

The AggreWell™ System Provides a Platform for Generation and Culture of Human Embryoid Bodies of Defined Sizes

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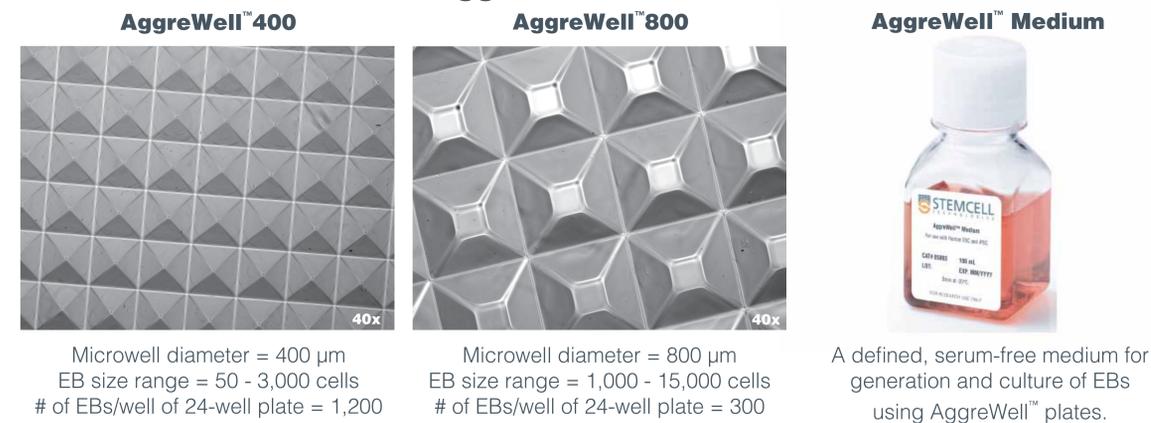
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Introduction

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 hPSCs, 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 mTeSR[®]1. EBs survive better in AggreWell™ Medium compared to traditional EB culture media with maintenance EB morphology. AggreWell™ Medium can be modified with additional factors to induce differentiation; results shown here demonstrate differentiation to mesenchymal & neural pathways. AggreWell™400 and AggreWell™800 tissue culture tools complemented with the new serum-free AggreWell™ Medium offer a complete system for size-controlled EB formation from 50 to 20,000 hPSCs per EB, and for the maintenance of viability and morphology of EBs in culture prior to inducing specific differentiation.

