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APPLICATION NOTE No. 485

Human Induced Pluripotent Stem Cell (hiPSC) Aggregate Expansion in Stirred-tank Bioreactors Using SciVario® twin Bioprocess Controller

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Abstract

Cell therapy is an advanced and promising field with the potential to transform modern medicine as we know it and bring therapeutic benefits to patients in different disease areas with unmet medical needs. Autologous cell therapy is a novel intervention in which cells are collected from an individual patient, expanded/engineered *ex vivo*, and reinfused into the same patient. This is a promising approach that reduces the risk of immune rejection and the need for immunosuppressive drugs. However, the high costs, the scale-up complexity, and the prolonged time to manufacture the final product for each patient present several challenges that prevent it from reaching its full potential. Off-the-shelf cell therapy (allogeneic cell therapy) utilizes the cells collected from a healthy donor (avoiding, for instance, possible patient cell mutations that could lead to a treatment with limited success) and is used in patients with different medical conditions. In this sense, induced pluripotent stem cells (iPSCs) could provide an inexhaustible cell source, expanding their applicability to new medical areas. Contrary to the autologous approach, this manufacturing process becomes more standardized with a high success rate and lower costs. Nevertheless, scalability requirements are some of the main challenges in supporting cell growth for a large group of patients. In this work, the potential, and benefits of stirred-tank bioreactors in the iPSC culture process were explored and technical details on iPSC aggregates expansion in 1 L bioreactors controlled by SciVario® twin bioreactor control system were provided. Specifically, the aggregate formation of human iPSCs (hiPSCs) was controlled (by using well-defined parameters) from single cell inoculation obtaining 6.4 x 10^9 hiPSCs in a working volume of 0.8 L (16-fold) after 5 days of culture. In addition, the metabolites derived from the process, as well as the morphology and expression of pluripotency markers were monitored and analyzed.

Introduction

Cell and gene therapy (CGT) represents a new and constantly evolving frontier in advanced therapies with the potential to provide breakthroughs in curing diseases with no or very limited treatment options [1-4]. It also enables personalized approaches to patients not responding anymore to conventional treatments. In terms of cell therapy strategies, two main categories are known, autologous and allogeneic cell therapy.

In autologous cell therapies, patients donate their own cells which then undergo expansion and sometimes also *ex vivo* genetic modification before being reinfused back into the same patient as a therapeutic intervention. Autologous cell therapy provides many benefits due to its immunological advantages but also faces challenges related to product stability, manufacturing, and logistics [5-7]. The dependence on sick patients as a source of starting material is a key challenge as not all patients have adequate cells to start the process leading to delayed treatments. However, allogeneic therapies follow a similar manufacturing workflow as autologous therapies but with the difference that the starting material consists of cells derived from healthy donors, stem cells or cell banks, as well as the producing material to stock in "bigger" volumes. Therefore, they are often referred to as "off-the-shelf" cell therapies [8-13]. The allogeneic strategy offers great advantages such as reduced manufacturing costs and increased accessibility to a large group of patients compared to its autologous counterparts. Despite all the benefits, one of the key challenges of allogeneic therapies is the allo-immune response that leads to graft versus host disease (GvHD) [14-16]. Although several cell types have been identified to treat GvHD, the use of human induced pluripotent stem cells has paved the way for developing cell therapies with very low immunogenicity [17-20].

hiPSCs, unlike embryonic pluripotent stem cells, are adult cells that have been genetically programmed back into a pluripotent state, capable to differentiate into almost all cell types. Moreover, hiPSCs are amenable to gene modification and can be expanded limitlessly, making them the ideal cell platform for developing and manufacturing off-the-shelf cell products [21-23]. iPSCs derived from human leukocyte antigen (HLA)-homozygous individuals and hiPSCs genetically engineered for HLA depletion are among the most promising sources of starting material for the manufacture of cell-based therapeutic products [24-26].

However, expansion of iPSC presents several challenges that need to be considered to ensure efficient large-scale manufacturing, for example process consistency and reproducibility [27-30]. Developing well-standardized protocols is essential to guarantee consistent results and this implies reducing variations in culture conditions, medium formulations, and handling techniques that can affect cell pluripotency and differentiation potential. This is the case with the hiPSC subcloning process. By isolating and expanding a single hiPSC colony from a heterogeneous culture (due to iPSC spontaneous differentiation), it is possible to obtain a more consistent and high quality hiPSC population (or inoculum) with identical genetic characteristics to the parent cells, thereby reducing variability within culture and providing consistency in pluripotency. Another important aspect to consider is the scalable culture system. The proper mix of nutrients and oxygen becomes crucial to support cell growth while maintaining pluripotency, which is why adopting scalable systems such as bioreactors or culture vessels with increased surface area or volume is imperative. Furthermore, feeding strategies must be optimized to efficiently deliver essential nutrients, growth factors, and signaling molecules to the cell culture. To support iPSC expansion, cost-effective processes including a batch, fed batch, or perfusion system should be considered in each expansion process case. Finally, iPSC expansion often involves transitioning from cell monolayer cultures to aggregate-based systems. Consistent aggregate formation as well as a controlled dissociation process into single cells are among the biggest challenges in maintaining cell viability and pluripotency.

In this study, a robust and consistent monitored culturing process was performed for bioreactor expansion of hiPSCs using the SciVario® twin bioreactor control system and DASGIP® 1 L Spinner Vessels. With the here described protocol, cell densities of 8×10^6 cells/mL were obtained in 5 days. Important process parameters like cell growth as well as viability, and metabolic activity (glucose, ammonia, and lactate levels in the culture) were analyzed throughout the run. In addition, hiPSC aggregate morphology and cell pluripotency markers expression was studied.

