Enhanced single-use bioreactor performance for scalable Vero cell culture on microcarriers in serum-free medium

Introduction

Adherent cell cultures, such as those with Vero cells, are often more challenging than their suspension-based counterparts. While microcarriers greatly increase the surface area available for adherent cells and offer flexibility for expansion into bioreactors, scale-up methods require optimization of agitation and other operating parameters. Likewise, parameter selection and optimization surrounding the detachment and reattachment of cells through the scale-up process is vital for success. This application note presents how Thermo Scientific™ HyPerforma™ Single-Use Bioreactors (S.U.B.s) can be enhanced for scaled-up growth of adherent cells on GE Healthcare Cytodex™ 1 microcarriers in serum-free medium, in culture volumes of 50 L, 250 L, and 500 L. The methods for the deployed bead-to-bead transfer of the cells at each scaling step and in subsequent cultures are also outlined. The enhanced S.U.B. was optimized for adherent cell growth with several modifications. The S.U.B. BioProcess Container (BPC) was also modified to allow for easy media exchanges in the vessel.

Materials and methods

Vero cells were maintained in VP-SFM medium throughout incubator and bioreactor culture. Key materials for this study are listed in Table 1. Initial cell cultures were begun on flasks and were eventually scaled to Nunc Cell Factory systems, followed by a 5 L glass bioreactor, and then into S.U.B.s.

Table 1. Materials used for adherent Vero cell culture and scale-up.

Components	Description
Cell line	Vero cells (ATCC™ CCL-81™ cells)
Microcarrier type	GE Healthcare™ Cytodex™ 1 beads
Composition of medium	Gibco™ VP-SFM AGT™ Medium with 6 mM glutamine and 1 g/L Pluronic™ F-68 surfactant
Wash solution	Gibco™ DPBS
Dissociation agent	Gibco™ TrypLE™ Express Enzyme
Quenching agent	Gibco™ Soybean Trypsin Inhibitor
Base solution	0.5 N NaOH
Antifoam agent	Sigma-Aldrich™ Antifoam C Emulsion at 10,000 ppm in DPBS
Tissue flasks	• Thermo Scientific™ Nunc™ EasYFlask™ Cell Culture Flasks • Nunc™ EasyFill™ Cell Factory™ systems
Benchtop bioreactors	5 L glass bioreactor
S.U.B. hardware	• HyPerforma 50 L S.U.B. with 4:1 drive shaft • HyPerforma 250 L S.U.B. with 4:1 drive shaft • HyPerforma 500 L S.U.B. with 4:1 drive shaft
BPCs (Aegis5-14 film)	• HyPerforma 50 L S.U.B. BPC for microcarriers (Cat. No. SH31150.01) • HyPerforma 250 L S.U.B. BPC for microcarriers (Cat. No. SH31150.03) • HyPerforma 500 L S.U.B. BPC for microcarriers (Cat. No. SH31150.04)

Cell culture material preparation

Microcarriers were rehydrated and washed with DPBS per product instructions prior to autoclave sterilization. VP-SFM AGT Medium was reconstituted per product instructions using Thermo Scientific™ Single-Use Mixer (S.U.M.) systems and sterilized by filtration. S.U.B. BPCs used a Hamilton™ OneFerm™ single-use sensor for pH, and a Thermo Scientific™ TruFluor™ probe for dissolved oxygen (DO).

Culture monitoring

Samples were taken daily to assess cell attachment and growth rate. Daily samples consisted of taking 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 Nova Biomedical™ Bioprofile FLEX2™ analyzer to measure nutrient/metabolite consumption and waste production. A few drops were placed on a microscope slide to visually inspect the culture, and approximately 8–9 mL was used for cell counting using the crystal violet/citric acid nuclei staining method on a hemocytometer.

Bioreactor seeding, cultivation, and scale-up

Vero cells were progressively scaled up from flasks to 4-layer and 10-layer Nunc Cell Factory systems. Cells were then dissociated and seeded into a 5 L glass benchtop bioreactor containing 15 g of microcarriers. Cell attachment to the beads was achieved through continuous mixing by slow agitation until they were attached. Cells were allowed to grow for grow for 4-5 days at each volume level before the introduction of fresh microcarriers and medium for future intermittent mixing operations.

The beads and cells were transferred to the HyPerforma 50 L S.U.B. vessel at an initial volume of 16 L with an additional 33 g of beads for a total of 48 g in fresh VP-SFM. Intermittent mixing was performed (cycles consisting of 45 minutes of settling and 5 minutes of mixing) for 8 hours without enabling gassing. After this period, the cells were attached and the agitation and gassing parameters were set for the run as outlined in Table 2.

Table 2. Recommended bioreactor conditions for enhanced HyPerforma S.U.B.

