PROCESS DEVELOPMENT STRATEGIES TO ENABLE LARGE SCALE EXPANSION OF MESENCHYMAL STEM CELLS FOR CELLULAR THERAPY

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ABSTRACT

The potential demand for clinical and commercial scale human mesenchymal stem cells (MSC) for cellular therapies requires a large-scale and well characterized culture system for MSC production. Currently, the majority of processes to produce MSCs rely on 2-dimensional, planar technologies that are expensive, labor intensive, and limited in scale potential. Strategies for process development have been used successfully in other industrial therapeutic markets such as the monoclonal antibody and vaccine industries to increase product yield. Many of these approaches such as Design of Experiment-based optimization (DOE) strategies can be used to develop cell culture media, reagents, and scale-up methods that may make cost effective and efficient manufacturing processes possible for cell therapy-relevant cells.

Here we report the results of DOE-based optimization studies to develop a microcarrier-based expansion system for human MSCs. Spinner flask studies demonstrated the ability of a xeno-free system to support expansion of MSC from bone marrow (BM MSC) and adipose tissue (ADSC) while maintaining the expected phenotype and differentiation potential. After 14 days of culture, BM MSC reached a maximum cell density of $2x10^5$ cells/ml (fold-increase of 18) while ADSC expanded to $1.4x10^5$ cells/ml (fold-increase of 14). Medium and process optimization strategies and the incorporation of fed-batch and perfusion approaches were used to increase the efficiency of the system. Human MSCs were expanded to a cell density of greater than $5x10^5$ cells/ml in DASGIP bench-top bioreactors. The cells maintained tri-lineage differentiation potential and retained the MSC immunophenotypic profile.

This work demonstrates the ability of a serum-free and xeno-free medium to support large-scale expansion of human MSC. This system can produce large numbers of high quality MSC, representing an efficient alternative to the traditional cell expansion protocol for clinical-scale manufacture of MSC.

INTRODUCTION

The large cell numbers required for MSC clinical applications (cell doses up to 5 million MSC/kg body weight) will require a fast and reproducible ex-vivo expansion protocol. However, the clinical-scale expansion of MSC has been traditionally performed under static conditions using t-flasks or cell factories, which are limited in terms of cell harvest efficiency and culture monitoring. Moreover, most of the studies focusing on scale-up systems for the expansion of human MSC have used culture media supplemented with fetal bovine serum which raises a concern among clinicians, since it may be a source of animal proteins, bacteria, viruses, or xenogeneic antibodies that might trigger an immune response upon MSC infusion. This can be a potential hurdle to obtain the approval from regulatory agencies. Recently developed clinical-grade medium formulations have been shown to support high MSC proliferation rates while maintaining immunophenotype and multipotency. These formulations along with a novel and robust scale-up system can enable a commercial scale process for cell therapy.

MATERIALS AND METHODS

Human BM MSC and ASC (2 donors each) were expanded in 80 ml spinner flasks (equipped with 90º paddles) using plastic microcarriers (Solohill Engineering) pre-coated with a CELLstart™ CTS ™ solution in StemPro® MSC SFM XenoFree medium. Agitation was set to 40 rpm and 25% of culture medium was renewed everyday (after day 3).

Throughout time in culture, cell expansion was determined daily (cells were detached from the microcarriers using TrypLE™ Express) and culture medium samples were stored for metabolic analyses. Immunophenotype analysis was also performed before and after the spinner flask expansion and the multilineage differentiative potential (osteogenesis, adipogenesis and chondrogenesis) of expanded cells was assessed. In order to determine if microcarrier culture had an effect of priming BM MSC and ASC differentiation to a particular lineage, RT-PCR analyses were performed for three early-differentiation genes: RGC32, FABP4 and SPP-1 that are early cell markers for osteogenesis, adipogenesis and chondrogenesis, respectively.

The scale-up of the microcarier-based culture system was performed using Bioflo® 110 (New Brunswick) or Cellferm-pro® (DASGIP) bioreactors at 20% of dissolved oxygen and a pH of 7.2.

