



Induction of Human iPSC-Derived Neural Crest Cells Using the CellPet 3D-iPS System

Our proprietary culture system, the CELLFLOAT series, is built around the CellPet 3D-iPS (3D-iPS), a 3D rotational floating culture device. It supports a wide range of research applications, including large-scale spheroid culture and organoid culture.

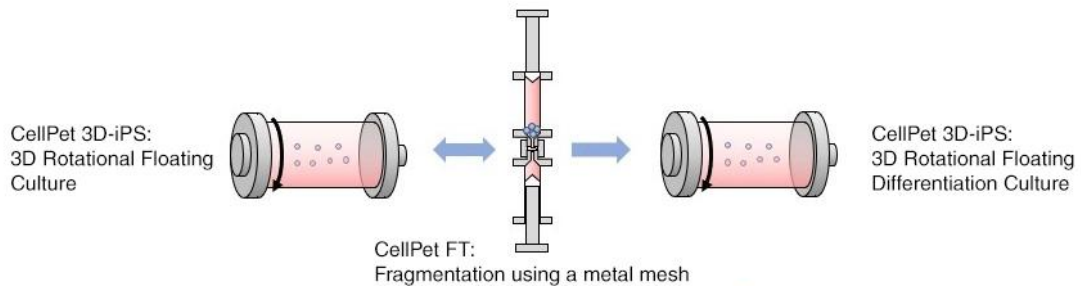
The CELLFLOAT series also includes the CellPet FT (FT), a device that fragments spheroids into smaller pieces. Alternating between the 3D-iPS and the FT allows 3D rotational floating culture to be repeated, which enables long-term culture of spheroids.

In this application note, we used the 3D-iPS and the FT to induce differentiation of iPSC spheroids. This process yielded iPSC-derived neural crest cell spheroids of uniform size that exhibited high expression levels of a differentiation marker. Differentiation efficiency was evaluated on days 7–9 based on CD271 expression.

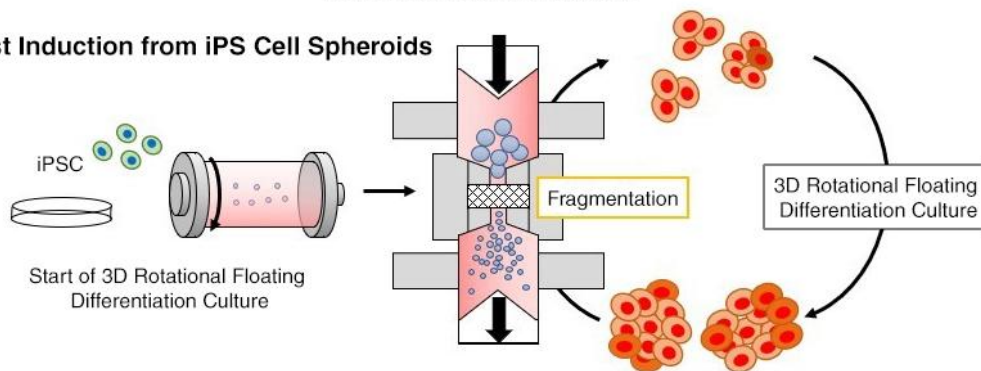
The CELLFLOAT system

Proprietary 3D Rotational Floating Culture Technology“CELLFLOAT”