Material and Methods

SciVario twin bioreactor control system

The SciVario twin bioreactor control system was used to perform two medium exchange batch culture runs simultaneously using DASGIP Spinner Vessels, with a working volume range of 0.35 to 1.0 L and equipped with eight-bladed impellers (60° pitch), developed and optimized especially for stem cell culture [31]. Each bioreactor control system is equipped with three universal port connectors for pH and dissolved oxygen (DO) sensors, a temperature control block that combines electrical heating and water cooling, agitation control and a gassing module that includes a Thermal Mass Flow Controller (TMFC) with gas flow rates of 0.1 – 1,200 standard liter per hour (SLPH) (resulting in an ultra-high turndown ratio of 1:12,000), as well as four solenoid valves (see Figure 1).

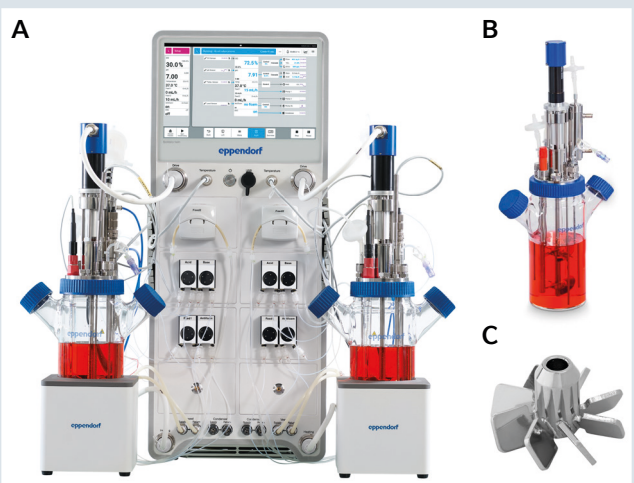

Fig. 1: (A) The SciVario twin bioreactor control system allows the control two single-use or glass bioreactors, either individually or in parallel, at the same time across a wide range of vessel sizes from small- to bench-scale. In this study, the **(B)** DASGIP Spinner vessel equipped with an **(C)** eight-blade pitched impeller specifically designed for the culture of shear-sensitive cells in suspension or as aggregates was employed.

To learn more about the possibilities of the SciVario twin bioreactor controller, please visit www.eppendorf.group/sci-vario

Sensor calibration

Prior to the preparation of the DASGIP Spinner Vessels (1 L), ISM® gel-filled pH sensors (Mettler Toledo®) were connected to the SciVario twin bioreactor control system where they were automatically detected by the software. The calibration process was performed according to the operations manual using buffer solutions of pH 7 and pH 4 as "zero" and "span", respectively. Hereafter, the pH sensors were disconnected and inserted into the headplate of the DASGIP Spinner Vessel.

The DO sensors (Mettler Toledo®) were inserted into the DASGIP Spinner Vessel's headplate as well and after autoclave sterilization calibrated under the same iPSC culture conditions in accordance with the SciVario twin operations manual.

Bioreactor preparation and process parameters

Each vessel was assembled with pH and DO sensors, 2 dip tubes along with a compression probe adapter inserted in a spare Pg 13.5 port (one for inoculation/medium addition and the second one for medium exchange) and two liquid addition ports on each vessel headplate (one for base (sodium bicarbonate, Thermo Fisher Scientific®) addition and another for the addition of 0.1 % antifoam (Pluronic F-68 surfactant, Thermo Fisher Scientific).

The vessels were sterilized and placed in their respective temperature control block to keep the system at a constant temperature. Then, direct drives as well as DASGIP Peltier exhaust condensers were assembled to each vessel and

Table 1: Process parameters and setpoints of the batch culture experiments.

Parameters	Setpoints
Working volume	0.8 L
Agitation	80 rpm (tip speed 0.4 m/s)
Temperature	37 °C
Inoculation density	0.5 × 10^6 cells/mL
Cell culture medium	Stem Scale PSC culture medium
DO setpoint	40 % (P = 0.1; I = 3.6/h)
pH setpoint	7.4 (deadband = 0.1), cascade to CO_2 (acid) and cascade to 0.45 M sodium bicarbonate (base)
Gassing range	0.1 SLPH – 60 SLPH
Gassing cascade	Set O_2 % at 30 % controller output to 21 % and at 100 % controller output to 80 %. Set flow at 0 % controller output to 0.1 SLPH, and at 100 % controller output to 60 SLPH

the gas sparge lines (from the controller) were connected to the submerged sparge filter on the vessels. Finally, each 1 L glass vessel was filled with 600 mL of Stem Scale PSC culture medium (Thermo Fisher Scientific) supplemented with 0.1% of Pluronic F-68 surfactant and 10 µM ROCK inhibitor Y27632 dihydrochloride (Thermo Fisher Scientific) and conditioned for at least 24 hours using the parameters and setpoints listed in Table 1.

hiPSC cell culture

An overview of the complete iPSC expansion workflow scheme is shown in Figure 2. The individual steps are detailed in the following.

