Human Mesenchymal Stem Cell Growth on Corning® Denatured Collagen Dissolvable Microcarriers in a 5L Bioreactor

Application Note

CORNING

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Introduction

Microcarriers are 100 to 300 micron-sized beads that support the attachment and growth of adherent cells in stirred culture. They enable more cost-effective cell production by reducing the fixed costs (e.g., footprint and labor) and variable costs (e.g., media consumption) in large-scale manufacturing.

One challenge associated with microcarrier-based expansion is that microcarriers eventually must be removed or separated from the final product (e.g., cell, virus, antibody, etc.)[1,2]. Typically this is accomplished through filtration and/or centrifugation methods. Because of the broad size distribution of traditional microcarriers and the potential for bead breakage with use, several downstream microcarrier separation processes are required to remove residual microcarriers. These processes may create additional challenges resulting in filter fouling and loss of product.

To address these concerns, Corning developed a dissolvable microcarrier made from a polygalacturonic acid (PGA) polymer that is cross-linked via calcium ions. As shown in Figure 1A, microcarrier dissolution is achieved through the addition of a harvest solution containing EDTA, pectinase, and a standard cell culture protease. When calcium ions are chelated by EDTA, the PGA polymer destabilizes. Subsequent PGA polymer degradation is achieved by pectinase. The additional cell culture protease (e.g., trypsin, TrypLE™, Accutase®) breaks down cell-cell interactions and extracellular matrices, resulting in a single cell suspension in a solution of small sugar oligomers (Figure 1B).

This application note describes the use of Corning dissolvable microcarriers for the expansion and recovery of human mesenchymal stem cells (hMSCs) in 5L bioreactors. We demonstrate 7-fold expansion of hMSCs on 2 g/L denatured collagen dissolvable microcarriers in serum-containing medium. Continuous agitation was used to achieve uniform cell attachment and expansion, and microcarrier aggregate size was maintained through continuous gas sparging. Cells were recovered from

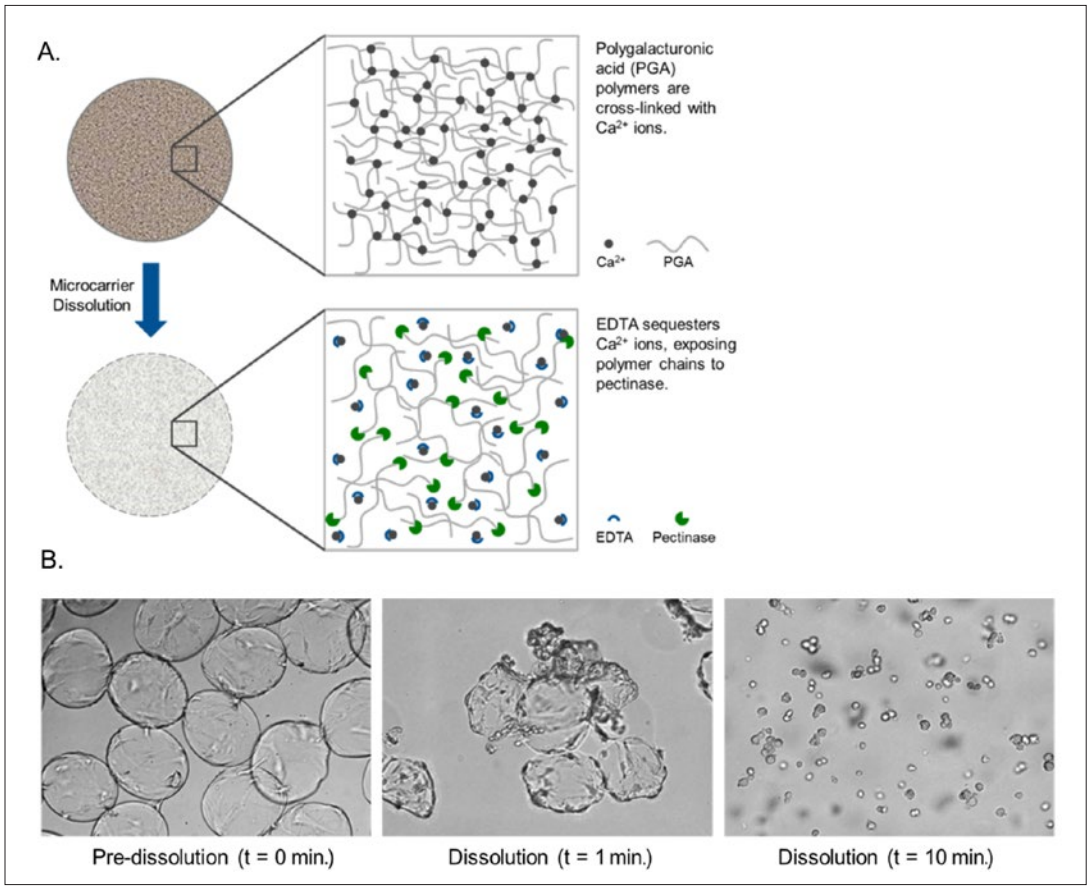

Figure 1. Description of Corning Dissolvable Microcarriers. Dissolvable microcarriers are made of polygalacturonic acid (PGA) polymer chains cross-linked via calcium ions. Dissolvable microcarriers have a surface area of 5,000 cm^2/gram; density of 1.02 to 1.03 g/cm^3, and diameter of ~250 micron. (A) Microcarrier dissolution is achieved with the addition of EDTA, pectinase, and a standard cell culture protease. (B) Microcarriers are completely dissolved within 10 to 15 minutes. Microscopy images show bead dissolution and hMSC release using a harvest solution of EDTA, pectinase, and TrypLE.

bioreactors using a harvest solution of EDTA, pectinase, and TrypLE™ with stirring for 20 minutes. The entire harvest process was completed by one operator in 1.5 hours and resulted in >95% cell recovery. Cells harvested from dissolvable microcarriers retained standard elongated morphology, normal karyotype, and multipotency, as measured by FACS analysis of phenotype and directed differentiation into osteocytes, chondrocytes, and adipocytes.

