Effective Bead-to-Bead Transfer of Cells in Serum-free Medium using Polystyrene Corning® Enhanced Attachment Microcarriers

Application Note

CORNING

Wang Xuebin, Liu Jian
Corning Life Sciences Asia Technology Center
Corning Incorporated
Shanghai, China

Introduction

Microcarrier-based stirred culture in a bioreactor system provides an ideal format for high yield culture of anchorage-dependent cells for bioprocess applications such as vaccine production, recombinant protein production, and stem cell therapy.

To achieve large-scale cell production, a microcarrier culture scale-up strategy is necessary. Several approaches have been developed to achieve the transfer of cells from one microcarrier to another including enzymatic dissociation and bead-to-bead transfer. However, enzymatic dissociation introduces additional process steps (washing, inactivation, and removal of the enzyme) that are not feasible at a large scale. Bead-to-bead cell transfer provides an easy scale-up process that involves the addition of fresh microcarriers and fresh medium to a confluent culture.

The success of bead-to-bead transfer depends on the adhesive property of the cells and microcarrier surface characteristics[1]. Only a limited number of microcarriers and cell types meet the requirements[2-5].

This report describes a method for expansion of anchorage-dependent cells on polystyrene Corning microcarriers with an enhanced attachment surface treatment in serum-free cell culture medium. Culture scale-up was accomplished via bead-to-bead expansion by the addition of fresh microcarriers without the need for enzymatic dissociation.

Materials and Methods

Cell Culture

Vero cells (ATCC® CCL-81™) were cultured in serum-free VP-SFM medium (ThermoFisher Cat. No. 11681020) supplemented with 4 mM L-glutamine (Corning Cat. No. 25-005-CI) and 0.5% penicillin/streptomycin (Corning Cat. No. 30-002-CI) at 37°C in a humidified atmosphere with 5% CO_2. The cells were maintained in T-75 flasks (Corning Cat. No. 3275) and subcultured with 1X TrypLE™ enzyme prior to microcarrier inoculation.

Microcarrier Preparation

Sterile, ready-to-use Corning enhanced attachment microcarriers, with Corning CellBIND® surface treatment (Corning Cat. No. 3779), were reconstituted in water according to manufacturer's instructions and used directly. The water was removed and the microcarriers were diluted in culture medium prior to cell seeding.

Fresh microcarriers were identified during bead-to-bead transfer by fluorescence staining. The fresh microcarriers were stained with 1 mg/mL rhodamine (MilliporeSigma Cat. No.83695, 250 mg) in 99.9% ethanol at room temperature for at least 2 hrs. They were used directly after five washes with 10 mL PBS.

Bead-to-bead Transfer of Vero Cells

Vero cells were grown in the presence of 1 g (6 cm^2/mL) Corning enhanced attachment microcarriers in a 125 mL Corning ProCulture glass spinner flask (Corning Cat. No. 4500-125) containing 60 mL of VP-SFM medium supplemented with 2 mM L-glutamine. The medium was seeded with 10,000 cells/cm^2 that were cultured under stirring at 35 rpm. After 4 days of culture, the culture was transferred to a 500 mL Corning glass spinner flask (Corning Cat. No. 4500-500) and scaled up by bead-to-bead transfer after gentle homogenization. Microcarriers in the spinner flask were allowed to settle and approximately 80% of the culture medium was then removed. Subsequently, fresh fluorescent labeled microcarriers (2 g [12 cm^2/mL]) were added to the spinner flask and the volume was adjusted to 150 mL with VP-SFM medium. The culture was stirred intermittently at 35 rpm for 3 min. every 45 min. for 24 hrs. and then continuously stirred at 40 rpm. In total, three bead-to-bead transfers were conducted, increasing the working volume from 60 mL to 1L, while stirring was increased from 35 rpm to 50 rpm to reduce cell aggregation (Figure 1). Cell growth was quantified by fluorescence staining of the adherent cells.

