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SCALED EXPANSION OF HUMAN PLURIPOTENT STEM CELLS (hPSC) IN SUSPENSION CULTURE FOLLOWED BY HIGH-YIELD FACTOR-DRIVEN GENERATION OF INDUCED NEURONS FROM 3D PSC SPHEROIDS

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ABSTRACT

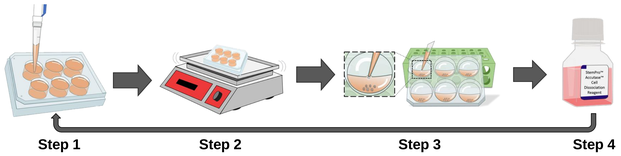
Culture systems for PSC expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While two-dimensional (2D) feeder-free expansion of PSC is well established, the scale at which PSCs and subsequent PSC-derived 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. Therefore, 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. Here we describe a new system for highly scalable expansion of human hPSC as three-dimensional (3D) spheroids in suspension, followed by rapid conversion of hPSC spheroids to functional cortical neurons by forced expression of a single transcription factor NGN2 (hPSC-iN). Generation of hPSC-iNs is traditionally done in 2D and can produce highly pure neurons from PSC in <10 days.
While 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 culture protocols. When determining whether to move from 2D 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 cells derived using 2D and 3D methodologies compare and contrast to each other. Here, we demonstrate the feasibility of generating hPSC-iN's from expanded hPSC 3D spheroids. Key parameters for both hPSC expansion and hPSC-iN generation are presented and discussed, which include scalability, neuronal yield, and differentiation efficiency. Notably, conversion in 3D resulted in significantly higher yield of hPSC-iNs compared to standard 2D method. Finally, the impact of 2D vs 3D induction on hPSC-iN maturation will be presented.

INTRODUCTION

It has been shown that efficient cortical neurons can be obtained from NGN2 inducible iPSC lines (1)(2). We also found NGN2 iPSC lines can differentiate to neurons with cortical phenotypes quite efficiently within 2 weeks. The highly pure population of these cortical phenotype neurons is valuable especially for assays requiring reproducibly homogenous high purity cortical neurons. However, a two-dimensional differentiation system has a limitation such as massive cell death during the rapid induction process. The resulting low-yield makes scale-up cell production difficult. Suspension culture has been developed for bioproduction cell lines whose logic was extended to such as pluripotent stem cells. Gibco™ StemScale™ PSC Suspension Medium is specifically designed to enable hPSC to proliferate in three-dimensional suspension culture. In this study, we cultured an NGN2 inducible hiPSC line in Gibco™ StemScale™ PSC Suspension Medium, and performed the induction system as spheroids. First we optimized 3D differentiation protocol and compared hPSC-iNs derived from 3D to one from 2D protocol in terms of efficiency and phenotype. Further, we investigated whether derived hPSC-iNs can be cryopreserved to generate working cell batch which facilitates large-scale production and is important to control and reduce variation coming from cell model in assay development.

METHODS

Figure 1. The Gibco™ StemScale™ PSC Suspension Medium workflow simplifies suspension culture initiation and maintenance



Step 1: single cells are seeded into culture vessels containing Gibco™ StemScale™ PSC Suspension Medium.
Step 2: place culture vessels onto a platform capable of providing constant agitation.
Step 3: periodically feed cultures with 50% medium replacement.
Step 4: passage spheroids using StemPro Accutase.

Figure 2. Workflow for generating cortical hPSC-iNs

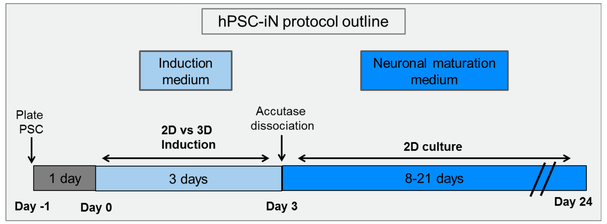
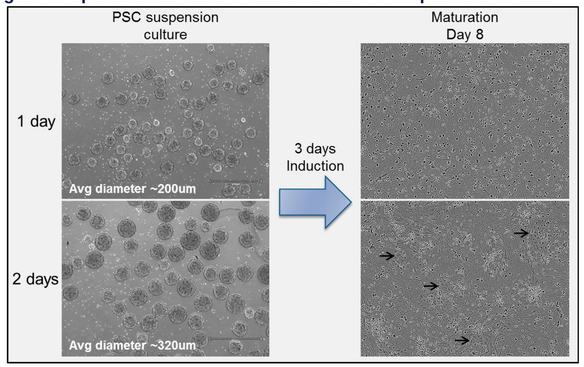


