Application Note

CELLFLOAT

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

Publication Date: September 30, 2021

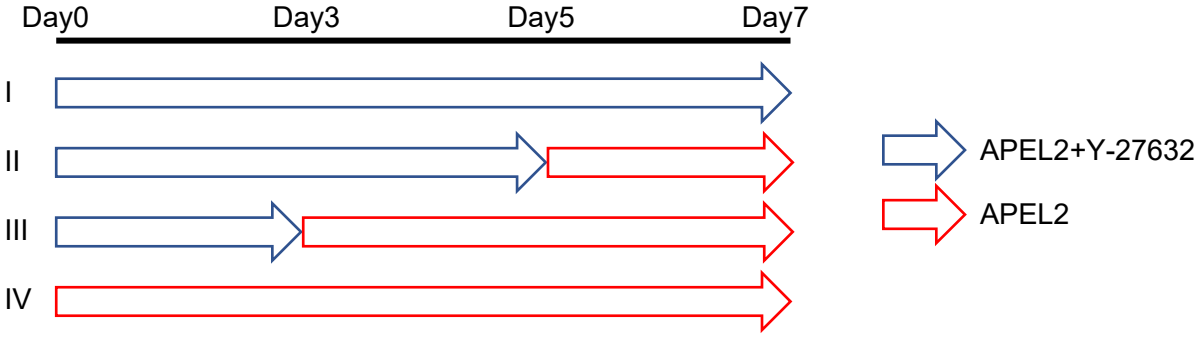
CellPet 3D-iPS Protocol

Trilineage differentiation derived human iPS cells

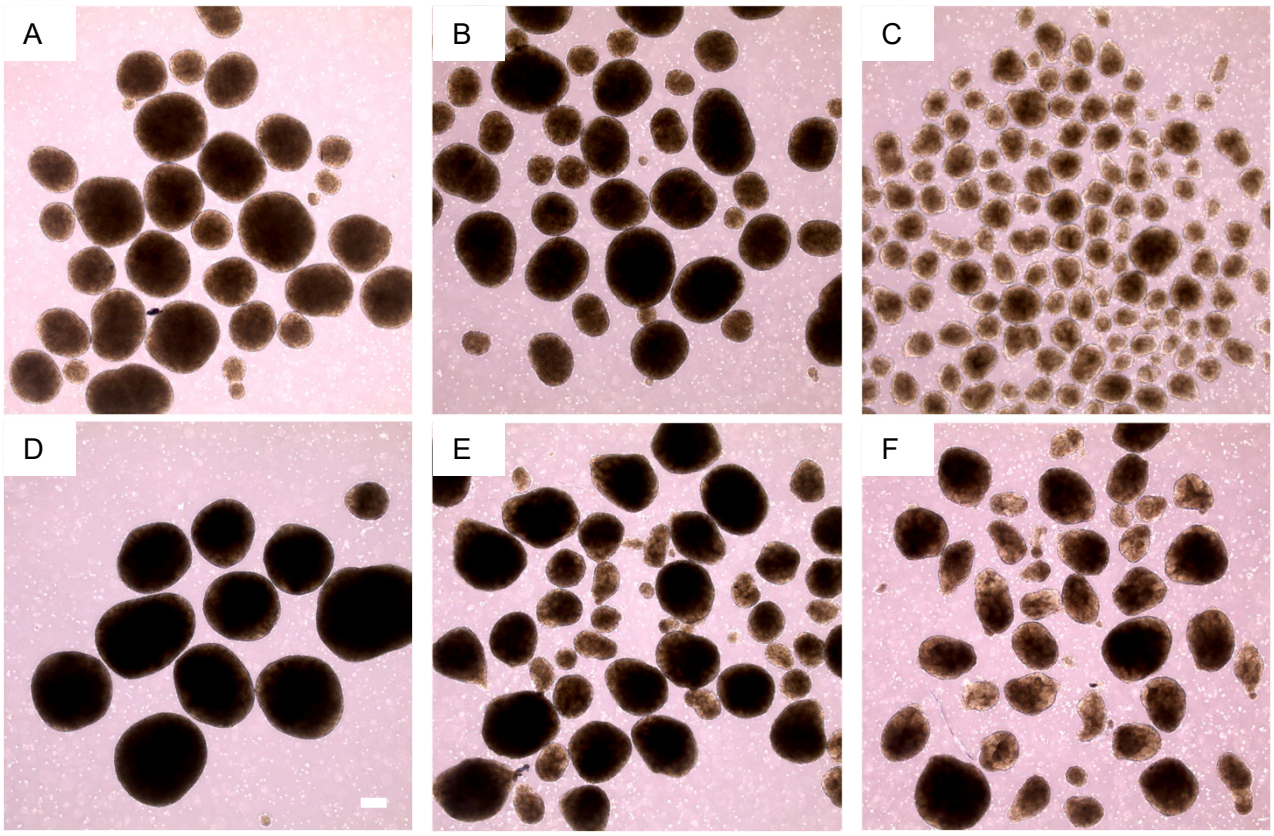
MATERIALS

Cell line: 253G1 (Riken BDR)
ECM coated: iMatrix511 (Nippi)
Medium: STEMdiff APEL2LI medium (STEMCELL Technologies), w/wo Y-27632 (Wako)
Seeding cell number; 1 x 10^5 cells/ml
Rotational speed: 40 rpm

Cultured condition



RESULT



Bar = 200μm

Figure 1
Cultured spheroids. A, B, C; Day5, D, E, F; Day7.
A and D; condition I, B and E; condition II, C and F; condition III
Condition IV did not make any spheroids.

