Application Note

CELLFLOAT

Data courtesy of Specially Appointed Associate Prof. Matsui, The University of Osaka.

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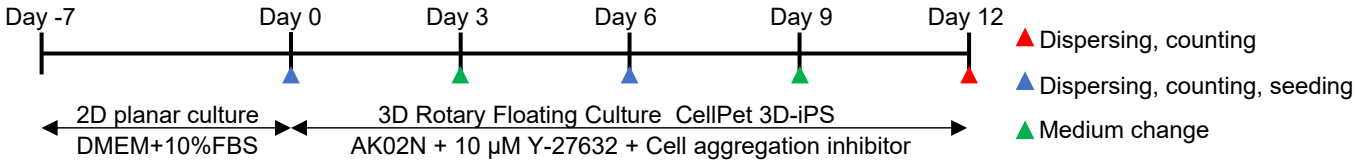
Spheroid culture of human mesenchymal stem cells using CellPet 3D-iPS

With the development of cell therapy using human mesenchymal stem cells, mass culture technologies are being developed to ensure a stable supply of human mesenchymal stem cells. Currently, 2D culture using multilayer flasks and 3D suspension culture using microcarriers made of various materials have been proposed. On the other hand, spheroid culture of human mesenchymal stem cells, such as iPS cells, has also been used, but it is not as popular as iPS cell spheroid culture due to the nature of mesenchymal stem cells themselves. Therefore, we performed spheroid culture of human mesenchymal stem cells using CellPet 3D-iPS in a 3D rotary floating culture. In the process of examining various culture media, excessive aggregation of human mesenchymal stem cells was observed during the rotary floating culture. When Company A's cell aggregation inhibitor reagent was added, spheroids of uniform particle size were formed without large agglomerates forming until 6 days after seeding after monodispersion. On day 6 of culture, the spheroids were monodispersed with a dispersing reagent, the cells were divided into two equal portions and transferred to two culture vessels, and the culture was continued until day 12. The number of cells reached approximately 8 times the initial seeding number.
The expression rate of CD44, a surface positive marker of mesenchymal stem cells, and CD34, a negative marker, was evaluated by flow cytometry and was unchanged before and after rotary floating culture.
This indicates that spheroid culture using CellPet 3D-iPS can be cultured while maintaining the properties of mesenchymal stem cells.

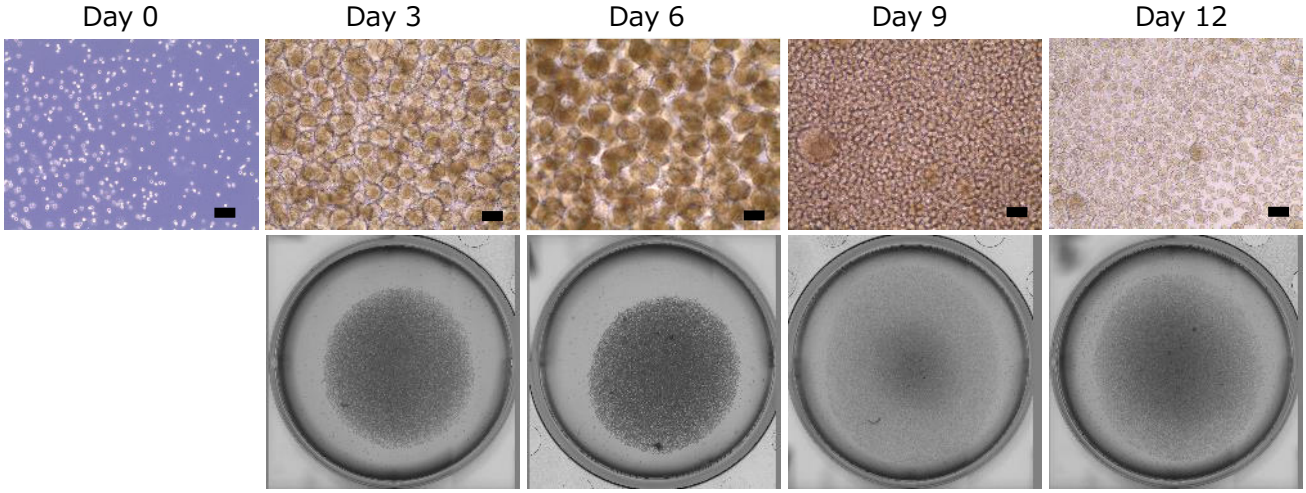
METHOD

- Cell type:Human bone marrow-derived mesenchymal stem cell immortalized cell line UE7T-13 (JCRB Cell Bank)
- 3D rotary floating culture medium: AK02N (Ajinomoto) + 10 μM Y-27632 (Wako) + cell aggregation inhibitor reagent (Company A)
- Vessel: Disposable culture vessel 50 ml (JTEC Corp.) / Rotational speed: 40 rpm
- Cell count: Countess 3 (ThermoFischer) / Imager: Cell3iMager (SCREEN)
- Flow cytometer: Attune Flow Cytometer (ThermoFischer),CD34 antibody (BD, Clone 581), CD44 antibody (BD, Clone L178)

Schedule for medium changing



RESULTS



Upper: Phase contrast microscopy image of human mesenchymal stem cell spheroids (Bar = 200 μm)
Bottom: Spheroid (6well plate) as seen by imager (Cell3iMager, SCREEN)