Methods

Dissolvable Microcarrier Preparation

The 10 g denatured collagen dissolvable microcarriers (Corning Cat. No. 4981) were aseptically hydrated in 1.5L of sterile water (Corning Cat. No. 25-055) in sterile, 2L glass bottles (Corning Cat. No. 1395-2L) that have been siliconized, for each bioreactor experiment according to the manufacturer's protocol. Water was replaced with culture medium by allowing microcarriers to settle and aspirating residual water without disturbing the microcarrier bead pack. Microcarriers were diluted in culture medium containing 1:1,000 antifoam (Thermo Fisher Cat. No. A10369-02) to a concentration of 2 g/L (surface area: 5,000 cm^2/gram) and aseptically transferred via a glass funnel into pre-sterilized, single-walled 5L glass bioreactors (Sartorius Biostat B® Twin bioreactors) fitted with one downward pumping 70 mm pitched blade impeller (Sartorius Cat. No. BB-8847401) and ring sparger with 1 mm downward bores (Sartorius Cat. No. UNIVESSEL-00013). Bioreactor cultures were allowed to equilibrate to 37°C at 90 rpm for 12 to 18 hours prior to cell addition. The pH (~7.4) and dissolved oxygen (DO, ~80%) levels were confirmed through offline measurements of samples (Nova Biomedical BioProfile® 400 Analyzer) before cell seeding.

Cell Preparation

Human mesenchymal stem cells (RoosterBio Cat. No. MSC-001) were thawed in Corning® CellBIND® surface-treated HYPER*Flask*® cell culture vessels (Corning Cat. No. 10034) and expanded in serum-containing medium (RoosterBio Cat. No. KT-001) for one passage to create a working cell bank according to manufacturers' protocols. For each bioreactor experiment, cells from the working cell bank were thawed into Corning CellBIND surface-treated HYPER*Flask* vessels and were harvested on day 4 using TrypLE (Thermo Fisher Cat. No. 12563029) for 12 minutes. Recovered cells were pelleted at 260 x g for 10 minutes, resuspended in fresh culture medium at 400,000 cells per mL (200 billion in 500 mL), and seeded directly (4,000 cells per cm^2) into equilibrated bioreactors containing dissolvable microcarriers. The final bioreactor working volume was 5L.

Cell Attachment to Dissolvable Microcarriers (0 to 24 hours)

Continuous agitation was used for cell attachment to dissolvable microcarriers. We observed cell attachment, but not complete spreading, after 10 cycles of 5 minutes at 100 rpm, followed by 20 minutes at 20 rpm. After ~5 hours, agitation was maintained at 100 rpm until 24 hours.

Cell Expansion on Dissolvable Microcarriers (24 to 96 hours)

To control microcarrier aggregate size with continued cell expansion, the agitation rate was increased to 120 rpm, and the DO was maintained at 50% using equal volumes of air and nitrogen sparge (25 to 40 ccm), with minimal supplementation of oxygen (<30 ccm) when needed. The pH was maintained at 7.4 through CO_2 sparge. The rate of gas addition at any given time was maintained at <0.02 vessel volumes per minute (vvm).

Sampling

The 10 mL samples were removed each day from the 5L bioreactor through an 1/8" ID dip tube with a syringe and transferred to an Ultra-Low Attachment (ULA) 6-well plate (Corning Cat. No. 3471) for microscopy (Zeiss Axiovert 40C) and spent media analysis (Nova Biomedical BioProfile 400 Analyzer). Cells were removed from dissolvable microcarriers for enumeration with a ViCell™ automated cell counter. Spent media was removed and samples were washed with 4 mL DPBS (Corning Cat. No. 21-031) and 2 mL of pre-warmed (>25°C) harvest solution was added. To prepare the harvest solution, a 5X solution of TrypLE was made from equal volumes of a 10X stock solution (Thermo Fisher Cat. No. A1217702) and DPBS. Pectinase (Sigma Cat. No. P2611) and EDTA (Corning Cat. No. 46-034-CI) were directly supplemented to the 5X TrypLE solution to a final concentration of 100 U/mL and 10 mM, respectively. Plates containing microcarrier cultures in harvest solution were placed in a 37°C incubator for 10 minutes. Cultures were gently mixed using a 5 mL Stripette™ serological pipet (Corning Cat. No. 4487) and observed under the microscope to confirm complete microcarrier dissolution and a single cell suspension. The sample was again mixed and 700 µL was transferred to a ViCell cup for enumeration.

Microcarrier Dissolution and Cell Recovery

Cells were harvested from day 4 bioreactor cultures by turning off all bioreactor controls and allowing microcarriers to settle for 15 minutes; this resulted in a settled bead pack volume of ~1L. Spent culture medium was removed through 1/8" ID dip tube, and 2 washes with 1L DPBS were performed to remove residual serum. A pre-warmed, filter sterilized (500 mL vacuum filtration system, Corning Cat. No. 430769) harvest solution was prepared as a 3X concentrate as follows: pectinase and EDTA were added to 500 mL of 10X TrypLE to a final concentration of 200 U/mL and 20 mM, respectively. The 500 mL of 3X harvest solution was added to the ~1L packed bead bed, resulting in a final volume near 1.5L allowing for a completely submersed impeller. Microcarrier dissolution was facilitated through gentle mixing at 80 rpm for 10 minutes; then agitation was increased to 100 rpm for an additional 10 minutes, resulting in a near single cell suspension. Cell suspensions were removed from bioreactors through a dip tube and total cell yields were calculated using a ViCell automated cell counter.

Cell Characterization

Aliquots of recovered cells were pelleted at 260 x g, washed with DPBS, and resuspended in CryoStor® CS5 freeze media (BioLife Solutions Cat. No. 205102) for later analysis.

For cell differentiation into chondrocytes, osteocytes, and adipocytes, cells were thawed, washed in culture medium to remove freezing medium, and re-seeded in Corning CellBIND surface 6-well plates (Corning Cat. No. 3335) in StemPro® differentiation media according to manufacturer's protocols (Thermo Fisher

Cat. Nos. A10071-01, A10072-01, and A10070-01). After 2 to 3 weeks, differentiated cells were fixed in 2% paraformaldehyde and stained for markers of differentiation: chondrocytes (chondrogenic pellets via Alcian Blue, Sigma Cat. No. TMS-010-C), osteocytes (calcium deposits via Alizarin Red, Sigma Cat. No. TMS-008-C), and adiptocytes (lipid droplets via Oil Red O, Sigma Cat. No. O1516-250ML).