Quantification of Vero Cell Growth

Cell growth was evaluated by fluorescence staining with Calcein AM dye (Corning Cat. No. 354216). The 10,000-fold stock was diluted to prepare a 2-fold staining solution. Adherent cells were stained for 5 min. at room temperature in the dark. After staining, the dye was removed, and the cells were rinsed twice with PBS before imaging using a fluorescence microscope (Olypums, X-100).

Vero Cell Viability Assay

The microcarrier suspension was transferred into a centrifuge tube and the microcarriers were allowed to settle. Then, the medium was removed and the microcarriers were washed with PBS. Pre-warmed TrypLE was added and the mixture was incubated at 37°C for 5 to 10 min. The viability of the harvested cells was determined by Trypan blue staining.

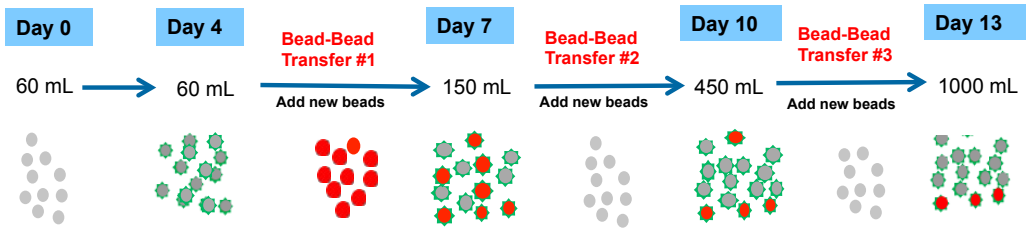
Results

Vero Cells Efficiently Attach to Corning® Enhanced Attachment Microcarriers

The microcarrier cultures in spinner flasks were mixed continuously for 2 hrs. during the cell attachment phase. Figure 2 shows microscopic images of a culture at the time of cell seeding (0 hr.) and 2 hrs. after seeding. At 0 hr., nearly all cells were unattached. After 2 hrs., very few unattached cells were visible in the medium; more than 95% attachment was achieved within 2 hrs. of cell seeding. Thus, Vero cells efficiently attach to Corning enhanced attachment microcarriers in serum-free medium.

Vero Cells Effectively Expand on Corning Enhanced Attachment Microcarriers

To be applicable in biopharmaceutical production, microcarrier cultures must demonstrate efficient cell expansion and high cell yield. Figure 3 illustrates the expansion of Vero cells cultured on Corning microcarriers in spinner flasks. The upper panels are microscopic images of Vero cells obtained over 4 days of cell expansion. The cells were stained with Calcein AM to better visualize cell coverage on the microcarriers. As shown in the lower panels of Figure 3, nearly all the microcarriers were entirely covered with cells on day 4, demonstrating the suitability of the protocol for Vero cell expansion.



	Seeding	Bead-Bead Transfer #1 Days 4-7	Bead-Bead Transfer #2 Days 7-10	Bead-Bead Transfer #3 Days 10-13	Final Volume/ Surface Area
Microcarrier	1g/360 cm^2	3g/1080 cm^2	9g/3240 cm^2	20g/750 cm^2	20g/7200 cm^2
Volume	60 mL	150 mL	450 mL	1000 mL	1000 mL
Stirring	35 rpm	40 rpm	45 rpm	50 rpm	N/A
Surface area/ mL	6 cm^2/mL	7 cm^2/mL	7 cm^2/mL	7 cm^2/mL	7 cm^2/mL

Figure 1. Work flow for scaling up Vero cell culture from 60 mL to 1L by bead-to-bead transfer using polystyrene Corning enhanced attachment microcarriers.