CELLFLOAT system was observed to make spheroid using APEL2 medium with Y-27632 (Figure 1). However, the culture medium without Y-27632 (condition IV) did not make spheroid.

Application Note

Pluripotent marker

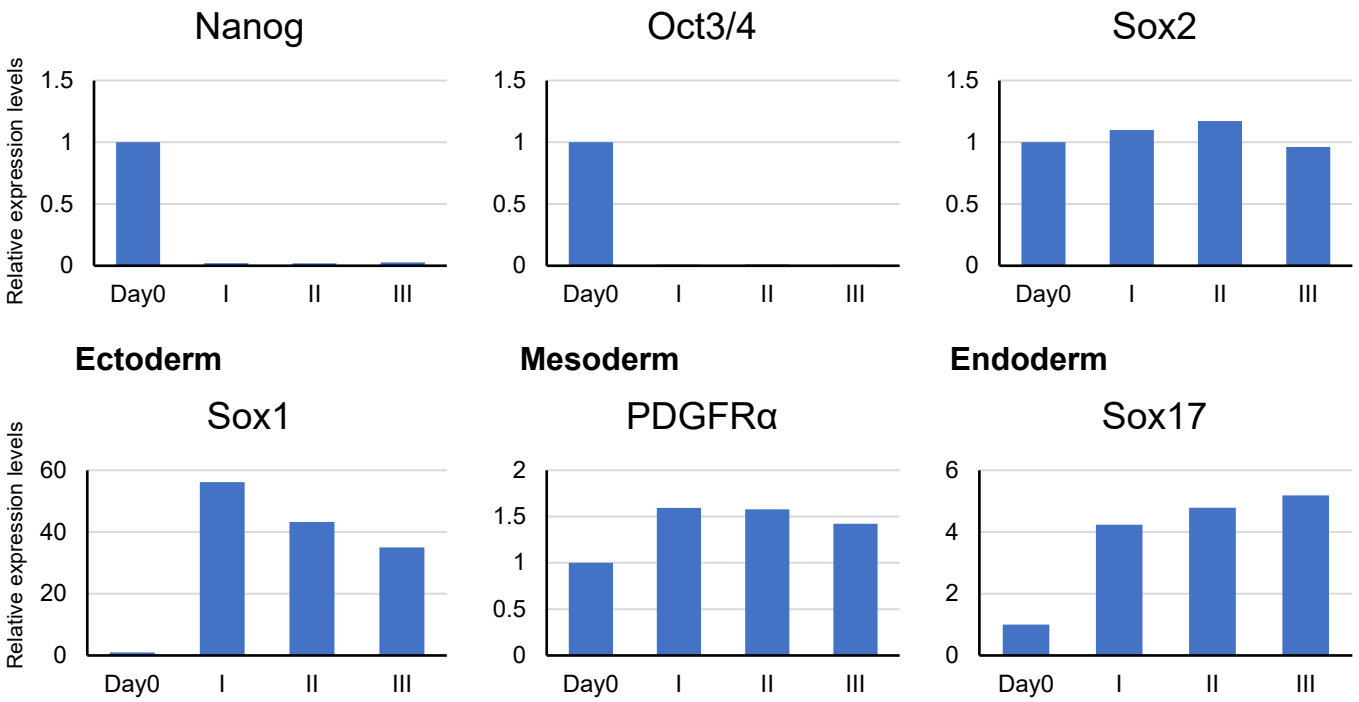


Figure 2
Quantitative PCR analysis. Gene expression levels were determined relative to 18S and presented as fold change relative to the expression in day 0 hiPSCs.

We analyzed mRNA expression by RT-qPCR (Figure 2). The pluripotent marker, Nanog and Oct3/4, was decreased in APEL2 conditions. In addition, trilineage markers, Sox1 (ectoderm), PDGFRα (mesoderm) and Sox17 (endoderm), were increased in these conditions.

CONCLUSIONS

For spheroid formations, it is essential to add Y-27632 until at least third day. However, after spheroid formation, it is possible to remove Y-27632 and maintain the spheroid. Depending on the presence or absence of Y-27632, the size of the spheroid differs.

We observed the spheroids formed had reduced expression of pluripotent markers by qPCR methods. The expression of the Yamanaka factor, Sox2, was not altered, which is also known as an early neuroectodermal marker. In addition, the expression of tri-differentiation markers was increased by the present culture conditions.

With these results, we were able to demonstrate how the CELLFLOAT system can be used to induce differentiation. The different efficiency of differentiation may be due to the lack of differentiation activator. It is expected that a certain activators and small molecule compounds will be required to preferentially induce differentiation into each lineages.

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