Upscaling and Neural Differentiation of Human Induced Pluripotent Stem Cell Spheroids in 3D

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INTRODUCTION

The efficacy of current treatments targeting neurological diseases of the brain is affected by their inability to cross the blood-brain barrier (BBB). Innovative study models to find a solution to this problem is beneficial. Human induced pluripotent stem cells (hiPSCs) are capable of indefinite cultivation and differentiation into nearly any cell type, including traditionally inaccessible cells critical for BBB function like astrocytes. hiPSCs thus can represent an effective alternative screening tool aiding in determining the efficacy of new treatments.

The challenge in using this approach for screening is the need for billions of high-quality cells. Obtaining high cell numbers is not impossible with traditional monolayer culturing, but laborious and inefficient. Bioreactor technology can limit the labor demand and simultaneously provide optimal growth conditions for hiPSCs. However, process development is required for the transition to such new cell culture devices, as multiple parameters can affect growth and quality. Thus, we aimed to develop methods for large-scale hiPSC culture for subsequent differentiation to astrocytes in 3D. To achieve this, we investigated iPSC spheroid upscaling using the SCINUS Cell Expansion System (SCES) and direct differentiation of suspension-grown iPSCs towards astrocytes.

METHODS AND MATERIALS

Adherent hiPSCs were adapted as self-assembled spheroids in 6-well plates and maintained on an orbital shaker agitating at 70 rpm. Single cells were seeded at a density of 1 – 1.5x10^5 cells/ml in StemScale medium. To scale-up, critical culture parameters were investigated by imposing variations in refreshing regimes, seeding densities and agitation to evaluate favorable parameter combinations. Process development for bioreactor culture was performed by assessing growth profiles with successive changes to culture formats from 6-well plates, to Permalife bags and finally largescale culture in the SCES. Spheroid homogeneity and differentiation capacity, was attained by astrocytic lineage induction over a 49-day period followed by an adherent maturation phase (Figure 1).

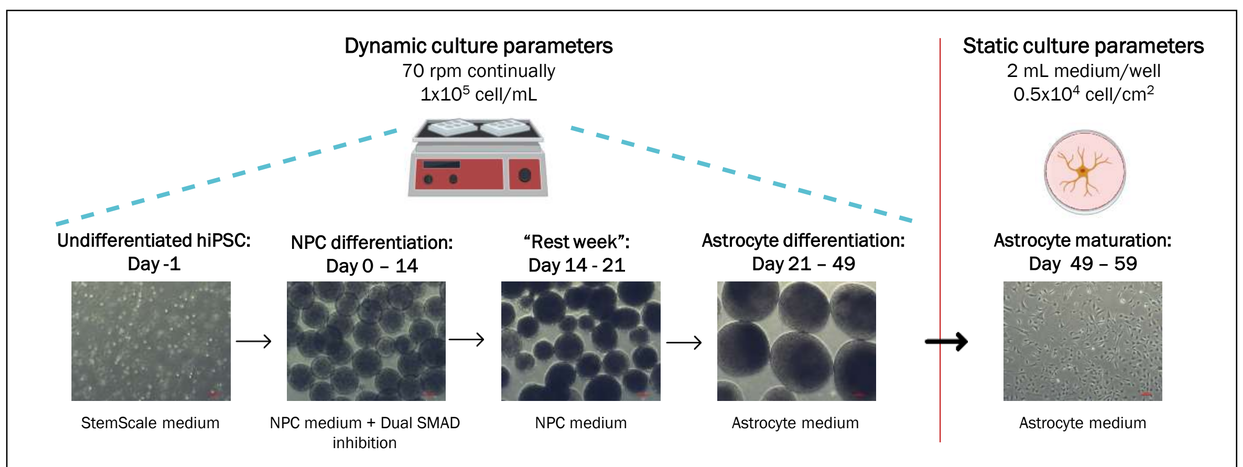

Figure 1 – *3D differentiation of hiPSC spheroids to Neural Progenitor Cells (NPCs) and finally to astrocytes.*

RESULTS

Upscaling hiPSC spheroids in suspension

The balance in cell density, medium refreshment and agitation regime was improved during this study to maintain the growth and proliferation of hiPSC spheroids during the adaptation period (Figure 2). Agitation speed was essential to spheroid formation after single cell seeding and subsequent growth until harvest. Rocker speeds upwards of 300˚/s were key to controlling spheroid size and homogeneity, ensuring nutrient availability and cell survival until harvest. Results show proof-of-principle for a successful adaptation of small-scale static- to large-scale suspension hiPSC cultures for the first time in the *SCES*.

