

SOLOHILL Microcarriers in a 25 L PadReactor™ Single-Use Bioreactor

INTRODUCTION

Microcarriers provide a large surface area for growth of anchorage-dependent cell types and make possible the use of bioreactors for generation of large numbers of adherent cells under current good manufacturing processes (cGMP). Presently, microcarriers are used in a wide array of applications with a diverse range of cell types for the production of biologicals and vaccines, and for the generation of stem cells for cell therapies and regenerative medicine. Within each application, there exists the challenge of providing environmental conditions that promote optimal cell growth and quality end products. To meet this challenge, SoloHill Engineering offers an assortment of microcarrier types with varying densities and unique combinations of surface chemistries and coatings. This portfolio provides users with options to select a microcarrier that excels in their bioreactor platform. Microcarriers for serum-containing or animal protein-free (APF) applications are available.

The recent emergence of disposable bioreactor technology, such as the PadReactor™ (ATMI LifeSciences, Figure 1), has provided a useful platform for the manufacture of biologics. These systems expedite cGMP clinical and commercial production by improving process reliability and reducing costs with greater flexibility. The bioreactor vessel, which offers comparable functionality to classic stirred tank bioreactors, is a single use cube-

shaped bag integrating an internal paddle mixer with a sparger system. The PadReactor offers an open architecture controller platform which gives the end user the opportunity to use the companion control system offered by ATMI or to choose a preferred controller.



Figure 1. ATMI disposable PadReactor system.

The purpose of this study was to demonstrate compatibility and performance of SoloHill's Collagen microcarriers in ATMI Lifesciences' single-use bioreactor.

The solid polystyrene core of the collagen-coated microcarriers prevents absorption of serum or cell products into the core of the microcarrier, and the 1.03 specific gravity and 125-212 micron diameter allows them to be maintained in suspension with a gentle low stirring speed. These solid microcarriers provide an excellent substrate for cell growth (Figure 2). They can be sterilized by autoclaving at temperatures up to 130°C, or via gamma irradiation doses of 25 to 40 kGy with no deleterious effects on function.



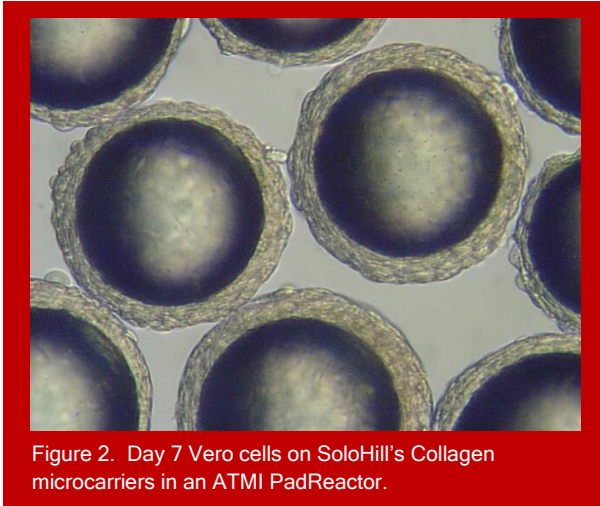


Figure 2. Day 7 Vero cells on SoloHill's Collagen microcarriers in an ATMI PadReactor.

MATERIALS AND METHODS

Microcarriers, Bioreactor and Spinners

SoloHill Collagen microcarriers (Product # C102-1521) were used at a final concentration of 30 g/L.

A 25 L Single-use ATMI PadReactor (p/N 704782) disposable bioreactor bag retrofitted with a perfusion filter was employed using the conditions described in this report.

Corning™ Brand 250 mL Glass Spinner Flasks (p/N 4500-250) were used for serial passaging.

Cells and Media

Vero Monkey Kidney cells (ATCC CCL-81) were used at Passage 131.

