
SOLOHILL Microcarriers for MDCK Cell Expansion



INTRODUCTION

Common production platforms employed for manufacture of vaccines, biologics, and cell therapeutics include roller bottles, cell cubes/factories or suspension cultures. These systems are typically used for expansion of cells to seed large bioreactors. Although well-established, these formats occupy a large footprint, are labor intensive, and are susceptible to frequent contamination problems due to numerous open handling steps. With suspension cultures, problems can arise because cells grown in suspension display characteristics that differ from their adherent phenotypes. Furthermore, downstream processing becomes more challenging because of the need for cell retention.

Closed-impeller bioreactor systems utilizing microcarriers provide a logical alternative to these systems. The advantages of bioreactors 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.

Microcarriers offer a large surface area for growth of anchorage-dependent cell types, thereby facilitating the use of bioreactors for propagation of attachment-dependent cells. Because specific cell types have unique requirements for attachment and growth, the optimal microcarrier should be selected empirically. The most cost-effective pathway to this end is to perform small-scale studies with multiple microcarrier types to identify the best microcarrier for the application. To streamline manufacturing processes, consideration should be given to the physical characteristics of the microcarriers and the bioreactor platform to be used for final production. For example, durable and rigid microcarriers facilitate efficient harvest of cells from bioreactors. Additionally, some microcarrier types require minimal preparation steps prior to use. Consideration should also be given to media composition, bioreactor configuration and proposed harvest procedures if applicable.

MDCK cells are a kidney epithelial cell line derived from a healthy adult female dog. These cells are currently employed for production of Influenza A and B viruses used in trivalent vaccines. Preliminary studies performed using SoloHill’s microcarriers indicated that MDCK cells exhibit excellent growth characteristics on Collagen (SoloHill C102-1521), FACT III (SoloHill F102-1521), Plastic Plus (SoloHill PP102-1521) and Hillex® II (SoloHill H112-170) microcarriers.

In this application note, we characterize MDCK cell (ATCC CCL34™) growth on four SoloHill microcarriers in stirred vessels.



MATERIALS AND METHODS

For this study, MDCK cells purchased from ATCC (CCL34) were expanded to create working cell banks (P3 and P4) and cells from the working cell banks were used for these experiments. Corning™ brand 250 mL spinner vessels (Fisher Scientific 10-203B) containing 200 mL of MEM media (Sigma M3024) supplemented with 5% fetal bovine serum (FBS) (Thermo Scientific HyClone SH3007103) and antibiotic/antimycotic (Fisher MT-30-004-CI) were used for MDCK cell propagation. For initial growth experiments on various SoloHill microcarriers, a total surface area of 1030 cm² was used per spinner culture (2.0 grams Hillex II and 2.86 grams for all other microcarriers). For Collagen microcarrier concentration experiments, 10, 20, and 50 g/L (2, 4, and 10 grams per spinner) were employed in spinner vessels. All microcarriers were prepared according to manufacturer's instructions by autoclaving at 121°C in deionized water. Spinner cultures were essentially performed as described in the microcarrier technical briefs⁽¹⁾.

For growth on microcarriers, MDCK cells were harvested from roller bottles (Fisher 09-761-113) using 0.25% trypsin EDTA (Sigma T4049) or TrypLE Express (Invitrogen 12604-021) after two washes with Dulbecco's phosphate buffered saline (DPBS), (HyClone SH30028.03). Cell counts were performed using standard assays to quantify cell numbers and assess viability. Spinners were seeded (Ni) at 2.0 x 10⁴ cells/cm² (approximately 18 to 19 cells per microcarrier). Cells in spinners containing Hillex II microcarriers were stirred at 60 rpm, whereas, all other microcarrier spinners were kept at 40 rpm. Incubation conditions were 37°C with 5% CO₂. Media exchanges of 100 mL (50%) were performed on Days 3, 4, and 5 of the culture.

During culture, samples were retrieved daily for nuclei counts using the citric acid/crystal violet method.

Nuclei were counted using a Nexcelom counter with associated software and the number of nuclei per cm² surface area was calculated for each sample.

For trypsinization of cells from microcarriers, 3 or 10 mL samples were taken from spinner culture and transferred to a 15 mL tube. Cell-laden microcarriers were allowed to settle and media was removed. The microcarrier bead pack was washed with DPBS for 5 minutes at room temperature, occasionally rocking back and forth by hand to re-suspend microcarriers. After 5 minutes, microcarriers were allowed to settle and the DPBS was removed. One to three mL of 0.25% porcine trypsin was added (1 mL trypsin for a 3 mL sample and 3 mL trypsin for a 10 mL sample). The samples were gently pipetted up and down 1 to 2 times to thoroughly mix, and then incubated at 37°C for 30 minutes with occasional rocking by hand. Cells and microcarriers were pipetted after 15 minutes, and again after 30 minutes to achieve a single-cell suspension that could be used to reseed fresh microcarriers.

