Application note

CELLFLOAT

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

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Analysis of Exosome Secretion from Human Mesenchymal Stromal Cell Spheroids

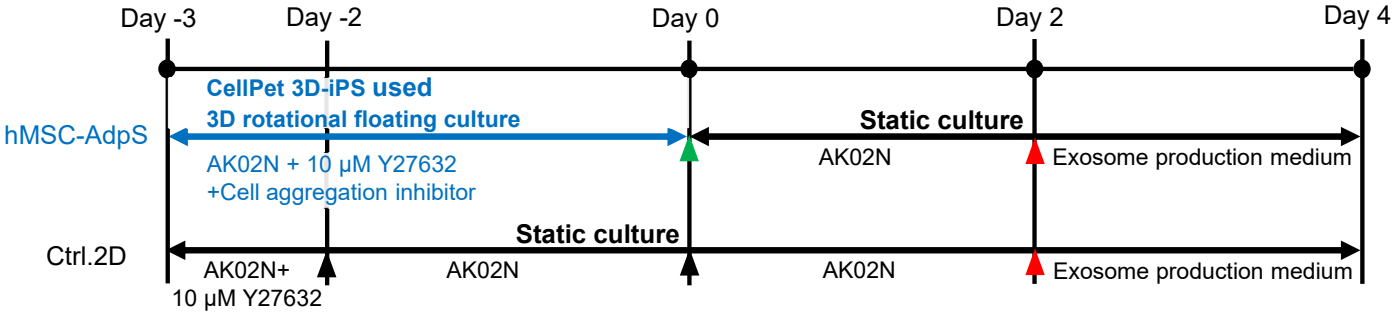
To widely implement cultured cell-based therapies in the field of regenerative medicine, it is necessary to develop technologies for the stable and large-scale production of functional human mesenchymal stromal cells (hMSCs).In this study, adipose-derived human mesenchymal stromal cell spheroids (hMSC-AdpS) were maintained using a three-dimensional rotational floating culture equipment, the CellPet 3D-iPS. Subsequently, the cells were transferred to static culture conditions to induce exosome production, and exosome secretion levels were quantitatively compared as a functional evaluation.

METHOD

- Cell type: Adipose-derived human mesenchymal stromal cells (seeding density: 1 × 10^5 cells/mL), expanded and maintained as spheroids using the CELLFLOAT CellPet 3D-iPS. After expansion culture, spheroids were enzymatically dissociated and seeded onto culture vessels.
- Maintenance Medium for Undifferentiated Cells: AK02N (Ajinomoto)
- Additives: Y-27632 (Fujifilm Wako), Cell aggregation inhibitor (Fujifilm Wako)
- Vessel: 12-well plates (Falcon) coated with iMatrix-511 (Nippi)
- Exosome Production Medium: EV-Up™ MSC Exosome Production Basal Medium including additives (Fujifilm Wako)
- Analysis: Human exosome quantification CD9 × CD63 ELISA kit (Cosmo Bio)

Culture Schedule

Note: Cells were seeded onto iMatrix-511-coated surfaces for static culture.

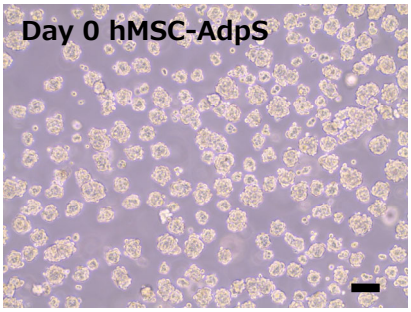


▲ : AK02N medium change

▲ : hMSC-AdpS were seeded in a planar vessel at the same cell density as Ctrl.2D using AK02N medium

▲ : Medium was changed to exosome production medium

RESULTS and DISCUSSION



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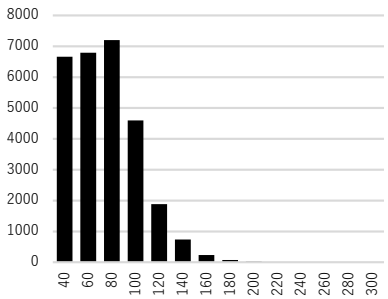
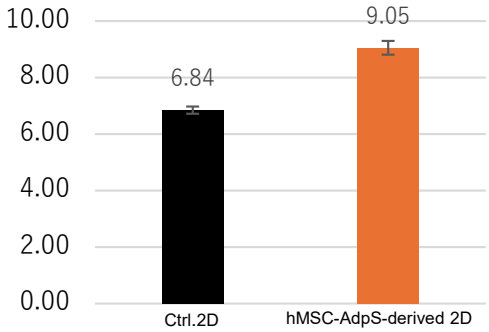


Particle Size (μm) and Count of hMSC-AdpS



Exosome Production per 1×10^6 Cells (pg)

- Using the CellPet 3D-iPS, a large-scale culture of spheroids derived from adipose-derived mesenchymal stromal cells was performed, followed by static culture for exosome production.
- The hMSC-AdpS spheroids had a uniform size distribution, peaking at approximately 80 μm in diameter. After enzymatic dissociation of the spheroids and subsequent static culture in exosome production medium, exosome yield was approximately 1.3 times higher compared to the control.
- These results suggest that spheroid formation using the CellPet 3D-iPS may enhance the exosome production capacity of hMSC-AdpS.

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