Expansion of adipose derived mesenchymal stem cells using shake flasks

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Abstract

The expansion of stem cells, including mesenchymal stem cells (MSCs), has been successfully demonstrated using microcarrier-based small bioreactors such as spinner flasks. In this study, we explored a simple alternative for microcarrier-based MSC expansion using conventional shake flasks. This method relies on a new type of shaker with built-in CO_2 gas control capability (incubator shaker). The expansion of adipose-derived mesenchymal stem cells (AdMSCs) was compared between shake flasks and spinner flasks using microcarriers. The AdMSCs were seeded at a density of 3x104 cells/cm^2 in either a baffled shake flask or a spinner flask, each containing 0.5 g plastic microcarriers (cross-linked polystyrene) and 40 ml of stem cell growth medium (serum and growth supplement-free). For the initial attachment of cells, the agitation speed of the incubator shaker and rotation speed of the spinner (housed inside of a CO_2 incubator) were set at 50 rpm and both systems were set to maintain CO_2 at 5%. After 2 hours of incubation, the cell culture volume was adjusted to 50 ml of total volume with 10 ml of medium containing serum (4% final concentration of FBS) and growth supplement (10 ng/ml final concentration of rh FGF basic, rh FGF acidic, rh EGF and 4.8 mM final concentration of L-Alanyl-L-Glutamine). Following the addition of FBS and growth supplements, the impeller speed of the spinner flask and rotation of the incubator shaker were set at 70 rpm. Cells attached to the microcarrier beads and entered into the stationary phase of growth within 3 days of culture. During early growth phase (on day 2), AdMSCs had 2.44-fold higher cell numbers under shake flask culture as compared to spinner flask culture. Another experiment was performed under both shake and spinner culture conditions where cells were inoculated at 10x lower density (3x103 cells/cm^2) in order to avoid overpopulation and to retain the differentiation properties of stem cells. This experiment was performed for 12 days and each day samples were collected for cell number and metabolite measurements. Cell number measurements revealed that AdMSCs grown under shake culture conditions had 1.8-fold and 1.6-fold higher cell numbers than spinner flasks during early log (on day 4) and stationary (on day 9) phases of growth. Extracellular metabolite measurements revealed that glucose concentrations decreased from 1.09 g/l to 0.548 g/l (for shake flasks) and 0.798 g/l (for spinner flasks), whereas lactate concentrations increased from 0.042 g/l to 0.396 g/l (for shake flasks) and 0.259 g/l (for spinner flasks) after 12 days of cells growth. Further, during early growth phase (day 4); the ammonium concentration in spinner flask culture was 1.8 fold higher as compared to shake flask culture. Finally, the growth of MSCs under shake flask conditions did not alter their stem cell properties, which was evident by their ability to differentiate into adipocytes and osteocytes.

Introduction

Stem cells are undifferentiated cells which have the capability of self-renewal and the potential to divide for a long period of time. They have the ability to differentiate into various specialized cells when appropriate growth factors and conditions are provided. Stem cells can be broadly classified as: embryonic, adult and induced pluripotent stem cells (iPS). Adult stem cells can be further characterized by their tissue of origin, such as: hematopoietic, mammary, intestinal, mesenchymal, endothelial, neural, and hair follicle stem cells. Recently, most of the studies performed on adult stem cells are either hematopoietic or adipose-derived mesenchymal stem cells[1]. Like any other adult stem cell, adipose-derived mesenchymal stem cells (AdMSCs) express all of the common stem cell markers and can be differentiated into various types of specialized cells under appropriate growth conditions. AdMSCs have advantages over other mesenchymal stem cells (MSCs), since they can be isolated in large quantities from fat tissue and are resistant to apoptosis[2].

Although MSCs have enormous advantages for regenerative medicine, drug screening and drug discovery, their applications are limited by the quantities required for industrial or clinical applications[3]. In this study, we developed a simple shake flask culture technique to expand MSCs on microcarrier beads; which can be used to scale-up into large scale bioreactors.

Methods

Cell culture

AdMSCs were obtained from ATCC® (PCS-500-011) at passage 2 and cells were seeded at a density of 5,000 cells/cm^2 to expanded the cells in a T-75 cm^2 flask.

