APPLICATION NOTE No. 334 I May 2014

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. Although successful expansion of MSCs *in vitro* has been well established, the large-scale production of MSCs remains a bottleneck. In this study, we demonstrate a successful large-scale bioprocess application of adipose-derived mesenchymal stem cells (AdMSCs) in an industrial single-use vessel at 3.75 liter (L) scale (working volume). The vessel offers a precision controlled environment for the ideal growth of stem cells under simulated physiological conditions. Stem cells and culture media were monitored, analyzed, and controlled, thus allowing us to produce AdMSCs in large-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, 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, mesenchymal stem cells (MSCs) are the most studied stem cells [1 – 3].

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 [2, 4 – 8].

Although MSCs have enormous potential for regenerative medicine, drug screening, and drug discovery, their applications are limited by the quantities required for industrial applications [8]. Here in this study, we scaled-up AdMSC culture from shake flasks, a method previously developed in our lab [9], into a BioBLU 5c (Eppendorf) single-use vessel. In the vessel, cell samples, and medium can be analyzed throughout the expansion process and the growth process can be tightly controlled (e.g., oxygen, pH, temperature, glucose, glutamine, lactate, ammonia, etc.), thus allowing us to produce AdMSCs in large-scale quantities.

Materials and Methods

Initial cell culture in T-flasks
AdMSCs were obtained from American Type Culture Collection (ATCC®, PCS-500-011™) at passage 2 and cells were seeded at a density of 5,000 cells/cm² into a T-75 cm² flask (USA Scientific®, CC7682-4175) using 15 mL of mesenchymal stem cell basal medium (ATCC, PCS-500-030™). The medium was supplemented with components of the Mesenchymal Stem Cell Growth Kit (ATCC, PCS-500-040™) at the following concentrations: 2 % fetal bovine serum (FBS), 5 ng/mL rh FGF basic, 5 ng/mL rh FGF acidic, 5 ng/mL rh EGF, and 2.4 mM L-alanyl-L-glutamine.

Preparation of microcarrier
Prior to the start of the experiment, polystyrene (SoloHill® Engineering, P-221-040) and collagen coated microcarriers (SoloHill Engineering, C102-1521) were prepared according to the manufacturer's instructions, including sterilization.

Cultivation of cells on microcarriers in shake flasks
Cultivation of AdMSCs on microcarriers in shake flask culture was performed as described previously [9].

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 Xing, Kenty, Li, and Lee [10]. Briefly, a pH sensor was calibrated using different standard buffer solutions and placed inside a bioreactor containing PBS buffer. 4 N NaOH at 0.5 % vessel working volume (3.75 L) was added to the bioreactor which created a pH disturbance. The pH value was continuously recorded until reaching a steady state. After each run, the pH value of the bioreactor was brought back to initial pH using 4 N HCl. The homogeneity (H) of pH mixing was calculated and plotted against elapsed time using the following equation:

$$H(t) = \frac{pH(t) - pHi}{pHf - pHi} \times 100$$

> H(t) = homogeneity at time t
> pH(t) = pH value at time t
> pHf = final pH value under the complete homogenized condition
> pHi = initial pH value upon trace (NaOH) addition

Optimization and cultivation of AdMSCs in BioBLU 5c single-use vessels
Two independent large-scale experiments were performed in BioBLU 5c single-use vessels using two different microcarriers: the first experiment was performed with polystyrene and the second experiment with collagen coated microcarriers. The New Brunswick™ CelliGen® BLU benchtop bioreactor used for each independent experiment was configured with low-flow thermal mass flow controllers (TMFCs) providing a gas flow range of 0.002 – 1.0 Standard Liters per Minute (SLPM) and an included overlay with a control range of 0.01 – 5.0 SLPM.

For the first experiment, polystyrene 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 1.5 L of AdMSC complete medium with microcarriers at a concentration of 15 g/L. Following the day of inoculation, another 1.5 L of AdMSC complete growth medium was added to the vessel with microcarriers at a concentration of 45 g/L, to reach the final concentration of microcarriers (30 g/L). The agitation speed was set at 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 CO_2 gas and 7.5 % sodium bicarbonate ($NaHCO_3$) solution. During the experiment, the dissolved oxygen (DO) level was set to 10 % and the controller was set to 4-gas mode to automatically maintain the DO setpoint by delivering 4 gases (air, CO_2, N_2, and O_2) through the overlay (vessel head space). The overlay gas flow was maintained at 0.1 SLPM during the first 10 days of the experiment. After 10 days, the overlay gas flow was increased to 0.3 SLPM. A 25 % medium exchange was performed at day 5 and an additional 0.75 L AdMSC complete medium was added at day 11 to reach the maximum working volume of the vessel (3.75 L). Furthermore, a 50 % medium exchange was performed at day 14.

