Improved differentiation of human pluripotent stem cell-derived neurons through reduction of progenitor proliferation

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Introduction

Neurons derived from human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are excellent resources for disease modeling and drug screening. Neural stem cells (NSCs) deirved from hPSC can be expanded and further differentiated into neurons for various experiments. In classical culture medium, typically including a basal medium, B27/N2, brain-derived neurotrophic factor, glial cell-derived neurotrophic factor and other reagents, differentiated cells often contain differentiated neurons and undifferentiated NSCs. Due to the continuing proliferation of NSCs, very high cell densities and cell aggregation are usually observed during the differentiation, which poses challenges for long-term maintenance and end-point quantification. We have developed a CultureOne™ supplement which can be added into conventional neuronal differentiation media to greatly reduce the proliferation of undifferentiated NSCs. Differentiated neurons treated with CultureOne™ supplement are evenly distributed across the culture surface with extensive neurite networks but without the formation of clumping or aggregation. Immunocytochemical staining showed that differentiated neurons treated with CultureOne™ supplement expressed neuronal marker MAP2 with very few SOX1 positive undifferentiated NSCs. The long-term differentiated neurons express mature neuronal maker Neurofilament and Synaptophysin. Upon depolarization with KCl, the signals of calcium influx of the differentiated neurons with the treatment of CultureOne™ supplement are much greater than untreated neurons, indicating the treatment with CultureOne™ supplement accelerates the maturation process of differentiating neurons. By using CultureOne™ supplement, differentiated neurons can be maintained for longer time in culture for more mature neurons. Furthermore, the evenly distributed neurons are more favorable to manual or automated imaging for quantification.

Results

1. CultureOne™ supplement induces even distribution of differentiated neurons and enables long-term maintenance of differentiated neurons in culture.

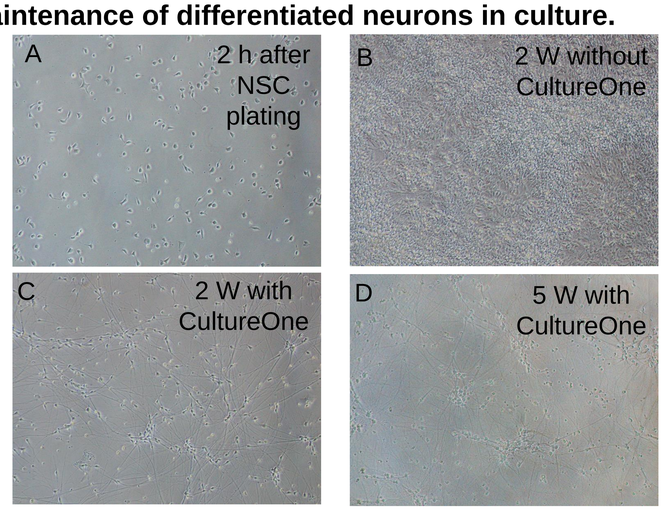

Fig. 1. The neuronal differentiation of hPSC-derived NSCs. A: H9 ESC-derived NSCs at 2 hours after plating at $5x10^4$ cells/cm^2. **B:** Very high density of cells with cell clump formation at 2 weeks of differentiation in conventional neuronal differentiation medium without CultureOne™ supplement. **C:** Evenly distributed neurons with extended neurites at 2 weeks of differentiation with CultureOne™ supplement**. D:** Differentiated neurons at 5 weeks of differentiation with CultureOne™ supplement.

2. CultureOne™ supplement significantly enhances the purity of differentiated neurons.

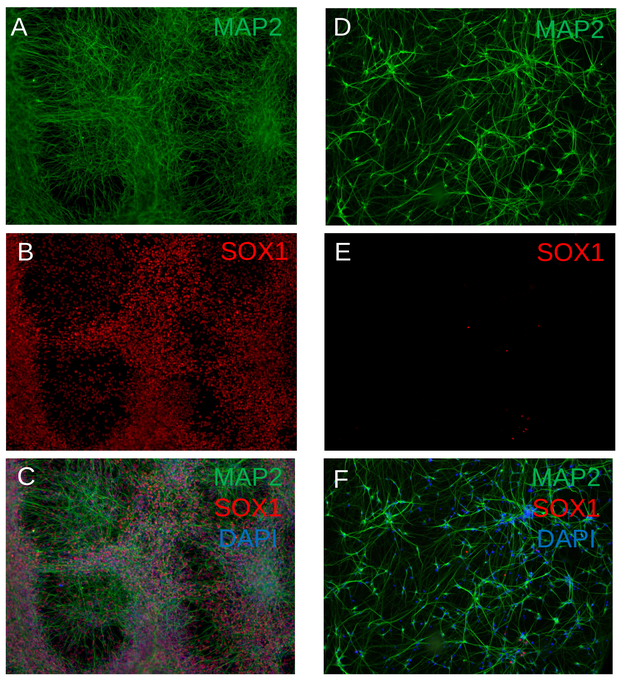

Fig. 2. Neural marker expression of differentiated neurons. A-C: Without CultureOne™ supplement treatment, differentiated cells expressed neuronal marker MAP2 and contaminated with a large number of SOX1 positive undifferentiated NSCs at 2 weeks of differentiation. **D-F:** At 2 weeks of differentiation, almost all cells expressed neuronal marker MAP2 with very few SOX1 positive NSCs in the culture treated with CultureOne™ supplement. Cell nuclei were stained with DAPI (C, F).

