Cytotherapy, 2015; 17: 163–173

International Society for Cellular Therapy
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Increasing efficiency of human mesenchymal stromal cell culture by optimization of microcarrier concentration and design of medium feed

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Abstract

Background aims. Large amounts of human mesenchymal stromal cells (MSCs) are needed for clinical cellular therapy. In a previous publication, we described a microcarrier-based process for expansion of MSCs. The present study optimized this process by selecting suitable basal media, microcarrier concentration and feeding regime to achieve higher cell yields and more efficient medium utilization. *Methods.* MSCs were expanded in stirred cultures on Cytodex 3 microcarriers with media containing 10% fetal bovine serum. Process optimization was carried out in spinner flasks. A 2-L bioreactor with an automated feeding system was used to validate the optimized parameters explored in spinner flask cultures. *Results.* Minimum essential medium-α–based medium supported faster MSC growth on microcarriers than did Dulbecco's modified Eagle's medium (doubling time, 31.6 ± 1.4 vs 42 ± 1.7 h) and shortened the process time. At microcarrier concentration of 8 mg/mL, a high cell concentration of 1.08×10^6 cells/mL with confluent cell concentration of 4.7×10^4cells/cm^2 was achieved. Instead of 50% medium exchange every 2 days, we have designed a full medium feed that is based on glucose consumption rate. The optimal medium feed that consisted of 1.5 g/L glucose supported MSC growth to full confluency while achieving the low medium usage efficiency of 3.29 mL/10^6cells. Finally, a controlled bioreactor with the optimized parameters achieved maximal confluent cell concentration with 16-fold expansion and a further improved medium usage efficiency of 1.68 mL/10^6cells. *Conclusions.* We have optimized the microcarrier-based platform for expansion of MSCs that generated high cell yields in a more efficient and cost-effective manner. This study highlighted the critical parameters in the optimization of MSC production process.

Key Words: *bioreactor, fed-batch, metabolism, mesenchymal stromal cells, microcarriers*

Introduction

Human mesenchymal stromal cells (MSCs) are anchorage-dependent, fibroblast-like cells isolated from a variety of tissues and organs including bone marrow, skeletal muscle connective tissue, human trabecular bones, adipose tissue, periosteum, fetal blood and liver, umbilical cord tissue, amniotic fluid, placental tissues and blood [1,2]. MSCs can also be derived from human embryonic stem cells and induced pluripotent stem cells [3–5]. These cells are identified by their ability to express mesenchymal markers (CD29, CD44, CD73, CD90 and CD105) and not hematopoietic ones (eg CD31, CD34 and CD45), and their ability to differentiate down mesodermal lineages including adipocytes, osteoblasts, chondrocytes, myocytes and vascular tissue. The differentiation versatility, immune modularity properties and the limited or non-immunogenic properties of MSCs make them the leading cell type for research and clinical applications in the field of tissue regeneration [1]. There are more than 186 open US clinical trials targeting different diseases [2]. Some MSC treatments have already been approved for clinical applications (eg, Prochymal from Osiris Therapeutics is approved in Canada and New Zealand for treating acute graft-versus-host disease).

The number of MSCs required for treatment depends on the disease indication, ranging from 15 million cells in cartilage regeneration to 6000 million cells for treating osteogenesis imperfecta [2]. Current MSC production for clinical application under Good Manufacturing Practice conditions is carried out with the use of multiple planar plastic tissue culture plates, which is labor-intensive, lacks control and monitoring of environmental conditions (eg, pH and dissolved oxygen) and requires multiple subculturing steps [6–8]. This method can only support production of MSCs for low treatment doses or limited

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(Received 17 June 2014; accepted 24 August 2014)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2014.08.011

number of applications [8]. Because this planar expansion platform cannot supply the expected amounts of MSCs needed for high-dose treatment [8], the microcarrier cultivation systems, which offer several additional advantages including larger surface area per unit of medium volume, single-vessel operation with sampling options for process monitoring and control, automation to reduce manpower needs and smaller footprint have been suggested as a scalable alternative [2]. Moreover, these advantages of microcarrier cultures pave the way to generate robust and reproducible processes that allow better adaptation to Good Manufacturing Practice conditions. In this system, MSCs can be cultured on the surface of suspended small beads (microcarriers) in controlled large stirred bioreactors that enables economical production of the large doses of MSCs needed for clinical treatments [6,8,9].

