Accepted Manuscript

Title: Development of a process control strategy for the serum-free microcarrier expansion of human mesenchymal stem cells towards cost-effective and commercially viable manufacturing

Authors: Thomas R.J. Heathman, Alvin W. Nienow, Qasim A. Rafiq, Karen Coopman, Bo Kara, Christopher J. Hewitt

PII: S1369-703X(18)30383-8
DOI: https://doi.org/10.1016/j.bej.2018.10.018
Reference: BEJ 7069

To appear in: *Biochemical Engineering Journal*

Received date: 28-4-2018
Revised date: 8-8-2018
Accepted date: 16-10-2018

Please cite this article as: Heathman TRJ, Nienow AW, Rafiq QA, Coopman K, Kara B, Hewitt CJ, Development of a process control strategy for the serum-free microcarrier expansion of human mesenchymal stem cells towards cost-effective and commercially viable manufacturing, *Biochemical Engineering Journal* (2018), https://doi.org/10.1016/j.bej.2018.10.018

Development of a process control strategy for the serum-free microcarrier expansion of human mesenchymal stem cells towards cost-effective and commercially viable manufacturing

Thomas R.J. Heathman , Alvin W. Nienow , Qasim A. Rafiq , Karen Coopman , Bo Kara , Christopher J. Hewitt, ,

Thomas R.J. Heathman

Hitachi Chemical Advanced Therapeutic Solutions (HCATS), 4 Pearl Court, Allendale, NJ, 07401

Centre for Biological Engineering, Loughborough University, Leicestershire, LE11 3TU, UK

Alvin W. Nienow

Centre for Biological Engineering, Loughborough University, Leicestershire, LE11 3TU, UK

Centre for Bioprocess Engineering, University of Birmingham, B15 2TT, UK

Qasim A. Rafiq

Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, London, WC1E 6BT, United Kingdom

Aston Medical Research Institute, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET

Centre for Biological Engineering, Loughborough University, Leicestershire, LE11 3TU, UK

Karen Coopman

Centre for Biological Engineering, Loughborough University, Leicestershire, LE11 3TU, UK

Bo Kara

FUJIFILM Diosynth Biotechnologies, Billingham, TS23 1LH, UK

Christopher J. Hewitt,

School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET

Centre for Biological Engineering, Loughborough University, Leicestershire, LE11 3TU, UK

Author for correspondence:

Professor Christopher J. Hewitt, Aston Medical Research Institute, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, B4 7ET. Email: c.j.hewitt@aston.ac.uk, Tel: +44 (0) 121 204 4949

Highlights

- Superior control in the bioreactor led to improved hMSC quality, consistency and yield
- At 25% dissolved oxygen in serum-free media, there was a 500 % increase in the hMSC yield
- Process control significantly reduced the variation in hMSC yield to < 15%

Abstract

Human Mesenchymal Stem Cells (hMSCs) are advancing through clinical development with the first allogeneic adult hMSC therapy receiving approval in Europe. To enable successful large-scale manufacture of hMSC therapies, increased product consistency and yield, and a reduced batch-to-batch variation must be achieved. This paper addresses ways to reduce variation by controlling the processing conditions, in particular the dissolved oxygen concentration (dO_2), and the culture medium. Bone marrow derived hMSCs were cultured in DASGIP DASbox bioreactors on Plastic P-102L microcarriers in FBS-containing and serum free (SFM) media at various dO_2 values from 100% to 10%, experiencing the same dO_2 value throughout the culture process. The superior control of pH and dO_2 in the bioreactor led to improved performances compared to poorly controlled spinner flasks, particularly at reduced dO_2 concentrations. At 25% dO_2, there was a 300 % increase in the BM-hMSC yield in the bioreactor across the two donor BM-hMSCs in SFM compared to FBS-containing medium. Overall, the process yield increased by an average of around 500% for both donors under controlled conditions in SFM at 25% dO_2 in the bioreactor compared to the poorly controlled expansion at atmospheric conditions in FBS-containing medium in spinner flasks. Process control significantly reduced the BM-hMSC variation in yield from 79.1% in FBS-containing medium in spinner flasks to < 15% in controlled SFM bioreactor culture.

Keywords

Process control, serum-free, human mesenchymal stem cell, microcarrier expansion, harvest, dissolved oxygen

1. Introduction

Bone marrow-derived human mesenchymal stem cells (BM-hMSCs) have generated much interest in the field of regenerative medicine (RM), with the potential to treat and in some cases, cure human disease. This interest has been largely driven by their ability to proliferate under appropriate culture conditions and their capacity to secrete a range of trophic factors which regulate host immune response and initiate tissue repair [1]. Consequently, hMSCs are advancing through clinical development targeting indications such as cardiac repair, neurological disease and immune disorders [2]. This progress is exemplified by the first allogeneic adult mesenchymal stem cell therapy to receive central marketing authorization approval in Europe [3].

The addition of microcarriers has been used to culture adherent cells such as BM-hMSCs in suspension [4] allowing for process scale-up, where online monitoring and control systems can be used to deliver a consistent and cost-effective BM-hMSC therapy. Further to this, stirred-suspension bioreactors are currently employed in biopharmaceutical production and therefore their design and operation are well-understood [5], with the potential to meet the expected manufacturing demands of large-scale BM-hMSC therapies [6]. The use of serum-free medium with microcarriers for the expansion of BM-hMSCs has previously been demonstrated for uncontrolled processes [7-9] and therefore represents a viable alternative for large-scale serum free manufacture of BM-hMSCs.

One of the key aspects of a successful manufacturing process is in the reduction of product variation, which is particularly challenging when the cell is the final product. Variation can be introduced into the product by both the process input material and the process conditions [10]. The input to the process

must be controlled by strict BM-hMSC isolation techniques and potentially cell selection steps to improve product input consistency [11], although appropriate isolation methods are typically poorly defined for BM-hMSCs. The US Food and Drug Administration (FDA) has released guidance documents on the use of process control and real time release testing for pharmaceutical manufacturing [12]. This guidance has highlighted the importance regulators are placing on continual manufacturing process improvement and enhanced understanding as a fundamental aspect of manufacturing development and control. Process analytical technology or PAT is a system for analyzing and controlling manufacturing processes through measurement of product attributes to ensure final product quality, proposed by the FDA [13]. It will therefore be desirable to utilize these PAT technologies and measure online parameters in order to develop control systems to ensure that the product characteristics remain consistent. These relevant process parameters are likely to include a combination of cell growth, medium temperature, pH, pO_2 and pCO_2, which are commonplace in current biopharmaceutical production processes [14].

Of these parameters, the level of dO_2 is the one that is usually of most concern when cultivating cells in bioreactors [15]. With respect to BM-hMSCs, our earliest work conducted in an oxygen-controlled incubator for both monolayer [16] and spinner flask culture [17] indicated that with the same donor cells as cultured here, the performance with respect to growth kinetics was better at 100% dO_2 than at lower values when cultured over 3 passages. Whilst some researchers have also shown 100% dO_2 gives enhanced growth kinetics, others have found that lower dO_2 values [17] are advantageous. Much of that early literature, including our own studies, did not offer very close control of dO_2 in the actual medium in which the cells are growing. Therefore, it was hypothesized that this rather loose control might account for the literature differences, always recognizing too that it might be associated with cell to cell variability. However, very tight dO_2 control can be achieved by an in-situ dO_2 probe throughout the culture in the closed environment offered by a bioreactor. Therefore, it was decided to investigate the effect of dO_2 on culture as a major part of this study.

Thus, whilst the impact of dO_2 is a major feature, the overall aim is to develop a process control strategy for the microcarrier culture of BM-hMSCs in suspension to drive increased consistency and yield into the process. The impact of this process control strategy on process economics and product consistency will also be assessed here for two BM-hMSC donors in both FBS-based and serum-free medium and compared to previously developed, loosely controlled monolayer and microcarrier-based suspension BM-hMSC expansion processes [8]. Of course, agitation intensity and aeration strategy (sparged, with or without protective surfactants, or headspace) are also relevant and these aspects have also been addressed for the same cells elsewhere [18].