Experiments were performed using hiPSC line KYOU-DXR0109B derived from dermal fibroblasts obtained from a healthy donor (ACS1023, ATCC). Prior to suspension culture, hiPSCs expansion process was performed as described earlier [32], but with some modifications.

hiPSC were seeded in vitronectin-precoated 6 well plates using pre-warmed StemFlex medium (Thermo Fisher Scientific) supplemented with RevitaCell supplement (Thermo Fisher Scientific) at a density of 4×10^4 cells/cm^2. Pre-coating of the 6 well plates was achieved by mixing 60 µL of thawed vitronectin (Thermo Fisher Scientific) with 6 mL of sterile Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium and magnesium (Ca_2^+/Mg_2^+) at room temperature into a 15 mL conical tube to a final concentration of 0.5 µg/cm^2. StemFlex medium was changed daily. At day 5, the 6 well plate containing hiPSC colonies was removed from the CellXpert® C170i incubator and placed into the laminar hood under the EVOS FL microscope (Thermo Fisher Scientific) (Figure 3).

hiPSC were picked by tracing a circle around the colonies border with a needle. A 200 µl Eppendorf Research® plus pipette was used to remove the hiPSC colonies from the surface of the plate. The colony picking process was continued until enough hiPSC were available. Then, hiPSC were seeded in vitronectin-precoated T-75 flask (Thermo Fisher Scientific) and incubated at 37°C and 5 % CO_2 in a CellXpert C170i Incubator. StemFlex medium was changed the day after seeding, and then every other day until cells reached the desired confluence. For passaging at a hiPSC confluence of 60-80 %, StemFlex medium was aspirated, the cells were washed with PBS without Ca_2^+/Mg_2^+ and subsequently detached by incubating with ReLeSR (Stem Cell Technologies) at 37°C and 5 % CO_2 for 5 min. Afterwards, StemFlex medium was added to each flask to dilute the ReLeSR. Hereafter, the cells were transferred into a 15 mL conical tube and counted using a Vi-CELL XR cell viability analyzer (Beckman Coulter®). Finally, the hiPSC suspension was centrifuged at 200 × g (Centrifuge 5430R) for 4 min, followed by supernatant removal, resuspension in StemFlex medium and subsequent seeding of cells at a density of 4×10^4 cells/cm^2 in vitronectin-precoated T-175 flasks (Thermo Fisher Scientific).

In the following, the above procedure of cell expansion and culture splitting was repeated until an inoculum cell density of 0.5×10^6 cells/mL was reached.

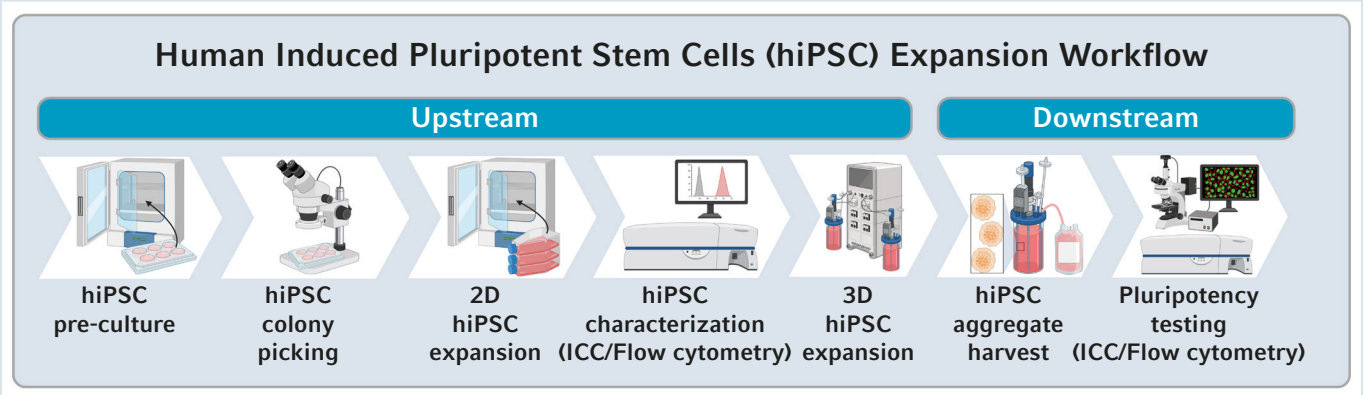

Fig. 2: hiPSC expansion workflow as aggregates in stirred-tank bioreactors (STB). Flow cytometry (FC) and immunocytochemistry (ICC) techniques were used to characterize hiPSC pluripotency. Created with BioRender.com.

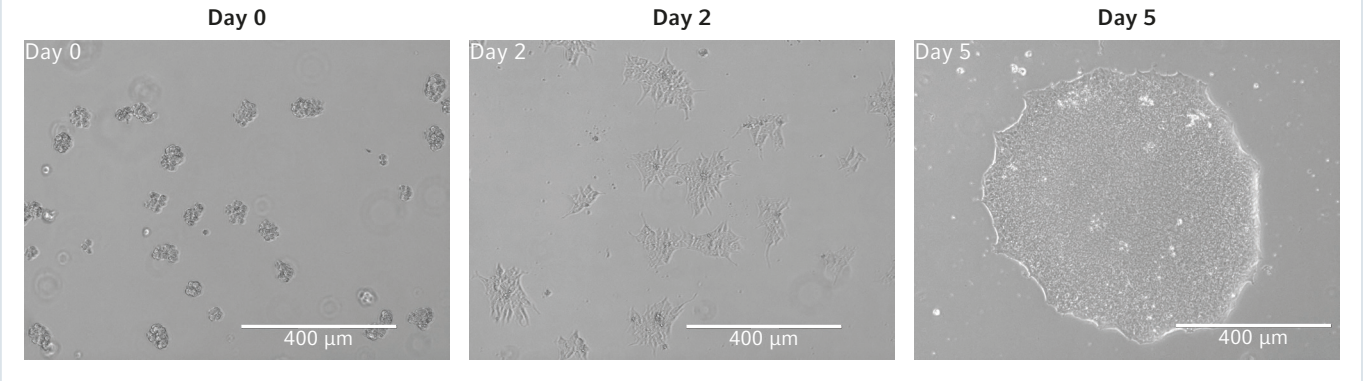

Fig. 3: Growth and morphology of hiPSC colonies in 6 well plates over time. hiPSC colony (Day 5) consists of tightly packed cells and well-defined borders. The scale bar corresponds to 400 µm.