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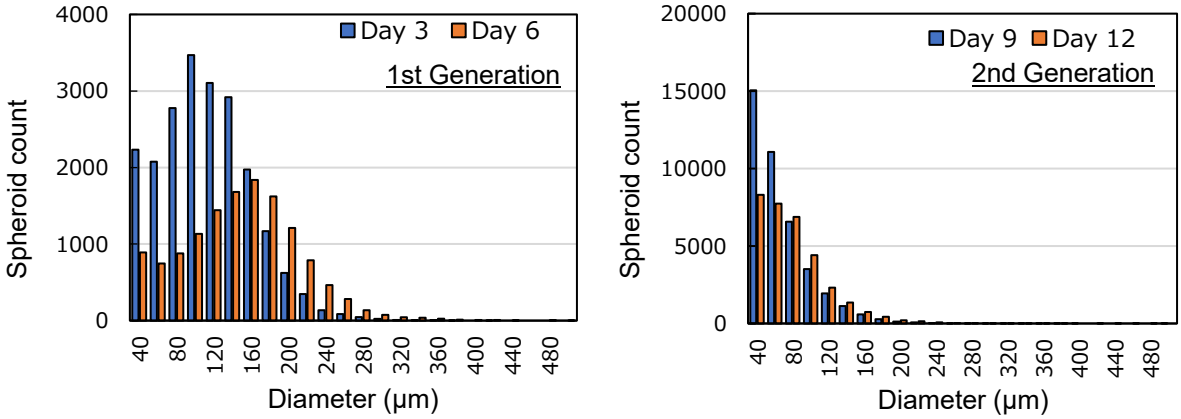
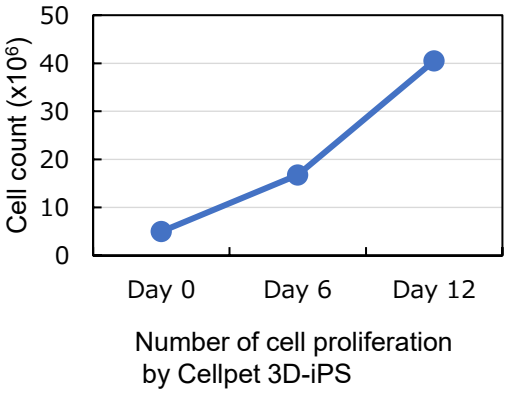
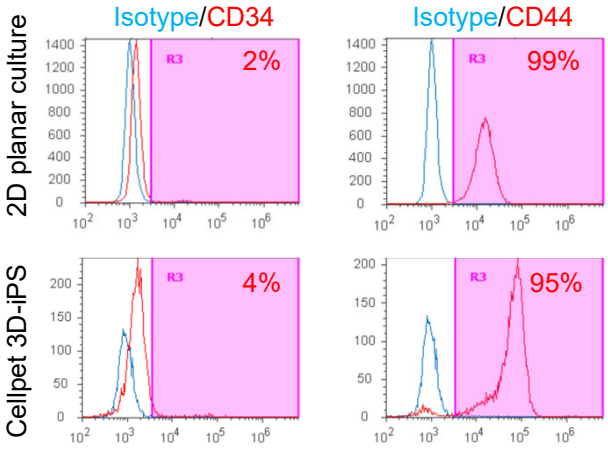


Particle diameter distribution of human mesenchymal stem cell spheroids



Number of cell proliferation by Cellpet 3D-iPS



Percentage of human mesenchymal stem cell markers expressed

2D planar cultured human mesenchymal stem cells were monodispersed and seeded into CellPet 3D-iPS culture vessels to 3D rotary floating culture. Phase contrast microscopy images and imager analysis showed that almost uniform spheroids with a diameter mode of about 100 μm were formed on Day 3. At Day 6, the diameter mode was about 160 μm, indicating that the spheroids were growing as the incubation period was extended. After monodispersion of spheroids by enzymatic dispersion on Day 6, they were evenly divided and transferred to two culture vessels for 3D rotary floating culture. Microscopic images and imager analysis on Day 9 and Day 12 showed that the spheroid diameter mode was less than 60 μm, but the particle size increased from Day 9 to Day 12, indicating that the spheroids grew as the number of incubation days increased. The cell counts on Day 6 and Day 12 were 16.8 x 10^6 cells (3.4x) and 40.5 x 10^6 cells (8.1x, total of two culture vessels), respectively, compared to seeding cell number 5.0 x 10^6 on Day 0. The expression rate of surface markers was evaluated by flow cytometry before and after 3D rotary floating culture, and the positive marker CD44 was maintained at 95% and the negative marker CD34 was 4%, suggesting that the human mesenchymal stem cell spheroids can be cultured while maintaining their characteristics.

DISCUSSION

Human mesenchymal stem cell spheroids were successfully formed using the CellPet 3D-iPS which is 3D rotary floating culture system. Spheroids with almost uniform particle size were formed, with a maximum frequency of 100 μm and 160 μm on Day 3 and Day 6, respectively. The cell proliferation rate was 8.1-fold higher than the number of cells seeded on Day 0 by culturing cells up to Day 12. In the future, we will conduct differentiation induction experiments using human mesenchymal stem cell spheroids formed by CellPet 3D-iPS and evaluate the effect of initial seeded cell density on spheroid culture.

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