Efficient Production of Neural Progenitors from Human Pluripotent Stem Cells (hESC and iPSC) in AggreWell™400 and AggreWell™800 in a Novel Induction Medium

STEMCELL TECHNOLOGIES

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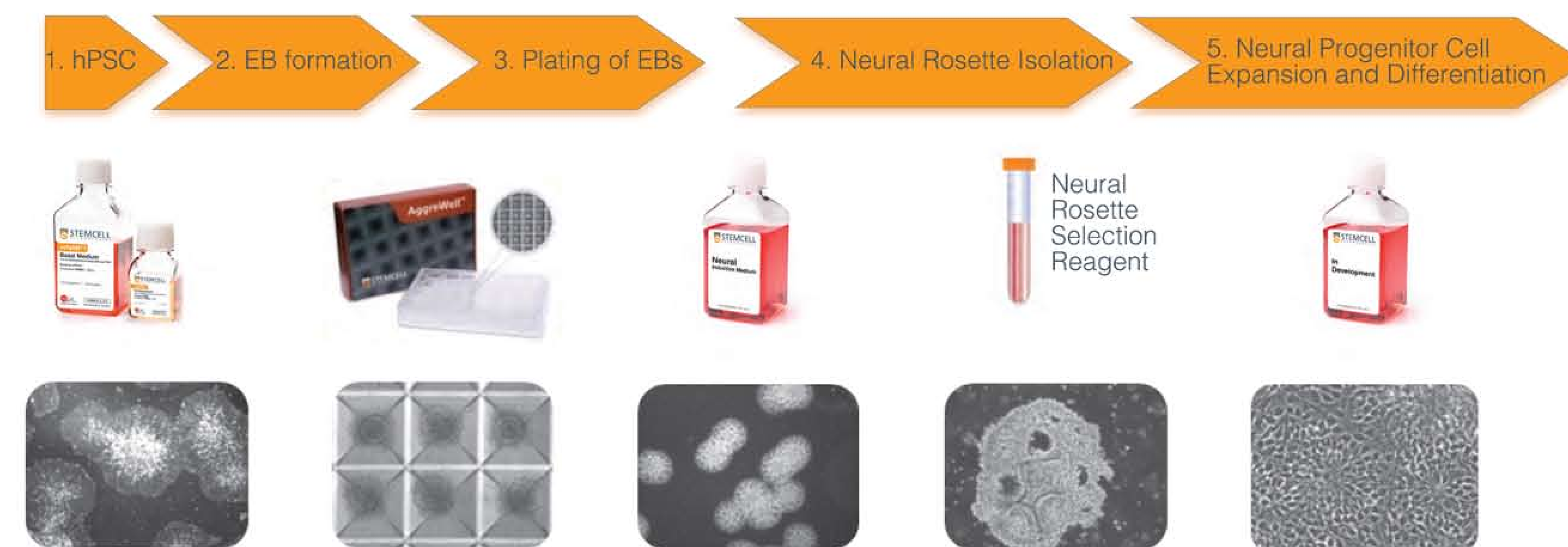
Introduction

Induction of neuroectoderm from human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) is the first phase in differentiation protocols used to produce neural progenitor cells and more specialized cell types of the CNS such as neurons, astrocytes and oligodendrocytes. However current neural induction protocols and media are not standardized within and between labs and therefore reproducibility of the protocols and results is an on-going problem for researchers. The formation of embryoid bodies (EBs) from undifferentiated human pluripotent stem cells (hPSC) is the first step in many neural induction protocols. Furthermore, there also exists contradictory evidence on the effect of EB size on induction of neural fate. In an attempt to standardize protocols for neural induction from hPSC, we used the new AggreWell™ system (STEMCELL Technologies) to generate EBs of defined sizes and a novel Neural Induction Medium. Varying numbers of undifferentiated hESC were suspended in Neural Induction Medium and aggregated using the AggreWell™ protocol in either AggreWell™400 or AggreWell™800 plates to generate EBs that contained 500-20,000 cells per EB. After 5 days of culture in AggreWell™ plates, EBs were plated in Neural Induction Medium and neural differentiation was evaluated using morphological and immunocytochemistry (ICC) criteria. The results showed that 90-100% of the colonies formed from EBs of all sizes cultured contained rosette structures that consisted of cells which expressed the early neural differentiation markers: Pax6, Sox1, and Nestin, thus confirming the presence of early neural progenitors. These early neural progenitor cells were easily isolated from rosette structures using Neural Rosette Selection Reagent. The resulting pure populations of neural progenitors were terminally differentiated into mature neurons and astrocytes as determined by expression of the markers TUJ1 and GFAP, respectively. This work describes a highly efficient protocol for the induction of neural progenitor cells and their progeny from hESCs and iPSCs.

