Biotechnology Journal
www.biotechnology-journal.com

Scalable Manufacturing of Human Mesenchymal Stromal Cells in the Vertical-Wheel Bioreactor System: An Experimental and Economic Approach

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Mesenchymal stromal cells (MSC) hold great promise for tissue engineering applications and cell-based therapies. Large cell doses (>1 × 10^6 cells kg^{-1}) and Good Manufacturing Practices (GMP)-compliant processes are however required for clinical purposes. Here, a serum- and xenogeneic-free (S/XF) microcarrier-based culture system is established for the expansion of human umbilical cord matrix (UCM)- and adipose tissue (AT)-derived MSC using the Vertical-Wheel system (PBS-0.1 MAG; PBS Biotech). UCM and AT MSC are expanded to maximum cell densities of 5.3 ± 0.4 × 10^5 cell mL^{-1} ($n = 3$) and 3.6 ± 0.7 × 10^5 cell mL^{-1} ($n = 3$), respectively, after 7 days of culture, while maintaining their identity, according to standard criteria. An economic evaluation of the process transfer from T-flasks to PBS-0.1 MAG shows a reduction in the costs associated with the production of a dose for an average 70 kg adult patient (i.e., 70 million cells). Costs decrease from $17.0 K to $11.1 K for UCM MSC and from $21.5 K to $11.1 K for AT MSC, proving that the transition to Vertical-Wheel reactors provides a cost-effective alternative for MSC expansion. The present work reports the establishment of a scalable and cost-effective culture platform for the manufacturing of UCM and AT MSC in a S/XF microcarrier-based system.

1. Introduction

Mesenchymal stromal cells (MSC) have been receiving great interest in clinical settings due to their self-renewal capacity, differentiation potential, and immunomodulatory and regenerative properties.[1] By using the minimal criteria proposed by the International Society for Cellular Therapy (ISCT),[2] MSC have been isolated from different human tissues, including bone marrow (BM), adipose tissue (AT), synovial membrane, periosteum, umbilical cord matrix (UCM), placenta, and amniotic fluid.[3–5] Numerous studies have shown that MSC isolated from different sources present different biological features, such as distinct expression of cell surface markers, proliferative capacity, differentiation ability, and immunomodulatory and regenerative features.[6–8]

The use of MSC in clinical settings usually requires large cell numbers per dose (>10^6 cells kg^{-1} of body weight) and eventually administration of several doses, depending on the type of disorder.[9–11] It is thus crucial to establish culture systems

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DOI: 10.1002/biot.201800716

capable of enabling efficient isolation and ex vivo expansion of MSC, while complying with Good Manufacturing Practices (GMP) guidelines. In this context, cell culture media and cultivation platforms represent key parameters for the successful manufacturing of MSC. Over the past few years, humanized products have been exploited as serum-/xenogeneic-free (S/XF) culture supplements for the isolation and expansion of MSC, like thrombin-activated platelet rich plasma, autologous and allogeneic human serum, and pooled human platelet lysate (hPL).[3] Although representing a feasible alternative to conventional culture supplement (fetal bovine/calf serum [FBS/FCS]), hPL still presents limited availability, batch-to-batch variability, and ill-definition[3] and, thus, chemically defined S/XF formulations should be adopted. Moreover, MSC expansion has been traditionally performed on static planar polystyrene culture systems. Limitations as low surface-area-to-volume ratio, lack of monitoring, and controlling of culture parameters, which most likely result in variability regarding cell number and quality,[3] are driving MSC manufacturing to move toward 3D culture systems operating under dynamic conditions, namely microcarrier-based suspension systems.

Different bioreactor configurations and modes of operation have been exploited in this context.[3] Stirred-tank reactors (STR) represent the most common bioreactor configuration for scalable expansion of MSC under dynamic conditions.[12–14] However, these systems present some limitations, including limited capacity for microcarrier suspension, leading to non-homogeneous particle distribution, and thus the necessity of using higher impeller velocities at larger scales, which may generate detrimental shear rates and hamper cell growth on the microcarriers.[15] In an attempt to overcome such limitations, PBS Biotech Inc. developed an innovative type of bioreactor using the Vertical-Wheel (VW) technology. These systems consist of U-shaped vessels incorporating a vertically rotating wheel, resulting in faster and more efficient mixing at lower agitation rates and power input, compared to the traditional STR.[15] Additionally, this type of bioreactor features scalability to industrial volumes, including 500 L bioreactors.[16] Therefore, there is the need for a cost-effectiveness analysis using VW technology in comparison to planar culture systems, the culture platforms routinely used for the expansion of MSC.

Bioprocess economic models have been employed to determine the optimal cost-effective upstream[17–20] and downstream[19–21] processing technologies and evaluate the current technical bottlenecks of the process for allogeneic (i.e., donor and recipient are different individuals) cell therapies. The models were previously used to evaluate the cost-effectiveness of replacing FBS by hPL lysate culture supplements on a scale-out MSC manufacturing strategy for autologous (i.e., using cells from the patient) cell therapies.[22]

The present study aims at establishing a S/XF microcarrier-based, cost-effective culture system for the manufacturing of MSC derived from different human sources, in a PBS-0.1 single-use bioreactor system (working volume of 100 mL; PBS Biotech Inc.) combined with a fibrinogen-depleted hPL-based culture supplement (UltraGRO-PURE; AventaCell Biomedical). The economic feasibility of the small-scale process transition from static to dynamic conditions was assessed through the utilization of a bioprocess economics tool previously reported by our group.[22] The results are expected to contribute to the development of a cost-effective and scalable platform to obtain clinical meaningful MSC numbers for therapeutic settings.