RESULTS

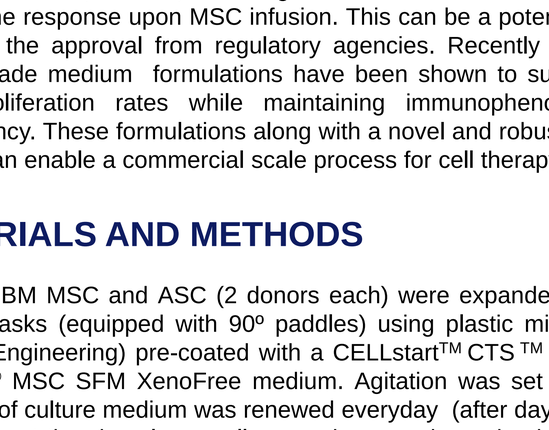

Figure 1. *Ex-vivo* expansion of BM MSC and ASC in a xeno-free microcarrier-based culture system in spinner flasks.

After 14 days of culture (employing 25% medium renewal every two days), BM MSC reached a cell density of $(2.0\pm0.2)x10^5$ cells/ml, which corresponded to a fold increase in total cell number of 18±1, while ASC expanded to a density of $(1.4\pm0.5)x10^5$ cells/ml (fold increase 14±7).

Table 1. Immunophenotype analysis of BM MSC and ASC before and after the xeno-free spinner flask culture.

BM MSC	Day 0	Day 14
CD 31	0.6±0.5	1.1±0.2
CD 73	98.4±0.8	99.1±0.1
CD 80	0.6±0.0	0.1±0.0
CD 90	97.1±2.0	91.7±2.0
CD 105	96.8±2.4	95.4±0.0
HLA-DR	0.9±0.7	0.6±0.2

ASC	Day 0	Day 14
CD 31	0.0	0.3±0.2
CD 73	98.9±0.2	98.1±0.6
CD 80	0.4±0.1	0.7±0.0
CD 90	97.3±1.4	82.4±0.0
CD 105	98.6±0.8	96.6±0.9
HLA-DR	0.0±0.0	0.0

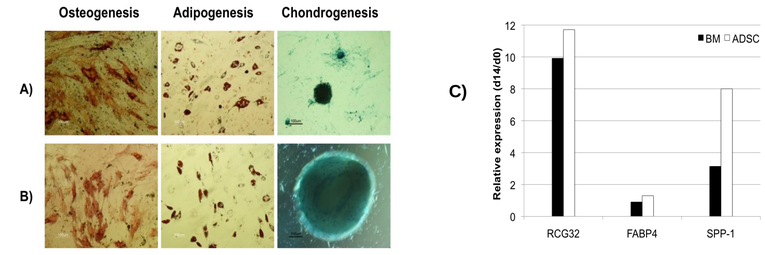

Figure 2. Multilineage differentiative potential of (A) BM MSC and (B) ASC after spinner flask expansion. Cell differentiation was induced for 14 days and was assessed by staining for osteogenesis (ALP and von Kossa), adipogenesis (Oil Red-O) and chondrogenesis (Alcian blue). (C) Relative expression of early differentiation markers of expanded cells.

RT-PCR analysis showed an up-regulation of early osteocyte (approximately 10- and 12-fold) and chondrocyte (approximately 3- and 8-fold) cell markers for both BM MSC and ASC, while no difference was observed for the expression of the early adipocyte cell marker. Even though RT-PCR analysis indicated a cell priming to osteogenic and chondrogenic lineages, expanded BM MSC and ASC were able to differentiate into osteocytes, adipocytes and chondrocytes. Moreover, MSC characteristic immunophenotype was maintained after the expansion in the spinner flasks.

