Parameters to Consider When Expanding Cells on Corning® Microcarriers

Application Note

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Introduction

Microcarriers are often used to culture larger quantities of adherent cells in a fraction of the space of traditional static culture systems. They combine the advantages of traditional flask technology with those of suspension cultures. Currently, large scale expansion of cells using microcarriers may be achieved in bioreactors (stainless steel or single-use) in a controlled environment, which allows precise control and monitoring of multiple parameters such as pH, dissolved oxygen (dO), and temperature.[1] Additionally, the constant mixing (whether in a bioreactor or spinner flask) provides researchers with the ability to reduce the amount of medium per growth area from 0.2 to 0.5 mL/cm^2 (recommended in traditional flask technology) to <0.2 mL/cm^2, thereby decreasing overall medium costs and increasing cell yields. Researchers can employ bioreactors up to the 2,000L range to generate larger quantities of cells in one batch run to reduce variability and the risk of contamination.[1] This larger surface area-to-volume ratio and scalability makes microcarriers an ideal choice for many cell culture applications. Corning microcarriers offer the same advantages of traditional microcarriers, but with the added benefits of sterile and ready-to-use without any swelling, washing, or other preparatory steps (Figure 1).

It is important to keep in mind that many parameters can impact cell attachment and expansion on microcarriers. For instance, the type of microcarrier (e.g., positively-charged vs. collagen- or peptide-coated), pH level, and medium selected can greatly impact cell yields. Changes in agitation rate, sparger choice (e.g., micro vs. macrosparger), sparging rate, and impeller design can also impact cell attachment and subsequent expansion on microcarrier beads depending on the growth vessel.[2] In this review, we will briefly cover how parameters such as choice of microcarrier, medium, serum, and agitation rate can impact cell growth.

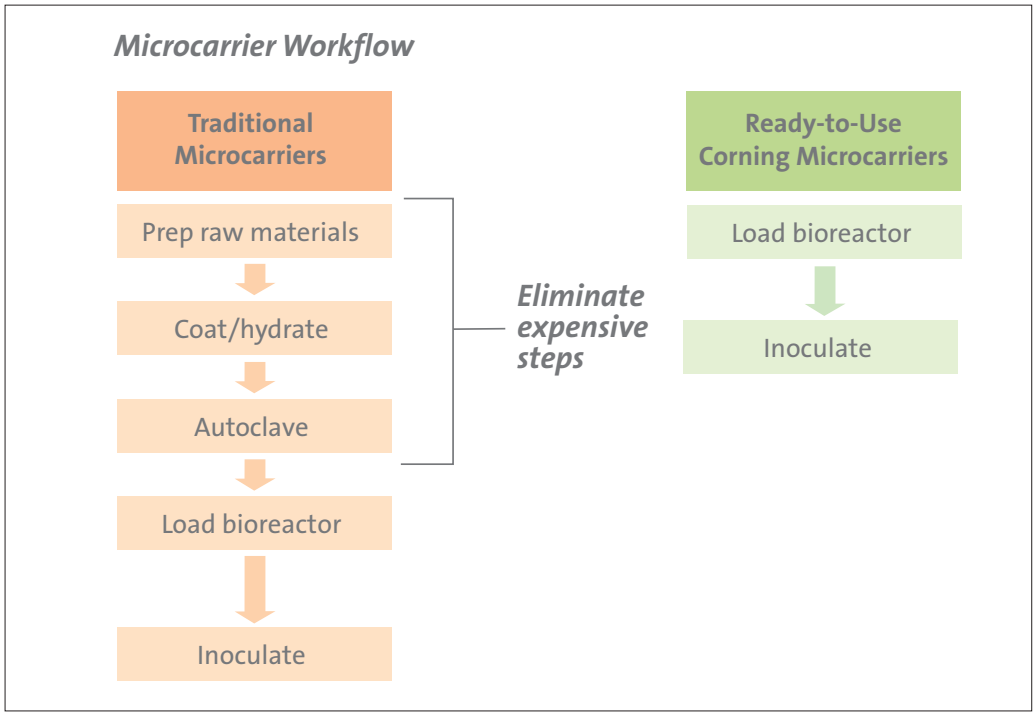

Figure 1. Corning microcarriers simplify the workflow process.

