Microcarrier bead separation and cell harvesting using the Harvestainer large-scale system

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 between-use cleaning and sterilization. Until now, single-use options were restricted to disposable spin filters and hollow-fiber microfiltration systems.

The Thermo Scientific™ Harvestainer™ BioProcess Container (BPC) is a new, single-use product designed to simplify and economize the cell-bead separation process. The Harvestainer system is used to harvest the cells cultured on microcarrier beads, after they have been dissociated from the beads, by separating the beads from the cells and culture media. The large-scale or 3D Harvestainer system uses a bag-within-a-bag design for full containment of trapped microcarrier beads within the inner microbarrier of the Thermo Scientific™ Labtainer™ BPC. The spent cell culture medium and detached cells are collected in the outer BPC. The collected supernatant can then be pumped into a separate 200 L Labtainer BPC for further holding, or it can be pumped on to another

process, with little-to-no loss due to the dip well that is built into the system. 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.

ThermoFisher SCIENTIFIC

Goal

To demonstrate its effectiveness, the 25 L Harvestainer system was 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.

Materials and methods

Cell culture growth conditions

Vero cells (CCL-81™ cells, ATCC) were grown on Cytodex™ 3 microcarrier beads using Gibco™ DMEM, high glucose 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 manufacturer's instructions, and then placed in fresh culture medium prior to being added to the bioreactors.

Table 1. Materials for growth and dissociation of cells.

Description	Material	Supplier
S.U.B. 250 L BPC (Cat. No. SH3B1145.03)*	Thermo Scientific™ CX5-14 film	Thermo Fisher Scientific
S.U.B. 250 L (custom for microcarriers)**	Bioreactor hardware	Thermo Fisher Scientific
DMEM, high glucose	Medium	Thermo Fisher Scientific
Gibco™ FBS, certified	Medium supplement	Thermo Fisher Scientific
Cytodex 3 beads	Microcarrier beads	GE Healthcare Life Sciences
Vero cells	Cell line	ATCC
Gibco™ DPBS	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., modifications to the BPCs were made, including:
  – Replacement of standard impeller with a larger impeller (500 L impeller for 250 L S.U.B.)
  – Sample/resistance temperature detector (RTD) port location being moved to the probe belt
  – Addition of a harvest line in the standard sample port position to facilitate the decanting and rinsing process 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	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 for the 250 L S.U.B. was found to be sufficient for microcarrier beads to remain in suspension throughout the study.

Samples were taken daily to assess attachment and growth rate 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 consumption and waste production. A few drops were placed on a microscope slide to visually inspect the culture, and approximately 8–9 mL of sample was used to count the cells using a crystal violet/citric acid solution for nuclei staining.

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. A 200 L DPBS rinse was added to the system, and the bioreactor's agitation was restarted for 5 minutes. The cells and microcarriers were then allowed to settle again.

The DPBS was then decanted through the designated port and 75 L of TrypLE Express 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 the detachment progress. After the cells detached from the microcarrier beads, 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 removed to assess culture density and viability. 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 process allowed the cells to become less sticky as they passed by the beads and through the Harvestainer mesh.

Harvestainer preparation

The 25 L Harvestainer system was set up as instructed in the user's guide by positioning it into a 200 L drum and inflating the system with air. The top port transfer line set was then connected to the bioreactor's bottom harvest line, and the dip well line was connected to a 200 L Labtainer system. Both the inlet and outlet lines of the Harvestainer system were connected to peristaltic pumps that were set at a rate of greater than 6.5 L/min.

Microcarrier bead trap

The bioreactor's fluid contents were pumped through its harvest port, through the Harvestainer transfer-line set, and into the 25 L microbarrier Labtainer system. The Labtainer system trapped and captured the microcarrier beads. The cells and media supernatant flowed through the Labtainer system and were collected in the 200 L outer BPC. They were then pumped out into the attached 200 L Labtainer system.

Results and discussion

Transfer time from bioreactor to Harvestainer system

A total volume of 200 L was transferred from the bioreactor through the 25 L Harvestainer system in an average of 24.5 minutes.

Microcarrier bead trap efficiency

All of the final, filtered cell supernatant material was passed through a microsieve to ensure that no microcarrier beads or bead fragments passed through the Harvestainer system. Filtrate from the Harvestainer system was free of microcarrier beads and fragments.

Cell viabilities and recoveries

The 25 L Harvestainer system showed cell recovery yields of 98.2% with very little cell viability loss. Figure 1 shows these results.

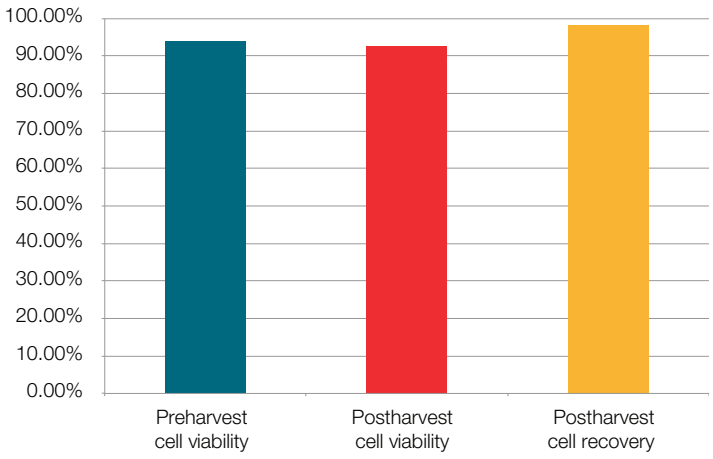

Figure 1. Cell viability and recovery. Preharvest cell viability was 93.8%, postharvest cell viability was 92.8%, and cell recovery was 98.2% for the 25 L Harvestainer system.

thermo scientific

Conclusion

The large-scale Harvestainer system quickly and efficiently segregated the microcarrier beads from cells and media. 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 cell culture medium to flow through without any impedance in flow. The 200 L outer BPC collected the cell culture medium and cells without loss of viability or yield.

For bioproduction processes utilizing more than 12 L of swelled beads (an equivalent of over 600 g of dry-weight Cytodex 3 beads) to grow adherent cells, the 25 L and 50 L Harvestainer systems provide customers with a single-use BPC for harvesting and separating the cells from the used microcarrier beads. The system is designed for fast throughput to save process time. It is delivered sterile to reduce costs and time needed for setup and clean-up. Additionally, it is configured as a closed system for full product containment and easy disposal. The Harvestainer system readily integrates into existing processes and equipment; it effectively eliminates the need to invest in expensive equipment typical of traditional systems.

Related publications

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

Please see your sales representative for more information about these application notes.

Find out more at **thermofisher.com/harvestainer**

ThermoFisher SCIENTIFIC