Then, 400 × 10^6 cells in 200 mL of Stem Scale PSC culture medium supplemented with 0.1% of Pluronic F-68 surfactant and 10 µM ROCK inhibitor Y27632 dihydrochloride was added to each inoculation bottle.

Bioreactor inoculation

Two DASGIP 1 L Spinner Vessels were inoculated as described in the section "hiPSC cell culture" for a total working volume of 0.8 L with a cell density of 0.5 × 10^6 cells/mL and 97 % cell viability. In both cases, hiPSCs were cultivated at 37 °C and an impeller stirring speed of 80 rpm. DO and pH setpoints were controlled by the cascades described in Table 1. Medium exchange was performed daily 24 hours after non-aggregate cell inoculation until day 5. For that, the agitation and gas flow were stopped for 5 min, allowing the aggregates to settle at the bottom of the vessel, and 40 % of medium was exchanged using a dip tube to remove the cell culture medium from the surface and the feeding port to add fresh and pre-warmed (37°C) Stem Scale PSC culture medium without supplementation.

Aggregates sampling and analysis

In order to monitor the aggregates formation and diameter, 20 mL of culture per bioreactor were collected daily by welding a Labtainer bag with line sets (Thermo Fisher Scientific) to one of the liquid addition ports without interruption of gassing and stirring. The culture samples were then transferred into a 50 mL conical tube. After allowing the aggregates to settle to the bottom of the tube, the supernatant was removed, and 1 mL of Stem Scale PSC culture medium was added. Up to 10 brightfield images from both samples were taken and the mean average of the aggregate diameter was obtained from around 20 single aggregates using ImageJ (National Institutes of Health, USA). The collection and analysis processes were repeated every day until day 6 of the culture.

Cell viability and metabolic activity

Samples were collected twice a day from the bioreactors to determine the cell viability, cellular density, and the concentration of metabolites like ammonia (NH_3), lactate, and nutrients like glucose. For that, a sterile 5 mL syringe was connected to the Luer Lock sample port and 3 mL of dead volume were discarded before another 3 mL were collected with a new 5 mL sterile syringe as a viable sample for analysis. The sample was transferred into a 15 mL conical tube. After allowing the aggregates to settle to the bottom of the tube, the supernatant was collected for pH monitoring and metabolite analysis. Glucose, ammonia, and lactate were measured using a CEDEX® Bio Analyzer (Roche). Aggregates were washed with DPBS, filtered through a 37 µm Strainer (Stem Cell Technologies) and incubated with 500 µl of enzyme-free Gentle Cell Dissociation Reagent (GCDR, Stem Cell Technologies) at 37°C for 5 min. Then, 2 mL of Stem Scale PSC culture medium were added, and the aggregates were gently dissociated into single cells using 1 mL serological pipette. hiPSC were centrifuged at 200 × g for 4 min, the supernatant was discarded, and cells resuspended in 3 mL of Stem Scale PSC culture. Cell density and viability

were measured via the trypan blue exclusion method using a Vi-CELL XR Viability Analyzer (Beckman Coulter).

pH values were monitored offline by using an Orion Star 8211 pH-meter (ThermoFisher Scientific). Using the offline pH value, the pH calibration on the controller was re-standardized daily to prevent any discrepancy between online and offline measurements.

Pluripotency expression

Flow cytometry

Cells expanded in T flasks (iPSC colonies) and from both bioreactors (iPSC aggregates) were dissociated into a single cell suspension and incubated with conjugated antibodies (diluted in FACS buffer (PBS, 5% FBS) against TRA 1-60 (1:20, 330609, BioLegend), SSEA4 (1:20, 330405, BioLegend) and SSEA1 (1:20, 323005, BioLegend) (pluripotency markers) and corresponding isotypes control for 30 min at 4° C. The cells were washed twice with FACS buffer (PBS containing 1-5% FBS (fetal bovine serum)) and centrifuged at 200 × g for 4 min. Samples were acquired with a Guava easyCyte flow cytometer (Luminex Corporation) and analyzed by the FlowJo software (BD).

Immunocytochemistry

iPSC suspension from T flasks and aggregates from both bioreactors were fixed with paraformaldehyde fixation solution (4% in PBS) for 15 min at room temperature and washed twice with DPBS. For intracellular marker staining, cells were incubated with DPBS containing Sigma-Aldrich® 0.3% Triton X-100 (Merck), washed twice with DPBS, and incubated for 30 min in blocking buffer (10 % normal goat serum in DPBS (Thermo Fisher Scientific) at room temperature. Primary antibodies directed against pluripotency markers (rabbit anti OCT-4 (MA514845, Thermo Fisher Scientific), rabbit anti Nanog (PA1097X, Thermo Fisher Scientific) and mouse anti TRA 1-60 (MA1-023, Thermo Fisher Scientific)) were diluted in blocking buffer and samples were incubated overnight at 4°C. Cells were then washed twice with DPBS and incubated 1h at room temperature with their respective fluorescently labelled secondary antibodies (goat anti-rabbit IgG, Alexa Fluor 488, (A-11008, Thermo Fisher Scientific), goat anti-rabbit IgG, Alexa Fluor 568 (A-11011, Thermo Fisher Scientific), and rabbit anti-mouse IgG, Alexa Fluor 594 (A-11062, Thermo Fisher Scientific)). Nuclei were counterstained with DAPI. Images were taken with a confocal microscope (LSM 780, ZEISS).