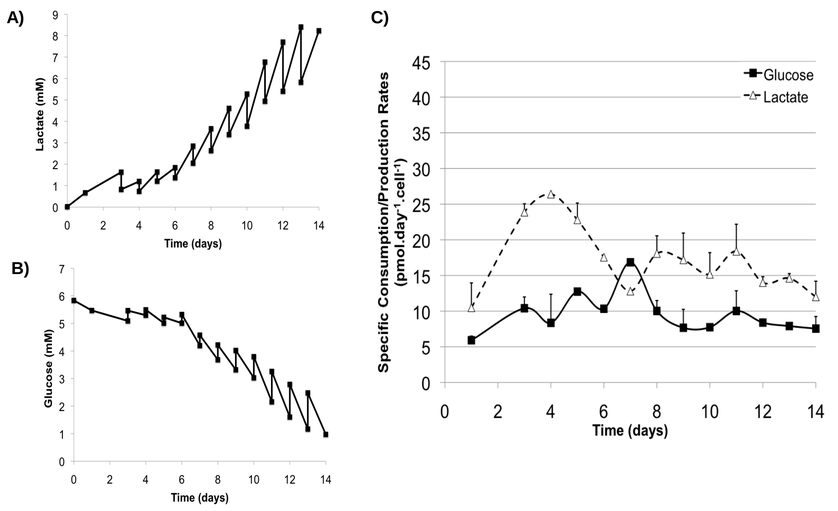

Figure 3. Metabolic analysis of BM MSC expansion in spinner flasks. A) Lactate and B) Glucose profiles are shown. C) BM MSC exhibited a constant specific consumption rate of glucose, with values between 5 and 15 pmol.cell-1.day-1 (with exception of day 7) and an average value of 10±1 pmol.cell-1.day-1. However, a higher specific production rate of lactate between days 3 and 5 (with values over 20 pmol.cell-1.day-1, average of 23±1 pmol.cell-1.day-1) was observed, followed by lower values for the remaining culture time (average of 15±1 pmol.cell-1.day-1)

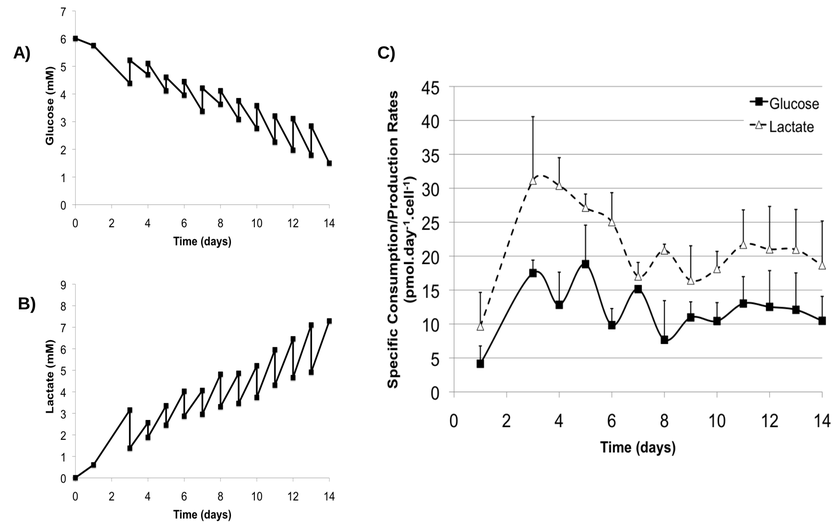

Figure 4. Metabolic analysis of ASC expansion in spinner flasks. A) Lactate and B) Glucose profiles are shown. C) ASC displayed a specific glucose consumption rate with small variations throughout the culture time (between 10 and 20 pmol.cell^{-1}.day^{-1}), with an average value of 12±2 pmol.cell^{-1}.day^{-1}. On the other hand, the lactate specific production rate exhibited a trend similar to BM MSC: between days 3 and 6 with a higher average value of 28±3 pmol.cell^{-1}.day^{-1}, followed by lower values after day 7 (average of 19±2 pmol.cell^{-1}.day^{-1}).

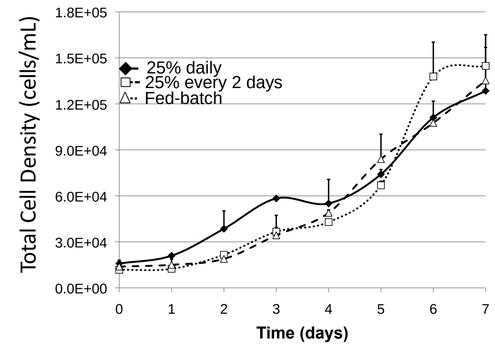

Figure 5. *Ex-vivo* expansion rates of BM MSC in fed-batch mode are comparable to the partial medium exchange regime: i) 25% daily medium renewal (black diamonds), ii) 25% medium renewal every two days (white triangles) and iii) addition of concentrated nutritional feed every two days (white squares). Results are presented as mean±SD of two independent samples.

