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Scaled expansion of pluripotent stem cells in suspension culture followed by direct neural differentiation from 3D cell aggregates

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ABSTRACT

Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While two-dimensional (2D) feeder-free expansion of PSCs is well established, the scale at which PSCs and subsequent PSC-derived cell types can be efficiently manufactured using traditional methods is limited without a significant increase in hands-on time, as well as a potential risk of contamination. To overcome this challenge, expansion of PSC as three-dimensional (3D) spheroid in suspension as self-assembled aggregates was introduced for large scale cell proliferation. And it will be important to fully realize the potential of PSCs in downstream applications where large numbers of cells are required, such as cell therapy and high-throughput screening applications, alternative expansion methodologies may be beneficial. In this study we demonstrated the feasibility of directing expanded PSC 3D aggregates to neurons using reagents designed originally for monolayer applications. Key parameters and considerations were identified for both PSC expansion and neural differentiations. The new strategy demonstrates expedited differentiation and expansion steps, scalable expansion of NSCs and neural progenitors, and earlier onset of functional network activity, compared to standard 2D protocol.

INTRODUCTION

While PSC expansion potential is an important parameter for assessing a fit for purpose medium system (i.e., 2D vs. 3D), another important consideration is compatibility with downstream differentiation protocols. In recent years, 3D aggregate cell culture has been gaining traction as an enhanced culture technique which provides more physiologically relevant cell-cell interactions over the traditional 2D cell culture protocols. When determining whether to move from 2D culture environments to 3D culture environments, a number of considerations need to be made; including the quantity of desired cell type(s) required for downstream applications, compatibility of reagents and experimental endpoints designed for 2D, and importantly, how neurons derived using 2D and 3D methodologies compare and contrast to each other. To explore applications of PSC aggregates we optimized 3D protocols for two existing monolayer neural differentiation kits: Gibco® PSC Neural Induction Medium kit and Gibco® PSC Dopaminergic Neuron Differentiation kit. One major challenge of 3D culture is robust quantitative analysis of 3D structures. Our strategy to bypass this challenge was to harness the benefits of the 3D environment for the differentiation and expansion of neural stem and progenitor cells while dissociating these aggregates for quantitative endpoint analysis in 2D. Using this approach we were able to directly compare and contrast 2D vs 3D differentiation with established quantitative assays for differentiation efficiency and neuronal maturation.

