The 18th Takeda Science Foundation Symposium on Bioscience

AGC

Efficient Embryoid Body Formation from Human iPS Cells using Novel Microfabric Vessels

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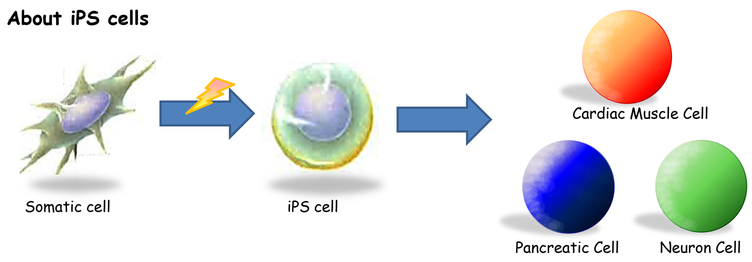
ABSTRACT

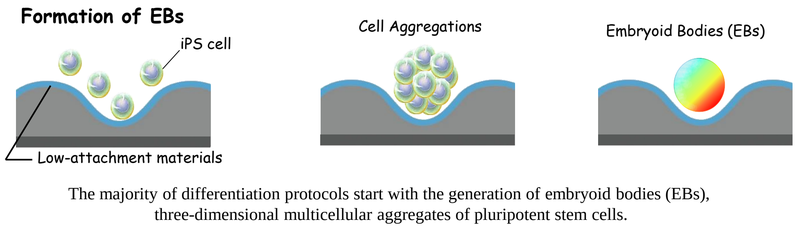
Human iPS cells (iPSCs) have high potential applications in regenerative medicine and drug discovery with their ability of differentiating into a wide variety of somatic cells. In the differentiation process of the iPSCs, embryoid body (EB) formation is known to be a major bypass in most cases. Furthermore, EB size and uniformity are known to be one of critical factors affecting the differentiation efficiency. However, there are still technical limitations in control of EB size and its uniformity using conventional EB formation methods, such as hanging drop, static suspension culture or using multi-well culture plates. To solve such problems, we attempted to apply novel microfabricated vessels, in which a large number of micro-wells, approximately 200-9,000 per 35 mm culture dish, were solely created by laser beam, followed by coating with non-cell-adhesive reagents. The diameter and depth of wells are 200-1,000 μm and 100-400 μm, respectively. Using such specific vessels, uniformly formed EBs in the micro-wells were achieved after optimizing cultivation, dissociation and inoculation methods of the hPSCs. In addition, it was also confirmed that EBs formed in the micro-wells were able to be maintained for 8 days by replacing half of culture media in every two days and possessed high differentiation potential, which was tested by induction of nerve cells. These results indicated that the novel microfabricated vessels (named as EZSPHERE) are useful tool for creating large amounts of EBs with uniform size at low cost with simple method.

INTRODUCTION

Regenerative medicine using pluripotent stem cells



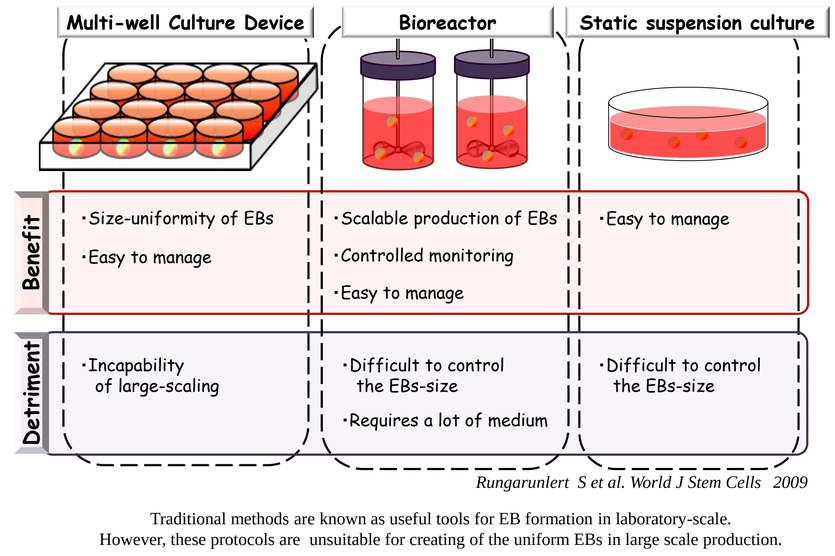
Human pluripotent stem cells (hPSCs) have some potential applications in regenerative medicine, such as drug discovery, organ transplantation and examining congenital disorders.



The majority of differentiation protocols start with the generation of embryoid bodies (EBs), three-dimensional multicellular aggregates of pluripotent stem cells.