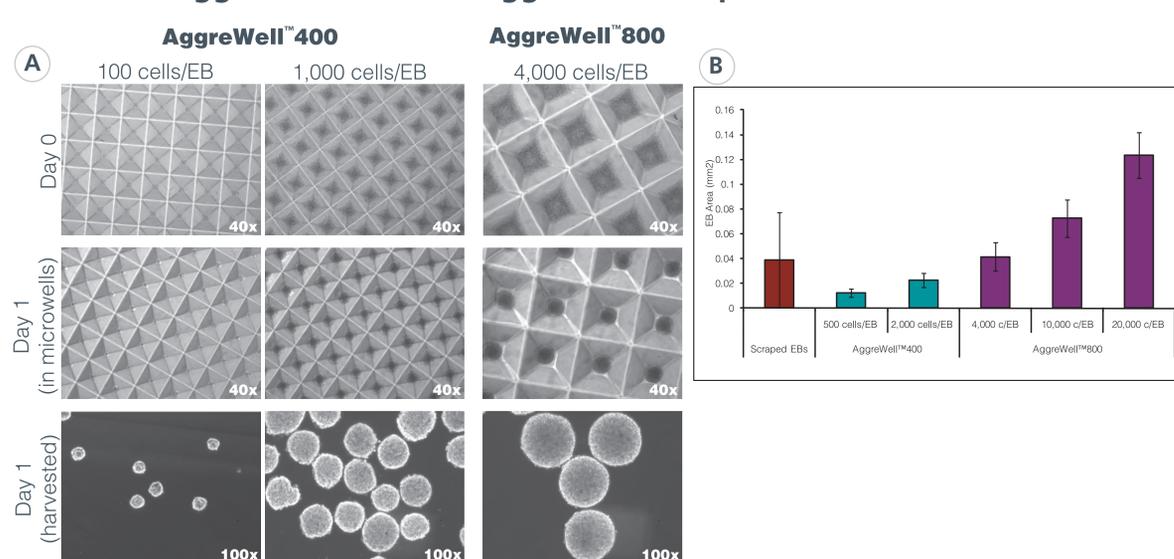
Materials

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



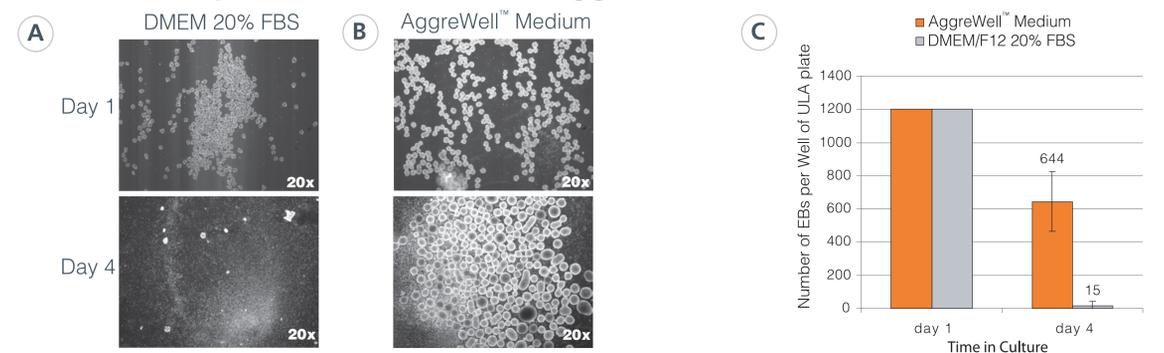
Results

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



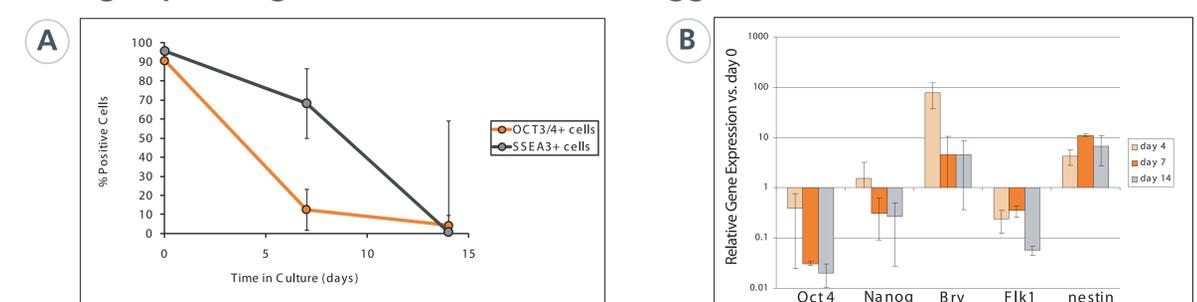
(A) AggreWell™400 plates (left & middle columns) were used to form EBs at low and high cell densities by inoculation with 240,000 cells per well (100 cells per microwell) or 2,400,000 cells per well (1,000 cells per microwell). AggreWell™800 (right column) was inoculated with 1,200,000 cells per well (4,000 cells per microwell). After 24 hrs, EBs are visible inside each microwell. These were harvested and transferred to ultra-low adherence plates. EBs are well-formed, spherical, and uniform in size and shape. (B) EB size correlates well with cell densities inoculated in AggreWell™. Shown are EB sizes (n=16-40; mean ±st. dev) after inoculation of approximately 500 or 2,000 cells per microwell into AggreWell™400; and 4,000, 10,000 or 20,000 cells per microwell into AggreWell™800. Conversely, EBs made by the scraping method had large variations in size.

FIGURE 3: Improved EB survival in AggreWell™ Medium



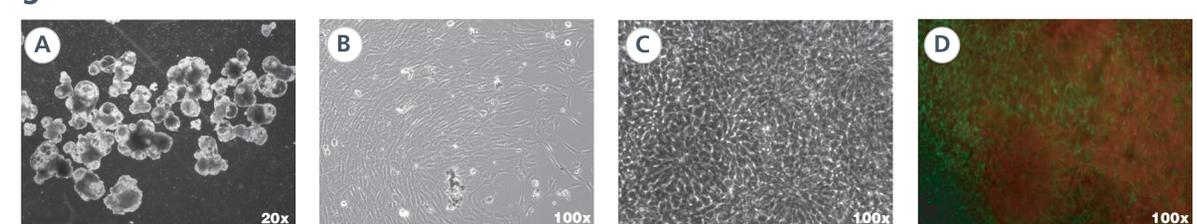
EBs of 2,000 cells each were generated using AggreWell™400 plates. In standard culture conditions, most EBs do not survive (A,C; n=9, mean ±st. dev) and rapidly disintegrate into single cells. Conversely, in AggreWell™ Medium (B,C; n=6) at least 50% of the EBs survived, and retained spherical EB structure.

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



(A) FACS analysis for surface expression of SSEA-3 (n=1) and intracellular expression of Oct4 (n=2, mean ±st. dev) shows rapid down-regulation in cells of EBs during culture in AggreWell™ Medium, indicating loss of stem cell pluripotency. (B) Gene expression assessed by quantitative PCR (n=2, each 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 (Bry) and ectodermal (nestin) genes, suggesting a multi-lineage priming for differentiation toward mesodermal or ectodermal fates. Endoderm-specific genes (Flk1) are not expressed under these conditions without additional growth factors added.

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



(A) EBs generated in AggreWell™400 and cultured for 8 days in AggreWell™ Medium supplemented with mesodermal induction factors (BMP4, Activin, VEGF) acquired the cavitated morphology characteristic of self-organizing EBs. (B) EBs generated in AggreWell™400 and cultured for 8 days in AggreWell™ Medium supplemented with 15% FBS were dissociated and plated onto tissue culture-treated dishes in MesenCult™ Medium for 4 days before being passaged as single cells. Cells have the morphology typical of mesenchymal cells. (C,D) EBs were generated in AggreWell™400 and cultured for 4 days in AggreWell™ Medium for 4 days, followed by plating onto poly-L-ornithine/laminin-coated plates. The cells formed neural rosette-filled colonies (C,D; 32, 8, and 4% of total colonies containing >50% rosettes; n=3) which expressed Nestin (green) and Sox1 (red) 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.