AGC News Release

August 12, 2016

AGC ASAHI GLASS

Japan Agency for Medical Research and Development

AGC ASAHI GLASS Develops Mass Culture Platform for iPS cells for regenerative medicine

—AGC publishes a research paper in the *Scientific Reports* (Nature Publishing Group, UK)—

Tokyo, August 12, 2016—AGC Asahi Glass (AGC) , a world-leading manufacturer of glass, chemicals and high-tech materials, announced today that it has developed a microfabricated culture vessel (EZSPHERE®)[note 1] for iPS cell [note 2] culture, and has succeeded in not only mass culture of iPS cells in uniform cell aggregates in this vessel but also differentiation into a target cell type in the same vessel with support from the "Program for Research Center Network for Realization of Regenerative Medicine" (principal investigator: Hiromichi Kumagai, Fellow Researcher, Technology General Division, Innovative Technology Research Center, Asahi Glass Co., Ltd.) of the Japan Agency for Medical Research and Development (AMED).

A paper describing this research achievement was published on-line in an electronic journal of the Nature Publishing Group (UK) in August 2016 (http://www.nature.com/articles/srep31063).

To achieve clinical application of pluripotent stem cells[note 3] such as iPS cells, mass production of cell aggregates is necessary, but conventional culture techniques have limitations in the production of uniform cell aggregates.

The AGC Group has developed a unique type of plastic culture vessel, EZSPHERE®, which has high-density microwells with several hundred micron pore size on the surface. These microwells are formed by laser-based microfabrication technology, followed by further treatment with a special coating designed to minimize cell adherence. As a result of iPS cells cultivation in this EZSPHERE® culture vessel, cell adhesion to the culture vessel surface was suppressed and uniform cell aggregates were rapidly obtained with high efficiency. Furthermore, differentiation into a target cell type was successfully achieved in the same culture vessel.

Applications of the pluripotent stem cells such as iPS cells to regenerative medicine and drug discovery (drug screening) are anticipated to expand further. The mass production method of obtaining uniform cell aggregates established by this research is expected to serve as the essential basic technology for achieving advances in stem cell application.

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For a summary of the research paper, see the annex.

Under the management policy termed ***AGC plus***, the AGC Group manufactures products that provide "safety, security, and comfort" to society, and creates "new value and functions" for customers. In the life-science field, one of the target areas encompassed by our business strategy, the Group will continue to promote technical innovations that yield products and solutions satisfying our customers' needs.

[Glossary]

(Note 1) EZSPHERE®:

EZSPHERE® is a microfabricated culture vessel developed, manufactured and marketed by AGC Techno Glass Co., Ltd. In this vessel, the bottom surface is microfabricated employing a CO_2 laser to form microwells approximately 200 to 1400 μm in diameter and approximately 100 to 400 μm in depth at regular intervals without gaps over the entire surface, and then treated with a special polymer coating that suppresses cell adhesion. This microfabrication technology can be applied to the already available polyethylene dishes and plates.

(Note 2) iPS cells:

The term "iPS cells" is an abbreviation for "induced pluripotent stem cells", which are prepared from somatic cells through transfer of multiple genes. As with ES cells, iPS cells have multipotency, the capability of differentiating into many types of cells and replication competence. Shinya Yamanaka, Professor, Kyoto University, firstly reported this technique in 2006.

(Note 3) Stem cells:

Stem cells are defined as cells that are capable of replicating themselves via division (replication competence), differentiating into other types of cells and unlimited proliferation. Various stem cells have been reported, including iPS, ES and somatic stem cells.

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<Reference Information>

About the AGC Group

AGC Asahi Glass (or also called AGC, Registered Company name: Asahi Glass Co., Ltd., Headquarters: Tokyo, President & CEO: Takuya Shimamura) is the parent company of the AGC Group, a world-leading glass solution provider and supplier of flat, automotive and display glass, chemicals, ceramics and other high-tech materials and components. Based on more than a century of technical innovation, the AGC Group has developed a wide range of cutting-edge products. The AGC Group employs some 50,000 people worldwide and generates annual sales of approximately 1.3 trillion Japanese yen through business in about 30 countries. For more information, please visit www.agc-group.com.