Parameter	50 L S.U.B.	250 L S.U.B.	500 L S.U.B.
Working volume	16 L*/50 L	250 L	500 L
Temperature	37°C	37°C	37°C
pH	7.3 (CO_2/0.5 N NaOH)	7.3 (CO_2/0.5 N NaOH)	7.3 (CO_2/0.5 N NaOH)
Agitation	35 rpm*/42.8 RPM	26.2 rpm	20.5 rpm
Tip speed	0.27*/0.33 m/s	0.34 m/s	0.34 m/s
DO setpoint	30%	30%	30%
DO cascade	Air/O_2 through standard drilled-hole sparger	Air/O_2 through standard drilled-hole sparger	Air/O_2 through standard drilled-hole sparger
Headspace sparge	Air at 10 L/m^2/min, 1.0 LPM	Air at 10 L/m^2/min, 2.8 LPM	Air at 10 L/m^2/min, 4.5 LPM
Antifoam	As needed	As needed	As needed
Target seeding density	2.5 x 10^5 cells/mL or 1.89 x 10^4 cells/cm^2	2.5 x 10^5 cells/mL or 1.89 x 10^4 cells/cm^2	2.5 x 10^5 cells/mL or 1.89 x 10^4 cells/cm^2
Microcarrier concentration	3 g/L	3 g/L	3 g/L
Intermittent mixing parameters	45 min off/5 min on, for 8 hours	45 min off/5 min on, for 8 hours	45 min off/5 min on, for 8 hours

* Denotes initial operation at lower volume for in-vessel scale-up.

Starting on day 2, 50% of the medium was exchanged daily. To exchange the medium, gases and agitation were turned off, and the microcarriers and cells were allowed to settle. Half of the culture medium was decanted through a decant port directly above the microcarrier bead pack level. Fresh medium was then added through a feed line in the top of the BPC, and reactor parameters were resumed. During any process when agitation was stopped, temperature control was also suspended to avoid temperature fluctuations in the vessel. Instead, the external temperature control unit was set at a steady 37°C during these operations. Once those were completed, temperature control was resumed.

Once the cells were confluent, the volume was brought up to the 50 L working volume of culture medium and fresh beads were added to a final microcarrier concentration of 3 g/L. Intermittent mixing was performed after the additional beads were added to the reactor, to allow for bead-to-bead cell transfer. Cells were again grown for 4–5 days with 50% exchanges of the medium each day.

The contents of the 50 L reactor were then added to the 250 L S.U.B. at full volume, with sufficient fresh beads added for a final load of 3 g/L of microcarriers. After completion of 250 L growth, approximately half of the 250 L volume was used to seed the 500 L reactor to maintain similar split ratios. At each step where new beads were introduced, an intermittent mixing step was completed to allow for bead-to-bead transfer of the Vero cells.

Enhanced S.U.B. for microcarriers: vessel modifications

The impeller size in these bioreactors was increased by using the impeller from a vessel one size larger. This size change served to reduce fluid shear while increasing the amount of power input per rotation to provide sufficient mixing. The impeller placement was also modified by a combination of drive shaft length and angle to bring the impeller closer to the bottom and center of the vessel. Due to the placement of sensing probes that fell within the bead pack, turning off the gas control during intermittent mixing is important to avoid perturbing the settled cells and microcarriers.

Gassing was controlled with the standard laser drilled-hole sparger incorporated into the BPC. While agitation is an obvious point of concern for shear, gassing must also be considered to minimize shear on the cells. To aid in this, cascading control with pure oxygen as an available gas allows for lower overall gas flow rates while maintaining sufficient oxygen delivery. To further minimize shear concerns, surfactant was also added to the base medium as outlined in Table 1.

Results

Optimizing both the culture settings and the intermittent mixing steps for each scale-up step was pivotal to the success of the study. If the cells do not flatten properly during these transfer steps, they can potentially come off the beads within hours or days once standard agitation has resumed. Ensuring that the DO remains high enough so that no gassing is needed during the first several hours of culture while performing these steps contributes to optimal conditions for cell attachment. This was accomplished by using fresh medium that was aerated to 60–80% DO prior to adding the cells and beads from the previous scale. Completing the intermittent mixing for 8 hours was aided by use of the Thermo Scientific™ TruBio™ software, allowing for discrete motor control on a scheduled calculation block. Cell attachment should be verified through microscopic observations prior to enabling the final control parameters for the culture.

Scale-up principles

Scaling in these microcarrier cultures requires the consideration of many factors. While overall geometric similarity can be conserved, it is impossible to scale all operating parameters at the same time, as they can be mutually exclusive. Of the available scaling criteria, power input per volume (PIV), impeller tip speed, and Kolmogorov length scales around impeller eddies are all viable options to consider. In the present work, tip speed was largely conserved across scales, and the initial tip speed value was dictated by a worst-case Kolmogorov length in the smallest 50 L vessel. The 50 L vessel had lengths around 90 µm, which arises from targeting length scales near 50% of the particle diameter. That same tip speed correlates with eddy length scales closer to 120 µm in the 500 L vessel. Maintaining a tip speed caused the PIV to change from around 1.0 W/m^3 in the 50 L vessel to 0.57 W/m^3 in the 500 L vessel.