For the second experiment, 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 (Figure 1) containing 3.5 L AdMSC complete medium with collagen coated microcarriers at a concentration of 17 g/L. The initial agitation speed was again set to 25 rpm. 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 ng/mL final concentration of rh FGF basic, rh FGF acidic, and rh EGF, and 2.4 mM final concentration of L-alanyl-L-glutamine). Most of the bioreactor control parameters were the same as the first bioreactor run, except that the agitation speed was increased to 35 rpm after 6 days of cell culture. In addition, the overlay gas flow was increased to 0.3 SLPM and N_2 gas was introduced at 0.01 SLPM through the macrosparger to maintain the DO level at 15 %. A 50 % medium exchange was performed at days 4, 8, and 12 with AdMSC complete medium containing 0.1 % Pluronic®-F68 surfactant (Thermo Fisher Scientific®, 24040-032) and 0.5 g/L of glucose was added to the vessel at day 15 to sustain cell growth without additional media exchange.

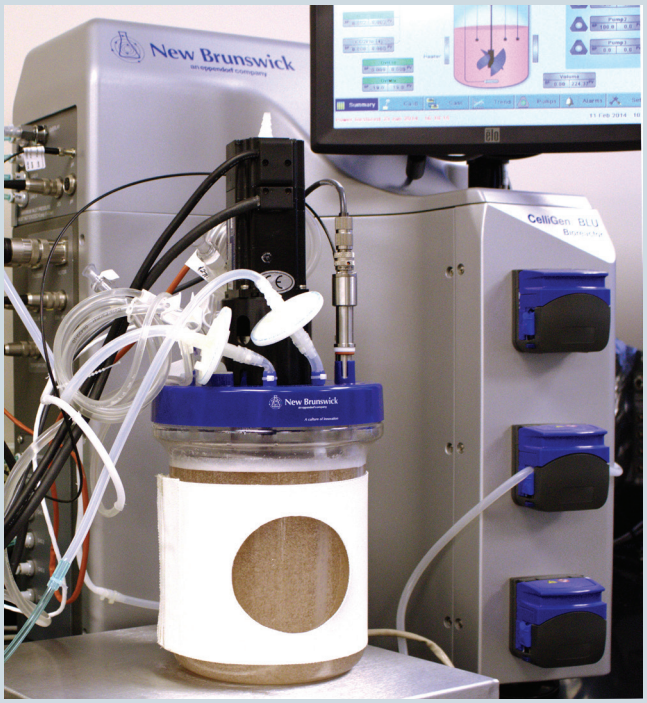

Figure 1: New Brunswick CelliGen BLU benchtop bioreactor combines single-use technology with the trusted performance and true scalability of a traditional stirred-tank design. CelliGen BLU has been engineered for high-density animal cell culture in research or production, using interchangeable, single-use, stirred-tank BioBLU 5c, 14c, and 50c vessels. A compact controller enables advanced process management for research or cGMP manufacturing.

Cell counting and metabolite measurement

Cells on microcarrier beads were counted by NucleoCounter® NC-100™ (ChemoMetec® A/S) according to the manufacturer's protocol. The supernatants collected during cell counting were used for metabolite measurement using the automated Cedex® Bio Analyzer (Roche®). In addition to the NucleoCounter, a Vi-CELL® XR (Beckman Coulter®) was also used to count the cells that were collected from T-75 cm^2 flasks. Vi-CELL was not used for counting cells from the microcarrier culture due to the risk of jamming the Vi-CELL's needle sipper with microcarriers.

Stem cell surface marker assays

To assess the quality of AdMSCs after expansion and to confirm that the stem cell markers were retained during cultivation in the vessel, CD44, CD90, and CD105-specific fluorescent immunoassays were performed using the following procedure: cells on the microcarrier beads were fixed with 4 % paraformaldehyde for 30 min, followed by Dulbecco's PBS (DPBS), Ca^{2+} and Mg^{2+} free (ATCC, 30-2200™) wash 3 times, and blocked with 5 % FBS at room temperature for 1 h. Immunostaining was performed using BioLegend® FITC-conjugated anti-human CD44 antibody solution containing the nuclear stain 4', 6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific, P36935) for 1 h at room temperature. For immunostaining of the CD90 and CD105 markers, cells were fixed and blocked using the same protocol as described above. The cells were incubated with mouse anti-human CD90 and CD105 antibodies (Abcam®, ab23894 and ab44967) for 1 h and washed 5 times with room temperature DPBS for 5 min each. The cells were further incubated with Alexa-Fluor® 546 and Alexa-Fluor 594 anti-mouse secondary antibodies (Thermo Fisher Scientific, A21123 and A21125) and DAPI solutions at room temperature for 1 h. The cells were washed 5 times with room temperature DPBS for 5 min each and visualized under an EVOS® FL LED-based fluorescence microscope (Thermo Fisher Scientific).

