

# AggreWell™ 400 generates a uniform population of embryoid bodies from human embryonic stem cells: Effects of modified mTeSR™ formulations on stability and growth of AggreWell™ generated embryoid bodies

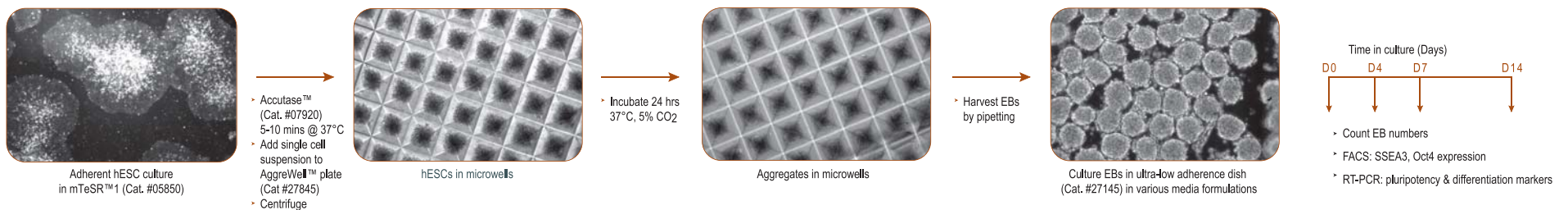
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## INTRODUCTION

Most differentiation protocols for human embryonic stem (hES) or induced pluripotent stem (iPS) cells involve the formation of embryoid bodies (EBs) as a first step. Current methods of generating EBs from pluripotent hES cells involve enzymatic dissociation followed by mechanical scraping of the hES colonies. These rather crude methods result in heterogeneously sized EBs, with poor viability and lineage heterogeneity. AggreWell™ 400 is a unique tool that allows standardization of hES or iPS cell differentiation protocols within and between laboratories, as well as scale-up of differentiation systems. Controlling the size of EBs during differentiation of hES cells facilitates the process of mimicking early embryonic development *in vitro*. Here we show that culture of AggreWell™ EBs follows early development, with down-regulation of pluripotency markers, and up-regulation of lineage restricted markers.

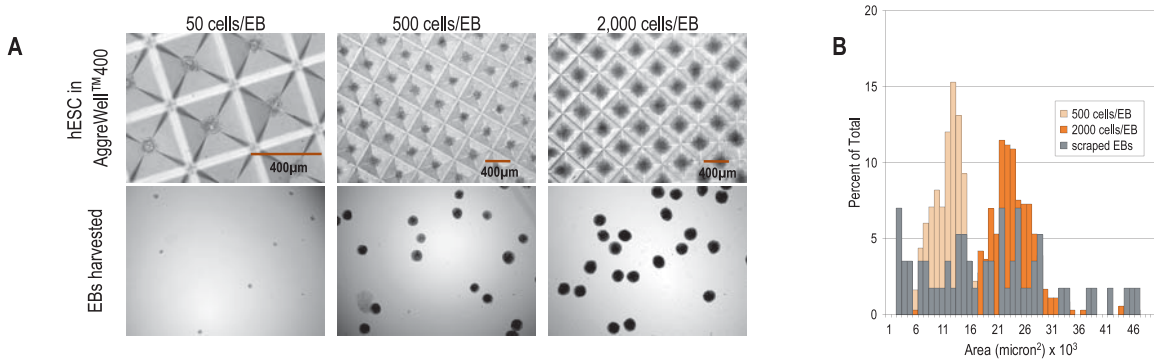
### Figure 1: Method of EB generation in AggreWell™ and subsequent culture

Each well of an AggreWell™ 400 multiwell plate contains an array of 1,200 microwells on the bottom surface. EBs are formed within the microwells by filling the overlying AggreWell™ 400 well with a single cell suspension of hES cells. Upon centrifugation of the plate, hES cells distribute evenly among the individual microwells of the AggreWell™ 400, where they will form individual EBs within 24 hours. In this study, AggreWell™ EBs were grown in suspension culture for up to 14 days in various media formulations. EB survival and morphology, as well as expression of pluripotency and differentiation markers were assessed at day (D) 0, 4, 7 and 14 of culture.