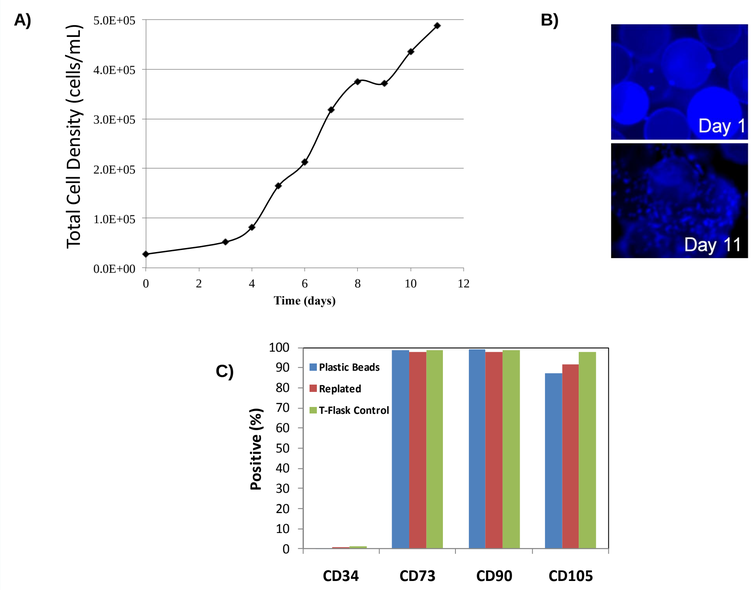

Figure 6. BM MSCs reach a cell density of $5x10^5$ cells/ml xeno-free perfusion bioreactor system. (A) Cell growth kinetics in the 11-day culture process. (B) Fluorescence microscopic images showing the increasing cell densities on microcarriers throughout the culture process. Media and process optimization resulted in an up to 2.5 fold improvement in cell yield when compared to the un-optimized serum-free process (as shown in Figure 1). (C) Cells harvested from microcarriers, re-plated cells, and control cells grown exclusively in T-flasks showed equivalent expression of CD90, CD73, and CD105 markers.

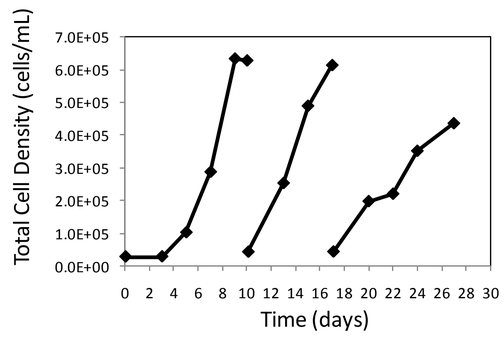

Figure 7. Bead-to-bead transfer enables multiple passage scale-up expansion under xeno-free and serum-free conditions. Cells were grown on microcarriers for one passage, then the confluent microcrocarriers were used to seed another vessel without the use of cell dissociation. Cells from occupied beads successfully seeded fresh empty beads in the subsequent passage. This process was demonstrated for 3 continuous passages. This methodology resulted in a theoretical fold expansion of greater than 3000 fold (assuming full scale-out of all produced cells).

CONCLUSIONS

These results demonstrate that the microcarrier-based culture system described here is suitable for the efficient expansion of MSC from different sources under xeno-free conditions. This serum-free and xeno-free microcarrier-based stirred system resulted in cell densities greater than $5x10^5$ cells/ml, while maintaining cell characteristics, immunophenotype and differentiation potential. The ease of scalability of microcarrier-based cultures combined with cell densities obtained in bioreactors demonstrates the potential for using this system to generate commercially-relevant cell numbers.

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