Optimizing the HyPerforma Single-Use Bioreactor for adherent cell culture on microcarriers

Introduction

The scale-up of culture of adherent cells, such as Vero cells and fibroblasts, can be difficult. This study shows a process for scaling up Vero cell culture from static T-flasks and Thermo Scientific™ Nunc™ Cell Factory™ Systems to a stirred-tank system, utilizing Thermo Scientific™ HyPerforma™ Single-Use Bioreactors (S.U.B.s) and Cytodex™ 3 microcarriers (GE Healthcare). The study then demonstrates necessary processes for scaling up from the 50 L S.U.B. to the 250 L S.U.B. For each scale-up step from T-flasks through S.U.B.s, the cells were detached by trypsinization before going to the next step. To go from 50 L S.U.B. to 250 L S.U.B., the detached cells were collected by harvesting the contents of the bioreactor through a Thermo Scientific™ 3 L Harvestainer™ separation system to separate the cells from the microcarriers. The collected cells were then added to the prepared 250 L S.U.B. with new microcarriers.

To demonstrate process reproducibility, the 50 L S.U.B. study was performed in duplicate.

Goal

The performance of 50 L and 250 L HyPerforma S.U.B.s was studied with respect to achieving optimal cell growth of adherent cells on microcarriers.

Materials and methods

Vero cells (CCL-81™ cells, ATCC) were maintained in Gibco™ DMEM medium with 10% fetal bovine serum (FBS) throughout the scale-up and culture in the single-use systems. Materials used in this study are described in Table 1.

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

Description	Material	Supplier
S.U.B. (50 L)	Bioreactor hardware	Thermo Fisher Scientific
S.U.B. 50 L BPC (Cat. No. SH3B1145.01)*	Thermo Scientific™ CX5-14 film	Thermo Fisher Scientific
S.U.B. 250 L BPC (Cat. No. SH3B1145.03)*	CX5-14 film	Thermo Fisher Scientific
S.U.B. 250 L (custom for microcarriers)**	Bioreactor hardware	Thermo Fisher Scientific
Gibco™ DMEM, high glucose	Media supplement	Thermo Fisher Scientific
Gibco™ fetal bovine serum, certified	Media supplement	Thermo Fisher Scientific
Cytodex 3 beads	Microcarrier beads	GE Healthcare Life Sciences
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 50 L and 250 L S.U.B.s, customizations to the Thermo Scientific™ BioProcess Containers (BPCs) were made, including:
- Replacement of standard impeller with a larger impeller (100 L impeller for 50 L S.U.B. and 500 L impeller for 250 L S.U.B.)
- Sample/resistance temperature detector (RTD) port location moved to the probe belt
- Additional harvest line added in the standard sample-port position to facilitate decanting and rinsing during detachment of cells

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

Bioreactor setup and microcarrier preparation

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

Bioreactor seeding, cultivation, and scale-up

Cultures grown in Nunc Cell Factory Systems were used to seed a 50 L microcarrier culture in the 50 L S.U.B. The 50 L culture was subsequently used to seed a 250 L culture by detaching the cells from the beads through trypsinization using TrypLE Express Enzyme, harvesting the detached cells through a 3 L Harvestainer system, and adding the collected cells to the prepared 250 L S.U.B. with new microcarriers.

After initially inoculating the S.U.B.s, intermittent mixing was performed to allow the cells to attach to the microcarriers. This was done by having multiple settling cycles of 20–30 min followed by 3–5 min of agitation (on-cycle). During the on-cycle, the impeller was briefly agitated at 85.6 rpm (~30 sec) for the 50 L S.U.B. and 60–80 rpm for the 250 L S.U.B. to help the bead pack lift off the bottom of the reactor, followed by mixing at the standard set point. During the off-cycle, the impeller and gassing cascades were disabled to allow sufficient settling of the bead pack.

After 8–10 on/off cycles, the cells were attached, and the agitation and gassing parameters were turned on. The reactor was sampled to verify the attachment of cells under a microscope. Once the cells attached, reactor parameters were set for the run as shown in Table 2.

