Reproducible Production of Well-defined and Uniform Embryoid Bodies using Corning® Elplasia® Plates

Application Note

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Introduction

Human induced pluripotent stem cell (hiPSC)-derived embryoid bodies (EBs) are three-dimensional multicellular aggregates that show significant advantages in hiPSC differentiation efficiency and organoid establishment[1]. When hiPSCs are cultured in a suspension environment with relevant media, cells spontaneously differentiate and form EBs comprising the three developmental germ layers: endoderm, mesoderm, and ectoderm. Compared with conventional 2D planar hiPSC culture, this multicellular structure better recapitulates *in vivo* cell-cell interactions, improves intercellular communication, and enhances substance exchange[2]. 3D cell culture systems such as spheroids and organoids are gaining traction in drug discovery applications as improved models of disease. iPSC-derived organoids are invaluable resources that can provide more predictive screens to aid in personalized medicine. It has been demonstrated that the quality of EBs can impact the stability and feasibility of iPSC-derived organoids[3,4]. Therefore, to further advance organoids for functional screening, the need for high throughput production of homogenous EBs with uniform shape and size has emerged.

A variety of techniques can be used to form EBs from iPSCs with EB-specific medium. Conventional methods such as static suspension culture in dishes or hanging-drops can lead to variable EB production and unstable EB-derived differentiation[5,6]. Newer platforms based on size-defined micropatterned wells, also known as "micro-space" or "microcavity" culture, have been developed to generate EBs with uniform size and morphology using single hiPSCs[7]. The Corning Elplasia plate is an example of a microcavity plate that can produce replicate spheroids of uniform size in mass quantities. The plate is black with a clear bottom and features the Corning Ultra-Low Attachment (ULA) surface, a biologically inert hydrogel that supports the self-aggregation of cells to form 3D structures. Corning Elplasia plates are available in 6- to 384-well formats enabling reproducible scale-up of EBs to support basic research to screening applications. In this study, we demonstrate how the Corning Elplasia plate can enable spontaneous formation of spheroids from hiPSCs and subsequent reproducible production of uniform differentiated EBs (Figure 1).

Materials and Methods

Spheroid Formation

Corning Elplasia 24-well round bottom plates with ULA surface (Corning Cat. No. 4441) were pre-wet prior to seeding cells by adding 1 mL mTeSR™1 medium (STEMCELL Technologies Cat. No. 85850) per well and centrifuging at 500 x g for 1 minute to remove trapped air. Human iPSCs (DYR0100; ATCC® Cat. No. ACS-1011™) were harvested from a Corning Matrigel® matrix-coated 6-well plate (Corning Cat. No. 354671) and cells counted using a Corning Cell Counter (Corning Cat. No. 6749). Cells were seeded into plates at increasing densities of 100, 250, 500, and 1000 cells per microwell in a volume of 1 mL mTeSR1 medium containing 10 µM Y27632 (R&D Systems Cat. No. 1254) per well. Cells were incubated and media (mTeSR1 + Y27632) was changed daily for 3 days. The study was independently repeated three times.

EB Formation

On day 3, the media was changed to 1 mL AggreWell™ EB Formation Medium (STEMCELL Technologies Cat. No. 05893) containing 10 µM Y27632. Cultures were incubated for an additional 3 days with daily media (EB Formation Medium + Y27632) change.