RESULTS

Figure 1. Diagram of Standard 2D protocols and 3D optimization strategies

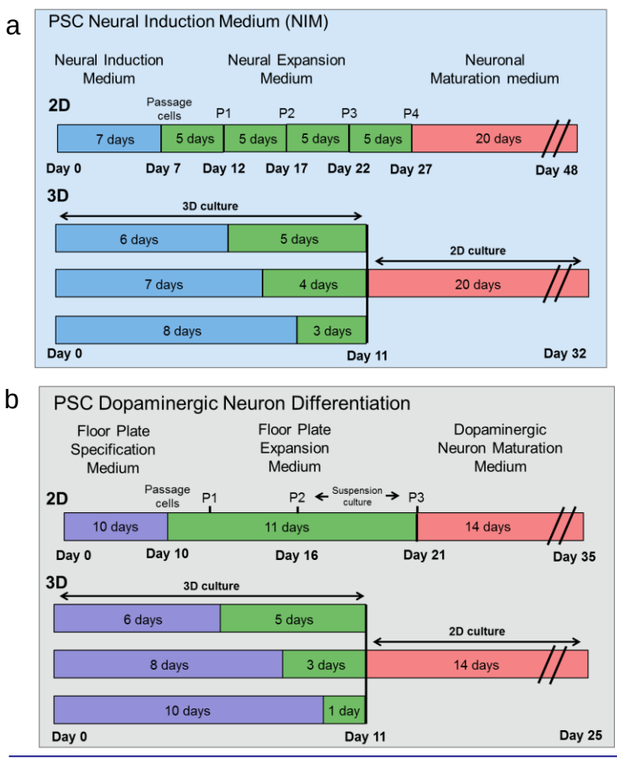
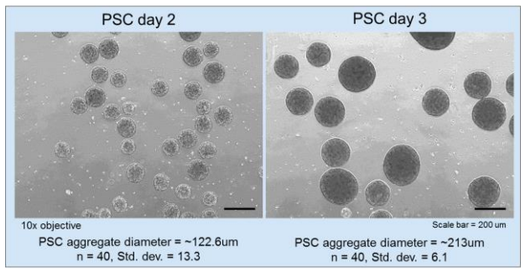
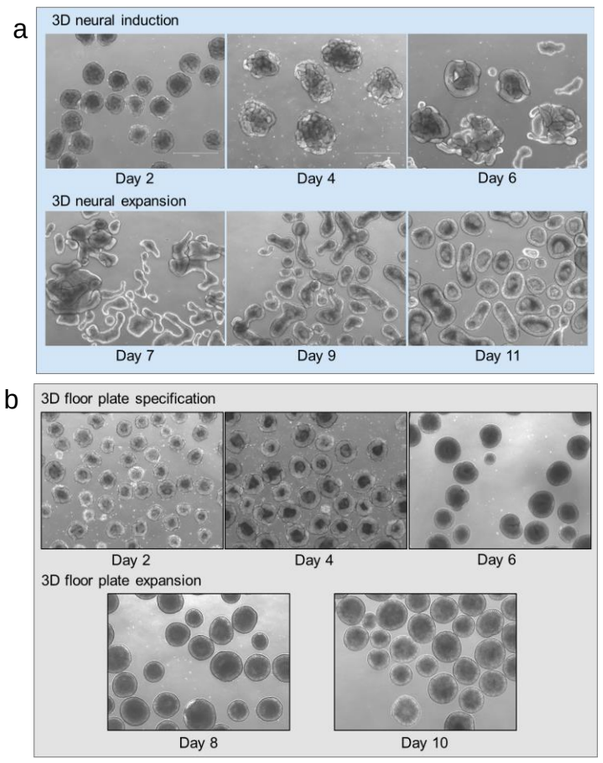


Figure 2. Diameter of PSC aggregates at start of differentiation (2 days vs. 3 days)



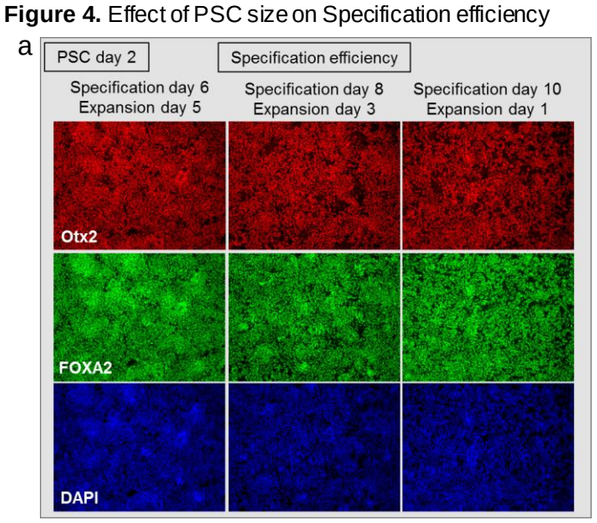
- Sphere size at the start differentiation is an important variable in 3D differentiation protocols and proved to be key in our optimization.
- Gibco Human Episomal iPSCs (GEPI) or WTC11 iPSCs were plated in 6 well non-tissue culture treated plates in PSC suspension culture medium and placed on an orbital shaker at 70 rpm in a standard cell culture incubator at 37C and 5% CO_2. All subsequent 3D steps were carried out in this environment.