For quantitative analysis of cell phenotype, recovered cells were thawed, immunostained for positive (CD73, CD90, and CD105 [BD Biosciences Cat. Nos. 550256, 555593, 555690, respectively]), and negative (CD14 [MilliporeSigma Cat. No. MAB1219], CD34 [BD Biosciences Cat. No. 555820]) surface markers, and the percent of viable cells expressing each marker were assessed via flow cytometry. We observed more consistent expression of CD105 when thawed cells were replated in flasks for a few hours until fully spread; replated cells were harvested from flasks using TrypLE™ and directly immunostained according to standard protocols. Briefly, cells were diluted to 1 x 10^6 cells/mL in 1X DPBS supplemented with 10% heat-inactivated serum (Corning Cat. No. 35-011-CV) (blocking buffer) and 1:1,000 propidium iodide (Millipore Sigma Cat. No. P4864) and incubated in the dark for 20 minutes at room temperature. Cells were washed twice with 1X DPBS, resuspended in blocking buffer at 0.5 x 10^6 cells/50 µL, and incubated with primary antibodies (10 µg/mL) or corresponding IgG1 isotype control (BD Biosciences Cat. No. 554121) for 30 minutes at 4°C in the dark. Cells were washed in staining buffer containing BSA (BD Biosciences Cat. No. 554657) and then incubated with secondary antibody (1:1,000 of Alexa Fluor® 488 goat anti-mouse IgG1 [Thermo Fisher Cat. No. A11001]) for 30 minutes at 4°C in the dark. Stained cells were washed once and resuspended in staining buffer for processing on a BD FACSCalibur™ flow cytometer. During acquisition, 30,000 events/sample were collected and analyzed using the BD CellQuest™ Pro software.

For analysis of cell karyotype, cells were submitted as live cultures in flasks for G-banded karyotype analysis at WiCell Cytogenetics Laboratory.

Results

Cell Attachment to Dissolvable Microcarriers

To demonstrate attachment of human mesenchymal stem cells (hMSCs) on microcarriers in bioreactors, we first determined the best agitation conditions that would support a uniform cell attachment across all microcarriers. Based on preliminary results with dissolvable microcarriers in spinner flasks, several agitation protocols in the bioreactor were investigated for the cell attachment phase: no agitation, intermittent cycles of settling and mixing, and completely continuous agitation. A minimum agitation speed that supported complete suspension of all microcarriers was determined experimentally by observing the microcarrier suspension throughout the working volume at different agitation speeds. Based on the following bioreactor configuration: one 70 mm pitched blade impeller positioned as low as possible on the impeller shaft, 5L working volume, and 2 g/L microcarrier concentration (10 cm^2/mL), a minimum agitation speed of 100 rpm supported complete suspension of the microcarriers throughout the working volume.

When cells were added to bioreactors containing suspended microcarriers, the culture was mixed at 100 rpm for 5 minutes before initiating one of the three agitation protocols described above: no agitation (0 rpm), continuous agitation (100 rpm), or intermittent cycles of 20 rpm for 20 minutes then 100 rpm for 5 minutes. It was expected that no agitation during cell attachment would promote an attachment rate similar to what is observed in planar cultureware; however, because the microcarriers would settle in the absence of agitation, cells would primarily attach to only those microcarriers exposed at the top of the bead pack, resulting in a non-uniform distribution of cells across the microcarrier population. In contrast, it was expected that a completely continuous agitation at 100 rpm would support the most uniform cell attachment to all suspended microcarriers, but the cell attachment period may be prolonged or potentially inhibited due to the hydrodynamic shear. For these reasons, intermittent cycles of low- and high-speed stirring were investigated to promote phases of bead settling and mixing. Based on the observation in planar flasks that cell attachment occurs within 20 minutes and complete spreading is achieved within a few hours, a 20-minute period of low agitation (20 rpm) was implemented to encourage cell attachment.

To achieve a more uniform attachment across all microcarriers, the culture was mixed at the minimum speed (100 rpm) required to suspend all the microcarriers for the minimum time (5 minutes) required to achieve a complete microcarrier suspension. This intermittent mixing protocol was continued until cell spreading was observed. To evaluate the effect of each agitation protocol on cell attachment, 10 mL samples were removed every hour to monitor cell attachment and spreading via microscopy. We recommend investigating different intermittent mixing cycles depending on the cell type, media condition (+ or - serum), microcarrier surface (charged vs. peptide/protein), cell seeding density, or microcarrier concentration[1,3,4].

As expected, no agitation (0 rpm) and constant agitation (100 rpm) did not support the desired uniform cell attachment with spreading. The culture exposed to no agitation contained both sparse, large cell-microcarrier aggregates with fully spread cells and numerous single, bare (no cells) microcarriers. This could be a result of cell-cell clustering and subsequent settling to the top of the microcarrier bead pack before attachment and spreading. The culture exposed to continuous agitation, in contrast, had better cell attachment uniformity, but very few cells had attached to the microcarriers even after 24 hours. The attachment protocol involving intermittent mixing supported the most uniform attachment of the majority of cells. Based on hourly microscopy images from samples, cyclic periods of mixing were continued for a total of 5 hours; at this point, very few non-attached cells were observed, and spreading of attached cells had begun (Figure 2). After the 5-hour attachment period, the culture was exposed to constant agitation at the minimum agitation speed (100 rpm) required to suspend the microcarriers. By 24 hours post-seeding, fully elongated cells (Figure 3) were observed and nearly 100% of cells were attached, based on cell enumeration from representative 10 mL samples.

Cell Expansion on Dissolvable Microcarriers

Based on preliminary experiments in small-scale spinner flasks, hMSC expansion was expected to be most impacted by cell-microcarrier aggregate size and aggregate settling as a result of increasing size. Several methods were investigated to reduce microcarrier aggregate size by promoting better mixing such as: increasing agitation speed, utilizing different impeller shapes or designs, adding a second impeller, and controlling gas sparge rate[2]. Limited success was observed when increasing the agitation speed of a single impeller and with adding a second impeller; in both situations, microcarrier aggregate size and distribution were able to be maintained early in the culture period but could not be sustained at higher cell confluence without a negative effect on cell viability, most likely resulting from an increase in hydrodynamic shear.