Isolation of cDNA and polymerase chain reaction (PCR) amplification of stem cell markers

Total RNA was isolated from the AdMSCs grown on the microcarrier beads and T-75 cm^2 flasks using TRIzol® reagent (Thermo Fisher Scientific, 15596-018). cDNA was synthesized using the High-capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, 4374966) in a Mastercycler® pro thermocycler (Eppendorf). The primer sequences and PCR conditions used for the CD45, CD105, and beta actin genes were described previously [11]. The Oct3/4 and Sox2 genes were amplified using primer pair kits from R&D Systems® (RDP-321 and RDP-323). The Human CD44 gene was amplified using forward 5´ AGAAGAAAGCCAGTGCGTCT 3´ and reverse 5´ GGGAGGTGTTGGATGTGAGG 3´ primers, which were designed using the BLAST program with Entrez Gene: 960 human as a template. The following program was used for amplification: Step 1: 94 °C for 4 min; 35 cycles of Step 2: 94 °C for 45 sec, 60 °C for 45 sec, 72 °C for 45 sec; Step 3: 72 °C for 10 min and Step 4: 4 °C hold. All the primers were validated by aligning with respective gene sequences using the BLAST program.

Stem cell differentiation assays

AdMSCs were harvested from the bioreactor into 50 mL conical tubes (USA Scientific, 1500-1200). Once the microcarrier beads settled to the bottom of the tube, the supernatants were removed and cells were washed with DPBS. Afterwards, the microcarrier beads were treated with 5 mL of prewarmed trypsin-EDTA solution (ATCC, PCS-999-003™) at 37 °C for 10 min. During incubation, the tubes were occasionally vortexed for 2 s and then neutralized by adding an equal volume of trypsin neutralizing solution (ATCC, PCS-999-004™). Microcarrier beads were allowed to settle to the bottom of the

tube and the supernatants were collected as soon as possible. Microcarrier beads were washed 2 – 3 times with DPBS and the supernatant was combined into a 50 mL tube. Following washing, AdMSCs were collected from the bottom of the tube by centrifugation at 120 x *g* for 5 min and resuspended into 5 mL of mesenchymal stem cell medium. Cells were seeded at a density of 18,000 cells/cm^2 into 6-well plates (USA Scientific, CC7682-7506). Differentiations were induced with Adipocyte (ATCC, PCS-500-050™) and Osteocyte (ATCC, PCS-500-052™) Differentiation Toolkits. Following manufacturer's instructions, differentiated adipocytes were identified by Oil Red O staining (ScienCell™, 0843) and osteocytes were identified with Alizarin Red S staining (ScienCell, 0223). Both were visualized using an Olympus® CK40 inverted microscope equipped with an Infinity2 CCD camera (Lumenera®).

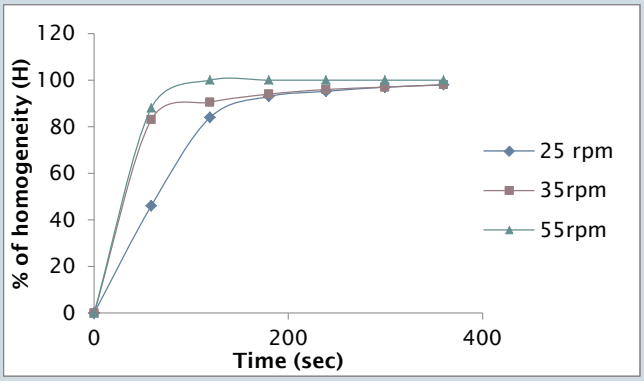

Figure 2: Homogeneity curves during the pH-based mixing study at various rpm in a BioBLU 5c single-use vessel

Results and Discussion

From the mixing study, it was found that 100 % homogeneity was achieved by 120 s with agitation at 55 rpm, whereas 90.4 % and 84 % homogeneity were achieved by 35 and 25 rpm agitation, respectively (Figure 2). MSCs are very sensitive to shear force damage; gentle agitation at lower rpm is preferred whenever possible. Since a significant amount (≥ 84 %) of homogeneity was achieved in the BioBLU 5c at 25 or 35 rpm within 2 min, the bioreactor agitation speed was maintained between 25 and 35 rpm during the entire experiment.

AdMSCs were initially expanded under shake flask culture conditions using single-use polycarbonate flasks. Microcarriers containing AdMSCs were collected from these flasks and used to inoculate the BioBLU 5c single-use vessel with an initial cell density of 5,000 cells/mL. For the first experiment, 30 g/L of microcarrier was used in order to explore the maximum microcarrier concentration for AdMSCs cultured under a controlled environment. Although AdMSCs quickly expanded in the bioreactor within 24 h of inoculation, there was a 4 day lag phase in cell growth following the addition of high concentration of microcarriers. This might be due to collisions between microcarriers and shear forces resulting from the ultra-high density microcarrier use. The initial culture also showed that the DO level could not be maintained at the 10 % setpoint. Thus, the overlay gas flow was increased to 0.3 SLPM after 10 days of cell growth. However, the 0.3 SLPM overlay gas flow was still not enough to bring the DO down to the 10 % setpoint. Direct gas sparging was not used in this experiment, but was subsequently used in later experiments. The actual DO fluctuated around 20 % throughout the bioreactor run. After the 50 % medium exchange on day 14, cell growth increased and reached its maximum density of 3.9 x 10^4 cells/mL by day 18. The final density was ~7-fold higher than the initial cell density (Figure 3).

