Scalability of microcarrier bead separation using the Harvestainer systems

Introduction

Current techniques used to separate adherent mammalian cells from microcarrier beads include sedimentation using conical or inclined settlers, centrifugation, acoustic resonance, spin filtration, and microfiltration. These techniques often use sophisticated equipment, requiring significant capital expenditure as well as routine maintenance, and cleaning and sterilization between uses. Until now, single-use options were restricted to disposable spin filters and hollow-fiber microfiltration systems.

The Thermo Scientific™ Harvestainer™ Microcarrier Separation System is a single-use system designed to simplify and economize on the cell–bead separation process. The Harvestainer system is used to harvest the cells that have been cultured on microcarrier beads, after they have been dissociated from the beads, by separating the microcarrier beads from the cells and culture medium. The systems come in two different styles: 2D systems that can filter up to 3 L or up to 12 L of swelled bead volume (equivalent to 150 g and 600 g, respectively, of dry Cytodex™ 3 microcarrier beads), and larger 3D systems that use a bag-within-a-bag design for full containment of trapped microcarrier beads within the inner Thermo Scientific™ Labtainer™ BioProcess Container (BPC) with the spent cell culture medium, and detached cells collected in the outer BPC. These 3D systems can collect and separate up to 25 L or up to 50 L of swelled bead volume (equivalent to 1.25 kg and 2.5 kg, respectively, of dry Cytodex 3 beads).

The collected supernatant from each of these systems can be pumped into a separate BPC for further holding, or to other processes with little to no loss of cell yield or viability. The Harvestainer system can be integrated with either stainless steel or single-use bioreactors. The system is also customizable for optimal plug-and-play convenience, and the self-contained design simplifies disposal.

Goal

To demonstrate the effectiveness and scalability of the small-scale and large-scale Harvestainer systems, the 3 L, 12 L, and 25 L systems were used to collect the cells and isolate the microcarrier beads in a model system that employed a 250 L Thermo Scientific™ Hyperforma™ Single-Use Bioreactor (S.U.B.) and an anchorage-dependent cell line grown on dextran-based microcarrier beads. Results of these harvests were compared to show scalability of the Harvestainer systems.

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Materials and methods

Cell culture growth conditions

Vero cells (CCL-81™ cells, ATCC) were grown on Cytodex 3 microcarrier beads in Gibco™ high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a 250 L S.U.B. Materials used to grow and dissociate the cells are described in Table 1.

The S.U.B. system was set up according to the Thermo Scientific™ HyPerforma™ S.U.B. User Guide, and controlled by a DeltaV™ system (Finesse Solutions), utilizing TruFluor™ single-use dissolved oxygen (DO) sensors (Finesse Solutions) and single-use pH sensors for DO and pH control (Mettler-Toledo). Operating parameters are listed in Table 2. Cytodex 3 microcarrier beads were utilized at 3 g/L for the cultures. The beads were prepared and autoclaved according to the supplier's instructions, and then placed in fresh culture medium prior to being added to the bioreactors.

Samples were taken daily to assess attachment and growth rate. This was done by pulling a sample of approximately 10 mL from the reactor to clear the sample line, discarding it, and then pulling a fresh 10 mL sample. Of that 10 mL sample, 1 mL of the supernatant was run on the BioProfile™ FLEX cell culture analyzer (Nova Biomedical) to measure nutrient and/or metabolite consumption and waste production. A few drops were placed on a microscope slide to visually inspect the culture, and an 8–9 mL sample was used to count the cells using a crystal violet/citric acid nuclear staining method.

Table 1. Materials used for scale-up and culture of Vero cells.

