Neural progenitor cells can be generated efficiently using STEMdiff™ Neural Induction Medium by either Embryoid body or Monolayer Culture Methods

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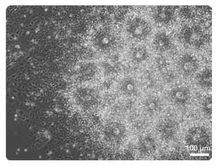
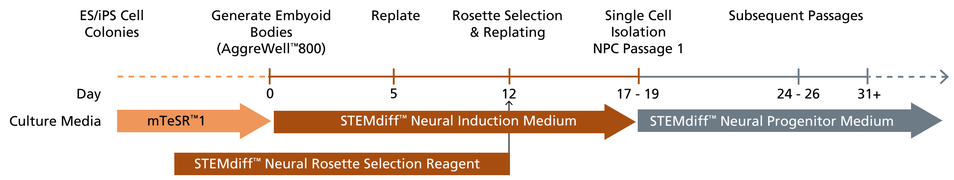
1. Introduction

Neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs), are extensively used for studying human central nervous system (CNS) development, modeling of neurological disorders and screening for therapeutic molecules. We have previously demonstrated that CNS-type NPCs can be produced efficiently using STEMdiff™ Neural Induction Medium (NIM) in an embryoid body (EB)-based protocol. When used in conjunction with AggreWell™800 for EB formation from hPSCs, STEMdiff™ NIM induces up to 100% neural rosette formation in all the EBs harvested and replated on Corning® Matrigel® or poly-L-ornithine/laminin coated plates. These neural rosettes can then be isolated using STEMdiff™ Neural Rosette Selection Reagent (STEMdiff™ NRSR) to obtain an enriched population of CNS-type NPCs for further studies. Recent publications have reported that neural induction from hPSCs can also be accomplished using a single-step monolayer culture protocol. This method does not require EB formation thereby simplifying the experimental procedures. Here, we describe the efficient derivation of NPCs using STEMdiff™ NIM in a monolayer culture protocol. Briefly, hPSCs maintained in mTeSR™1 or TeSR™-E8™ were dissociated using Gentle Cell Dissociated Reagent (GCDR) into single cells, seeded at 200,000 cells/cm^2 on Matrigel® or PLO/L coated plates, and cultured as a monolayer in STEMdiff™ NIM for up to 9 days. Neural induction was assessed qualitatively by immunocytochemistry at different time points for the presence of PAX6$^+$OCT4$^-$ cells in culture, as well as, quantitatively by qPCR for PAX6 and OCT4 transcripts. The timing of induction varied between hPSC lines tested. hESC lines typically required 6 days for induction whereas some hiPSC lines could take up to 9 days. Neural induction (>95% PAX6$^+$OCT4$^-$ cells detected in 15 random, non-overlapping areas on at least two coverslips per condition) was achieved by day 6 for hESC (H1, H9; n = 6) and day 8 - 9 for hiPSC (STiPS-M001, STiPS-F016, WLS-4D1; n = 6) lines. Data from qPCR analyses corroborated the immunocytochemistry results and showed an increase in PAX6 expression during neural induction with a concomitant decrease in OCT4. These data show that CNS-type NPCs can be rapidly induced from hPSCs maintained in mTeSR™1 and TeSR™-E8™ media using STEMdiff™ NIM in a monolayer protocol.

2. Methods

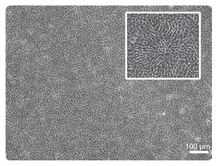
Embryoid Body Protocol

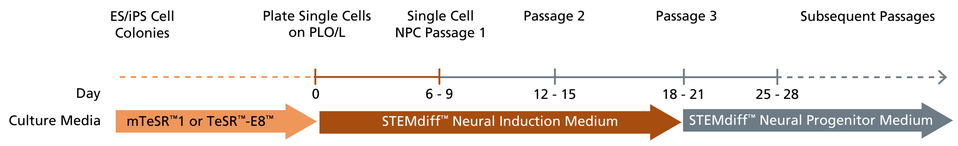




Embryoid bodies (EBs) are formed from hPSCs previously maintained in mTeSR™1 or TeSR™2, in AggreWell™800 with STEMdiff™ Neural Induction Medium (NIM) and cultured for 5 days wihtin the microwells. EBs are harvested and plated (day 5) and colonies with abundant neural rosettes are formed. Colonies containing rosettes can be isolated (day 12) using STEMdiff™ Neural Rosette Selection Reagent (NRSR) and replated in STEMdiff™ NIM. Once this NPC-enriched culture is established (days 17 - 19), single cells can be isolated and further cultured in STEMdiff™ Neural Progenitor Medium (NPM) every 7 days.

Monolayer Protocol





Human ES or iPS cells are harvested from mTeSR™1 or TeSR™-E8™ cultures and plated at 200,000 - 250,000 cells/cm^2 in STEMdiff™ NIM onto poly-L-ornithine/laminin (PLO/L) or Matrigel®-coated plates. After 6 (for hESC) or up to 9 (for hiPSC) days, NPCs are generated, which are then sub-cultured in STEMdiff™ NIM for an additional 2 passages. Starting at passage 3 (days 18 - 21), NPCs are isolated as single-cell suspensions and further expanded in STEMdiff™ Neural Progenitor Medium (NPM) every 7 days.

