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

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Abstract

Human iPS (induced pluripotent stem) cells have high potential applications in regenerative medicine and drug discovery with their ability of differentiating into a wide variety of cell types.Suspension culture of iPS cell aggregates, named as embryoid bodies (EBs), is one of effective methods for propagation and differentiation of the iPS cells. Furthermore, size and uniformity of the EBs are known to be one of critical factors affecting the differentiation efficiency. However, there are still technical limitations in the generation method of large-number of EBs with uniform size by simple and easy handling. To solve such problems, we attempted to apply novel micro-fabricated culture vessels (named EZSPHERE), on which large-number of micro-wells are solely created by laser, followed by low-cell-adhesive coating. The diameter and depth of each micro-well can be altered around 200-1,000 and 100-400 micrometer, respectively. We confirmed that the EZSPHERE is very useful for generating large-number of uniformly-sized EBs, when we inoculate 2.3 x 10^5 iPS cells into a standard type of the EZSPHERE (35 mm dish with approximately 2,400 micro-wells) in differentiation medium. After cultivation for 4 days, a typical Gaussian distribution was obtained for diametric size (108.3± micrometer) of the generated EBs with the total number of over 2,200. It was found that shape of the micro-wells is suitable for gathering inoculated cells and most of the EBs were formed within 3-6 hours. In addition, it was also confirmed that the obtained EBs could propagate at a good rate and maintain uniformity in growth medium. Differentiation tendency of the EBs was also confirmed by induction into cardiomyocyte or nerve cells. These results indicate that EZSPHERE is a useful tool for the controlled large-scale generation of EBs with uniform size and the differentiation capacity in a reproducible manner. This study was performed as a part of the AMED (Japan Agency for Medical Research and Development) project “Research Center Network for Regenerative Medicine”

Introduction

Novel micro-fabricated vessels: EZSPHERE

Methods and Materials

IPS cells & media

IPS cells: Human iPS cell lines 201B7 and 293G1 (passage until 50), were used for this study.

On-feeder culture medium: PRIMeE medium (REPROCELL), which used for iPS cell culture on S2 feeder cells with mytomycin C for inactivation.

Feeder-free culture medium: mTeSR1 medium (STEMCELL Technologies) was used for iPS cell culture on Matrigel (CORNING) or Laminin-521 (BioLamina).

Results

Figure 1. High efficient generation of EBs with uniform size on the EZSPHERE

Figure 2. EB size control with micro-well sizes or inoculating cell densities

Figure 3. Improvement of EB viability by optimizing culture condition on the EZSPHERE

Figure 4. Efficient proliferation of EBs in undifferentiating condition on the EZSPHERE

Figure 5. High performance of maintaining undifferentiation state on the EZSPHERE

Figure 6. Differentiation potency of EBs evaluated by neural cell induction

Figure 7. Differentiation potency of EBs evaluated by cardiac differentiation

Summary

The novel microfabricated vessels EZSPHERE, produced by laser-fabrication of usual plastic dishes and plates, are useful for mass-production of EBs with uniform size. We confirmed that the obtained EBs could propagate at a good rate and maintain uniformity in growth medium. Differentiation tendency of the EBs was confirmed by induction into cardiomyocyte or nerve cells. These results indicate that EZSPHERE is a useful tool for the controlled large-scale generation of EBs with uniform size and differentiation capacity in a reproducible manner.

Considering utilization of iPS cells for regenerative medicine, developing large-scale and efficient iPS cell producing techniques are required. In this study, we demonstrated that the novel micro-fabricated culture vessels, EZSPHERE, enable to culture of EBs for both cell expansion and differentiation processes as well as useful tool for the controlled large-scale generation of EBs with uniform size in a reproducible manner and easy handling. Furthermore, we are trying to attempt EZSPHERE techniques to more large-scale culture in the future.