For the early realization of the stem cell-based therapies, it is problems how to easy production of uniform EBs in large scale.

Conventional methods of EBs formation



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Traditional methods are known as useful tools for EB formation in laboratory-scale. However, these protocols are unsuitable for creating of the uniform EBs in large scale production.

Development of the novel culture vessels for supplying size-uniformly of and inexpensive EBs in large scale is necessary.

AIM OF STUDY

Background-1

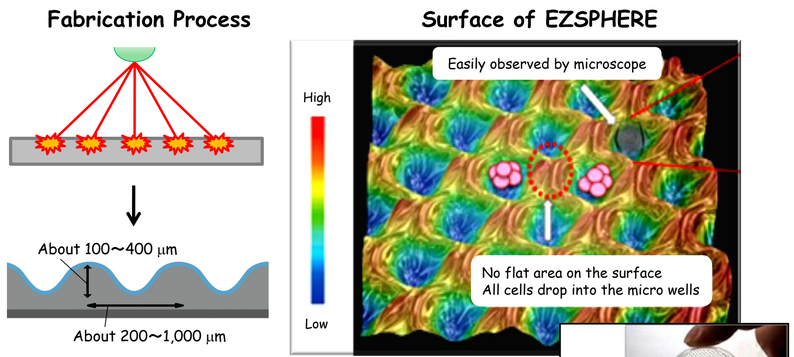
For the early realization of regenerative medicine, it is essential that EBs are stably and inexpensively supplied in large scale.

Background-2

The majority of differentiation protocols start with the generation of EBs. However, the traditional methods are unsuitable for creating of the uniform EBs in large scale production.

We attempted to develop the new EB-formation methods using the novel microfabricated vessels (named as EZSPHERE). Here, we report some problems of EZSPHERE when using iPS cells in conventional culture conditions and, to resoluble these problems, the optimum condition to form EBs on EZSPHERE.

EZSPHERE, a novel culture vessel for spheroid formation



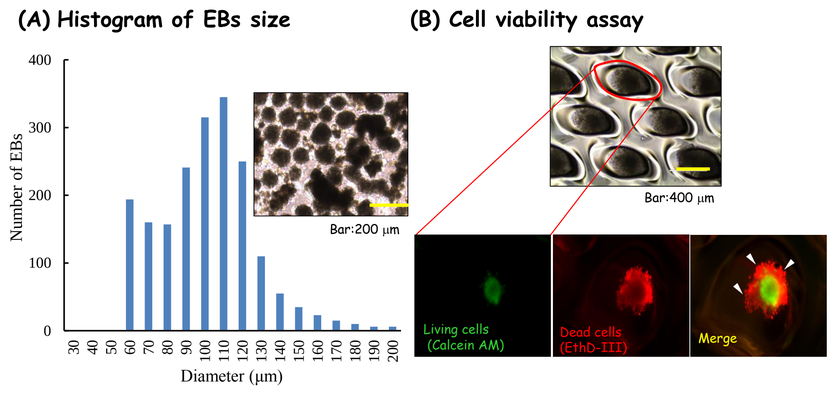
Micro-wells of EZSPHERE are solely created by CO_2 laser beam, followed by coating with low-cell adhesive reagents (MPC polymer).

Because micro-wells are closely positioned in culture ware, inoculated cells equally drop into these wells.

EZSPHERE is specifically designed to form a large number of uniformly sized spheroids and EBs.

RESULTS

Fig.1 EBs formation on EZSPHERE using iPS cells



When iPS cells were inoculated on EZSPHERE using conventional conditions, the EBs were scarcely formed to uniform size (A). In the result of cell viability assay, the single cells dropped out from EBs were almost dead (B arrow heads).

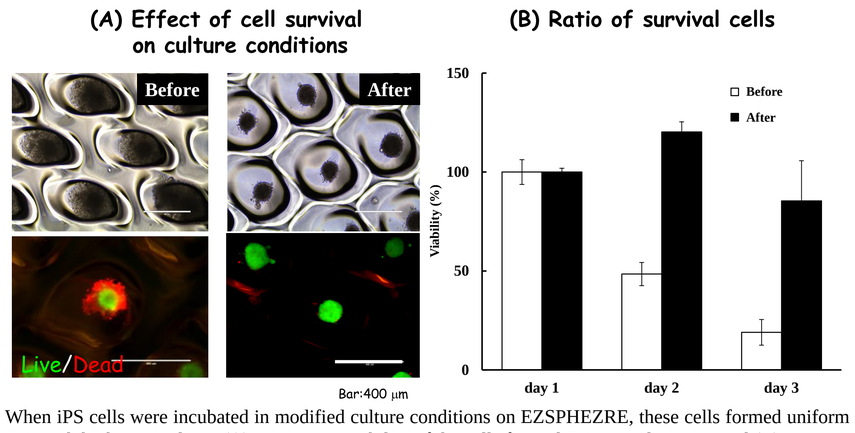
When culturing iPS cells using EZSPHERE, improvement of culture condition was necessary.