Methods and Materials

Preparing Microcarriers for Use

To prepare 10 g vials of Corning® Enhanced Attachment (Cat. No. 3779), Collagen-coated (Cat. No. 3786), or Positively charged (Cat. No. 3787) microcarrier were aseptically transferred to either a 150 mL storage bottle (Cat. No. 431175) or a Corning 1L autoclaved, siliconized, glass spinner flask (GSF) (Cat. No. 4500-1L) and resuspended in either tissue culture grade water (Cat. No. 25-055-CV) (final volume, 100 mL) or culture medium (for more details, see 1L GSF optimization studies), respectively.

MRC-5 Medium Optimization

MRC-5 cells (ATCC® CCL171™) were seeded at 15,000 cells/cm^2 onto Corning Collagen-coated microcarriers at either 0.2 mL/cm^2 (14 g/L) or 0.1 mL/cm^2 (28 g/L) into a Corning 125 mL disposable spinner flask (DSF)[a] (Cat. No. 3152) at a final volume of 50 mL. MRC-5 cells were cultured in Corning MEM medium (Cat. No. 10-010-CM) with 5% Corning FBS (Cat. No. 35-010-CV), 1X Corning MEM Nonessential Amino Acids (NEAA) (Cat. No. 25-025-CI), and 2 mM Corning L-glutamine (Cat. No. 20-005-CI). Cultures were incubated at 5% CO_2 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.

1L Glass spinner flask (GSF) optimization studies

Vero cells (ATCC™ CCL81) or HEK293-AD cells (Cell Biolabs Cat. No. AD-100) were seeded at 15,000 cells/cm^2 onto either Corning Collagen-coated (28 g/L), Enhanced Attachment (28 g/L), or positively-charged (19 g/L) microcarriers at 0.1 mL/cm^2 in 1L glass spinner flasks.[b] Both cell types were maintained in 400 mL of Corning DMEM (Cat. No. 10-013-CM) with 5% FBS, 1X NEAA, and extra L-glutamine (Final concentration = 4 mM).

Microcarriers (4,000 cm^2) were added to an autoclaved, siliconized 1L GSF. Pre-warmed medium was added to the spinner flask to bring the volume to 290 mL. GSFs containing medium and microcarriers were equilibrated in the incubator for at least 15 minutes prior to seeding[c]. Following incubation, 50 mL of the cell suspension (1.2 x 10^6 cells/mL) was added to the GSFs (Volume = 340 mL; 85% of total culture volume; cell concentration ~1.8 x 10^5 cells/mL). Cell attachment was monitored over the course of 8 hours. Once 90% of the cells attached, or cells were in culture for 8 hours, the final volume was increased to 400 mL. All studies were carried out under continuous agitation.[d,e] Cultures were incubated at 5% CO_2 at 37°C with half media changes performed as indicated by the nutrient and metabolic profiles (typically on days 3 and 5). To evaluate the effect of high protein-containing medium (e.g., serum) on cell attachment, Vero and HEK293-AD cells were seeded under either serum-containing or serum-free conditions. For serum-free conditions, cell inoculum was also void of serum, and serum was added back to the culture when 90% of the cells were attached (typically between 1 and 2 hours post seed, cell-line dependent).

Monitoring Cell Attachment

Hourly samples (~1 mL) of each, well-mixed, microcarrier culture was collected and analyzed for cell attachment by visualizing and enumerating cells that remained unattached in the medium. To assess unattached cells, half of the sample was passed through a test tube with Corning cell strainer snap cap (Cat. No. 352235) to separate attached cells (on microcarriers) from unattached (in the medium). The medium was analyzed via the NucleoCounter® NC-200™ analyzer (ChemoMetec). The percentage of cell attachment was quantified by comparing the hourly cell densities of the medium to the density at 0 hours. To visualize cells on microcarriers, the other half of the sample (microcarriers and medium) was transferred to a Corning 24 Multiple Well plate (Cat. No. 3524) and visualized using the AMG EVOS® Fl microscope.