### Figure 2: AggreWell™ generates uniform EBs of defined size

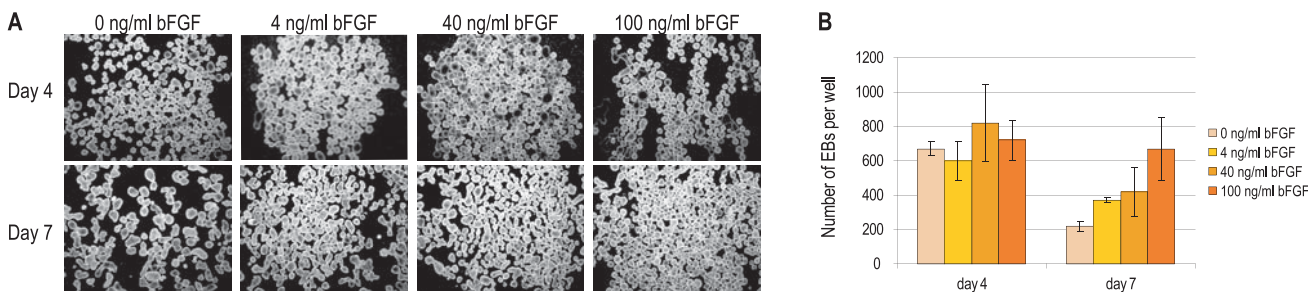
(A) The size of the EBs formed can be controlled by adjusting the concentration of hES cells in the single cell suspension prior to spinning cells into microwells, and the EBs retain that size differential after harvesting. (B) Tight size distributions were demonstrated with AggreWell™ EBs of 500 or 2,000 cells/EB, in contrast to EBs formed by scraping methods, which showed wider size distributions.



- AggreWell™ 400 generates a uniform population of EBs.
- EB size can be easily controlled using AggreWell™ 400.

### Figure 3: Enhanced survival of EBs in bFGF containing medium

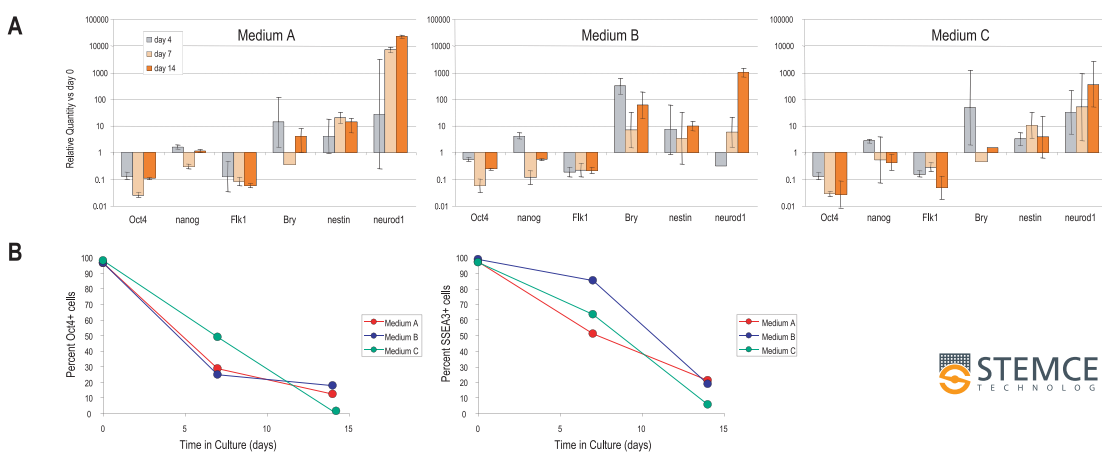
EBs of 2,000 cells each were formed using AggreWell™ 400 in modified mTeSR™ containing 0, 4, 40 or 100 ng/ml bFGF, and cultured in the same medium in suspension conditions for a further 7 days. Complete media changes were carried out at days 1 and 4, and EBs were scored at days 1, 4, and 7. (A) photos of EBs in culture, (B) EB yields per well (n=2 ± st. dev.)



- Survival of AggreWell™ EBs in suspension culture correlates with bFGF concentration.

### Figure 4: Loss of pluripotency and initiation of differentiation in EBs cultured in test media

Three new test media were assessed for their ability to support multi-lineage differentiation. AggreWell™ EBs were grown in test medium for up to 14 days, with periodic assessment of differentiation by real-time PCR (A) and FACS (B). With varying kinetics, all three media supported down-regulation of pluripotency markers (Oct4, nanog, SSEA3), and up-regulation of both ectoderm (neurod1, nestin), and mesoderm (Bry) markers, without the addition of lineage-inducing factors. (n=1, Q-PCR in triplicate ± st. dev.)



- Three new test media support EB growth with down-regulation of pluripotency markers, and up-regulation of lineage-restricted markers.
- Kinetics and magnitude of marker expression depend on the EB culture media formulation.

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