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Article

Easy Cell Detachment and Spheroid Formation of Induced Pluripotent Stem Cells Using Two-Dimensional Colloidal Arrays

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Experimental Section

Materials

Ethylenediaminetetraacetic acid (EDTA, 0.5 M, pH 8.0), phosphate buffer solution (PBS)(-), StemFit AK02N, CultureSure Y-27632, and 4% paraformaldehyde in PBS were purchased from Wako Pure Chemical Industries (Osaka, Japan). Alexa Fluor 488 antihuman stage-specific embryonic antigen (SSEA)-4 antibody (cat. no.: 330412), Alexa Fluor 647 antihuman SSEA-4 antibody (cat. no.: 330407), and Alexa Fluor 647 mouse IgG3, κ isotype ctrl antibody (cat. no.: 401321) were purchased from BioLegend (San Diego, CA, USA). The iMatrix-511 solution was purchased from Nippi (Tokyo, Japan). TrypLE Express Enzyme 1× was purchased from Thermo Fisher Scientific K.K. (Tokyo, Japan). Veritas (Tokyo, Japan) supplied the STEMdiff Trilineage Differentiation Kit. All reagents were used without further purification.

Coculture of induced pluripotent stem cells (iPSCs) and mesodermal cells

StemFit AK02N (0.2 mL/cm^2) culture medium with Y-27632 (10 μM) and iMatrix-511 solution (2.5 μL/mL) was added to a 35-mm culture dish, and iPSCs (cell line 201B7, 260 cells/cm^2) were seeded. The cells were cultured at 37°C in an atmosphere containing 5% CO_2. At 24 h after cell seeding, the culture medium was replaced with StemFit AK0$_2$N without Y-27632 or iMatrix-511 solution. After 6 days of culture from iPSC seeding, mesoderm cells (1110 cells/cm^2) were seeded. The mesoderm cells were created using a STEMdiff Trilineage Differentiation Kit according to the manufacturer's instructions and cultivated for 1 day at 37°C in a 5% CO_2 environment. After removing the medium and rinsing the cells with PBS(-), 1-mL TrypLE-EDTA (1:1 volume mixture of TrypLE Express Enzyme 1× and 0.5-mM EDTA solution) was added, and the cells were incubated at 37°C for 2 min. The TrypLE-EDTA solution was removed, and the cells were washed with PBS(-). StemFit AK02N with Y-27632 at 4°C was added and gently pipetted several times. For polystyrene culture dishes that were not coated with colloids, the cells were detached by scraping the surface with a cell scraper.

Flow cytometry analysis

The cell suspension was centrifuged and treated with 4% paraformaldehyde before being centrifuged again and washed with PBS(-). A solution of PBS(-) with bovine serum albumin (1 wt%), EDTA (0.25 mM), and Alexa Fluor 488 antihuman SSEA-4 antibody (0.25 μg/mL) was added. The fluorescent staining process was performed at room temperature for 30 min, followed by centrifugation and washing with PBS(-). The same procedure was used to prepare samples stained with isotype antibodies. A flow cytometer (BD Accuri C6 Plus; Becton, Dickinson and Company, New Jersey, USA) was used to determine the proportion of iPSCs in the cell suspensions. Pure iPSCs stained with anti-SSEA-4 antibodies were used as a positive control, and iPSCs stained with isotype antibodies were used as a negative control.

Results

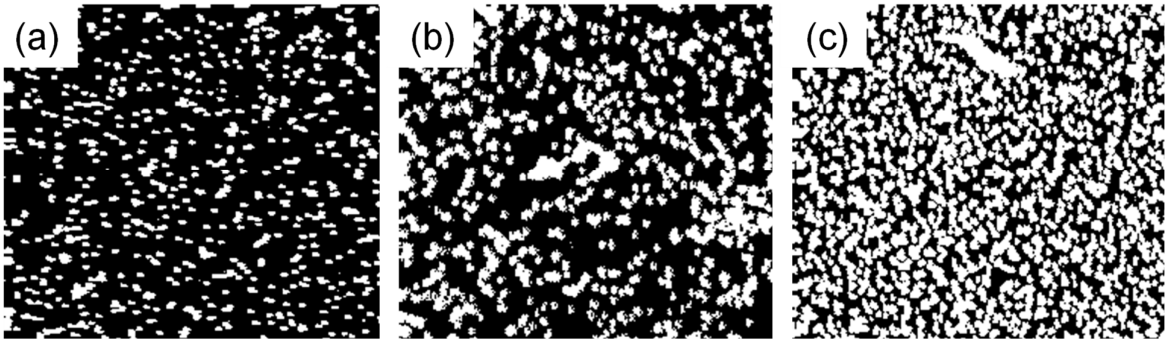

Figure S1. Binarized atomic force microscopy images of colloid arrays. The colloid arrays were prepared using (a) 2 wt%, (b) 5 wt%, and (c) 10 wt% core–shell colloid dispersion.