Fig.2 Improvement of culture methods

The culture conditions were optimized to improve the EBs formation efficiency using EZSPHERE as follows.

i: The basal medium was supplemented with Knockout Serum Replacement (KSR) (final concentration: 5%).

ii: A half volume of culture medium was exchanged with fresh medium in every two days.

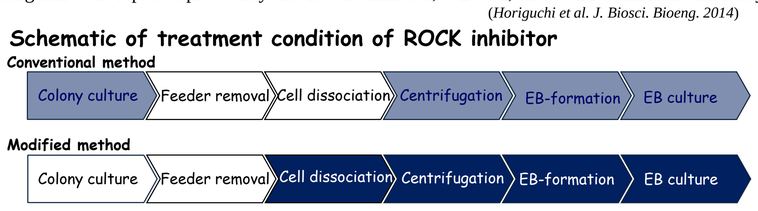


When iPS cells were incubated in modified culture conditions on EZSPHEZRE, these cells formed uniform EBs with high survival rate (A). Moreover, viability of the cells formed EBs was also improved (B).

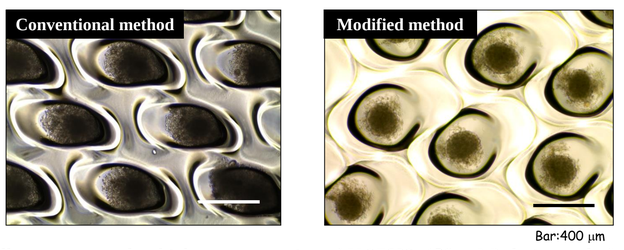
By the addition of KSR and replacing half of culture media, survival rate of iPS cells was improved compared with conventional conditions.

Fig.3 Effect of Y-27632 concentration on EB-formation

Horiguchi *et al.* reported previously that ROCK inhibitor, Y-27632, affects EB-formation efficiency. (*Horiguchi et al. J. Biosci. Bioeng. 2014*)



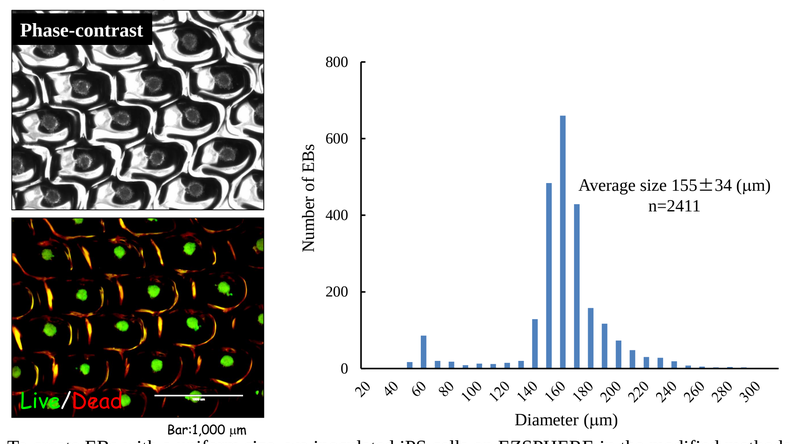
10 (▷) or 50 (▶) μM of Y-27632 was added.



When iPS cells were exposed to higher concentration of Y-27632, (50 μM) during and after cell dissociation, the efficiency of EB-formation was improved compared with conventional method.

Treatment with higher concentration of Y-27632 during and after cell dissociation significantly promoted formation of EBs.