Monitoring Cell Growth

A daily aliquot (between 2 to 3% of total culture volume) from each, well-mixed, microcarrier culture was obtained. To monitor nutrient and metabolite profiles, ~1.5 mL of the sample was passed through a test tube with cell strainer snap cap and analyzed via the Nova Biomedical BioProfile® FLEX. To visualize cells on microcarriers, 400 µL of microcarriers and medium were transferred to a multiple well plate and visualized using the AMG EVOS® Fl microscope. To perform cell enumeration, microcarriers were allowed to settle, medium was removed, cells attached to the microcarriers (viable cells) were lysed, passed through a Corning 40 µm cell strainer (Cat. No. 431750) and loaded into a Via1-Cassette™ (ChemoMetec Cat. No. P0820-5220) for analysis via the NucleoCounter NC-200 analyzer. To assess viability, the remaining sample from the nutrient/metabolite analysis was saved and the non-viable cells in the medium were quantified using the Nucleocounter NC-200.

Results

Choice of Microcarrier

Different cell lines require different surfaces and conditions for attachment and growth, especially when maintained under low-serum 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. Table 1 has a list of microcarriers with general guidelines for selecting a suitable microcarrier for your application. However, optimization is critical for any application and multiple surfaces may need to be evaluated to select the most appropriate surface for a specific cell type, medium selected, and application.

[a] If performing studies in a spinner flask, it is critical to verify that the selected stir plate is certified for a humidified (greater than 95%) incubator at 37°C. If the stir plate is not certified for high humidified incubators, excess heat can be generated beyond 37°C, leading to cell death.

[b] To ensure an even distribution of the cells on the microcarriers, a single cell suspension with a minimal amount of cell clumps in the inoculum is recommended at the time of seed. A cell strainer may be required to prepare a single cell suspension.

[c] To promote cell attachment, it is important to pre-equilibrate the culture medium and vessel in the incubator prior to cell seeding. For smaller volumes (<500 mL), 5 to 15 minutes (depending on volume) of pre-equilibration will be sufficient. However, for larger volumes (e.g., 50 to 100L) an overnight incubation may be necessary to ensure proper temperature, pH, and gas equilibrium. These variables are factors that can impact cell attachment, and should be considered when developing the protocol.

[d] If seeding cells onto the positively-charged microcarriers, it is recommended to add the cells under continuous agitation to ensure an even distribution. Most cells attach to the positively-charged microcarriers at a faster rate compared to other microcarriers.

[e] For cell lines that do not attach readily, a static period or overnight incubation with intermittent agitation may enhance cell attachment.

Table 1. General Guidelines for Selecting a Suitable Microcarrier

Microcarrier	General Guidelines
Positively-charged (Cat. No. 3787)	Cell line is typically cultured on a positively-charged surface (e.g., Poly-D-Lysine, Corning® PureCoat™ Amine).
Low Concentration Corning Synthemax® II (Cat. No. 3781)	Cells cultured under serum free conditions (e.g., MSC).
High Concentration Corning Synthemax II (Cat. No. 3784)	iPSC, ESC, or other specialized cell types cultured under serum-free conditions.
Enhanced Attachment (Cat. No. 3779)	Cell line is typically cultured on a negatively charged surface (e.g., TC-treated or Corning CellBIND® Surface).
Collagen-coated (Cat. No. 3786)	Cell lines with low attachment efficiency (less than 50%) to other microcarriers or cell lines typically cultured on collagen-coated surfaces.
Untreated (Cat. No. 3772)	If a different coating is required than those described above, these microcarriers may be coated. Cell line is difficult to detach from other microcarriers, cells may detach easier from untreated surface.