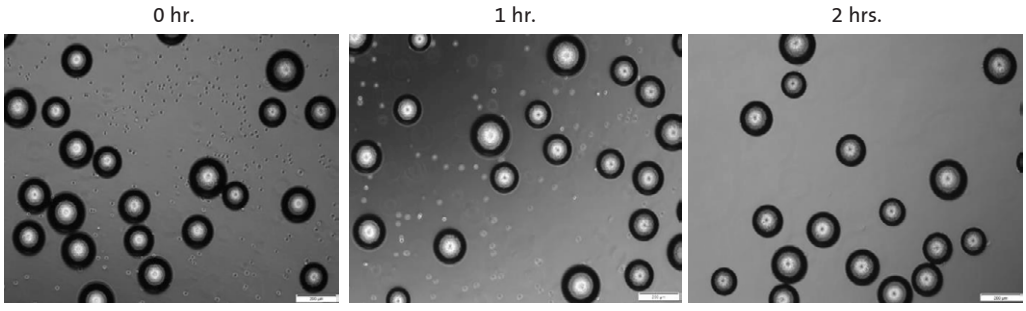


Figure 2. Vero cells efficiently attach to Corning enhanced attachment microcarriers with serum-free medium. The microscope images reveal that nearly all cells attached to microcarriers within 2 hrs. of cell seeding. Representative images were selected from 3 cultures, all with similar results. Scale bar = 200 µm.

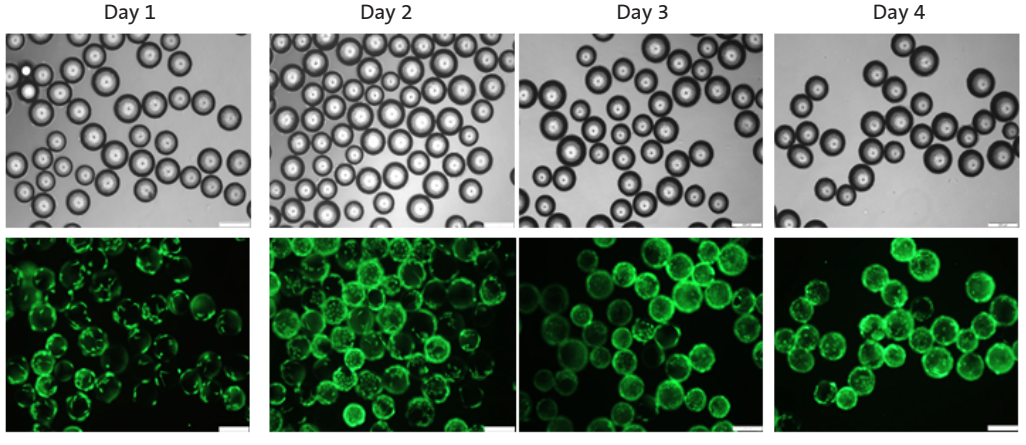


Figure 3. Vero cells efficiently expand on Corning enhanced attachment microcarriers in a spinner flask with serum-free medium. Calcein AM staining on days 1 to 4 indicate consistent and uniform cell confluence on microcarriers in serum-free medium. Scale bar = 200 µm.

Bead-to-bead Transfer of Vero Cells in Serum-free Medium

Traditionally, cells cultured on two-dimensional surfaces are subcultured using enzymes that digest protein interactions between cells and the surface. However, the repeated use of enzymes during cell passaging can damage the cellular receptors required for cell attachment and intracellular communication. Therefore, we investigated cell expansion by the addition of new microcarriers to promote cell migration and growth in serum-free medium without enzymatic dissociation. Three bead-to-bead transfers were carried out, increasing the working volume from 60 mL to 1L. Cells were seeded onto non-labeled microcarriers in a spinner flask, as described above. Rhodamine-labeled microcarriers were added to the culture as indicated in Figure 4. After 24 hrs. of intermittent agitation, cells had colonized the rhodamine-labeled microcarriers (indicated in red in Figure 4), indicating that cells migrate to fresh microcarriers with intermittent agitation. After 13 days, the cell number increased from 3.6 x 10^6 to 6.3 x 10^8, corresponding with a fold expansion of 175. We observed two common bead-to-bead transfer strategies: either the Vero cells detached from a confluent microcarrier and reattached to a freshly prepared unoccupied microcarrier, or they formed bridges between confluent and fresh microcarriers (Figure 5).

