

# Corning® Enhanced Attachment Microcarriers Show Improved Expansion of Vero Cells for Bioprocess Applications Compared to Competitor

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## Application Note

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### Introduction

Corning microcarriers provide an ideal format for high yield culture of anchorage-dependent cell lines for bioprocess applications. The microcarriers are sterilized and ready-to-use, thereby greatly reducing the time-consuming preparatory steps that are required with many other microcarriers. Here we use Vero cells to compare the performance of Corning enhanced attachment microcarriers to a leading commercially available microcarrier. Vero cells are an ideal candidate for microcarrier culture because they are anchorage-dependent and are popular for cell line-based vaccine production.

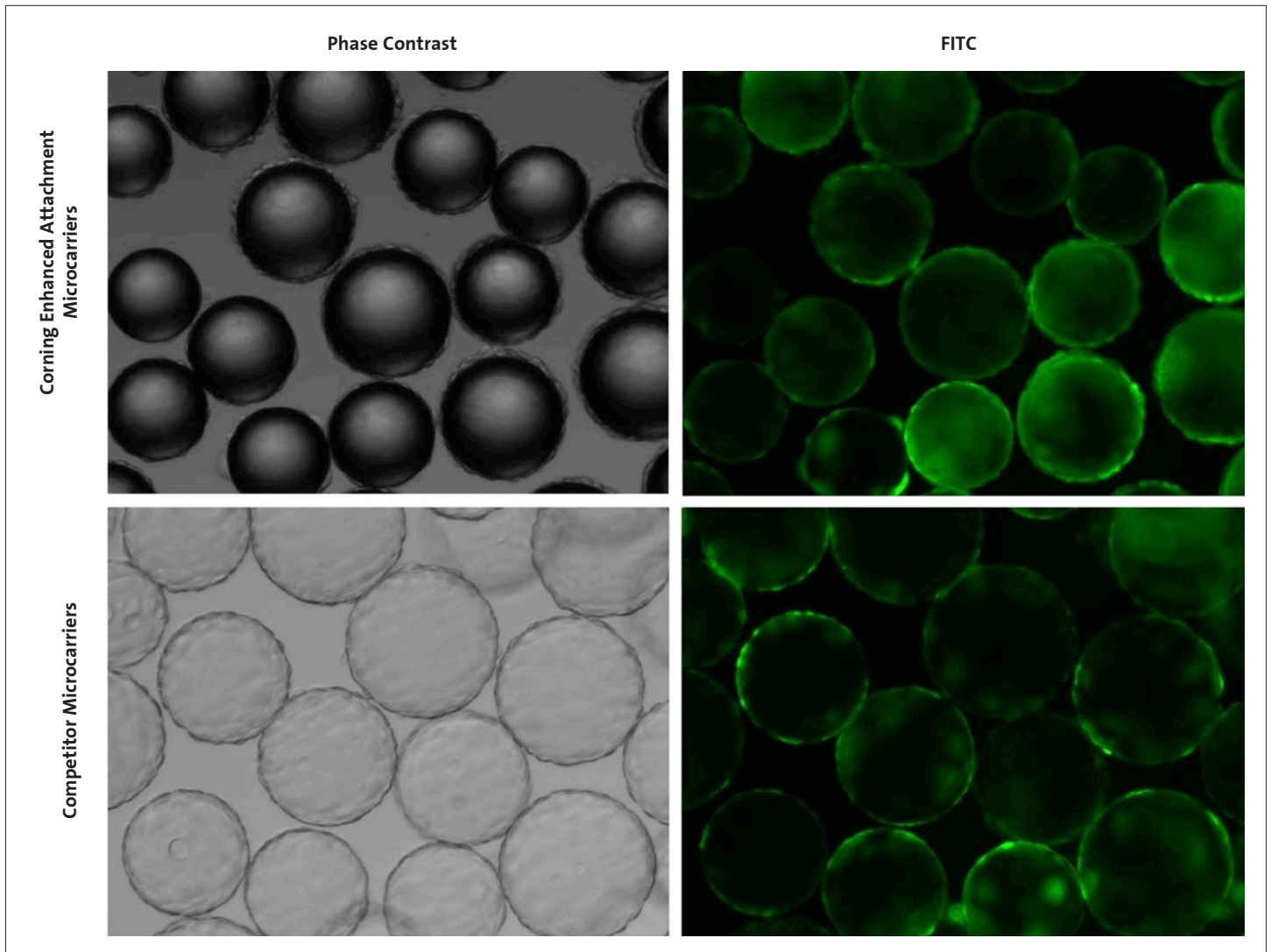
### Materials and Methods

Vero cells (ATCC® CCL81™) were cultured in DMEM (Corning Cat. No. 10-013) supplemented with 10% FBS (Corning Cat. No. 35-010), 1X MEM NEAA (Corning Cat. No. 25-025), and 2mM L-glutamine (Corning Cat. No. 25-005). 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 HYPERFlask® cell culture vessels (Corning Cat. No 10030).

Corning enhanced attachment microcarriers (Corning Cat. No. 3779) and competitor microcarriers were used to expand Vero cells in Corning 1L glass spinner flasks (Corning Cat. No. 4500-1L). In order to accurately compare the results, the optimal cell culture protocol for each respective microcarrier was used. Table 1 summarizes the conditions that were used for each microcarrier. Microcarriers were added to a Corning 1L glass spinner flask in DMEM and allowed to equilibrate in a 5% CO<sub>2</sub> incubator at 37°C