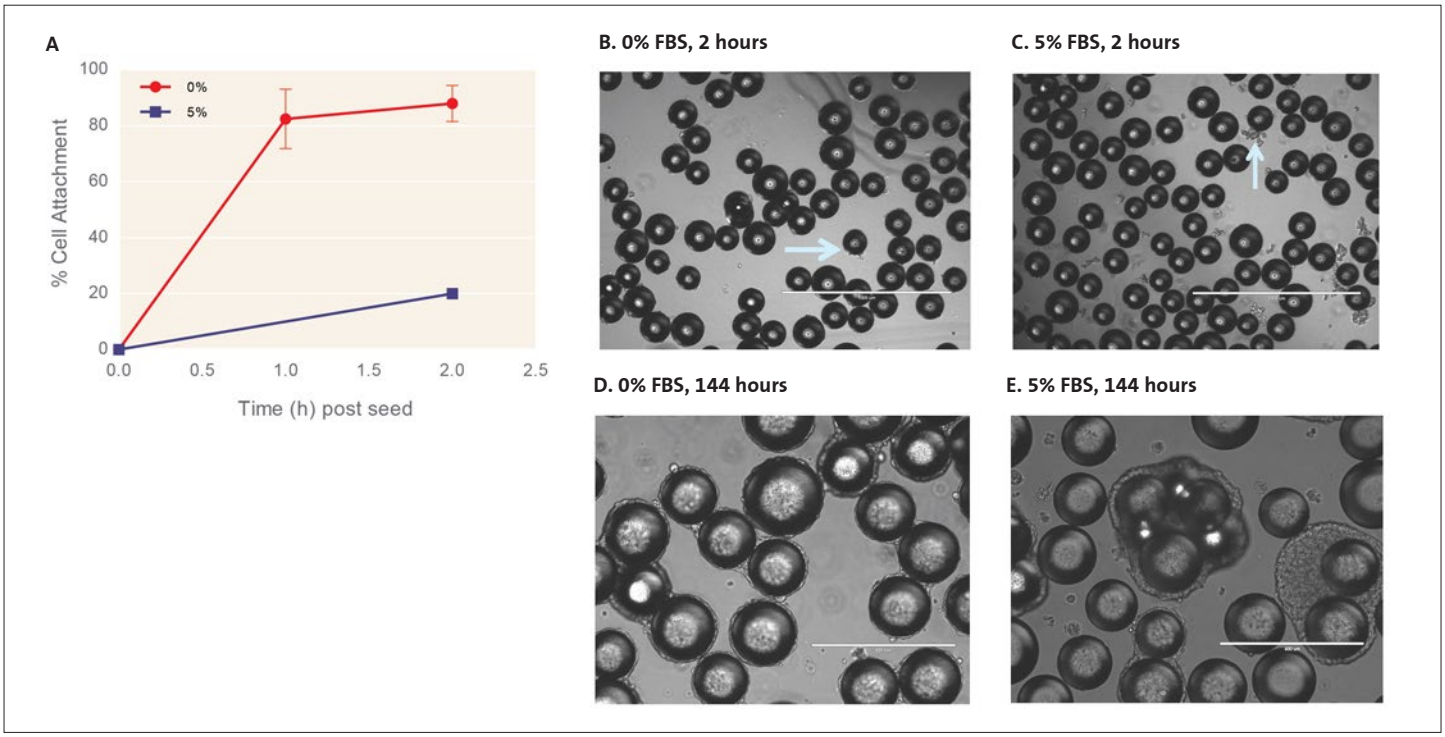

Figure 2. The presence of serum at the time of seeding significantly impacts HEK293-AD cell attachment and expansion on Corning Enhanced Attachment microcarriers. (A) 90% of the cells attached within two hours when cells were seeded under serum free conditions, whereas only 20% of cells attached in the presence of serum. (B, C) Representative photographs demonstrating either (B) efficient and uniform cell attachment in the absence of serum (arrow pointing to cells attached to microcarrier) or (C) poor and uneven cell attachment in the presence of serum (arrow pointing to cell aggregates in the medium). Scale bar represents 1000 µm. (D, E) Representative photographs demonstrate an uniform distribution of cells on microcarriers under serum-free conditions (D) compared to serum containing (E). Scale bar represents 400 µm.