3. Mature neuronal maker expression in long-term cultured neurons with CultureOne™ supplement.

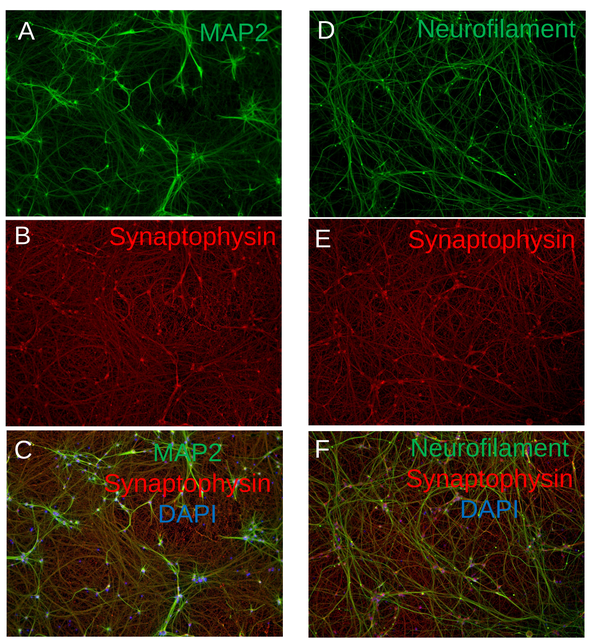

Fig. 3. Long-term cultured neurons treated with CultureOne™ supplement expressed mature neuronal markers. Human PSC-derived NSCs were plated and differentiated for 5 weeks. **A-C:** Differentiated neurons expressed neuronal marker MAP2 and Synaptophysin**. D-F:** Differentiated neurons expressed neuronal marker Neurofilament and Synaptophysin**.** Cell nuclei were stained with DAPI (C, F).

4. CultureOne™ supplement suppresses NSC proliferation without inducing cell death.

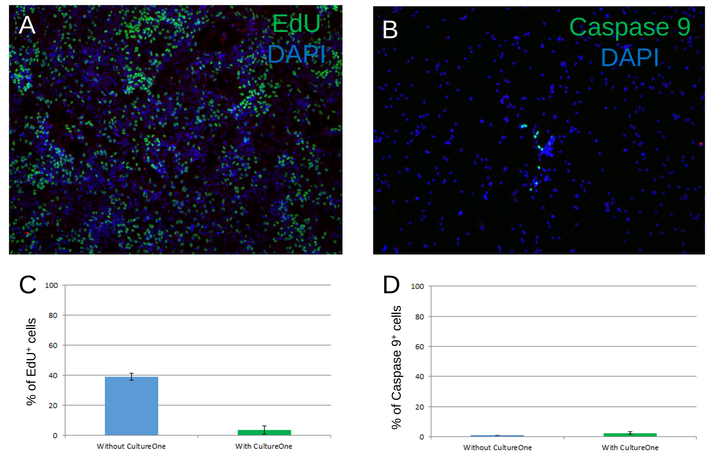

Fig. 4. Detection of proliferation and apoptotic cell death in neuronal differentiation with or without CultureOne™ supplement. EdU was introduced into culture medium and incubated for 2 hours at day 6 of differentiation**.** At day 7 of differentiation, cells were fixed for staining**. A & B:** EdU positive cells of neuronal culture without (A) or with (B) CultureOne™ supplement. **C & D:** Quantification of EdU (C) and cell death marker Caspase 9 (D) positive cells. Cell nuclei were stained with DAPI (A, B).

5. The treatment with CultureOne™ supplement accelerates the maturation of differentiating neurons.

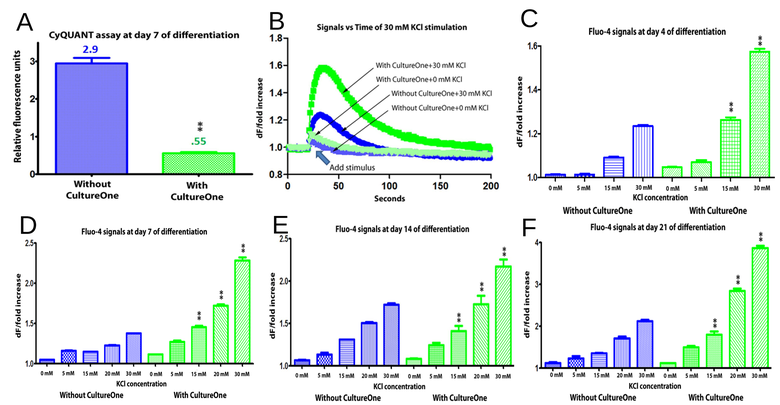

Fig. 5. Calcium influx after depolarization of differentiating neurons induced by KCl. A: Quantification of total cell number in the culture with or without CultureOne™ supplement at day 7 of differentiation. B: A plot of signal vs time for measuring calcium flux using the Fluo-4 Calcium Imaging Kit. **C**-**D**. Tabular data showing the averaged peak calcium responses in cultures with or without CultureOne™ supplement at day 4 (C), 7 (D), 14 (E) and 21 (F) after differentiation.

Conclusions

CultureOne™ supplement can be added into conventional neuronal differentiation for the differentiation of hPSC-derived NSCs to achieve:

1. Even distribution and high purity of differentiated neurons without contaminated undifferentiated NSCs and cell clump formation for easier analyses and quantification
2. Longer maintenance of neuronal culture for more mature functional neurons.
3. Acceleration of neuronal maturation process.

Acknowledgments

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