Complete media for cell growth was Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific SH30585.02), 1% Non Essential Amino Acids (Thermo Fisher Scientific SH30238.01), 2 mM L-Glutamine (Thermo Fisher Scientific SH30034.01) and 1% antibiotic/antimycotic (Mediatech 30-004-C1)

supplemented with 5% fetal bovine serum (FBS; Thermo Fisher Scientific SH30071.03) unless otherwise specified.

Microcarrier Mixing

To evaluate the mixing capabilities of the ATMI PadReactor system containing SoloHill 1.03 density Collagen microcarriers, the microcarrier distribution was mapped at various impeller speeds by collecting samples from multiple positions and depths in the bioreactor bag. Samples from the top, middle and bottom of the bag at all four corners of the bioreactor were retrieved and dried overnight in a oven and the resultant microcarrier weight for each sample was measured on an analytical balance. To determine percent mixing, the empirically-determined mass of microcarriers in each sample was compared to the predicted microcarrier concentration if 100% mixing occurred. Thus, the equation used to determine percent mixing was:

$$\% \text{ mixing} = (\text{measured microcarrier weight} / \text{predicted weight at 100\% mixing}) \times 100.$$

Bioreactor Culture Conditions

Temperature was maintained at 37°C, agitation rate was continuous (35-45 rpm), dissolved oxygen (DO) concentration was maintained at 30-40% of air saturation, and pH controlled at 7.0-7.4. During the culture period DO was controlled by oxygen overlay (100 mL/min) as well as through a 2 mm macrosparger that was fixed on the mixing paddle (100-200 mL/min). The culture pH was controlled by air overlay and addition of 2.5 N NaOH as needed (Figure 3). Media perfusion began two days after culture initiation and continued through Day 6 at ~150% volume changed per day for a total utilization of 92 L as demanded by an escalating high density Vero culture.



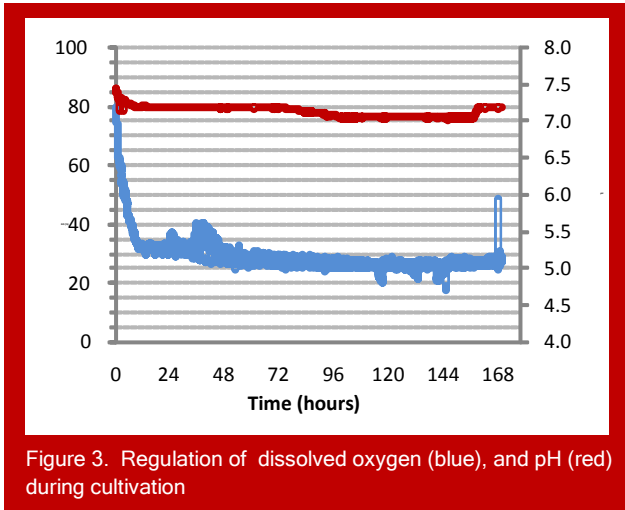


Figure 3. Regulation of dissolved oxygen (blue), and pH (red) during cultivation

On the day of culture initiation, 450 g of the collagen-coated microcarriers (equivalent to 162,000 cm²) were pre-autoclaved in 900 mL of deionized water and added to the disposable bioreactor bag containing 12.5 L of complete media without FBS. The DO probe was calibrated using standard methods. The bioreactor was then seeded at 40 rpm with a single cell suspension (12.23 x 10⁶ cells/mL) at a final concentration of 0.2 x 10⁶ cells/mL (1.7 x 10⁴ cells/cm²; approximately 16 cells per microcarrier) when the DO content of the medium was 80% of air saturation and pH was 7.45. Once cells attached to > 90% of the collagen microcarriers, FBS was added to the culture to a final concentration of 5% and the final volume of culture (microcarrier + media) was adjusted to 15 L by addition of complete DMEM media without FBS. Post cell seeding, the DO concentration and pH both drifted down within the bioreactor operating range (DO concentration; 30-40% of air saturation, pH 7.0-7.4). These conditions were maintained for the remainder of the bioreactor run.