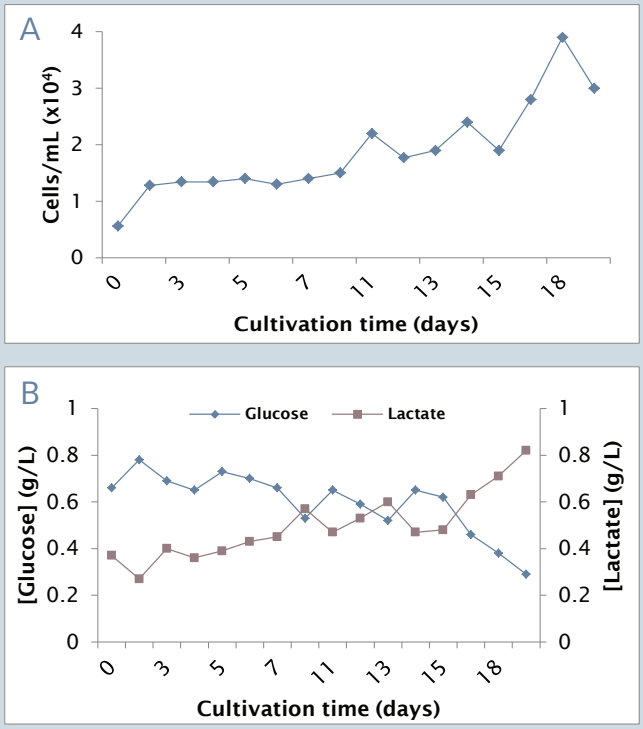

Figure 3: Growth profile of AdMSCs in BioBLU 5c single-use vessel with polystyrene microcarrier beads
A: Cell density in single-use vessel
B: Glucose and Lactate concentrations over time

Since the maximum expected AdMSC density was not achieved from the first bioreactor experiment using polystyrene microcarriers, a second experiment was performed using collagen coated microcarriers. Recent studies have shown that collagen coated microcarriers may support higher MSC density in single-use vessels [12, 13]. In the second experiment, microcarriers containing AdMSCs were collected from shake flasks and inoculated into the bioreactor for a final density of 17,500 cells/mL. Medium exchanges were performed every 4 days during the experiment. The DO was set to a more controllable 15 % and maintained using N_2 addition through the overlay. Beginning on day 6, N_2 gas was also introduced through the sparger at 0.01 SLPM. Since 100 % DO was calibrated using 100 % air, 15 % DO setpoint represents only ~3 % O_2 in the medium, still within the targeted hypoxic physiological conditions (2 – 5 % O_2). Furthermore, the agitation speed of the bioreactor was increased to 35 rpm to support the complete suspension of AdMSCs containing microcarriers in the BioBLU vessel. Pluronic-F68 surfactant (0.1 %) was also introduced into the medium to reduce foaming resulting from N_2 sparging. Pluronic-F68 is also known to protect cell membranes and reduce the shear force during cell culture agitation [14]. Cell growth steadily increased in the bioreactor from day 6 which was accompanied by an increase in glucose consumption and lactic acid production. Although cells were still metabolically active at day 15 as seen from continued glucose consumption and lactic acid production, the addition of 0.5 g/L glucose at day 15 did not result in a significant increase in cell growth (Figure 4), which indicated that AdMSCs reached a stationary state. This might be due to cell growth being limited by either space for propagation or exhaustion of certain essential nutrients other than glucose. After 16 days of cell culture, AdMSCs in the vessel reached a maximum density of ~2.4 X 10^5 cells/mL (0.24 million cells/mL), which was about 14-fold higher than initial seeding density.

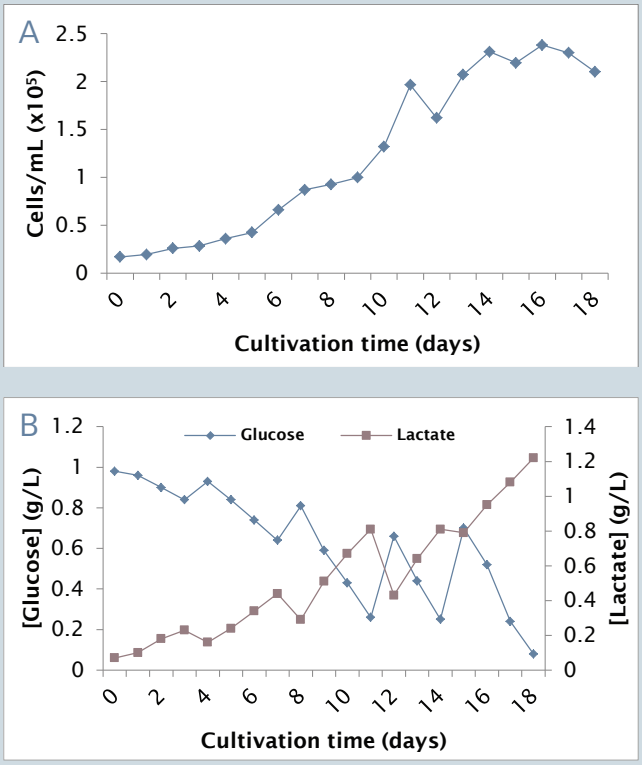

Figure 4: Growth profile of AdMSCs in BioBLU 5c single-use vessel with collagen coated microcarriers
A: Cell density
B: Glucose and Lactate concentrations over time: 50 % medium exchanged was performed every 4 days and 0.5 g/L glucose was added at day 15