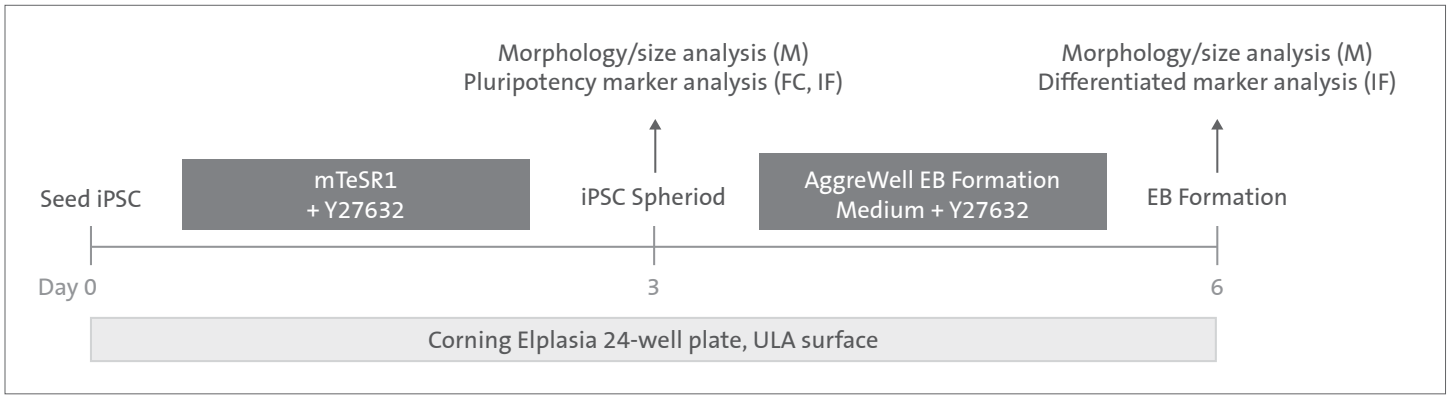

Figure 1. Schematic of the iPSC spheroid and EB formation protocol.

Spheroid and EB Consistency

On days 3 and 6, 1 µL of 1 mM Calcein AM (Corning Cat. No. 354216) was added to each well and incubated for 15 minutes. Once cells were completely stained, the 3D structures were imaged with the Olympus IX53 microscope to assess size and morphology (M). Spheroid and EB diameters were measured with Olympus cellSens Standard software.

Spheroid and EB Surface Marker Expression

On day 3, iPSC spheroids were stained with SSEA4 (BD Cat. No. 560128) and Oct4 (Abcam Cat. No. ab19857) antibodies for flow cytometry (FC) and immunofluorescence analysis (IF), respectively. For flow cytometry analysis, spheroids were digested into single cells with Accutase® (Thermo Fisher Cat. No. A1110501). The BD Accuri™ flow cytometer was used. On day 6, EBs were stained with alpha-fetoprotein (C3; BD Cat. No. 563002), alpha smooth muscle Actin (Alexa Fluor® 647; Abcam Cat. No. ab196919), and Alexa Fluor 555 β-Tubulin, Class III (BD Cat. No. 560339) antibodies to characterize endoderm, mesoderm, and ectoderm layer formation, respectively. Immunofluorescence data was captured used the BioTek Cytation™ 5.

Results and Discussion

Uniform iPSC Spheroid Formation

To assess the ability of Corning® Elplasia® plates to generate uniform iPSC spheroids, DYR0100 cells were seeded at four different densities into plates and incubated for 3 days with daily media changes. Once iPSC spheroids were generated, they were stained with Calcein AM and viewed using brightfield and fluorescence microscopy to examine morphology and size. The data show single, circular iPSC spheroids formed in each microcavity (Figure 2) that were consistent in size using diameter measurements (Figure 3). At each seeding density, spheroid diameter increased with longer culture duration from 1 to 3 days. The spheroids could also be size-controlled by adjusting the initial seeding density. Spheroid size ranged from 160 to 275 µm with seeding densities of 100 to 1000 cells per microwell at day 3 (Figure 3).