2. Materials & Methods

2.1 Monolayer Culture

BM-hMSCs were isolated from bone-marrow aspirate purchased from Lonza (Walkersville, USA) obtained from two healthy donors with informed consent; BM-hMSC 1 (lot: 071313B) and BM-hMSC 2 (lot: 071281D). The local Ethical Committee approved the use of the sample for research. Cells from passage 1 were cryopreserved at a density of 1-2 x 10^6 cells.ml^{-1} in a freeze medium containing 90% (v/v) FBS (Hyclone, Belgium) and 10% (v/v) dimethylsulphoxide (Sigma-Aldrich, UK). Prior to the experimental work, cells were grown in T-flasks seeded at 5,000 cells.cm^{-2} at 37°C in humidified air (100% dissolved oxygen, dO_2) containing 5% CO_2. For serum-free culture, the growth surface of T-flasks was coated with 0.4 µg.cm^{-2} PRIME-XV human fibronectin (FN) (Irvine Scientific, USA) and cultured in PRIME-XV MSC Expansion SFM (Irvine Scientific, USA) as per manufacturer's instructions. On passage, the hMSCs were washed with phosphate buffered saline without Ca^+ or Mg^+ (PBS) and incubated for 4 min with TrypLE Express (Invitrogen, UK). Dissociation reagents were inactivated by the addition of appropriate growth medium and the cell suspension was centrifuged at 220g for 5 min. The supernatant was discarded, and the remaining pellet was re-suspended in an appropriate volume of

culture medium. BM-hMSCs underwent one adaptation passage in serum-free medium, prior to experimental work taking place.

2.2 DASGIP DASbox bioreactor microcarrier culture

The glass surfaces of 250 ml DASGIP DASbox bioreactors (diam. T = 60 mm) (Eppendorf, Germany) were siliconized with Sigmacoat (Sigma-Aldrich, UK) according to manufacturer's instructions. The bioreactors were equipped with a 3-blade 30°-pitch, down pumping impeller (diameter, D = 30.25 mm), a temperature probe, a Hamilton dO_2 probe, a Hamilton pH probe, an off-gas analyser and two sterile sample ports. Solid, non-porous Plastic P-102L microcarriers of 160-200 microns (Solohill, USA) were prepared at 500 or 1000 cm^2 following manufacturer's instructions. Extensive physical characterization of these Plastic P-102L microcarriers and 18 other commercially available microcarriers has been performed previously by our group [19]. In addition, culture of hMSCs on each of them was undertaken and the SoloHill Plastic P-102L microcarrier was selected as the optimal microcarrier based on the following criteria, (i) extent of cell proliferation on the microcarrier, (ii) amenability for xeno-free processing and (iii) the ability to effectively harvest the cells from the microcarrier without any detrimental effect on cellular immunophenotype and differentiation capacity. They have since been shown to perform consistently well in our studies [20].

Microcarriers were or coated with 0.1 $\mu g.cm^{-2}$ PRIME-XV FN prior to hMSC inoculation at 6,000 $cells.cm^{-2}$ and cultured in 100 mL of either Dulbecco's Modified Eagle Medium (DMEM; Lonza, UK) supplemented with 10 % (v/v) foetal bovine serum (FBS) (Lot: RVC35874, Hyclone, Belgium) and 2 mM ultraglutamine (Lonza, UK), or PRIME-XV MSC Expansion SFM with bioreactor control set-points of 37 °C and pH 7.4. As discussed earlier, various dissolved oxygen concentrations (dO_2) were used as set out in detail below. A 50 % medium exchange was performed every two days as per manufacturer's instructions, with fresh culture medium equilibrated to the appropriate dO_2, temperature and pH using a controlled bioreactor vessel on the DASGIP system prior to medium exchange. Following inoculation,

the culture was static for one hour, after which the culture was agitated constantly at the minimum rate for just complete suspension of microcarriers (N_{JS}) found to be 115 rpm as recommended previously [2, 3, 4], with daily medium and cell-microcarrier samples of 1 mL taken for analysis. The DASbox system was set up, calibrated and controlled using the DASGIP DASbox control unit (Eppendorf, Germany) and was operated with headspace aeration to achieve sufficient gas supply, which gave good dO_2 control.

2.3 DASGIP DASbox bioreactor harvest

BM-hMSCs were harvested using a method previously developed by us [21, 22]. Briefly, the DASbox control was switched off and the microcarriers allowed to settle. The spent culture medium was removed and the microcarriers were washed twice in 50 ml of Ca^{2+} and Mg^{2+} free PBS. The PBS was removed before adding 80 mL of TrypLE Express dissociation reagent for 10 minutes at an impeller speed of 375 rpm (ending with 5 seconds at 400 rpm). The dissociation reagent was then quenched with 70 ml of culture medium and a 2 mL sample placed in a single well of a 6 well plate for analysis under a light microscope to assess cell detachment. The remainder of the solution was filtered through a 60 µm Steriflip Filter Unit (Merck Millipore, Germany) to separate the microcarriers from the BM-hMSCs. The cell suspension was centrifuged, the supernatant aspirated and the cell pellet re-suspended in culture medium. A cell count was then performed using a NucleoCounter NC-3000 to assess the overall hMSC growth and the culture harvest efficiency.

2.4 Spinner flask culture and harvest

Full details of this aspect have been published previously [7]. In essence, the culture was undertaken in 100 mL spinner flasks (BellCo, USA) in an incubator in the presence of ambient air with the same type and concentration of microcarriers, with the cell concentration used in the bioreactor. All other conditions were also as in the bioreactor except that the agitation speed, $N_{JS,}$ was 30 rpm. Daily medium samples of 1mL taken for analysis. BM-hMSCs were harvested using the same previously

developed method [21, 22] as in the bioreactor, except that the agitation speed was 150 rpm for 7–10 min and 250 rpm for the final few seconds. More details are given elsewhere [7].

2.5 Analytical Techniques

Analysis of glucose and lactate concentrations in the spent medium was performed using a Cedex Bio-HT (Roche, Germany). Cell counting, mean cell diameter and viability (via acridine orange uptake and DAPI exclusion) was performed using a NucleoCounter NC-3000 automated mammalian cell counter (Chemometec, Denmark). The following parameters were obtained:

1. Specific Growth Rate

$$Specific\ growth\ rate, \mu = \frac{\ln\left({C_x(t)}/{C_x(0)}\right)}{\Delta t}$$

where μ is the net specific growth rate (h^{-1}), $C_x(t)$ and $C_x(0)$ are the cell numbers at the end and start of the exponential growth phase, respectively and t is time (h).

2. Population Doublings

$$Population\ Doublings, P_d = \frac{1}{\log\ (2)} \cdot \log\left(\frac{C_x(t)}{C_x(0)}\right)$$

where P_d is the number of population doublings, $C_x(t)$ and $C_x(0)$ are the cell numbers at the end and start of the exponential growth phase, respectively.

3. Specific Metabolite Consumption/Production Rate

$$Specific\ metabolite\ flux, q_{met} = \left(\frac{\mu}{C_x(0)}\right) \cdot \left(\frac{C_{met}(t) - C_{met}(0)}{e^{\mu t} - 1}\right)$$

where q_{met} is the net specific metabolite consumption or production rate, μ is the specific growth rate (h^{-1}), $C_x(0)$ is the cell number at the end of the exponential growth phase, $C_{met}(t)$ and $C_{met}(0)$ are the metabolite concentrations at the end and start of the exponential growth phase, respectively and t is time (h).