**Table 1.** Summary of cell attachment and cell expansion conditions for Corning enhanced attachment microcarriers and competitor microcarriers.

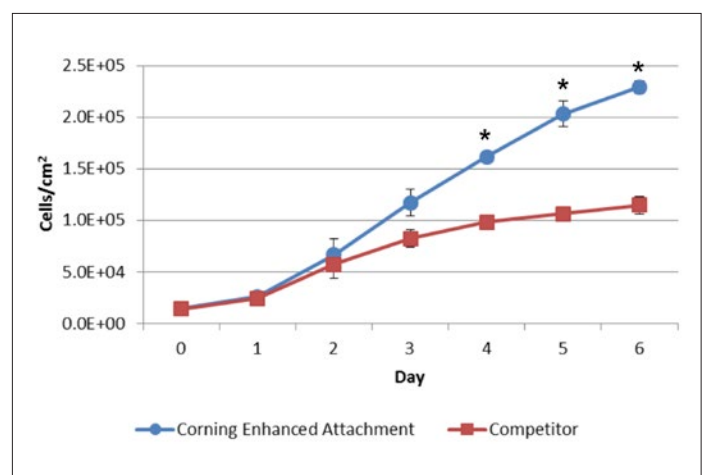
	Corning Enhanced Attachment Microcarrier	Competitor Microcarrier
<b>Microcarrier Preparation</b>		
Requirement for swelling dry microcarriers	Not required	Required (at least 3 hours)
Requirement for washing microcarriers	Not required	Required
Requirement for sterilization of microcarriers	Not required (sterile, ready-to-use)	Required (autoclave or ethanol sterilization)
Microcarrier surface area (dry weight)	360 cm <sup>2</sup> /g	4,400 cm <sup>2</sup> /g
<b>Cell Attachment Conditions</b>		
Medium	DMEM	DMEM
Serum concentration during attachment	0.1% to 0.5% FBS	5% FBS
Culture vessel	1L glass spinner flask	1L glass spinner flask
Final culture volume during attachment	500 mL	500 mL
Culture volume	425 mL (85% final culture volume)	250 mL (50% final culture volume)
Final microcarrier concentration in culture	10 cm <sup>2</sup> /mL; 0.1 mL/cm <sup>2</sup> ; 27.8 g/L	13.2 cm <sup>2</sup> /mL; 0.076 mL/cm <sup>2</sup> ; 3.0 g/L
Total surface area	5,000 cm <sup>2</sup>	6,579 cm <sup>2</sup>
Cell seeding density	15,000 cells/cm <sup>2</sup>	15,000 cells/cm <sup>2</sup>
Agitation	Continuous mixing (40 rpm)	Intermittent mixing (1 min. at 40 rpm, 45 min. static, repeat)
Duration	2 to 3 hours	2 to 3 hours
<b>Cell Expansion Conditions</b>		
Medium	DMEM (5% FBS)	DMEM (5% FBS)
Agitation	Continuous mixing (40 rpm)	Continuous mixing (40 rpm)
Media replenishment	½ volume media exchange on days 3 and 5	½ volume media exchange on days 3 and 5
Duration	5 to 7 days	5 to 7 days



