Culture of MSCs on Polystyrene Microcarriers in VueLife® Bags

INTRODUCTION

Mesenchymal Stromal (Stem) Cells

MSCs can be derived from bone marrow, adipose tissue, umbilical cord and a variety of other tissues. ISCT defines MSCs by the following[1]:

1. Plastic-adherence under standard culture conditions
2. Expression of CD105, CD73 and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79 or CD19 and HLA-DR
3. Ability to differentiate into osteoblasts, adipocytes and chondrocytes *in vitro*

2D vs. 3D Culture of MSCs in Bags

MSCs need to adhere and spread on an *in vitro* culture surface in order to trigger normal cell processes such as proliferation. Achieving the clinical autologous cell doses – 10^9 cells/patient – requires expansion of cells *in vitro* and requires significant surface area.

3D expansion of MSCs using microcarriers or "beads" is common for allogeneic therapies using traditional bioreactors, and there are a wide-range of plastic- and hydrogel-based beads commercially available from a variety of suppliers. However, bioreactors are typically operated at a much larger scale than what is required for personalized medicine. A solution combining mid-size, fully closed and single-use bags as provided by Saint-Gobain's VueLife® range with microcarriers is therefore a promising alternative.

VueLife® bags are made of FEP film, which is transparent, remains flexible between -200°C and +200°C, and provides high permeability to oxygen and carbon dioxide, allowing for efficient gas exchange during culture. This makes them an excellent choice for suspension culture applications and for use with microcarriers for adherent culture by minimizing adhesion of cells to the bag material.

MATERIALS & METHODS

MSC Culture

For this study, human bone marrow-derived mesenchymal stromal cells were purchased from RoosterBio Inc. and cultured in RoosterBio's serum-free RoosterNourish™-MSC-XF medium. To assess donor-dependent variability in the cell culture performance and outcome, three individual donors (referenced as lots 164, 172 and 227) were compared.

To generate cells for microcarrier cultures, MSCs were thawed and cultured in T175-flasks in RoosterNourish™-MSC-XF medium at 37°C and 5% CO_2, until cells were in the log expansion phase. To harvest, cells were washed once with DPBS and then incubated with TrypLE™ Select Enzyme (10X) for 8 minutes. Following cell dissociation, cells were centrifuged at 200 x g for 5 minutes and then re-suspended in RoosterNourish™-MSC-XF medium.

[1] Dominici, M. et al (2006). "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy Position Statement." *Cytotherapy*, 8(4), 315-317.

Microcarrier Cell Culture Protocol

Corning® CellBIND® microcarriers (#4620) are made of USP Class VI polystyrene material, providing a known and consistent platform to users familiar with tissue-culture treated polystyrene-based culture systems, such as T-flasks and multiwell plates. The proprietary CellBIND® treatment incorporates oxygen-containing functional groups into the polystyrene surface, rendering it more hydrophilic and increasing cell attachment. These beads do not require any special handling or storage for use in closed systems.

Addition of Microcarriers to VueLife® 32-C Bags for 3D Culture

First, the microcarriers were resuspended in RoosterNourish™-MSC-XF medium to a final volume of 15 mL prior to seeding into VueLife® 32-C bags. Then, 5 mL of the microcarrier suspension was transferred into the bag using a sterile syringe via the FLV port. The conical tube was then washed with of 10 mL of fresh medium and the remaining contents were added to the VueLife® 32-C bag, bringing the total volume to 32 ml. To allow for medium equilibration, the bag was incubated in a cell culture incubator for 30 min. During this step, MSCs were harvested from T -flasks as described above.

Cell Seeding and Attachment Phase

MSCs (donors 164, 172 and 227) were seeded at a concentration of 0.6 x 10^6 cells/bag into VueLife® 32-C bags with Corning® CellBIND® Microcarriers (360cm^2/gram microcarriers, 0.4 gram = 150 cm^2 surface area) and cultured for six days.