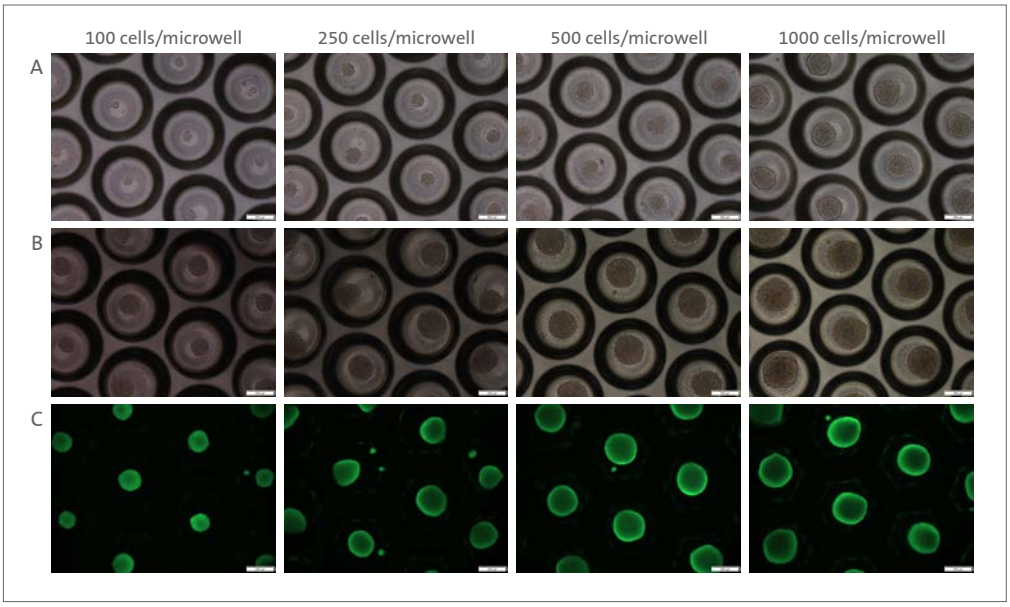

Figure 2. Uniform, single iPSC spheroids formed in each microcavity. Representative images of a digitally zoomed field from one well of a 24-well Corning Elplasia plate using brightfield (A = Day 1; B = Day 3) and fluorescence (C = Day 3) imaging. DYR0100 cells were initially seeded at four different densities into the plate (100 to 1000 cells/microwell). Images were taken with Olympus IX53 microscope using a 100X objective. Scale bar is 200 µm.

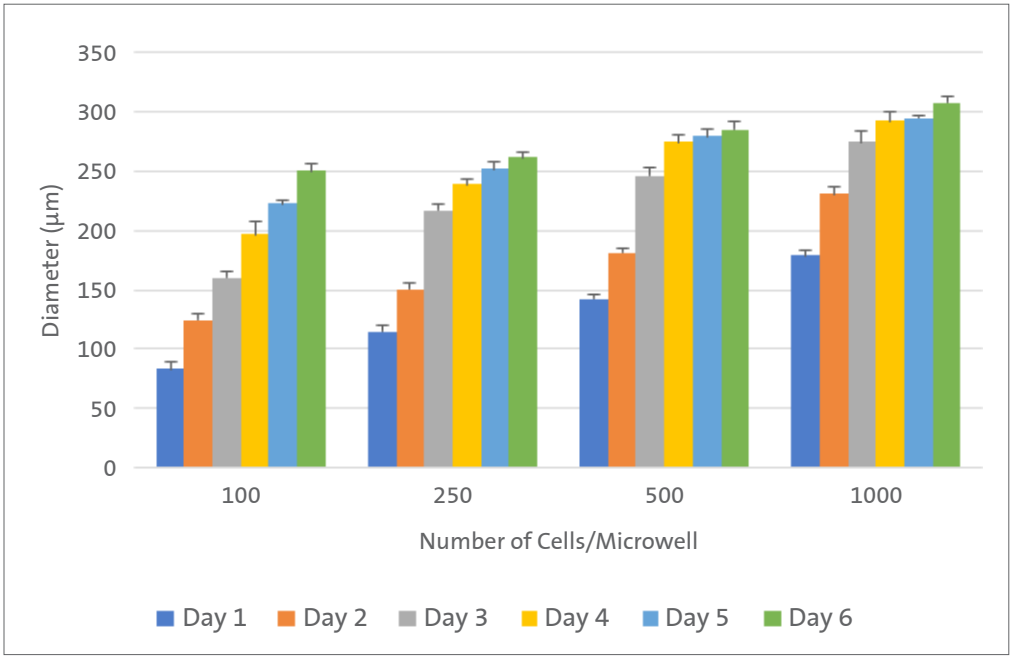

Figure 3. iPSC spheroids and EBs formed in each microcavity were consistent in size. DYR0100 cells were seeded at four different densities (100 to 1000 cells/microwell) into the Corning Elplasia plate and spheroid (days 1 to 3), and EB (days 4 to 6) diameters were measured using Olympus cellSens Standard software. Data shown with standard error of the mean from 20 independent microwells.