Discussion

The subculture method is one of the critical factors to be considered in the scaling up of microcarrier culture of anchorage-dependent mammalian cells. Bead-to-bead cell transfer provides an attractive approach to the scale-up operation. Not only does it minimize the physiological damage caused by traditional cell-harvesting methods, it also simplifies the scale up process and enhances the applicability of the microcarrier technology in the commercial production of biological products.

We demonstrated that Vero cell culture can be scaled up by bead-to-bead transfer in serum-free medium. Intermittent agitation allowed the beads to fully contact with each other and the cells to migrate from confluent microcarriers to bare ones. Increasing the agitation speed after intermittent agitation effectively prevented excessive cell-seeded bead aggregation. While we observed aggregation as the cell density of the culture increased, the aggregate size decreased after the addition of fresh microcarriers. The formation of small cell-seeded microcarrier aggregates is crucial for bead-to-bead migration and should not be eliminated or prevented.

Cell characteristics such as migration and cell-surface binding properties affect the efficiency of bead-to-bead transfer[6]. Cells could detach from the microcarrier surface during cell division and re-attach to empty carriers. In addition, we observed that cells formed bridges between microcarriers. This is only possible when the cells migrate during the exponential growth phase[1]. Therefore, efficient bead-to-bead transfer requires rapidly proliferating cells and must be carried out under conditions where the cell division capacity is not reduced by overconfluence and/or metabolite inhibition.