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AGC ASAHI GLASS Develops Mass Culture Platform for iPS cells for regenerative medicine [Annex: Summary of Research Paper]

Major Points

- AGC develops a three-dimensional culture technology using a microfabricated culture vessel that leads to "highly efficient, rapid mass production" of cell aggregates from human iPS cells with "uniform size", thereby achieving the goal of "stable proliferation".
- AGC succeeds in reducing the time required for iPS cells to differentiate into neural stem cells[note 4] by controlling the size and density of iPS cell aggregates, based on the microwell size of the microfabricated culture vessel and seeding density of iPS cells.
- AGC establishes a sequential process from the formation of human iPS cell aggregates to differentiation and maturation of dopaminergic neurons[note 5] in the same microfabricated culture vessel.

Outline of the research

Pluripotent stem cells such as iPS cells are expected to serve as an important cell source in regenerative medicine, and low-cost mass production of such cells with high quality is thus needed. Cell aggregates (spheroid[note 6]) or an embryoid body[note 7] of pluripotent stem cells formed in three-dimensional culture can be grown to high density with pluripotency (undifferentiated state), but such three-dimensional cultures have limitations including decreases and variations in the subsequent growth and differentiation efficiency due to non-uniform sizes of the cell aggregates obtained.

To address the above problems, in this research, three-dimensional culture and differentiation of iPS cells were carried out using a plastic culture vessel, EZSPHERE®, which has high-density microwells with several hundred micron pore size on the surface. These microwells are formed by laser-based microfabrication technology, followed by further treatment with a special coating designed to minimize cell adherence. As a result of the suppressed adhesion of iPS cells to the culture vessel surface, uniform cell aggregates were rapidly obtained with high efficiency. This study demonstrated that the size of cell aggregates can be controlled by changing the cell seeding density or microwell size. Furthermore, this technology enables cells to differentiate into a target cell type in the same vessel. For example, AGC succeeded in achieving the differentiation of iPS cells into dopaminergic neurons.

Social impacts of this research achievement (significance of this research achievement to society)

Applications of pluripotent stem cells such as iPS cells to regenerative medicine and drug discovery (drug screening) are anticipated to expand, at an ever-increasing pace. The technology established in this research allows not only highly efficient mass production of uniform cell aggregates (spheroids/embryoid bodies) from iPS cells but also efficient differentiation, and is thus expected to serve as the essential basic technology in three-dimensional culture of various pluripotent stem cells in the future.

Description of the research and achievement

1. Highly efficient formation of iPS cell aggregates in microfabricated culture vessels

This research achieved the landmark development of iPS cell aggregate (spheroid) formation technology using a unique microfabricated culture vessel (EZSPHERE®). The EZSPHERE has microwells with several hundred micron pore size on the surface, and is also treated with a special coating designed to minimize cell adherence to the vessel surface. This laser-based microfabrication technology can be applied to the surfaces of various plastic dishes and plates. When human iPS cells (hiPSC line 201B7 provided by iPS Academia Japan, Inc.) were seeded into this vessel, the cells spontaneously dropped into each microwell without adhering to the vessel surface and promptly formed cell aggregates within a short period of time (approximately 3-4 hours). Compared with other vessels, formation efficiency and size uniformity were excellent (Figure 1). Furthermore, high proliferation capability and pluripotency were confirmed. Because the microfabrication technology is laser-based, microwell size (diameter and depth) can be controlled. This research further showed that this novel technology allowed uniform formation of cell aggregates with an appropriate size.