After the addition of cells to the VueLife® 32-C bag and removal of air bubbles, the culture was mixed manually by tilting the bag back and forth several times. Next, the culture was incubated in static phase (0 rpm) for 1 hour. This process was repeated for a total of 3 cycles times followed by incubation in static conditions overnight. Cell attachment was monitored the next day via optical microscopy, and dynamic cell culture was initiated for cell expansion.

Cell Expansion Phase

Following the cell attachment phase, the microcarrier culture was mixed continuously at 16 rpm. During the cell expansion phase, the mixing speed was increased daily in an attempt to minimize microcarrier aggregation. The mixing speed was increased as follows: 16 rpm (following cell attachment), 16 rpm (day 1), 22 rpm (day 2), 27 rpm (day 3), 32 rpm (days 4- end of culture). Half-volume media exchanges were performed on day 3 and day 5 of culture.

To exchange the media, the microcarriers were allowed to settle in the VueLife® 32-C bag for several minutes. Next, half spent media was removed and fresh medium was added. The bag was then returned to the orbital shaker in the cell culture incubator.

Cell Harvest

For cell harvest, the entire cell/microcarrier suspension was collected from the bag into a conical tube, and cells were released from the microcarriers using TrypLE™ Express Enzyme, a recombinant trypsin (enzyme) solution. For removal of the microcarriers from the cell suspension, the solution was filtered using cell strainers (0.4µm pore size).

Cell Characterization

Cell viability and cell number was assessed using the Vi-CELL™ XR Cell Viability Analyzer (Beckman Coulter, Inc.). This system utilizes the Trypan Blue dye exclusion method for quantification of viable cells in the measured cell population.

MSC Characterization by Flow Cytometry

Expression of hallmark MSC surface markers CD73, CD90 and CD105, as well as absence of expression of CD14, CD20, CD34 and CD45 were confirmed by flow cytometry using Miltenyi Biotec's MSC Phenotyping Kit (#130-095-198). Cell viability was assessed using the Fixable Viability Stain FVS510 (BD Biosciences, #564406).

MSC Tri-lineage Differentiation Assay

Tri-lineage differentiation capacity of the MSCs used in this study was confirmed using Miltenyi Biotec's StemMACS™ AdipoDiff and OsteoDiff media for adipogenic and osteogenic differentiation, and Gibco's StemPro® Chondrogenesis Differentiation Kit. All kits were used following the manufacturers' recommendations.

Optical Microscopy

Changes in cell morphology and the optical appearance of the cultured cell population was assessed using an inverted microscope, the Axio Observer 7 (Carl Zeiss, Inc.) equipped with Zen Pro 2.3 software.

RESULTS & ANALYSIS

MSCs were seeded into VueLife® 32-C bags with microcarriers and cultured for six days.

As depicted in **Figure 1**, microcarriers showed an increasing level of aggregation in the bags towards the end of culture, which was concurrent with high cell expansion in the VueLife® FEP bags.

Cell expansion results are summarized in **Table 1** and show strong donor-dependent variability in the outcome. While donor 164 showed strong expansion (~21 fold increase), the two other tested donors #172 and #227 showed only low levels of proliferation (4 and 9-fold, respectively). However, since the beads are not transparent, this evolution cannot be easily tracked via microscopy (**Figure 2**).