Serial Passage Conditions

Cells harvested from the ATMI bioreactor were used to seed a spinner containing fresh collagen-coated microcarriers. After seeding, the culture was maintained at 37°C, 5% CO₂, with a continuous agitation rate of 35 rpm in a water-jacketed incubator. The spinner was prepared and autoclaved according to the protocol found in SoloHill's microcarrier technical briefs⁽¹⁾, with 6 g of microcarriers suspended in 25 to 30 mL of deionized (DI) water. After autoclaving, the DI water was carefully decanted through the side arm of the spinner flask without loss of microcarriers. Afterwards, 150 mL of the DMEM medium without FBS was added to sterile flasks and spinners were stirred at a constant rate of 40 rpm.

The spinner was seeded with a single cell suspension (12.23 x 10⁶ cells/mL) at a final concentration of 0.2 x 10⁶ cells/mL (1.7 x 10⁴ cells/cm²; ~16 cells per microcarrier). Once cells attached to > 90% of the microcarriers, FBS was added to the culture to a final concentration of 5% and the final volume (microcarrier + medium) of culture was adjusted to 200 mL by addition of the DMEM medium without FBS.

Bioreactor and Spinner Culture Sampling

Bioreactor culture samples were retrieved daily at 40-50 rpm to evaluate cell growth on microcarriers. Prior to sampling, the sample line was purged with 60 mL of culture using a 60 mL syringe attached to the front port (port 11). A 20 mL sample was then immediately collected in a 20 mL syringe for counts. Spinner culture samples were also retrieved daily at 40-50 rpm to evaluate cell growth on microcarriers. Three aliquots (1 mL each) of culture were retrieved with a Finnpiptette (Fisher T21346) at the 150 mL mark on the spinner and aliquots were pooled and images of



cell-laden microcarriers were captured via phase contrast microscopy.

Cell Enumeration

Immobilized cell density was estimated by cell lysis with an aqueous Triton® X-100 (0.5%) and citric acid (0.1 M) solution; subsequent counting of released intact cell nuclei was performed with a Cellometer® Auto T4 cell counter (Nexcelom Bioscience.)

RESULTS

Microcarrier Mixing

SoloHill Collagen microcarriers with a relative density of 1.03 exhibited an optimal distribution between 30 and 50 rpm, as seen in Figure 4. At these speeds the PadReactor’s mixing performance was very high and therefore these conditions were chosen for subsequent cell growth studies.

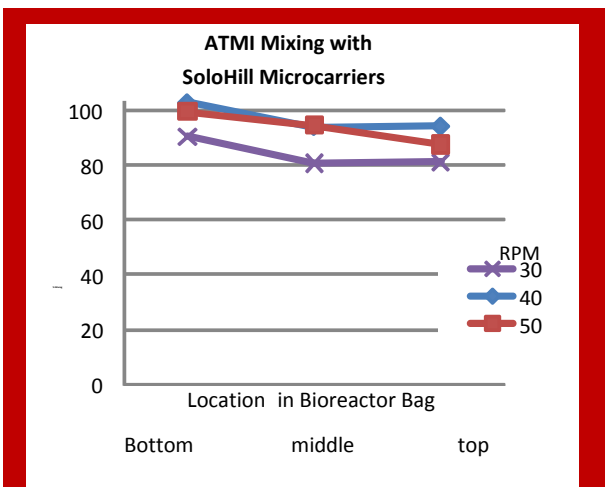


Figure 4. Mixing profile of SoloHill’s 1.03 density Collagen microcarriers in an ATMI PadReactor.

Bioreactor Culture

Microscopic examination of a representative sample retrieved from the bioreactor one hour after culture initiation revealed that cells had attached and begun to spread onto > 90% of the microcarriers. At Day 1, it was apparent that cells had begun to grow on ~90% of the microcarriers (Figure 5).

