Magnetic Bead Removal Using Microcarrier and Cell Separation System

Compared to conventional small-scale cell harvesting methods

Entegris' microcarrier and cell separation system can effectively separate cells from microcarriers and provide maximum cell recovery.

INTRODUCTION

The criticality of reproduceable manufacturing in cell and gene therapies underlines the cGMP regulation requirement for quality by design in every step of the process. Complex manufacturing processes call for new manufacturing tools to drive closed-system processing, reduction in operator error, and standardization across therapy modalities. Product quality considerations are not new; even with recent technology advancements, numerous challenges surrounding industry standardization remain, heightening the hurdles on the path to commercialization.

An extremely variable unit operation in cell therapy processing involves the complete removal of magnetic beads from target cells. The FDA holds strict requirements for magnetic bead removal prior to therapeutic use making consistent results paramount.[1] Current accepted methods for separation of microcarrier beads from mammalian cells include tangential flow filtrations, counter-flow centrifugation elutriations, conical or inclined settlers, and magnet-based instruments.[2] Such techniques require expensive equipment that involves complex, costly infrastructure and systems to support compliance, as well as highly skilled personnel with expertise in GMP manufacturing and specialized training to operate the equipment. Despite this specialized training, it still relies on this single individual to operate the process flawlessly every time. As an added level of risk, many of the currently employed methods involve open processing steps and can result in contamination.

An efficient, closed system, GMP-compliant microcarrier bead removal method is crucial to deliver consistent, controlled results when the starting material is precious and highly variable.[3] Entegris' solution? The microcarrier and cell separation system.

MICROCARRIER AND CELL SEPARATION SYSTEM

Entegris' microcarrier and cell separation system is designed to overcome the user-interface complexity and economic process challenges of single-use systems while maximizing simplicity, ease of use, and affordability. The system facilitates a separation process using a streamlined, single-use filter/mesh system and peristaltic pump; no other equipment is required. Simply pump the unfiltered fluid to the inlet port, through the filtration mesh, and out the second port. Manufactured in an ISO-7 cleanroom, USP <788> compliant, and composed of raw materials and components approved for clinical use, there is little risk of particulate introduction, critical to downstream cell therapy manufacturing.

By customizing the chamber size, mesh size (10 – 200 µm), and pump settings, the microcarrier and cell separation system can be used for a wide array of applications such as retaining aggregate cells or microcarrier beads, cell washing, and harvest, all while achieving the same or better performance as alternative separation methods.

GOAL

Through the *Magnetic Bead Removal Using Microcarrier and Cell Separation System Study*, Entegris' microcarrier and cell separation system was incorporated into an optimized bioreactor subculturing process to maximize viable cell recovery and obtain a high split ratio between bioreactors during scale-up. Small-scale microcarrier experiments demonstrate the ability to subculture anchorage-dependent cells between vessels in a biological safety cabinet (BSC). A logical extension of this technology is to apply a similar process to commercial-scale bioreactor systems. One strategy for accomplishing this is to employ a chain of bioreactors of gradual increasing size to obtain large quantities of seed cells to inoculate into a final bioreactor culture. When implementing this approach, it is beneficial to utilize a simple and robust process to separate cells from microcarriers and transfer them into the next vessel in a sterile, closed system, without the need of a laminar flow hood.

MATERIALS

- SoloHill® microcarriers: collagen coated, plastic, and Hillex®-II microcarriers
- Entegris microcarrier and cell separation system: 150 µm, 65 µm, and 33 µm mesh pore sizes
- Vero (African green monkey kidney) cells
- Dulbecco's Modified Eagle's Medium (DMEM) with 5% FBS, 2 mm L-glutamine, 1% NEAA and 1% antibiotic-antimycotic solution
- Porcine trypsin (1x)
- New Brunswick™ 5 L Celligen® 310 bioreactor
- Corning® 250 mL glass spinner

METHODS

Cell recovery through the microcarrier and cell separation system was compared to conventional small-scale cell harvesting methods performed in conical tubes in a BSC. For the conventional method a small portion of culture was transferred into a 15 mL conical tube, the slurry was trypsinized, and cells were enumerated on a haemocytometer. The cell recovery obtained using this method is designated as 100% yield because the process is performed with very few manipulative steps and data shows that there is negligible loss during the process.

At the end of each bioreactor run a small volume of culture was taken as a representative sample of the entire culture. This sample was processed using the conventional tube method previously stated and the cell yields obtained were considered as 100% cell recovery. The same trypsinization method used in the tube method was employed for the remaining whole bioreactor culture. Once a single cell suspension was achieved, the whole cell-microcarrier trypsin slurry was passed through Entegris' microcarrier and cell separation system that was attached to the next vessel through a sterile connection, assisted by a peristaltic pump. Following this initial transfer, the microcarriers captured in the system were washed once with complete medium containing serum to remove any residual cells both from the bioreactor and the bag. At the end of this process, the cell recovery count for the microcarrier and cell separation system approach was compared to the tube method.

