Supplementary Data

Supplementary Materials and Methods

Human pluripotent stem cell differentiation

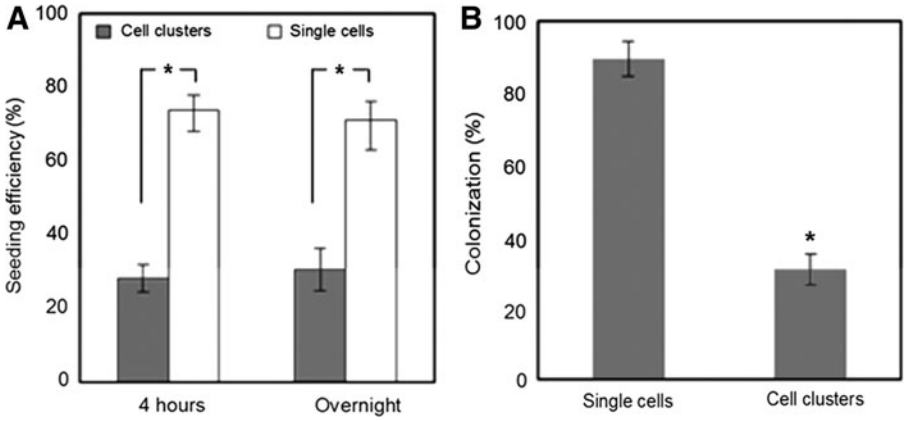
Definitive endoderm differentiation. Differentiation of human pluripotent stem cells (hPSCs) harvested from microcarriers to definitive endoderm (DE) was performed as described.[1,2] Briefly, cells on microcarriers were dissociated into single cells with Accutase and seeded on Matrigel-coated dishes with mTeSR medium supplied with 10 μM ROCK inhibitor and cultured for 1 day. Differentiation was carried out in RPMI (Gibco) supplemented with 100 ng/mL activin A (R&D Systems) from days 0 to 4 with various amount of knockout serum replacer (KSR; Life Technologies), that is, no KSR (day 1), 0.2% KSR (day 2), and 2% KSR (days 3–4).

Mesoderm differentiation. Before mesoderm differentiation, hPSCs were harvested from beads and plated on Matrigel-coated dishes as described above. Mesoderm differentiation was carried out in RPMI medium supplemented various amount of activin A, BMP4, and KSR for 5 days.[2,3] On day 1, RPMI was supplemented with 100 ng/mL activin A. For the second and third days, the medium was replaced by RPMI containing 0.2% KSR, 10 ng/mL activin A, and 10 ng/mL BMP4 (R&D Systems). Medium with the same composition was utilized on days 4 and 5 of the differentiation except that the KSR concentration was increased to 2%.

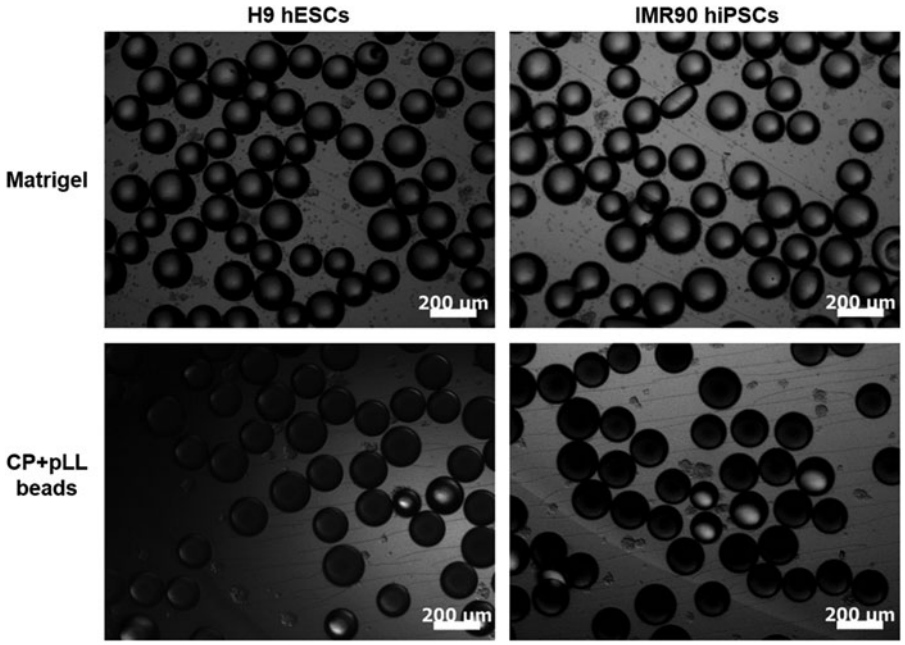
Neuroectoderm differentiation. Cells collected and separated from microcarriers were plated on Matrigel-coated dishes for ectoderm differentiation as described before.[2,4] On day 1, mTeSR was replaced by neural induction medium [NIM: DMEM/F12:Neurobasal medium (1:1), 1×N2 supplement, 1×B27 supplement without vitamin A (all from Life Technologies) and 2 mM Glutamax (Mediatech)]. The next day, cells were treated with collagenase IV (Life Technologies) and seeded in low-attachment dishes (BD Biosciences) to form embryoid bodies (EBs). EBs were cultured in NIM for 4 days with daily medium change. Then, the medium was switched to neural proliferation medium [NPM: DMEM/F12:Neurobasal (1:1), 0.5×N2 supplement, 0.5×B27 supplement, 2 mM Glutamax and 20 ng/mL FGF2 (R&D Systems)] and the cells were cultured for 3 days with daily medium change. After that, EBs were replated on Matrigel-coated dishes and cultured for another 2 days before characterization.