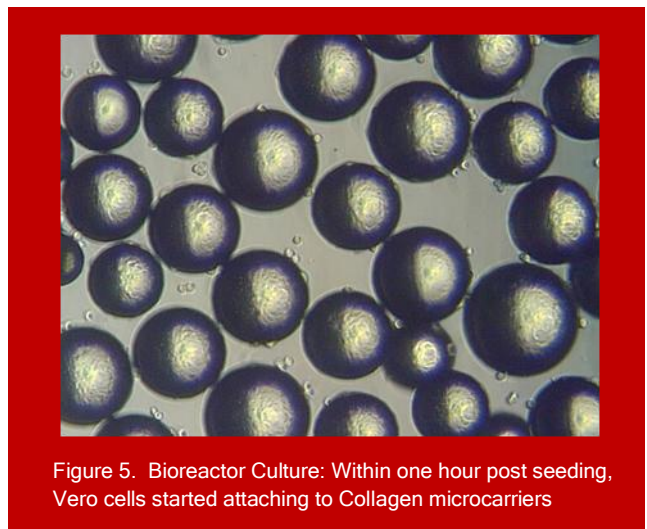


Figure 5. Bioreactor Culture: Within one hour post seeding, Vero cells started attaching to Collagen microcarriers

By 72 hours most microcarriers were confluent (Figure 6) and at Day 7, Vero cells had grown 5.9 generations, reaching a cell concentration of 11.2×10^6 cells/mL (Figures 7, 8). Glucose concentration was maintained with perfusion through Day 6 (Figure 8). The decline in glucose levels measured on Day 7 was due to terminating medium perfusion on Day 6.



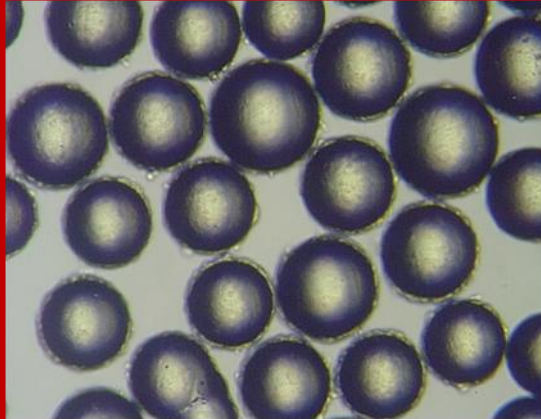


Figure 6. Bioreactor Culture: $\geq 90\%$ Collagen microcarriers reached confluency by 72 hours

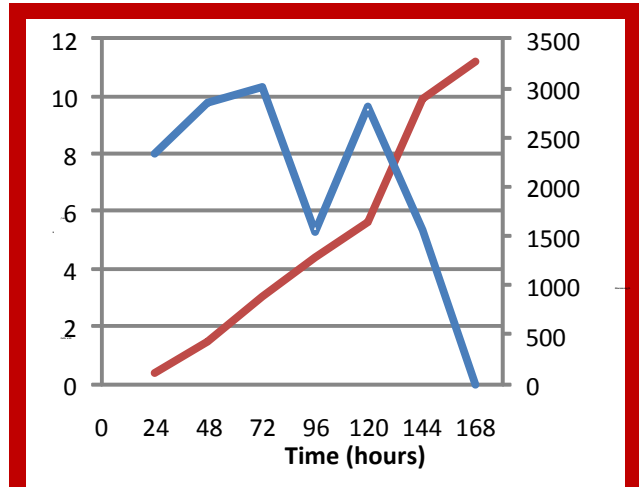


Figure 8. High density growth curve (red) of Vero cells on Collagen microcarriers in a 25 L PadReactor. Glucose concentration (blue) was maintained with media perfusion through Day 6.