To confirm that AdMSCs retained their stem cell properties during expansion in the bioreactor, immunostaining of stem cell surface markers was performed. MSCs express various cell surface markers such as: CD73, CD90, CD105, and CD44 [3 – 5, 7]. Microcarrier beads that contained AdMSCs were characterized based on surface marker expression using CD44, CD90, and CD105-specific antibodies followed by fluorescence imaging. The results revealed that AdMSCs retained stem cell surface markers during the experiment (Figure 5).

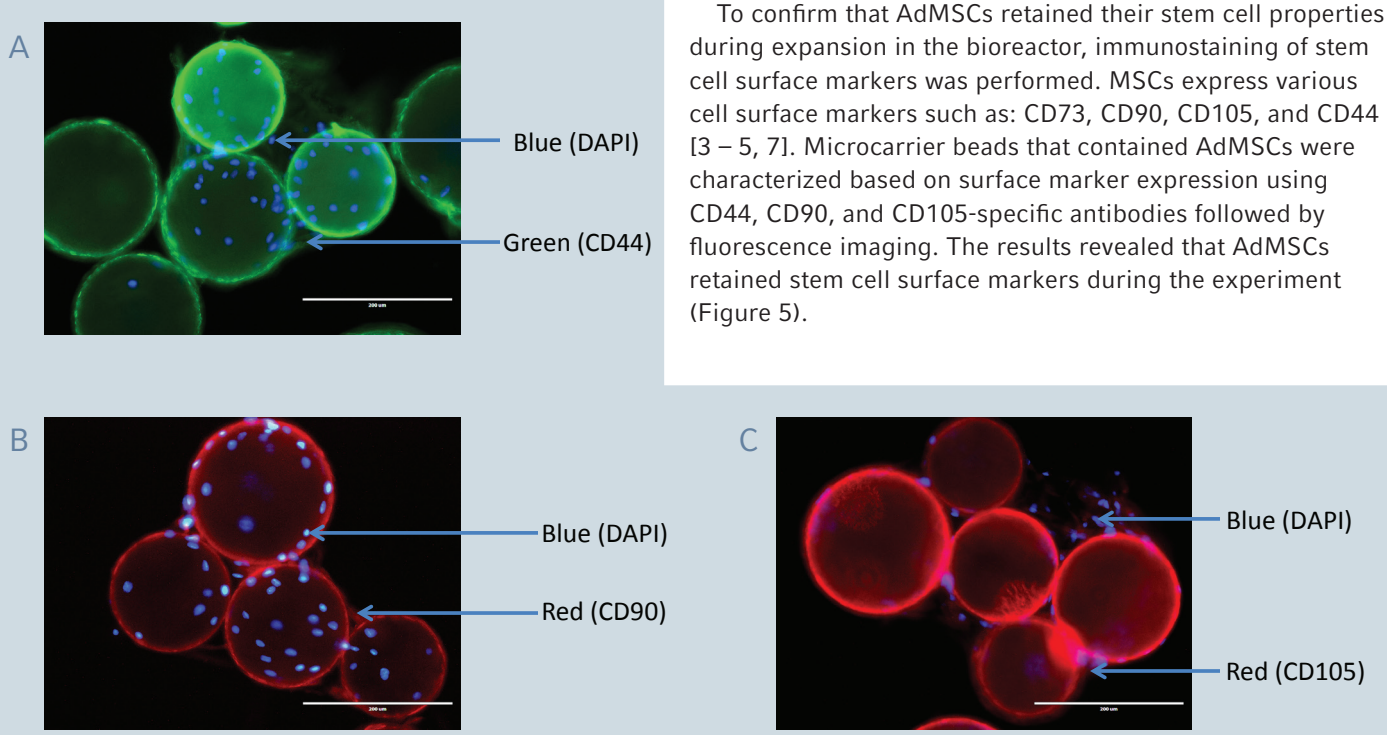

Figure 5: Stem cell marker identification immunoassays for AdMSCs expanded on microcarriers in bioreactor
A: AdMSCs on microcarrier beads are positive for CD 44 stem cell marker, as indicated in green by fluorescence imaging
B: AdMSCs on microcarrier beads are positive for CD90 stem cell marker, as indicated in red by fluorescence imaging
C: AdMSCs on microcarrier beads are positive for CD105 stem cell marker, as indicated in red by fluorescence imaging

In addition to immunostaining, PCR was also performed to monitor gene expression of additional stem cell markers. PCR data revealed that AdMSCs collected towards the end of the bioreactor culture were positive for CD44, CD90, CD105, Oct3/4, and Sox2 gene expression, whereas they were negative for CD45 gene expression. The post-bioreactor stem cell marker gene expression was compared to cells cultured on T-75 cm^2 flasks. From the comparison, it was observed that AdMSCs collected from the bioreactor and T-75 cm^2 flasks prior to bioreactor culture had the same stem cell marker gene expression pattern (Figure 6).