RESULTS AND DISCUSSION

Vero-Hillex-II (10 g/L) 2 L Bioreactor Culture

The performance of two different microcarrier and cell separation systems containing either a 150 µm or 65 µm mesh with collagen-coated microcarriers was evaluated to determine if the microcarrier and cell separation system assemblies were compatible with a current bioreactor configuration and to ensure the cells could be efficiently separated from the microcarriers.

CULTURE 1	CULTURE 2
The post-trypsinization cell culture slurry was passed through 65 µm microcarrier and cell separation system	The post-trypsinization cell culture slurry was passed through 150 µm microcarrier and cell separation system
Cell Recovery	**Cell Recovery**
Tube method: 19.5 × 10^4 cells/cm²	Tube method: 17.6 × 10^4 cells/cm²
Microcarrier and cell separation system: 20 × 10^4 cells/cm²	Microcarrier and cell separation system: 22.2 × 10^4 cells/cm²

The above data demonstrates that Entegris' microcarrier and cell separation system can effectively separate cells from microcarriers and provide maximum cell recovery. In the next step of the study, cells were passed to a fresh spinner of microcarrier culture.

Vero-Collagen (30 g/L) 2 L Bioreactor Culture (N=3)

We evaluated the ability to separate cells from collagen microcarriers with three different microcarrier and cell separation system configurations with discrete mesh pore sizes. After trypsinization, the cell culture slurry passed readily through all assemblies tested (150 µm, 65 µm, and 33 µm mesh sizes). 100% cell recovery was achieved. Recovered cells were passaged to subsequent spinner microcarrier cultures and samples were retrieved daily to generate the growth curve depicted in Figure 1.

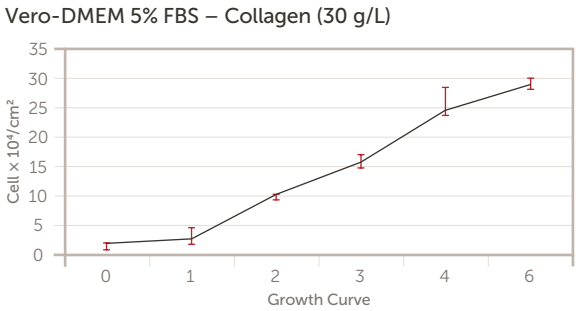

Figure 1. Entegris' microcarrier and cell separation system recovered cells subculture to 30 g/L collagen microcarrier spinner culture. The growth curve is the average result of three experiments.

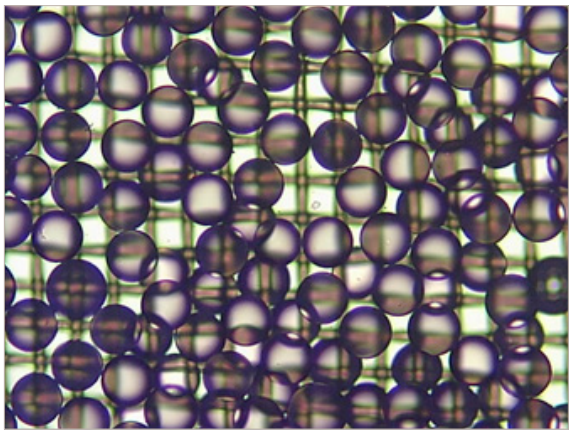

Figure 2. (160 – 180 µm) microcarrier retain on 150 µm microcarrier and cell separation system.

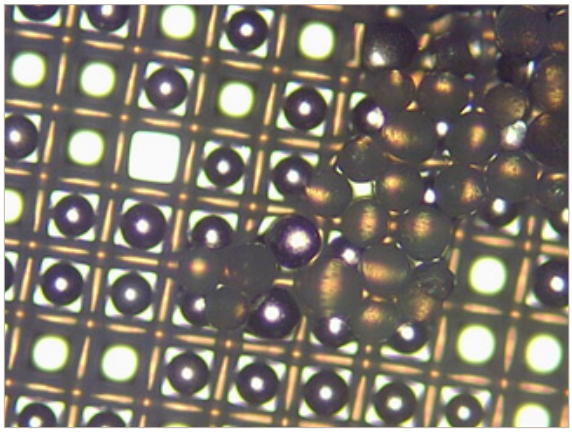

Figure 3. Collagen (125 – 212 µm) microcarrier can pass through 150 µm microcarrier and cell separation system.