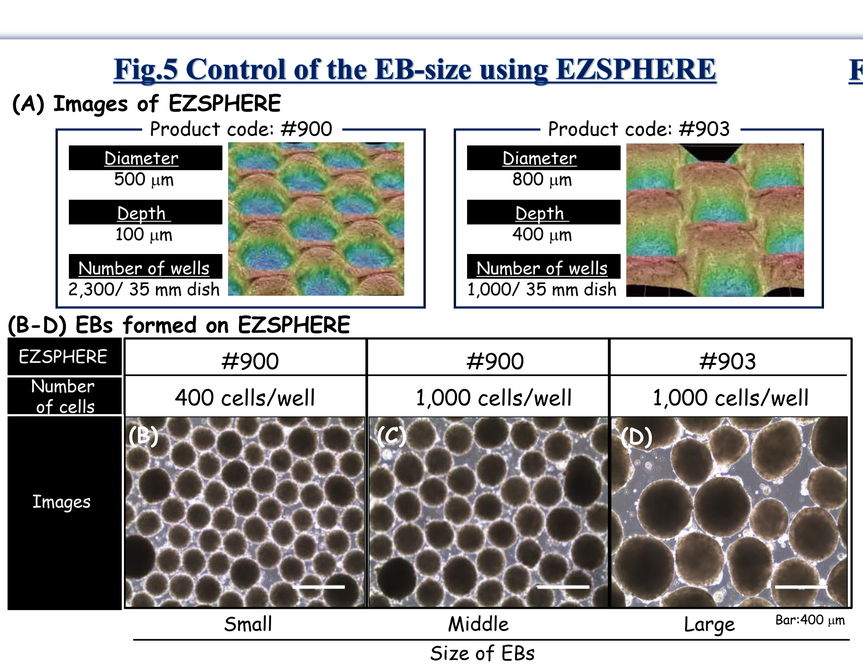
Fig.4 Uniform EBs formation using EZSPHERE



To create EBs with a uniform size, we inoculated iPS cells on EZSPHERE in the modified methods. In phase-contrast observation, an EB with uniform size was formed into each micro-well. Fluorescence microscopy revealed that every EBs were alive (A) and with uniform size (B).

Optimization of culture conditions resulted in the formation of EBs with a uniform size on EZSPHERE.

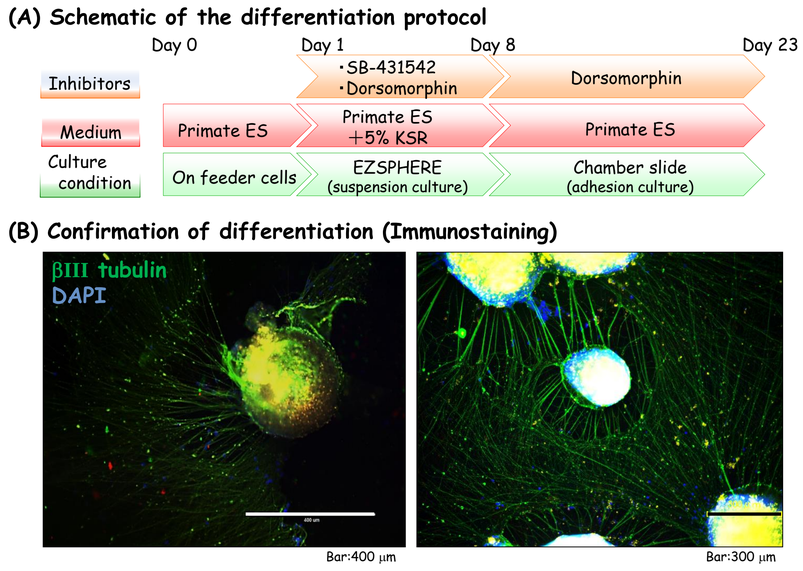
Fig.5 Control of the EB-size using EZSPHERE



EZSPHERE #900 and #903 have micro-wells about 500 μm and 800 μm in diameter, respectively (A). The inoculation of 400 or 1,000 cells per micro-well on EZSPHERE #900 resulted in the formation of EBs with a small and middle size, respectively (B,C), and in a case of EZSPHERE #903, larger size of EBs (D).

By choosing the number of inoculation cells and size of micro-wells of EZSPHERE, the size of EBs was easy to be controlled.

Fig.6 Neural differentiation assay using EBs formed by EZSPHERE



After the EBs formation with a uniform size using EZSPHERE, EBs were transferred to a Matrigel coated chamber slide to evaluate their differentiation abilities to neural cells (A). Immunofluorescence staining demonstrated that βIII tubulin-positive neurites were detected (B).

The EBs formed by EZSPHERE were capable of differentiation to nerve cells (especially neurons).

CONCLUSION

- Culture conditions were improved to achieve EBs with a uniform size and higher viability using EZSPHERE.
- The addition of KSR, medium change and treatment of higher concentration of Y-27631 were necessary to allow EBs of uniform size using EZSPHERE in high survival rate.
- EZSPHERE could create > 2,000 EBs per 35 mm culture dish at once.
- The size of EBs created by EZSPHERE were able to adjust freely by choosing the number of inoculation cells and appropriate type of EZSPHERE.
- EBs were capable of differentiation to nerve cells efficiently.

These results indicated that EZSPHERE is useful tool for creating large amounts of EBs with uniform size at low cost with simple method.

COI Disclosure Information

Hiroki Sato

No relevant conflicts of interest to declare.

This study was performed as a part of the JST (Japan Science and Technology Agency) project "Research Center Network for Realization of Regenerative Medicine".