2. Experimental Section

2.1. Cultivation of Human MSC in the PBS-0.1 MAG System

Previously isolated and expanded human MSC (see the Supporting Information) were inoculated in PBS-0.1 MAG (PBS Biotech Inc.) with a working volume of 100 mL. Two grams of plastic microcarriers (Pall SoloHill) per culture were prepared according to manufacturer's instructions and then incubated with 20 mL of low glucose Dulbecco's modified Eagle's medium (Sigma) supplemented with GlutaMAX (Life Technologies) (referred to as DMEM) and 50% v/v UltraGRO-PURE (AventaCell Biomedical) for 1 h at 37 °C under intermittent agitation (2 min at 750 rpm, 10 min nonagitated), using a Thermomixer comfort (Eppendorf AG). Inoculation in the PBS-0.1 MAG was performed in 60 mL of DMEM + 5% v/v UltraGRO-PURE, with an initial MSC density of 0.83×10^5 cell mL^{-1} and a microcarrier concentration of 33 g L^{-1}. MSC previously expanded under static conditions were added to the microcarrier suspension inside the PBS-0.1 MAG. During the first 6 h of culture, agitation cycles of 1 min agitating at 25 rpm followed by 20 min with no agitation were used, in line with what has been previously reported.[15] After 6 h, a continuous agitation mode at 25 rpm was adopted. At day 1 of culture, the number of cells attached to the microcarriers was determined by the Trypan Blue (Life Technologies) exclusion method, as previously described,[23] to estimate initial cell adhesion. At day 2 of culture, 40 mL of fresh culture medium with a glucose pulse to a final glucose concentration of 3 g L^{-1} was added to the PBS-0.1 MAG (final working volume of 100 mL). For AT MSC culture, agitation was set to 30 rpm, to overcome an increased medium viscosity and excessive cell aggregation. From day 2 onward, exchange of 25% v/v of culture medium was performed every 12 h. Addition of fresh culture medium supplemented with glucose to a final concentration of 3 g L^{-1} was performed at days 3, 4, 5, 6 (for both UCM and AT MSC cultures), and 7 (only for UCM MSC culture), whereas fresh medium with no glucose supplementation was carried out at days 2.5, 3.5, 4.5, 5.5, 6.5 (for both UCM and AT MSC cultures), and 7.5 (only for UCM MSC culture). Agitation rate was increased to 35 rpm on AT MSC culture when cell aggregates started to form. Conversely, agitation rate was maintained at 25 rpm throughout the culture period. Cell growth, viability, and metabolite analysis were assessed every day, as described in de Soure et al.[24] The specific growth rate during exponential growth phase was calculated as described in Hanga et al.[25] Cell visualization on microcarriers was performed from day 1 onwards, by staining the cells with 4′,6-diamidino-2-phenylindole (DAPI, 1.5 μg mL^{-1} in phosphate-buffered saline [PBS]), as previously described in de Soure et al.[24]

2.2. Harvesting and Characterization of Expanded MSC

At the end of expansion, UCM, and AT MSC were detached from the microcarriers in the PBS-0.1 MAG by transferring the

culture to 50 mL tubes (BD Falcon), washing with PBS (1×), and incubating with TrypLE Select CTS (1×) (Life Technologies) for 15 min at 37 °C and 750 rpm, in a Thermomixer comfort. The cell suspension was then filtered through a 100 µm cell strainer (BD Biosciences).

Before and after PBS-0.1 MAG culture, cells were characterized in terms of immunophenotypic profile,[24] while cells after PBS-0.1 MAG culture were also tested for multilineage differentiation capacity.[24]

2.3. Metabolite Analysis

Glucose and lactate concentrations were determined in the supernatant of the samples collected throughout the experiments by using an automatic analyzer (YSI 7100MBS; Yellow Spring Instruments). Specific glucose consumption and lactate production rates were calculated by normalizing the amount of glucose consumed/lactate produced to cell growth at each day of culture. The apparent yield of lactate from glucose was calculated by the ratio of the specific production rate of lactate and the specific consumption rate of glucose.

2.4. Economic Evaluation

A bioprocess economics tool, TESSEE - Tool for Early Stem Cells Economic Evaluation (http://github.com/catiabandeiras/TESSEE) was previously implemented by our group.[22] Briefly, the tool accounts for the isolation, expansion, downstream processing (DSP) and quality controls, with different operation times and reagent requirements on a simulated GMP facility. This tool was modified to account for the expansion of UCM and AT MSC considering an allogeneic setting, as well as to include intermediate banking steps and allow estimation of cell expansion in the VW system. The isolation yields, growth rates, seeding densities, and harvesting densities are modeled as inputs for each expansion scheme based on the experimental data presented in this study for each cell source.

