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Expansion of human mesenchymal stem cells in fibrous bed bioreactor

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ABSTRACT

Expansion of human mesenchymal stem cells (hMSCs) in bioreactor while preserving their innate properties is important in translation of hMSC-based therapy to clinical applications. The present study investigates the feasibility of hMSC expansion in a 2.5 L CelliGen® 310 Bioreactor packed with Fibra-Cel® disks. After 9 days of expansion, a 9.2-fold increase in cell number with the population doubling (PD) time of 2.8 days (67.2 h) was achieved and that the specific glucose consumption and lactate production were measured to be 12.48 pmol/cell/day and 20.95 pmol/cell/day, respectively. hMSCs harvested from the bioreactor maintained their properties based on the analysis of phenotypic surface markers, colony forming unit-fibroblasts (CFU-F) number, and multilineage differentiation ability. The results demonstrate the feasibility and the potential of the fibrous bed bioreactor for large scale hMSC expansion.

1. Introduction

Human mesenchymal stem or stromal cells (hMSCs) isolated from various adult tissues emerge as primary candidate in cell therapy for a wide range of diseases. The translation of hMSC into clinical application depends on the delivery of therapeutically competent hMSCs in sufficient quantity. While most cell therapy products to date are based on autologous cells for immunologic compatibility [1,2], hMSCs have been used in allogenic transplantation as a potential "off-the-shelf" product [3–5]. In autologous transplantation, rapid expansion of patient specific cells in a fully enclosed and automated bioreactor system is important to ensure product isolation and to improve product yield with reduced cost. In the production of cells for allogenic transplantation, a scalable production process is crucial for the commercial scale cell production with required quality attributes.

Scalable production of adherent cells such as hMSCs for cell therapy requires large cell adhesion surface in a well-controlled culture environment to support efficient cell expansion while preserving their innate biology properties. Among the major adherent cell production platforms, planar technologies are most commonly used but are limited in lot size and productivity to 100–400 $\times 10^9$ cell range [6]. Suspension platform such as microcarrier culture developed for therapeutic protein production is scalable and has high surface area to volume ratio that greatly increases production efficiency. However, challenges remain in the adaption of suspension bioreactor for hMSC expansion. While the stirred-tank bioreactor is well-mixed, the hydrodynamic environment is non-homogeneous due to mechanical agitation, especially at high agitation rate. As a result, hMSCs adhered on microcarrier surfaces are continuously exposed to the hydrodynamic stress with non-even spatial distribution. As hMSCs are highly sensitive and responsive to the mechanical forces, the potential impact of the biomechanical stimuli on hMSC phenotype and therapeutic potency is yet to be determined. As stromal cells, hMSC have extensive potential to secrete endogenous macromolecules and are strongly influenced by their microenvironment. The microenvironment in the bioreactor system is expected to play an important role in hMSC expansion in large scale bioreactors.

To date, hMSC expansion in liter-scale vessels has been primarily conducted in microcarrier bioreactors [7,8] (Table 1). As an alternative, fibrous bed bioreactors (FBB) provide a uniform hydrodynamic environment and high surface to volume ratio in a protective and low-shear 3D environment. FBB have the capacity to fully control cell culture parameters such as pH and dO_2 and have been used in the expansion of embryonic stem cells as well as human amniotic fluid-derived mesenchymal stem cells [9,10]. Our previous studies have shown that 3D fibrous polyethylene terephthalate (PET) scaffolds support long-term hMSC expansion under various flow conditions [11]. Under low shear stress, hMSCs have the capacity to secrete an extensive extracellular matrix (ECM) network in the 3D fibrous scaffolds that better preserve their stemness and proliferation potential compared to planar culture [12]. The objective of the current study is to demonstrate the potential of an automated,

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Table 1
Lists of liter-scale bioreactor for mesenchymal stem cell expansion.

Cell type	Bioreactor volume (L)	Bioreactor type	Material	Fold-increase; Culture duration	Population doubling time (hours)	Reference
Human BM-MSC	3.0	Mobius cellready 3-L Bioreactor (EMD Millipore)	Cytodex 1 and 3, collagen-coated and Hillix microcarrier, Cultispher G and S	~40.0; 12 days	~54	[29]
Human MSC	3.0	Mobius cellready 3-L Bioreactor (EMD Millipore)	Collagen coated microcarrier	5.2; 5 days	~48	[30]
Human MSC	2.5	Biostat B Plus bioreactor	Non-porous plastic P-102L microcarriers (Solohill)	7.0; 12 days	76.8	[25]
Human ASC	2.0	UniVessel SU 2 L/ (Sartorius StedimBiotech)	ProNectinF-coated microcarriers (Solohill)	~35.0; 7 days	~25.6	[31]
Human BM-MSC/ ASC	1.0	Bioflo® 110 bioreactor (Eppendorf)	Non-porous plastic microcarriers (SoloHill)	~7.0; 7 days ~3.0; 7 days	~60/~105	[32]
Human BM-MSC	2.5	Bioflo®310 bioreactor (Eppendorf)	Fibra-Cel® disks (Eppendorf)	9.2; 9 days	67.2	Current study

Abbreviations: BM-MSCBone marrow-derived MSC; ASCAdipose tissue-derived MSC.