Impact of Serum on Cell Attachment

With some types of microcarriers, the presence of serum may negatively impact cell attachment. To evaluate the effect of serum with Corning® microcarriers, HEK293-AD and Vero cells were seeded under equivalent conditions in the absence or presence of serum. For serum-free cultures, serum was added back to the culture when 90% of the cells were attached to the microcarriers. Results demonstrated that in the presence of serum, HEK293-AD cells attached at a slower rate (Figure 2A), and if attached, the population was unevenly distributed on the microcarriers (Figure 2C, 2E vs. Figure 2B, 2D). Additionally, cells seeded in the presence of serum exhibited large spheroid-like clumps of cells in suspension throughout the culture (data not shown). However, under serum-free conditions, greater than 90% of the cells attached within the first two hours, and an even distribution of the cells on the microcarriers was observed throughout the culture (Figure 2A, 2B, 2D). Not all cell lines, however, are as sensitive to the presence of serum during the seeding phase. For example, Vero cells will still attach to and evenly distribute on the microcarriers in the presence of serum (Figure 3). However, the rate of attachment is likely to be slower and not as efficient compared to serum free conditions (Figure 3A).

Additionally, the rate of attachment is also dependent on the microcarrier selected. Typically, cells will attach to the positively-charged microcarriers at a faster rate compared to other microcarriers. Figure 3F demonstrates that approximately 90% of the Vero cells attached to the positively-charged microcarriers within 10 minutes when seeded under serum free conditions, and within 2 hours if seeded in serum containing medium.

Recommendation: Perform a side-by-side experiment to evaluate the effect of serum on cell attachment and expansion for your particular cell line, microcarrier, and application.

Agitation Rate

Agitation rate plays an important role in cell attachment and expansion on microcarriers. If the agitation rate is too high, cells may suffer from shear stress leading to a decreased growth rate, and eventually cell death. However, if the agitation rate is too slow, the microcarriers will settle and clump together, leading to aggregation, which may greatly impact cell yields and desired products. Determining the optimal agitation rate depends on (a) cell type, (b) density of selected microcarriers, (c) impeller design, and (d) vessel (or bioreactor). Ideally, the optimal agitation rate is the minimum speed necessary to resuspend all of the microcarriers into solution without causing shear stress.

Depending on the cell line, the agitation during the first 24 hours may need to be adjusted to promote cell attachment. Some cell lines may require a static phase, intermittent agitation, or significantly lower than normal speeds for the initial 24 hours to promote cell attachment. For example, MSC and MRC-5 cells require slower speeds (≤20 rpm) during the initial seeding phase to promote cell attachment on Corning® Enhanced Attachment microcarriers. However, it is recommended to increase the agitation rate to 30 rpm on the following day.

Selecting the correct agitation rate for the microcarrier is also important. Some microcarriers are denser than others, and therefore, require a higher agitation rate to remain in solution. For example, the Corning® positively charged microcarriers have a density of approximately 1.1 g/cm^3; whereas the other Corning microcarriers have a density of approximately 1.03 g/cm^3. As a result, the positively-charged microcarriers are agitated at higher speeds (40- to 60 rpm) compared to the other Corning microcarriers (20 to 40 rpm).

Selecting the wrong agitation rate for the microcarriers will also impact how and when the cells attach. Figure 4A demonstrates that Vero cells attached to the Corning Enhanced Attachment microcarriers within 4 hours in the presence of serum at agitation rates between 20 to 30 rpm; however, when the rate was