Methods

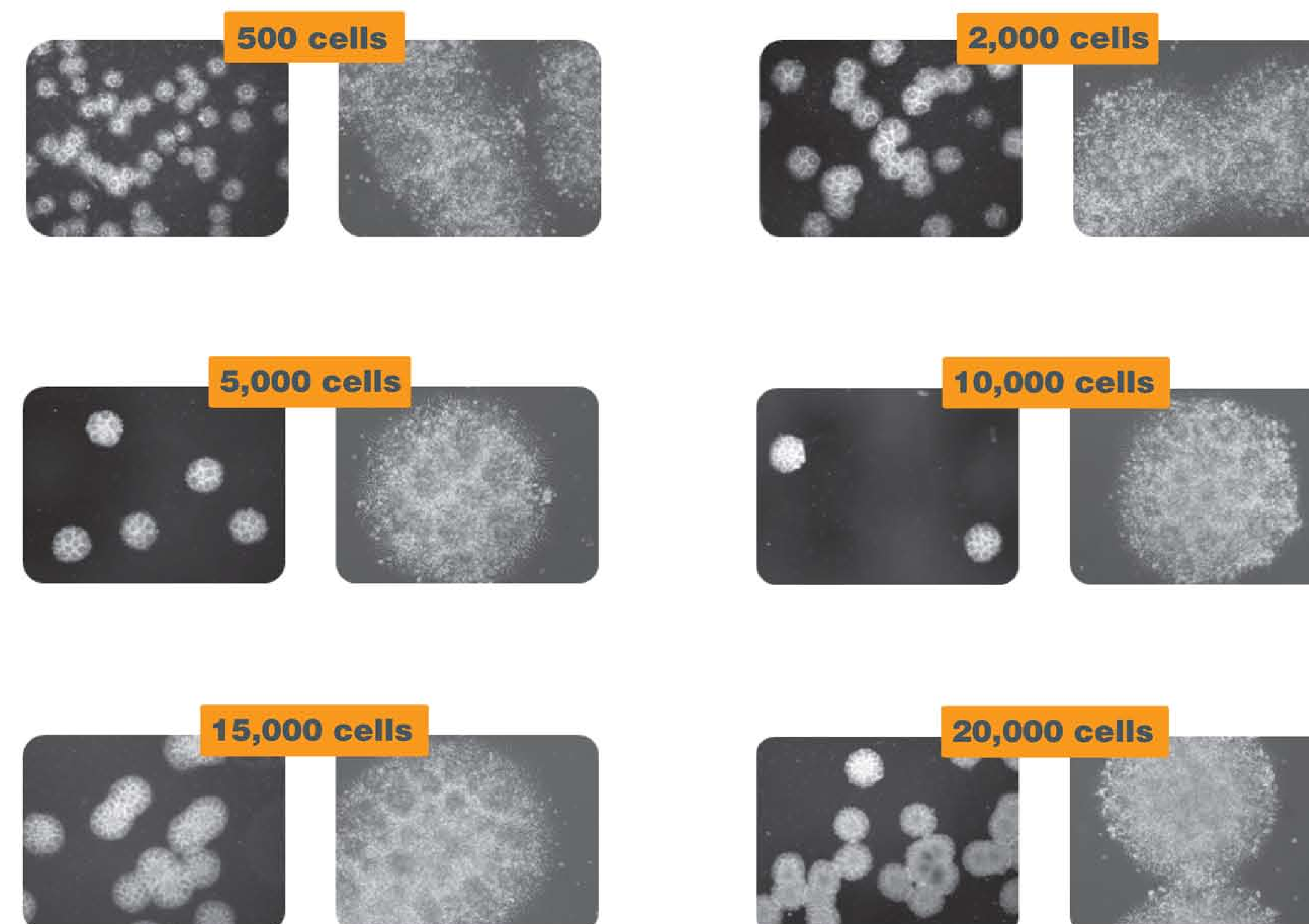
FIGURE 1: A Complete Neural Induction System: Media, Tools and Reagents to Generate Neural Progenitor Cells



We have developed a novel efficient system to produce pure populations of neural progenitor cells (NPCs). Step 1: Human pluripotent stem cells (PSCs) are maintained in mTeSR®1 or TeSR™2. Step 2: AggreWell™400 or AggreWell™800 plates are used to form uniform, size-controlled EBs, which were cultured in a novel Neural Induction Medium. Steps 3: On day 5, EBs are harvested from the AggreWell™ plates and sub-cultured on poly-L-ornithine/laminin (PLO/Lam) coated plates containing Neural Induction Medium for an additional 2 days to generate neural colonies. Step 4: Neural rosettes containing putative neural progenitor cells (NPCs) are selectively detached using "Neural Rosette Selection Reagent" and then mechanically dissociated into a single cell suspension. Step 5: NPCs can then be expanded and maintained using various methods. NPCs can also be differentiated into neurons, astrocytes, and oligodendrocytes.

