as cardiac muscle cells and nerve cells. By replacing the culture medium with one used for differentiation, we believe that the culture and differentiation processes could be performed sequentially, with the cells kept in the same culture tank.

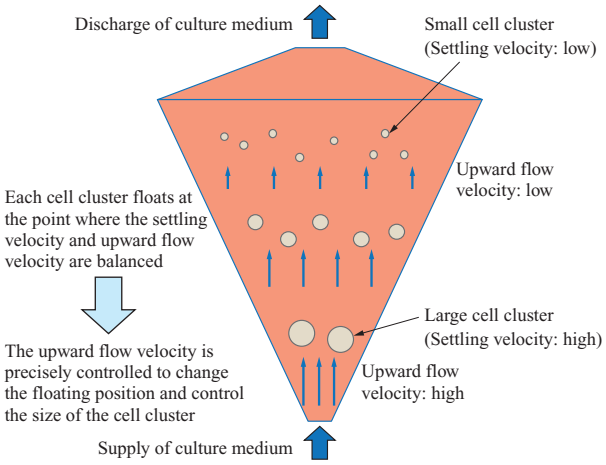
(2) Controlling the size of cell clusters

As previously stated, cell clusters become increasingly large as cell division proceeds, and as clusters combine together. This has an adverse effect on cell quality, since it becomes difficult for the nutrient components to penetrate the interior of the cell medium, and for the interior waste products to be discharged. In existing suspension culture systems, large cell clusters are shredded using mesh, but there are concerns that the mesh may become clogged or that cell quality may be degraded by the force generated when the cells pass through it. For this reason, a method is required for shredding large cell clusters without the use of mesh.

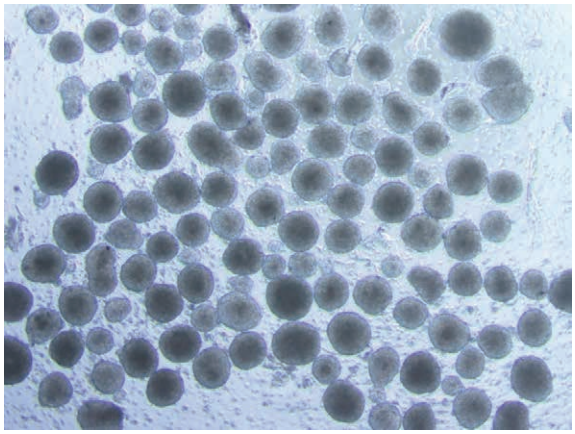
In addition, it has been found that cell cluster size also affects differentiation. It is therefore recognized that, even during the differentiation process, it is important to maintain suitable cell cluster size.

As described in (1), in IHI's culture system, the floating position (vertical height) changes depending on the size of the cell cluster. In addition, by utilizing the property by which larger, heavier cell clusters settle out more quickly, and by precisely controlling the flow velocity of the culture medium, such clusters can be made to selectively settle out in the lower part of the culture tank. The lower part of the tank is designed to have a higher flow velocity, and it was found that shredding cell clusters may be promoted by exposing large cell clusters to this strong flow.

Using this method, shredding force is applied only to large cell clusters, and small cell clusters are not exposed to unnecessary force or further divided. Therefore, it may be possible to adjust cell cluster size to a certain range



Principle of IHI's culture tank



iPS cell clusters cultured with IHI's automated cell culture system (microscopic image)

and, at the same time, minimize deterioration of cell quality caused by exposure to external force.

Availability of IHI's technologies for cell culture

These features are world firsts, but many people may wonder why IHI, a heavy industry manufacturer, is developing such a cell culture system. However, there is a surprising relationship between cell culture systems and IHI's technical strengths.

The IHI Group provides water treatment and pharmaceutical plants. Some water treatment plants make good use of microorganisms. In addition, some pharmaceutical plants perform animal cell culture. Through the experience of these plant designs, the IHI Group possesses fundamental technologies for cell culture. Furthermore, advanced fluid analysis and design techniques, which are required for jet engines, etc., constitute one of IHI's specialty fields.

We wondered whether we could apply these technologies to the field of medicine — which is, going forward, expected to be a growing market — and this was the stimulus that prompted us to develop our own unique cell culture system. Needless to say, the development of a system that can actually culture cells required a great deal of equipment design and control technology, together with much trial, error, and ingenuity.

Future expectations — Eyeing the global contribution

These are the characteristics of our automated cell culture system. When we conducted interviews concerning this equipment with pharmaceutical companies and research institutions, we felt that they regarded it with significant expectation.

However, in order for the system to be accepted by the pharmaceutical industry, more test data must be collected to demonstrate its safety and effectiveness. Therefore, we hope to discover the needs of worldwide universities, research institutions, and pharmaceutical companies, and acquire opportunities for the use of IHI's culture technologies.

IHI had been conducting joint research with Hideki Taniguchi, Professor of Department of Regenerative Medicine, Graduate School of Medicine, Yokohama City University since 2014, and in 2019 conducted joint research with promising research institutions in Asia in cooperation with IHI ASIA PACIFIC PTE. LTD., which is an IHI subsidiary in Singapore. This constitutes a first step towards attracting the world's attention to IHI's culture system.

Going forward, we will explore worldwide needs and verify the concept of this system with various types of cells — in addition to iPS cells — with the aim of acquiring opportunities to apply the system to regenerative medicine.

Research on regenerative medicine is underway all over the world, and its speed is accelerating. However, in order for regenerative medicine to become a commonplace treatment, and to widely benefit people around the world, it is still necessary to overcome several hurdles, including mass-production technology for cells.

We are confident that IHI's cell culture technology can serve as a powerful tool for overcoming these hurdles. We hope to work with universities, research institutes, and pharmaceutical companies around the world to save more people from serious diseases as soon as possible.

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