4. Inter- and intra-donor coefficient of variation

$$c_v = \frac{\sigma}{\mu}$$

where c_v is the coefficient of variation, σ is the standard deviation of the Relative Process Yield for each experimental run and each hMSC donor, and μ is the average (mean) of the Relative Process Yield for each experimental run and each hMSC donor.

2.6 Colony-forming unit fibroblast (CFU-f) Efficiency

To assess the CFU-f efficiency, BM-hMSCs were seeded in a T-flask at 10 cells.cm^{-2} and cultured with a medium exchange every 3-5 days. Following 14 days culture, cells were washed with PBS and fixed in 4% formaldehyde (v/v) (Sigma, UK) for 30 minutes. Colonies were stained with 1% crystal violet (Sigma, UK) in 100% methanol (w/v) for 30 minutes. Stained colonies that were made up of more than 25 cells were recorded as CFUs.

2.7 hMSC Characterization

Immunophenotype analysis was performed by multiparameter flow cytometry before and after the hMSC expansion process using a previously developed protocol [23]. Morphology images were obtained using a light microscope (Nikon Eclipse TS-100, UK).

The hMSC differentiation was induced using PRIME-XV Differentiation SFM (Irvine Scientific, USA) as per manufacturer's instructions. After 21 days the differentiation medium was removed, cells rinsed with PBS then fixed with 4% (v/v) PFA at room temperature. Adipocytes were stained with 1% (w/v) Oil Red O (Sigma-Aldrich, UK) in isopropanol at room temperature and rinsed with distilled water. Osteoblasts were incubated with 2.5% (v/v) silver nitrate (Sigma-Aldrich, UK) under ultraviolet light (30 minutes exposure), rinsed with distilled water and stained with fast violet solution (Sigma-Aldrich, UK) containing 4% (v/v) napthol AS-MX phosphate alkaline (Sigma-Aldrich, UK) for 45 minutes at room

temperature in the dark. Chondrocytes were stained with 1% (w/v) Alcian blue (Sigma-Aldrich, UK) in 0.1 M hydrochloric acid (Sigma-Aldrich, UK). After 30 minutes incubation, cells were rinsed three times with 0.1 M HCl. After staining, differentiated cells were visualized under a light microscope (Nikon Eclipse TS-100, UK). Previously published short tandem repeat analysis of these two BM-hMSC lines showed a retention of the characteristic genotype of BM-hMSCs throughout an extended culture process [24].

2.8 Statistical Analysis

Results were deemed to be significant if $p < 0.05$ using an unpaired two-tailed Students t-test.

3. Results & Discussion

3.1 Effect of dissolved oxygen concentration on BM-hMSC expansion on microcarriers

Dissolved oxygen concentration has been previously shown to have an impact on BM-hMSC growth and cell characteristics [25], with much debate around the use of the terms “normoxia” (100 % dO_2) and “hypoxia” (typically < 25 % dO_2) [16]. Almost all of the current technology for BM-hMSC expansion utilize open, laboratory scale processes such as T-flasks (or occasionally spinner flasks) wherein controlling, monitoring, and evaluating the impact of key parameters on target cell output and productivity is difficult [26]. Control of process parameters such as dissolved oxygen during the manufacturing process will be vital in maximizing product consistency and ensuring that the process is scalable. A large part of this ability to control the process conditions is in maintaining a closed process during expansion, which not only reduces contamination risk but is vital in maintaining the desired culture environment of the BM-hMSCs and avoid fluctuations in these critical process parameters. Much of the current literature on low oxygen culture of BM-hMSC use hypoxic incubators that maintain a low oxygen environment (an oxygen concentration in the gas phase lower than that found in atmospheric air) rather than controlling the dO_2 conditions in the actual bioreactor medium in T-flasks

(or spinner flasks), with process manipulations taking place within the atmospheric oxygen concentrations.

Figure 1 shows the impact of exposing low dissolved oxygen (dO_2) experiments to atmospheric oxygen within an agitated bioreactor process simulating the exposure to such conditions during culture involving media exchange when using hypoxic incubators. This figure demonstrates that for a dO_2 set-point of 5 % (1.05 % atmospheric oxygen), after a one-hour exposure of the culture to atmospheric conditions (21 % atmospheric oxygen equivalent to 100 % dissolved oxygen at saturation), creates a deviation to a maximum dO_2 concentration of 77.9 %, which takes a further 294 minutes to return to 5 % dO_2 with continual headspace aeration with 1.05% atmospheric oxygen. Considering the number of manipulations that occur during culture, for example, daily sampling and medium exchange, this mode of operation causes large and prolonged fluctuations in dissolved oxygen concentrations experienced by the BM-hMSCs. This experiment has taken place in agitated conditions with headspace aeration, which represents a best-case scenario, as in static monolayer culture without headspace gassing these deviations in dO_2 would be larger with an increased set-point recovery time. It is suggested that this variation in dO_2 during processing is a potential reason why there is so much discrepancy within the published literature of BM-hMSCs cultured in low oxygen concentration atmospheres, with some groups suggesting that the optimal BM-hMSC culture condition occurs when the cells are never exposed to atmospheric oxygen levels, even during the initial isolation and banking phase [27]. In addition, of course, when “normoxic” (21 % atmospheric oxygen) is used, such variations do not occur.

Figure 2A shows the effect of various controlled dissolved oxygen concentrations on BM-hMSC 1 growth on microcarriers over six days of culture in FBS-containing medium, with increased growth kinetics at lower dissolved oxygen concentrations where each dO_2 value was held constant throughout that time by performing culture sampling and medium exchange without exposing the culture to atmospheric conditions. This effect of dO_2 is confirmed by Figure 2B, which shows the post-harvest cell

number at each dO_2 concentration, with significantly higher BM-hMSC numbers ($p < 0.05$) at 10 and 25 % dO_2 compared with 100 % dO_2. There is currently no common consensus in the literature on whether low oxygen concentrations are beneficial for BM-hMSCs, with some studies demonstrating improved growth [28] and function [29], whilst others, including our previously published work, demonstrates diminished BM-hMSC characteristics [16] in uncontrolled monolayer culture. These results suggest that for these donor cells (BM-hMSC 1), BM-hMSC growth characteristics are improved at low dissolved oxygen concentrations, when undertaken in a fully dO_2 controlled microcarrier expansion process. More work of this type with fully controlled dO_2 is required to establish whether low values are generally beneficial for BM-hMSC culture.

It is again important to assess the effect of changing dO_2 concentrations on the relative metabolite flux of the BM-hMSC during microcarrier expansion. Supplementary figure 1 shows the concentrations of glucose, lactate and ammonia over the six days of expansion, with increased consumption of glucose and production of lactate in low dissolved oxygen concentrations, in accordance with the increased BM-hMSC growth rate. The production of ammonia in 10 and 25 % dO_2 was reduced compared with higher dO_2 concentrations, despite the increased BM-hMSC number at the lower dissolved oxygen concentration. The LDH and total protein concentrations have been measured to assess whether any BM-hMSC stress or damage has been caused by the different dO_2 concentrations, which did not change significantly through culture in any of the conditions (supplementary figure 2). The per cell flux of ammonia and the yield of lactate from glucose can be seen in figure 2A and 2B respectively for BM-hMSC 1 culture on microcarriers at various controlled dO_2 concentrations. The per cell flux of ammonia in figure 2A shows a significant reduction ($p < 0.05$) for both 25 and 10 % dO_2 to around 2.5 $pmol.cell^{-1}.day^{-1}$, compared to 100 % dO_2. These findings are again in agreement with a previous study [28] and suggests a more efficient cell metabolism under low dO_2 culture conditions. A reduction in the production in ammonia at low dO_2 concentrations for microcarrier culture is also advantageous for process operation, as the build-up of toxic compounds to inhibitory levels will become less of an issue [30]. The yield of lactate production from glucose can be seen in figure 2B, which shows a reduction in

yield to 1.21 ± 0.37 mol.mol^{-1} at 25% dO_2, but a subsequent increase at 10 % dO_2 to 2.27 ± 0.26 mol.mol^{-1} compared with 100 % dO_2. This result is in agreement with studies of BM-hMSC culture at low oxygen concentrations in monolayer involving BM-hMSCs [16, 28] but is significantly higher than BM-hMSCs derived from the umbilical cord at both high and low dO_2 concentrations [31]. Considering the importance of the *in vivo* niche environment on hMSC characteristics, it is possible that this difference in cell metabolism at different dissolved oxygen concentrations is due to relative differences in the *in vivo* oxygen concentrations in the bone-marrow and umbilical cord tissues [32]. This difference may also help explain the varying results in the literature.

