

SOLOHILL Microcarriers for Vero Cell Expansion

INTRODUCTION

Bioreactor systems provide a logical platform for expansion of the large numbers of cells required for production of therapeutic molecules or for cellular therapies. Arguments for employing bioreactors for these purposes include the ability to precisely control and optimize cell growth conditions, ease of use, and avoidance of contamination due to the “closed” nature of the system along with the recent emergence of disposable bioreactor technology. In addition, large numbers of cells can be produced under current good manufacturing practices (cGMP) in a small space.

For anchorage-dependent cells, microcarriers provide a large surface area for propagation and growth. Because specific cell types possess unique requirements for attachment and spreading, the optimal microcarrier should be selected empirically. It is practical to perform small-scale studies with multiple microcarrier types to identify the best microcarrier for the application and to minimize costs during development. To streamline manufacturing processes, consideration should be given to the physical characteristics of the microcarriers. For example, durable and rigid microcarriers are amenable to long production runs and facilitate efficient harvesting of cells from bioreactors.

Additionally, production processes can be simplified by employing microcarriers that require minimal preparation steps prior to use.

Vero cells (ATCC CCL-81) are commonly used in veterinary and human medicine. Preliminary studies performed using SoloHill’s microcarriers with Vero cells in spinner culture demonstrated that Hillex® II microcarriers (SoloHill H112-170) support excellent cell growth⁽¹⁾.

In this application note we present data demonstrating feasibility of serial passaging Vero cells on SoloHill Hillex II microcarriers in bioreactors. Three serial passages were performed proceeding from a 0.2 L spinner through a 10 L culture in a bioreactor (Figure 1).

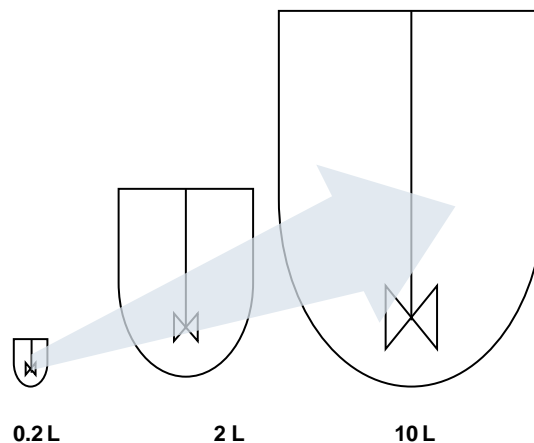


Figure 1. Diagram detailing bioreactor-based expansion scheme used in these studies.



MATERIALS AND METHODS

Vero cells purchased from ATCC (CCL-81; P121) were expanded to create master (P125) and working cell banks (P127). Cells from the working cell bank were used for these experiments. Cell suspensions at Passage 136 were used to seed initial spinner cultures for scale up.

Spinner Culture

For the first passage on Hillex II (HP1), Vero cells were harvested from vented T150 tissue culture flasks (Corning 430825) with trypsin (0.05% 1X Sigma T 3924) after two washes with Dulbecco's phosphate buffered saline (DPBS) (Hyclone SH30028.03).

Corning™ brand 250 mL spinner vessels (Fisher Scientific 10-203B) containing 200 mL of DMEM media (HyClone SH30585.02) supplemented with 5% fetal bovine serum (HyClone SH3007103), 1% Non Essential Amino Acids (Hyclone SH302.38.01), 2mM L-Glutamine (HyClone SH 30034.01) and 1% antibiotic/antimycotic (MediaTech 30-004-C1) were used for Vero cell propagation. Microcarrier concentrations equivalent to a total surface area of 5150 cm²/L (10 g/L) were used and all microcarriers were prepared according to manufacturer's instructions by autoclaving at 121°C in deionized water for 30 minutes. Spinner cultures were essentially performed as described in SoloHill's microcarrier technical briefs⁽²⁾.

A 200 mL spinner containing 2 g of Hillex II (1,030 cm²) was seeded (Ni) at a density of 2.0 x 10⁴ cells/cm² (18 to 19 cells per microcarrier). Incubation conditions were 37°C with 5% CO₂ and batch volume exchanges of 150 mL media were performed on Days 3 and 4. One mL of a 2 mg/mL stock solution of folic acid (Sigma F8758-5g) was added daily to

Hillex II cultures to promote growth during expansion and after passage to fresh substrate. In the biological safety hood, with the spinner on the stir plate and the impeller mode set at 60-70 rpm, the single-cell suspension was added to seed the spinner and warmed media was added to bring the final spinner volume to 200 mL. The spinner flask was then immediately transferred to an incubator equipped with a stir plate set at 55-60 rpm.