A therapeutic dose of 1 million cells kg^{-1} was considered, in agreement with typical doses in clinical trials using MSC as therapeutic interventions.[26] Doses containing 70 million cells (i.e., average adult patient weighting 70 kg) were used as a proxy.

The economic evaluation of the VW system is hereby performed against the use of planar cell cultivation technology (T-175 flasks), the current standard system for MSC expansion. The model was not set to deliver a specific yield, and the output is calculated instead. This value is determined in terms of the number of doses of 70 million cells possible to obtain from a single donor of either AT-MSC or UCM MSC following two different process options. Therefore, each process corresponds to the cells expanded from one single donor. Such cells are first used to prepare a master cell bank (MCB), and from this, a working cell bank (WCB) is established using planar technology (i.e., T-flasks). The WCB is prepared maximizing facility capacity use. Then the WCB is completely used in planar or bioreactor batches according to the process evaluated. The model workflow is described in detail in the Supporting Materials and Methods Section in the Supporting Information.

The total costs per dose are obtained by dividing the sum of total process costs by the number of doses produced. The total process costs are obtained by considering several categories (consumables, labor, quality controls, and facility costs).

The consumables category includes the disposable components of the process, such as single-use cell culture flasks (T-175) and bioreactor disposable vessel (PBS-0.1MAG), microcarriers, and other reagents, such as isolation, culture and centrifugation media, and harvesting agents. Note that the PBS costs include a disposable PBS vessel and the microcarriers, which contributes to the consumables associated with the use of this expansion technology.

The consumables costs for wet materials are obtained by multiplying the total volume of culture medium, buffers, and harvesting agent by the cost per volume of each reagent. The costs for disposable consumables include the disposable vessels for cell expansion (T-175 and PBS-0.1MAG), microcarriers for expansion support in PBS-0.1MAG, and accessories, such as cryovials. The total cost is determined by accounting for the total number of units used in the process and multiplying by the unit cost.

The labor costs include the contribution of the manufacturing personnel, with a fixed daily rate, multiplied by the total duration of the process.

The facility costs include the fixed and operational costs related with the GMP facility and the equipment required for cell culture processing (incubators, biosafety cabinets [BSC], and centrifuges). These fixed and operational costs are input on a daily basis and included in the costs proportionally to the duration of the process.

The testing contribution is obtained by multiplying the number of tests for MCB, WCB, and final product release incurred during the process, by the unit costs of each of these tests.

The parameters associated with the setup of the modeling case study are depicted in **Tables 1** and **2**.

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software). Results are presented as mean + standard

Table 1. Characteristics of the cultivation systems used.

	T-175 flask	PBS-0.1 MAG
Culture medium volume [mL]	35	100
Harvesting reagent volume [mL]	7	20
Mass of microcarriers [g]	—	2
Type of microcarrier	—	Plastic SoloHill
Incubator capacity	24	6
Expansion area [cm^2]	175	720
Seeding density [cells cm^2]	3000	6944
Unit costs [$]	7.38	181.55
Ancillary equipment costs [$]	—	2306.25
DSP yield [%]	90	75

The parameters work as inputs for the bioprocess economics model and are derived from the characteristics of the experimental process.

Table 2. Key facility, labor, quality control, and reagent assumptions for the bioprocess economics modeling.

Parameter	Value	References
GMP facility area	180 [sq m]	This work
% Clean room area	20	This work
# Clean rooms	1	This work
# Incubators	4	This work
# Biosafety cabinets	1	This work
# Centrifuges	1	This work
Incubator unit cost [$]	10 000	This work
Biosafety cabinet (BSC) unit cost [$]	10 500	This work
Centrifuge unit cost [$]	8500	This work
T-175 unit cost [$]	7.38	This work
PBS-0.1 MAG unit cost [$]	181.55	This work
PBS-0.1 MAG ancillary equipment unit cost [$]	2306.25	This work
Microcarrier cost [$ g^{-1}]	3.00	This work
Facility depreciation period [years]	15	Expert opinion
Equipment depreciation period [years]	5	Expert opinion
# Operators	4	This work
Daily worker rate ($)	$100	This work
Quality control cost/MCB	$100 000/batch	Expert opinion
Quality control cost/WCB	$10 000/batch	Expert opinion
Quality control cost/final product release	$10 000/batch	Expert opinion
Culture medium cost mL^{-1} [$]	0.34	This work
Harvesting reagent cost mL^{-1} [$]	0.21	This work
# Cells per dose	70 million cells	This work
Initial # P0 cells	5 million AT MSC; 500 000 UCM MSC	This work
Cells per vial (MCB, WCB, and final product)	500 000	This work
Batch failure per quality control step	10%	[27]

error of the mean (SEM) of the values obtained for the different MSC donors. A Mann–Whitney *U* test was used to evaluate the statistical significance of the differences regarding specific glucose consumption/lactate production rates and apparent yields among UCM and AT MSC.

