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Expansion of 3D human induced pluripotent stem cell aggregates in bioreactors: Bioprocess intensification and scaling-up approaches

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ABSTRACT

Human induced pluripotent stem cells (hiPSC) are attractive tools for drug screening and disease modeling and promising candidates for cell therapy applications. However, to achieve the high numbers of cells required for these purposes, scalable and clinical-grade technologies must be established.

In this study, we use environmentally controlled stirred-tank bioreactors operating in perfusion as a powerful tool for bioprocess intensification of hiPSC production. We demonstrate the importance of controlling the dissolved oxygen concentration at low levels (4%) and perfusion at 1.3 day^{-1} dilution rate to improve hiPSC growth as aggregates in a xeno-free medium. This strategy allowed for increased cell specific growth rate, maximum volumetric concentrations (4.7×10^6 cell/mL) and expansion factors (approximately 19 in total cells), resulting in a 2.6-fold overall improvement in cell yields. Extensive cell characterization, including whole proteomic analysis, was performed to confirm that cells' pluripotent phenotype was maintained during culture.

A scalable protocol for continuous expansion of hiPSC aggregates in bioreactors was implemented using mechanical dissociation for aggregate disruption and cell passaging. A total expansion factor of 1100 in viable cells was obtained in 11 days of culture, while cells maintained their proliferation capacity, pluripotent phenotype and potential as well as genomic stability after 3 sequential passages in bioreactors.

1. Introduction

Human pluripotent stem cells (hPSC), including embryonic (hESC) and induced pluripotent (hiPSC) stem cells, have a huge potential for clinical application due to their inherent capacity of self-renewal and ability to differentiate into cells of the three germ layers (Robinton and Daley, 2012). In spite of all hurdles and challenges posed in the last decades, clinical trials using either hESC or hiPSC derivatives for the treatment of spinal cord injury, macular degeneration of retina, heart failure and type 1 diabetes are already ongoing (www.clinicaltrials.gov). In particular, hiPSC are also powerful cell-based tools for application in drug screening and toxicology testing (Nishikawa et al., 2008; Robinton and Daley, 2012) as well as for disease modeling and organ generation (Takebe et al., 2013).

Since high numbers of hiPSC and derivatives are required for both therapeutic (e.g. 10^9–10^{10} of hPSC-derived progenitors are needed per patient) and drug screening purposes (reviewed in Serra et al., 2012), the use of cost-effective methods for scalable and reproducible cell expansion is inevitable. Classical small scale culture systems, such as planar technologies (well plates, T-flasks) or even shake flasks (Erlenmeyer), present several drawbacks regarding cell production yields, limited scalability and lack of control of culture parameters (Steiner et al., 2010). To overcome these limitations, several attempts have been made in the last years to expand hiPSC (as well as hESC) in stirred suspension culture systems (spinner vessels and stirred-tank bioreactors) using microcarriers (Kehoe et al., 2010; Silva et al., 2015; Serra et al., 2010), hydrogel capsules (Kerscher et al., 2015) or by culturing the cells as three-dimensional (3D) aggregates (Olmer et al., 2012).

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The culture of hiPSC as 3D aggregates offers key advantages over the use of microcarrier and microencapsulation technology, requiring no attachment surfaces, adhesion molecules, microcarriers or hydrogels, thus impacting on reducing the cost-of-goods (CoGs) (Jenkins et al., 2015). One of the technical challenges of this culture approach is the need for controlling the aggregate size, in initial generation and long-term culture, as it affects cell phenotype and differentiation potential (Sart et al., 2016). Recent progresses made on the use of controlled methods, such as forced aggregation, micro-contact patterning and agitation through the use of bioreactors, show the capability to modulate cell aggregate size and morphology while minimizing heterogeneity (Sart et al., 2016; Tostões et al., 2012) and thus maintaining the pluripotent phenotype of hPSC (Olmer et al., 2010; Singh et al., 2010). It is well established today that the use of bioreactors is critical for hPSC bioprocessing, providing dynamic and temporal biochemical and biophysical signaling for hiPSC cultures (Serra et al., 2012). In particular, the use of environmentally controlled stirred-tank bioreactors, operating in perfusion, allows the fine-tuning of cell culture environment, ensuring the control of key factors, such as the concentration of dissolved oxygen, soluble growth factors, small molecules, nutrients and metabolites, that showed to affect hPSC fate (Kropp et al., 2016; Serra et al., 2012, 2010).

During the last years, some studies have already demonstrated the expansion of hiPSC and hESC aggregates in stirred culture systems, reporting maximum cell concentrations of approximately 2-2.8 $\times 10^6$ cell/mL and expansion factors of approximately 5–6 within a time scale of 7 days (Kropp et al., 2016; Serra et al., 2012). Taking into account the cell numbers required for therapeutic applications, this implies that significant process improvement is required together with the implementation of efficient scaling-up strategies for continuous cell expansion in bioreactors. Since enzymatic dissociation procedures have been reported as a major cause of low cell recovery yields in aggregate cultures (Zweigerdt et al., 2011), mechanical dissociation techniques appear as a suitable cost-effective alternative (Otsuji et al., 2014).

This work aimed to develop a microcarrier- and xeno- free protocol for the continuous expansion of hiPSC in stirred-tank bioreactors. Different culture parameters (dissolved oxygen concentration, dilution rate) and protocols (aggregate dissociation) were tuned in order to boost stem cell expansion factors and facilitate process scale-up in bioreactors. The outline of our study is schematically represented in Fig. 1.