It is also important to assess whether microcarrier expansion at reduced dO_2 concentrations is not unduly affecting the post-harvest BM-hMSC characteristics, despite the improved growth kinetics seen previously. Figure 3A shows the outgrowth rate of BM-hMSC 1 after harvesting from the microcarriers at the various dO_2 concentrations compared to pre-expansion (prior to expansion in the bioreactor) and post-harvest from the spinner flasks in a controlled environment cabinet with atmospheric air (~ 100 % dO_2). This comparison shows significantly higher ($p < 0.05$) outgrowth kinetics for BM-hMSCs across all controlled bioreactor expansion conditions including 100% dO_2 compared to pre-expansion and post-harvest from the spinner flasks. In addition to this, BM-hMSCs cultured at 10 % dO_2 demonstrated significantly higher ($p < 0.05$) outgrowth kinetics compared to all other controlled dO_2 concentrations. These results suggest that the controlled (pH and dO_2) expansion of BM-hMSCs on microcarriers in bioreactors at 100 % dO_2 is having a positive impact on the outgrowth kinetics of BM-hMSCs compared to the less-well controlled conditions in spinner flasks. In addition, for these cells, low dissolved oxygen concentrations further enhances that improvement.

In accordance with the increase in outgrowth kinetics, the mean cell diameter of BM-hMSCs cultured in the controlled bioreactor conditions in figure 3B is significantly ($p < 0.05$) lower than pre-expansion and post-harvest from spinner flasks. The benefits to the manufacturing process of producing smaller BM-hMSCs has previously been discussed [33] and smaller cells are commonly associated with

increased proliferation, greater colony-forming efficiency and longer telomeres [34]. Likewise, BM-hMSC culture at low dO_2 from different sources may also display different functional characteristics and can modulate the autocrine or paracrine activity of a variety of cytokines and growth factors in BM-hMSCs [35]. The post-harvest colony-forming (CFU) efficiency of BM-hMSCs from the controlled bioreactor process at various dO_2 concentrations can be seen in figure 3C, which shows a similar level of CFU efficiency for BM-hMSC harvested form 50 and 100 % dO_2 conditions, compared with pre- and post- expansion spinner flask culture. In contrast, the post-harvest CFU efficiency of BM-hMSC from 10 and 25 % dO_2 was significantly higher ($p < 0.05$) than pre-expansion and post-harvest from spinner flasks. An increase in CFU efficiency of BM-hMSCs at low dissolved oxygen has previously been reported and has been shown to be independent of hypoxia inducible factor (HIF) expression in BM-hMSCs [36].

3.2 Controlled microcarrier expansion of BM-hMSC donors in serum-free

Following the development of a process control strategy in FBS-based culture, it is important to assess the effect of this new control strategy on the serum-free (SFM) microcarrier expansion of multiple BM-hMSC donors. A DO_2 concentration of 25% was selected for the controlled expansion in SFM, due to the previous data that showed increased culture performance at low DO_2 concentrations. Specifically, at DO_2 concentration of 25% was selected over 10% DO_2 due to the increased lactate production experienced at 10% DO_2, which could be detrimental to the culture, particularly as the BM-hMSC density increases. Figure 4A shows the growth rate of the two donor BM-hMSC lines, BM-hMSC 1 and 2, in controlled bioreactors in FBS and SFM, with a significantly increased growth rate for both BM-hMSC donors in SFM to a final cell concentration of $7.11 \pm 0.90 \times 10^5$ cell.mL^{-1} for BM-hMSC 1 and $6.13 \pm 1.82 \times 10^5$ cell.mL^{-1} for BM-hMSC 2. This improvement represents a 300 % increase in the BM-hMSC yield across the two donors in SFM compared to that in FBS, which will be advantageous in driving cost-effective BM-hMSC manufacturing processes. The increase in growth kinetics in a controlled SFM

microcarrier process presented here is in accordance with our previously published data for monolayer culture [33] and uncontrolled spinner flask culture [8].

The harvest efficiency of BM-hMSC from the FBS-based process was 91.5 ± 8.9 % across both donors, which is comparable to previous studies which demonstrated > 95% harvest efficiency for a spinner flask based harvesting process [22]. This result also demonstrates the effectiveness of the scalable harvesting method developed on sound engineering principals, as despite the change in bioreactor platform, maintaining the same detachment protocol for the DASbox bioreactor platform has yielded a similar BM-hMSC harvest efficiency [21] to that in spinner flasks. The overall harvest efficiency of the BM-hMSCs from the SFM process, however, was 76.5 ± 2.9 %, significantly lower than the previous FBS-based microcarrier processes. This drop is most likely due to the significantly increased BM-hMSC densities achieved under serum-free conditions, reducing the effectiveness of the separation process. Supplementary figure 3 shows that the harvest procedure employed has successfully removed the BM-hMSCs from the microcarriers in the SFM process. Therefore, the reduction in harvest efficiency can be attributed to losses during the subsequent cell-microcarrier separation by filtration followed by cell concentration. This separation problem will clearly have to be addressed moving forward, as increased cell densities will be required in order to drive cost-effective manufacturing processes and harvesting efficiencies will have fundamental impact on final product yields [37]. Part of this success will be in developing scalable downstream technology, capable of reducing losses in the process step of separating the BM-hMSCs from the microcarriers after detachment [38].

Metabolite analysis of the controlled bioreactor microcarrier culture of BM-hMSCs showed differences in the metabolic pathway usage relating to lactate and ammonia production between FBS-containing and serum-free cultures. Figure 4 shows the relative consumption of glucose (B), production of lactate (C) and ammonia (D), as well as the yield of lactate from glucose (E) in FBS and SFM for two donor BM-

hMSC lines. In FBS-based expansion the consumption of glucose was 9.86 ± 1.49 pmol.cell^{-1}.day^{-1} for BM-hMSC 1 and 8.37 ± 1.94 pmol.cell^{-1}.day^{-1} for BM-hMSC 2, whereas for SFM expansion the consumption of glucose was significantly lower ($p < 0.05$) for both BM-hMSC donors with 4.41 ± 0.14 pmol.cell^{-1}.day^{-1} for BM-hMSC 1 and 3.54 ± 1.40 pmol.cell^{-1}.day^{-1} for BM-hMSC 2. Similarly, the production of lactate in the controlled microcarrier process was significantly lower ($p <0.05$) in the SFM process compared to the FBS-based process for both donor BM-hMSC lines. This result is once again comparable to the levels of glucose and lactate flux measured during monolayer [33] and uncontrolled microcarrier culture [8] using the same donor BM-hMSCs. As with these previous studies, the production of ammonia is more similar between the FBS and SFM conditions indicating that the metabolite utilization under SFM conditions is more efficient, with a switch from energy production predominantly *via* anaerobic glycolysis in FBS culture to utilization of the oxidative phosphorylation pathway under SFM. The metabolite utilization between BM-hMSC donors is also more consistent in controlled SFM microcarrier culture, with a range in the yield of lactate from glucose between BM-hMSC 1 and 2 of 0.12 mol.mol^{-1}. In contrast, the yield of lactate from glucose in controlled FBS culture between donors showed a range of 1.32 mol.mol^{-1}. This increase in consistency of BM-hMSC characteristics between donors in controlled SFM culture offers significant advantages for bioprocess development and has been maintained from monolayer culture of BM-hMSCs, demonstrating comparable process transfer throughout development.