Next, the effect of gas sparging rate on microcarrier cluster formation was investigated, based on methods implemented in large-scale vaccine processes with suspension cells5. Two scenarios for gas sparging were evaluated to maintain a dissolved oxygen (DO) level of 50%: (1) intermittent sparging of air or oxygen gas when the DO level reached the setpoint; and (2) constant gas sparging starting 24 hours post-seeding using a mixture of air and nitrogen with oxygen supplementation when needed. As shown in Figure 4, a constant flow of gas delayed the formation of cell-microcarrier aggregates and promoted a more uniform microcarrier aggregate size and distribution throughout the working volume of the culture. Specifically, the air/nitrogen flow rate was adjusted relative to the oxygen flow rate to ensure that the combined gas flow from 24 to 96 hours post-seeding was 0.01 to 0.02 vessel volumes per minute (vvm). Using this approach, hMSC growth on denatured collagen dissolvable microcarriers was evaluated in 3 independent 5L bioreactor runs. As shown in Figure 5, a cell density of 35,000 cells/cm^2 (350,000 cells/mL) was achieved by day 4. Nutrient depletion and metabolite accumulation in the culture medium were monitored daily; these results indicated that medium replenishment was not required during the 4-day culture period. Representative images of cell confluence and microcarrier aggregate size are shown.

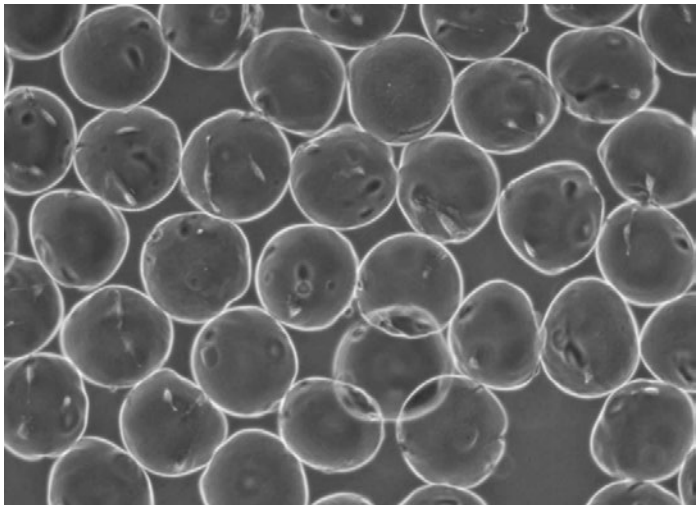

Figure 2. hMSC attachment to Corning® dissolvable microcarriers after 5 hours. Five hours after cell seeding, a 10 mL sample from the bioreactor was removed and transferred to an Ultra-Low Attachment (ULA) 6-well plate. Cell attachment and uniformity of distribution were assessed via microscopy. A representative image at 5X magnification is shown.

Even though continuous gas sparging was implemented to better control microcarrier aggregate size, a gradient of microcarrier clusters was still observed throughout the 5L liquid volume, which changed based upon the daily rate of cell growth. Therefore, acquiring a representative sample from the bioreactor at a defined dip tube location during the 4-day culture period was challenging. To address this concern and obtain a more accurate assessment of total yield on harvest day, samples were removed at different dip tube locations within the bioreactor. 10 mL samples were removed from 7 dip tube locations (Figure 6A). Cells were harvested from each sample and averaged to obtain a cell concentration (~27,000 cells/cm^2 or 7-fold expansion) more representative of the full culture volume (Figure 6B). This value was then compared to the number of cells recovered from the full 5L volume after microcarrier dissolution.

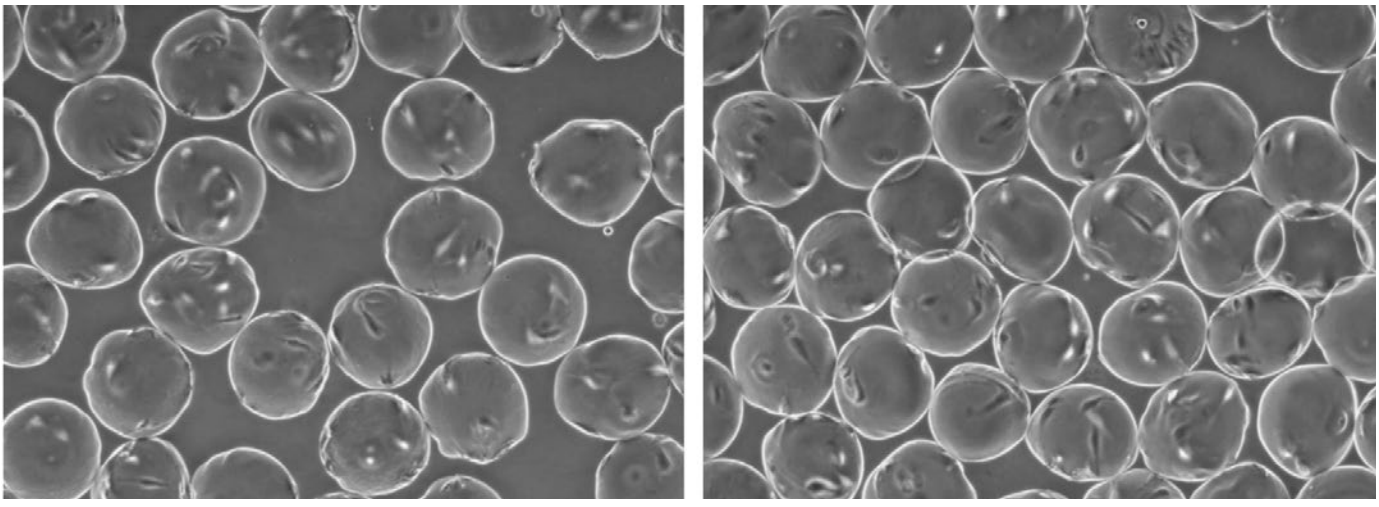

Figure 3. hMSC attachment to Corning dissolvable microcarriers after 24 hours. Twenty-four hours after cell seeding, a 10 mL sample from the bioreactor was removed and transferred to an Ultra-Low Attachment (ULA) 6-well plate. Cell attachment and uniformity of distribution were assessed via microscopy. Representative images at 5X magnification are shown.