Studies have indicated that various types of microcarriers can support the growth of MSCs on their surfaces, including polystyrene [10], glass [11], decellularized adipose tissue [12], gelatin [6,13–18] and dextran [9,19,20]. The most commonly used microcarriers in MSC cultures are Cultispher S (macroporous gelatin), Cytodex 1 (positively charged dextran beads) and Cytodex 3 (collagen-coated dextran beads). Depending on culture conditions as well as microcarrier and cell type, concentrations of $2-8.3 \times 10^5$ cells/mL and 4- to 20-fold expansion have been reported [2].

To date, MSC microcarrier cultures have been carried out mainly in small-scale spinner flasks with minimal process controls (eg, stirring speed and temperature) and low concentrations of microcarriers [21]. Excessive feeding regimes consisting of frequent partial medium refreshment are usually implemented (25–50% medium exchange been carried out every 1–3 days) (Supplementary Table I). These conditions are not suitable for large-scale production [2] because of the low cell yields, lack of monitoring and control of key environmental growth conditions (eg, pH and dissolved oxygen control) and high cost of serum-containing growth medium caused by excess feeding. Limited studies have been carried out to address these issues. Eibes *et al.* [13] propagated human bone marrow–derived MSCs on Cultispher S microcarriers in a stirred bioreactor, achieving 4.2×10^5 cells/mL (8.4 ± 0.8-fold expansion) through a feeding regime of 25% daily medium exchange [13]. The daily feeding regime aimed to address the glucose starvation and accumulation of ammonium observed in their previous study [13]. The same medium feeding regime was later implemented in microcarrier-based (plastic) expansion of MSCs in xeno-free medium and achieved $2.0 \pm 0.2 \times 10^5$cells/mL with $18 \pm$ 1-fold expansion [6]. To the best of our knowledge, there are no reports describing further development and optimization of medium feeding regime for volumetric cell yield enhancement.

Human fetal-derived MSCs (hfMSCs) have significant advantages in cell expansion when compared with other MSCs derived from umbilical cord, adult adipose and bone marrow tissues; they have shorter doubling times (32 h versus 54–111 h) and can be maintained for more than 70 population doublings before senescence [22,23]. Moreover, hfMSCs also performed better than other MSCs in bone regeneration *in vitro* and *in vivo* in a rat femoral critical-size bone defect model [22,24]. The expansion of hfMSCs on microcarriers was first reported by Goh *et al.* [9]. The authors investigated the expansion capacity of 4 commercially available microcarriers with different surface properties, scaling up of the microcarrier process in controlled bioreactors, development of a cell harvesting method and the osteogenic differentiation potential of cells harvested from microcarriers cultures. With the use of Cytodex 3 (4.17 mg/mL) in 1 L bioreactor under controlled conditions (pH at 7.2–7.3 and minimal 30% air saturation) with 50% medium exchange every 2 days, a cell concentration of $6.0 \pm 0.2 \times 10^5$ cells/mL and 12-fold expansion was obtained at day 8. The amount of medium used was estimated to be 4.55 mL/10^6 cells. In an osteogenic differentiation study, the authors showed that hfMSCs expanded on microcarriers generated 64.5% more calcium in scaffolds *in vitro* and 47.2% more ectopic bone volume *in vivo* in mice when compared with hfMSCs expanded in planar platform [9]. In this study, we focused on further improvement of volumetric cell productivity and medium utilization efficiency by selecting a better basal medium, increasing microcarrier concentrations and designing of an efficient medium feeding regime.

Methods

Cells and media

hfMSC line (batch No. S27) was kindly provided by Jerry Chan, MD, PhD from National University Hospital, Singapore. Fetal tissues were obtained from 13-week–old, clinically terminated pregnancies. hfMSCs were isolated from the fetal bone marrow by plastic adherence and characterized through immunophenotyping, colony-forming assays and tri-lineage differentiation into osteoblasts, adipocytes and chondrocytes as described previously [22,25].

hfMSCs were propagated in Dulbecco's modified Eagle's medium (DMEM, 4.5 g/L glucose, Life Technologies, Grand Island, NY, USA Cat. No. 11965084) or minimum essential medium-alpha (αMEM, 1 g/L glucose, Life Technologies Cat. No.