Figure 3. Phase images of 3D aggregates in Induction and expansion medium

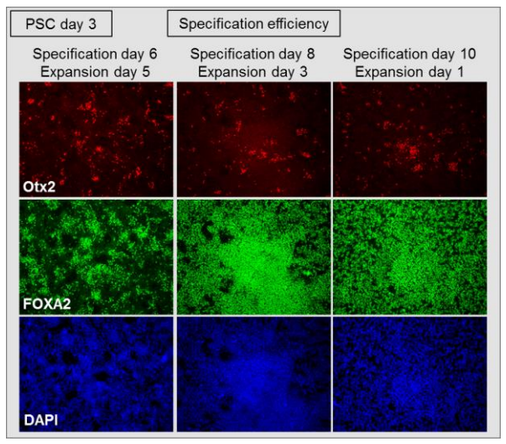


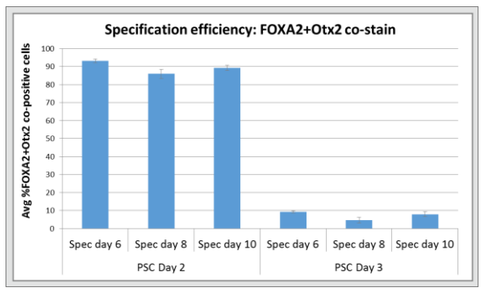
a) Phase images of morphology changes over the duration of neural neural induction and expansion aggregates initially grow in size before forming smaller aggregates with neuroepitheal-like characteristics.
b) Specification aggregates demonstrate visible folding characteristics with less expansion observed during differentiation. Aggregates remain intact and grow in size during expansion.

Figure 4. Effect of PSC size on Specification efficiency



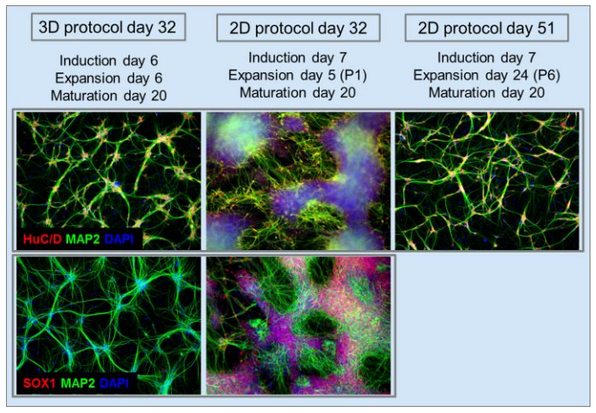
a) Three specification conditions tested from the PSC day 2 starting point showed high levels of FOXA2+Otx2 co-positive cells. The presence of these markers together are used to characterize floor plate progenitor cells.





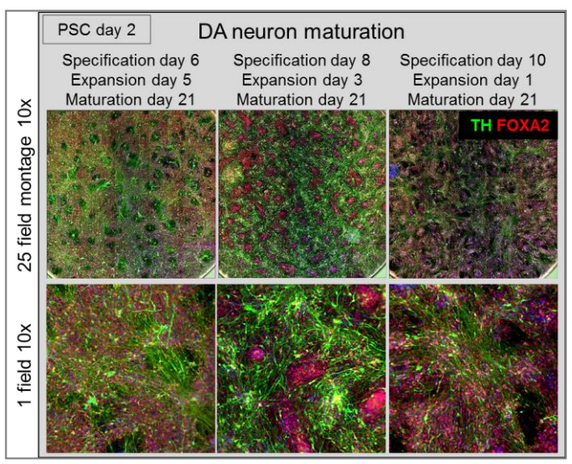
b) Three specification conditions from PSC D3 aggregates showed low levels of FOXA2 and Otx2 co-postivie cells.
c) Quantitative image analysis was performed to using the CellInsight CX5 high-content screening platform. The specification conditions from PSC day 2 all showed over 80% co-stained cells, equivalent to the 2D protocol

Figure 6. Comparison of neurons from 3D NSC vs. 2D NSC (early and later passage)



Neurons from 3D protocol showed similar phenotype marker expression profile to later passage 2D NSC rather than early passage 2D NSC. Upon 20 days maturation of 3D NSC, population is enriched with neurons (HuCD and Map2) with a few expanded progenitors (Sox1)

Figure 7. Effects of PSC size on DA maturation efficiency



a) High levels of Tyrosine hydroxylase (TH) positive dopaminergic neurons (30-50%) were generated during maturation from the PSC day 2 derived progenitor cells.
b) (Top of next column) Significantly lower levels of TH positive neurons were generated from PSC day 3 progenitors.