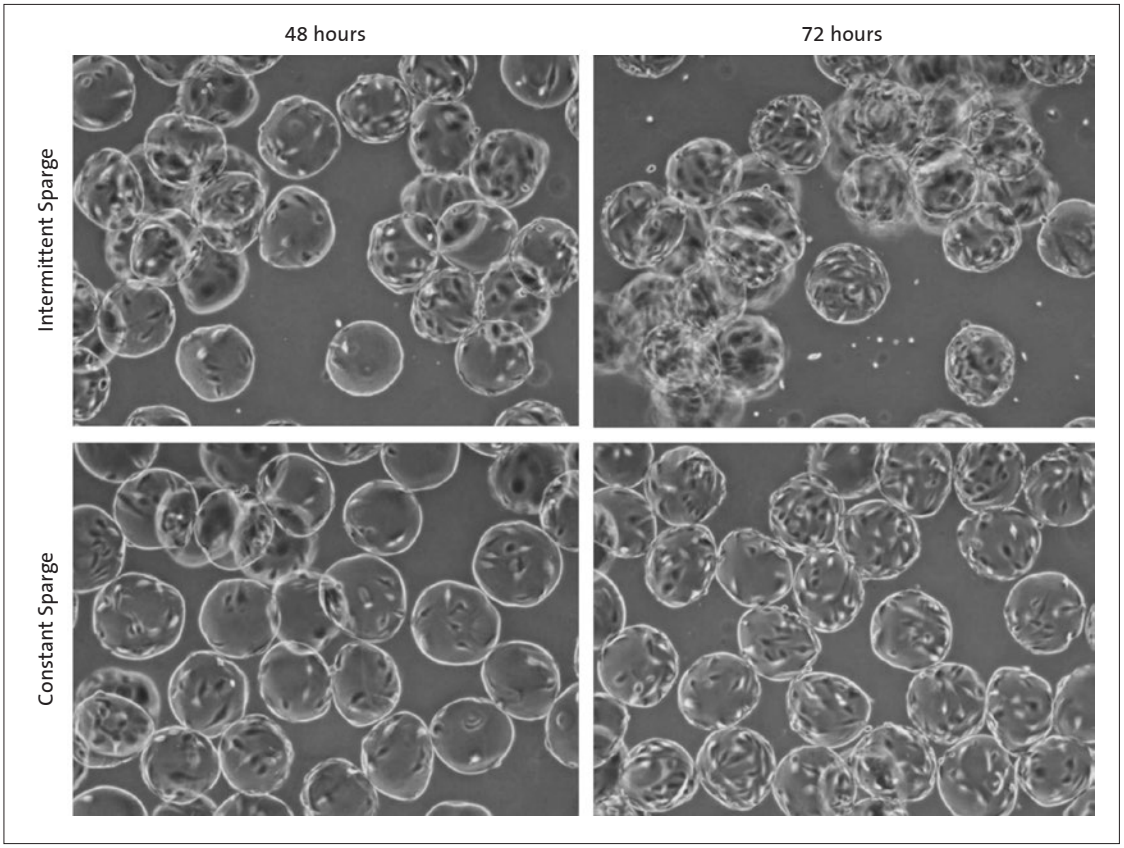

Figure 4. A comparison of gas flow rate on microcarrier aggregate size. Two gas flow rate protocols, intermittent sparge and constant sparge, were investigated to control microcarrier aggregate size with continued cell expansion. As shown, intermittent injections of gas through a ring sparger to maintain a DO set point resulted in microcarrier aggregates (~10 beads) by 48 hours, and the aggregate size continued to increase with cell growth by 72 hours (top images). In contrast, continuous gas sparging decreased the amount and size of microcarrier aggregates (bottom images), resulting in better cell surface area utilization of individual microcarriers. Representative images are shown at 5X magnification.

