Corning® Enhanced Attachment Microcarriers Offer an Ideal Solution for Bioprocess Applications

Application Note

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Introduction

The manufacturing of biological therapeutics is a complex and important part of the biopharmaceutical industry. Many biological drugs such as antibodies, hormones, and other factors are produced using engineered cell lines.[1-4] Microcarriers have been used as an alternative to traditional static cultures because of the ability to culture large quantities of adherent cells in a fraction of the space using methods typical of suspension culture. The ability to decrease surface area to volume ratio, increase scalability, and easily separate protein product from cells makes microcarriers an ideal choice for many cell therapies.[5] The new Corning microcarriers offer the same advantages of traditional microcarriers with the added benefits of being sterile and ready to use without any additional steps (e.g., swelling, washing, or other preparation steps).

In this study, Corning Enhanced Attachment Microcarriers are used to effectively culture an M-CSF (macrophage colony stimulating factor) secreting engineered Chinese Hamster Ovary (CHO) line. The data demonstrates how the Enhanced Attachment Microcarriers are an effective and user-friendly tool compared with an equivalent commercially available brand.

Materials and Methods

Microcarrier Preparation

Corning Enhanced Attachment Microcarriers (Corning Cat. No. 3779) were transferred to a 150 mL storage bottle (Corning Cat. No. 431175) and reconstituted in sterile cell culture water (Corning cellgro® Cat. No. 25-055-CM) to a volume of 100 mL (36 cm^2/mL). Corning Enhanced Attachment Microcarriers are treated with Corning CellBIND® surface, which infuses more oxygen into the plastic for improved cell attachment.

Competitor microcarriers (Sigma Cat. No. C0646) were prepared per manufacturer's recommendations. Briefly, microcarriers were reconstituted in a siliconized glass container in 300 mL of phosphate buffered saline (PBS) (Corning cellgro Cat. No. 21-031-CM) and swelled overnight at room temperature. After swelling, microcarriers were washed once with PBS and then autoclaved. Finally, the PBS was removed and the microcarriers were reconstituted in PBS to a concentration of 24 mg/mL (105 cm^2/mL). Both Corning and competitor microcarriers equivalent to 75 cm^2 were transferred to Corning 15 mL centrifuge tubes (Corning, Cat. No. 430791) in duplicate. Unused microcarriers were stored at 4°C.

Cell Seeding

After the microcarriers settled, the buffers from each sample were gently aspirated, and each tube of microcarriers was resuspended in IMDM (Corning cellgro Cat. No. 10-016-CM) supplemented with 10% FBS (Corning cellgro Cat. No. 35-010-CV) containing $3.75x10^5$ 5/9 m alpha3-18 cells (ATCC® Cat. No. CRL-10154™). Cells and microcarriers were transferred to Corning 125 mL disposable spinner flasks (Corning Cat. No. 3152). The volume in each spinner flask was brought up to 30 mL by adding additional media. The spinners were stirred at 30 rpm for 2 minutes every 30 minutes for 3 hours to allow sufficient time for cell attachment. After the attachment period, an 15 additional mL of media was added to each vessel and the stir rate was changed to continuous stirring at 30 rpm. Cells were cultured in a 37°C incubator at 5% CO_2 for 4 days. The entire study was repeated 3 independent times.

M-CSF Production

To evaluate M-CSF production, 1 mL of media was collected from each sample prior to harvest on day 4. Samples were centrifuged at 300 x g for 4 minutes to remove debris (cells and microcarriers). The supernatants were stored at -20°C until they were assessed using a human M-CSF ELISA kit (R&D Systems Cat. No. DMC00B).

Nuclei Assessment

Total adherent cells were assessed by collecting 10 mL of microcarriers and centrifuging at 300 x g for 6 minutes in order to separate microcarriers from media. A sample from the media was enumerated on the BioProfile® Flex Analyzer (Nova® Biomedical) to assess the number of cells in suspension. One mL of cell lysis buffer (Chemometec Cat. No. 910-0003) was added to microcarriers for less than 1 minute, followed by 3 mL of stabilizing buffer (Chemometec Cat. No. 910-0002). Lysed cells were than separated from microcarriers using a Falcon® 40 µM cell strainer (Corning Cat. No. 352340) with a 50 mL centrifuge tube. An additional 2 mL PBS rinse was used to wash the cell strainer. Cells were stained with Hoechst (Invitrogen, Cat. No. H21486) and enumerated using a hemacytometer.