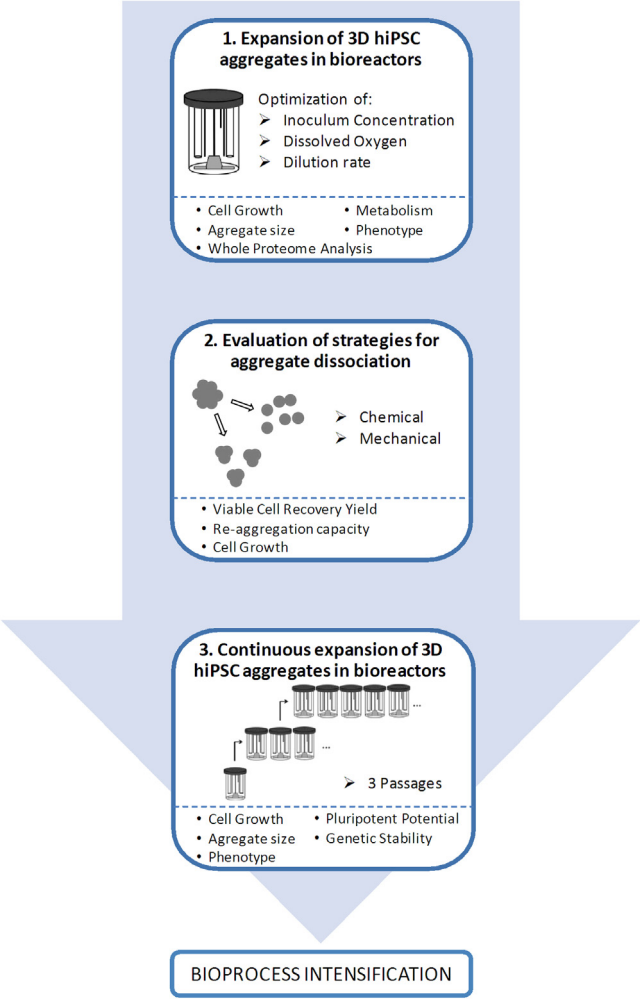

Fig. 1. Representative scheme of the experimental design of the study: 1) establishment and intensification of the bioprocess for expansion of 3D hiPSC aggregates in bioreactors; the impact of different process variables (inoculum concentration, dissolved oxygen concentration and dilution rate) on hiPSC growth and phenotype was evaluated; 2) assessment of chemical and mechanical strategies for dissociation of 3D hiPSC aggregates; and 3) implementation of continuous expansion of 3D hiPSC aggregates in bioreactors; three sequential passages were carried out. For each stage of the bioprocess workflow, different readouts (depicted in the bottom of each box) were considered for characterization and quality control analysis of hiPSC/cultures.

2. Materials and methods

2.1. Human iPSC culture

In this work, 9 hPSC lines were used, including 5 hiPSC (ChiPS C4, ChiPS C12, ChiPS C15, ChiPS C18, ChiPS C22) and 4 hESC (SA121, SA167, SA181, SA461) lines (all from Takara Bio Europe AB, Tokyo, Japan). Cells were routinely propagated in static adherent culture systems (T-flasks or 6-well plates), coated with Synthemax® (Corning, New York, USA), in Cellartis® DEF-CS™ Xeno-Free Culture Medium (Cat.no. Y30040, http://www.clontech.com/SE/Products/Stem_Cell_Research/Culture_and_Cryopreservation/Xeno-Free_Stem_Cell_Culture_System?sitex=10120:22372:US, Takara Bio Europe AB, Tokyo, Japan), and were placed at 37 °C in a humidified atmosphere with 5% (v/v) CO_2. Briefly, hPSC were inoculated at 4–6 $\times 10^4$ cell/cm^2 and cultured for 34 days; culture medium was replaced daily. At 80% cell confluence, hiPSC were detached from the T-flasks by rinsing with Dulbecco phosphate-buffered saline (DPBS, Thermo Fisher Scientific, Waltham, Massachusetts, EUA), adding Versene (Thermo Fisher Scientific), and incubating the cells for 8 min at 37 °C. Cells were detached with cell scrapers (Sarstedt AG, Nümbrecht. Germany), resuspended in Cellartis® DEF-CS™ Xeno-Free culture medium and counted using Trypan Blue exclusion method to determine cell number and viability, as described elsewhere (Serra et al., 2011).

2.2. Human iPSC culture in computer-controlled stirred-tank bioreactors

2.2.1. Human iPSC expansion as 3D aggregates

Human iPSC (ChiPSC4 cell line, P18) were inoculated as single cells in stirred-tank bioreactors (DasGip cellferm-pro bioreactor system, Eppendorf AG, Hamburg, Germany) in 200 mL of Cellartis® DEF-CS™ Xeno-Free 3D Spheroid Culture Medium (Cat. No. Y30047, http://www.clontech.com/SE/Products/Stem_Cell_Research/Culture_and_Cryopreservation/Xeno-Free_3D_Stem_Cell_Culture_Medium?sitex=10120:22372:US). Flat bottom bioreactor vessels equipped with trapezoid shaped paddle impellers with long arms were used in this work. The choice of the impeller design relied on several previous publications from our group (Correia et al., 2014; Simão et al., 2016) and others (Kropp et al.,