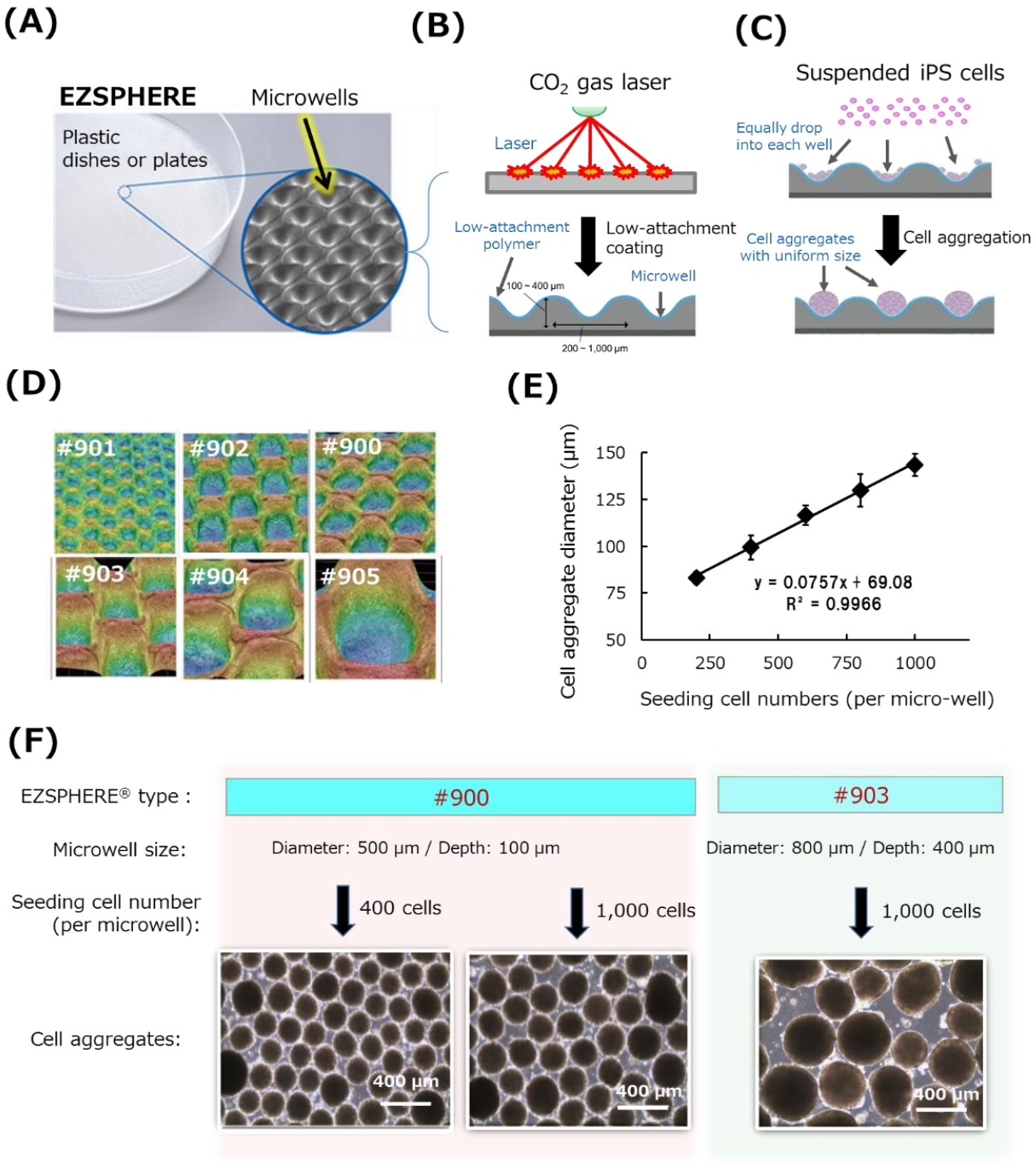

Figure 1. Microfabricated culture vessel, EZSPHERE®, and highly efficient formation of iPS cell aggregates in this vessel

(A) Configuration of microfabricated microwells; (B) Microwell processing method; (C) Method for the formation of cell aggregates in microwells; (D) Variety of microwell sizes; (E) Correlation between seeding cell density and cell aggregate size (EZSPHERE® #900); (F) Size adjustment of cell aggregates by combining the optimal microwell size and seeding cell density

2. Control of neural differentiation efficiency corresponding to iPS cell aggregate size

Size control of cell aggregates is known to be important for optimal differentiation of iPS cells into a target cell type. To investigate the correlation between cell aggregate size and differentiation efficiency, experiments on neural differentiation were carried out. The experiments using high and low seeding cell densities per microwell (1000 cells/microwell and 400 cells/microwell, respectively) revealed the formation of cell aggregates of different sizes to depend on cell density. Interestingly, the differentiation of large cell aggregates into neural stem cells was observed to occur in a remarkably short period of time (Figure 2).