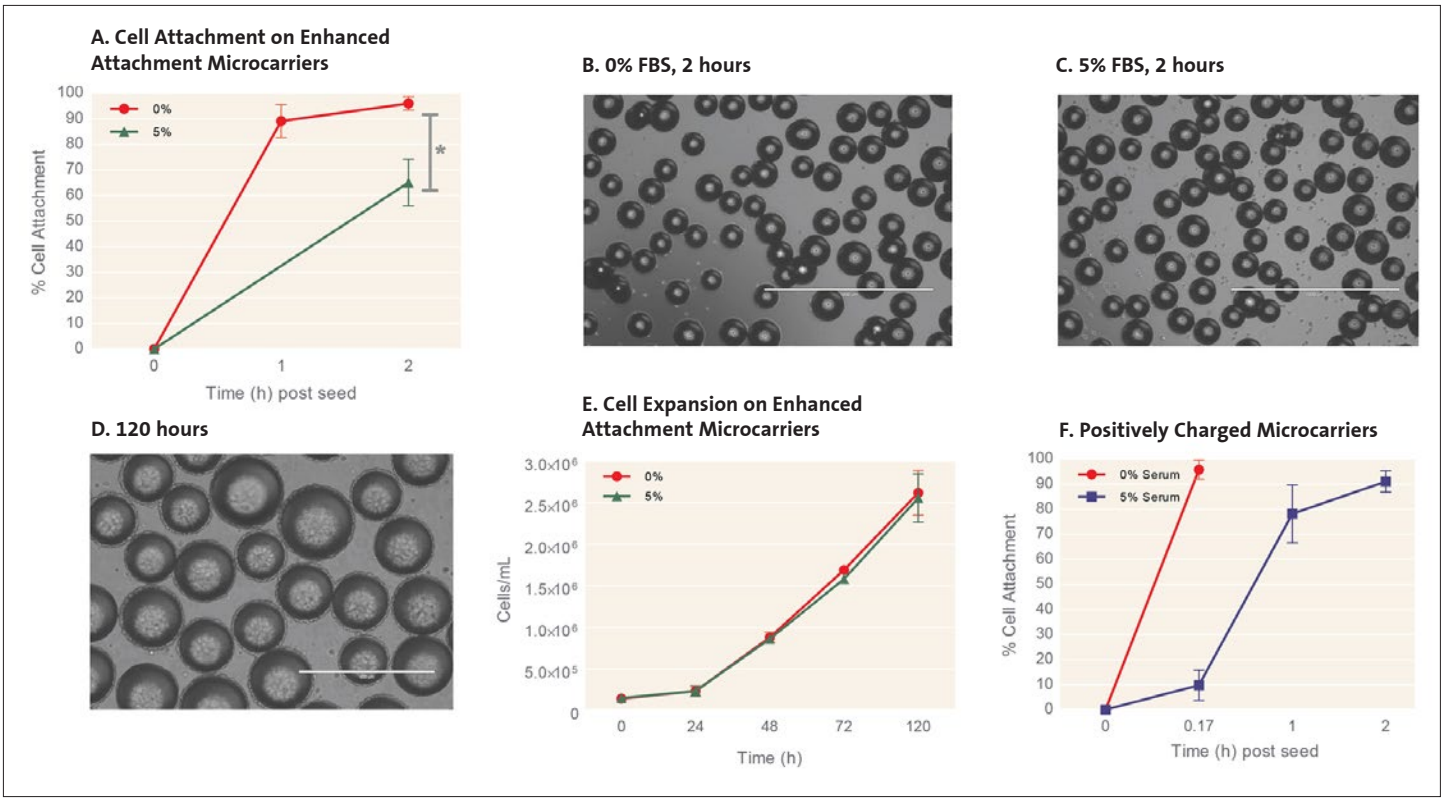

Figure 3. Impact of serum on Vero cell attachment and expansion. (A-E) Vero cells cultured on Enhanced Attachment microcarriers at 30 rpm. (A) 90% of the cells attached within two hours when cells were seeded under serum free conditions, whereas only 60% of cells attached in the presence of serum. (B, C) Representative photographs demonstrating either (B) efficient and uniform cell attachment in the absence of serum or (C) inefficient cell attachment in the presence of serum. Scale bar represents 1000 µm. (D) Representative photograph demonstrating uniform distribution of cells on microcarriers under serum-free conditions. Scale bar represents 400 µm. (E) Similar cell expansion was depicted in cells seeded under serum-containing or serum-free conditions. (F) Vero cells attached to the positively-charged microcarriers within 2 hours in the presence of serum (and attached within approximately 10 minutes under serum-free conditions) at an agitation rate of 50 rpm. 2-Way ANOVA, $p \leq 0.05$.

increased to 40 rpm, only 40% of the cells attached within 8 hours. Additionally, after 5 days in culture, the cells exhibit a more uniform attachment and expansion on the microcarriers when cultured at 20 rpm (Figure 4B) compared to 40 rpm (Figure 4C) (~90% of microcarriers covered at 20 rpm, compared to ~50% at 40 rpm).