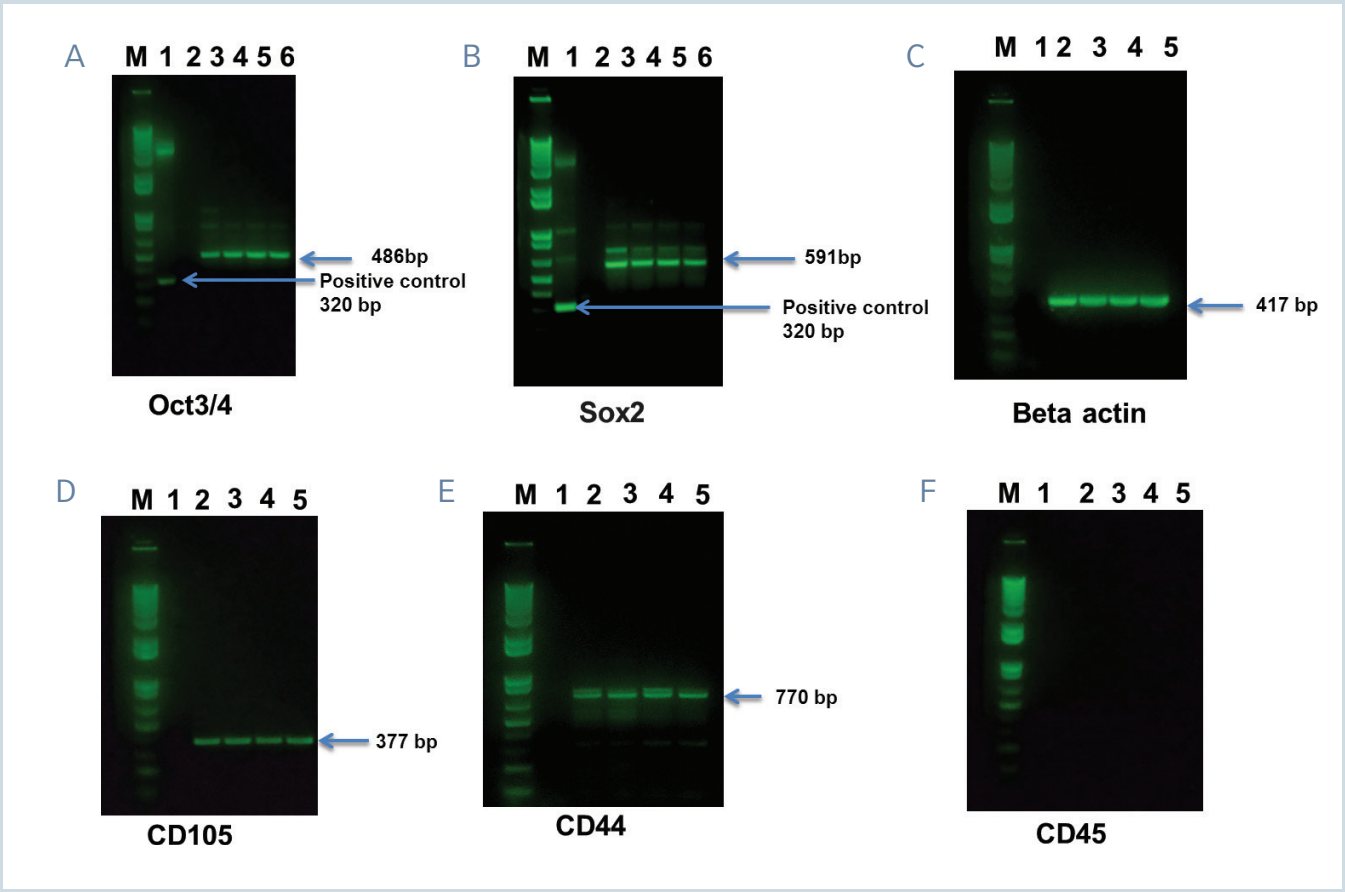

Figure 6: PCR analysis of multipotency markers in AdMSCs cultured in T-flasks and in BioBLU single-use vessels
For gel A & B: M: DNA ladder; Lane 1: Positive control synthetic DNA sequence for Oct3/4 or Sox2; Lane 2: PCR negative control; Lane 3: Sample at 0.2 million cells/mL; Lane 4: Sample at 0.24 million cells/mL; Lane 5: Sample from T-75 cm^2 flask at passage 4; Lane 6: Sample from T-75 cm^2 flask at passage 5
For gel C, D, E, & F: M: DNA ladder; Lane 1: PCR negative control; Lane 2: Sample at 0.2 million cells/mL; Lane 3: Sample at 0.24 million cells/mL; Lane 4: Sample from T-75 cm^2 flask at passage 4; Lane 5: Sample from T-75 cm^2 flask at passage 5

To further confirm that the AdMSCs cultured in the bioreactor retained their differentiation capacity, adipocyte and osteocyte differentiation assays were performed. AdMSCs were collected from the microcarrier beads and seeded into 6-well plates containing either adipocyte or osteocyte differentiation media. In the osteocyte differentiation medium, cells transformed into long polygonal shaped osteocytes and produced calcium deposits in the extracellular matrix.