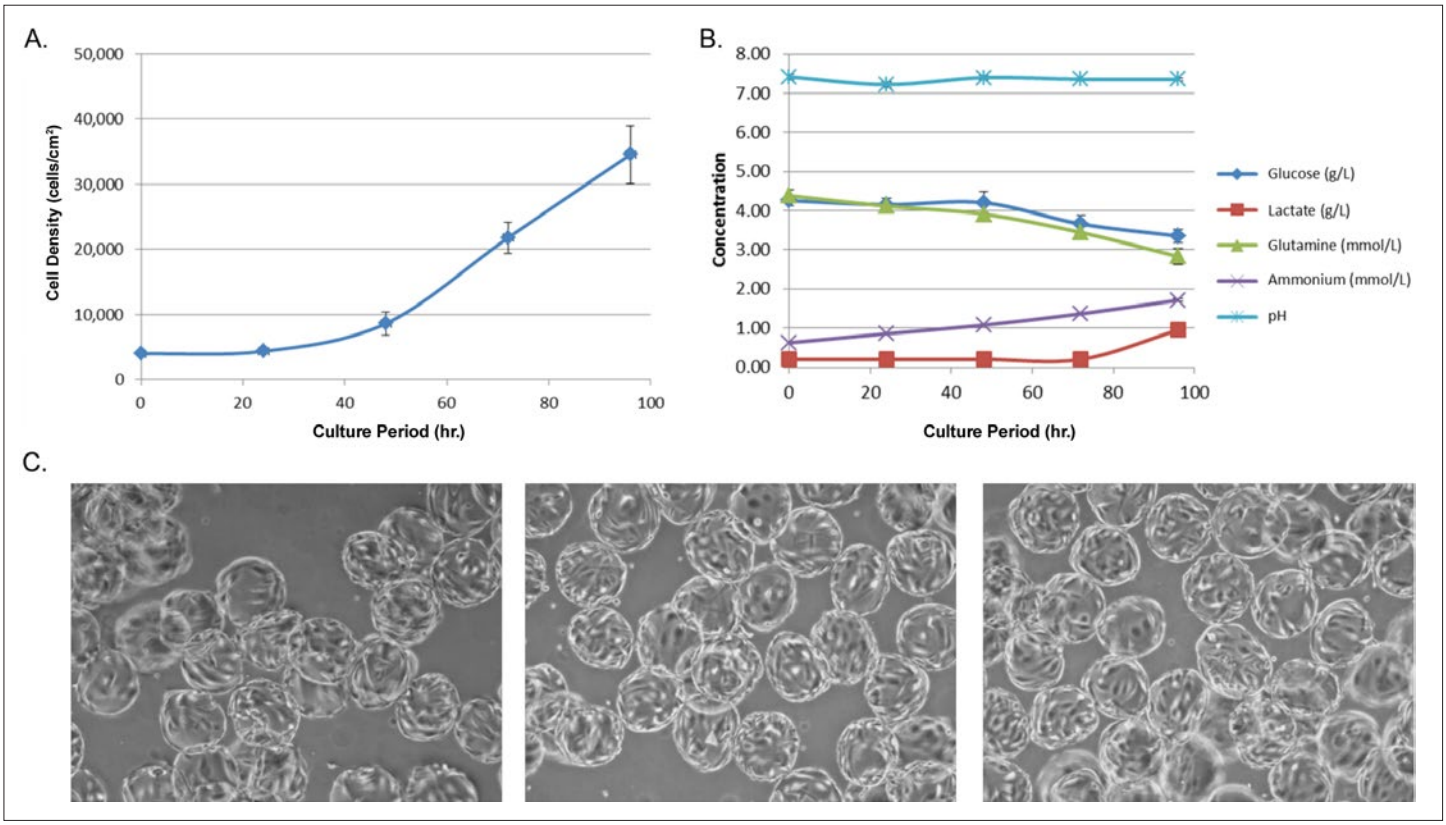

Figure 5. Cell expansion on dissolvable microcarriers in 5L bioreactors. (A) hMSCs were seeded on denatured collagen dissolvable microcarriers in 5L bioreactors, and daily samples were removed to assess cell growth; error bars represent the standard deviation of the average cell density per day from 3 independent bioreactor experiments. (B) Daily spent media samples were analyzed for pH, nutrient (glucose, glutamine) consumption, and metabolite (lactate, ammonium) accumulation. (C) Representative 5X images from the 3 bioreactor cultures show ~70% confluent hMSCs on dissolvable microcarriers at day 4.

Microcarrier Dissolution and Cell Recovery

Cells were harvested from day 4 bioreactor cultures by allowing microcarriers to settle, removing spent culture medium, and washing twice with DPBS to reduce the residual serum concentration. Next, a concentrated harvest solution containing pectinase, EDTA, and TrypLE™ were added, and stirring at 80 rpm to gently mix the culture. After 10 minutes, a sample was removed to check the dissolution process; no remaining microcarriers were seen, but cells remained in small clusters and slightly elongated. The agitation speed was increased to 100 rpm for an additional 10 minutes to promote a single cell suspension and fully rounded cells (Figure 7). The cell suspension was removed from the bioreactor, and the total cell yield and viability were calculated using a ViCell™ automated cell counter. The entire harvest process was completed by one operator in 1.5 hours. Comparison of the final cell yield from the full volume harvest to that based on the calculated average from the 7 dip tube locations, an average cell recovery of 96% (~1.4 billion cells/bioreactor) and cell viability of 93% was calculated for 3 independent bioreactor experiments.

Characterization of Recovered Cells

To confirm that hMSCs grown on dissolvable microcarriers retain their standard elongated morphology and ability to migrate in planar cultureware, a sample of cells on microcarriers (before microcarrier dissolution) was re-plated in 6-well plates, and cells were allowed to migrate from microcarriers to the plate surface (Figure 8). Similarly, a sample of cells recovered after microcarrier dissolution were directly replated in 6-well plates. As shown in Figure 8, cells successfully migrated from the dissolvable microcarriers and displayed elongated cell morphology similar to those cells seeded directly after microcarrier dissolution.

Secondly, to confirm the multipotency of cells recovered from dissolvable microcarriers, cells were immunostained for phenotypic markers (positive: CD73, CD90, CD105; negative: CD34, CD14) and also re-plated for directed differentiation into adipocytes, osteocytes, and chondrocytes (Figure 9). Cells recovered from dissolvable microcarriers in 3 independent bioreactor experiments (red bars) retained expression of phenotypic markers, as measured by flow cytometry, comparable to both the input cells from Corning® HYPER*Flasks*® used to seed the bioreactor cultures (blue bars) and to same-passage control cells harvested from T-flasks (green bars). Similarly, cells exposed to differentiation media for 2 to 3 weeks positively stained for markers of differentiation: lipid droplets via Oil Red O (adipocytes), calcium deposits via Alizarin Red (osteocytes), and chondrogenic pellets via Alcian Blue (chondrocytes) as shown. Lastly, recovered cells retained normal 46, XX karyotype (data not shown).

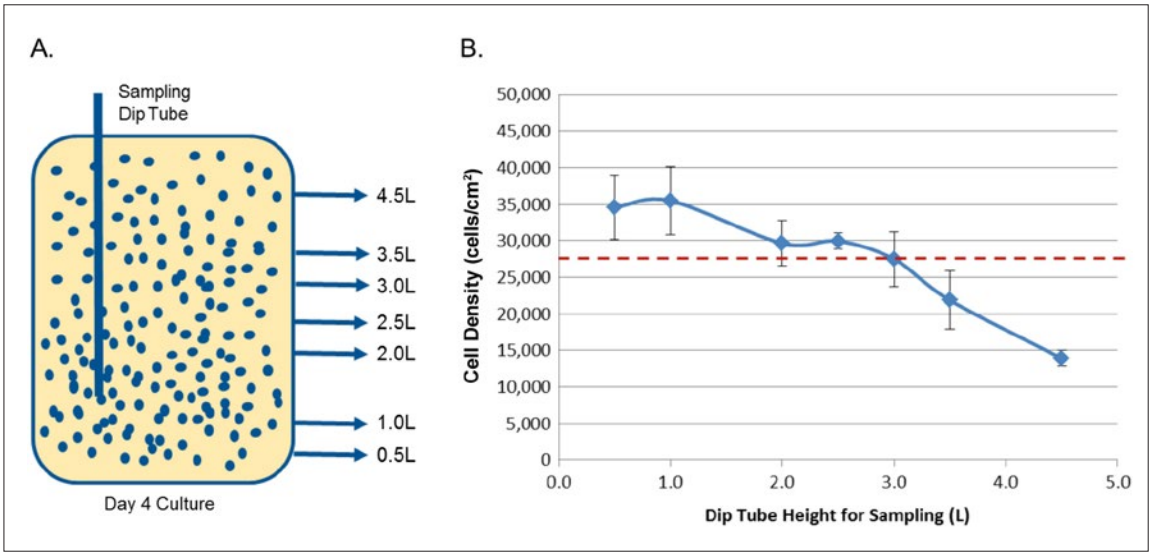

Figure 6. Investigation of dip tube position for bioreactor sampling. (A) Day 4 bioreactor cultures were sampled at various locations (or volume heights) within the bioreactor as shown by the position of the 7 blue arrows. (B) cells were enumerated from samples taken at these locations; error bars represent the standard deviation of 3 independent bioreactor runs. The average cell density across the 7 samples was calculated as 27,000 cells/cm² and shown by the red dotted line.