Recommendation: Optimizing the agitation rate for cell attachment and growth will depend on the microcarrier selected, cell type, growth medium conditions (serum vs. serum free), and vessel type (e.g., impeller design). Generally, recommended agitations rates in Corning® spinner flasks for Corning positively charged microcarriers are 40 to 60 rpm, and 20 to 40 rpm for Corning Collagen-coated, Untreated, Enhanced Attachment, and Corning Synthemax® II microcarriers.

Medium Optimization

As stated above, one major advantage of microcarriers compared to traditional flask technology is the ability to decrease the volume of medium per cm^2. However, media that supports cultures at 0.2 mL/cm^2 efficiently may not adequately support higher cell density cultures and will need to be optimized. In Figure 5A, MRC-5 cells were cultured in MEM and seeded at either 0.2 mL/cm^2 (~250 cm^2 in 50 mL) or 0.1 mL/cm^2 (~500 cm^2 in 50 mL). These data demonstrate that the cultures failed to expand at the same growth rate when seeded at 0.1 mL/cm^2 (28 g/L) compared to 0.2 mL/cm^2 (14 g/L). Upon analysis of the nutrient/metabolite profiles, it was determined that the glucose and glutamine levels were too low and the nutrients were completely utilized within 72 hours when seeding at 0.1 mL/cm^2 (data not shown). There-

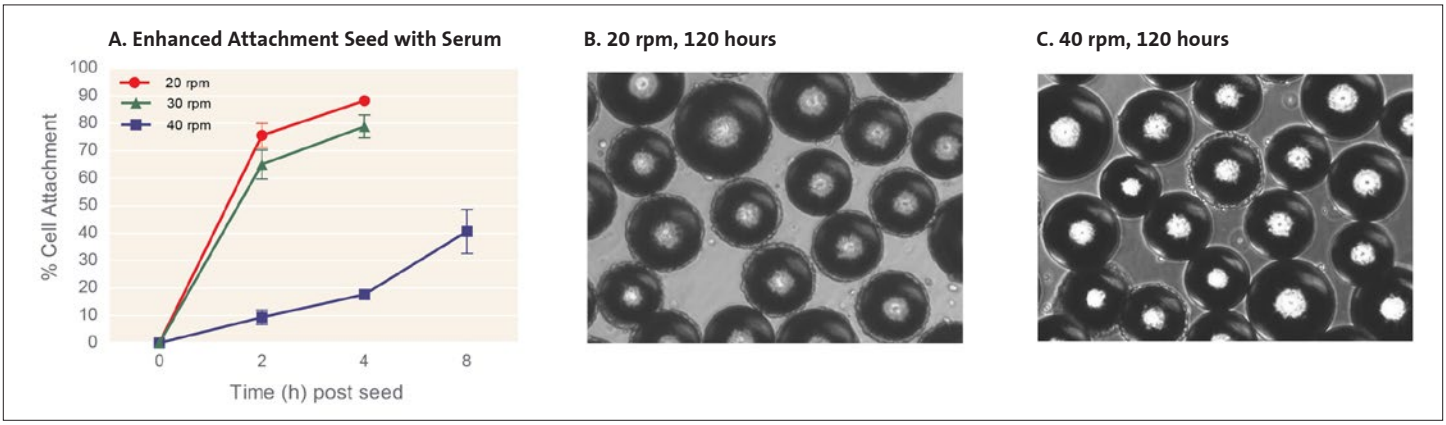

Figure 4. Agitation rate impacts cell attachment. (A) Vero cells attached to the Corning Enhanced Attachment microcarriers within 4 hours in the presence of serum at agitation rates between 20 to 30 rpm. If the agitation rate was increased to 40 rpm, only 40% of the cells attached within 8 hours. Representative photographs of Vero cells cultured on the microcarriers for 5 days at either 20 rpm (B) or 40 rpm (C). These pictures demonstrate a more uniform cell attachment and expansion when cultured at 20 rpm compared to 40 rpm (~90% of microcarriers covered at 20 rpm, compared to ~50% at 40 rpm). Cells visible along the periphery of the microcarriers.