The concentrations of LDH and total protein for controlled bioreactor culture on microcarriers in FBS and SFM for two BM-hMSC donors can be seen in figure 5. The FBS-based process demonstrated little increase in LDH or total protein over the six days of culture, suggesting no significant cell damage is occurring during the expansion process. The total protein concentration in controlled SFM expansion also showed no significant increase over the six days of culture. However, the LDH concentration did see a slight increase on day six, when the cell density is at a maximum, up to the baseline level during

FBS culture. Considering that this occurs at increased BM-hMSC densities, this outcome should be evaluated as the final cell density of BM-hMSC bioreactor processes increase, to ensure that increasing the yield does not impact the quality of the product.

It is evident from figure 6A that the attachment efficiency of BM-hMSCs in the bioreactor to the microcarriers is improved for both donors in the SFM expansion process, with > 75 % attachment efficiency after one day. In contrast, the FBS-based process had an attachment efficiency of < 70 % for both donors after one day, with a reduced attachment in the subsequent four days of expansion. This is likely due to the use of fibronectin to coat the microcarriers in the SFM process, which has been shown to improve cell attachment [39]. Given the implications of poor cell-microcarrier attachment described above, particularly as the process increases in scale, this higher level of attachment represents a key advantage of the SFM compared to the FBS-based microcarrier process.

The specific outgrowth rate of BM-hMSCs from both the FBS and SFM controlled bioreactor processes can be seen in figure 6B which shows that the post-harvest outgrowth kinetics of BM-hMSC have been maintained. Figure 6C shows the post-harvest BM-hMSC diameter which has been significantly reduced ($p < 0.05$) compared to pre-expansion, which for the FBS process represented a reduction of 2.1 µm for BM-hMSC 1 and 2.2 µm for BM-hMSC 2. This is in contrast to the uncontrolled spinner flask process presented previously [8] which demonstrated a slight increase in mean cell diameter of BM-hMSC 1 .(1.2 µM) and BM-hMSC 2 (0.4 µm) between post-harvest and pre-expansion. The post-harvest colony-forming potential of the BM-hMSCs can be seen in figure 6D, which shows that the CFU potential has either increased or been maintained compared to pre-expansion. This is particularly apparent for the controlled FBS-based process, which showed a significant increase ($p < 0.05$) in the post-harvest CFU potential for both BM-hMSC donor lines. Verification of post-harvest immunophenotype and differential potential of BM-hMSCs used during this study can be seen in Supplementary Figure 4.

3.5 Process control to drive yield and consistency into BM-hMSC manufacture

For these BM-hMSC therapies to successfully progress through to commercial production, the manufacturing cost of goods should be minimized, and the process robustness maximized throughout development. Driving yield and consistency into the BM-hMSC manufacturing process at an early stage of development will be critical to reduce the overall cost of goods and increase the cost-effectiveness of the final BM-hMSC product. Increasing the consistency of the final product will reduce process costs by demonstrating a level of control over the product and reducing the risk of batch failure. For large-scale off the shelf processes, where the capital invested per batch is high, increasing the consistency of product quality will reduce the risk of product batch failures and significantly reduce the overall production costs. This means that assessing the final BM-hMSC yield and donor consistency for various process iterations, would be highly informative on the direction of future manufacturing development. Currently, two of the key cost drivers for the production of off-the-shelf cell-based therapies are in the culture medium and the time it takes to manufacture a product batch in a facility [6]. Therefore, to make a basic yield comparison of each of the processes developed, the relative process yield in terms of number of BM-hMSCs produced as a function of the volume of culture medium and process time has been calculated for each of the controlled and uncontrolled systems.

In addition to this process yield estimation, a combination of the inter- and intra-donor variation has been assessed to get an understanding of the relative consistency within each process. The results of this can be seen in figure 7**Error! Reference source not found.**, which demonstrates the increased process yield that is achieved in controlled SFM microcarrier culture. This improvement represents an increase in process yield of over 300 % for the SFM process compared to the FBS based process under the controlled conditions of the bioreactor. As the process scale increases and the manufacturing costs of the SFM are reduced, this improvement is likely to increase further, due to the issues associated with serum supply at a large-scale [40]. It can also be seen from figure 7 that the process yield is also increased by an average of around 500% for both donors under controlled conditions compared to the

poorly controlled expansion in spinner flasks, highlighting the importance of systematically developing a process control strategy to increase the yield of BM-hMSC production on microcarriers. It is important to note that the large difference in process yield between monolayer and microcarrier culture at the bench-scale evaluated here, will increase as the scale increases, due to the improved economies of scale achievable in a suspension bioreactor-based process.

The percentage coefficient of variation has been calculated for each of the expansion processes to assess the relative amount of variation in cell yield between each condition. This parameter is a combination of inter-donor variation, (the variation between each donor) and intra-donor variation (the variation between batch runs of each donor), as seen in figure 7, which shows the lowest coefficient of variation for the controlled SFM microcarrier process with 14.7 %. A coefficient of variation of 15 % or less is in alignment with the established regulatory guidance [41, 42], demonstrating control over the product. There is also a clear difference between the controlled and uncontrolled processes in FBS, with process control reducing the coefficient of variation from 79.1 % for the spinner flask to 37.5 % for the bioreactor experiments. In addition to the scalability of stirred-tank bioreactors *versus* alternative BM-hMSC culture technology, this further demonstrates that stirred-tank bioreactors can achieve effective process control across a number of process parameters, utilizing online and non-destructive methods to maintain the process in a state of control for the duration of the culture. This outcome represents a significant reduction in process variation, *via* the introduction of a process control system, once again demonstrating the importance of developing a process control strategy for BM-hMSC production.

Conclusions

The systematic development of a process control strategy in a stirred-tank bioreactor for the microcarrier expansion of BM-hMSCs has significantly increased the yield and consistency achievable in the process compared to the relatively-uncontrolled conditions in a spinner flask. Even operating the microcarrier expansion process for BM-hMSCs in closed system with controlled pH and at 100 %

dO_2, has demonstrated improved growth and post-harvest BM-hMSC quality characteristics compared to those produced from a spinner flask in atmospheric air in biological safety cabinets.

Studies looking at the effect of exposing cells grown at low dissolved oxygen ("hypoxic") conditions during bioreactor culture to atmospheric conditions during the culture period, as can occur during media exchange, etc., has highlighted the importance of operating closed and controlled processes to avoid large fluctuation of the dissolved oxygen concentration of the medium. Thus, for closed conditions for these donor cells grown at 10 and 25 % dO_2 in FBS-based medium, a further improvement was achieved compared to 100 % dO_2.

The introduction of serum-free medium into such controlled low dO_2 conditions has further increased the yield across two BM-hMSC donors, however, with reduced harvest efficiency compared to the FBS-based process that has lower cell numbers at harvest. It is argued that this drop was primarily due to cell losses in the cell-microcarrier separation step (filtration). Thus, it will be imperative to develop specific downstream technology to effectively separate the cells from the microcarriers, especially as the number of BM-hMSCs per volume is increased under serum-free conditions.

The development of a process control strategy has significantly increased the yield and consistency between BM-hMSC donors with a coefficient of variance between and within these donors under serum-free conditions of less than 15 %. This increase in consistency is in conjunction with a significant increase in the BM-hMSCs produced per volume of medium per unit time under serum-free conditions, which will be critical in increasing the economies of scale and reducing the cost of BM-hMSC therapies. Furthermore, the introduction of the process control strategy has significantly reduced the BM-hMSC inter- and intra-donor variation in FBS-based culture from 79.1 to 37.5 %, which will be critical for the future development of both patient-specific and off-the-shelf BM-hMSC manufacturing processes.