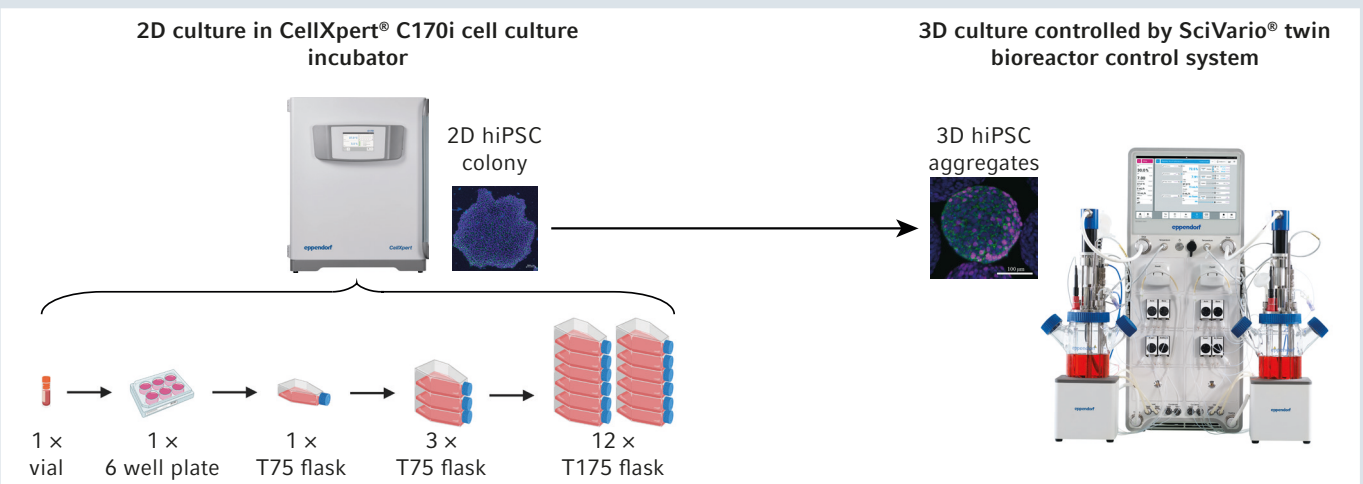

Fig. 4: Controlled expansion process of hiPSC from T flasks to bioreactor.

Results and Discussion

Expansion of hiPSC in DASGIP 1 L Spinner Vessels

A 3D bioreactor culture system using DASGIP 1 L Spinner Vessels controlled by SciVario twin bioreactor control system was evaluated for expansion of hiPSCs. Following the schematic representation depicted in Figure 4, hiPSC were first expanded in two-dimensional (2D) culture systems until a cell density of 4 × 10^4 cells/cm^2 followed by 6 days of suspension-based expansion in 2 parallel bioreactors at 0.8 L process scale.

Before proceeding with the bioreactor's inoculation process, hiPSCs were detached from monolayer cultures and analyzed for pluripotency properties. As shown in Figure 5A, cells expressing different pluripotency-associated markers were identified using flow cytometry and confirmed by immunocytochemistry staining of seeded hiPSCs (Figure 5B).