Table 2. 50 L and 250 L S.U.B. operating conditions.

Parameter	50 L settings	250 L settings
Working volume	50 L	250 L
Temperature	37°C	37°C
pH	7.3 (no base)	7.3 (no base)
Agitation	42.8 rpm*	26.2 rpm*
Tip speed	0.33 m/sec	0.34 m/sec
DO set point	30%	30%
DO cascade	Oxygen through standard drilled-hole sparger	Oxygen through standard drilled-hole sparger
Headspace sparge	1 L/min air	5 L/min air
Antifoam	5–10 ppm added prior to DO calibration	5–10 ppm added prior to DO calibration

* The agitation speeds of 42.8 rpm for the 50 L S.U.B. and 26.2 rpm for the 250 L S.U.B. were found to be sufficient for microcarriers to remain in suspension throughout the study.

When the cell density in the 50 L S.U.B. had reached the desired level of confluency, agitation was turned off, and microcarriers and cells were allowed to settle to begin the dissociation step. The culture medium was decanted through the top harvest port directly above the microcarrier level, and a rinse was performed by adding 50 L of DPBS to the system, mixing for 5 min, and then letting the cells and microcarriers settle again. The DPBS was then decanted through the designated port and 15 L of TrypLE enzyme was added to the system. After the cells detached from the microcarriers, DMEM with 10% FBS was added to the system to bring the volume back up to 50 L.

The cells were counted and then transferred to the 250 L S.U.B. through the 3 L Harvestainer system by pumping through the Harvestainer system and pumping into the 250 L S.U.B. at about 2–3 L/min. After the 250 L S.U.B. was seeded, intermittent mixing was once again performed to allow the cells to attach to the microcarriers, as outlined above. Once the cells attached, reactor parameters were set for the run as shown in Table 2.

Culture monitoring

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 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 a sample was pulled for counting the cells using a crystal violet/citric acid solution for nuclear staining.

Results and discussion

Bioreactor control and operation

During the run, the culture temperature was maintained at 37°C. The pH of the culture was maintained by addition of CO_2 only, and remained within allowable limits for the duration of the culture. Additionally, the DO was maintained at the desired level of 30% by a cascade control of small amounts of oxygen and nitrogen through the drilled-holed system of the S.U.B., as well as a manual overlay of air through the system. The bioreactor system was microscopically evaluated and remained free of contamination for the duration of the experiment.

Microcarrier culture evaluation

The cells were consistent through scale-up to the 50 L S.U.B and into the 250 L S.U.B. (Figure 1), with cell densities above 100,000 cells/cm^2, which is what is typically seen with this cell line.

Intermittent mixing was a very important step in allowing the cells to attach to the beads in the most efficient way possible. The intermittent mixing usually was completed in 5 hours or less, and attachment was verified through microscopic observations prior to setting the final parameters for the culture.