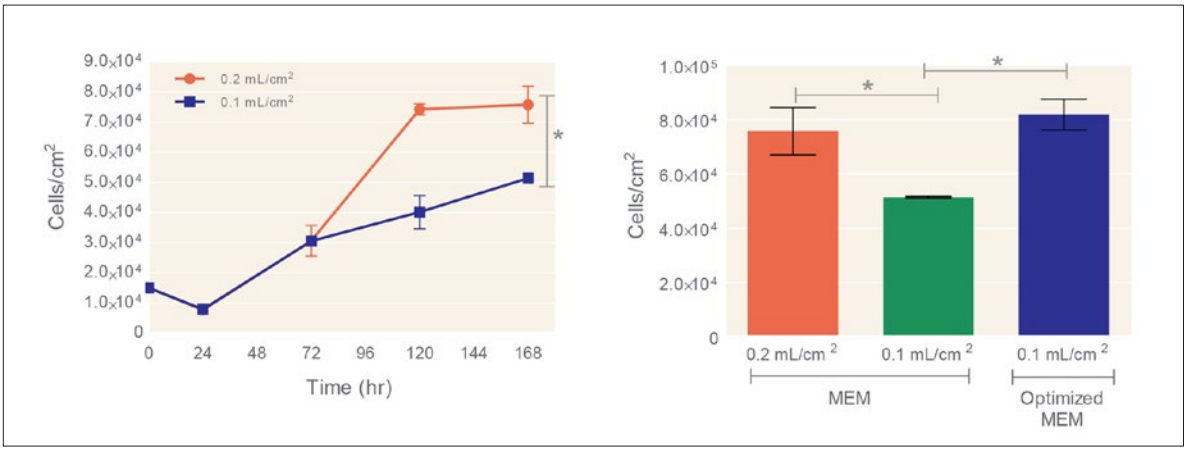

Figure 5. Medium optimization is critical when decreasing mL/cm^2 (A) MRC-5 cells were cultured in MEM at either 0.2 mL or 0.1 mL/cm^2 on Corning Collagen-coated microcarriers. MRC-5 cells failed to expand at the same rate in MEM media at 0.1 mL/cm^2. 2-Way ANOVA, *$p \leq 0.05$ (B) Following medium optimization (Optimized MEM), similar cell yields per cm^2 were obtained at 0.2 (Standard MEM) and 0.1 (Optimized MEM) mL/cm^2. ANOVA, *$p \leq 0.05$.

fore, when the medium was optimized by increasing the glutamine concentration from 2 mM to 4 mM, and increasing the glucose concentration from 1 g/L to 2 g/L, and also adding sodium pyruvate to the MEM medium, cell growth at 0.1 mL/cm^2 on day 7 was similar to cultures seeded in standard MEM at 0.2 mL/cm^2 (Figure 5B). In summary, to optimize medium conditions, various parameters should be considered including concentrations of glucose, glutamine, vitamins, and minerals.

Recommendation: If using microcarriers for the first time, perform initial studies at the same mL/cm^2 that was used for T-flask experiments. Therefore, the study will focus on optimizing cell attachment and growth and eliminate media as a potential factor for reduced cell growth. Once the conditions for cell attachment and expansion have been optimized, then proceed to decreasing mL/cm^2 and media optimization.

Summary

In summary, multiple parameters can affect cell attachment and expansion on microcarriers. These parameters include, but are not limited to, the choice of microcarrier (and surface properties), serum concentrations, impeller design, agitation rate, and medium. Therefore, it is critical to re-optimize cell attachment and expansion parameters if any of these variables are modified.

References

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