Microcarrier bead separation and cell harvest using Thermo Scientific HyQ Harvestainer

Introduction:

Techniques used to separate anchorage-dependent mammalian cells from microcarrier beads include: sedimentation using conical or incline settlers, centrifugation, acoustic resonance, spin filtration and microfiltration. These techniques often use sophisticated equipment that require a 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 HyQ Harvestainer is a new single-use product designed to simplify and economize the cell-bead separation process. The HyQ Harvestainer system is used to harvest the cells cultured on microcarrier beads and to separate the microcarrier beads from the cells and culture media. The Harvestainer uses a bag-within-a-bag design for full containment of trapped microcarrier beads within the inner Thermo Scientific Microbarrier Labtainer with the spent cell culture media and detached cells collected in the outer BioProcess Container (BPC). The Harvestainer can be integrated with either stainless steel or single-use bioreactors, is customizable for optimal plug-and-play convenience and is self-contained for easy disposal.

Objective:

To demonstrate its effectiveness, the Harvestainer was used to collect the cells and isolate the microcarrier beads in a model system that employed a 250 L Thermo Scientific HyClone 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

Anchorage-dependent Vero cells (ATCC CCL 81) were grown on Cytodex 3 microcarrier beads using a serum supplemented Thermo Scientific HyClone DMEM/High Glucose medium formulation (Table 1) in a 250 L S.U.B. The working volume was 225 L with daily samples taken to assess cell attachment and growth. Cells were harvested after six days.

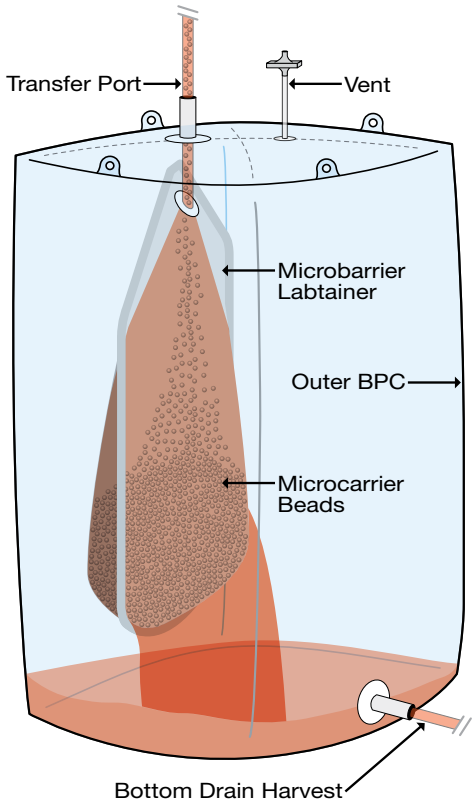

Figure 1. Thermo Scientific HyQ Harvestainer

Component	Amount
DMEM/High Glucose	13.38 g/L
Sodium Bicarbonate	3.4 g/L
Iron-Supplemented Bovine Calf Serum	50 mL/L

Table 1. Sterile filtered (0.22 µm) cell culture medium formulation used to grow Vero cells

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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.

Approximately 130 L of cell culture medium were aseptically decanted from the bioreactor and stored for later use.

60 L of Thermo Scientific HyClone Dulbecco's Phosphate Buffered Saline (DPBS) were added to the bioreactor to bring the volume to 130 L. The bioreactor's agitator was re-started and the contents were agitated at normal operating rate. After 5 minutes, agitation was stopped and the microcarrier beads were allowed to settle for 15 minutes. This step was repeated once more with fresh DPBS.

After the second settling step, the DPBS was again decanted and replaced with 60 L of a 0.25% trypsin solution for cell detachment. Agitation was resumed at 50% the normal agitation rate and samples were removed every 5 minutes to assess the detachment progress.

At 30 minutes post-trypsin introduction, cell detachment was satisfactory (>90%). At this time the stored cell culture medium was added back to the bioreactor for a final volume of 200 L. The final culture density and viability measurements were taken at this time.

Harvestainer Preparation

The Harvestainer was positioned into a 200 L drum with the top port transfer lineset connected to the bioreactor's harvest port.