On the other hand, when cells were treated with adipocyte differentiation medium, cells became oval shaped and accumulated lipid droplets. After 21 days of culture, the plates were stained with Oil Red O or Alizarin Red S staining solutions. Microscopic observation revealed that the AdMSCs from the bioreactor were successfully differentiated either into adipocytes or osteocytes (Figure 7).

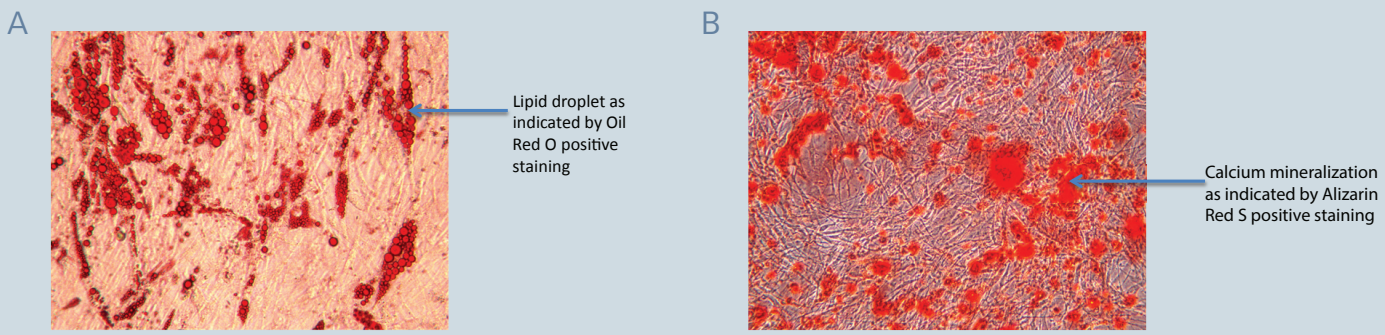

Figure 7: Differentiation assays for AdMSCs expanded on microcarriers in bioreactor
A: Adipogenic differentiation formed lipid droplets as indicated by Oil Red O positive staining
B: Osteogenic differentiation caused calcium mineralization of extracellular matrix as indicated by Alizarin Red S positive staining

Since the Vi-CELL could not be used for cell counting in the presence of microcarriers, the NucleoCounter NC-100 was used to conduct daily cell counts throughout the bioreactor run. However, the NC-100 appears to have a smaller dynamic range as compared to Vi-CELL, thus giving inaccurate readings at higher cell densities. In order to provide more accurate cell counts, a comparative study was performed between the NC-100 and the Vi-CELL. For this purpose, AdMSCs were collected from T-75 cm^2 flasks and counted with both the Vi-CELL and the NC-100 counter after a 3-fold dilution. The cell count results indicated that at high cell concentrations, the NC-100 undercounts the cells significantly as compared to the industry standard Vi-CELL. In the high cell concentration range, Vi-CELL reported on average 1.8-fold higher than the NC-100 from the same sample (Figure 8).

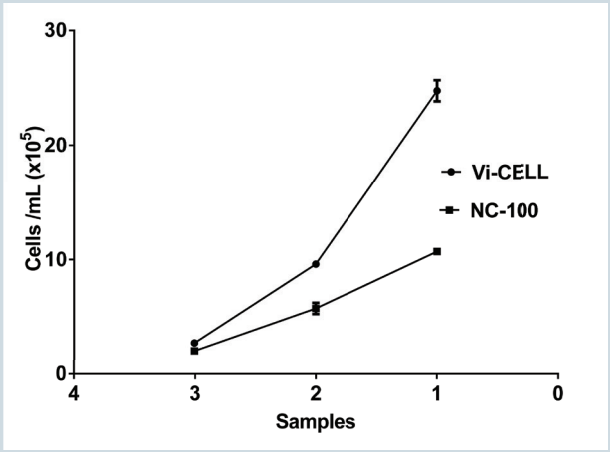

Figure 8: Comparison between Vi-CELL and NC-100 for measuring cell counts from the same AdMSC samples

A corrected bioreactor cell growth profile was provided based on NC-100 to Vi-CELL correlation using the averaged correction factor of 1.8 (Figure 9). The peak cell density reached ~0.43 million cells/mL in the BioBLU single-use vessel's 3.75 L maximum working volume, resulting in a total cell number yield of ~1.6 billion cells (1.62 x 10^9) on day 16. Such a large quantity is necessary for stem cell therapy using MSCs. It was estimated that the average human would require approximately 1 billion cells per treatment dose [15].

