

Dynamic Expansion of Vero and MRC-5 Cells Using Corning® Microcarriers

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A brief technical report
from the Corning
Applications Group

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Introduction

Microcarriers have often been used to culture large quantities of adherent cells in a fraction of the space of more traditional static culture systems. The large surface area to volume ratio and scalability make them an ideal choice for many biotherapeutic applications. Different cell lines require different surfaces and conditions for attachment and growth, especially when maintained under low or serum-free conditions. To that end, Corning's microcarrier product line offers several different surface treatments and coatings to help support optimal growth of a variety of cell types. Corning microcarriers offer the same advantages of traditional microcarriers, but with the added benefits of being sterile and ready-to-use without any swelling, washing, sterilizing or other preparatory steps. This study demonstrates the dynamic expansion of MRC-5 (human lung fibroblast) and Vero (monkey kidney epithelial) cells, two of the most commonly used cell lines for vaccine production, using untreated, Collagen-coated and Positively Charged Corning microcarriers.

Methods and Materials

The conditions for MRC-5 and Vero cell expansion were modified from commercially available protocols (SoloHill Engineering, Inc APP-003B and APP-006B) and reformatted to a 125 mL disposable spinner flask (DSF) format (Corning Cat. No. 3152). All studies were performed using three independent experiments.

MRC-5 (ATCC® CCL171™) cells were cultured in MEM (Corning Cat. No. 10-010-CM) supplemented with 5% FBS (Corning Cat. No. 35-010-CV), 1X Non-essential amino acids (NEAA, Corning Cat. No. 25-025-CI) and 2 mM L-glutamine (Corning Cat. No. 25-005-CI). These cells were used to evaluate Corning Untreated (Cat. No. 3772) and Collagen-coated (Cat. No. 3786) microcarriers, as well as competitor microcarriers with comparable surface treatments (SoloHill, Plastic Cat. No. P102-1521 and Collagen-coated, Cat. No. C102-1521). Cells were seeded into a 125 mL DSF at a density of 15,000 cells/cm², 260 cm² of microcarriers, in a final volume of 50 mL with growth medium for a seeding concentration of 0.192 mL/cm². Cultures were incubated at 5% CO₂ at 37°C with constant agitation at 20 rpm for the first 48 hours, and then increased to 30 rpm for the remaining time in culture (7 days). Half volume media changes were performed on days 3 and 5.

Vero (ATCC® CCL81™) cells were cultured in DMEM (Corning, Cat. No. 10-017-CM) supplemented with 5% FBS and 1x NEAA. These cells were used to evaluate positively charged Corning Microcarriers, (Corning Cat. No. 3787) and competitor microcarriers with comparable surface treatments (SoloHill Hillex® II Cat. No. H112-170). Cells were seeded into a 125 mL DSF at a density of 14,000 cells/cm², 371 cm² of microcarriers, in a final volume of 50 mL with growth medium for a seeding concentration of 0.135 mL/cm². Cultures were incubated at 5% CO₂ at 37°C with constant agitation at 50 rpm for the entire culture period (7 days). To promote cell growth, 0.25 mL of folic acid [2 mg/mL stock] (Sigma Cat. No. F8758-5g) was added once a day. Half volume media changes were performed on days 3 and 5.

Cell enumeration was performed through nuclei counts using a NucleoCounter® NC-200™ analyzer (ChemoMetec). Once a day, 1 mL samples were aliquoted from each microcarrier culture, the cells were lysed, and then loaded into a Via1-Cassette™ (ChemoMetec Cat. No. P0820-5220) for analysis.

Results

Cell Attachment and Morphology

Calcein-AM staining of live cells demonstrates typical cell distribution throughout the microcarrier beads. Figure 1 shows micrographs of Vero and MRC-5 expansion cultures (day 7) on Positively Charged, Collagen-coated and Untreated Corning Microcarriers (Fig. 1).