RESULTS

To provide feasibility data for growing MDCK cells in bioreactors we examined cell growth on four different microcarrier types in spinner cultures.

Data presented in Figure 1 shows that MDCK cells exhibit excellent growth on Collagen, Fact III, Plastic Plus, and Hillex II microcarriers, reaching a maximum density of ~50 to 60 x 10⁴ nuclei per cm² by Day 5 of culture at 1030 cm² per spinner.

These cells grew very rapidly with a population doubling time (PDT) of ~20 hours through the five-day growth experiment. We optimized cell attachment and uniform growth on all four microcarrier types by switching from 0.25% trypsin to TrypLE and omitting serum from the media for the initial 4-6 hours of attachment. Images in Figure 2 show MDCK cell growth achieved on all four microcarrier types after 4



▶ **SOLOHILL APPLICATION NOTE APP-002A** EXPANSION OF MDCK CELLS ON MICROCARRIERS VIA GROWTH IN STIRRED VESSELS

days of spinner culture. Under all conditions, MDCK cells have such adherent cell-to-cell junctions that clumps of 2 to 5 microcarriers were seen after 24 hours in culture.

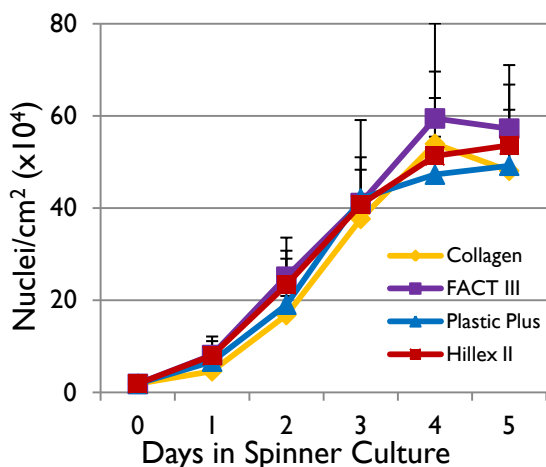


Figure 1. Growth curves of MDCK cells in various microcarrier spinner cultures. Data is presented as means ± SEM (n = 3).

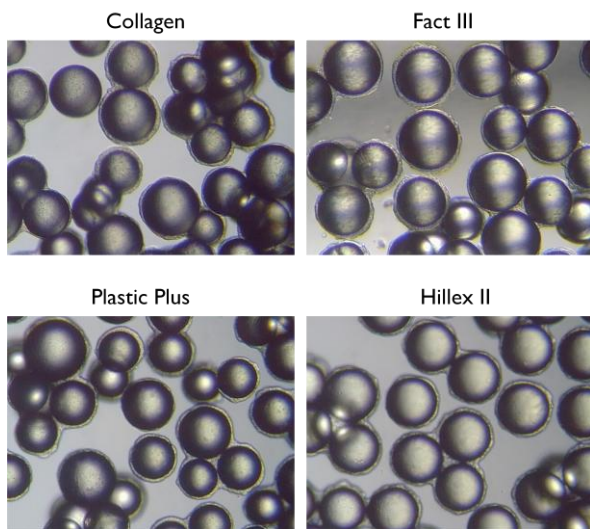


Figure 2. Images from MDCK spinner cultures on SoloHill microcarriers suggest potential for scale-up on Collagen, Fact III, Plastic Plus and Hillex II.

Figures 1 and 2 demonstrate the ability to expand MDCK cells very rapidly on SoloHill microcarriers in

stirred vessels with satisfactory growth on Collagen, FACT III, Plastic Plus and Hillex II microcarriers.

To determine whether an increase in cell concentration per unit volume of media could be obtained, Collagen microcarrier spinners were prepared at increasing concentrations of 10, 20, and 50 g/L. Figure 3 shows that by Day 5, the lowest concentration of microcarriers tested reached a cell density of 3.1×10^6 nuclei/mL (10 g/L Collagen microcarrier), and the highest density tested (50 g/L) reached 6.0×10^6 nuclei/mL. This demonstrates that by increasing microcarrier concentration in spinner vessel formats the number of MDCK cells per unit volume media can be significantly increased.