System Overview

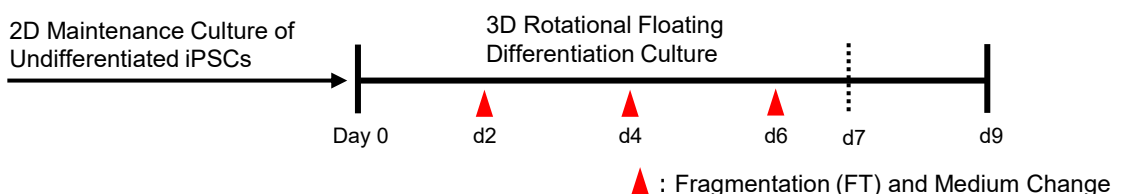


Neural Crest Induction from iPSC Cell Spheroids

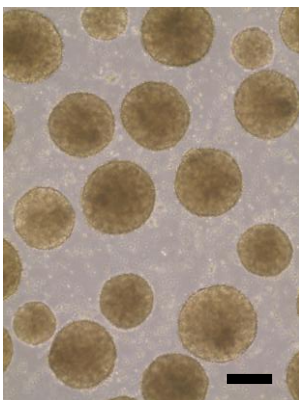
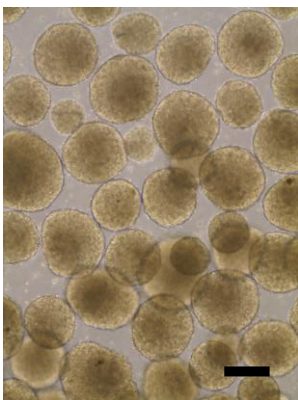
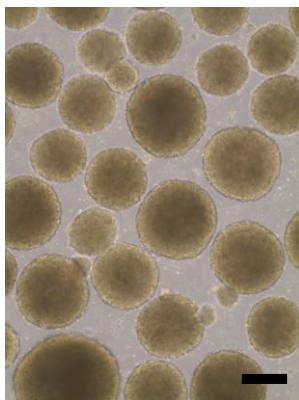
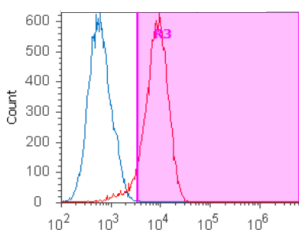
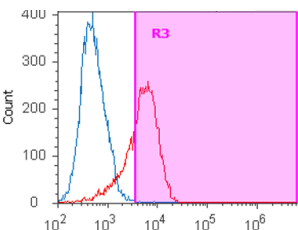
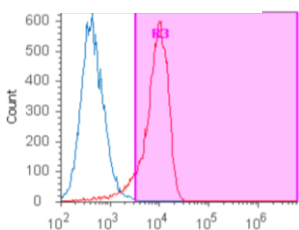


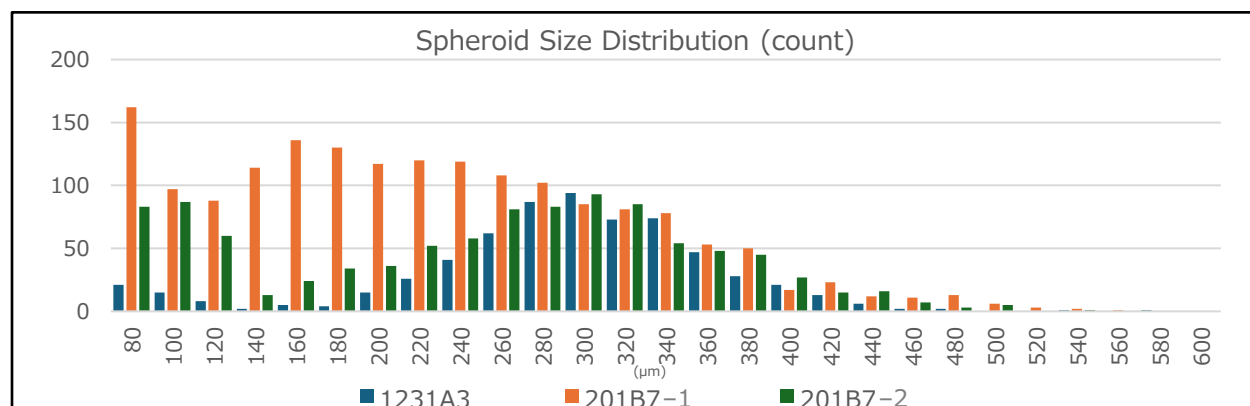
METHODS

- Cells: Human iPSC lines 1231A3 and 201B7 (seeding density: 5×10^5 cells/mL)
- Maintenance medium: AK02N (Ajinomoto)
- Materials: 60 mm dish (Falcon), iMatrix-511 (Nippi)
- Differentiation medium: STEMdiff Neural Crest Differentiation Kit (STEMCELL Technologies)
- Supplement: Y-27632 (FUJIFILM Wako Pure Chemical)
- Differentiation culture vessel: Plastic culture vessel for the CELLFLOAT system (10 mL)
- Flow cytometer: Attune Flow Cytometer (Thermo Fisher Scientific)
- Antibody: Anti-CD271 antibody (BD)
- Differentiation period: 7–9 days



RESULTS

Cell Lines Used	1231A3	201B7-1	201B7-2
Culture Period (days)	9	9	7
Spheroids Scale bar: 200 μ m			
CD271-Positive Cells(%) Blue line: Isotype control Red line: CD271	92.2 	70.1 	90.4 



DISCUSSION

In validation experiments using the iPSC lines 1231A3 and 201B7, 3D rotational floating culture combined with spheroid fragmentation using the CELLFLOAT system produced spheroids of highly uniform size, approximately 300 μ m in diameter. Notably, differentiation efficiency was very high, with more than 90% of cells positive for the neural crest cell marker CD271. These results suggest that precise control of spheroid size by this system may contribute to efficient nutrient supply and penetration of differentiation-inducing factors into the spheroids.

The CELLFLOAT system is a highly effective tool for the stable, large-scale culture of high-quality iPSC-derived neural crest cell spheroids, and it strongly supports innovation in differentiation protocols for regenerative medicine research.