3. Results

3.1. Expansion of Human UCM and AT MSC in a PBS-0.1 MAG Microcarrier-Based Culture System

Dynamic culture systems have been exploited in the context of MSC manufacturing to overcome the limitations associated with planar systems, including limited surface area-to-volume ratio, lack of ability to monitor and control culture parameters, cost-effectiveness, and handling.[3] An innovative approach, the VW bioreactor, was developed recently, consisting of a U-shaped vessel with a large vertical impeller.[15] In our study, the PBS-0.1 MAG system was assessed for the expansion of UCM and AT MSC, under S/XF culture conditions (**Figure 1**).

Both UCM and AT MSC were successfully expanded in the PBS-0.1 MAG system, using hPL-supplemented culture medium (Figure 1A,B). Although initial cell adhesion has been higher for AT MSC (81 ± 4%) than for UCM MSC (49 ± 4%), the latest achieved a higher maximum cell concentration ($5.3 \pm 0.4 \times 10^5$ cells mL^{-1}) compared to AT MSC ($3.6 \pm 0.7 \times 10^5$ cells mL^{-1}). These cell densities correspond to maximum fold increase values in the total cell number (i.e., normalized to the number of cells that successfully adhered to the microcarriers upon 24 h) of 21 ± 1 and 9 ± 1 for UCM and AT MSC, respectively. MSC obtained from both sources showed similar specific growth rates during the exponential growth phase (UCM: 0.50 ± 0.04 day^{-1}; AT: 0.49 ± 0.04 day^{-1}, data not shown). Cell viability was in a range of 94 ± 3% and 96 ± 2% for UCM and AT MSC, respectively, throughout the culture time (data not shown). The maximum cell density was achieved at day seven of culture for both MSC sources (Figure 1C).

Importantly, MSC isolated from both UCM and AT sources maintained the characteristic MSC immunophenotype after culture in the PBS-0.1 MAG system (Figure 1D,E). Both CD73 and CD90 biomarkers are expressed in ≥95% of the cells. However, CD105 expression is not only heterogeneous among sources (98.8 ± 0.4% in UCM MSC and 93.9 ± 3.5% in AT MSC), but also decreases after culture in the PBS-0.1 MAG system (84.0 ± 7.3% in UCM MSC and 74.3 ± 11.2% in AT MSC). Additionally, both UCM and AT MSC cultured in the PBS-0.1 MAG system retain their multilineage differentiation ability toward the osteogenic (Figure 1F), adipogenic (Figure 1G), and chondrogenic (Figure 1H) lineages.

Glucose and lactate concentrations were monitored throughout the culture period of cells obtained from both sources (Figure 1I,J). Of particular importance is the fact that glucose was nearly exhausted at day 2 (UCM MSC: 1.0 ± 0.6 mM; AT MSC: 0.6 ± 0.2 mM), but medium addition, with glucose supplementation, increased concentrations up to 10 mM. Conversely, lactate reached high concentrations (up to 26 mM) at the end of both cultures. In fact, similar glucose and lactate profiles were observed among the two MSC sources studied. No statistically significant differences were seen on average specific glucose consumption rates (UCM MSC: 56 ± 32 pmol $cell^{-1}$ day^{-1}; AT MSC: 54 ± 18 pmol $cell^{-1}$ day^{-1}) and average specific lactate production rates (UCM MSC: 115 ± 53 pmol $cell^{-1}$ day^{-1}; AT MSC: 105 ± 35 pmol $cell^{-1}$ day^{-1}), during the exponential growth phase (data not shown). Similarly, no statistically significant differences were observed among the apparent lactate to glucose yields (UCM MSC: 2.12 ± 0.43 mol lact. mol^{-1} gluc; AT MSC: 1.94 ± 0.01 mol lact. mol^{-1} gluc) (data not shown).

3.2. Economic Evaluation of the Process

The economic model developed by our group[22] was used to determine the economic feasibility of the process. A total of five