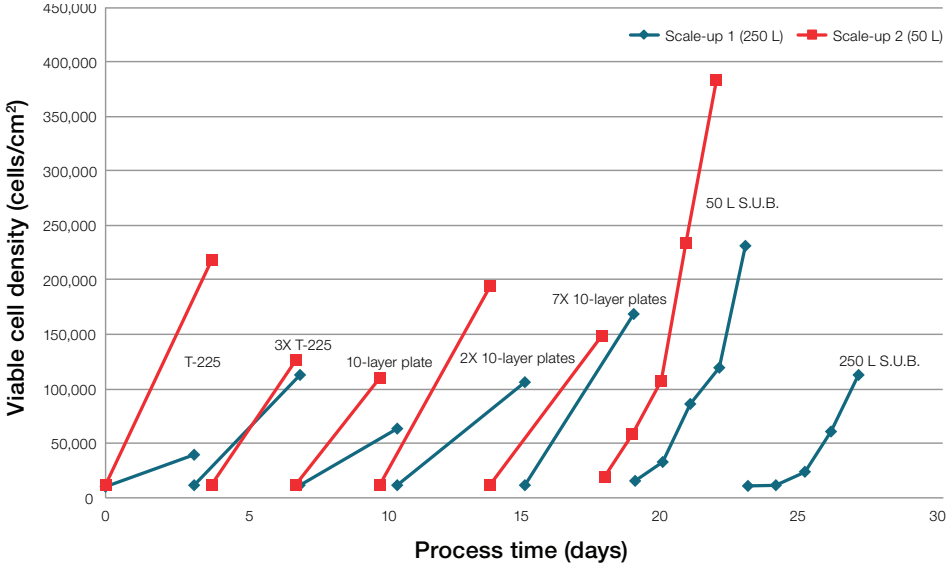

Figure 1. Cell density of passages scaling up from one T-225 flask to a 250 L S.U.B. cell run with 3 g/L microcarriers.

Figure 2 illustrates that the cells in each of the cultures reached at least 9 x 10^5 cells/mL within the 4-day culture. In vaccine cultures, Vero cells are typically grown to at least 70% confluency within 4 days, before virus is added to the culture. With each of the cultures in this study, the cells were allowed to achieve at least 70% confluency before harvesting. The difference in growth rates is due to the seed concentration: the 250 L had the lowest seed concentration at 11,000 cells/cm^2 or 90,000 cells/mL, the first 50 L had a seed concentration of 15,000 cells/cm^2 or 120,000 cells/mL, and the second 50 L had a seed concentration of 20,000 cells/cm^2 or 180,000 cells/mL. This demonstrates how important it is to have at least 15,000 cells/cm^2 when seeding, to decrease the lag phase in the culture.

The culture composition (Figures 3–5) was comparable to other cultures of Vero cells in this culture medium. The level of lactate reached close to 2–2.5 g/L, which is consistent with the utilization of glucose during this time. Typically, the medium would be exchanged before virus inoculation to remove the waste products.

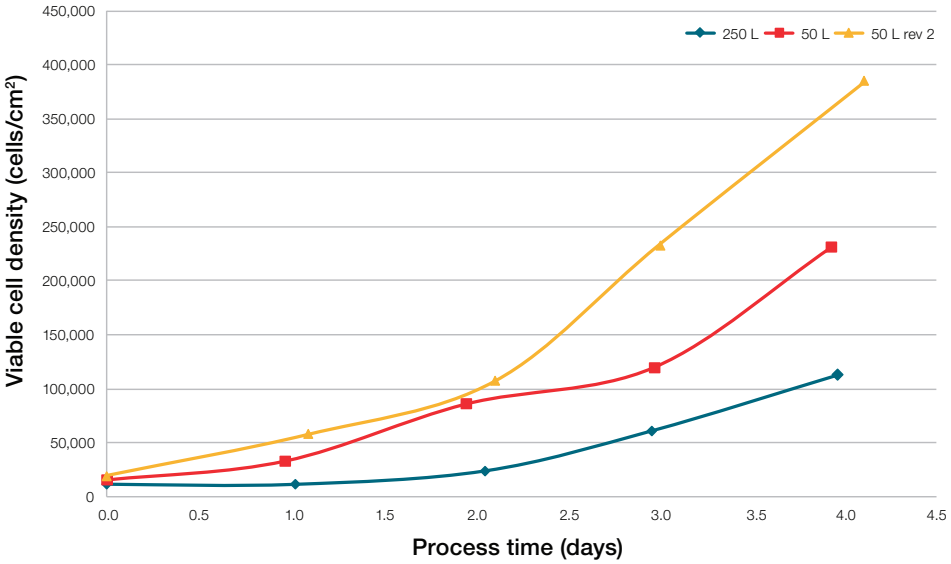

Figure 2. Cell density of 3 different Vero cultures grown in S.U.B. systems.