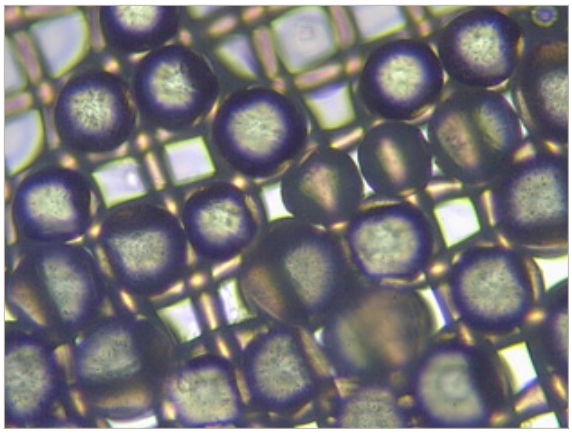

Figure 4. Collagen (125 – 212 µm) microcarrier stuck in 65 µm microcarrier and cell separation system.

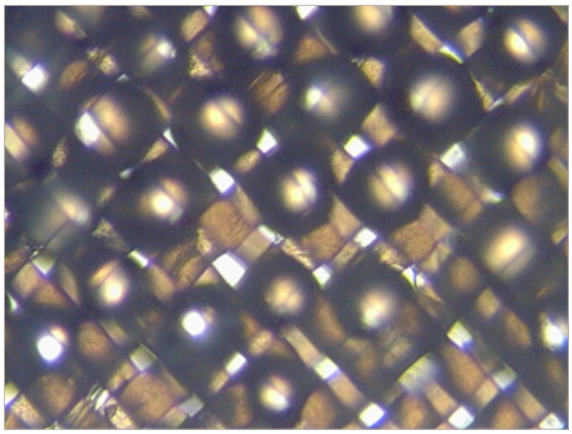

Figure 5. Plastic (125 – 212 µm) microcarrier retain on 33 µm microcarrier and cell separation system.

CONCLUSION

Entegris' microcarrier and cell separation system can be used to easily separate microcarriers from cell suspension during cell subculture process. Excellent cell recovery was obtained with the system, which increased the speed of the scale-up process in larger scale bioreactors (2 – 20 L). The growth curve in Figure 1. demonstrates that cells harvested from the microcarrier and cell separation system possess the same growth rate and viability as those without microcarrier treatment. Cellular health is critical in cell therapy applications where further processing is required post bead removal approaching final formulation, while being mindful of the limited incoming starting material.

Cytodex™, collagen-based, and other variations of microcarriers can be used, (~160 – 200 µm) with both 65 µm and 150 µm pore size mesh without clogging the screen, Figure 2. In this study, microcarriers ranging from 125 – 212 µm in size were shown to pass through the 150 µm pore size microcarrier and cell separation system, Figure 3. The data in Figure 4. demonstrates that there is a small percentage of collagen microcarriers lodged into the pores of a 5 µm size.

Plastic microcarriers ranging from 125 – 212 µm were retained on 33 µm microcarrier and cell separation system without being wedged into screen pores, Figure 5. However, a microcarrier and cell separation system with pore size 33 µm, which is smaller than standard spin filter 40 µm, can either trap cell clumps or break cell clumps with the force generated by a peristaltic pump, which can maximize cell recovery.

The growing cell therapy market needs to adopt closed system process tools that enable efficient operation, reproducibility, and lower the training required for proper execution. Entegris' microcarrier and cell separation system is a low capital investment to employ, easy to use, and can scale-up with your process to the cGMP manufacturing scale. An efficient and sterile bead removal tool will reduce the risk of residual beads in your final product, avoiding OOS deviations, preventing terminated batches, and most significantly, contributing to a consistent process with a reduced vein-to-vein time for patients who depend on us to manufacture at the highest quality level.

For more information, please contact your Entegris representative.

Related Publications

- Lonza study utilizing microcarrier and cell separation system IJMS | Free Full-Text | End-to-End Platform for Human Pluripotent Stem Cell Manufacturing (mdpi.com)
- Entegris' microcarrier and cell separation system

Sources

[1] https://www.genengnews.com/magazine/268/overcoming-the-roadblocks-to-successful-t-cell-isolation/

[2] https://www.nature.com/articles/s41598-018-31019-y

[3] https://insights.bio/cell-and-gene-therapy-insights/journal/article/2347/Magnetic-selection-for-consistent-cellular-starting-material-in-autologous-cell-therapy-manufacture

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Entegris

Corporate Headquarters
129 Concord Road
Billerica, MA 01821
USA

Customer Service
Tel +1 952 556 4181
Fax +1 952 556 8022
Toll Free 800 394 4083