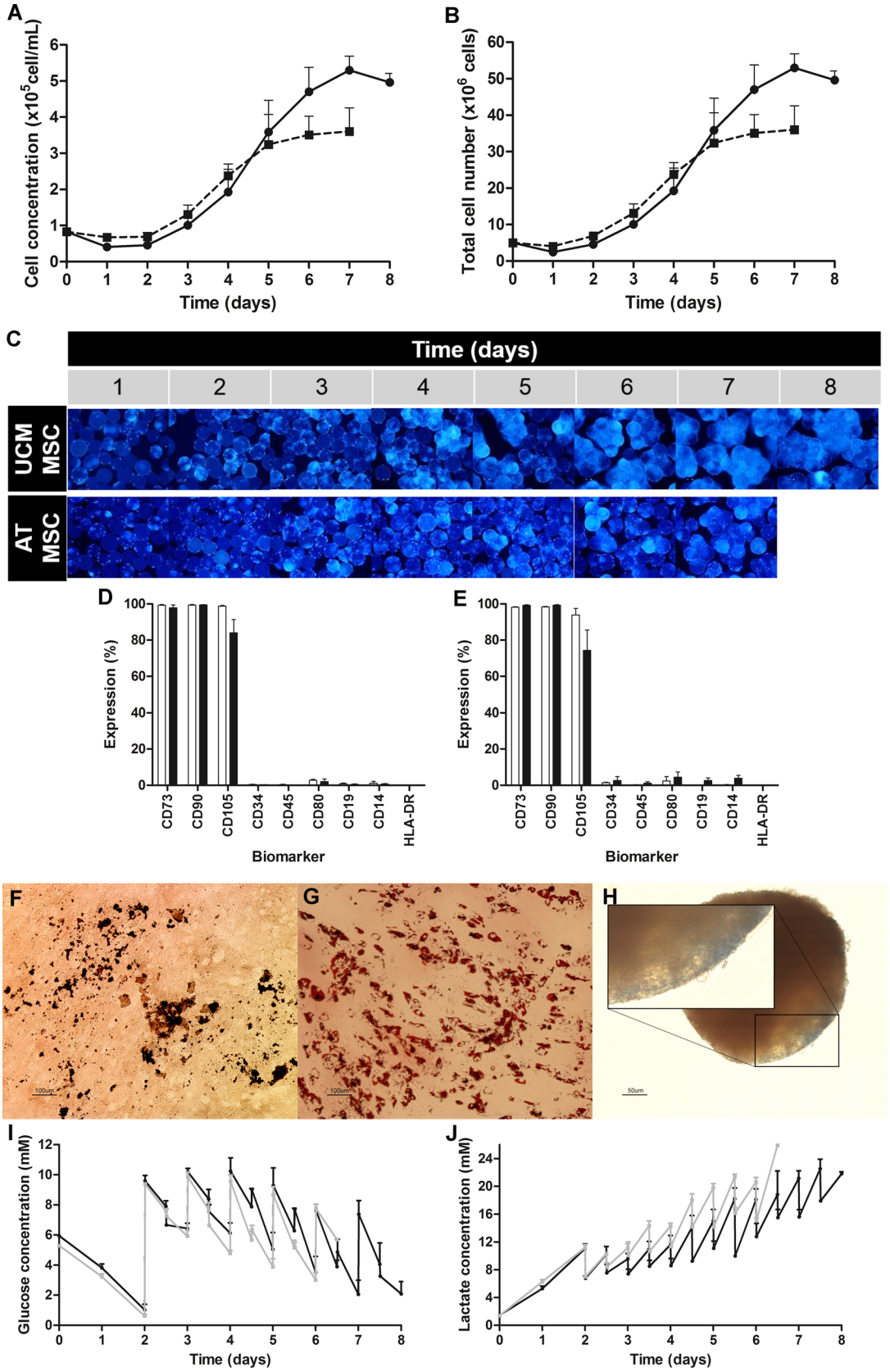

Figure 1 Continued.

passages (around 10–13 population doublings) were considered for the model to maximize cell numbers in the cell banks, without compromising cell quality (Table S1, Supporting Information).[28] The calculated number of cells expanded from a single donor after five passages (with intermediate MCB and WCB steps) was similar for UCM MSC (1.84 vs 1.97×10^9 cells) and considerably higher for AT MSC (3.49 vs 2.24×10^9 cells), when using PBS-0.1 MAG in comparison with the planar culture system (**Figure 2**A). The higher number of cells needed to seed the PBS-0.1MAG system for a full expansion capacity led to a higher number of cells per batch, where an increase from 245 to 460 million cells per batch for UCM MSC and from 172 to 388 million cells per batch for AT MSC was simulated, when introducing the PBS-0.1 MAG in the final expansion stage (Figure 2B). The number of total final product batches was reduced with the transition to PBS-0.1MAG from 13 to 9 (AT MSC) and from eight to four (UCM MSC). Finally, the number of total doses of 70 million cells produced increased from 26 to 45 (AT MSC) and from 23 to 25 (UCM MSC) (Table S1, Supporting Information).

The utilization of PBS-0.1 MAG also leads to a reduction in the total process costs of 11% and 30% for UCM MSC and AT MSC, respectively (Table S2, Supporting Information). Costs per dose decrease from \$17.0 K to \$11.1 K for UCM MSC and from \$21.5 K to \$11.1 K for AT MSC (**Figure 3**A). Moreover, the reduction of the quality control costs per dose (testing) and labor contribution are important factors leading to cost reduction (Figure 3B,C; Tables S2 and S3, Supporting Information). In absolute values, the higher contributors for cost reduction are labor and testing, in agreement with the increase in cell output provided by the VW system when compared with the use of T-175 flasks. As the seeding density of PBS-0.1MAG is considerably higher than for T-175, more WCB cells are seeded to initiate the expansion cycle. This leads to a faster consumption of the full WCB capacity, resulting in lower numbers of batches in the PBS-0.1MAG expansion (Table S1, Supporting Information). However, each batch of the PBS-0.1MAG has higher cell numbers (Figure 2B). The release testing costs are fixed per batch, regardless of the number of cells per batch. Thus, with less batches and, therefore, less instances of release testing required, the PBS-0.1 MAG expansion offers reduction in absolute the relative release testing contribution. The consumable costs of the total process are higher for the VW system, in agreement with the higher unit costs of PBS-0.1MAG, and the total consumable costs per dose of final product also show an absolute reduction, associated to the higher cell yield with the VW system (Table S2, Supporting Information). However, the relative contribution of consumable costs to the cost structure per dose is increased, as well as the quality control relative cost contribution (Figure 3D,E).

