

# AggreWell™ 400 and AggreWell™ 800 Provide a Unique Platform for Generation of Size-Controlled Aggregates Including Human Embryoid Bodies

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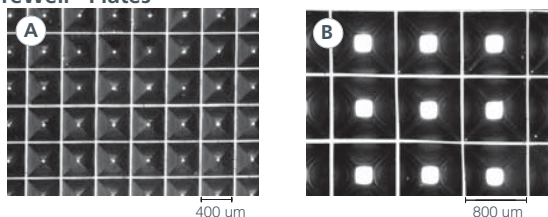
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## INTRODUCTION

Aggregation of cells and the formation of three-dimensional spheroid structures are useful in many types of tissue engineering strategies. For example, as a first step towards differentiation of pluripotent stem cells (PSCs), many protocols involve the formation of spherical embryoid bodies (EBs). Studies have shown that EB size can affect differentiation outcome<sup>1</sup>, so the ability to control EB size will allow for greater control of directed differentiation efficiencies. AggreWell™ plates are a unique cell culture tool for aggregation of a defined number of cells in a high-throughput format. In the application presented here, EB size is controlled in AggreWell™ by adjusting the density of human embryonic stem (hES) cells in the overlying cell culture well prior to distribution into microwells. AggreWell™ is available in two sizes: AggreWell™ 400 and AggreWell™ 800, which together provide for controlled EB formation over a range of 50 to 20,000 cells per EB. In addition, a large number of uniform EBs are generated from each AggreWell™ plate, allowing for scale-up of downstream differentiation protocols.

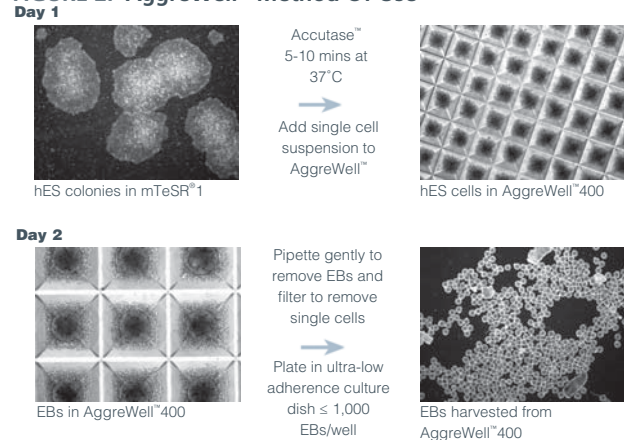
## METHODS

**FIGURE 1: Microwell Arrays On The Bottom Surfaces Of AggreWell™ Plates**



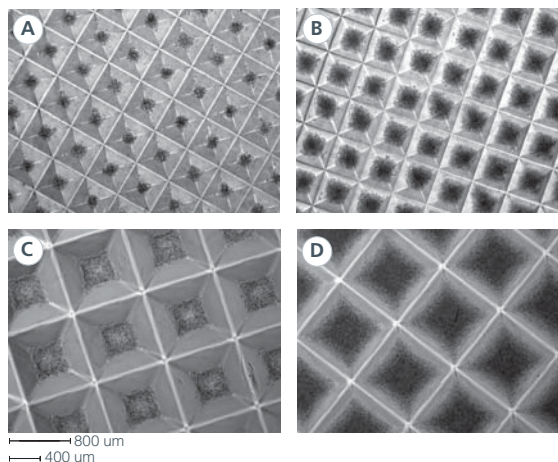
A) AggreWell™ 400 plates have inverse pyramidal microwells of 400 microns in diameter. B) AggreWell™ 800 plates have inverse pyramidal microwells with flat bottoms of 800 microns in diameter. Both are shown at 40x magnification without media or cells added.

