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Short communication

Investigating the feasibility of scale up and automation of human induced pluripotent stem cells cultured in aggregates in feeder free conditions☆

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ABSTRACT

The transfer of a laboratory process into a manufacturing facility is one of the most critical steps required for the large scale production of cell-based therapy products. This study describes the first published protocol for scalable automated expansion of human induced pluripotent stem cell lines growing in aggregates in feeder-free and chemically defined medium. Cells were successfully transferred between different sites representative of research and manufacturing settings; and passaged manually and using the CompacT SelecT automation platform. Modified protocols were developed for the automated system and the management of cells aggregates (clumps) was identified as the critical step. Cellular morphology, pluripotency gene expression and differentiation into the three germ layers have been used compare the outcomes of manual and automated processes.

1. Introduction

Human induced pluripotent stem cells (hiPSC) are generated from reprogrammed fibroblasts by overexpression of pluripotency factors (Takahashi et al., 2007; Yu et al., 2007). These pluripotent cells have the unique characteristic to self renew in vitro while maintaining the capacity to differentiate into a broad number of cell types. By combining these unique properties, hiPSC could enable the generation of large quantity of cells for clinical applications. Furthermore, the possibility of generating hiPSC from somatic cells using epigenetic reprogramming represents a unique opportunity for personalized regenerative medicine. Indeed, these pluripotent stem cells could enable the production of patient specific cell types that are fully immuno-compatible with the original donor thereby avoiding the need for immune suppressive treatment after cell transplantation. Nevertheless, the practical, financial and temporal obstacles in producing and validating personalized clinical-grade hiPSC and their differentiated progeny will almost certainly limit the feasibility of this approach. These limitations could selectively restrict patient access to autologous cell-based therapies (Faden et al., 2003). The creation of clinical banks of hiPSC from donors that can provide HLA matching to recipients is proposed as a strategy to attenuate the host immune response to transplanted tissue (Lin et al., 2009; Taylor et al., 2005, 2011).

Similarly, hiPSC can be used to develop in vitro disease models, allowing large scale studies otherwise restricted due to the limited availability of primary cells and biopsy material. This application has been proven useful to model neurodegenerative diseases, cardiac syndromes and metabolic disorders in vitro for basic studies and drug screening (Ebert et al., 2009; Moretti et al., 2010; Rashid et al., 2010). However, each of these applications requires large quantity of hiPSC produced in reproducible and standardized ways. Indeed, expansion of hiPSC remains time and resources consuming while experimental variability due to human intervention is almost systematic.

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Process automation has been a key mechanism to achieve controlled and standardized cell production. Successful automated protocols have been developed for the expansion of human mesenchymal stem cells (Thomas et al., 2007) and human embryonic stem cells (hESC) (Thomas et al., 2009b). Scale up automation enables scale out for conventional formats with predictable process variation and quality outcome by removing manual interventions. However, little work has been done in developing technologies for automation and scale up of hiPSC for healthcare applications. Several solutions and technologies have been developed for live cell production in suspension platforms (Ratcliffe et al., 2012). However these are not readily adapted for cells growing in adherent conditions. Furthermore, large scale production of hiPSC for clinical applications would require expansion in culture using clinically compatible conditions in a reproducible way without loss of function and in sufficient numbers to create reproducible and cost effective therapeutic products. Finally, passaging represent the main difficulty to develop an automation platform to expand hiPSC since these cells must be propagated as aggregates/clumps to maintain their integrity and quality (Beers et al., 2012). Indeed, evidence indicates that hiPSC grown and harvested as single cells are more likely to acquire genetic anomalies (Amps et al., 2011). Consequently, standardization of cell counting and cell clump size measurement has proven to be impractical. Here, we have addressed all these issues by transferring an established manual method to grow hiPSC in feeder free and chemically defined medium onto an automated platform compatible with large scale production. This study shows for the first time that large scale automated production of hiPSC is possible without the need of single cell dissociation thereby respecting their natural properties.