4. Discussion

MSC represent an attractive cell source in the context of cell-based therapies for a variety of disorders. However, large cell doses and eventually multiple doses are required to achieve meaningful clinical results. For instance, a meta-analysis of eight clinical trials testing MSC in promoting cardiac regeneration after an event of myocardial infarction showed an improvement of 1.47% in heart function after infusion of MSC,[29] highlighting the need of improving cell quality or increasing infused cell numbers. In this work, we successfully describe a cost-effective S/XF protocol for the expansion of UCM and AT MSC to clinically relevant cell concentrations, using a humanized cell culture supplement and the single-use, GMP-compliant, and scalable PBS-0.1 MAG system.

To establish the expansion platform, we first adapted the culture conditions previously reported by our group for the expansion of UCM MSC in spinner flasks, using plastic microcarriers combined with hPL-supplemented medium.[24] The same culture conditions were translated to the VW-PBS system, including the initial cell density, microcarrier type and concentration, coating solution, agitation protocol during cell adhesion and feeding regime. Later, culture parameters, including agitation and feeding regimes, as well as microcarrier concentration, were optimized and a new protocol was established. The optimized protocol led to a UCM concentration of $5.3 \pm 0.4 \times 10^5$ cell mL^{-1} (13.0-fold expansion), after 7 days of culture. Importantly, the process did not compromise UCM identity, since they maintained the typical MSC immunophenotype and multilineage differentiation potential.[2] To our best knowledge, the expansion results obtained are comparable, or even superior, to those described in the literature for MSC obtained from UCM.[24,30–32]

The platform herein described was also efficient in promoting the expansion of AT MSC up to $3.6 \pm 0.7 \times 10^5$ cell mL^{-1} (5.3-fold expansion), for a period of 7 days. AT is a valuable source of MSC and AT MSC have demonstrated their potential in different preclinical and clinical studies.[33,34] Similar or higher cell yields have been reported for AT MSC expansion.[35–37] AT MSC cultured in the PBS-0.1 MAG system also maintained their identity.

For cells of both tissue sources, the reduction in CD105 expression after the culture in the VW system may be explained due to longer times of exposure to the cell detachment reagent

Figure 1. Ex vivo expansion of UCM- and AT-derived MSC in a xeno-free dynamic culture system. Passage 4 UCM and AT MSC were seeded on plastic microcarriers pre-coated with 50% UltraGRO-PURE and cultured in 5% UltraGRO-PURE for 8 and 7 days, respectively, in a PBS-0.1 MAG system. A) UCM (continuous line) and AT (discontinuous line) MSC concentrations ($\times 10^5$ cell mL^{-1}) throughout the 8 (UCM MSC) or 7 (AT MSC) days of culture. B) Total UCM (continuous line) and AT (discontinuous line) MSC number ($\times 10^6$ cells) throughout the 8 (UCM MSC) or 7 (AT MSC) days of culture. Results are presented as mean + standard error of mean (SEM, $n = 3$). C) Cell nuclei were stained with DAPI for each day of the cultures and analyzed using a fluorescent microscope. D) Immunophenotypic characterization of UCM MSC before (white) and after (black) dynamic culture, analyzed by flow cytometry. E) Immunophenotypic characterization of AT MSC before (white) and after (black) dynamic culture, analyzed by flow cytometry. Results are presented as mean + SEM ($n = 2$). Representative images of multipotency characterization of UCM and AT MSC cultured in the PBS-0.1 MAG system through multilineage differentiation assays, upon 14 days under (F) osteogenic, (G) adipogenic, and (H) chondrogenic differentiating conditions. I) Glucose and (J) lactate concentration profiles of UCM (black) and AT (grey) MSC expansion in the PBS-0.1 MAG system throughout the culture period. Results are presented as mean + SEM ($n = 3$).

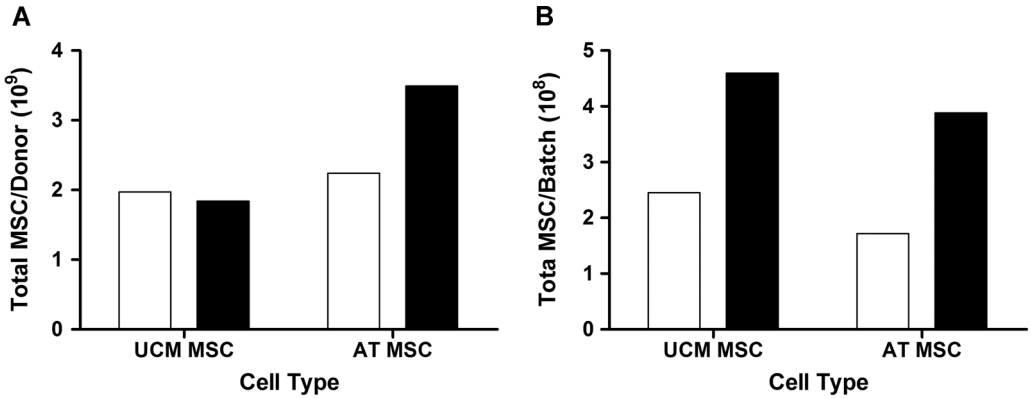

Figure 2. Total predicted number of UCM- and AT-derived MSC expanded in T-175 flasks versus PBS-0.1 MAG system, per donor and batch. A) Total predicted number of UCM- and AT-derived MSC expanded from a single donor using T-175 flasks (white) versus PBS-0.1 MAG (black) systems. B) Total predicted number UCM MSC and AT MSC expanded from a single batch using T-175 flasks (white) versus PBS-0.1 MAG (black) systems.