3. STEMdiff™ Neural Induction Medium promotes neural differentiation of human ES cells in a monolayer protocol

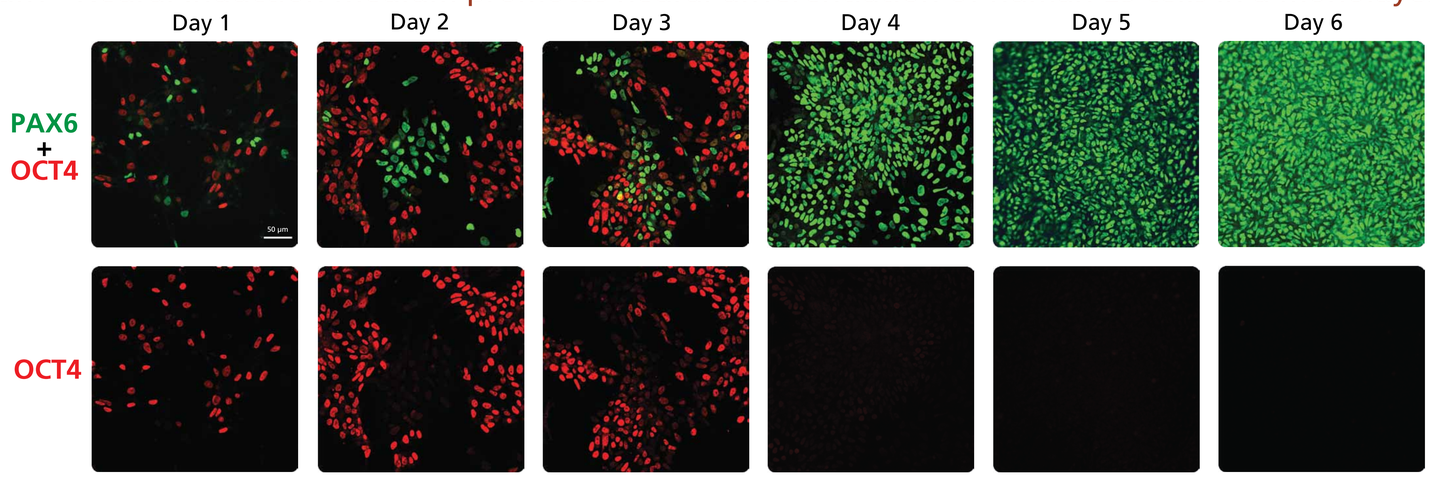


Figure 1. H9 cells maintained in mTeSR™1 or TeSR™-E8™ were dissociated, plated at 200,000 cells/cm^2 and cultured in STEMdiff™ Neural Induction Medium for 6 days. Cells were fixed on each day and processed for PAX6 **(green)** and OCT4 **(red)** immunostaining. Duplicate cultures were collected each day for RNA preparation and quantitative PCR analyses. **(Day 1)** A small number of PAX6$^+$ cells could be detected after one day in NIM, while majority of the cells were OCT4$^+$. **(Days 2 - 3)** The number of PAX6$^+$ cells gradually increased, by day 3 roughly 30% of the cells were PAX6$^+$. **(Day 4)** OCT4 expression in NIM decreased dramatically while PAX6$^+$ cells continued to increase. **(Days 5 - 6)** No OCT4$^+$ cells were detectable by day 5. At day 6, NIM cultures were confluent and most of the cells were PAX6$^+$OCT4$^-$. Scale bar = 50 µm; all panels were taken at the same magnification.

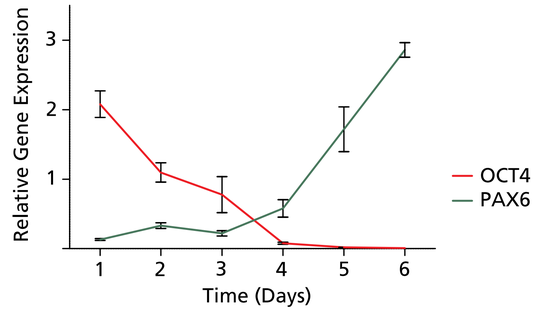


Figure 2. Quantitative PCR data from H9 cells revealed that PAX6 expression **(green)** was low at day 1 and increased as neural induction proceeded. PAX6 expression spiked sharply after day 4 and continued to rise on day 5 and 6. In contrast, OCT4 expression **(red)** was high on day 1 in NIM and decreased rapidly. Undifferentiated H9 cells cultured in mTeSR™1 or TeSR™-E8™ were used as controls where OCT4 remained high and there was no PAX6 expression (data not shown).