Trypsin Harvesting

To assess the utility of the microcarriers for cell scale-up culture, a trypsin harvest was performed. Ten mL of microcarriers from each vessel were centrifuged at 300 x g for 6 minutes in order to separate microcarriers from media. The microcarriers were washed once with 10 mL of PBS and then centrifuged again to remove the buffer. Two mL of trypsin (Corning® cellgro® Cat. No. 25-052-CV) were added to each sample. The samples were then transferred to wells of a Costar® 6 well plate (Corning Cat. No 3506) for monitoring cell dissociation. Once cells began to round up, a P1000 pipettor was used to further detach the cells and 2 mL of culture media were added to quench the sample. Cells were separated from microcarriers by using a 40 µM cell strainer with a 50 mL centrifuge tube. An additional 2 mL PBS rinse was performed to wash the 6 well plate and the cell strainer of any additional cells. Cells were then counted on the Nova Bioprofile Flex Analyzer.

Results and Discussion

5/9 m alpha3-18 cells were expanded on microcarriers for 4 days. Similar confluence and cell distribution were confirmed on day 4 by staining cells with Calcein AM (Corning Cat. No. 354216) (Fig. 1). Paired T-tests indicated that a greater number of cells attached to Corning Enhanced Attachment Microcarriers (Fig. 2) ($P<0.05$), and a greater number of viable cells were recovered with Trypsin from the Enhanced Attachment Microcarriers compared to the competitor microcarriers (Fig. 3) ($P<0.001$). Finally, taking into account adherent as well as any suspension 5/9 m alpha3-18 cells, there was no statistical difference in M-CSF production per cell regardless of which microcarrier was used (Fig. 4) ($P>0.05$).

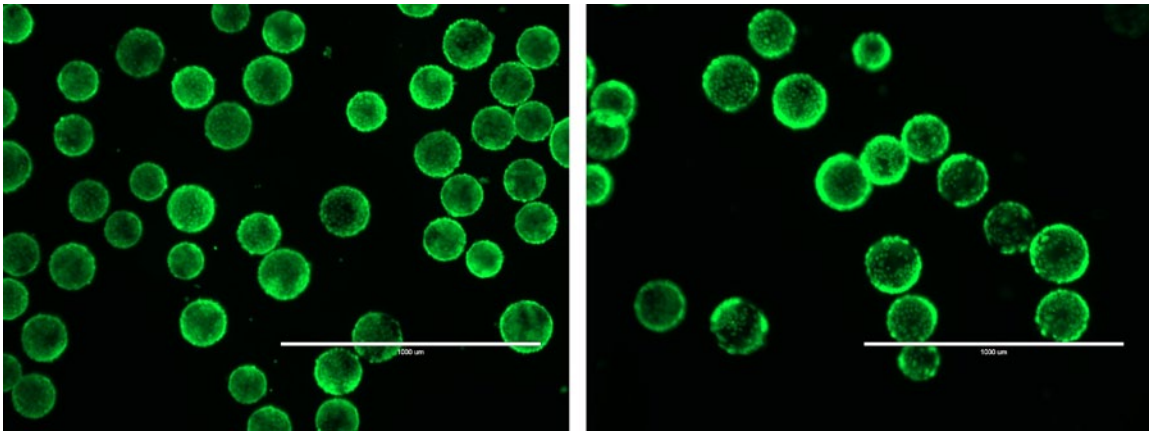

Figure 1. Calcein AM stained 5/9 m alpha3-18 cells on Corning Enhanced Attachment microcarriers (left) and competitor microcarriers (right). Similar confluence and distribution of 5/9 m alpha3-18 cells were observed on both microcarriers.