and higher agitation rates[38] required to dissociate large cell-carrier aggregates present at the end of the culture. Replating the harvested cells in tissue culture flasks can re-establish CD105 expression to values over 95%.[2,12]

Interestingly, MSC obtained from both sources showed similar specific growth rates during the exponential growth phase. Moreover, UCM and AT MSC demonstrated similar glucose consumption and lactate production profiles under the culture conditions studied and glucose/lactate did not reach exhausted/inhibitory levels (>35.4 mM of lactate[39]) throughout the process.

VW bioreactors have been previously used for the expansion of MSC, but isolated from a different source, the BM.[15] In that study, BM MSC were expanded in a 3 L VW bioreactor (PBS 3) combining Synthemax II microcarriers (Corning) and a XF formulation, MesenCult-XF medium (STEMCELL Technologies). This system allowed the expansion of BM MSC up to 3×10^5 cell mL^{-1} after 2 weeks of culture, with a higher percentage of proliferative cells and a lower percentage of apoptotic cells, compared to an STR system.[15]

A possible limitation regarding the scalability of the process is the feeding regime. At the industrial scale, the manufacturing of MSC should not rely on a protocol that requires medium change, since it may be labor- and cost-intensive and may raise some concerns in terms of GMP compliance. To avoid such manipulation of the culture system, a continuous, perfusion system should be applied. Moreover, at our lab, Dos Santos et al.[12] demonstrated an improved volumetric cell concentration when expanding BM MSC in a continuous perfusion bioreactor system.

Finally, the lack of system monitoring and control at the 100 mL scale also represents a limitation of the protocol. To achieve more consistent results and to be able to comply with GMP guidelines, process monitoring and control need to be included in the bioreactor system, which is present in VW bioreactors featuring working volumes higher or equal than 3 L.

The costs of goods per dose obtained within the scope of this study ($11 000–$21 000) are within the range of costs of goods obtained in other MSC bioprocess modeling studies.[20,40,41] Given that the final prices of commercially available ATMPs are in the range of $500–$850 000 per dose,[20,42,43] interventions aimed at reducing the costs of goods per dose are key to ensure sustainability of cell based products under reimbursement constraints.[42]

The higher cell seeding density requirements of microcarrier-based technologies is a consequence of the suboptimal MSC adhesion rates in microcarriers and impacts the process operation.[3] For AT MSC, the number of WCB vials (500 000 cells each) needed to seed the last expansion cycle increase from 27 to 62 with the process transfer from T-175 to PBS-0.1 MAG, while for UCM MSC the number of WCB vials used increases from 19 to 43. Only four or nine batches are sufficient to exhaust the WCB vials of UCM or AT MSC, respectively, using the PBS-0.1 MAG; while 8 or 13 batches are required for complete use of the WCB vials of UCM MSC or AT MSC (Tables S1 and S4, Supporting Information). Further technological improvements at the level of initial adhesion to microcarriers would offer shorter times to attain confluence.

In the current setup, the main cost driver is the quality controls. We assumed a fixed cost, irrespective of the number of MCB, WCB, and final batch vials. However, the scale of the banks simulated in this process is fairly small.[44] Therefore, it is estimated that in future studies concerning the scalability of expansion in bioreactors of higher volumes and in larger GMP facilities, the relative contribution of the quality controls to the cost structure will decrease.

The increase in total consumable costs in the microcarrier-based culture poses challenges in the scalability supply and cost of consumables.[17] Additionally, other challenges are related with the need to guarantee scalability of the proliferative benefits across higher volume platforms, and that quality attributes are maintained.[41,45,46] Finally, a current bottleneck of scalability is the volumes handled by current DSP systems,[19–21] for which DSP with microcarrier-based systems is generally less effective than for planar technologies.[18]

In conclusion, the establishment of such culture platform featuring easy scalability to higher volumes (i.e., PBS-3, PBS-80 and PBS-500, with maximum working volumes of 3 L, 80 L and 500 L, respectively) represents an important advance in obtaining safer, cost-effective, and clinically meaningful MSC numbers for clinical translation in a controlled and closed system.