Description	Material	Supplier
S.U.B. (250 L modified)*	Thermo Scientific™ Aegis™5-14 film	Thermo Fisher Scientific
S.U.B. (250 L modified)**	Bioreactor hardware	Thermo Fisher Scientific
DMEM, high glucose	Media supplement	Thermo Fisher Scientific
Gibco™ certified FBS	Media supplement	Thermo Fisher Scientific
Cytodex 3 beads	Microcarrier beads	GE Healthcare
Vero cells	Cell line	ATCC
Gibco™ DPBS (phosphate-buffered saline)	Saline solution	Thermo Fisher Scientific
Gibco™ TrypLE™ Express Enzyme	Dissociation reagent	Thermo Fisher Scientific
Antifoam C	Antifoam agent	Sigma-Aldrich

* For cultivation in the 250 L S.U.B. (Cat. No. SH3B1145.03), modifications to the BPCs were made, including:
- Replacement of the standard impeller with a larger impeller (500 L impeller for 250 L S.U.B.)
- Moving the sample/resistance temperature detector (RTD) port location to the probe belt
- Addition of a harvest line in the standard sample port position to facilitate decanting and rinsing during detachment of cells

** For cultivation in the 250 L S.U.B., use of a modified 250 L BPC required a custom motor mount and shaft (assembly number SV50237.914).

Table 2. 250 L S.U.B. operating conditions.

Parameter	250 L settings
Working volume	250 L
Temperature	37°C
pH	7.3 (no base)
Agitation	26.2 rpm*
Tip speed	0.34 m/sec
DO set point	30%
DO cascade	Oxygen through standard drilled-hole sparger
Headspace sparge	5 L/min air
Antifoam	5–10 ppm added prior to DO calibration

* The agitation speed of 26.2 rpm was found to be sufficient for microcarriers to remain in suspension throughout the study.

Cell harvest

When the cell population reached the desired density, the bioreactor's agitation was stopped and the microcarrier beads were allowed to settle for 15 minutes. The culture medium was decanted through the top harvest port directly above the microcarrier level. To rinse the system, 200 L of DPBS was added and the bioreactor's agitation was restarted for 5 minutes. The cells and microcarriers were then allowed to settle again.

The DPBS rinse was then decanted through the designated port, and 75 L of TrypLE enzyme was added to the system for cell detachment. Agitation was resumed at a normal rate, and samples were taken every 5 minutes to assess progress of the detachment. After the cells detached from the microcarriers, after approximately 15 minutes, fresh DMEM with 10% FBS was added to the system to bring the volume back up to 250 L. A final sample was pulled to assess culture density and viability at this time. The temperature control was then turned off while the agitation was still set at its normal set point, and the system was held for 1 hour prior to harvesting the cells. This allowed the cells to become less adherent as they passed by the beads and through the Harvestainer mesh.

Harvestainer system preparation

The 3 L and 12 L Harvestainer systems were run in sequence, while the 25 L Harvestainer system was run in parallel with the 3 L system. Each system was set up as instructed in the user's guide, and connected to one of the bioreactor's bottom harvest lines as well as to an appropriately sized Labtainer BPC. Both the inlet and outlet lines of the Harvestainer system were connected to peristaltic pumps that were set at a rate of >2.5 L/min for the 3 L system, >3.5 L/min for the 12 L system, and >6.5 L/min for the 25 L system.

Microcarrier bead trap

The bioreactor's fluid contents were pumped through its harvest port, through the Harvestainer's transfer-line set, and into the Harvestainer systems. For the 3 L and 12 L systems, the Harvestainer mesh film layer trapped and captured the microcarrier beads. For the 25 L system, the microcarrier beads were captured within the 25 L Microbarrier Labtainer BPC. The cells and media supernatant flowed through the film layers of each Harvestainer system, and then were pumped into the attached Labtainer BPC.

Results and discussion

Transfer time from bioreactor to Harvestainer system

A total volume of 25 L was transferred from the bioreactor through the 3 L Harvestainer system in an average time of 8 minutes. A total volume of 125 L was transferred from the bioreactor through the 12 L Harvestainer system in an average time of 22 minutes. A total volume of 200 L was transferred from the bioreactor through the 25 L Harvestainer system in an average time of 24.5 minutes.

