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Scalability and process transfer of mesenchymal stromal cell production from monolayer to microcarrier culture using human platelet lysate

THOMAS R.J. HEATHMAN[1,2], ALEXANDRA STOLZING[2,3], CLAIRE FABIAN[3,4], QASIM A. RAFIQ[2,5], KAREN COOPMAN[2], ALVIN W. NIENOW[2,5,6], BO KARA[7,a] & CHRISTOPHER J. HEWITT[2,5]

[1]PCT, a Caladrius Company, NJ, USA, [2]Centre for Biological Engineering, Loughborough University, Leicestershire, UK, [3]Interdisciplinary Centre for Bioinformatics, University of Leipzig, Leipzig, Germany, [4]Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany, [5]Aston Medical Research Institute, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK, [6]Centre for Bioprocess Engineering, University of Birmingham, UK, and [7]FUJIFILM Diosynth Biotechnologies, Billingham, UK

Abstract

Background aims. The selection of medium and associated reagents for human mesenchymal stromal cell (hMSC) culture forms an integral part of manufacturing process development and must be suitable for multiple process scales and expansion technologies. *Methods.* In this work, we have expanded BM-hMSCs in fetal bovine serum (FBS)- and human platelet lysate (HPL)-containing media in both a monolayer and a suspension-based microcarrier process. *Results.* The introduction of HPL into the monolayer process increased the BM-hMSC growth rate at the first experimental passage by 0.049 day and 0.127/day for the two BM-hMSC donors compared with the FBS-based monolayer process. This increase in growth rate in HPL-containing medium was associated with an increase in the inter-donor consistency, with an inter-donor range of 0.406 cumulative population doublings after 18 days compared with 2.013 in FBS-containing medium. Identity and quality characteristics of the BM-hMSCs are also comparable between conditions in terms of colony-forming potential, osteogenic potential and expression of key genes during monolayer and post-harvest from microcarrier expansion. BM-hMSCs cultured on microcarriers in HPL-containing medium demonstrated a reduction in the initial lag phase for both BM-hMSC donors and an increased BM-hMSC yield after 6 days of culture to $1.20 \pm 0.17 \times 10^5$ and $1.02 \pm 0.005 \times 10^5$ cells/mL compared with $0.79 \pm 0.05 \times 10^5$ and $0.36 \pm 0.04 \times 10^5$ cells/mL in FBS-containing medium. *Conclusions.* This study has demonstrated that HPL, compared with FBS-containing medium, delivers increased growth and comparability across two BM-hMSC donors between monolayer and microcarrier culture, which will have key implications for process transfer during scale-up.

Key Words: *bioprocess, cell-based therapy, comparability, harvest, human platelet lysate, manufacture, mesenchymal stromal cell, microcarrier expansion, process development, process transfer*

Introduction

The growing field of regenerative medicine (RM) aims to treat unmet clinical indications such as cardiovascular disease and neurological disorders by restoring or maintaining cell or tissue function. Cell-based therapies form a large part of this RM industry and have the potential to transform health care. Human mesenchymal stromal cells (hMSCs) are of particular interest with clinical trials currently underway for multiple indications [1]. For the majority of these clinical indications, however, the *in vitro* expansion of cells is required to deliver an effective therapeutic dose without impacting the quality of the cell. Understanding and defining the quality attributes of hMSC therapies will be critical for their successful manufacture. This is proving difficult, however, because of their complex, multifaceted and poorly understood *in vivo* mechanisms of action [2].

At present, the majority of hMSC expansion takes place in static monolayers (T-flasks or multilayer flasks), which are suitable for the manufacture of the cell

[a]Current affiliation: GSK R&D, Gunnels Wood, Stevenage, Herts, UK.
Correspondence: **Christopher J. Hewitt**, BSc, PhD, DSc, CEng, CBiol, CSci, FIChemE, FRSB, FHEA, Aston Medical Research Institute, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK. E-mail: c.j.hewitt@aston.ac.uk

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numbers required for early clinical development. However, it is widely recognized that these manual processes will not be sufficient to cost-effectively meet the needs for large-scale commercial production, where lot sizes will likely be on the order of trillions of cells [3]. For processes to drive toward the production of cost-effective therapies, they should be scalable, compliant with Good Manufacturing Practices and amenable to closed and automated process steps.