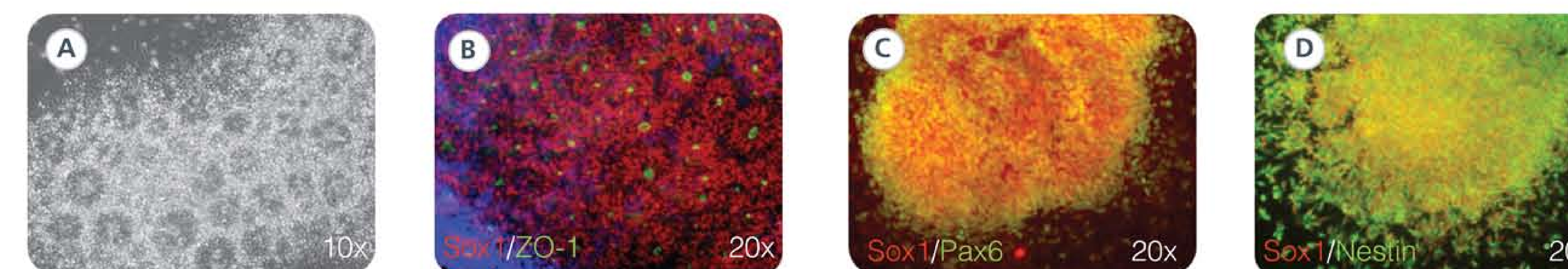
Results

STEP 2: Plated EBs Generated in AggreWell™400 or AggreWell™800 and Neural Induction Medium Display >90% Neural Rosette Structures



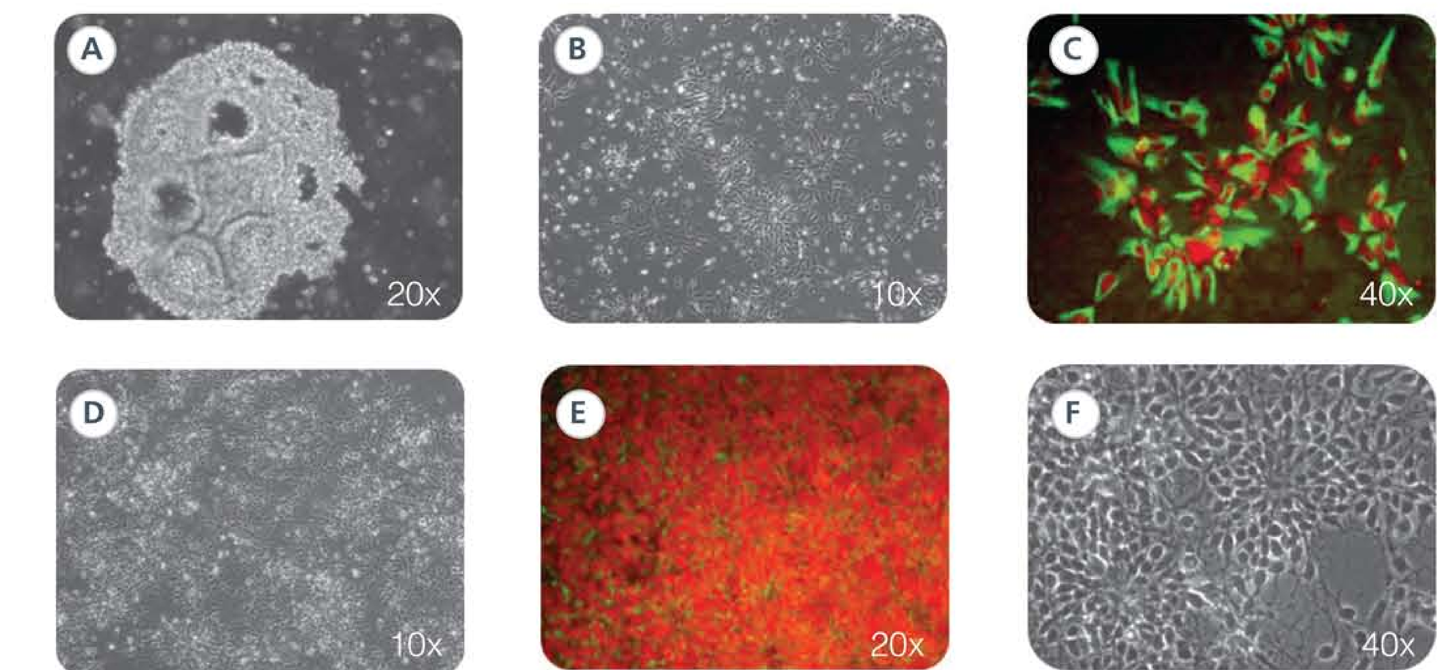
EBs were generated in AggreWell™400 (500 cells/EB) or AggreWell™800 (2000, 5000, 10000, 15000 and 20000 cells/EB) plates. After 5 days, EBs were released from the AggreWell™ plates and plated onto PLO/Lam coated dishes for attachment. Here, attached EBs of various sizes are shown 1 day after plating on PLO/Lam coated dishes.