4. Neural progenitor cells can be generated from human iPS cells using STEMdiff™ Neural Induction Medium

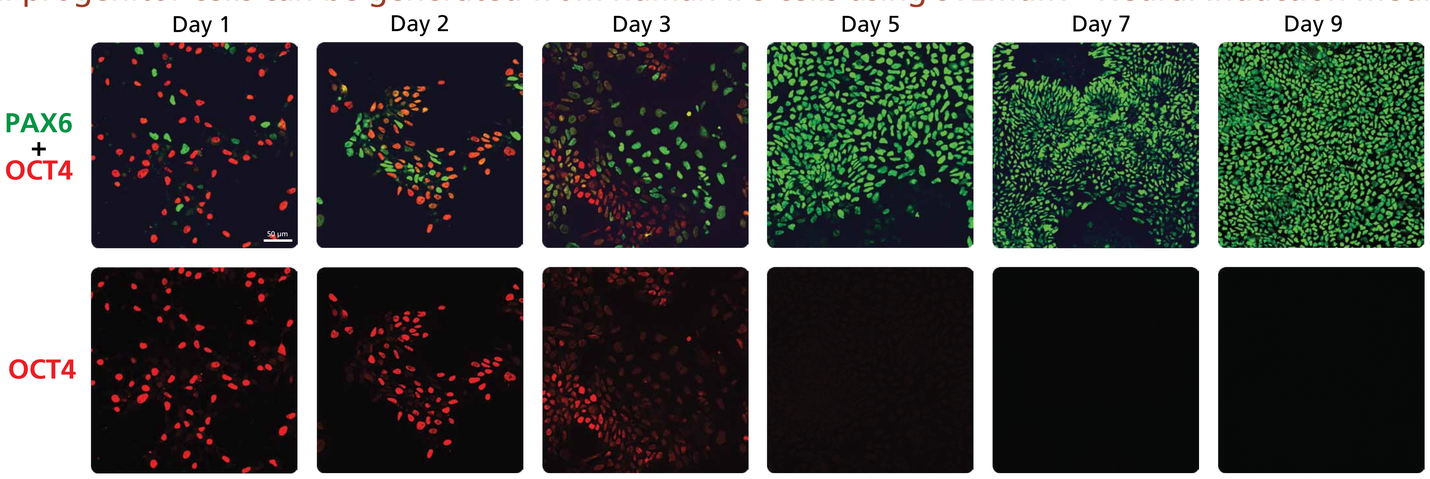


Figure 3. STiPS-M001 cells maintained in mTeSR™1 or TeSR™-E8™ were dissociated, plated onto Matrigel-coated coverslips at 200,000 cells/cm2, and cultured in STEMdiff™ Neural Induction Medium for 9 days. Cells were fixed on each day and processed for PAX6 **(green)** and OCT4 **(red)** immunostaining (data for day 4 and 6 not shown). Duplicate cultures were collected each day for RNA preparation and quantitative PCR analyses. **(Day 1)** A small number of PAX6$^+$ cells could be detected after one day in NIM, while majority of the cells were OCT4$^+$. **(Days 2 - 3)** The number of PAX6$^+$ cells gradually increased, by day 3 roughly 30% of the cells were PAX6$^+$. **(Day 5)** OCT4 expression was very low at day 5 while PAX6$^+$ cells continued to increase. **(Day 7)** Although not all the cells in cultures were PAX6$^+$ at day 7, no OCT4$^+$ cells could be observed. **(Day 9)** NIM cultures were confluent and virtually all cells were. PAX6$^+$OCT4$^-$. Scale bar = 50 µm; all panels were taken at the same magnification.

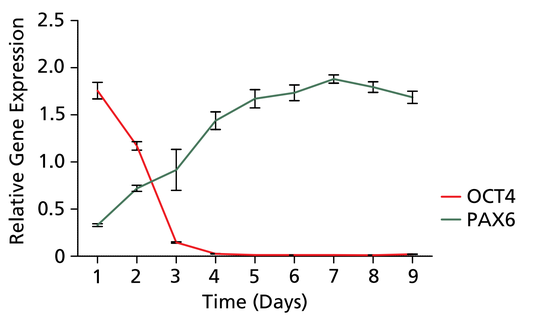


Figure 4. Quantitative PCR data from STiPS-M001 demonstrated that PAX6 expression **(green)** was low at day 1 and gradually increased during the course of neural induction. This is slightly different from H9 cells, which showed a surge in PAX6 expression (green line) between day 4 and 5 instead of a steady rise. Similar to H9 cells, OCT4 expression **(red)** in STiPS-M001 was high on day 1 in NIM and decreased rapidly. Undifferentiated STiPS-M001 cells cultured in mTeSR™1 or TeSR™-E8™ were used as controls where OCT4 remained high and there was no PAX6 expression (data not shown).

5. Conclusions

- STEMdiff™ NIM can support robust neural induction for both human ES and iPS cells in a single step monolayer protocol
- Immunostaining and qPCR data demonstrated that PAX6 expression increases whereas OCT4 decreases during the time course of neural induction
- STEMdiff™ NIM is a versatile medium that allows the efficient generation of NPCs from hPSCs using either the EB- or monolayer-based neural induction protocols