Acknowledgments

This study has been funded by the Engineering and Physical Sciences Research Council (EPSRC, EP/F500491/1) and FUJIFILM Diosynth Biotechnologies.

References

[1] A.I. Caplan, J.E. Dennis, Mesenchymal stem cells as trophic mediators, Journal of cellular biochemistry, 98 (2006) 1076-1084.
[2] T.R.J. Heathman, A.W. Nienow, M.J. McCall, K. Coopman, B. Kara, C.J. Hewitt, The translation of cell-based therapies: clinical landscape and manufacturing challenges, Regenerative medicine, 10 (2015) 49-64.
[3] TiGenix and Takeda announce Alofisel® (darvadstrocel) receives approval to treat complex perianal fistulas in Crohn's disease in Europe, Press Release, https://www.takeda.com/newsroom/newsreleases/2018/tigenix-and-takeda-announce-alofisel-receives-approval-in-europe/ (2018).
[4] Q.A. Rafiq, K.M. Brosnan, K. Coopman, A.W. Nienow, C.J. Hewitt, Culture of human mesenchymal stem cells on microcarriers in a 5 l stirred-tank bioreactor, Biotechnology letters, 35 (2013) 1233-1245.
[5] A.W. Nienow, Reactor engineering in large scale animal cell culture, Cytotechnology, 50 (2006) 9-33.
[6] A.S. Simaria, S. Hassan, H. Varadaraju, J. Rowley, K. Warren, P. Vanek, S.S. Farid, Allogeneic cell therapy bioprocess economics and optimization: Single-use cell expansion technologies, Biotechnology and bioengineering, 111 (2014) 69-83.
[7] F. dos Santos, P.Z. Andrade, M.M. Abecasis, J.M. Gimble, L.G. Chase, A.M. Campbell, S. Boucher, M.C. Vemuri, C.L. da Silva, J.M.S. Cabral, Toward a Clinical-Grade Expansion of Mesenchymal Stem Cells from Human Sources: A Microcarrier-Based Culture System Under Xeno-Free Conditions, Tissue Engineering. Part C, Methods, 17 (2011) 1201-1210.
[8] T.R.J. Heathman, V.A.M. Glyn, A. Picken, Q.A. Rafiq, K. Coopman, A.W. Nienow, B. Kara, C.J. Hewitt, Expansion, harvest and cryopreservation of human mesenchymal stem cells in a serum-free microcarrier process, Biotechnology and bioengineering, 112 (2015) 1696-1707.
[9] K.Y. Tan, K.L. Teo, J.F. Lim, A.K. Chen, S. Reuveny, S.K. Oh, Serum-free media formulations are cell line-specific and require optimization for microcarrier culture, Cytotherapy, 17 (2015) 1152-1165.
[10] D.J. Williams, R.J. Thomas, P.C. Hourd, A. Chandra, E. Ratcliffe, Y. Liu, E.A. Rayment, J.R. Archer, Precision manufacturing for clinical-quality regenerative medicines, Philosophical transactions. Series A, Mathematical, physical, and engineering sciences, 370 (2012) 3924-3949.
[11] M.W. Naing, D.A. Gibson, P. Hourd, S.G. Gomez, R.B.V. Horton, J. Segal, D.J. Williams, Improving umbilical cord blood processing to increase total nucleated cell count yield and reduce cord input wastage by managing the consequences of input variation, Cytotherapy, 17 (2015) 58-67.
[12] FDA, Guidance for Industry Q8(R2) Pharmaceutical Development in: U.S.D.o.H.a.H. Services (Ed.)www.fda.gov/downloads/Drugs/Guidances/ucm073507.pdf, 2009.
[13] FDA, PAT - A framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance, in: F.a.D.A. Center of Drug Evaluation and Research (Ed.), 2004.
[14] K. Cierpka, C.L. Elseberg, K. Niss, M. Kassem, D. Salzig, P. Czermak, hMSC Production in Disposable Bioreactors with Regards to GMP and PAT, Chemie Ingenieur Technik, 85 (2013) 67-75.
[15] A.W. Nienow, Scale-Up, Stirred Tank Reactors, In: Encyclopedia of Industrial Biotechnology, (Ed, M. C. Flickinger), John Wiley & Sons, Inc., Hoboken, NJ, USA, 7 (2010) 4328-4341.
[16] Q.A. Rafiq, K. Coopman, A.W. Nienow, C.J. Hewitt, A quantitative approach for understanding small-scale human mesenchymal stem cell culture - implications for large-scale bioprocess development, Biotechnology journal, 8 (2013) 459-471.
[17] A.W. Nienow, K. Coopman, T.R. Heathman, Q.A. Rafiq, C.J. Hewitt, Bioreactor Engineering Fundamentals for Stem Cell Manufacturing. In "Stem Cell Manufacturing", (Eds. J.M.S. Cabral, C.L. de Silva, L. G. Chase and M. M. Diogo), Elsevier Science, Cambridge, USA, Chapter 3 (2017) 43-76.
[18] T.R.J. Heathman, A.W. Nienow, Q.A. Rafiq, K. Coopman, B. Kara, C.J. Hewitt, Agitation and aeration of stirred-bioreactors for the microcarrier culture of human mesenchymal stem cells and