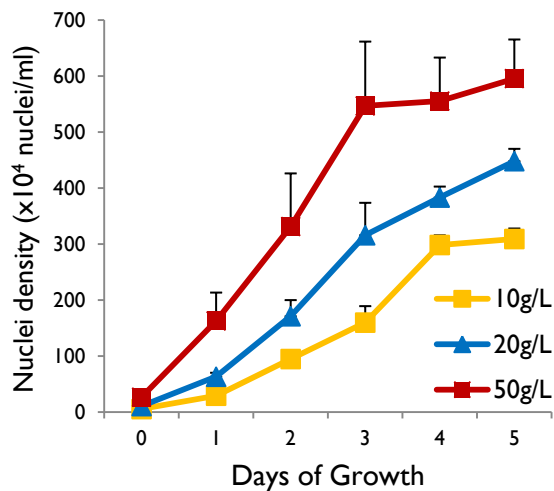


Figure 3. Growth curves of MDCK cells on Collagen microcarriers at increasing concentrations. All spinners were seeded at 1.5×10^4 cells/cm².

To establish feasibility for scale-up to large bioreactor volumes, trypsinization and cell harvest from confluent microcarriers must be attainable. This is particularly important for MDCK cells because in static cultures, harvesting becomes increasingly difficult as cell density increases.

▶ SOLOHILL APPLICATION NOTE APP-002A EXPANSION OF MDCK CELLS ON MICROCARRIERS VIA GROWTH IN STIRRED VESSELS

Figure 4 shows images of confluent MDCK cells on Collagen microcarriers undergoing trypsinization. Cells began to round and detach from microcarriers after two minutes incubation at 37°C and after 30 minutes cells could easily be pipetted up and down to create a single-cell suspension. This process efficiency is achieved because of the rigid core of all SoloHill microcarriers which facilitates cell detachment during trypsinization.

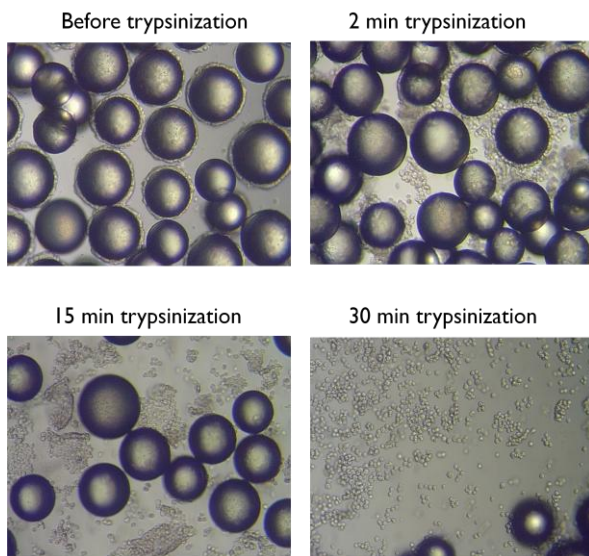


Figure 4. A single-cell suspension of MDCK cells trypsinized from Collagen microcarriers can be obtained by incubating at 37 °C for 30 minutes.

The higher relative density of Hillex II microcarriers at 1.1 provides an extra advantage during cell harvesting because Hillex II settles rapidly compared with other microcarrier types. This property facilitates more efficient harvest because cells remain in suspension much longer than the microcarriers.

Separation of Hillex II microcarriers is seen as early as 10 seconds after termination of mixing and after 1 minute they are completely settled (Figure 5).

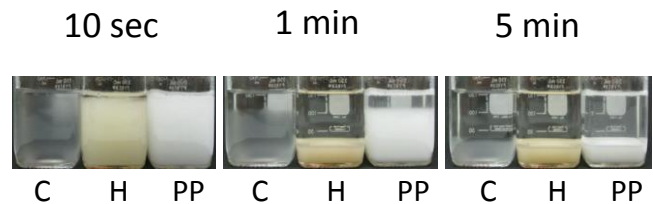


Figure 5. SoloHill Hillex II microcarriers exhibit rapid settling which expedites process times. Time course of Cytodex 1 (C), Hillex II (H) and Plastic Plus (PP) microcarrier settling in DPBS.

In addition, the opaqueness of all SoloHill microcarriers allows for easy visualization of microcarrier settling and facilitates cleaner process steps because microcarriers can be avoided during solution removal and transfer, unlike cells grown in suspension culture.

