



Conclusions

30.0

20.0

10.0

0.0

• Spheroids of human bone marrow-derived mesenchymal stem cells were generated using CellPet 3D-iPS and were successfully induced to differentiate directly into cartilage tissue.

8.0

6.0

4.0

2.0

0.0

29.5

Day 25

6.2

cont. Medium only

Chondrogenic differentiation

3D rotational floating Culture Differentiation

Day 25 GAG content in tissue (µg/mg wet tissue)

- In the early stage of differentiation induction, differentiation started with uniform spheroids of about 80 µm in diameter, and by the end of culture, the spheroids gradually aggregate to form dense tissues with integrated adhesions between spheroids.
- The spheroid staining revealed that cartilage cavities were formed in 3D rotational floating culture in differentiation medium. Additionally, it was observed that elastin and collagen were better stained than those in static culture.
- After 25 days of 3D rotational floating culture in chondrogenic differentiation medium, the hyaluronic acid concentration in the medium and the amount of glycosaminoglycan in the tissue increased compared to the control medium, indicating that chondrogenic differentiation was promoted.
- These data suggested that 3D rotational floating culture was better for chondrogenic differentiation.

Experimental Methods

Cell type: Human bone marrow-derived mesenchymal stem cell immortalized cell line UE7T-13(JCRB Cell Bank) 3D rotational floating culture medium:

AK02N (Ajinomoto) +10 μM Y27632 (Wako) + Cell aggregation inhibitor reagent (Company A)

Static culture medium: AK02N (Ajinomoto)

47

Medium only

47

Hyaluronic acid concentration in supernatant of

3D rotational floating culture medium (ng/ml)

- 3D Rotational Flotation Chondrogenic Differentiation Medium:
 - Human Mesenchymal Stem Cell(hMSC) Chondrogenic Differentiation Medium BulletKit (Lonza) + TGFβ3 (SIGMA) + Cell aggregation inhibitory reagent (Company A)

Static Culture Chondrogenic Differentiation Medium: hMSC Chondrogenic Differentiation Medium BulletKit (Lonza) + TGFβ3 (SIGMA)

Vessel: Disposable culture vessel 50ml (JTEC Corp.) Rotational culture 40 rpm

Imager: Cell3iMager (SCREEN) Photography: EVOS (Thermo Fisher), Axio Observer (Zeiss) Plate reader: Infinite® 200 PRO (TECAN)

Staining: Alcian blue stain: Alcian blue solution (Wako) + Cologne echtrode solution (Mutoh Chemical)

Elastica-van Gieson stain: Van Gieson's solution (Wako) + Iron hematoxylin solution (Wako)

+Resorcinol fuchsin solution (Mutoh Chemical)

Measuring Kits :Hyaluronic acid concentration /DuoSet Hyaluronan (R&D), GAG amount:/GLYCOSAMINOGLYCAN (biocolor)



2-5-38 Saito-Yamabuki, Ibaraki, Osaka 567-0086, Japan



Contact Phone: +81-72-655-2786 Mail: info@j-tec.co.jp

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