2.5 L CelliGen® 310 bioreactor using porous Fibra-Cel® scaffolds to support hMSC expansion and preservation of innate properties.

2. Material and method

2.1. hMSC culture

Standardized frozen hMSCs derived from human bone marrow were obtained from the Tulane Center for Gene Therapy and cultured following the method outlined in our prior publications [11,12]. hMSCs were expanded using α-MEM with 10% v/v FBS and 1% v/v penicillin/streptomycin (Life Technologies, Carlsbad, CA) (growth media) in a standard incubator (Thermo Scientific, Hudson, NH) with 37 °C and 5% CO_2. Cells at passage 6 were seeded for each bioreactor experiment and cells from multiple donors were used in the experiments. All reagents were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise noted.

2.2. hMSC expansion in bioreactor

A 2.5 L CelliGen® 310 bioreactor is used in the current study (Fig. 1). The 2.5 L glass vessel is jacketed by a water bath layer and capped with a stainless steel head plate. Ports on the head plate are designed for pH and dissolved oxygen (DO) probes, seeding and sampling tube, and gas transfer. The vessel was autoclaved with 25.0 grams of Fibra-Cel® disks (Eppendorf) fixed within the packed bed. After sterilization, growth media was delivered into the vessel with pump first, and then hMSCs obtained from plate culture were seeded into the vessel. In the seeding process, 100 rpm agitation speed was used for 1 h for even-distributed cell attachment. Temperature at 37 °C, pH at 7.4, and mixture gas (5% CO_2 and 95% air) were automatically controlled and monitored by the microprocessor control system. The agitation speed was adjusted to 50 rpm for cell expansion after seeding. 10% of fresh growth media was replaced daily and samples were collected before and after each media change. The bioreactor operation was repeated twice using identical operation procedure.

2.3. DNA assays and metabolic activity

Cell numbers in the CelliGen® 310 bioreactor system were determined by DNA assay following a method in a prior publication [11]. After 9 days of culture, the basket was removed from the control tower and multiple Fibra-Cel® disks were sampled from different positions in the basket. The disks with cells were washed with phosphate buffered saline (PBS) and lysed using the Tris-EDTATriton X-100 (TEX) solution with proteinase K at 50 °C overnight. Picogreen (Life Technologies), an ultra-sensitive fluorescent nucleic acid stain that quantifies DNA content in solution, was added to the triplicate samples and a series of DNA standards for 10 min and read using Fluoro Count (PerkinElmer, Boston, MA). In this assay, fluorescence intensity is proportional to DNA content and cell number with an average DNA content per cell of 9.3 pg/cell as determined from our previous study [11]. Media samples were collected daily through the sampling port and glucose and lactate concentrations were obtained by an YSI® 2500 Biochemistry Select Analyzer (Yellow Spring, OH). Three independent measurements were used for each data point.

To determine the relation between hMSC expansion and glucose consumption, hMSC seeded in Fibra-Cel disks were cultured in a 125 mL spinner flask bioreactor with daily sampling of media and disks for analyzing glucose concentration and cell number. The relation between cell number and glucose consumption is plotted in Fig. 2B (insert).

2.4. DAPI staining and scanning electron microscopy (SEM)

For DAPI staining, the Fibra-Cel® disks collected at day 9 from the CelliGen® 310 bioreactor culture were washed with PBS and stained with DAPI, using non-seed Fibra-Cel® disks as the control. For SEM, the harvested cells on the Fibra-Cel® disks were washed by PBS and fixed in 4% paraformaldehyde (PFA), dehydrated through a graded series of ethanol, incubated in hexamethyldisilazane, and vacuum dried overnight. The samples were mounted onto carbon-coated chucks, sputter-coated with gold in an argon atmosphere for 4 min at 2 kV, and analyzed on a SEM (JSM-7401F) (JEOL, Tokyo, Japan) [13].

2.5. Flow cytometry

Cells harvested from 9 days of CelliGen® 310 bioreactor system culture were washed by PBS and trypsinized from Fibra-Cel® disks. Cells from a parallel expansion from the tissue culture plates at the same population doubling were also prepared for comparison. Detached single cells were fixed in 4% paraformaldehyde (PFA) at room temperature for 30 min. Aliquots of 100 μL

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