**FIGURE 2: AggreWell™ Method Of Use**



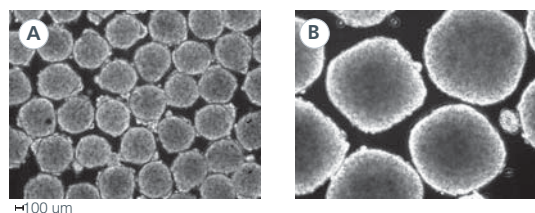
## RESULTS

**FIGURE 3: EB Size Is Controlled By Adjusting Cell Density**



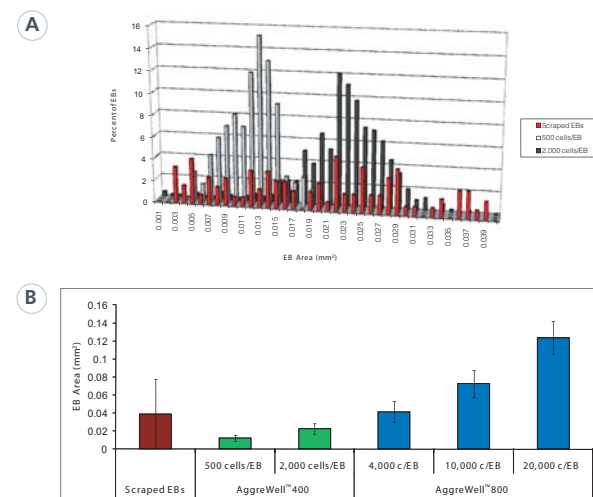
AggreWell™ 400 plates can be used to make EBs of 50 to 3,000 cells each. Shown are AggreWell™ 400 plates inoculated with cells at densities calculated to distribute approximately 500 (A) or 2,000 (B) cells into each microwell. AggreWell™ 800 plates can be used to make EBs of 1,000 to 20,000 cells each. Shown are AggreWell™ 800 plates inoculated with cells at densities calculated to distribute approximately 4,000 (C) or 15,000 (D) cells into each microwell. All photos were taken at 40x magnification immediately after distribution of cells into AggreWell™ plates (day 1).

**FIGURE 4: EBs Harvested from AggreWell™ Are Uniform In Size and Shape**



After 24 hours of culture, EBs of 2,000 cells each (A) or 15,000 cells each (B) were harvested from AggreWell™ 400 or AggreWell™ 800 plates, respectively. Each of the 8 wells on an AggreWell™ 400 plate can generate up to 1,200 EBs; each well of an AggreWell™ 800 plate can generate up to 300 EBs. EBs are well formed, spherical, and robust to handling.

**FIGURE 5: AggreWell™ Provides Tight Control Over EB Size**



A) hES cells were inoculated into AggreWell™ 400 at 500 (light grey) or 2,000 (dark grey) cells per microwell and EBs harvested after 24 hrs. Scraped EBs were obtained from aggregates collected after dispass treatment of hES cells, followed by 24 hr culture in an ultra-low adherence plate. EBs were measured by ImageJ analysis of microscopic images. Distinct EB sizes were generated from two different densities of cells in AggreWell™ (500 c/EB: n=1, 183 EBs measured; 2,000 c/EB: n=4, 424 EBs measured), versus a broad range of EB sizes by the traditional scraping method (red; n=2, 183 EBs measured).

B) Statistically significant increases in EB size are seen when AggreWell™ is inoculated at different densities. Shown are EB sizes (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 were heterogeneous in size.

## CONCLUSIONS

- The AggreWell™ system can be applied for rapid aggregation of defined numbers of PSCs or other cell types<sup>3</sup>
- AggreWell™ 400 and AggreWell™ 800 together provide for a broad range of defined EB sizes: from 50 to 20,000 cells
- AggreWell™ can be used to generate large numbers of aggregates, which are uniform in size & shape

1. Bauwens et al. Stem Cells. 2008 Sep;26(9):2300-10.  
2. Mohr et al. Biomaterials. 2010 Mar;31(7):1885-93.  
3. Markway et al. 2010. Cell Transplant 19(1): 29-42.