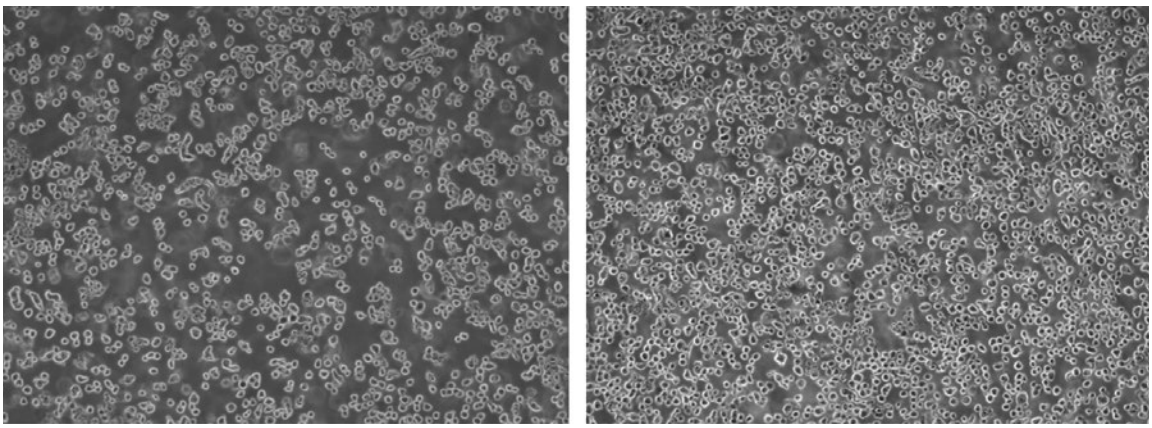

Figure 7. Cell suspensions after microcarrier dissolution. Microcarriers were dissolved in 5L bioreactors using a harvest solution of TrypLE, pectinase, and EDTA. Representative images of recovered hMSC suspensions post microcarrier dissolution are shown at 5X magnification.

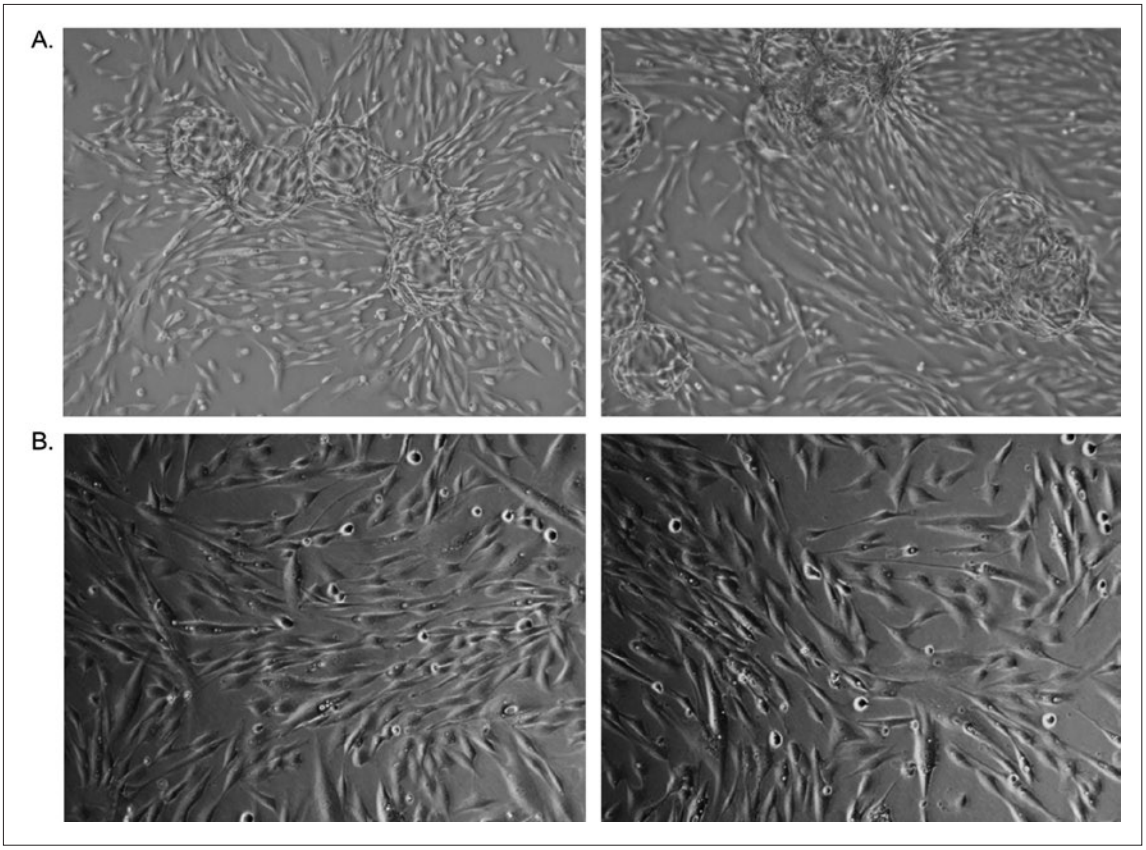

Figure 8. Standard hMSC morphology is maintained after culture on dissolvable microcarriers. (A) Before microcarrier dissolution, cells on microcarriers were transferred to 6-well plates, and cells migrated from microcarriers to the plate surface. (B) Similarly, cells recovered after microcarrier dissolution were directly re-plated in 6-well plates. Both cultures displayed typical hMSC elongated morphology. Representative images are shown at 5X (A) and 10X (B) magnifications.