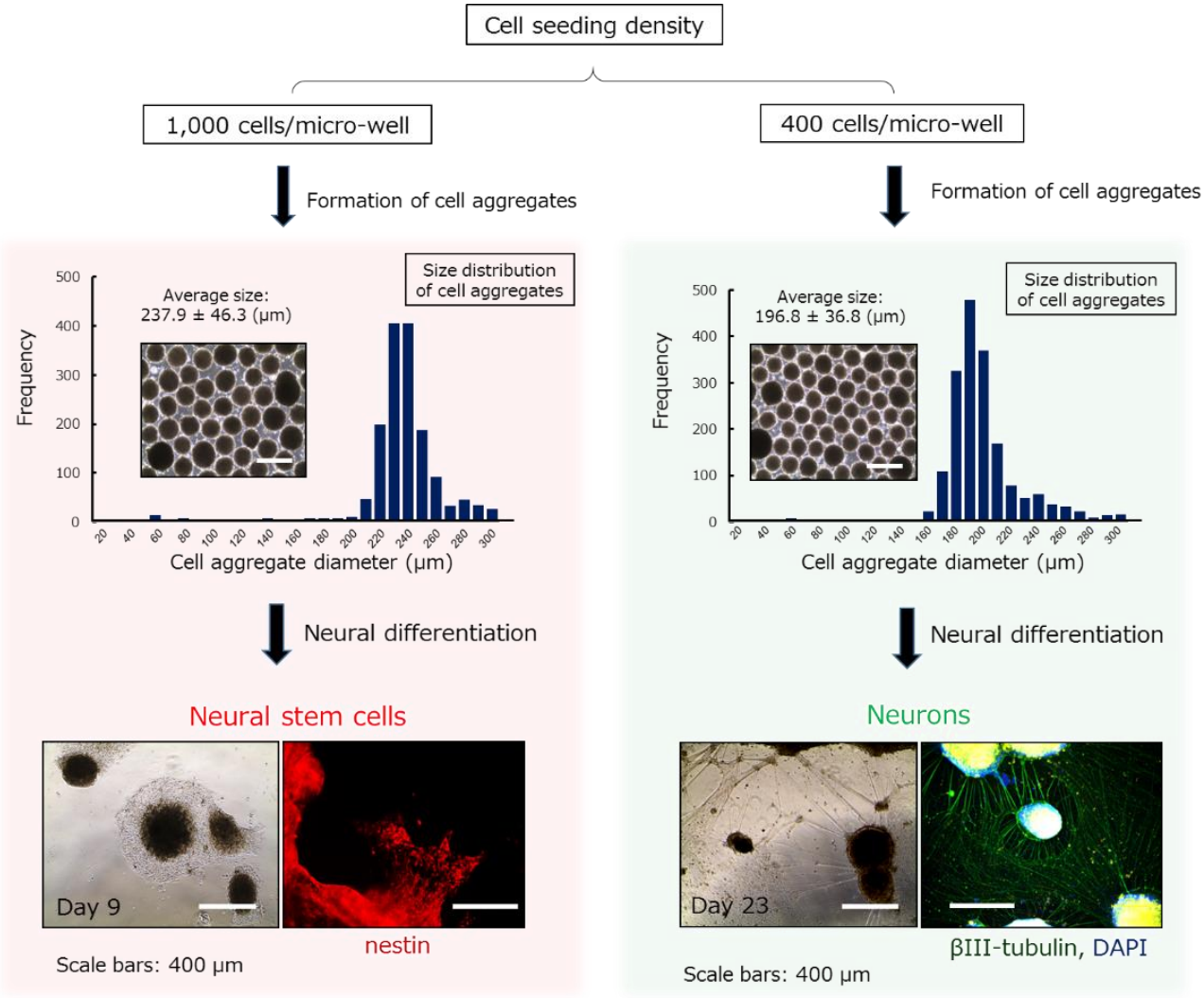

Figure 2 Control of neural differentiation corresponding to iPS cell aggregate size

3. Sequential process of achieving differentiation and maturation of dopaminergic neurons in the same microfabricated culture vessel

This research demonstrates that the sequential process from embryoid body formation through proliferation to differentiation into dopaminergic neurons and its maturation can be carried out in the same vessel by changing the culture medium (Figure 3).

