AggreWell™400, AggreWell™800, and AggreWell™ Medium Provide a Platform for Generation and Culture of Human Embryoid Bodies of Defined Sizes

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Introduction

As a first step towards differentiation of pluripotent stem cells (PSCs), many protocols involve the formation of spherical embryoid bodies (EBs). AggreWell plates are a unique cell culture tool for aggregation of a defined number of cells into EBs by forced aggregation in microwells. EBs of a defined size are formed within the microwells by filling the overlying well with a desired number of iPSCs, followed by centrifugation to distribute the cells evenly into the microwells, where they will aggregate into EBs during the subsequent 24-hour incubation period. A pool of EBs with uniform and defined size can then be collected from the AggreWell microwells by gentle pipetting. AggreWell™ is available in two size formats: AggreWell™400 for the formation of EBs from 50 to 3,000 cells each, and AggreWell™800 for the formation of EBs from 2,000 to 20,000 cells each. Control over EB size can improve directed differentiation efficiencies and help standardize differentiation protocols within and between labs. AggreWell™ Medium was developed to support EB formation in AggreWell™ when cells were previously maintained in mTeSR1. EBs survive better in AggreWell™ Medium compared to traditional EB culture methods with maintenance EB morphology. AggreWell™ Medium may be modified with additional factors to induce differentiation; results shown here demonstrate differentiation to mesenchymal and neural lineages. AggreWell™400 and AggreWell™800 tissue culture tools complemented with the new serum-free AggreWell™ Medium offers a complete system for size-controlled EB formation from 50 to 20,000 iPSCs per EB, and for the maintenance of viability and morphology of EBs in culture prior to inducing specific differentiation.

Materials

FIGURE 1: AggreWell™400 and AggreWell™800 have microwell-textured surfaces and are used with AggreWell™ Medium for EB formation

![Image of AggreWell™400 and AggreWell™800 plates](image)

A defined, serum-free medium for generation and culture of EBs using AggreWell™ plates.

Results

FIGURE 2: AggreWell™400 and AggreWell™800 plates form EBs of defined size

![Image of EB formation](image)

FIGURE 3: Improved EB survival in AggreWell™ Medium

![Graph showing improved EB survival](image)

EBs of 2,000 cells each were generated using AggreWell™400 plates. In standard culture conditions, most EBs do not survive (A; n=9, mean ± SD) and rapidly disintegrate into single cells. Conversely, in AggreWell™ Medium (B; n=4) at least 50% of the EBs survived, and retained spherical EB structures.

FIGURE 4: Down-regulation of pluripotency genes and up-regulation of lineage-specific genes in EBs cultured in AggreWell™ Medium

![Graph showing gene expression](image)

(A) qPCR analysis for surface expression of SSEA-4 (hs1) and intracellular expression of Oct4 (hs2, mean ± SD). EBs show rapid down-regulation of pluripotency genes during culture in AggreWell™ Medium, indicating loss of stem cell pluripotency. (B) Gene expression assessed by quantitative PCR (in triplicate) in cells from EBs cultured in AggreWell™ Medium for up to 14 days, shows down-regulation of pluripotency genes (Oct4, Nanog), and up-regulation of mesodermal (Brachyury) and ectodermal (nestin) genes, suggesting a multi-lineage priming for differentiation toward mesodermal or ectodermal fate. Undifferentiated genes (Fgf4) are not expressed under these conditions without additional growth factors added.

FIGURE 5: Directed differentiation of EBs in AggreWell™ Medium with added growth factors

![Image showing EB differentiation](image)

(A) EBs generated in AggreWell™400 and cultured for 8 days in AggreWell™ Medium supplemented with mesodermal induction factors (BMP4, Activin, VEGF) acquired the characteristic morphology of mesoderm EBs. (B) EBs differentiated in AggreWell™400 (left) and AggreWell™800 (right) were cultured with mesoderm induction factors. (C) EBs grown in AggreWell™400 and cultured for 4 days before being passaged as single cells. (D) EBs have the characteristic morphology of mesodermal EBs. (E) EBs were generated in AggreWell™400 and cultured for 4 days in AggreWell™ Medium with 10% FBS before being passaged as single cells, and then cultured in mesoderm induction plates. The cells formed mesodermal-like colonies (C; Oct4, Nkx2.2, and 4% of total colonies staining >50% mesoderm; n=3), which expressed Nkx2.2 and Sox17 as assessed by immunocytochemistry (D).

Conclusions

- AggreWell™ plates are used for aggregation of PSCs into EBs within 24 hrs.
- AggreWell™400 and AggreWell™800 provide for a broad range of defined EB sizes: from 50 to 20,000 cells in size.
- AggreWell™ Medium supports EB formation & EB survival in suspension culture.
- With growth factor addition, EBs can differentiate in AggreWell™ Medium along a variety of directed differentiation pathways.