Vero Cell Expansion on Positively Charged Corning Microcarriers

Daily nuclei counts (plotted as cells/mL) demonstrated no initial difference in cell expansion between Corning and competitor microcarriers. However, cell expansion on competitor microcarriers reached lag phase after 5 days in culture, while the cells cultured on Positively Charged Corning Microcarriers continued to expand. Two-way ANOVA indicates that the increased cell yields observed with the Positively Charged Corning Microcarriers (day 7) were statistically significant (p value <0.001) compared to the competitor yields (Fig. 2).

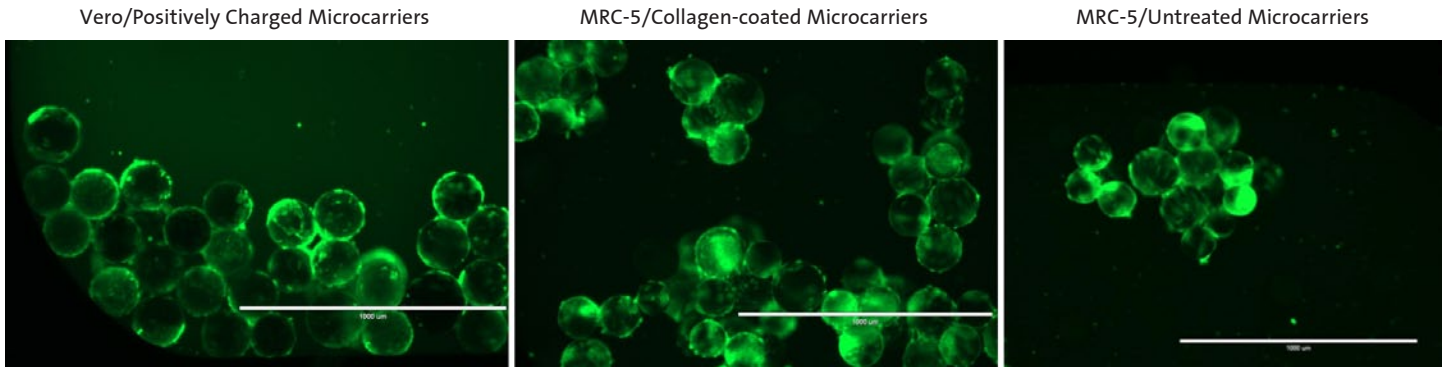


Figure 1. Cell attachment and distribution. Scale bar represents 1000 µm. Images were captured using the AMG EVOS® FL microscope.

MRC-5 Cell Expansion on Collagen and Untreated Microcarriers

Nuclei counts (plotted as cells/mL) demonstrated the advantage of using collagen-coated microcarriers compared to untreated microcarriers for optimal MRC-5 cell growth under dynamic conditions (Fig. 3A). Additionally, pre-sterilized, ready-to-use Corning® Microcarriers perform similarly to the competitor microcarriers with comparable surface treatments as shown in both Figures 3A and 3B, respectively.

Summary/Conclusion

- ▶ Vero cells exhibited an extended growth phase and higher cell yields when cultured on Positively charged Corning Microcarriers when compared to competitor microcarriers.
- ▶ MRC-5 expansion cultures demonstrate improved growth properties when cultured on Collagen-coated microcarriers, indicating that some cell types can benefit from surface optimization
- ▶ Pre-sterilized ready-to-use Collagen-coated and Untreated Corning Microcarriers perform similar to competitor microcarriers.
- ▶ The presterilized and ready-to-use Corning Microcarrier product line provides researchers with a variety of surface treatments including Enhanced Attachment, Low and High Concentration Synthemax® II, Collagen-coated, Positively charged, and Untreated Microcarriers. These Microcarriers enable improved optimization and culturing of adherent cells in dynamic cultures.