STEP 3: Neural Progenitor Cells are Detected Within Neural Rosette Structures



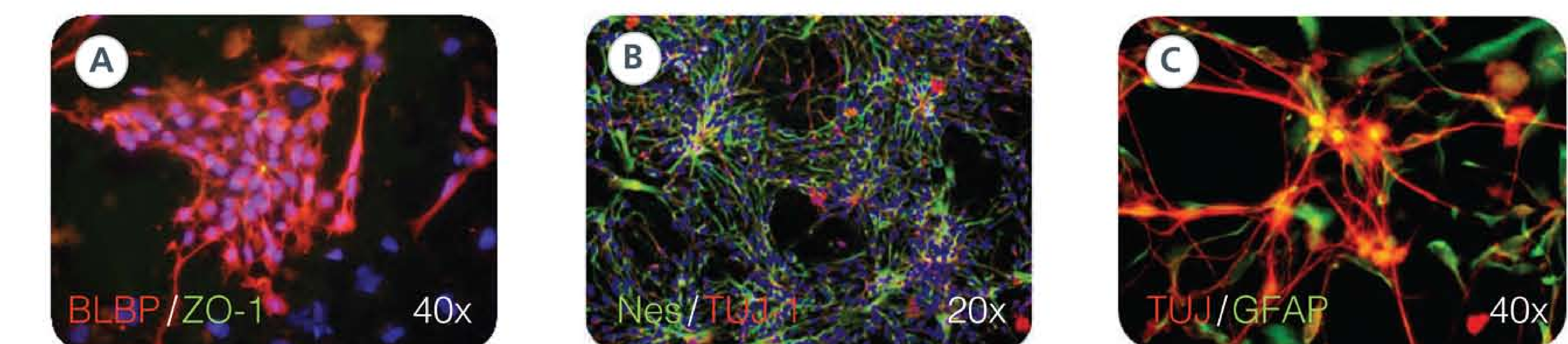
(A) Neural rosette structures were evident as early as day 1 within attached EB as shown by bright-field microscopy. (B) Cells co-express Sox1 (red) and ZO-1 (green), a marker for the lumen of the rosettes, confirming the presence of bona fide neural rosettes. (C) Cells co-express the early neural markers Sox1 (red) and Pax6 (green). (D) All cells co-express the neural markers Sox1 (red) and Nestin (green).

STEP 4: Pure Neural Progenitor Cell Populations can be Derived from Selectively Detached Rosette Structures Within Attached EBs



Detachment of rosette structures using Neural Rosette Dissociation Medium followed by mechanical dissociation produces single-cell populations of NPCs: (A) A detached rosette cluster after treatment with Neural Rosette Selection Reagent. (B) Morphology of cells, 2 days after re-plating in Neural Progenitor Expansion Medium. (C) Cells in (B) co-express Sox1 (red) and Nestin (green). (D) NPCs 8 days after re-plating, (E) co-express Sox1 (red) and Nestin (green). (F) Morphology of NPCs at passage 2 after treatment with TrypLE and re-plating on PLO/Lam coated dishes.

STEP 5: Neural Progenitor Cells Differentiate into Neurons and Astrocytes



NPCs expressed numerous neural markers at multiple passages. (A) Cells at passage 2, co-express the radial glia marker BLBP (red) and ZO-1 (green). (B) The presence of cells positive for Nestin (green) and the neuronal marker TUJ1 (red) indicates both neural and neuronal populations are detected in differentiation cultures. (C) Astrocytes, identified by GFAP (green) expression are also present in differentiation cultures (TUJ-1 in red).

Summary

We have developed a novel Neural Induction System consisting of AggreWell™ plates and a novel Neural Induction Medium (patent pending), which allows highly efficient formation of neural rosettes. We have also developed a Neural Rosette Selection Reagent which selectively detaches neural rosettes for subsequent isolation of neural progenitor cells at high purity.

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