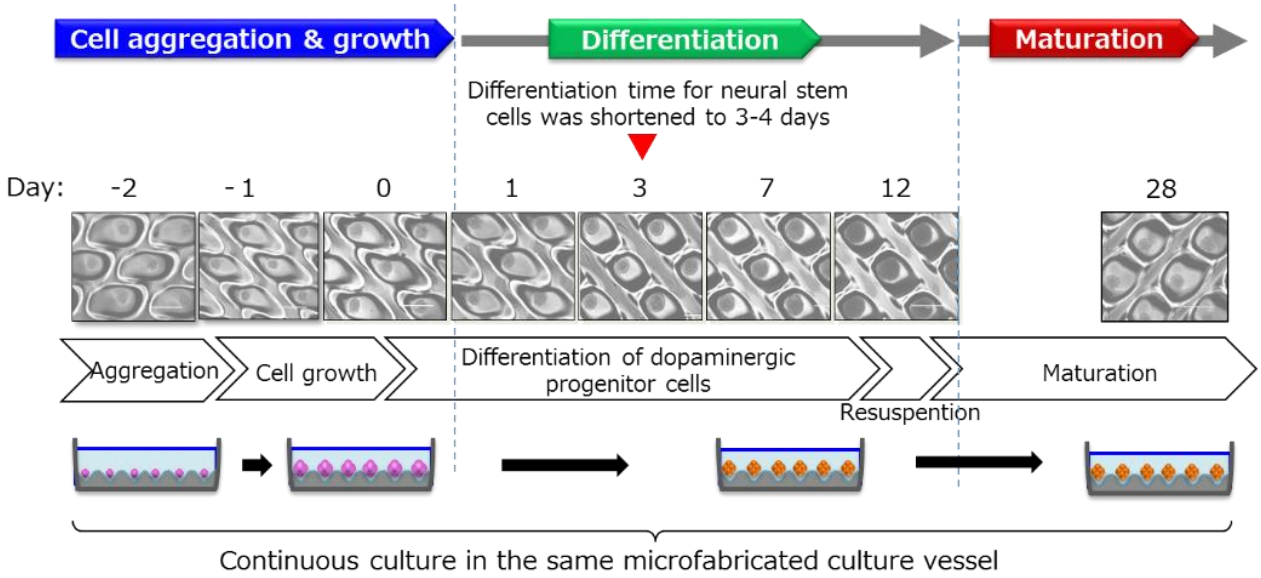

Figure 3: Sequential process from formation and proliferation of human iPS cell aggregates to differentiation and maturation of dopaminergic neurons in the same microfabricated culture vessel by three-dimensional culture

Conclusion and future developments

For application of iPS cells to regenerative medicine and drug discovery, technology for simple mass production of iPS cells with uniform properties and efficient differentiation is essential.

This research on a three-dimensional culture system using a unique microfabricated culture vessel, EZSPHERE®, showed that this system can efficiently achieve the sequential process from aggregate formation of stem cells, such as iPS cells, through cell proliferation and differentiation to cell purification and maturation. Furthermore, differentiation efficiency improved in a manner dependent on cell aggregate size.

The microfabricated culture vessel, EZSPHERE®, is anticipated to contribute not only to the development of a platform for low-cost and high-quality mass culture and differentiation of iPS cells but also to serve as a culture vessel essential for practical applications in the field of regenerative medicine.

Published paper

[Title]

Microfabric Vessels for Embryoid Body Formation and Rapid Differentiation of Pluripotent Stem Cells

[Authors]

Hiroki Sato, Alimjan Idiris, Tatsuaki Miwa & Hiromichi Kumagai

[Journal]

Scientific Reports

Glossary

(Note 4) Neural stem cells

Neural stem cells are a type of stem cells that is capable of differentiating into neurons and glial cells.

(Note 5) Dopaminergic neurons:

Dopaminergic neurons are one of the neuronal cell types that releases dopamine as a neurotransmitter.

(Note 6) Spheroid:

Spheroid indicates a spherical cell aggregate.

(Note 7) Embryoid body:

Embryoid body means a cell aggregate, in the shape of a sphere, of ES cells or iPS cells found in suspension culture under differentiation conditions, which can be induced to differentiate into various cell types. The term embryoid body is conventionally used in investigations examining differentiation into specific cell types and of the multipotency of cells.

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