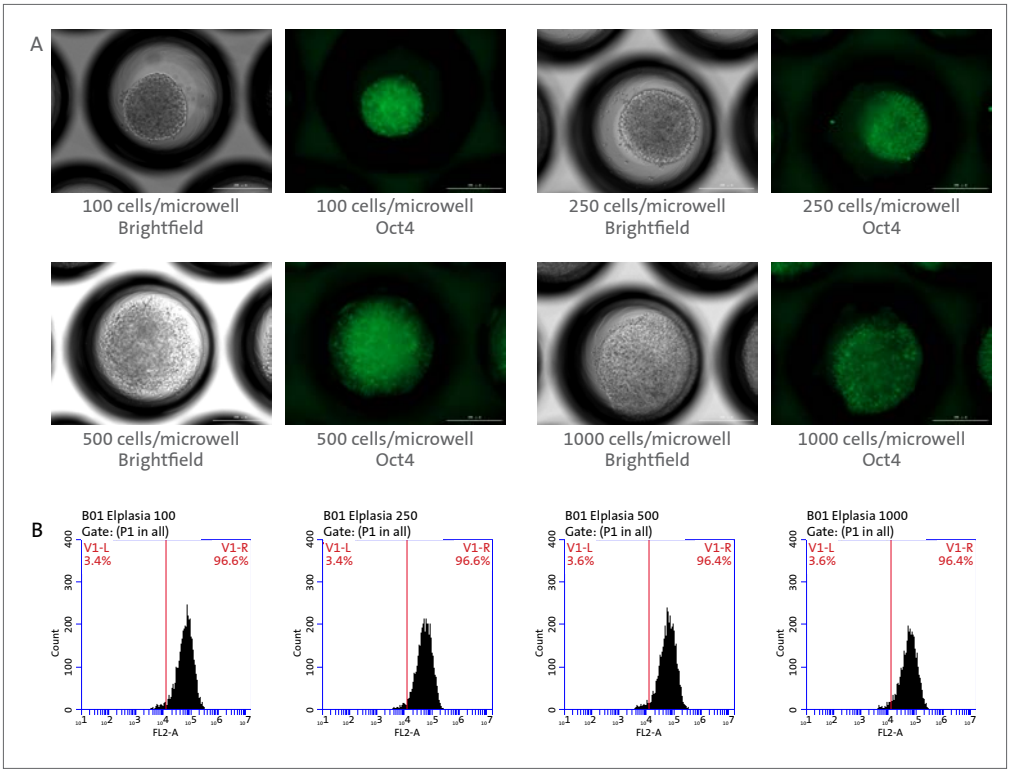

Figure 4. iPSC spheroids retained pluripotency marker expression. (A) Representative images of Oct 4-stained spheroids in individual Corning Elplasia plate microcavities. Images were taken using the BioTek Cytation 5 with a 400X objective. Scale bar is 200 µm. (B) Representative profile of flow cytometry analysis of SSEA+ cells in the iPSC spheroids using the BD Accuri flow cytometer. Data shown is for iPSC spheroids generated with 4 different cell seeding densities (100 to 1000 cells/microwell).

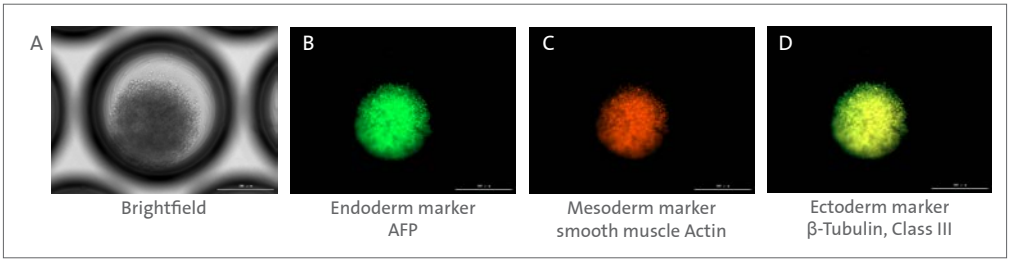

Figure 5. iPSC-derived EBs expressed typical germ layer-specific markers on day 6. (A) Brightfield, (B) Alpha-fetoprotein (endoderm), (C) smooth muscle Actin (mesoderm), (D) β-Tubulin, Class III (ectoderm). Images were taken using the BioTek Cytation 5 with a 400X objective. Scale bar is 200 µm.