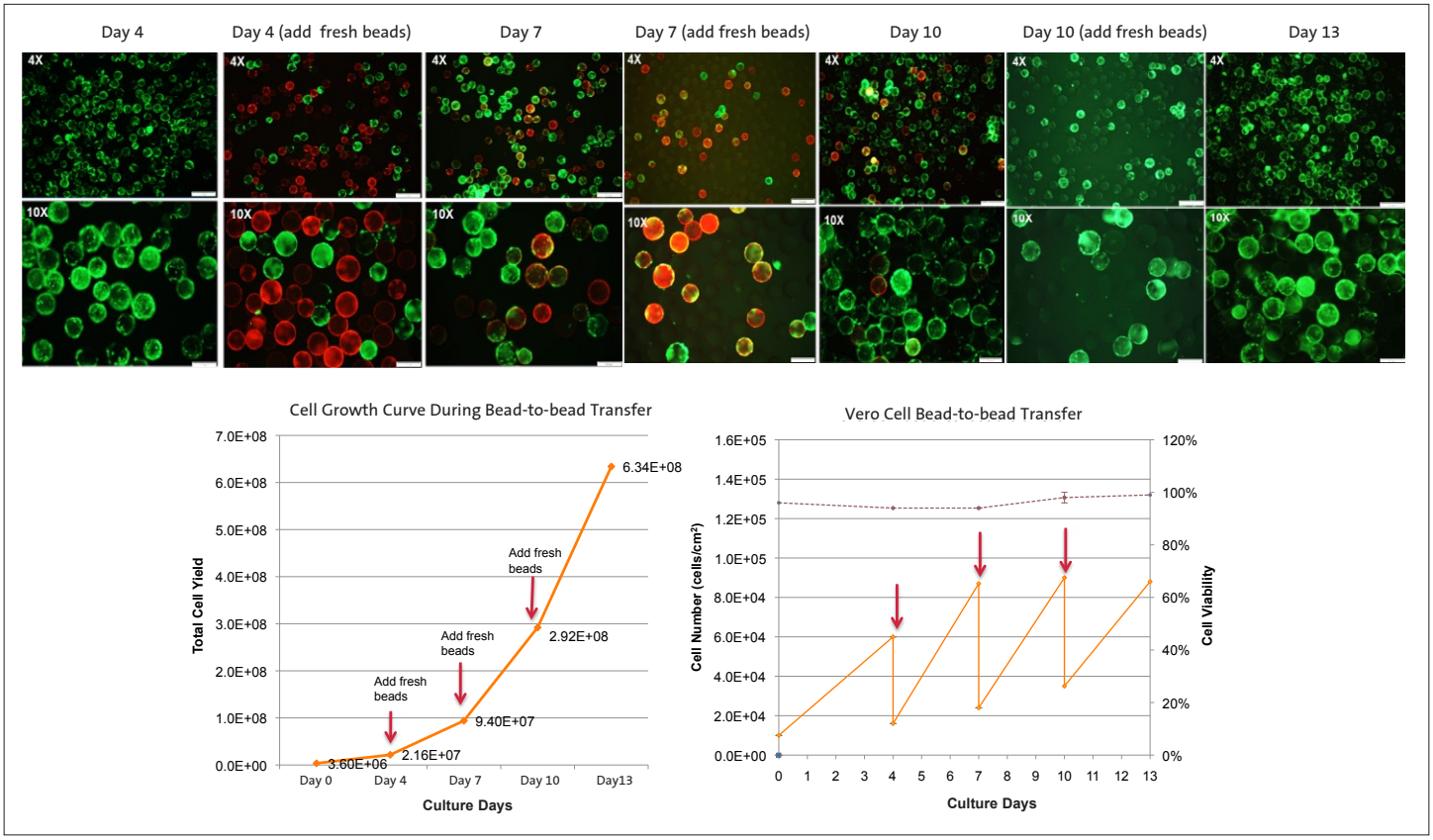


Figure 4. Vero cell culture scale up by bead-to-bead transfer on Corning® enhanced attachment microcarriers in serum-free medium. Freshly added microcarriers were stained with rhodamine (red fluorescence) and the cells were stained with Calcein AM (green fluorescence). The fluorescence microscopic images (upper panels) illustrate cell migration to freshly added microcarriers. Scale bar (4X) = 500 µm, Scale bar (10X) = 200 µm. The growth curve shows Vero cell expansion in serum-free media after three passages of healthy cells. The lower right panel shows cell numbers and cell viability during bead-to-bead transfer of Vero cells using polystyrene Corning microcarriers to increase the working volume from 60 mL to 1L, resulting in >175-fold cell expansion after 13 days of culture.