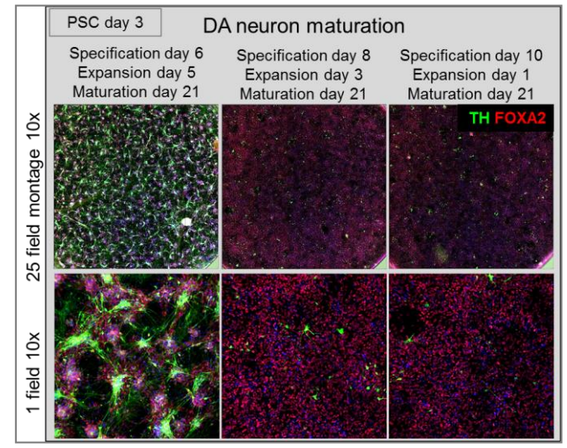
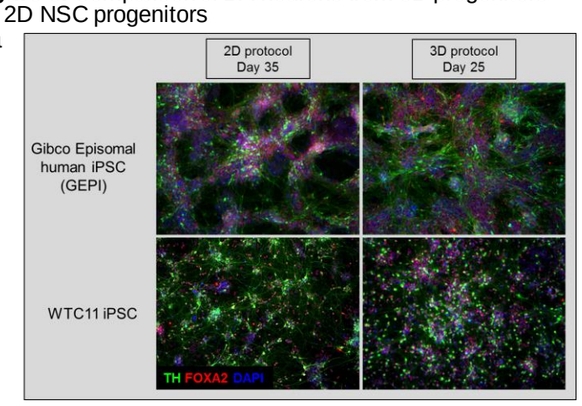
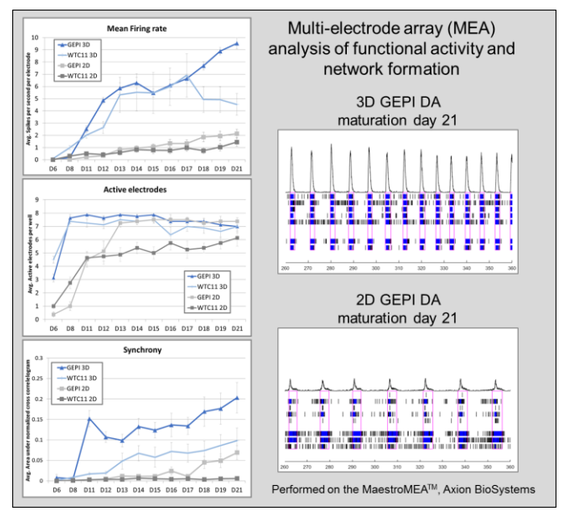


Figure 8. Comparison of DA neurons from 3D progenitors vs. 2D NSC progenitors





a) 2D vs 3D protocol neurons in the GEPI line showed a similar % of TH positive neuronal cell bodies, however significantly more TH expression was observed the neurites of 3D derived neurons. The 3D derived neurons in the WTC11 line were 45-60% TH positive compared to 30-40% for 2D derived.
b) MEA analysis of spontaneous activity showed significantly higher mean firing rate, active electrodes, and Synchrony in 3D derived DA neurons compared to 2D derived. These results suggest earlier onset of network activity and improved neuron functionality using the 3D protocol.

CONCLUSIONS

- PSCs expanded as 3D aggregates could differentiate to neurons and DA neurons using existing 2D differentiation reagents.
- Protocol has been optimized to reflect critical variables identified
- Differentiation in a 3D environment offers significant improvements over the standard 2D protocols: decreased differentiation and expansion steps, scalable expansion of NSCs and neural progenitors, and earlier onset of functional network activity.

TRADEMARKS/LICENSING

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