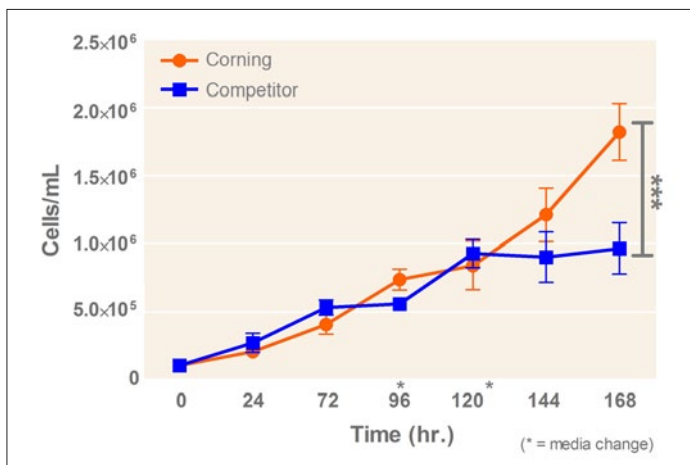


Figure 2. Vero cell expansion on positively charged microcarriers. The data are presented as the mean +/- SEM, N=6.

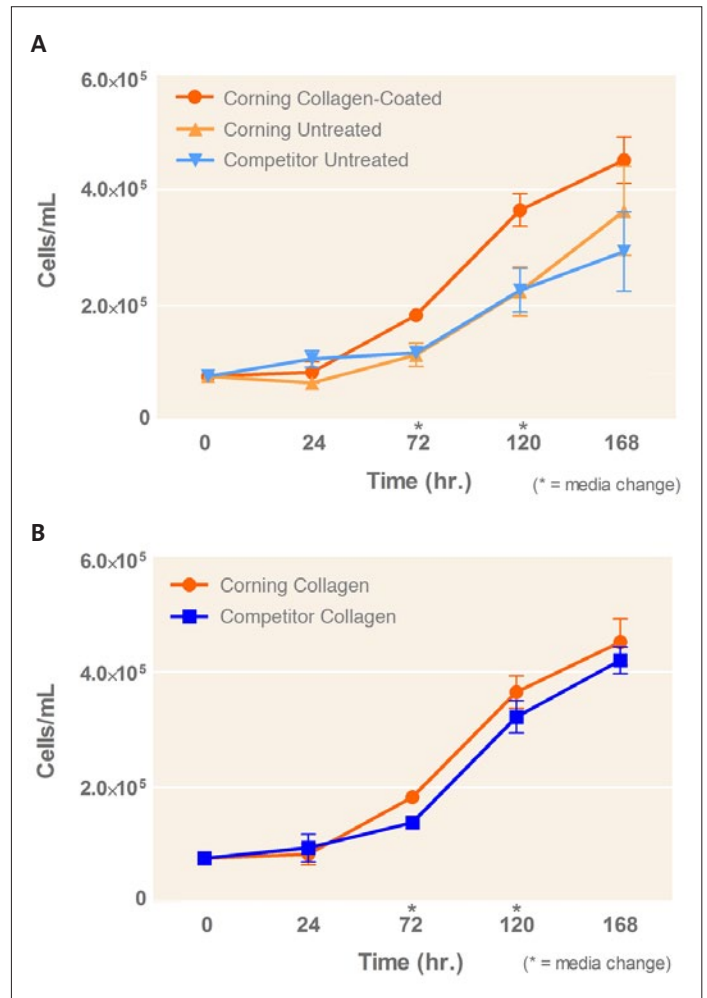
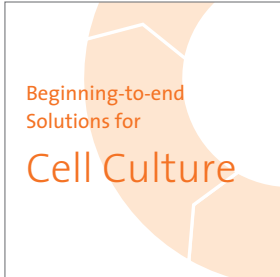


Figure 3A and 3B. MRC-5 cell expansion on Collagen-coated and Untreated microcarriers. The data represented as the mean +/- SEM, N=5.



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