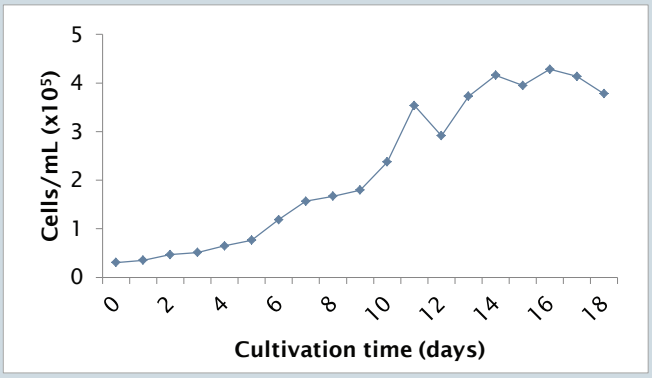

Figure 9: Vi-CELL corrected growth profile of AdMSCs in BioBLU 5c single-use vessel using collagen coated microcarriers

Conclusion

Our study clearly demonstrated the feasibility of using BioBLU 5c single-use vessels for the production of large-scale MSCs. The BioBLU 5c single-use vessel has a maximum working volume of 3.75 L, capable of producing large-scale MSCs in a single run. In addition, BioBLU 5c is equipped with a pitched-blade impeller which allows stem cells to be cultured under low rpm conditions to avoid shear force damages.

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. The above studies validated the general applicability of the CelliGen BLU benchtop bioreactor and BioBLU single-use vessels for large-scale process optimization and production of stem cells.

Besides the BioBLU 5c, Eppendorf also manufactures BioBLU 0.3c, BioBLU 1c, BioBLU 14c, and BioBLU 50c single-use vessels which are equipped with a range of reaction volumes (up to 40 L working volume). The larger single-use vessels will allow for the production of larger numbers of MSCs from a single bioreactor run.

Literature

[1] Comley J. Stem cells rapidly gaining traction in research and drug discovery. *Drug Discovery World (DDW)* 2013; Summer.

[2] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411):143-7.

[3] Zhang Y, Khan D, Delling J, Tobiasch E. Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. *The Scientific World Journal* 2012; 2012:793823.

[4] Caplan AI. Mesenchymal stem cells. *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society* 1991; 9(5):641-50.

[5] Ertas G, Ural E, Ural D, Aksoy A, Kozdag G, Gacar G, Karaoz E. Comparative analysis of apoptotic resistance of mesenchymal stem cells isolated from human bone marrow and adipose tissue. *The Scientific World Journal* 2012; 2012:105698.

[6] Mahmoudifar N, Doran PM. Osteogenic differentiation and osteochondral tissue engineering using human adipose-derived stem cells. *Biotechnology Progress* 2013; 29(1):176-85.

[7] Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. *Stem Cells and Development* 2011; 20(8):1297-308.

[8] Schop D, van Dijkhuizen-Radersma R, Borgart E, Janssen FW, Rozemuller H, Prins HJ, de Bruijn JD. Expansion of human mesenchymal stromal cells on microcarriers: growth and metabolism. *Journal of Tissue Engineering and Regenerative Medicine* 2010; 4(2):131-40.

[9] Siddiquee K, Sha M. Microcarrier-Based Expansion of Adipose-Derived Mesenchymal Stem Cells in Shake Flasks. *Bioprocessing Journal* 2013; 12(4):32-8.

[10] Xing Z, Kenty BM, Li ZJ, Lee SS. Scale-up analysis for a CHO cell culture process in large-scale bioreactors. *Biotechnology and Bioengineering* 2009; 103(4):733-46.

[11] Potdar P, Prasannan P. Differentiation of Human Dermal Mesenchymal Stem Cells into Cardiomyocytes by Treatment with 5-Azacytidine: Concept for Regenerative Therapy in Myocardial Infarction. *ISRN Stem Cells* 2013; 2013:Article ID 687282.

[12] Mesenchymal Stem Cells Expanded on SoloHill Microcarriers in Millipore Cell Ready Bioreactor. *SoloHill Application Note* 2014; App-007.

[13] Kehoe D, Schnitzler A, Simler J, DiLeo A, Ball A. Scale-up of Human Mesenchymal Stem Cells on Microcarriers in Suspension in a Single-use Bioreactor. *BioPharm International* 2012; 25(3):28-38.

[14] Murhammer DW, Goochee CF. Sparged animal cell bioreactors: mechanism of cell damage and Pluronic F-68 protection. *Biotechnology Progress* 1990; 6(5):391-7.

[15] Phillips BW, Lim RY, Tan TT, Rust WL, Crook JM. Efficient expansion of clinical-grade human fibroblasts on microcarriers: cells suitable for ex vivo expansion of clinical-grade hESCs. *Journal of Biotechnology* 2008; 134(1-2):79-87.

Ordering information

Description	Order no. International	Order no. North America
New Brunswick™ CelliGen® BLU Bioreactor Control Station	M1374-230-LSA (200 – 240 V)	M1374-120-LSA (100 – 120 V)
BioBLU® 5c Single-use Vessel, Macrosparge Working Volume 1.25 – 3.75 L	M1363-0121	M1363-0121
Mastercycler® Pro Thermal Cycler with Control Panel	6321 000.515 (230 V, 50/60 Hz)	950040015 (120 V, 50/60 Hz)

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