

# Expansion of Vero Cells on Corning® Enhanced Attachment Microcarriers and Demonstration of Scalability in a Benchtop Bioreactor

CORNING

## Application Note

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### Introduction

To address the need for increased cell yields, Corning has commercialized a panel of sterile, ready-to-use microcarriers for the culture of anchorage-dependent cell lines. Corning microcarriers are available with a variety of surface treatments to support the growth of different cell types. Microcarriers provide an ideal platform for improving the cell yield of anchorage-dependent cell lines by significantly increasing the culture surface area-to-volume ratio. Corning microcarriers provide many of the same advantages as traditional beads with the added benefit of being sterile and ready-to-use, which eliminates the need for time-intensive swelling, washing, and sterilization that is necessary when using other microcarriers.

Here we report on the expansion and scale-up of Vero cells on Corning enhanced attachment microcarriers for vaccine applications. Vero cells are an ideal candidate for microcarrier culture because they are anchorage-dependent and are required in large numbers for cell line-based vaccine production.

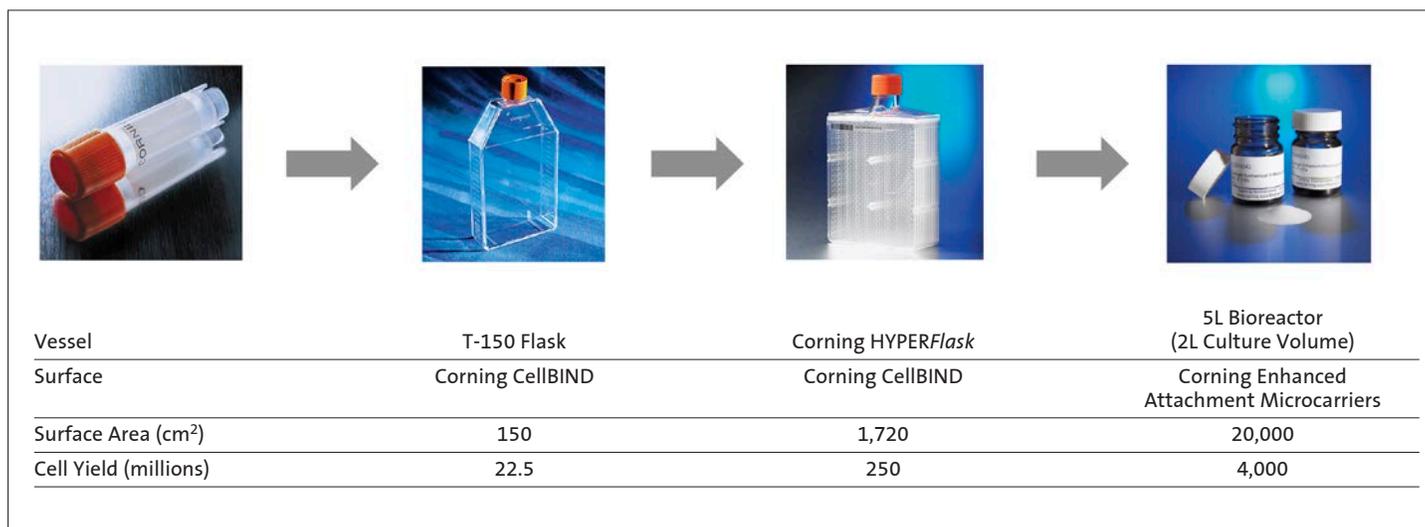
### Materials and Methods

#### Cell Culture

Vero cells (ATCC® CCL-81®) were cultured in DMEM (Corning Cat. No. 10-013-CV) supplemented with 10% FBS (Corning Cat. No. 35-010-CV), 1X MEM NEAA (Corning Cat. No. 25-025-CI), and 2 mM L-glutamine (Corning Cat. No. 25-005-CI). The cells were maintained in 150 cm<sup>2</sup> cell culture flasks with Corning CellBIND® surface (Corning Cat. No. 3291) and expanded in Corning CellBIND HYPERFlask® cell culture vessels (Corning Cat. No. 10030) prior to bioreactor inoculation (Figure 1).