potential implications for large scale bioprocess development, Biochemical Engineering Journal, In Press.
[19] Q.A. Rafiq, K. Coopman, A.W. Nienow, C.J. Hewitt, Systematic microcarrier screening and agitated culture conditions improves human mesenchymal stem cell yield in bioreactors, Biotechnology journal, 11 (2016) 473-486.
[20] Q.A. Rafiq, K. Coopman, C.J. Hewitt, Scale-up of human mesenchymal stem cell culture: current technologies and future challenges, Current Opinion in Chemical Engineering, 2 (2013) 8-16.
[21] A.W. Nienow, C.J. Hewitt, T.R.J. Heathman, V.A.M. Glyn, G.N. Fonte, M.P. Hanga, K. Coopman, Q.A. Rafiq, Agitation conditions for the culture and detachment of hMSCs from microcarriers in multiple bioreactor platforms, Biochemical Engineering Journal, 108 (2016) 24-29.
[22] A.W. Nienow, Q.A. Rafiq, K. Coopman, C.J. Hewitt, A potentially scalable method for the harvesting of hMSCs from microcarriers, Biochemical Engineering Journal, 85 (2014) 79-88.
[23] A.K.C. Chan, T.R.J. Heathman, K. Coopman, C.J. Hewitt, Multiparameter flow cytometry for the characterisation of extracellular markers on human mesenchymal stem cells, Biotechnology letters, 36 (2014) 731-741.
[24] T.R.J. Heathman, Q.A. Rafiq, A.K.C. Chan, K. Coopman, A.W. Nienow, B. Kara, C.J. Hewitt, Characterization of human mesenchymal stem cells from multiple donors and the implications for large scale bioprocess development, Biochemical Engineering Journal, 108 (2016) 14-23.
[25] W.L. Grayson, F. Zhao, R. Izadpanah, B. Bunnell, T. Ma, Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs, Journal of cellular physiology, 207 (2006) 331-339.
[26] D.C. Kirouac, P.W. Zandstra, The systematic production of cells for cell therapies, Cell Stem Cell, 3 (2008) 369-381.
[27] A.G. Kay, T.P. Dale, K.M. Akram, P. Mohan, K. Hampson, N. Maffulli, M.A. Spiteri, A.J.E. Haj, N.R. Forsyth, BMP2 repression and optimized culture conditions promote human bone marrow-derived mesenchymal stem cell isolation, Regenerative Medicine, 10 (2015) 109-125.
[28] F. Dos Santos, P.Z. Andrade, J.S. Boura, M.M. Abecasis, C.L. da Silva, J.M. Cabral, Ex vivo expansion of human mesenchymal stem cells: a more effective cell proliferation kinetics and metabolism under hypoxia, J Cell Physiol, 223 (2010) 27-35.
[29] L. Basciano, C. Nemos, B. Foliguet, N. de Isla, M. de Carvalho, N. Tran, A. Dalloul, Long term culture of mesenchymal stem cells in hypoxia promotes a genetic program maintaining their undifferentiated and multipotent status, BMC cell biology, 12 (2011) 12.
[30] D. Schop, F.W. Janssen, L.D.S. van Rijn, H. Fernandes, R.M. Bloem, J.D. de Bruijn, R. van Dijkhuizen-Radersma, Growth, Metabolism, and Growth Inhibitors of Mesenchymal Stem Cells, Tissue Engineering Part A, 15 (2009) 1877-1886.
[31] A. Lavrentieva, I. Majore, C. Kasper, R. Hass, Effects of hypoxic culture conditions on umbilical cord-derived human mesenchymal stem cells, Cell Commun Signal, 8 (2010) 18.
[32] A. Mohyeldin, T. Garzón-Muvdi, A. Quiñones-Hinojosa, Oxygen in Stem Cell Biology: A Critical Component of the Stem Cell Niche, Cell stem cell, 7 (2010) 150-161.
[33] T.R.J. Heathman, A. Stolzing, C. Fabian, Q.A. Rafiq, K. Coopman, A.W. Nienow, B. Kara, C.J. Hewitt, Serum-free process development: improving the yield and consistency of human mesenchymal stromal cell production, Cytotherapy, 17 (2015) 1524-1535.
[34] R.M. Samsonraj, B. Rai, P. Sathiyanathan, K.J. Puan, O. Rotzschke, J.H. Hui, M. Raghunath, L.W. Stanton, V. Nurcombe, S.M. Cool, Establishing criteria for human mesenchymal stem cell potency, Stem cells (Dayton, Ohio), 33 (2015) 1878-1891.
[35] R. Das, H. Jahr, G.J. van Osch, E. Farrell, The role of hypoxia in bone marrow-derived mesenchymal stem cells: considerations for regenerative medicine approaches, Tissue engineering. Part B, Reviews, 16 (2010) 159-168.
[36] K. Tamama, H. Kawasaki, S.S. Kerpedjieva, J. Guan, R.K. Ganju, C.K. Sen, Differential roles of hypoxia inducible factor subunits in multipotential stromal cells under hypoxic condition, Journal of cellular biochemistry, 112 (2011) 804-817.

[37] S. Hassan, A.S. Simaria, H. Varadaraju, S. Gupta, K. Warren, S.S. Farid, Allogeneic cell therapy bioprocess economics and optimization: downstream processing decisions, Regenerative medicine, 10 (2015) 591-609.
[38] B. Cunha, T. Aguiar, M.M. Silva, R.J. Silva, M.F. Sousa, E. Pineda, C. Peixoto, M.J. Carrondo, M. Serra, P.M. Alves, Exploring continuous and integrated strategies for the up- and downstream processing of human mesenchymal stem cells, J Biotechnol, (2015).
[39] M.J. Cooke, S.R. Phillips, D.S. Shah, D. Athey, J.H. Lakey, S.A. Przyborski, Enhanced cell attachment using a novel cell culture surface presenting functional domains from extracellular matrix proteins, Cytotechnology, 56 (2008) 71-79.
[40] D.A. Brindley, N.L. Davie, E.J. Culme-Seymour, C. Mason, D.W. Smith, J.A. Rowley, Peak serum: implications of serum supply for cell therapy manufacturing, Regenerative medicine, 7 (2012) 7-13.
[41] M. Streitz, T. Miloud, M. Kapinsky, M.R. Reed, R. Magari, E.K. Geissler, J.A. Hutchinson, K. Vogt, S. Schlickeiser, A.H. Kverneland, C. Meisel, H.-D. Volk, B. Sawitzki, Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study, Transplantation Research, 2 (2013) 17-17.
[42] FDA, Guidance for Industry. Potency tests for cellular and gene therapy products., in: U.S.F.a.D. Administration (Ed.)http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM243392.pdf, 2011.

Figures

Figure 1 – Implications of exposing low dissolved oxygen (dO_2) cultures (5% dO_2 and 25% dO_2) in a biological safety cabinet to normal atmospheric conditions for 1, 5 and 60 minutes. Demonstrating the importance of continuous closed process control during bioreactor culture. Bars represent the set-point recovery time to 5 or 25% dO_2 and diamonds represent the maximum dO_2 concentration reached during each exposure and recovery period.

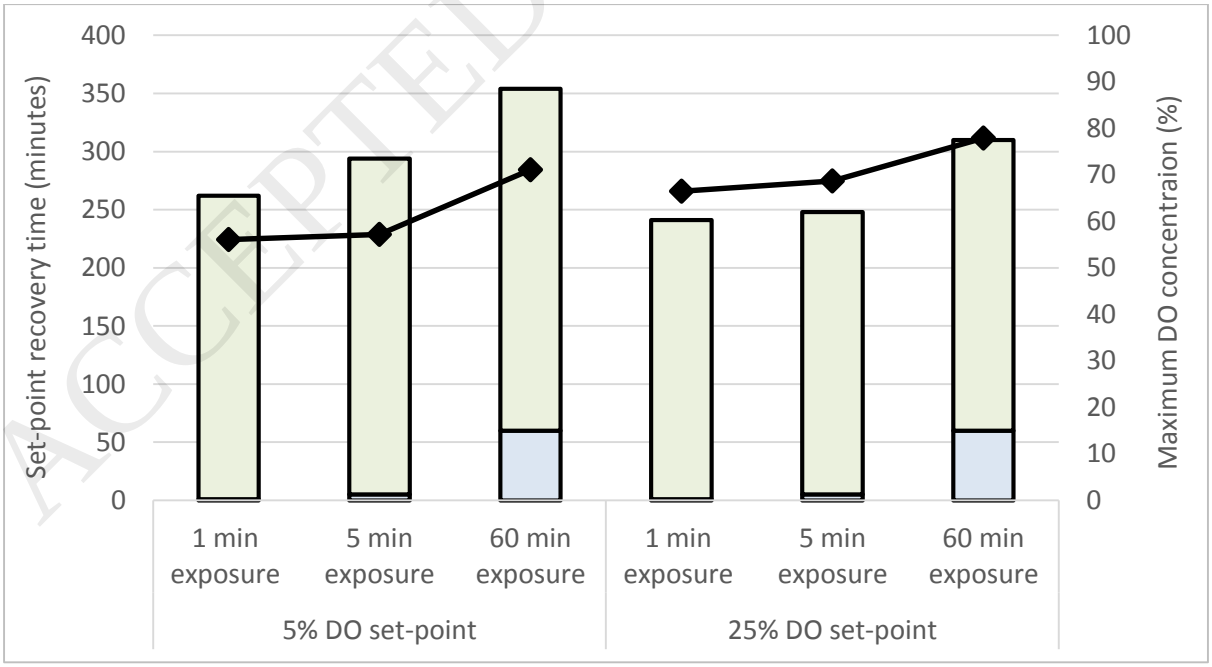

Figure 2 – Effect of dissolved oxygen (dO_2) on BM-hMSC 1 growth over six days of culture in FBS-containing medium in the DASbox controlled bioreactor, showing increased cell numbers over six

days at lower dissolved oxygen concentrations (A) pre-harvest, (B) post-harvest, (C) per cell flux of ammonia and (D) yield of lactate from glucose. Control set-points are 115rpm impeller speed and pH 7.4 with headspace aeration. Data shows mean ± SD, n = 3. * denotes a significant difference ($p < 0.05$) compared to 100% DO_2.