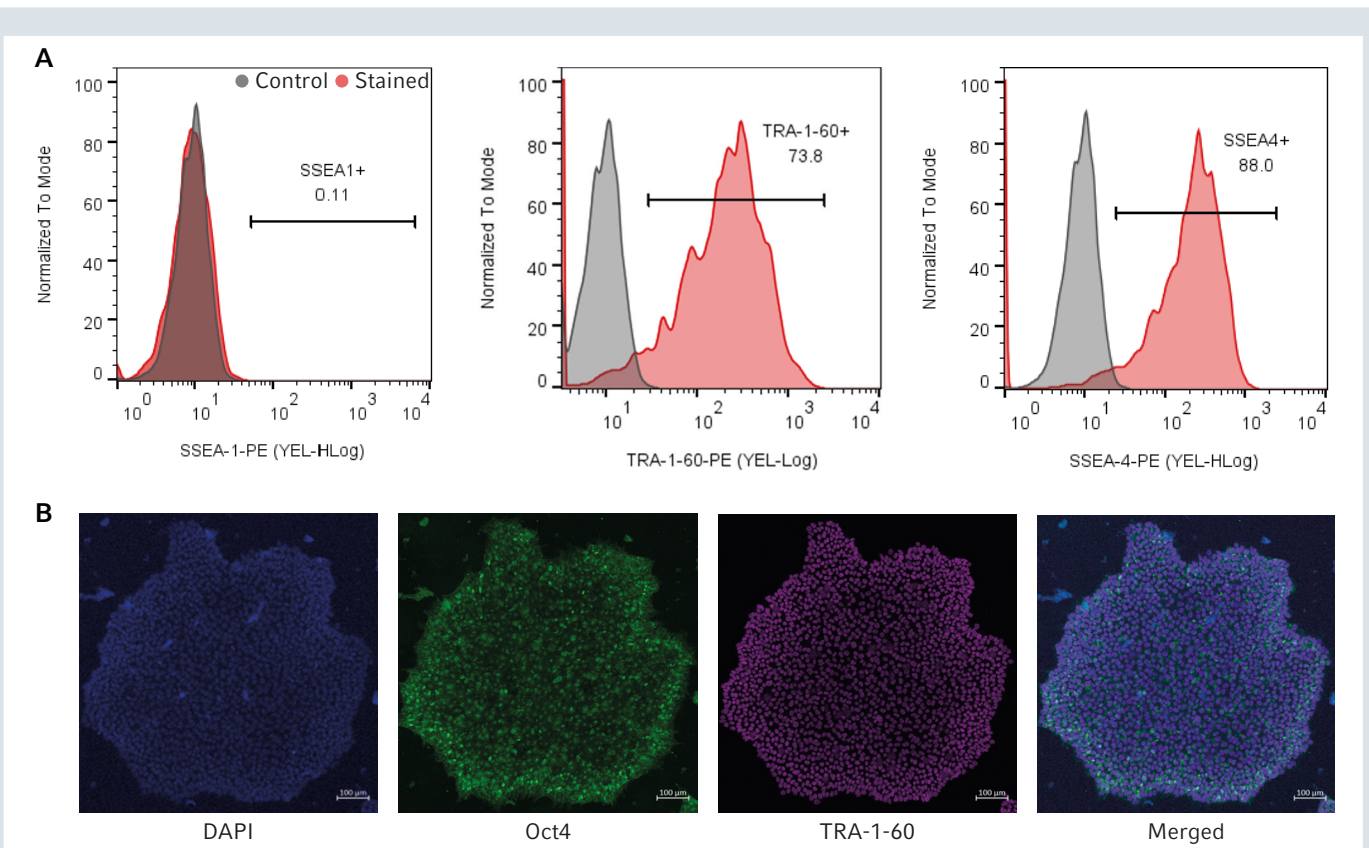

Fig. 5: hiPSC harvested after 2D expansion expressing pluripotency markers were analyzed by **(A)** flow cytometry (isotype controls shown in grey) and **(B)** immunocytochemistry.

Then, both DASGIP 1 L Spinner Vessels were inoculated with a cell density of 0.5 × 10^6 cells/mL at ~97 % cell viability under a controlled environment (see Table 1). An initial lag phase was observed within the first 24 hours (phenomenon already described in the literature [33]), followed by an exponential growth between day 2 and 5 of culture in both bioreactors. For both bioreactors, the cell viability and cell density curves were comparable, reaching a peak viable cell density of around 8 x 10^6 cells/mL (Figure 6A). Lactate and NH_3 levels were maintained below 2 g/L and 2 mmol/L respectively during the whole run (Figure 6B/C).

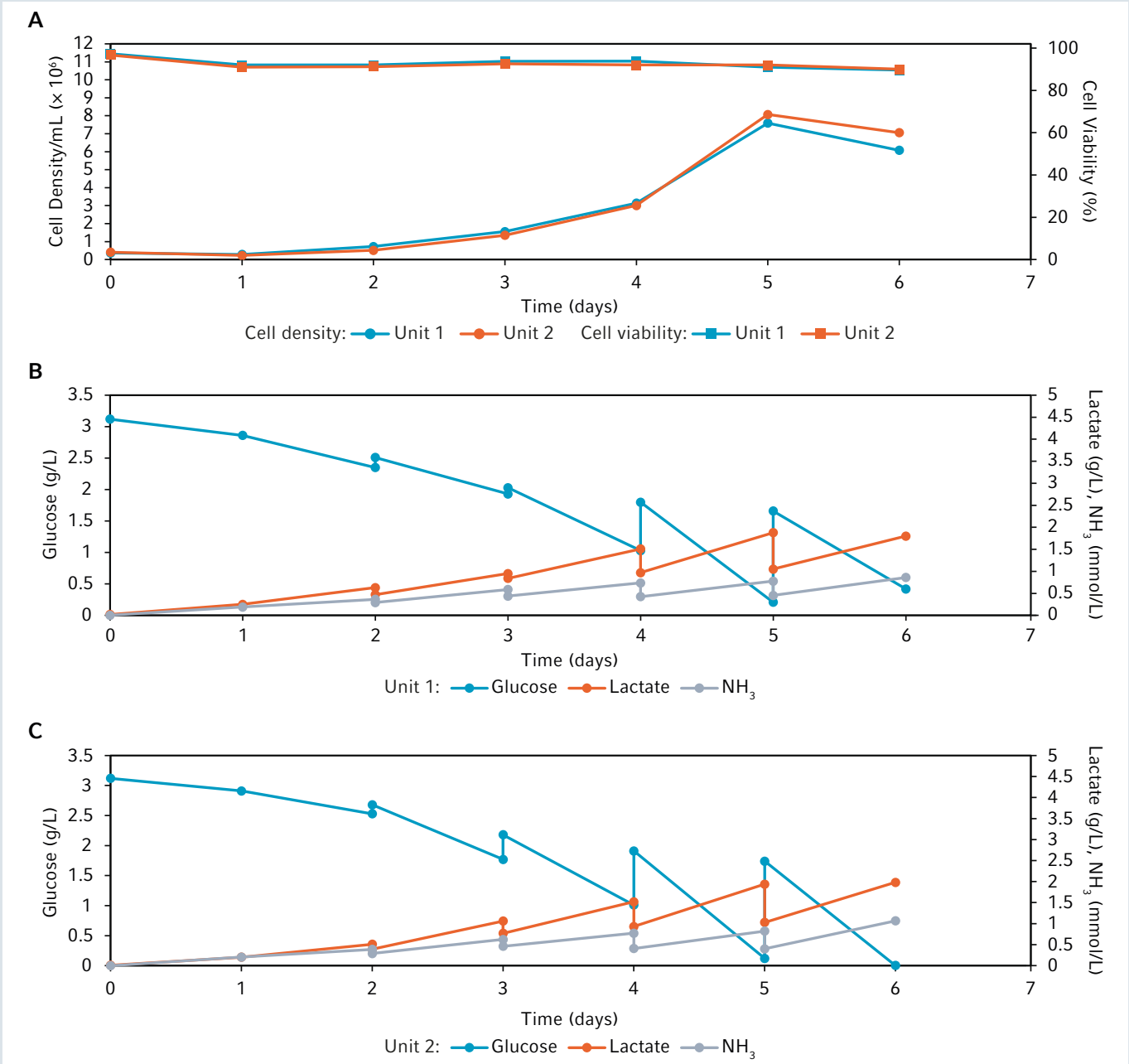

Fig. 6: hiPSCs growth profile in DASGIP 1 L Spinner Vessels in Stem Scale PSC culture medium. **(A)** hiPSCs cell density and viability. **(B)(C)** Metabolic profile from both bioreactors.