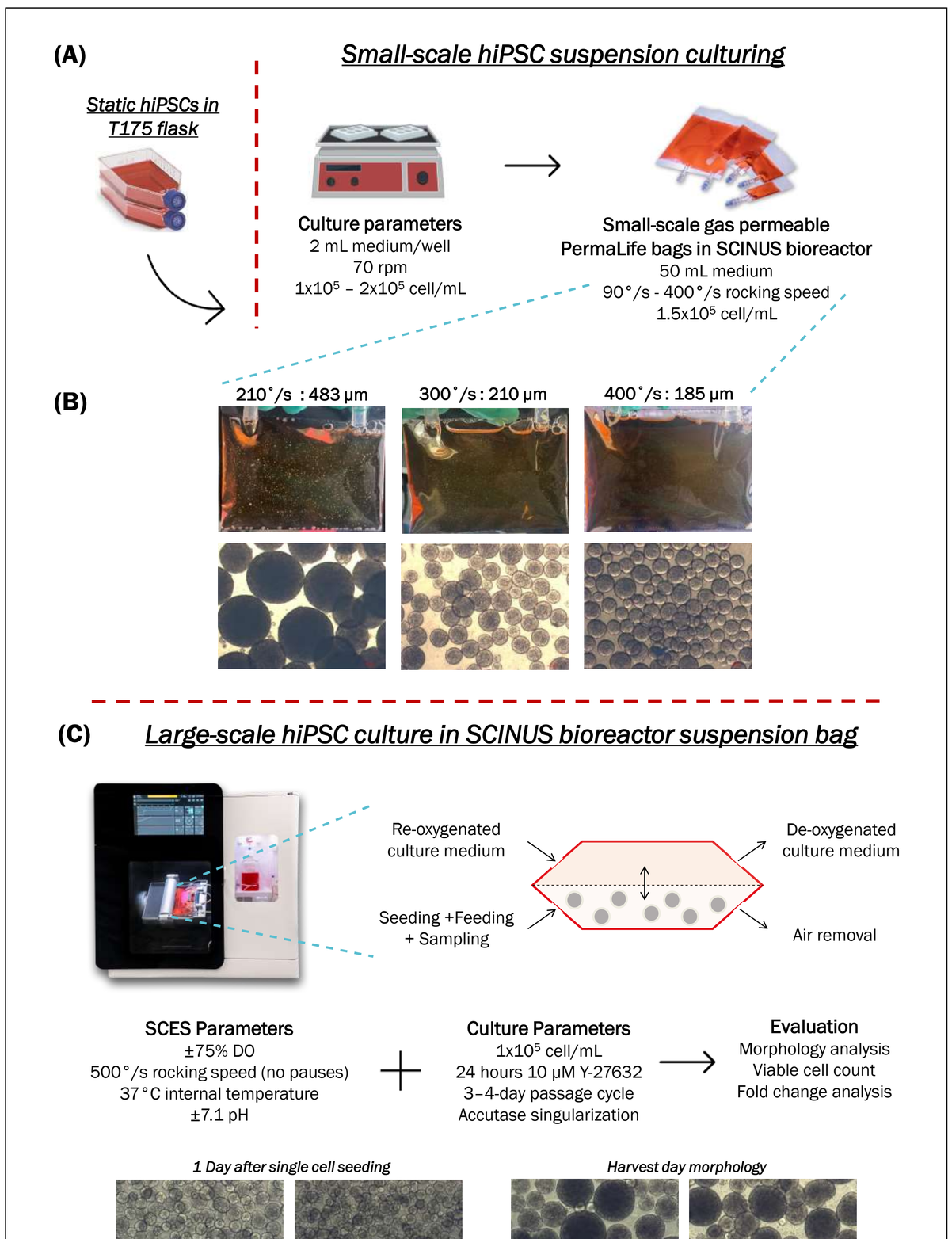

Figure 2 – *(A) Process development for upscaling and translating small-scale suspension models to the SCES- large-scale suspension bag. (B) Day 2 results in bioreactor pilot studies using Permalife suspension bags displaying how rocker speed can be used to control spheroid growth/size. (C) Day 1 and harvest day results of successful spheroid growth and maintenance in large-scale suspension bioreactor bags.*

hiPSC – Astrocyte spheroid differentiation in 3D

For 14 days, hiPSC spheroids were differentiated to NPCs via SMAD inhibition then allowed to rest for 1 week before feeding with Astrocyte medium for an additional 28 days (Figure 3). Spheroids remained intact in 3D until singularization and maturation on a 2D substrate. NPC marker expression was observed on day 18 indicating successful NPC differentiation and that nutrient availability was maintained, and finally, morphological analysis during maturation showed well adapted, *astrocyte-like cells.*