Cultivation of cells on microcarriers

In order to attach the AdMSCs to microcarrier beads (Solohill®), the cells were initially seeded at a density of 3x10^4 and 3x10^3 cells/cm^2 in either a pre-siliconized baffled shake flask or a spinner flask, each containing 40 ml of basal mesenchymal stem cell medium. For the initial attachment of cells, the agitation speed of the Eppendorf New Brunswick™ S41i incubator shaker and rotation speed of the spinner flask (housed inside a Galaxy® 170 CO_2 incubator) was set at 50 rpm and incubated for 2 hrs at 37 ^{0}C with 5% CO_2. After 2 hrs, the cell culture volume was adjusted to 50 ml 50 ml total volume with 10 ml of medium containing serum (4% final concentration of FBS) and growth supplement (10 ng/ml final concentration of rhFGF basic, rh FGF acidic, rh EGF and 4.8 mM final concentration of L-alanyl-L-Glutamine). Following the addition of FBS and growth supplements, the impeller speed of the spinner flask and rotation speed of the S41i incubator shaker were set at 70 rpm. After 18 to 24 hours incubation, 1 ml of samples (microcarriers and media) was collected for microscopic observations and to determine the attachments of cells to microcarriers.

Cell counting

Cells on microcarrier beads were counted by hemocytometer.

Metabolite measurement

The supernatants collected during cell counting were used for metabolite measurement using the automated YSI® 2950 bio-analyzer.

Stem cells surface markers identification assay

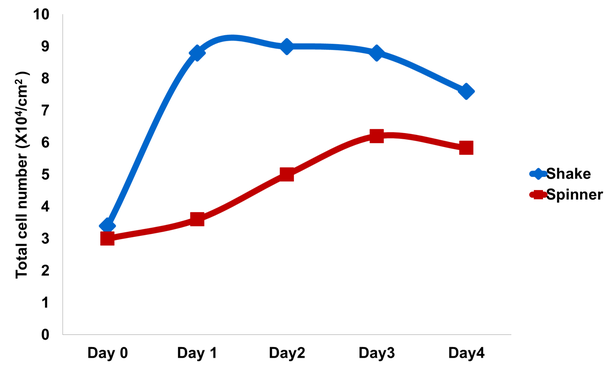
Identification of stem cell markers was done by immunostaining using antihuman CD44 and CD90 antibodies (BioLegend®) and visualized under EVOS® FL microscope.

Differentiation assays

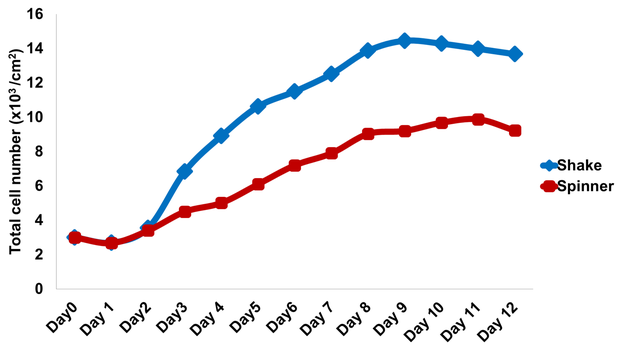
AdMSCs were harvested from both shake and spinner flasks into 50.0 ml tubes. Adipocytes and osteocytes differentiations were performed on microcarrier expanded cells using differentiation assay kits from ATCC®. Adipocyte and osteocyte differentiated cells were identified by staining with either Oil red O or Alizarin red S kits (ScienCell®) according to manufacturer instructions and visualized under OLYMPUS® CK40 microscope.

Results

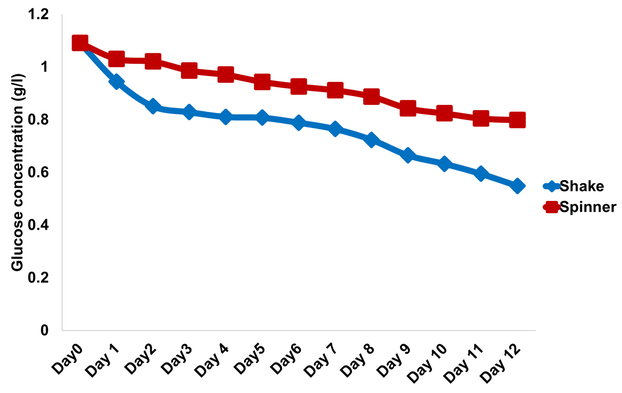
Growth curve of AdMSCs (High cell densities)



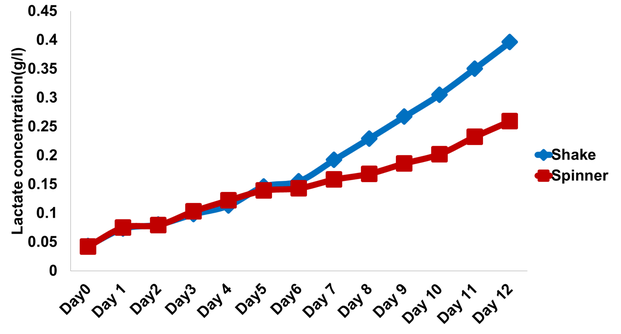
Growth curve of AdMSCs (Optimal cell densities)