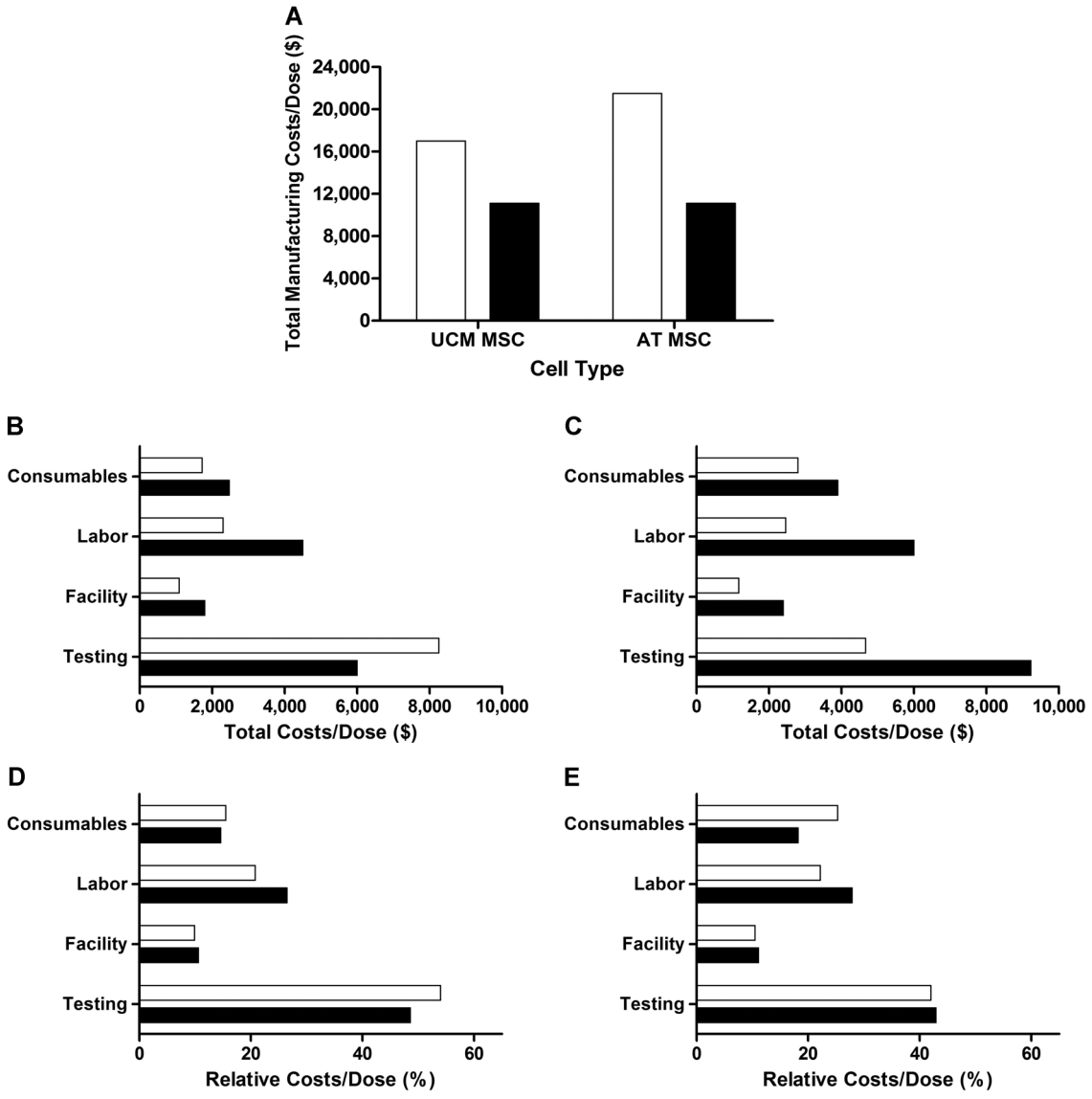

Figure 3. Total predicted manufacturing costs per dose and relative contribution of consumables, labor, depreciation and testing for the total cost-of-goods per dose and for the percentage of costs considering the expansion of AT- and UCM-derived MSC expanded using T-175 flasks versus PBS-0.1 MAG system. A) Total predicted manufacturing costs ($) per dose for UCM- and AT-derived MSC manufacturing using T-175 flasks (white) versus PBS-0.1 MAG system (black). Relative contribution of consumables, labor, depreciation, and testing for (B,C) the total cost-of-goods ($) per dose and for the (D,E) percentage (%) of costs for (B,D) UCM MSC, and (C,E) AT MSC manufacturing in T-175 flasks (white) versus PBS-0.1 MAG system (black).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Funding received by iBB—Institute for Bioengineering and Biosciences from FCT—Portuguese Funding for Science and Technology (UID/BIO/04565/2013) and from Programa Operacional Regional (POR) de Lisboa 2020 (Project N. 007317) is acknowledged. The authors also acknowledge the funding received from POR de Lisboa 2020 through the project PRECISE—Accelerating progress toward the new era of precision medicine (Project N. 16394) and to FCT through the project PTDC/QEQ-EPR/6623/2014. D.P., C.B., and M.A.F. acknowledge FCT for the Ph.D. fellowships PD/BD/52342/2013, PD/BD/105868/2014, and PD/BD/128328/2017, respectively. S.J., Y.H., and B.L. are employees of PBS Biotech Inc., Camarillo, CA, USA. R.-J.T., and W.M. are employees of AventaCell Biomedical Corp., GA, USA.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

economic model, ex vivo expansion, mesenchymal stromal cells, Vertical-Wheel Bioreactor

Received: December 6, 2018
Revised: March 12, 2019
Published online: May 31, 2019

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