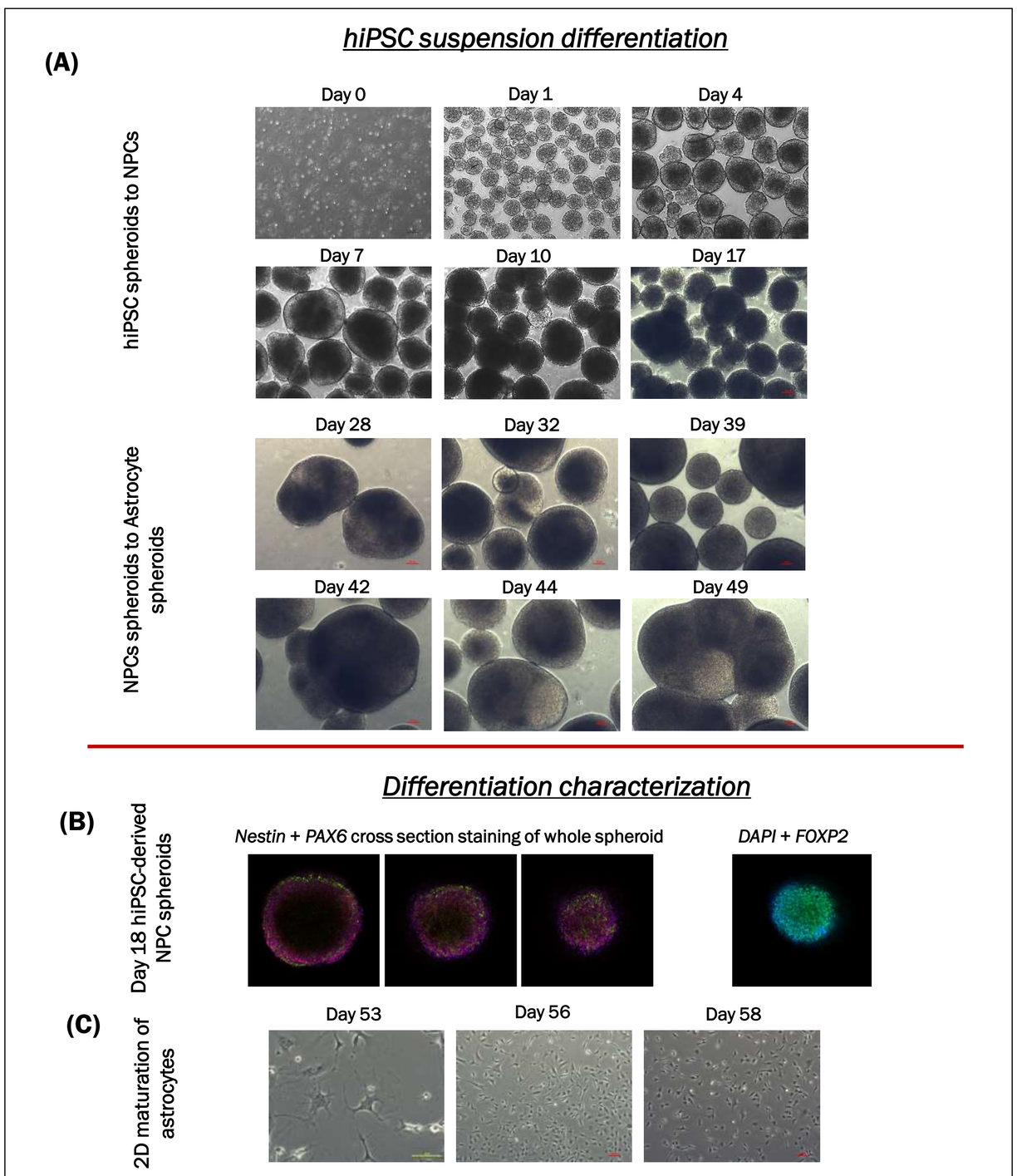

Figure 3 – *(A) Direct differentiation of hiPSC spheroids first to NPCs then to astrocytes in 6-well plates. (B) hiPSC-derived NPCs were stained with NPC-specific markers Nestin, Pax6 and FOXP2. (C) After spheroid singularization, cells adhered to uncoated plates revealing a star shaped, astrocyte-like morphological appearance.*

CONCLUDING REMARKS

We were able to successfully scale static hiPSCs to self aggregating spheroids in suspension. These could then be directly differentiated towards the neural lineage. This method for producing large-scale hiPSC cultures in the ***SCES*** is promising for obtaining large quantities of cells which can be employed for novel biomedical drug discovery and development approaches in fields like neurology.

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