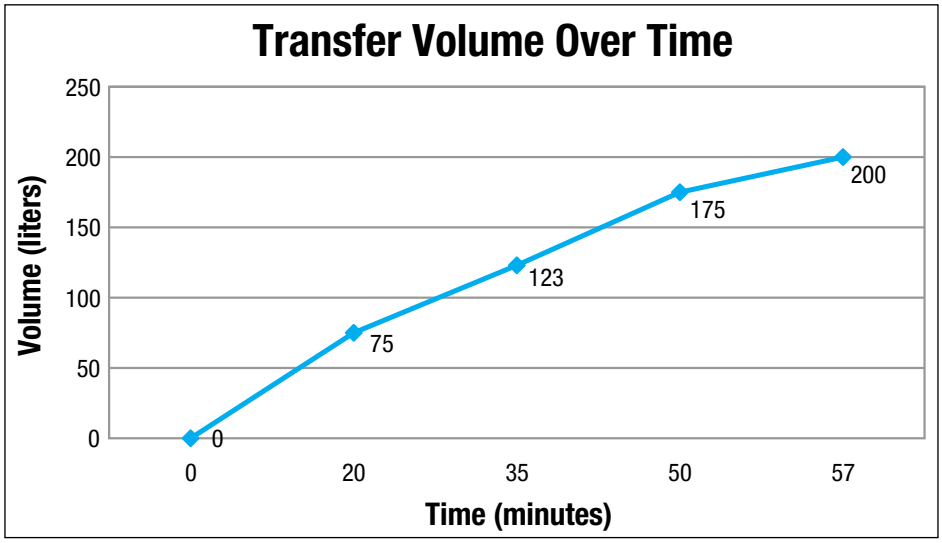
The Harvestainer was connected to a peristaltic pump (Table 2) with the pump's direction set to move the contents from the bioreactor to the Harvestainer at a rate of five liters per minute.

Microcarrier Bead Trap

The bioreactor's fluid contents were pumped through its harvest port, through the Harvestainer's transfer lineset and into the 25 L Microbarrier Labtainer. The Labtainer trapped and captured the microcarrier beads. The cells and media supernatant flowed through the Labtainer and were collected in the 200 L outer BPC.

Component	Make and Model
Peristaltic Pump	Cole Parmer Masterflex I/P Model 7591 60
Pump Head	Cole Parmer Model 77601 10

Table 2. Pump model used to transfer contents from the bioreactor to the Harvestainer



Graph 1. Shows the transfer rate at set intervals over the 57 minute period

Results and Discussion:

Bioreactor-to-Harvestainer Transfer Time

A total volume of 200 L was transferred from the bioreactor to the Harvestainer in 57 minutes.

Microcarrier Bead Trap Efficiency

Duplicate 50 mL samples were taken from the final filtered material which had passed through the Labtainer and was collected in the Harvestainer's 200 L outer BPC. Microscopic observation revealed the complete absence of microcarrier beads and microcarrier bead fragments.

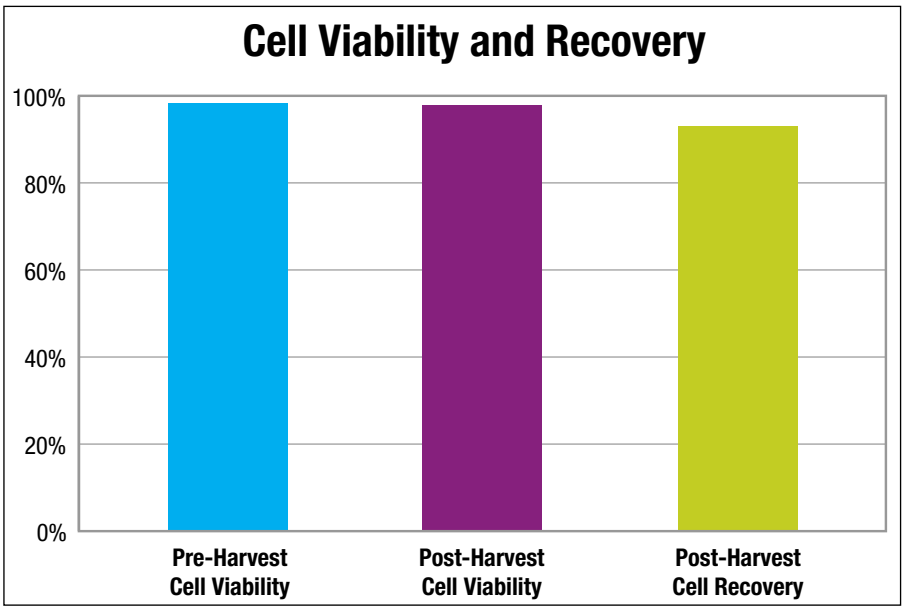
Cell Viabilities and Recovery

See Graph 2.

Summary:

The Thermo Scientific HyQ Harvestainer system quickly and efficiently segregated the microcarrier beads from cells and media. Transfer rates were consistent from start to finish without any decreases in flow rate due to plugging or fouling. The inner Microbarrier Labtainer captured all of the microcarrier beads and allowed the cells and the cell culture media to flow through it without any impedance in flow. The 200 L outer BPC collected the cell culture media and cells without loss of viability or yield.

For bioproduction processes using anchorage-dependent cells grown on microcarriers, the HyQ Harvestainer system provides the customer with a single-use BPC for harvesting and separating the cells from the used microcarrier beads. It is designed for fast throughput to save process time. It is delivered sterile to reduce costs and time needed for set-up and clean-up. And it is configured as a closed system for full product containment and easy disposal. The Harvestainer readily integrates into existing processes and equipment and eliminates the need to invest in expensive equipment typical to traditional system.



Graph 2. Pre-harvest cell viabilities were 98.3% and post-harvest cell viabilities were 97.8% and recovery was 93%

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