#### Microcarrier Protocol Development in 250 mL Glass Spinner Flask

Glass spinner flasks (250 mL; Corning Cat. No. 4500-250) were used for attachment and expansion of Vero cells on Corning microcarriers. The final working volume in the glass spinner flasks was 150 mL. Corning enhanced attachment microcarriers (Corning Cat. No. 3779) were added to the glass spinner flasks to provide a total culture surface of 1,500 cm<sup>2</sup> (10 cm<sup>2</sup>/mL). The microcarriers were added to each flask in 112.5 mL serum-free medium. Fifteen milliliters of Vero cells at 1.5 x 10<sup>6</sup> cells/mL were added to the culture for a seeding concentration of



**Figure 1. Scale up protocol for Vero cells.** After thawing from a cryogenic vial, cells were maintained in a 150 cm<sup>2</sup> cell culture flasks with Corning CellBIND surface. Cells were then expanded in a Corning HYPERFlask cell culture vessel to inoculate the bioreactor. Typical cell yields from culture flasks and microcarrier cultures were 1.5 x 10<sup>5</sup> and 2.0 x 10<sup>5</sup> cells/cm<sup>2</sup>, respectively.

15,000 cells/cm<sup>2</sup>. The FBS concentration during cell attachment was ~0.1% to 0.5%. Cells were allowed to attach for 2 to 3 hours with continuous agitation (40 rpm) in a 5% CO<sub>2</sub> incubator at 37°C. Following cell attachment, the medium was adjusted to 5% FBS and a final culture volume of 150 mL. The culture was incubated for 5 to 7 days at 40 rpm. The medium was replenished on days 3 and 5 by allowing the microcarriers to settle, removing 50% of the culture volume, and then replacing it with fresh medium.

### Bioreactor Runs

Scale-up experiments were performed in a Biostat® B bioreactor with a 5L single wall reaction vessel (Sartorius Cat. No. BB-39204774). For bioreactor experiments, the medium and microcarriers were incubated in the 5L reaction vessel overnight prior to cell seeding. For a 2L final culture volume, Corning® enhanced attachment microcarriers at 10 cm<sup>2</sup>/mL were incubated in 1.3L serum-free medium overnight. Compressed air was added through the overlay at 30%. The pH was set at 7.4 and maintained by CO<sub>2</sub> and sodium bicarbonate addition. Following the overnight incubation, 300 mL Vero cells were added at 15,000 cells/cm<sup>2</sup>. The FBS concentration during cell attachment was ~0.1% to 0.5%. The culture was mixed continuously at 40 rpm with a marine impeller for the entire attachment phase (2 to 3 hours). Following cell attachment, the culture was adjusted to 5% FBS and a final culture volume of 2L. The agitation rate was increased to 100 rpm for the remainder of the expansion phase. The medium was replenished on days 3 and 5 by allowing the microcarriers to settle, removing 50% of the culture volume, and then replacing it with fresh medium.

The culture was sampled daily in order to monitor cell growth and culture conditions. To measure cell growth, samples were lysed by incubating with Reagent A100 (Chemometec Cat. No. 910-0003) and stabilized with Reagent B (Chemometec Cat. No. 910-0002). Nuclei were counted using Via1-Cassettes™ (Chemometec Cat. No. 941-0012) on a Chemometec NucleoCounter® NC-200 automated cell counter. Microscope images were collected daily to evaluate cell growth on the microcarriers. Calcein AM (Life Technologies Cat. No. C1430) staining was used to visualize live cells. Metabolic analysis was performed daily with a Nova BioProfile® 400 analyzer to measure nutrient and metabolite concentrations.

### Results

#### Optimal Conditions for Expansion of Vero Cells on Corning Enhanced Attachment Microcarriers in Glass Spinner Flasks and a Benchtop Bioreactor

Table 1 summarizes the different process parameters that were optimized during the protocol development phase in 250 mL glass spinner flasks. Corning enhanced attachment microcarriers were identified as the optimal microcarrier surface for Vero cells based upon efficient cell attachment and cell expansion. Using the optimal conditions, cell yields routinely exceeded 2 x 10<sup>6</sup> cells/mL or 2 x 10<sup>5</sup> cells/cm<sup>2</sup> (>10-fold expansion). Equivalent performance was observed when the protocol was directly transferred to 1L glass spinner flasks. To demonstrate scalability, the protocol was transferred to a 5L benchtop bioreactor. The main parameters that required additional optimization for the bioreactor were the aeration and agitation conditions.

**Table 1.** Optimal cell attachment and cell expansion conditions for Vero cells on Corning enhanced attachment microcarriers in glass spinner flasks and a benchtop bioreactor.