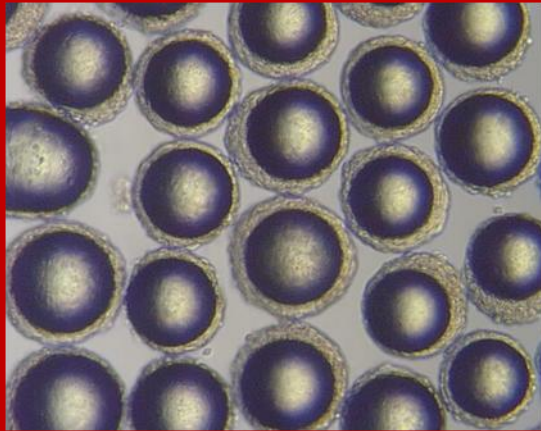


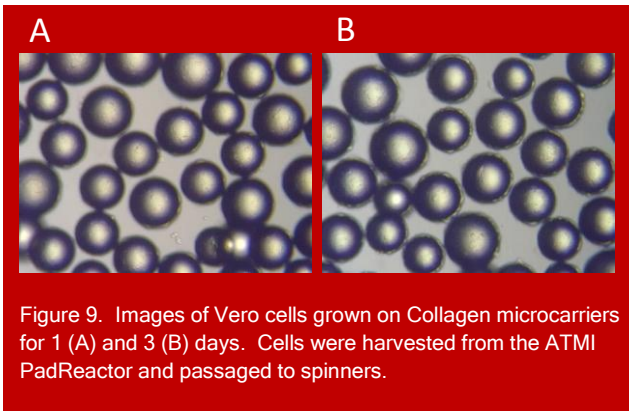
Figure 7. Bioreactor Culture: High-density culture on Day 7

Serial Passage

To demonstrate the feasibility of serial passaging high density cultures, Vero cells harvested from an aliquot of the entire bioreactor culture were used to seed a 250 mL spinner containing fresh collagen-coated microcarriers. Viability of this cell suspension was 98% and cells efficiently attached and spread on microcarriers when seeded into the spinner. A conservative estimate using cell numbers obtained from the bioreactor sample indicate that this 15 L culture could be used to seed at least one 300 L bioreactor containing an equivalent microcarrier concentration of 30 g/L providing a split ratio of 1:20. This estimate assumes an 80% cell recovery obtained from the entire bioreactor contents during harvesting.



Monitoring spinner cultures seeded with bioreactor-derived cells revealed that the cells attached and spread on over 90% of the collagen-coated microcarriers one day after seeding and by 72 hours had grown to near confluence (Figure 9).



CONCLUSIONS

The results reported here demonstrate compatibility and performance of SoloHill's Collagen microcarriers in an ATMI LifeSciences' PadReactor single-use bioreactor. In addition, the data show that cells derived from high-density culture can be serially passaged to fresh microcarriers for cell expansion.

The focus of our process development studies with Vero cells on collagen-coated microcarriers was to identify the best dynamic conditions for high-density cultures. This was accomplished in feasibility studies performed in small-scale spinners before transition into the bioreactor format (data not shown). These previously-identified culture conditions were employed in the bioreactor, and growth of Vero cells on the microcarriers in the disposable bioreactor was exceptional (Figures 7, 8 & 9).

These data show that SoloHill Collagen microcarriers employed in the ATMI PadReactor represent an attractive platform for generating large numbers of cells in a small footprint. The resultant cells could be harvested and used for subsequent passage into a larger independent reactor containing microcarriers or, with proper developmental efforts, for cell expansion in a single reactor through addition of fresh microcarriers and media. Additionally, the efficient attachment obtained when FBS is not present in the media and the subsequent rapid growth profile observed upon subsequent culture with FBS indicate that cells could be infected for virus production after as little as three days of culture.

The results obtained here lay the foundation for subsequent studies exploring virus production in this platform in both serum-containing and animal component-free systems. Additionally, these data demonstrate that the ATMI single-use PadReactor, coupled with SoloHill Collagen microcarriers, provides an attractive platform for expansion of cells required for seeding large bioreactors used for vaccine production.

REFERENCES:

¹ <http://www.solohill.com/Solohill/Microcarrier/Protocols/>

For additional information and technical assistance, either visit our web site at www.solohill.com or call our technical hotline at (734) 973-2956.