**Figure 1. Phase-contrast microscopy and Calcein AM staining of Vero cells on Corning® enhanced attachment microcarriers (top panels) and Competitor (bottom panels) microcarriers.** Vero cells exhibited similar confluency on both microcarriers. Images show Vero cell growth on day 4 post-seeding (10X magnification).

for 1 to 2 hours for media equilibration. Next, Vero cells were seeded at a density of 15,000 cells/cm<sup>2</sup>. For Corning® enhanced attachment microcarriers, the culture was continuously mixed at 40 rpm for the entire attachment phase. Per the manufacturer’s recommendations for the competitor microcarriers, the culture was mixed for 1 minute at 40 rpm followed by a 45-minute static incubation phase. This process was repeated for the entire cell attachment phase. Cell attachment was monitored hourly by quantifying the number of unattached cells in the culture. After 2 to 3 hours, the medium was adjusted to 5% FBS in a final volume of 500 mL. The culture was incubated for 5 to 7 days at 40 rpm in a 5% CO<sub>2</sub> incubator at 37°C. 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.

During the cell expansion phase, the cultures were sampled daily 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



**Figure 2. Expansion of Vero cells on Corning enhanced attachment microcarriers and competitor microcarriers.** Vero cell expansion was significantly higher on Corning enhanced attachment microcarriers compared to competitor microcarriers. Two way ANOVA \* p<0.05, n = 4.

(Chemometec Cat No: 910-0002). Nuclei were counted using Via1-Cassettes™ (Chemometec Cat No: 941-0012) on a 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

Corning® enhanced attachment microcarriers and competitor microcarriers were expanded for 6 days in Corning 1L glass spinner flasks. Microscopy was used to qualitatively evaluate cell attachment and cell expansion. Phase contrast images and Calcein AM staining indicated uniform and complete coverage of all microcarriers by Vero cells on day 6 (Figure 1). The absence of bare microcarriers for both conditions signified complete cell attachment and indicated the protocols were optimized. Quantitative analysis of the cell growth curves indicated statistically significant improvement in cell expansion on Corning enhanced attachment microcarriers compared to competitor microcarriers

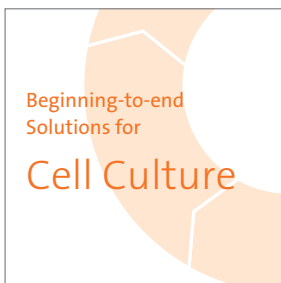
(Figure 2). Cell concentrations exceeded  $2 \times 10^5$  cells/cm<sup>2</sup> on Corning enhanced attachment microcarriers whereas cell concentrations plateaued at  $1.2 \times 10^5$  cells/cm<sup>2</sup> on the competitor microcarriers. At the end of a 6-day culture period, cell number/cm<sup>2</sup> was >50% higher for Corning enhanced attachment microcarriers than for competitor microcarriers.

## Conclusions

- ▶ Corning enhanced attachment microcarriers enable high yield expansion of Vero cells for bioprocess applications.
- ▶ Corning enhanced attachment microcarriers are sterile and ready-to-use, thereby reducing laborious preparatory steps required for competitor microcarriers (swelling, washing, and autoclaving).
- ▶ Corning enhanced attachment microcarriers allow efficient cell attachment of Vero cells under continuous mixing conditions without the need for burdensome intermittent mixing steps.
- ▶ Improved cell densities were achieved on Corning enhanced attachment microcarriers compared to competitor microcarriers.

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