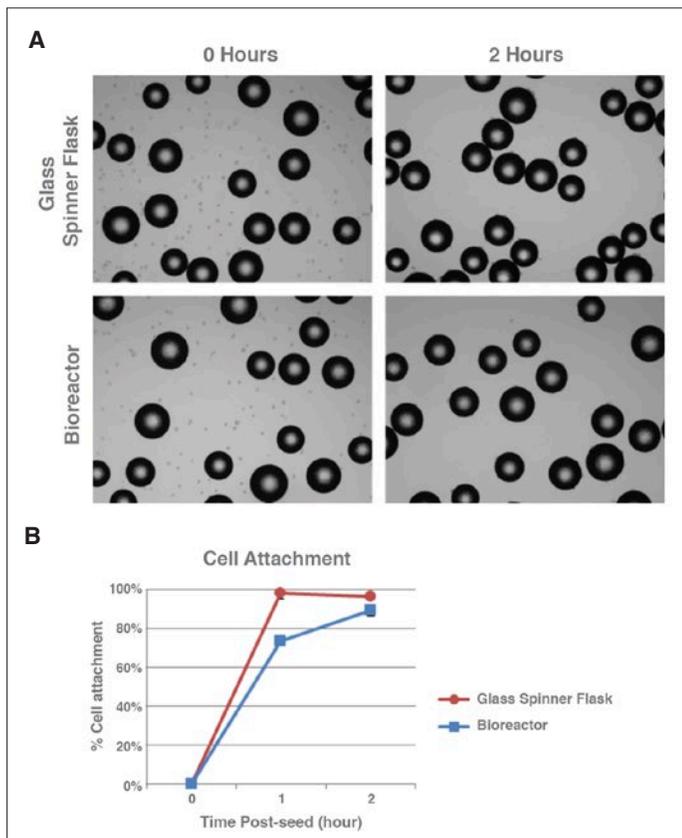
| Attachment Phase            |  |  |
|-----------------------------|--|--|
|                             | Glass Spinner Flask  | Bioreactor   |
| Medium                      | DMEM   | DMEM   |
| Serum                       | 0.1% to 0.5% FBS   | 0.1% to 0.5% FBS   |
| Microcarrier surface        | Enhanced attachment  | Enhanced attachment  |
| Microcarrier concentration  | 10 cm <sup>2</sup> /mL; 0.1 mL/cm <sup>2</sup> ; 27.8 g/L          | 10 cm <sup>2</sup> /mL; 0.1 mL/cm <sup>2</sup> ; 27.8 g/L  |
| Cell seeding density        | 15,000 cells/cm <sup>2</sup>                                       | 15,000 cells/cm <sup>2</sup>                               |
| Cell seeding culture volume | 85% final culture volume   | 85% final culture volume                                   |
| Agitation rate              | 40 rpm, continuous mixing  | 40 rpm, continuous mixing                                  |
| Impeller                    | N/A  | Marine   |
| pH                          | N/A  | 7.4 (controlled by CO <sub>2</sub> and sodium bicarbonate) |
| Aeration                    | N/A  | 30% compressed air (overlay)                               |
| Attachment duration         | 2 to 3 hours   | 2 to 3 hours   |
| Expansion Phase             |  |  |
| Medium                      | DMEM (+2 mM L-glutamine, 1X MEM NEAA)                              | DMEM (+2 mM L-glutamine, 1X MEM NEAA)                      |
| Serum                       | 5%   | 5%   |
| Agitation rate (GSF)        | 40 rpm, continuous mixing  | 100 rpm, continuous mixing                                 |
| Media re-feed               | ½ volume media exchange on days 3 and 5                            | ½ volume media exchange on days 3 and 5                    |
| Duration of culture         | 5 to 7 days  | 5 to 7 days  |
| Fold-expansion              | >10-fold   | >10-fold   |
| Cell concentration          | 1.5 x 10 <sup>5</sup> to 2 x 10 <sup>5</sup> cells/cm <sup>2</sup> | >2 x 10 <sup>5</sup> cells/cm <sup>2</sup>                 |

## Efficient Attachment of Vero Cells to Corning® Microcarriers in 1L Glass Spinner Flasks and a 5L Benchtop Bioreactor

In order to achieve robust cell expansion on the microcarriers, the cells must first attach efficiently. Figure 2 summarizes the cell attachment results for microcarrier cultures in a 1L glass spinner flask and a benchtop bioreactor. The microcarrier cultures were mixed continuously for 2 hours during the cell attachment phase for both vessels. Panel A shows microscope images of each culture at the time of cell seeding (0 hours) and 2 hours after seeding. At 0 hours, nearly all of the cells can be visualized as unattached cells in the medium. After 2 hours, very few unattached cells are visible in the medium and cells can be detected on the microcarriers. The cell attachment process can be quantified by counting the number of unattached cells in the microcarrier cultures. Panel B shows the cell attachment percentage following cell addition. Cell attachment exceeded 90% in both vessels approximately 2 hours after seeding.