Figure 3. Optimization of differentiation in PSC suspension culture

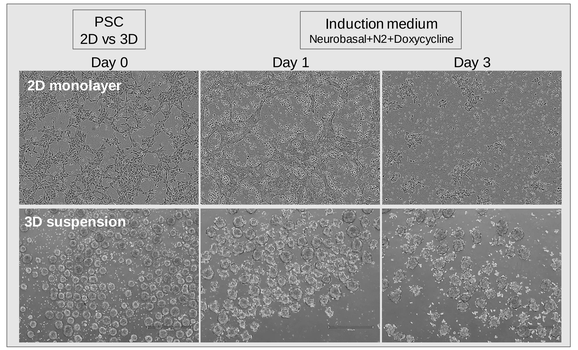


Sphere size at the start of induction is an important parameter to consider for most suspension culture differentiations. For generating hPSC-iNs, smaller PSC spheres at the start of induction lead to highly pure neurons (top). Larger starting sphere size results in decreased induction efficiency and non-neuronal populations during maturation.

RESULTS

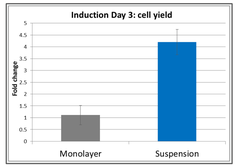
Figure 4. Induction of cortical hPSC-iNs in 3D improves cell survival

a



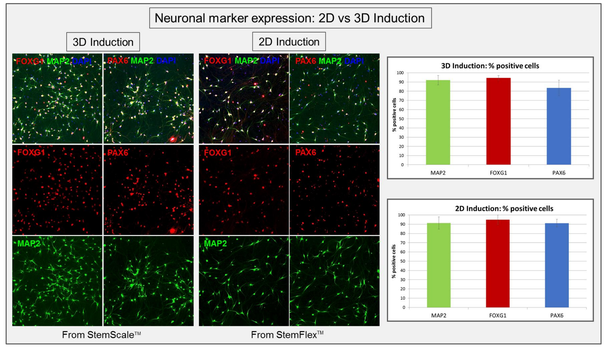
a) Significant cell detachment observed in 2D at day 3 of induction. 3D aggregates are maintained through induction with low levels of cell death

b



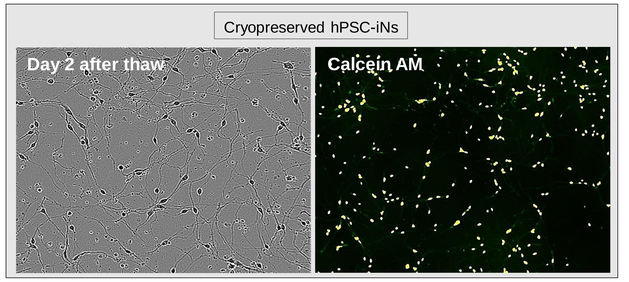
b) 3D Induction resulted in a 4.2 fold increase in cell yield at day 3 compared to a 1.1 fold increase for the 2D differentiation condition

Figure 5. Similar neuronal induction efficiency and cortical marker expression in 3D induced hPSC-iNs compared with 2D induction



Induction efficiency was measured by quantifying the %MAP2 positive neurons; 3D 92.1% neurons vs 91.3% for 2D. The cortical marker, FOXG1, was expressed in 94.5% of 3D derived neurons compared to 94.8% for 2D. Dorsal/ventral marker PAX6, 83.5% of 3D derived neurons compared to 91.4% for 2D.

Figure 6. hPSC-iN recovery from cryopreservation feasibility



After 3 days of Induction hPSC-iNs were cryopreserved. Upon thaw from cryopreservation and plating, cells recovered and at 2 days extensive neurite outgrowth is observed

CONCLUSIONS

- NGN2 PSC line can be expanded as 3D spheroids in Gibco™ StemScale™ PSC Suspension Medium.
- Differentiation protocol is optimized from 2D to 3D to generate hPSC-iNs.
- Combination of large scale PSC proliferation with direct 3D differentiation resulted in hPSC-iNs equivalent to those differentiated in 2D.
- Minimized stress during differentiation and improved yield in hPSC-iNs was obtained with 3D differentiation strategy.
- Obtained hPSC-iNs could be cryopreserved and recovered successfully
- Improved yields in the 3D culture method and the ability to cryopreserve are particularly enabling for large-scale production of hPSC-iNs.

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3. **ISSCR 2020 Poster:** DEVELOPMENT OF A NEW 3D PLURIPOTENT STEM CELL SUSPENSION CULTURE MEDIUM WITH A SIMPLIFIED PROTOCOL THAT YIELDS HIGHLY EFFICIENT SPHEROID NUCLEATION AND ROBUST EXPANSION
Poster board number: TSC337
Poster session: Poster Session VI
Poster session times: 12:00 to 14:00 (EDT, Boston, US)
Date: Saturday June 27, 2020

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