References

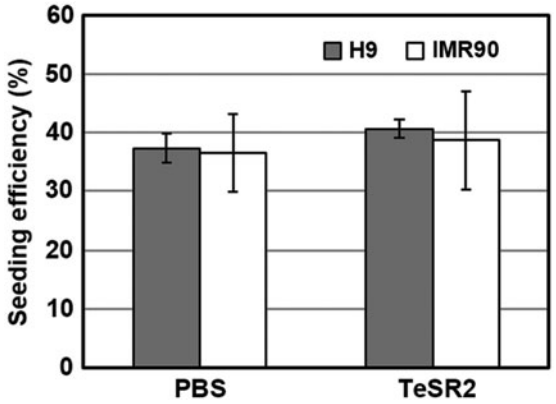
1. Lock, L.T., and Tzanakakis, E.S. Expansion and differentiation of human embryonic stem cells to endoderm progeny in a microcarrier stirred-suspension culture. Tissue Eng Part A **15,** 2051, 2009.
2. Wu, J., and Tzanakakis, E.S. Contribution of stochastic partitioning at human embryonic stem cell division to NANOG heterogeneity. PLoS One **7,** e50715, 2012.
3. Jing, D. Investigation of the cardiogenic differentiation of human pluripotent stem cells in static cultures and stirred-suspension bioreactors [Ph.D. 3423476]. State University of New York at Buffalo,New York, United States, 2010.
4. Nat, R., Nilbratt, M., Narkilahti, S., Winblad, B., Hovatta, O., and Nordberg, A. Neurogenic neuroepithelial and radial glial cells generated from six human embryonic stem cell lines in serum-free suspension and adherent cultures. Glia **55,** 385, 2007.



SUPPLEMENTARY FIG. S1. Seeding of IMR90 human induced pluripotent stem cells (hiPSCs) onto Matrigel-coated microcarriers as single cells or clusters. **(A)** Higher efficiency was noted when single cells were seeded instead of clumps ($^*p < 0.05$). **(B)** Colonization of microcarriers after seeding human pluripotent stem cells as single cells or clusters ($^*p < 0.05$).



SUPPLEMENTARY FIG. S2. Microcarrier seeding without ROCK inhibitor. H9 human embryonic stem cells (hESCs) and IMR90 hiPSCs were seeded as single cells without ROCK inhibitor on Matrigel-coated (top row) or CP + poly-L-lysine (pLL) (peptide conjugated/pLL-coated; Fig. 4) beads. Twelve hours after seeding, beads were mostly devoid of cells and only small clusters were observed with limited cell viability. Scale bars: 200 µm.



SUPPLEMENTARY FIG. S3. Seeding efficiency and equilibration medium. Human H9 and IMR90 cells were seeded on CP + pLL microcarriers equilibrated with phosphate-buffered saline (PBS) or TeSR2 for 30 min. The measured seeding efficiencies for each type of cell were similar regardless of the medium or buffer used for equilibration of the beads.