Another benefit of SoloHill microcarriers is that they do not require lengthy microcarrier preparation steps. In this regard, microcarriers suspended evenly in deionized water can be autoclaved and used immediately after sterilization. SoloHill microcarriers can also be gamma irradiated without adverse effects on performance.

CONCLUSIONS

The feasibility of using several types of SoloHill microcarriers for expansion of MDCK cells via microcarrier-based serial passage has been demonstrated in stirred vessels. Once adapted to suspension culture, MDCK cells will typically grow to a maximum density of 2×10^6 cells/mL⁽²⁾. Data presented here demonstrate that even at the lowest concentration of microcarriers tested, attachment-dependent MDCK cells will grow in spinner cultures to a density of 3.1×10^6 cells/mL. Additionally, higher cell densities can be reached by increasing microcarrier concentration. At the highest microcarrier concentration tested in these studies (50 g/L), cell density in spinner cultures reached 2×10^6 cells/mL by



▶ **SOLOHILL APPLICATION NOTE APP-002A** EXPANSION OF MDCK CELLS ON MICROCARRIERS VIA GROWTH IN STIRRED VESSELS

Day 1 and 6×10^6 cells/mL by Day 5, a three-fold increase compared to suspension cultures.

The experiments in this study were designed to establish a reproducible method for expansion of these anchorage-dependent cells in stirred tank reactors. The design was chosen to provide feasibility data for a scenario in which a 1:10 scale-up ratio between bioreactors would culminate in the seeding of a 1000 L bioreactor. Using this paradigm, the train would proceed from a 10 L bioreactor, to a 100 L bioreactor, to a final transfer to a 1000 L production bioreactor seeded with 2.0×10^4 cells per cm^2 surface area of microcarriers (Figure 6).



Figure 6. Diagram of a microcarrier-based scale up manufacturing

10 L bioreactor photo: Courtesy of New Brunswick Scientific website
 100 L and 1000 L bioreactor photos: Courtesy of Genetic Engineering News

The significance of the proposed bioreactor process is highlighted by the fact that a 10 L bioreactor containing 10 g/L Hillex II is equivalent to approximately $61 \times 850 \text{ cm}^2$ roller bottles. A 100 L bioreactor containing the same microcarrier concentrations replaces $606 \times 850 \text{ cm}^2$ roller bottles, given equivalent confluent cell densities are reached (Table 1).

Bioreactor Size (L)	10	100	1000
Grams Hillex II	100	1,000	10,000
Surface Area (cm^2)	51,500	515,000	5,150,000
Roller Bottles (850 cm^2)	60.6	606	6,060

Table 1. Hillex II and roller bottle-microcarrier surface area equivalents

Outside of direct material cost savings for large scale production, each bottle manipulation represents an “open” step with a potential for introduction of contaminants and the need for extensive labor to be eliminated.

An alternative approach for scale-up would entail performing microcarrier-based serial passage in a single bioreactor where fresh microcarriers and additional media are added to the vessel once cells have reached near confluence on the initial bolus of microcarriers. This process would require a versatile bioreactor that can function at turn-down ratios of at least 1 to 4 to make the process useful.

Future research is focusing on the optimization of a direct microcarrier-to-microcarrier cell migration either with or without the use of trypsin (or a trypsin-like enzyme). This would facilitate a simplified scale-up process into larger bioreactors and translation of these results into larger bioreactor formats.

For SoloHill microcarriers, the cell numbers were sufficient for serial passage, but may benefit from additional optimization, such as increased microcarrier concentration, supplementation of specific media components, improved harvesting procedures, etc. Results obtained with other cell types indicate that confluent cell densities reached in bioreactors exceeds those obtained in spinners, therefore, the yields reported here likely provide an underrepresentation of attainable cell numbers.

These feasibility studies demonstrate that SoloHill’s microcarriers provide an ideal substrate for expansion of MDCK cells in closed stirred-vessel systems and lay the groundwork for subsequent developmental studies in larger bioreactor formats.

▶ SOLOHILL APPLICATION NOTE APP-002A EXPANSION OF MDCK CELLS ON
MICROCARRIERS VIA GROWTH IN STIRRED VESSELS

REFERENCES:

1. <http://www.solohill.com/Solohill/Microcarrier/Protocols/>
2. [Lohr V](#), [Genzel Y](#), [Behrendt I](#), [Scharfenberg K](#), [Reichl U](#). "A New MDCK suspension line cultivated in a fully defined medium in stirred-tank and wave bioreactor." Vaccine. 2010. 28:6256-64.

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