2. Materials and methods

2.1. Manual maintenance and passage of hiPSC in feeder free and chemically defined medium

hiPSC were cultured in feeder-free conditions using chemically defined medium (CDM-PVA) with Activin A (10 ng ml^{-1}, R&D System) and FGF2 (12 ng ml^{-1}, R&D Systems) – iPSC medium, as previously described (Brons et al., 2007). The composition of CDM-PVA was 50% IMDM (Gibco) added to 50% F12 + GlutaMax-1 (Gibco), supplemented with 1% lipid concentrate (Gibco), 7 μg ml^{-1} of insulin (Roche), 15 μg ml^{-1} of transferrin (Roche), 450 μM of monothioglycerol (Sigma) and 1 mg ml^{-1} of Polyvinyl Alcohol (Sigma). Cells were harvested after 6 or 7 days of culture (dependent on visually confirmed confluence) using 1 mg ml^{-1} collagenase IV (Gibco) and 1 mg ml^{-1} dispase (Gibco). Detached colonies were aspirated and pooled into a conical tube and washed with CDM-PVA medium. A second wash step was performed before colonies were gently broken down into smaller cell aggregates (clumps) by pipetting and allowed to settle under gravity. It must be noted here that the aim was only to reduce the size of the aggregates and not to reduce them to single cells. Cell clumps were plated at 1:10 split ratio to 0.1% porcine gelatin plates (Sigma), pre-coated with mouse embryonic fibroblast medium containing 10% FBS (Biosera) for 24 h at 37 °C and 5% (v/v) CO_2. iPSC medium and 10 μM of Y27632 (Sigma) was added for the first 48 h. Following this, maintenance medium was replaced daily until readiness for the next passage. This protocol is routinely used for 6 well plates and T-25 flasks (Fig. 1).

2.2. Manufacturing platform and instrumentation

The CompacT SelecT (The Automation Partnership, UK) is a fully automated cell culture platform which incorporates a small six-axis anthropomorphic robotic arm (Cell Therapy Manufacturing Facility, 2011) that can access 90 T175 flask and plate incubators, controlled at 37 °C under an atmosphere of 5% (v/v) CO_2 and humidity. The system allows the automation of seeding, feeding and other cell culture processes in order to maintain cell lines in standard T175 cell culture flasks. Flasks are bar-coded for identification and cell process tracking. Two flask decappers and flask holders, automated medium pumping and an automatic cell counter (Cedex®, Roche Innovatis AG, Germany) are integrated within a high-efficiency particulate air (HEPA) filtered cabinet to ensure sterility. At Loughborough University, the CompacT SelecT has been successfully used to culture many different cell types including human mesenchymal cells and human embryonic stem cells (hESC) (Thomas et al., 2007, 2009a,b). The CompacT SelecT has also been shown to be successful at preventing contamination when the GMP version of the CompacT SelecT passed the "sterile fill" runs (Chandra et al., 2012). The CompacT SelecT allows activities during cell culture such as seeding, media changes and measurement cells in a controlled environment (Thomas, 2012). Thus this platform can be used to expand and differentiate batches of cells to a tighter specification than manual cell culture (Liu et al., 2010).

2.3. Automated passage of hiPSC in feeder free and chemically defined medium using CompacT SelecT

The automation enables scale out for conventional formats with predictable process variation and quality outcome by removing manual interventions. The CompacT SelecT is a preferred platform for development process friendly method of automating the culture of cells that grow in adherent conditions. The automation step mimics the manual process and is therefore demonstrably similar to the manual cell culture steps. For many manual cell culture protocols, there is a centrifugation step to concentrate the cell suspension and allow for cells to be washed. However, in this instance, cells grow in aggregates and do not require centrifugation as they settle under gravity.

In order to transfer the culture protocol to the CompacT SelecT it was necessary to scale up from a T25 to a T175 flask, media volumes were scaled proportionally to flask surface area, and work within the restricted set of plasticware and the allowable positioning of the plasticware within the automated system (Fig. 1). Operating conditions of the manual culture process were followed as closely as possible (temperature, timing, splitting ratio, mixing, volumes), however a number of detailed changes had to be made to the manual protocol as are discussed later in the manuscript.

2.4. Differentiation of hiPSC into endoderm, mesoderm and neuroectoderm

hiPSC were plated into gelatin plates pre coated with 10% FBS and maintained for 24 h in iPSC medium before inducing differentiation into the three germ layers: endoderm, mesoderm and neuroectoderm as described previously (Vallier et al., 2009). Differentiation was induced by supplementing CDM-PVA with Activin (R&D System), bFGF (R&D System), BMP4 (R&D System), LY294002 (Promega), SB431542 (Tocris) and CHIR99021 (Stemgent) at different times and concentrations (Supplementary Online Material).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2013.12.009.

2.5. Immunochemistry

hiPSC were fixed for 20 min at 4 °C in 4% paraformaldehyde (PFA) and washed three times in PBS. Cells were incubated 1 h at room temperature in PBS containing 10% donkey or goat serum