Large-scale Production of Human Mesenchymal Stem Cells in BioBLU® 5c Single-use Vessels

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Abstract

Stem cell-based regenerative medicine has great potential to revolutionize human disease treatments. Among the various stem cell platforms, mesenchymal stem cells (MSCs) represent one of the highest potentials as evidenced by clinical trial activities. Currently, there are over 400 clinical trials based on MSCs registered at clinicaltrials.gov. Although successful expansion of MSCs in vitro has been well-established, the large clinical-scale production of MSCs remains a bottleneck, potentially limiting the immediate clinical applications. Stem cells and culture media were monitored, analyzed, and controlled, thus allowing us to produce AdMSCs in large clinical-scale quantities while maintaining healthy stem cell properties as evidenced by stem cell marker assays and differentiation assays performed at the end of the culture. Furthermore, with clinical relevance in mind, every cell culture step from T-flask to shake flask to bioreactor vessel was conducted strictly using single-use consumables.

Introduction

Stem cells are undifferentiated cells which have the capability of self-renewal and the potential to differentiate into a variety of cell types, thus performing a critical role in tissue repair and regeneration. Stem cells can be broadly classified as: embryonic, adult, and induced pluripotent stem cells (iPSCs). 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. According to recent market reports, MSCs are the most studied adult stem cells (1).

Like other adult stem cells, 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 a unique advantage over other MSCs, since they can be isolated in large quantities from fat tissue and are resistant to apoptosis.

Although MSCs have enormous potential for regenerative medicine, drug screening, and drug discovery, their applications are limited by the quantities required for industrial or clinical applications. Here in this study, we scaled up AdMSC culture from shake flasks, a method previously developed in our lab, into a BioBLU 5c (Eppendorf) single-use vessel. In the vessel, cell samples, and medium can be analyzed throughout the expansion and tightly controlled (e.g., oxygen, pH, temperature, glucose, glutamine, lactate, ammonia), thus allowing us to produce AdMSCs in large clinical-scale quantities.

Materials and Methods

Cell culture: Cultivation of cells on microcarriers

Expansion of AdMSCs (ATCC®; PCS-500-011™) on microcarrier in shake flask culture were performed as described in previous study (2).

pH mixing study

In order to determine the lowest speed of agitation required for sufficient mixing, a pH-based mixing study was performed at various speeds such as: 25, 35, and 55 rpm according to King, Kenty, Li, and Lee (3).

Cell counting

Cells were counted by NuclEaseCounter® NC-100® (Chemosep Technologies A/S) and Vi-CELL® XR (Beckman Coulter®).

Metabolic measurement

The supernatants collected during cell counting were used for metabolic measurement using CellTiter® Bio-Marker (Becton®).

Stem cell marker immunoblot and stem cell differentiation assays

Both were performed as described in previous study (2).

Cultivation of AdMSCs in BioBLU 5c single-use vessel

Collagen-coated microcarriers containing AdMSCs were harvested from shake flask cultures after 15 days of growth and seeded directly into the BioBLU 5c single-use vessel containing 3.5 L AdMSCs complete medium with collagen coated microcarriers at a concentration of 7.5 g/L. The initial agitation speed was set to 25 rpm. The temperature was set at 37 °C. The pH of the bioreactor was maintained at 7.0 by the controller using automatic addition of CO₂ gas and 7.5% sodium bicarbonate (NaHCO₃) solution. After 1 h of incubation, the cell culture volume was adjusted to total 3.75 L with 0.25 L of serum-containing medium to reach a final FBS concentration of 4% and the targeted level of final concentration of growth supplements (10 mg/mL final concentration of rh FGF basic, rh FGF acidic and rh EGF, and 2.4 mg/mL final concentration of L-ascorbate-2-L-glutathione). The agitation speed was increased to 35 rpm after 6 days of cell culture. In addition, the overlay was adjusted to total 3.75/uni00A0L with 0.25/uni00A0L of serum-containing medium to reach a final FBS concentration of 4/uni00A0% and the targeted level of

Isolation of cDNA and PCR amplification of stem cells markers

A 50/uni00A0% medium exchange was performed at days 4, 8, and 12 with AdMSC complete medium.

15596-018). cDNA was synthesized using the High-capacity cDNA Reverse Transcription Kit (Life Technologies, 4374966) in a PCR based gene expression analysis of stem cell multipotency markers

In this study, we have also shown that AdMSCs cultured in BioBLU 5c single-use vessels retained their differentiation and multipotency properties as evident by immunostaining, PCR, and differentiation assays.

PCR based gene expression analysis of stem cell multipotency markers

For gel A & B: M: DNA ladder; Lane 1: Positive control synthetic DNA sequence for Oct3/4 or Sox2; Lane 2: metabolite control Lane 3: Sample at 0.2 ml culture in shake flask; Lane 4: 2 ml culture in shake flask; Lane 5: Sample at 0.2 ml culture in BioBLU 5c. For gel C: Oct3/4; Lane 1: Positive control synthetic DNA sequence for Oct3/4 or Sox2; Lane 2: Sample at 0.2 ml culture in shake flask; Lane 3: Sample at 0.2 ml culture in BioBLU 5c. For gel D: Sox2; Lane 3: Sample at 0.2 ml culture in BioBLU 5c.

Figure 3: For gel A & B: M: DNA ladder; Lane 1: Positive control synthetic DNA sequence for Oct3/4 or Sox2; Lane 2: metabolite control Lane 3: Sample at 0.2 ml culture in shake flask; Lane 4: 2 ml culture in shake flask; Lane 5: Sample at 0.2 ml culture in BioBLU 5c. For gel C: Oct3/4; Lane 1: Positive control synthetic DNA sequence for Oct3/4 or Sox2; Lane 2: Sample at 0.2 ml culture in shake flask; Lane 3: Sample at 0.2 ml culture in BioBLU 5c. For gel D: Sox2; Lane 3: Sample at 0.2 ml culture in BioBLU 5c.

Figure 2:

Cell density

Cell density was measured by CellTiter® Bio-Marker (Becton®).

Differentiation assays for AdMSCs expanded in BioBLU 5c vessel

Calcium mineralization was induced by Alizarin Red S positive staining. Lipid droplets were induced by Oil Red O positive staining.

Conclusion

Our study clearly demonstrated the feasibility of using BioBLU 5c single-use vessels for the production of clinical dose-scale numbers of MSCs. The BioBLU 5c single-use vessel has a maximum working volume of 3.75 L, capable of producing clinical dose-scale numbers of MSCs in a single run.

In this study, we have also shown that AdMSCs cultured in BioBLU 5c single-use vessels retained their differentiation and multipotency properties as evident by immunostaining, PCR, and differentiation assays. AdMSCs cultured in BioBLU 5c is equipped with a pitched blade impeller which makes cells to be cultured under low rpm conditions to avoid shear force damages. In addition, BioBLU vessels are produced from USP Class VI and animal-free component-free materials, and do not contain any toxic leachable/extractables, such as bis-2,4-di-tert-butylyphenylphosphate (bDtBPP).

The above studies validated the general applicability of the New Brunswick™ CelliGen® BLU benchtop bioreactor and BioBLU Single-use vessels for large-scale process optimization and production of stem cells in numbers appropriate for their clinical applications in regenerative medicine.

REFERENCES


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