Monitoring aggregates diameter over time

To evaluate the growth kinetic of hiPSCs under specific suspension culture conditions (see Table 1), daily samples were taken from both bioreactors (see section "Aggregates sampling and analysis") and analyzed by light microscopy. 24 hours post inoculation, aggregates formation was found with average aggregate diameters increasing from ~95 µm (day 1) to ~530 µm (day 5) (Figure 7A). Furthermore, a narrow distribution of aggregate diameter was found in the first few days after bioreactor inoculation, followed by a more variable aggregate size distribution after day 3 (Figure 7B).

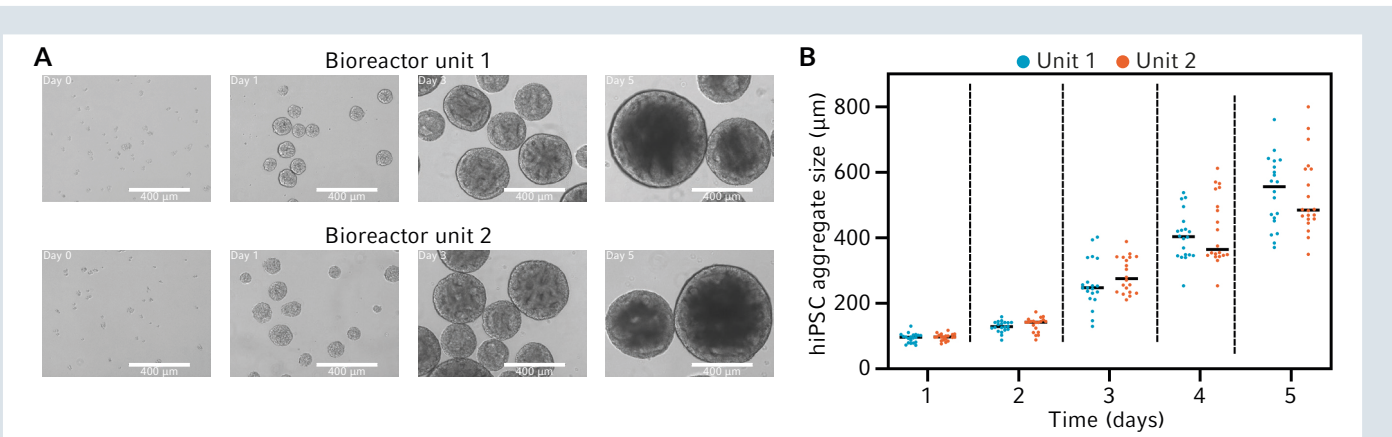

Fig. 7: hiPSCs aggregates formation, size, and morphology. **(A)** Light microscopy images of aggregates formation over time for bioreactor units (scale bar = 400 µm). **(B)** Distribution of aggregate diameters over cultivation time for bioreactor units.

Bioreactor expansion process is compatible with hiPSC pluripotency potential

Large-scale production of hiPSCs while maintaining their pluripotency is essential for clinical applications. In this regard, most of the cells harvested on day 4 from both bioreactors (aggregate diameters ~400 µm) expressed pluripotency-associated surface markers SSEA4 and TRA-1-60 as well as transcription factors Oct-4 and Nanog, just like the hiPSC cultured in monolayers (Figure 8).

Discussion

Cell-based therapies have revolutionized the treatment of various diseases by using cells as living drugs with the ability to regenerate tissues and restore cell functions [34-36]. However, limited availability of cells and challenges associated with process scalability make its implementation difficult. iPSCs have an enormous potential to be used as a universal cell source for cell therapy due to (i) their ability to expand as aggregates in suspension while generating large quantities of cells, making it crucial for a patient's demands, (ii) the possibility to create a stock of well-characterized iPSC lines to treat patients with different diseases at any time (off-the-shelf therapies), and (iii) the opportunity to reduce immunogenicity in order to make cell therapy treatments suitable for many patients by creating a stock of iPSCs that matches the HLA types of major portions of the population. In view of this, it is imperative to develop robust and reproducible procedures for iPSCs expansion and differentiation that allow for better standardization and quality control during the process as well as compliance with regulatory guidelines. Zweigerdt *et al.* [31] have shown