The cell attachment rate to Hillex II can be faster than other SoloHill microcarriers depending upon conditions used for cell seeding. We have observed rapid attachment to Hillex II within one hour. The cell attachment rate can vary based on the enzyme brand and type used for cell dissociation.

For subsequent passages on Hillex II (HP2 and HP3), cells were harvested from the microcarriers and passaged to fresh Hillex II microcarriers. For cell harvest, the 200 mL spinner was transferred to a biological safety hood, microcarriers were allowed to settle and the media was removed from the settled microcarrier pack by pouring through the side-arm of the vessel. Few microcarriers are discarded along with the media because of the higher specific gravity of Hillex II.

The cell-laden microcarriers were thoroughly washed by adding 50 mL DPBS to the vessel taking care not to dispense the liquid directly onto the microcarriers to avoid dislodging cells. This wash solution was decanted; an additional 50 mL of DBPS and the culture was then stirred at 40 rpm at room temperature for 10 minutes. After removing the final rinse, 22.5 mL of trypsin solution was added and the culture was incubated for 10 minutes. When cells were rounded and on the verge of dislodging, the microcarrier-cell suspension was triturated up and down to generate a single cell suspension. Cell counts were performed using standard assays to



assess cell numbers and viability and this cell suspension was used to seed a 2 L bioreactor containing 10 g/L of Hillex II.

Bioreactor Cultures

A New Brunswick CelliGen bioreactor Model 310 with a 5 L vessel was used for the second passage. The final working volume chosen for these experiments was 2 L. The vessel was equipped with a ring sparger, spin filter, 3-segmented pitched blade impeller (up-pumping), and 4-gas control at 100 mL/min (Air, N₂, CO₂, and O₂). Twenty grams of Hillex II (10 g/L) was combined with 125 mL of deionized water, and the slurry was added to the vessel. The reactor was autoclaved for 20 minutes at 100°C to warm the vessel and contents and then immediately autoclaved a second time for 40 minutes at 125°C. After autoclaving, 2 L media was added to the reactor. The vessel was maintained at 37°C while stirring at 60 rpm (impeller tip speed ~0.25 m/s) for 18 hours. Prior to cell addition, the pH was adjusted to 7.2, and the dissolved oxygen (DO) set to 30% air saturation. Trypsinized cells from the 200 mL spinner were added to the vessel via a peristaltic pump. The agitation rate was increased to 70 rpm during the cell addition, and returned to 60 rpm for the remainder of the run. Throughout the culture, pH was maintained with an air overlay and 1N sodium hydroxide (NaOH). DO was maintained with an oxygen overlay and sparging. Media perfusion at a rate of three quarter volumes per day began on the second day of culture and ended on Day 4 so that a total of 3 L of media was used for perfusion. Folic acid additions (10 mL of a 2 mg/mL stock solution) were performed daily. During culture, samples were retrieved daily and nuclei counts were performed using the citric acid/crystal violet method. Nuclei were enumerated using a hemocytometer and the nuclei per cm² surface area for each sample were calculated. On the

fifth day of culture, cells were harvested for passage to the next vessel. The harvest step began with turning off agitation and allowing the Hillex II microcarriers to quickly settle. The media was pumped out of the reactor via a dip tube and 1 L of DPBS was added to wash the cells. Microcarriers were re-suspended at an agitation rate of 55 rpm and the entire contents of the vessel were transferred to a sterile 1 L spinner vessel using air pressure.

Further processing took place in a biological safety hood. Microcarriers were allowed to settle and the DPBS was removed. An additional liter of DPBS was added and the microcarrier suspension was stirred at 40 rpm for 10 minutes. The microcarriers were then allowed to settle, wash solution was removed, 300 mL of trypsin was added and the vessel was incubated at room temperature for 10 minutes with occasional swirling by hand. The entire contents of the vessel were then stirred at 50 to 60 rpm for 5 minutes. The microcarriers and cells were then triturated up and down with a pipette and the resulting slurry was filtered through a 150 micron screen to separate cells from microcarriers.