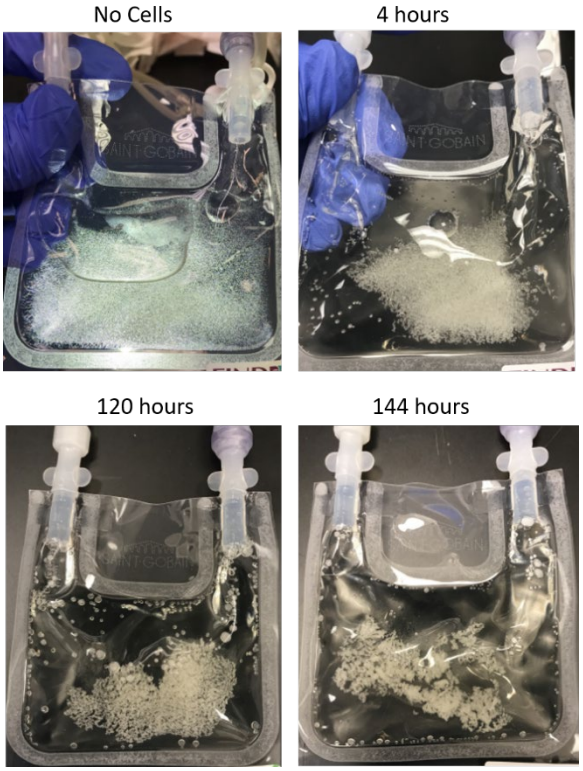

Figure 1. *Photographs of CellBIND® microcarriers in VueLife® 32-C bags, without cells (top left), and during MSC culture after 4, 120 and 144 hours. Cell/microcarrier aggregation is visible by eye towards the end of culture.*

Donor	Viability	No. viable cells harvested	Fold increase
164	96%	$12.5x10^6$	21
172	96%	$3.59x10^6$	4
227	95%	$5.1 x10^6$	9

Table 1. *Results of MSC expansion in VueLife® 32-C bags on Corning® CellBIND® microcarriers after 6 days of culture.*

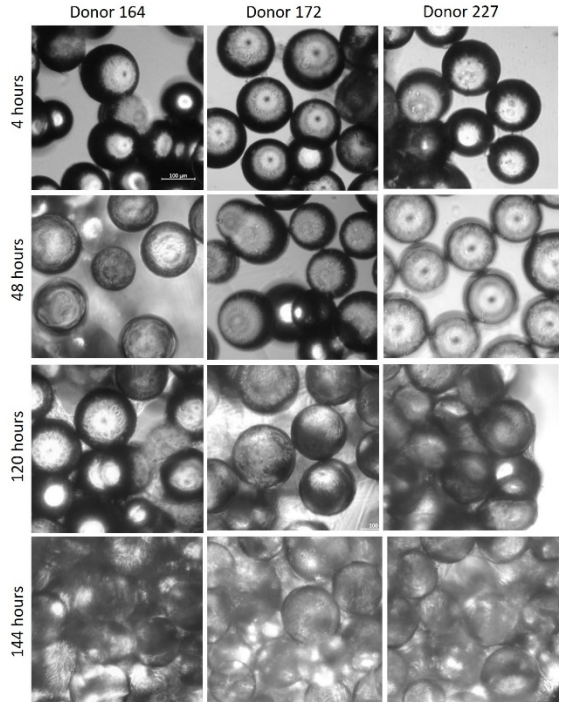

***Figure 2**. Microphotographs of MSCs adhering to Corning® CellBIND® microcarriers at the indicated time in culture. MSCs derived from three individual donors were expanded in VueLife® 32-C bags on microcarriers for a total of 6 days. Scale bar indicates 100µm.*

MSC surface marker expression levels were not affected by culture on Corning® CellBIND® microcarriers, as shown by analysis via flow cytometry. MSCs of all three tested donors showed the expected positive expression of MSC hallmark markers CD73, CD90 and CD105 and absence of CD14, CD20, CD34 and CD45. Expression levels of MSCs cultured in VueLife® 32-C bags on microcarriers showed were comparable to cells cultured in T-175 flasks (data not shown).

In summary, the results of this study indicate that MSC expansion can be facilitated by Corning® CellBIND® microcarriers in VueLife® 32-C bags. However, MSC expansion performance was variable, which may be a result of donor-dependent proliferation rates and/or a result of the inability of the operator to visualize and confirm cell attachment and detachment during the culture phase, thus hindering optimal use of the system. Optimization of culture protocols is currently ongoing to improve cell yields and performance.

ABOUT

SAINT-GOBAIN

Saint-Gobain Life Sciences is proud to take part in providing solutions for a multitude of cell therapy applications while collaborating with customers and industry partners to develop custom disposables, often for integration into automated systems. Through our material science expertise as well as our deep experience in bringing manufacturing technologies to scale, we are uniquely positioned to offer solutions to the numerous challenges faced by cell therapy manufacturers today.