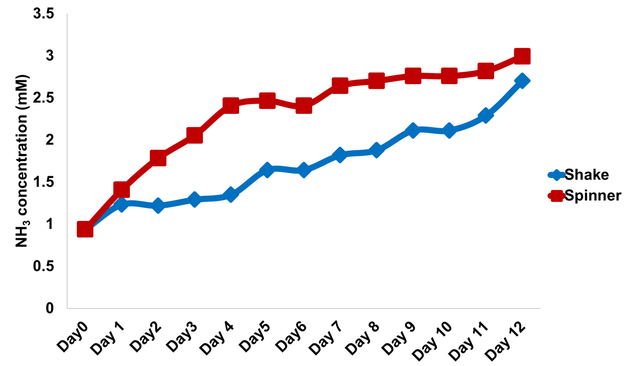
Concentration of glucose in AdMSCs cultures



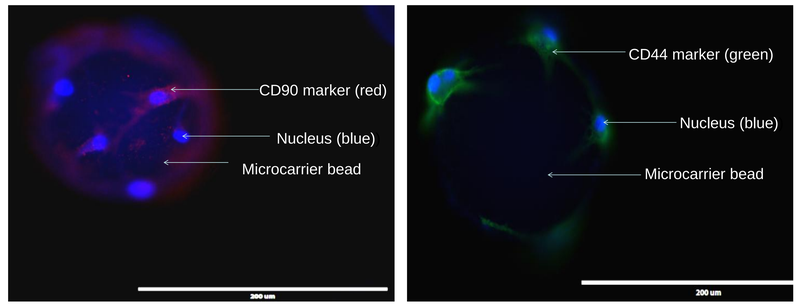
Concentration of lactate in AdMSCs cultures



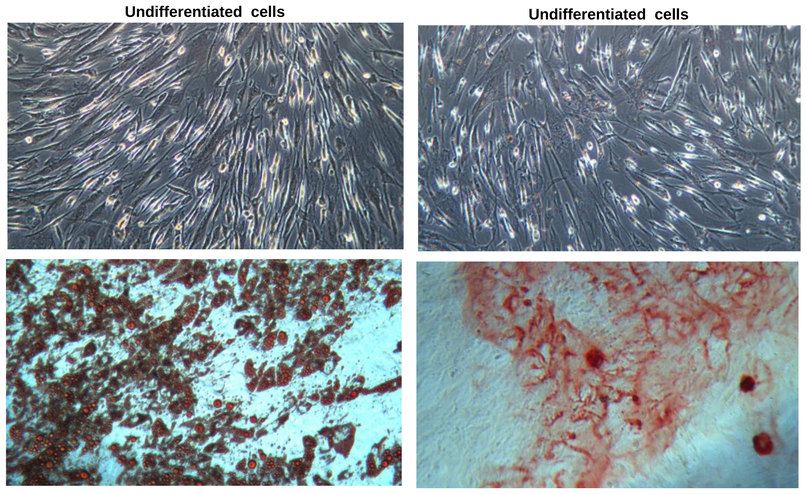
Concentration of ammonium in AdMSCs cultures



Immunostaining of AdMSCs expanded on microcarriers in shake flask culture



Multipotency assays for AdMSCs texpanded on microcarriers in shake flask culture



Conclusions

- Stem cell expansion using shake flask conditions appears to be a viable and simple alternative to the spinner flask system.
- Our newly developed shake flask culture conditions reduce shearing, elminate potential cell damage by the spinner rod, decrease the risk of contamination associated with inserting a magnetic stirrer base into the CO_2 incubator and as such, reduces experimental complexity.
- This method also greatly increases the cell culture capacity whereby multiple shake flasks can be placed in the incubator shaker simultaneously.
- In the case of spinner flask culture, a typical incubator without active cooling can only handle the heat emitted from a single magnetic stirrer base before causing temperature set-point overshoot, a significant limitation to the scale-up potential of the spinner method. This reinforces the superiority of using shake flasks in the incubator shaker for increased capacity.

REFERENCES: [1] Zhang et al. The Scientific World Journal (2012).Volume 2012; Article ID793823; [2] Nastran el al. Biotechnology progress (2013) Volume 29.No.1: 176- 185; [3] Schop et al. J. Tissue Eng Regen Med (2010); Volume 4. No. 4; 131-140.