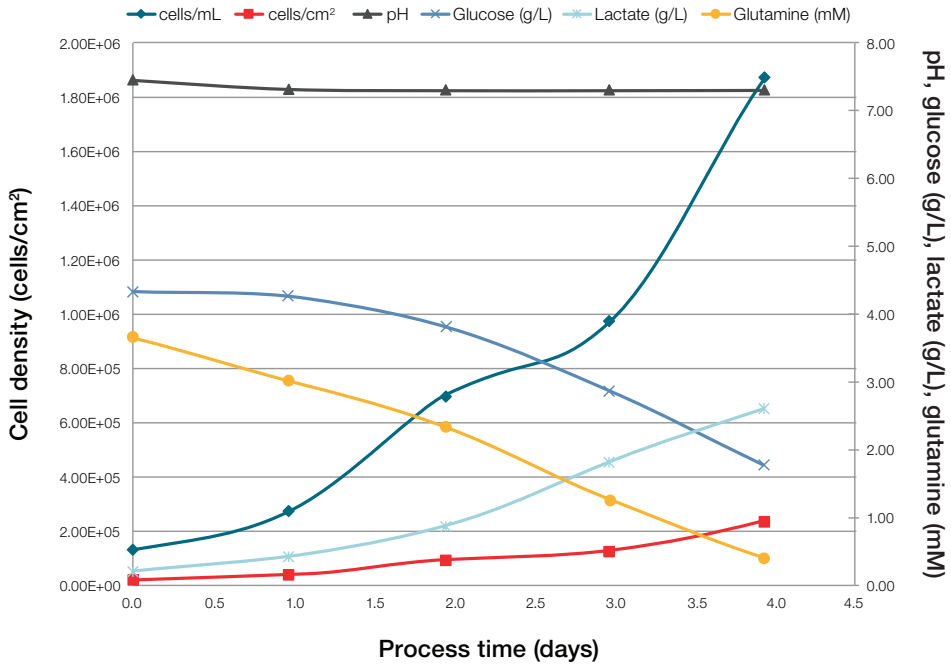

Figure 3. Vero cell culture growth using Cytodex 3 microcarriers and DMEM with 10% FBS over a 4-day period in the 50 L S.U.B. system. Culture substrate utilization and biochemical production rates were indicative of a normal metabolic pattern with this culture.

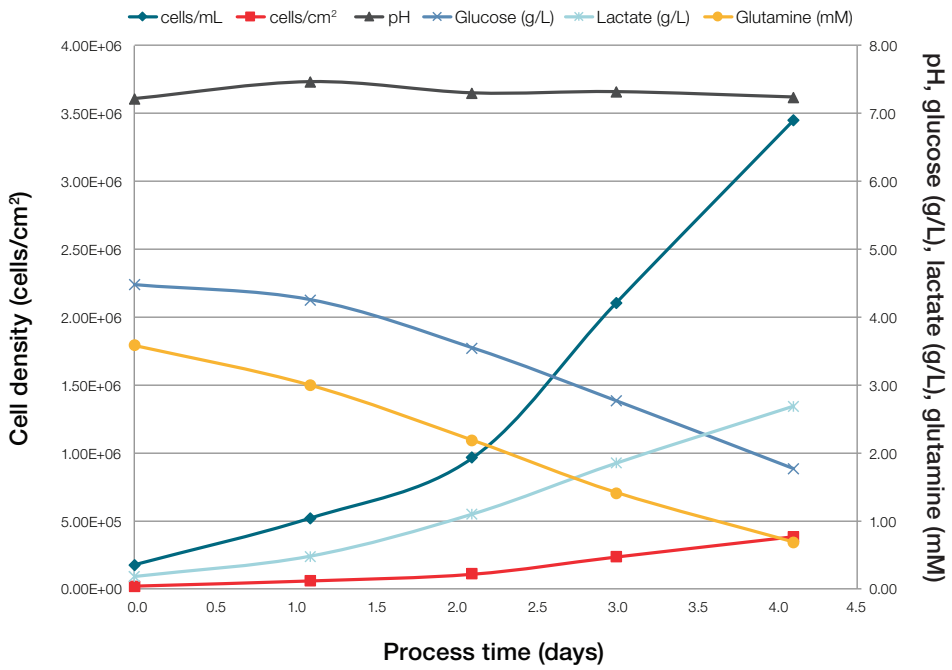

Figure 4. Vero cell culture growth using Cytodex 3 microcarriers and DMEM with 10% FBS over a 4-day period in the 50 L S.U.B. system, repeated as a duplicate run. Culture substrate utilization and biochemical production rates were indicative of a normal metabolic pattern with this culture.