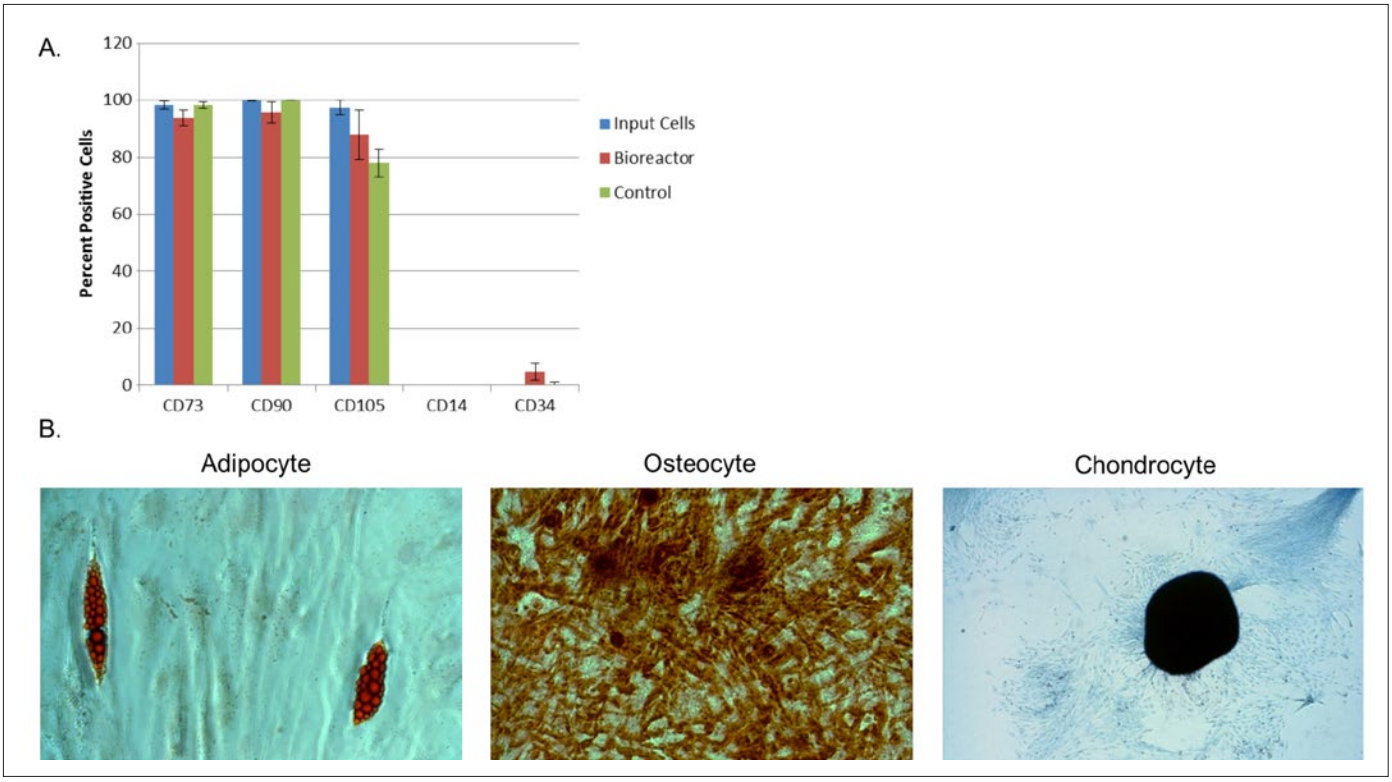

Figure 9. Characterization of cell multipotency after culture on dissolvable microcarriers. Cells recovered from dissolvable microcarriers in 3 independent bioreactor experiments were immunostained for phenotypic markers and analyzed by flow cytometry. (A) Cells recovered from microcarriers (red bars) retained high expression of positive markers of phenotype: CD73, CD90, and CD105 comparable to both the input cells from Corning® HYPER*Flasks*® used to seed the bioreactor cultures (blue bars) and to same-passage control cells harvested from T-flasks (green bars). Also, recovered cells were re-plated in 6-well plates for media-induced differentiation into adipocytes, osteocytes, and chondrocytes. (B) Cells exposed to differentiation media for 2 to 3 weeks positively stained for markers of differentiation: lipid droplets via Oil Red O (adipocytes, 20X magnification), calcium deposits via Alizarin Red (osteocytes, 10X magnification), and chondrogenic pellets via Alcian Blue (chondrocytes, 2.5X magnification).

Summary

We demonstrated 7-fold expansion of hMSCs on denatured collagen dissolvable microcarriers in 5L bioreactors. Continuous agitation was used to achieve uniform cell attachment and expansion, and microcarrier aggregate size was maintained through continuous gas sparging. We recovered 96% of cells from dissolved microcarrier cultures using a harvest solution of EDTA, pectinase, and TrypLE™, and the recovered cells retained standard elongated morphology, normal karyotype, and multipotency, as measured by FACS analysis of phenotype and directed differentiation into adipocytes, osteocytes, and chondrocytes.

References

1. Viswanathan S, Hematti P, eds. 2017. Mesenchymal stromal cells: Translational pathways to clinical adoption. Ch. 6,139-178.
2. Schnitzler AC, Verma A, Kehoe DE, Jing D, Murrell JR, Der KA, Aysola M, Rapiejko PJ, Punreddy S, Rook MS, 2016. Bioprocessing of human mesenchymal stem/stromal cells for therapeutic use: Current technologies and challenges. Biochemical Engineering Journal. 108:3-13.
3. de Soure AM, Fernandes-Platzgummer A, da Silva CL, Cabral JMS, 2016. Scalable microcarrier-based manufacturing of mesenchymal stem/stromal cells. Journal of Biotechnology. 236:88-109.
4. Strathearn KE, Pardo AMP, 2014. Parameters to consider when expanding cells on Corning microcarriers. Corning Application Note CLS-AN-243. https://www.corning.com/worldwide/en/products/life-sciences/products/bioprocess/microcarriers.html.
5. Hu W, Berdugo C, Chalmers JJ, 2011. The potential of hydrodynamic damage to animal cells of industrial relevance: Current understanding. Cytotechnology. 63:445-460.

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