A Sartorius BioStat C 15 L bioreactor containing a final working volume of 10 L media was used for the third and final passage. The bioreactor was equipped with a ring sparger, spin filter, paddle impeller (up-pumping), and 4-gas controller, which was set at 100 mL/min (Air, N₂, CO₂, and O₂). One hundred grams of Hillex II was combined with 700 mL of deionized water and added to the vessel. The vessel and contents were then in-situ steam sterilized for 40 minutes at 125°C and after sterilization, nine liters of media was added to the vessel. Media temperature was maintained at 37°C, pH set to 7.2, DO set to 30% of air saturation, and agitation set to 35 rpm (impeller tip speed ~0.29 m/s). The agitation



rate was increased to 45 rpm in preparation for cell addition and the single-cell suspension from the 2 L bioreactor was added to the bioreactor vessel via a peristaltic pump. After cell addition, media was added to bring the total working culture volume to 10 L. Agitation rate was maintained at 35 rpm for the remainder of the run. Throughout the culture, pH was maintained with an air overlay and addition of a 1N NaOH solution. DO was maintained with oxygen sparging. A 1.5 volume per day media perfusion began on Day 2 and ended on Day 4. During culture, samples were retrieved daily at a stir speed of 45 rpm and subsequently processed for nuclei counts using the citric acid/crystal violet method. Nuclei were counted using a hemocytometer and the number of nuclei per cm² surface area was calculated for each sample. Folic acid additions of 49 mL were performed daily.

RESULTS

To successfully implement a production scheme using microcarrier-based scale-up in large bioreactors it is important to obtain sufficient data to warrant transition into larger formats. The logical and most cost-effective path toward this goal is to obtain data in small-scale static and spinner-based feasibility studies prior to transitioning into costly studies in large bioreactors.

The maximum cell density achieved in the bioreactors was 2.75 x 10⁶ nuclei/mL and 53.7 x 10⁴ cells/cm² after seven days of culture. These results demonstrate the ability to expand Vero cells on Hillex II microcarriers in stirred vessels for three passages. (Figure 2.)

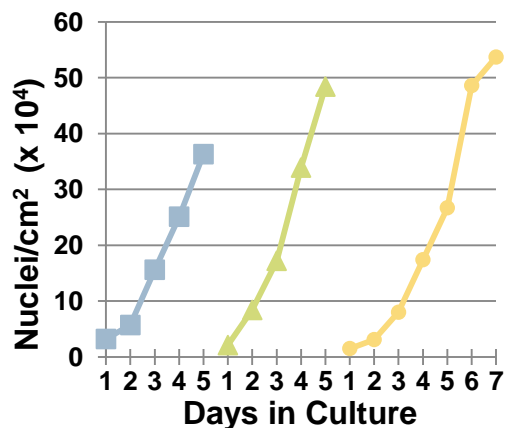


Figure 2. Vero cells serial passaged on Hillex II microcarriers. Passage 1 0.2L Spinner (squares), Passage 2, 2L bioreactor (triangles) Passage 3, 10 L bioreactor (circles) microcarriers.

Microscopic visualization of cells on microcarriers was consistent with these data (Figure 3).

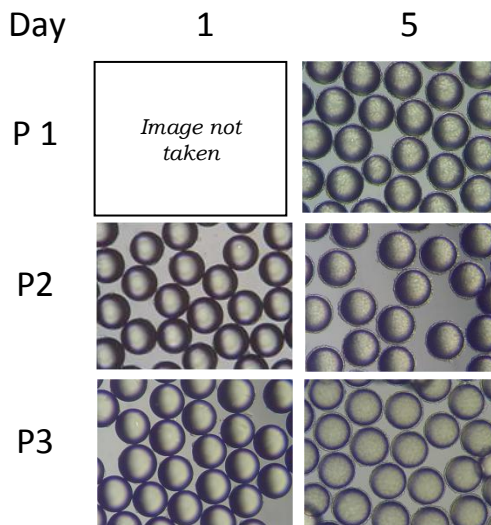


Figure 3. Images of Vero cells on Hillex II microcarriers (Passage 1 through 3) were captured after one and five days of culture. Photo of P3 cells taken after seven days of culture



▶ **SOLOHILL APPLICATION NOTE APP-003A** EXPANSION OF VERO CELLS ON SOLOHILL HILLEX II MICROCARRIERS VIA SERIAL PASSAGE IN STIRRED VESSELS

A critical feature of SoloHill microcarriers is the ability to easily and efficiently harvest cells from microcarriers. Standard enzymatic techniques can be used to readily harvest Vero cells in a single-cell suspension (Figure 4).