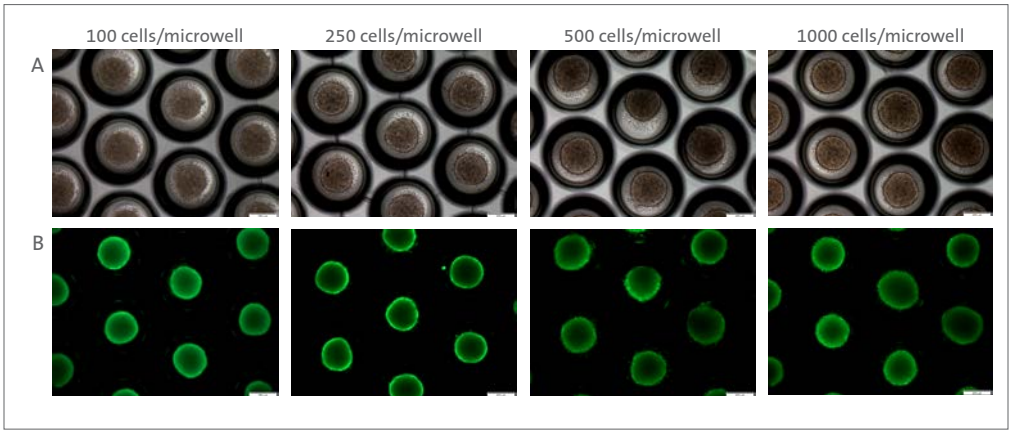

Figure 6. Uniform, single EBs formed in each microcavity. Representative images of a digitally zoomed field from one well of a 24-well Corning Elplasia plate using brightfield (A) and fluorescence (B) imaging on day 6. Images were taken with the Olympus IX53 microscope using a 100X objective. Scale bar is 200 µm.

As the Corning® Elplasia® 24-well plate contains 554 microcavities per well, the average number of replicate iPSC spheroids that can be generated per plate is over 13,000 spheroids. This demonstrates the effective scale-up of uniform spheroids that can be cultured under a single culture condition.

iPSC Spheroid Characterization

On day 3, pluripotency-associated markers of iPSC spheroids generated on the Corning Elplasia plate were characterized using immunofluorescence and flow cytometry. Regardless of cell seeding density, iPSC spheroids demonstrated strong Oct4 transcription factor expression throughout the spheroid (Figure 4A) and a high percentage of SSEA+ cells (>96%; Figure 4B) indicating retention of pluripotency characteristics.

EB Differentiation and Characterization

On day 3, culture medium was changed to EB Formation Medium to initiate EB differentiation in the Corning Elplasia plate. After a further 3 days of differentiated culture, cells were stained and expressed protein markers characteristic of the 3 developmental germ layers (Figure 5). The differentiated EBs were stable and uniform in size as observed within the individual microcavities

of a well (Figure 6). Similarly, to the iPSC spheroids, the EBs could also be size-controlled based on initial seeding density. EB size ranged from 250 to over 300 µm in diameter with seeding densities of 100 to 1000 cells per microwell at day 6 (Figure 3).

Following the same logic as earlier, the average number of reproducible differentiated EBs that can be generated per Corning® Elplasia® 24-well plate is over 13,000 EBs under a single culture condition.

Conclusions

- Corning Elplasia plates can support spontaneous uniform iPSC spheroid formation and progression to reproducible differentiated EBs using a simple 6-day protocol.
- The iPSC spheroids and differentiated EBs can be size-controlled in the Corning Elplasia microcavities depending on initial cell seeding density.
- The iPSC spheroids demonstrated strong pluripotency marker expression; EBs expressed endoderm, mesoderm, and ectoderm markers indicating differentiation of the specific cell lineages.
- The Corning Elplasia plates can be used for scale-up production of high quality, reproducible EBs, and as a tool in drug discovery and functional screening.

References

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