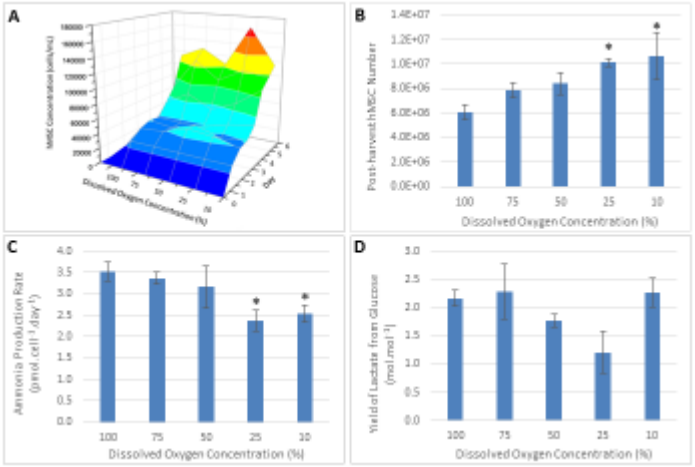

Figure 3 – Post-harvest characteristics of BM-hMSC 1 from controlled microcarrier culture in FBS-containing medium compared to pre-expansion and post-harvest from spinner flasks in a controlled environment cabinet with atmospheric air (~ 100 % dO_2). Showing (A) increased outgrowth rate; (B) reduced mean cell diameter; and (C) increased CFU efficiency at reduced dO_2 concentrations in the bioreactor and improved performance at all dO_2 concentrations compared to the spinner flasks. Data shows mean ± SD, n = 3. * denotes a significant difference ($p < 0.05$) compared to pre-expansion and post-spinners.

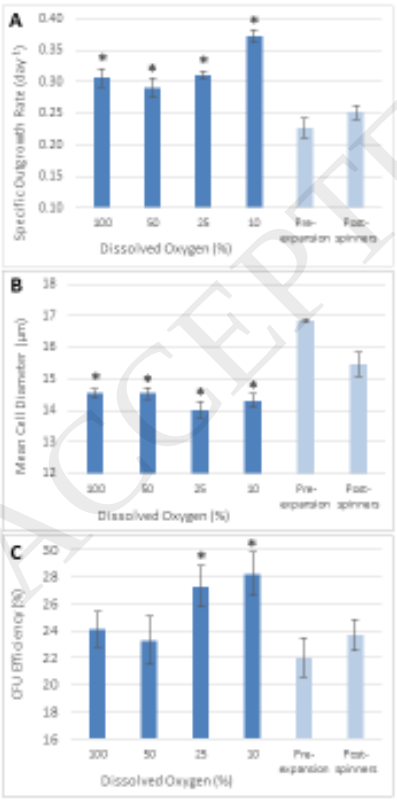

Figure 4 – Comparison of culture on microcarriers in the bioreactor in FBS and SFM across the two BM-hMSC donors at 115rpm, with controlled 25% dO_2 by headspace aeration and a pH of 7.4.Showing (A) increased growth rate for both donors in SFM, (B) reduced glucose consumption in SFM, (C) reduced lactate production in SFM, (D) reduced ammonia production in SFM and (E) yield of lactate from glucose. Data shows mean ± SD, n = 3. * denotes a significant difference ($p < 0.05$) compared with the same BM-hMSCs in FBS-containing medium.

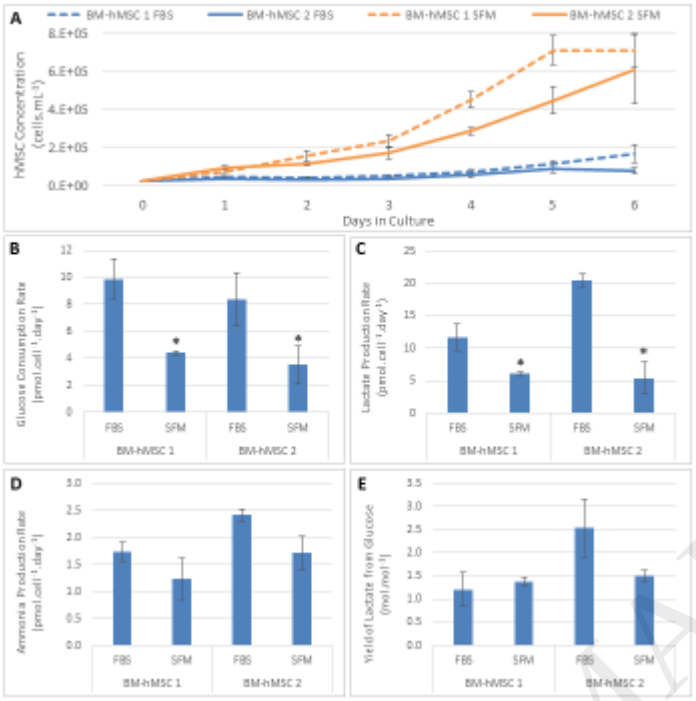

Figure 5 – Comparison of culture on microcarriers in the bioreactor in FBS and SFM across two BM-hMSC donors at 115rpm, with controlled 25% dO_2 by headspace aeration and a pH of 7.4. Showing (A) no increase in total protein concentration over six days and (B) increased LDH concentration at the end of SFM culture. Data shows mean ± SD, n = 3.

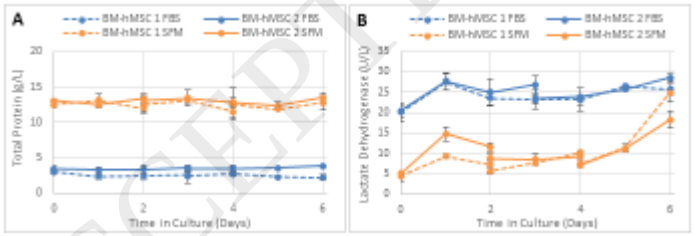

Figure 6 – Attachment rate and post-harvest characteristics during culture on microcarriers in the bioreactor in FBS and SFM across two BM-hMSC donors at 115rpm, with controlled 25% dO_2 by headspace aeration and a pH of 7.4. Showing (A) increased cell attachment to microcarriers in SFM, (B) increased outgrowth rate in SFM, (C) reduced mean cell diameter and (D) maintained CFU efficiency post-harvest. Data shows mean ± SD, n = 3. * denotes a significant difference ($p < 0.05$) compared with the same BM-hMSCs pre-expansion.

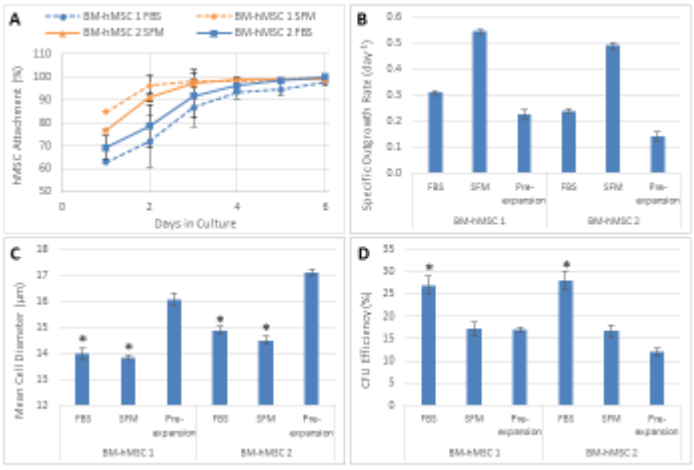

Figure 7 – Impact of process control on the process yield from a microcarrier expansion process at 25% dO_2 in the bioreactor compared poorly controlled monolayer and spinner flask culture under atmospheric conditions. Showing the controlled bioreactor process under serum-free conditions provides a much higher yield and more consistency between donors. Bars denote process yield in terms of number of cells produced per volume of medium per unit time and the line chart denotes the coefficient of variation between and within donor batch runs.