Microcarrier bead trap efficiency

All of the final filtered cell supernatant was passed through a microsieve to ensure that no microcarrier beads or bead fragments passed through the Harvestainer system. The filtrate was free of microcarrier beads and fragments for all of the systems.

Cell viabilities and recoveries

The Harvestainer systems showed cell recovery yields of >87% and viability loss of <2% post-harvest. Figure 1 shows these results.

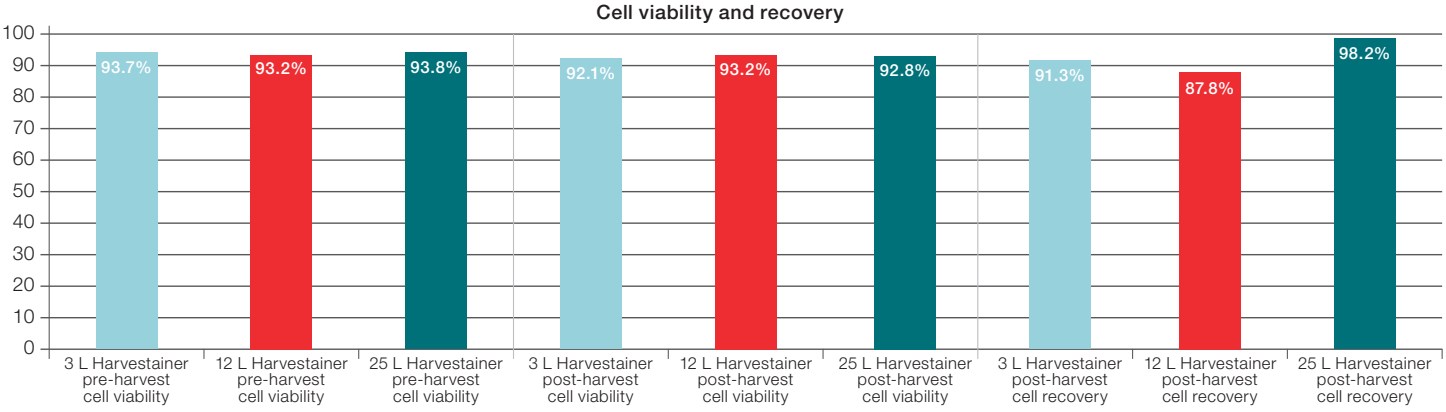

Figure 1. Cell viability and harvest yield of Harvestainer systems.

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Conclusion

Both the large-scale and small-scale Harvestainer systems quickly and efficiently segregated the microcarrier beads from the cells and medium. Transfer rates were consistent from start to finish without any decrease in flow rate due to plugging or fouling. The mesh film in the Harvestainer system captured all of the microcarrier beads and allowed the cells and the culture medium to flow through without any impedance in flow. The flow-through design of both the large-scale and small-scale systems allowed for collection of the culture medium and cells without loss of viability or yield.

Based on these results, the small-scale Harvestainer systems mimic the performance of the large-scale system, and both scales offer similarly high recoveries of cells. For bioproduction processes utilizing 12 L or less of swelled beads (equivalent to 600 g or less of dry Cytodex 3 beads) to grow adherent cells, the 3 L and 12 L Harvestainer systems provide a single-use BPC for harvesting and separating the cells from the used microcarrier beads. For bioproduction processes utilizing more than 12 L of swelled beads to grow adherent cells, the 25 L and 50 L Harvestainer systems provide comparable features and performance. Both styles of Harvestainer systems are designed for fast throughput to save process time. They are delivered sterile to help reduce costs and time needed for setup and cleanup. Additionally, they are configured as closed systems for full product containment and easy disposal. The Harvestainer systems readily integrate into existing processes and equipment; they effectively eliminate the need to invest in expensive equipment typical of traditional systems.

Related publications (application notes)

- Optimizing the single-use bioreactor for adherent cell culture on microcarriers
- Microcarrier bead separation and cell harvesting using Harvestainer small-scale separation system
- Microcarrier bead separation and cell harvesting using Harvestainer small-scale separation system

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