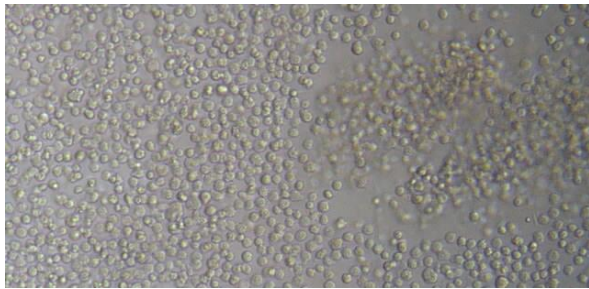


Figure 4. Single-cell suspension of cells trypsinized from a 2 L culture used to seed a 10 L culture.

When cells from these microcarriers are seeded into new vessels with fresh microcarriers the cells attached very quickly and evenly as seen in Figure 5.

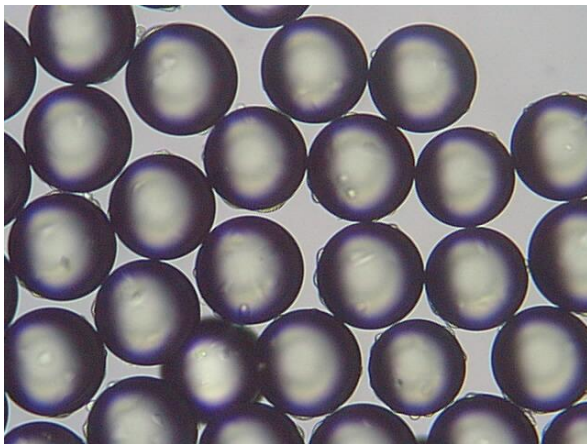


Figure 5. Initial cell attachment to Hillex II in a 2 L bioreactor (5 hours after seeding).

Subsequent culture reproducibly produced confluent layers of cells on the surface of the entire population

of microcarriers which are clearly identified by bright field images shown in Figure 6.

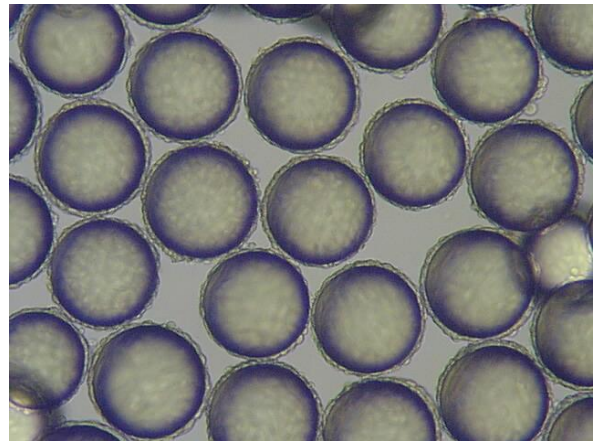


Figure 6. Confluent Vero cells grown on Hillex II microcarriers in a 15 L bioreactor.

CONCLUSIONS

Results presented here demonstrate the feasibility of using SoloHill's Hillex II microcarriers for expansion of Vero cells via microcarrier-based serial passage in stirred vessels.

The number of 850 cm² roller bottles required to produce equivalent cell yields is significant in that 45 or 450 roller bottles would be required to achieve the same cell yield as a single 10 L or 100 L bioreactor, respectively.

These feasibility studies demonstrate that SoloHill's Hillex II microcarriers provide an ideal substrate for expansion of Vero cells in closed stirred vessels and lay the groundwork for subsequent developmental studies in even larger bioreactor formats.



▶ SOLOHILL APPLICATION NOTE APP-003A EXPANSION OF VERO CELLS ON
SOLOHILL HILLEX II MICROCARRIERS VIA SERIAL PASSAGE IN STIRRED VESSELS

REFERENCES:

¹ Wallace B.L. and Hillegas, W.J. Method and Device
for Producing Vaccine (2009). United States Patent;
US 7,534,596B2

² <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.