## Efficient Expansion of Vero Cells on Corning Microcarriers in 1L Glass Spinner Flasks and a 5L Benchtop Bioreactor

To be used for biopharmaceutical production, microcarrier cultures must also demonstrate efficient cell expansion and high cell yield. Cell expansion should meet or exceed the performance observed in 2D cultures. Figure 3 shows the cell expansion data for Vero cells on Corning microcarriers cultured in 1L glass spinner flasks and a benchtop bioreactor. Panel A shows microscope

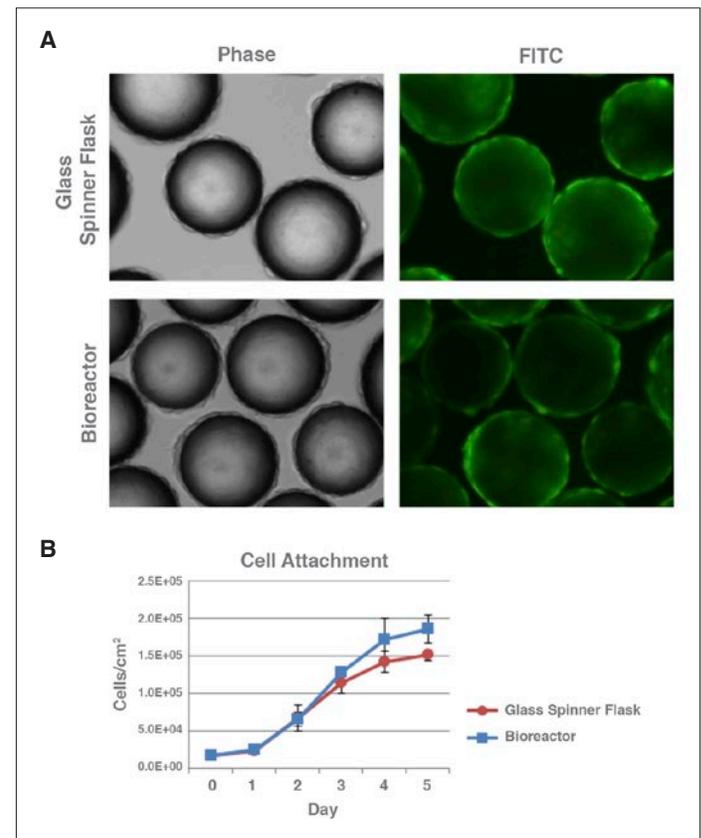


**Figure 2.** Efficient attachment of Vero cells to Corning enhanced attachment microcarriers in a 1L glass spinner flask and a 5L benchtop bioreactor. The final culture volume in each vessel was fixed at 1L. Microscope images (Panel A) and cell quantitation data (Panel B) indicate nearly all the cells were attached to microcarriers within 2 hours of cell seeding (n = 2).

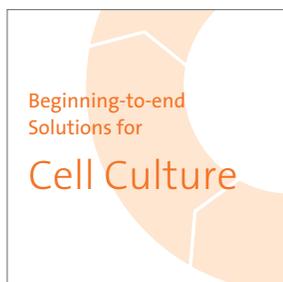
images of Vero cells on day 5 of cell expansion. Cells were stained with Calcein AM to better visualize cell coverage on the microcarriers (right panels). Cell expansion was quantified by lysing the cells and counting nuclei (Panel B). Vero cell expansion in both vessels demonstrated equivalent or improved performance relative to 2D cultures. Typical cell concentration for Vero cells in 2D culture ranges from  $1.2 \times 10^5$  to  $1.5 \times 10^5$  cells/cm<sup>2</sup> (data not shown). Additionally, the microscope images and Calcein AM staining indicated that nearly all of the microcarriers were covered with cells, which demonstrated the robustness of the protocol for Vero cell attachment and expansion.

## Conclusions

- ▶ Vero cells exhibited efficient cell attachment on Corning enhanced attachment microcarriers (>90% attachment was achieved within 2 hours of cell seeding).
- ▶ Vero cells exhibited robust cell expansion on Corning enhanced attachment microcarriers with cell concentrations ranging from  $1.5 \times 10^5$  to  $2.0 \times 10^5$  cells/cm<sup>2</sup> (>10-fold expansion) after 5 days in culture.
- ▶ Scalability was demonstrated by transferring the protocol from a 250 mL glass spinner flasks to a benchtop bioreactor.
- ▶ Corning enhanced attachment microcarriers provide an effective format for high yield expansion of Vero cells for biopharmaceutical applications.



**Figure 3.** Efficient expansion of Vero cells on Corning enhanced attachment microcarriers in a 1L glass spinner flask and a 5L benchtop bioreactor. Calcein AM staining on day 5 indicates consistent and uniform cell confluency on microcarriers in both culture vessels (Panel A). Quantitation data indicate >10-fold expansion in both vessels (Panel B, n = 2).



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