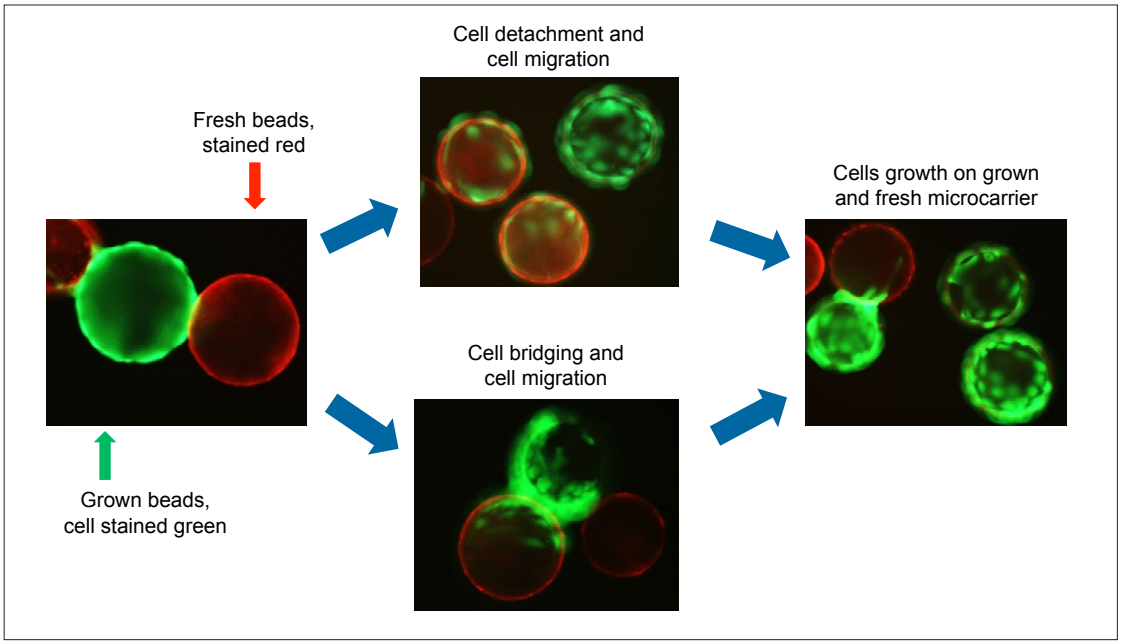


Figure 5. Bead-to-bead transfer strategies of Vero cells. Cells detached from a confluent microcarrier and re-attached to a freshly added microcarrier, or cells formed bridges between confluent and fresh microcarriers. Scale bar = 100 µm.

Conclusions

- Vero cells exhibit efficient cell attachment on Corning® enhanced attachment microcarriers in serum-free medium (>95% attachment was achieved within 2 hrs. of cell seeding).
- Vero cell culture can be scaled up to 1L of working volume by bead-to-bead transfer using intermittent agitation (stirring at 35 rpm for 3 min. every 45 min. for 24 hrs.).
- Cell-seeded microcarrier aggregates contained no more than 3 microcarriers per aggregate, which was achieved by increasing the agitation speed after each bead-to-bead transfer.
- Vero cell expansion was obtained by the addition of fresh beads without the need for enzymatic dissociation, and cumulative cell expansion of >175-fold was achieved after 13 days of culture.

References

1. Leber J, et al., (2016). Microcarrier choice and bead-to-bead transfer for human mesenchymal stem cells in serum-containing and chemically defined media. Process Biochemistry, 59:255-265.
2. Hervy M, et al., (2014). Long term expansion of bone marrow-derived hMSCs on novel synthetic microcarriers in xeno-free, defined conditions. PLoS One, 9:e92120.
3. Chen AK, Reuveny S, Oh SK (2013). Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: achievements and future direction. Biotechnology Advances, 31:1032-1046
4. Luo F, Sun H, Geng T, Qi N (2008). Application of Taguchi's method in the optimization of bridging efficiency between confluent and fresh microcarriers in bead-to-bead transfer of Vero cells. Biotechnology Letters, 30:645-649.
5. Wang Y, Ouyang F (1999). Bead-to-bead transfer of Vero cells in microcarrier culture. Cytotechnology, 21:211-221.
6. Merten O-W (2015). Advances in cell culture: anchorage dependence. Philosophical Transactions B 370:20140040.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

CORNING

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

ASIA/PACIFIC
Australia/New Zealand
t 61 427286832
China
t 86 21 3338 4338
f 86 21 3338 4300
India
t 91 124 4604000
f 91 124 4604099
Japan
t 81 3-3586 1996
f 81 3-3586 1291
Korea
t 82 2-796-9500
f 82 2-796-9300
Singapore
t 65 6572-9740
f 65 6861-2913
Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE
CSEurope@corning.com
France
t 0800 916 882
f 0800 918 636
Germany
t 0800 101 1153
f 0800 101 2427
The Netherlands
t 020 655 79 28
f 020 659 76 73
United Kingdom
t 0800 376 8660
f 0800 279 1117
All Other European Countries
t +31 (0) 206 59 60 51
f +31 (0) 206 59 76 73

LATIN AMERICA
grupoLA@corning.com
Brasil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400