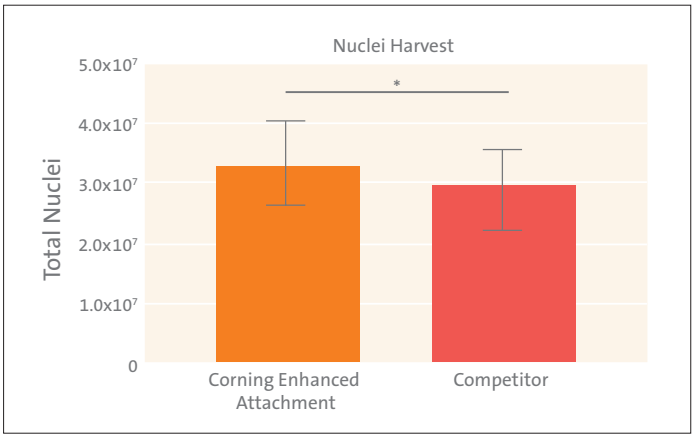

Figure 2. Total 5/9 m alpha3-18 nuclei from 75 cm^2 of Enhanced Attachment and competitor microcarriers. A Paired T-test shows that statistically more adherent nuclei were collected from 75 cm^2 of Enhanced Attachment Microcarriers compared to 75 cm^2 of competitor microcarriers. Unpaired t-test* $P<0.05$ (n=6).

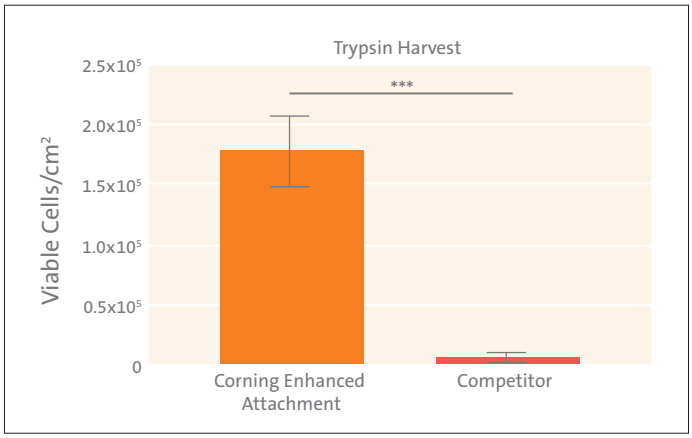

Figure 3. Adherent 5/9 m alpha3-18 cell densities harvested from 75 cm^2 of Enhanced Attachment and competitor microcarriers. A Paired T-test shows that statistically more adherent 5/9 m alpha3-18 cells were harvested from 75 cm^2 of Enhanced Attachment Microcarriers as compared to 75 cm^2 of competitor microcarriers. Unpaired t-test*** $P<0.0001$ (n=6).

Conclusions

- Corning® Enhanced Attachment Microcarriers are an ideal choice for use with engineered mammalian cell lines.
- Corning Enhanced Attachment Microcarriers can be used for biomanufacturing and scale-up for biotherapeutic production.
- Higher densities of 5/9 m alpha3-18 cells were achieved on Corning Enhanced Attachment Microcarriers as compared to competitor microcarriers.
- Viable 5/9 m alpha3-18 cells are easier to detach from Corning Enhanced Attachment Microcarriers as compared with competitor microcarriers.
- 5/9 m alpha3-18 cells produced equivalent M-CSF/cell when cultured in both microcarrier types that were tested.

References

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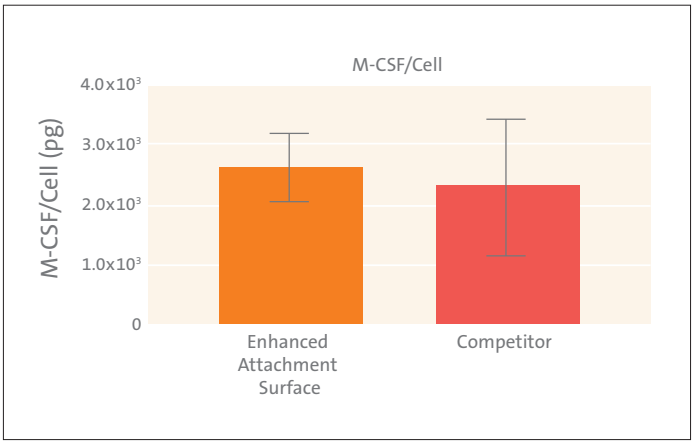

Figure 4. M-CSF production from 5/9 m alpha3-18 cells cultured on Corning Enhanced Attachment and competitor microcarriers. A Paired T-test shows no statistical difference in M-CSF production/cell between Enhanced Attachment Microcarriers and competitor microcarriers. *Calculated concentration includes cells found in suspension as well as cells attached to microcarriers. Unpaired t-test P>0.05 (n=6).

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