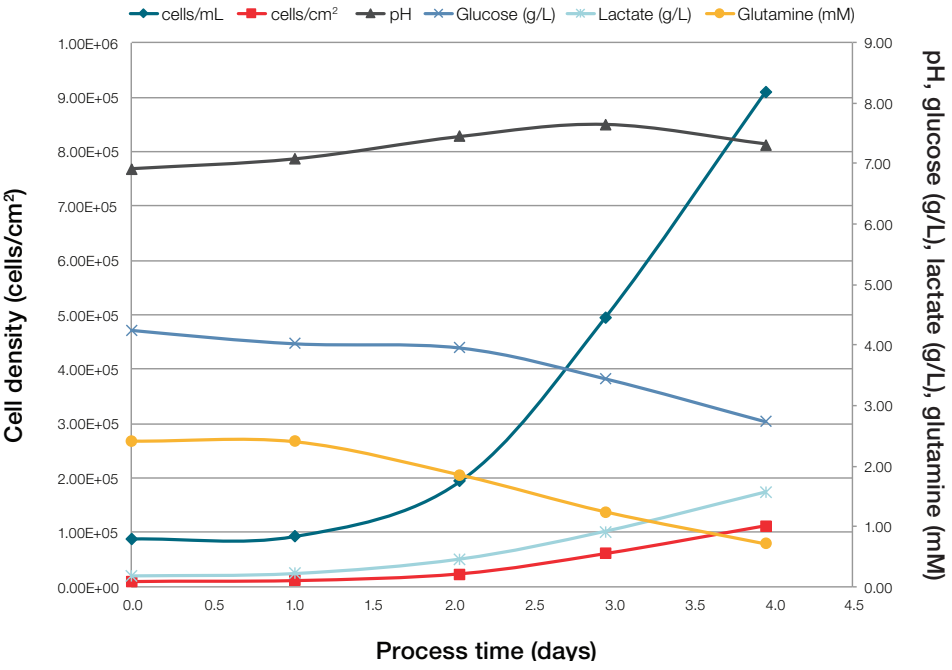

Figure 5. Vero cell culture growth using Cytodex 3 microcarriers and DMEM with 10% FBS over a 4-day period in the 250 L S.U.B. system. Culture substrate utilization and biochemical production rates were indicative of a normal metabolic pattern with this culture.

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Conclusion

The 50 L and 250 L S.U.B. systems can successfully be used for microcarrier cultivations of Vero cells. The key factors for successful microcarrier cultures include:

- The ability of the cells to attach and spread on the microcarrier surface—this was enabled by intermittent mixing over the course of approximately 5 hours.
- The ability to maintain a generally homogeneous suspension throughout the working volume while minimizing shear forces acting upon the cells—this was achieved by increasing the diameter size of the impellers, so lower agitation rates could be used.
- The ability to perform dissociation steps within the bioreactor itself—this was done by customizing the BPC with an additional harvest line directly above the bead-pack level, allowing decanting and rinsing steps prior to dissociation.
- The ability to separate dissociated cells from beads, and transfer them to fresh culture medium and beads for both harvest and scale-up steps—this was achieved utilizing our 3 L Harvestainer system.

Related publications (application notes)

- Microcarrier bead separation and cell harvest using Thermo Scientific Harvestainer large-scale separation system
- Scalability of microcarrier bead separation using Thermo Scientific Harvestainer systems
- Microcarrier bead separation and cell harvest using Thermo Scientific Harvestainer small-scale separation system

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

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