Culture of MSCs on Dissolvable 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 **(Figure 1)**.

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. In addition, the FEP films provide a non-adhesive surface, which in turn supports efficient use of microcarriers by minimizing adhesion of cells to the bag material.

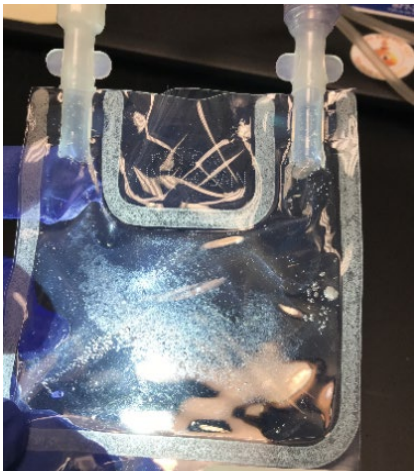

Figure 1. *MSC culture in VueLife® 32-C bag using dissolvable microcarriers.*

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.

[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.

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.

Microcarrier Cell Culture Protocol

Corning® offers Synthemax™ II-coated dissolvable microcarriers, which provide a convenient alternative to conventional carriers that require enzymatic release of the cells for harvest. The microcarriers are comprised of polygalacturonic acid (PGA) polymer chains cross-linked via calcium ions.

The microcarriers are dissolved using a soluton of EDTA and pectinase for 10 to 20 minutes. The narrow size distribution (1.02-1.03 g/cm^3 density, 200-300 µm diameter when fully hydrated) and large surface area (5000 cm^2/g) of the beads support homogenous cell culture. In addition, these dissolvable microcarriers are optically clear, which is a benefit for visualizing cell morphology, attachment and detachment to support process development and quality control for cell manufacturing.

Hydration of Dissolvable Microcarriers

Corning's dissolvable microcarriers are supplied as a sterile, dry powder and must be hydrated prior to use. To hydrate microcarriers for use in a VueLife® 32-C bag, 0.03 g of dissolvable microcarriers were aseptically transferred using a smartSpatula® to a sterile conical tube, and rehydrated in sterile water (UltraPure™ DNase/RNase-Free Distilled Water). The quantities of microcarriers and liquids used to fill a 32-C bag are listed in **Table 1**.

Beads	Water for hydration	Total media volume	Surface area	Cell seeding concentration
0.03 g	4.5 mL	30 mL	150 cm^2	4000-7500 cells/cm^2

Table 1. *Quantities of microcarriers and liquids used for VueLife® 32-C bag*

The microcarriers were incubated for 10 min to allow complete hydration and then the water was removed from the settled microcarriers. A sample of the hydrated beads (100 µL) was transferred onto a microscope slide for visual inspection and confirmation of the completed hydration.

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

The hydrated microcarriers were resuspended in RoosterNourish™-MSC-XF medium prior to seeding into VueLife® 32-C bags. The microcarrier suspension was then transferred into the bag using a sterile syringe via the FLV port. To allow for equilibration of culture medium, the bag was incubated in a cell culture incubator for 30 min. During this step, MSCs were harvested from T - flasks as described above and seeded into VueLife® bags in fresh medium, bringing the total volume to 32 mL per bag.

Cell Attachment Phase

After the addition of cells to the VueLife® 32-C bag and removal of air bubbles via syringe, 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

Cells were harvested from the dissolvable microcarriers using the procedure recommended by Corning®. First, a harvest solution was prepared per the manufacturer's recommendations in order to fully dissolve the microcarriers and release the attached cells. The recommended harvest solution for MSCs is: 100 U/mL pectinase, 10 mM EDTA, 5X TryPLE™ Select diluted in DPBS. The harvest solution was filter-sterilized and pre-warmed to 37°C prior to use.

Figure 2. *Dissolution of Corning® dissolvable microcarriers during cell harvest.*

The bag was removed from the 3D orbital shaker, placed in an upright position in the biosafety cabinet and allowed to rest for 3 minutes in order to allow the microcarriers to settle. Then, the spent media was removed via a sterile syringe and the microcarriers were washed with 25 mL of DPBS. The microcarriers were allowed to settle after the addition of DPBS, and the supernatant was removed.

To dissolve the microcarriers, 7.5 mL of harvest solution was added to the VueLife® 32-C bag, and the bag was incubated on a stir platform for 5 minutes at 32 rpm. Once a single-cell suspension was confirmed via optical microscopy (**Figure 2**), the cells were harvested into a 50 ml conical tube, and the bag was washed a final time with 10 ml of fresh medium or PBS. The harvested cells were centrifuged at 200 x g for 5 minutes and then resuspended in fresh medium for cell counting and further processing.

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 at a concentration of 6 x 10^5 cells/bag (4,000 cells/cm^2) and cultured on Corning® Synthemax II™-coated dissolvable beads.

As shown in **Figure 3**, cell attachment could be observed within the first 4 hours after seeding, and cells continued to spread across adjacent microcarriers rapidly and evenly. After 4 days of culture, all microcarriers were covered and 100% confluence levels were reached.

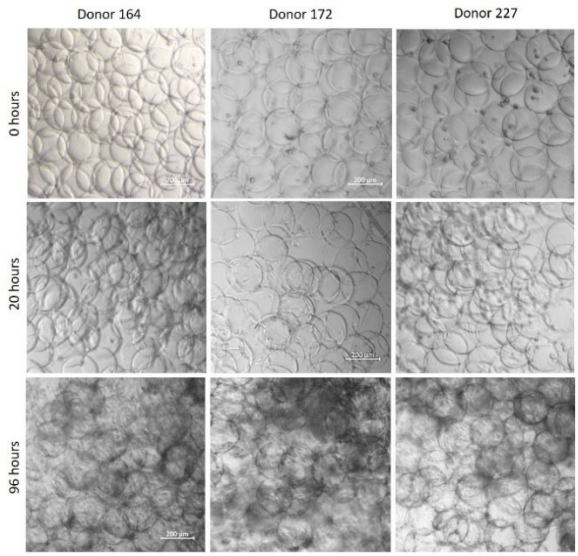

Figure 3. *MSC expansion on Corning® Synthemax™ II-coated dissolvable microcarriers in 32-C bags. Shown are microphotographs of MSCs derived from three individual donors adhering onto Corning® microcarriers at the indicated time points. Scale bar indicates 200 µm.*

MSC proliferation rates (**Table 2**) showed ~24-26-fold increase of the three tested MSC lines in VueLife® 32-C bags using dissolvable beads (**Table 2**). These results demonstrate that MSCs can rapidly and robustly be expanded using a combination of VueLife® FEP bags and Corning® dissolvable microcarriers.

Donor	Viability %	Harvested viable cells/bag	Fold increase
164	97.8	$16x10^6$	26
172	97.7	$15x10^6$	26
227	97.2	$15x10^6$	24

Table 2. *Results of MSC expansion on Corning's Synthemax™ II-coated microcarriers after 6 days in culture in VueLife® 32-C bags. Results are shown for three tested MSC donors (#164, #172 and #227).*

To support further cell expansion towards clinically-relevant doses, additional beads and media may be added to the bags. This can easily be achieved by starting the culture in a larger bag (i.e. VueLife® 197-C or larger) and/or using culture clamps to contain the culture into a smaller compartment at the start of culture, and then gradually opening up additional space in the bag as the cells expand.

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.