Microcarriers have been used to culture adherent cells such as BM-hMSCs in suspension in stirred bioreactors [4], allowing for process scale-up in which online monitoring and control systems can be used to deliver consistent and cost-effective BM-hMSC products [5]. Stirred tank bioreactors are currently employed for mammalian cell culture in biopharmaceutical production, and therefore their design and operation are well understood [6], with the potential to meet the expected manufacturing demands of large-scale BM-hMSC therapies. However, considerable work is required to demonstrate a satisfactory level of comparability between the traditional monolayer processes and these suspension-based systems in terms of cellular growth and quality.

A key aspect of these manufacturing processes is the culture medium in which the cells are to be expanded, which is typically supplemented with fetal bovine serum (FBS) [7]. In addition to lot-to-lot variability, there are further process constraints on the use of FBS, such as limited supply, spiraling costs [8], potential for pathogen transmission and immunological reactions against bovine antigens [9]. Human platelet lysate (HPL) has been proposed as a viable alternative in which blood platelets are lysed to release growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF), which then supplement the BM-hMSC growth medium [10]. This can be used as a patient specific supplement from their own blood plasma or pooled from multiple donors—for example, Stemulate manufactured by Cook Regentec. Furthermore, HPL has already been reported as a superior substitute to FBS for the *in vitro* expansion of BM-hMSCs [11] and has been shown to maintain the key cell characteristics and multipotent capacity [12]. Despite this, little work has been done to demonstrate the amenability of HPL to a scalable BM-hMSC manufacturing process, such as microcarrier suspension culture in stirred tank bioreactors.

Therefore, the aim of this study was to compare the relative performance of HPL to FBS for the monolayer expansion of BM-hMSCs and the amenability of HPL to transfer to a microcarrier-based suspension culture.

Methods

Monolayer culture

Human MSCs were isolated from bone-marrow aspirate purchased from Lonza obtained from two healthy donors with informed consent: BM-hMSC 1 (lot 071313B) and BM-hMSC 2 (lot 071281D). These two BM-hMSC donors were selected after pre-screening of five potential donors because of their differing growth and characteristics [13], representing the two extremes in terms of culture performance (±5.2 population doublings over 30 days of expansion). The local ethics committee approved the use of the samples for research. Cells from passage 1 were cryopreserved at a density of $1–2 \times 10^6$ cells/mL in a freeze medium containing 90% (v/v) FBS (Hyclone) and 10% (v/v) dimethylsulphoxide (Sigma-Aldrich). Cells were grown in T-flasks seeded at 5000 cells/cm^2 at 37°C in humidified air containing 5% CO_2. Dulbecco's Modified Eagles Medium (1 g/L glucose; Lonza) supplemented with 10% (v/v) FBS (Hyclone) or 10% (v/v) non-heparin requiring (PL-NH) Stemulate (Cook Regentec) and 2 mmol/mL UltraGlutamine (Lonza) was exchanged every 3 days. The FBS batch used in this study was selected from a number of tested FBS batches for its favorable growth performance, while retaining key BM-hMSC characteristics. On passage, the BM-hMSCs were washed with phosphate-buffered saline (PBS) without Ca^+ or Mg^+ and incubated for 4 min with trypsin (0.25%)/ethylenediaminetetraacetic acid (EDTA; Lonza) for FBS-based culture or TrypLE Express (Invitrogen) for PL-NH Stemulate-based culture. Dissociation reagents were inactivated by the addition of appropriate growth medium, and the cell suspension was centrifuged at 220*g* for 5 min. The supernatant was discarded and the remaining pellet was re-suspended in an appropriate volume of culture medium. For PL-NH Stemulate culture, BM-hMSCs underwent one adaptation passage in medium containing 10% (v/v) PL-NH Stemulate. Data represents four experimental repeats for each condition.

Spinner flask culture

The glass surfaces of 100-mL spinner flasks (diameter T = 60 mm; BellCo) with a magnetic, horizontal stirrer bar and a vertical paddle (diameter D = 50 mm) were siliconized with Sigmacoat (Sigma-Aldrich) according to the manufacturers' instructions. Solid, non-porous Plastic P-102L microcarriers (Solohill) at 500 cm^2/100 mL were prepared following the manufacturer's instructions. Microcarriers were preconditioned in 50 mL FBS or PL-NH Stemulate-containing growth medium for 1 h before BM-hMSC inoculation at 6000 cells/cm^2 and cultured in 100 mL of FBS-containing or PL-NH Stemulate-containing medium at 37°C in humidified