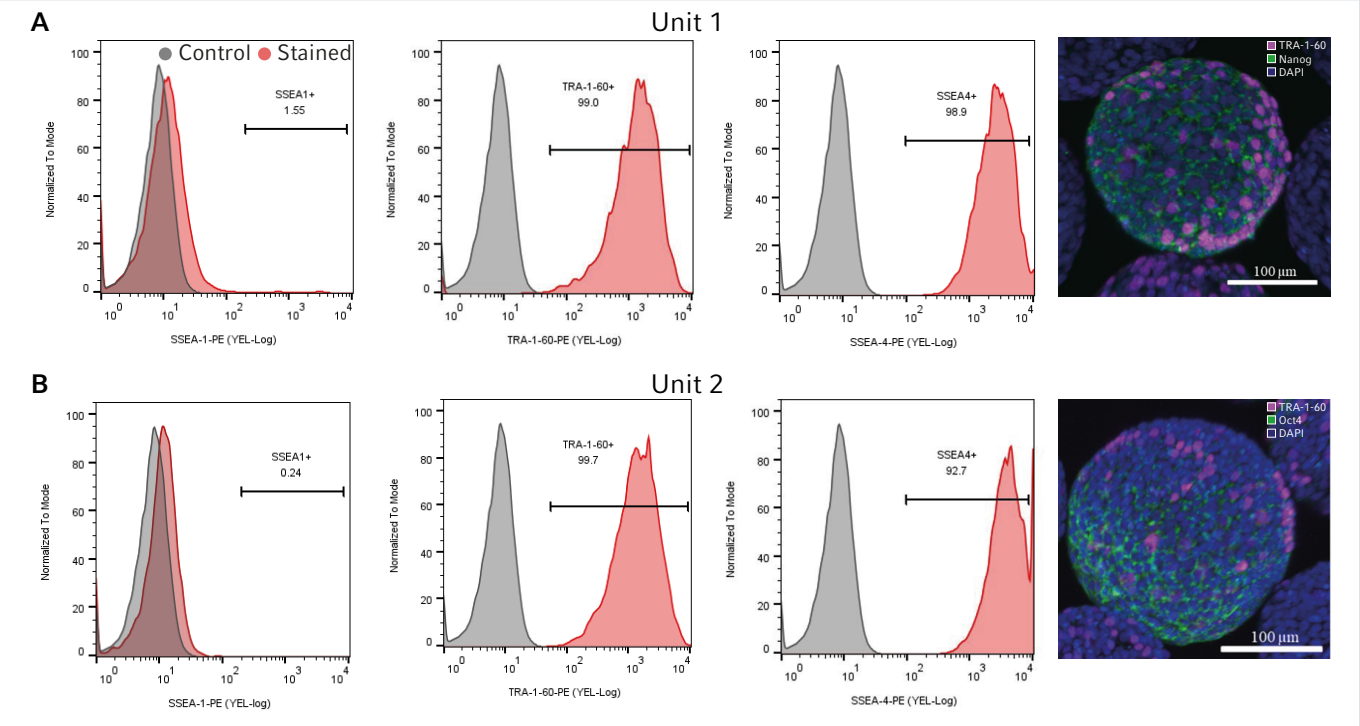

Fig. 8: Pluripotency marker expression on hiPSC aggregates after 4 days of 3D culture analyzed by flow cytometry **(A)** and immunocytochemistry **(B)**.

that conditions attuned to a stem cell's needs should be applied such as gentle mixing conditions, achieved for example by an eight-blade impeller configuration (60° pitch), low agitation speed to avoid cell stress (e.g. 60-80 rpm), and fitting inoculation cell densities. Thus, improving homogeneous distribution of cells, nutrients, oxygen, and growth factors and minimizing shear stress are crucial to maintain high cell viability of pluripotent stem cells in a suspension culture.

Building on previous studies [31,33], a robust hiPSC suspension culture process was developed, using the SciVario twin bioreactor system [37-38]. The system was used in combination with 1L DASGIP Spinner Vessels equipped with an eight-blade impeller (60° pitch) to create a precisely controlled growth environment. To reduce impurities and keep the subsequent iPSC culture as homogeneous as possible, the cell picking method was employed as a technique to exclude spontaneously differentiated cells. As a result, only a small number of cells were negative for the stem cell markers TRA-1-60 and SSEA4 during the inoculum preparation step in our workflow. Both bioreactor runs were showing comparable results, yielding around 6.4×10^9 hiPSCs in 0.8 L working volume (20-fold) after 5 days of culture while maintaining the cell viability above 90% throughout the experiments.

Aggregate size distribution is another important parameter to consider in iPSC suspension cultures. The Eppendorf eight-blade impeller (60° pitch) is specifically designed to control the formation of hiPSC aggregate, improving cell expansion rate, aggregate size, and homogeneity of aggregate size distribution [31]. At 80 rpm in the 0.8 L working volume, the average distribution of aggregates was below 280 µm for the first three days with a relatively narrow range and between 400-550 µm during days 4 and 5 with a broader distribution. While small aggregate size (50-200 µm) promotes self-renewal and maintains pluripotency, larger iPSC aggregates prevent diffusion of nutrients and growth factors causing cell apoptosis or heterogeneous cell

populations [39]. Further insight on controlling aggregate size with the 8-blade impeller in stirred-tank bioreactor iPSC culture can be found in the Eppendorf Application Note 472, summarizing one of the latest publications by Manstein *et al.* [33, 40].

Under a conventional and uncontrolled 2D culture system, iPSCs may face challenges in maintaining their pluripotency. Factors such as limited spatial constraint, uneven nutrient distribution, or inadequate signaling cues may contribute to their spontaneous differentiation. However, stirred-tank bioreactors offer controlled culture conditions such as optimized nutrient supply, adequate oxygen levels, pH as well as better cell distribution and enhanced mass transfer of gasses and nutrients into the iPSC aggregates, which contributes to a more physiologically relevant environment for iPSC expansion and preservation of pluripotency [41-44]. hiPSC pluripotency capacity in aggregates even with diameter larger than 200 µm was obtained, by retaining 99 % of expression of key markers such as TRA-1-60 and SSEA4 in unit 1 after 4 days of culture, indicating that developing a large-scale expansion process is compatible with iPSC ability to self-renew and pluripotency maintenance.

Conclusion

In summary, the study presented here highlights the bioreactor-based hiPSC expansion as a promising platform with vast potential for numerous cell therapy applications. The study described above demonstrates a 16-fold cell expansion of hiPSCs in a stirred-tank medium exchange batch culture using DASGIP 1 L Spinner Vessels equipped with an eight-blade impeller under control of a SciVario twin bioreactor control system. The results indicate the potential of large-scale production of hiPSCs in bioreactors while preserving their stem cell properties. The utilization of advanced suspension stirred-tank bioreactors and precise bioreactor control systems, as demonstrated in this study, provides a solid solution for further investigation and advancement of hiPSC-based therapies.

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