In principle, increasing the agitation rate of the larger vessels (thus, not maintaining tip speed) for greater PIV is acceptable for cultures as long as the Kolmogorov length scales are not reduced to sub-radius sizes for the suspended particles. With the enhancements described here, PIV values around 1 W/m^3 were suitable for the given microcarrier system in terms of eddy length scale across volume sizes. Thus, PIV is a viable alternative scaling basis.

Figure 1 shows the comparative cell density results for the 50, 250, and 500 L S.U.B. cultures. Each vessel reached over 7 x 10^4 cells/cm^2, with the 50 L culture having somewhat lower density than the other sizes. These values equate to ranging from 0.94 to 1.3 x 10^6 cells/mL of this 3 g/L culture. Microcarrier densities can be adjusted to modify total cell yields. One potential influence on the 50 L culture having the lowest growth rate is that it was operating at the most aggressive Kolomogorov length scale.

Potentially reducing agitation in the 50 L system or increasing agitation in the others may lead to growth profiles that are even more similar.

Media biochemistry data are presented in Figures 2–4. Samples from directly before media exchanges were analyzed. The 50% exchanges served to remove waste, such as lactate (Lac), and provide fresh nutrients, such as glutamine (Gln), glutamate (Glu), and glucose (Gluc).

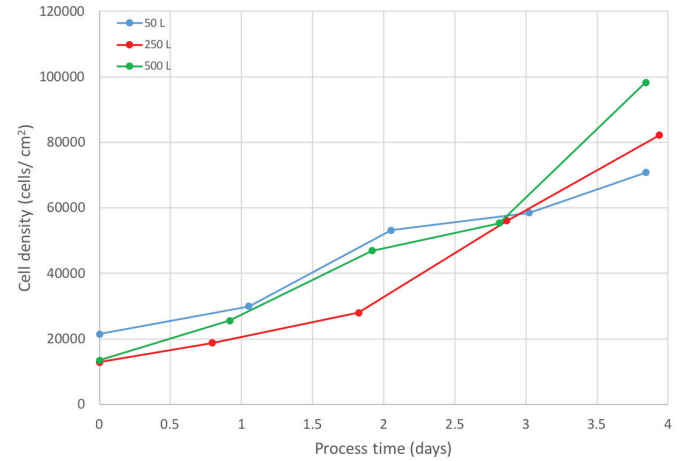

Figure 1. Viable cell density of 50, 250, and 500 L Vero cell cultures in enhanced S.U.B.s.

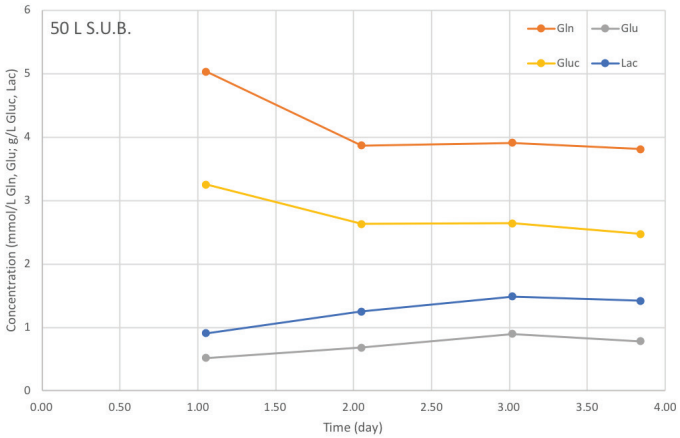

Figure 2. Media analysis data showing metabolic markers for Vero cell culture on microcarriers in an enhanced 50 L S.U.B.

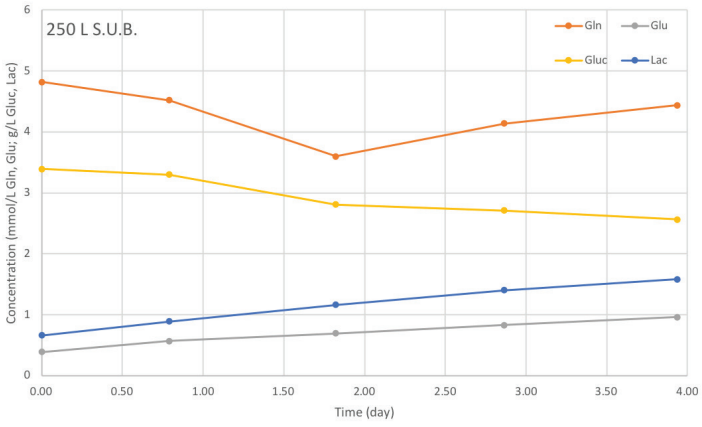

Figure 3. Media analysis data showing metabolic markers for Vero cell culture on microcarriers in an enhanced 250 L S.U.B.

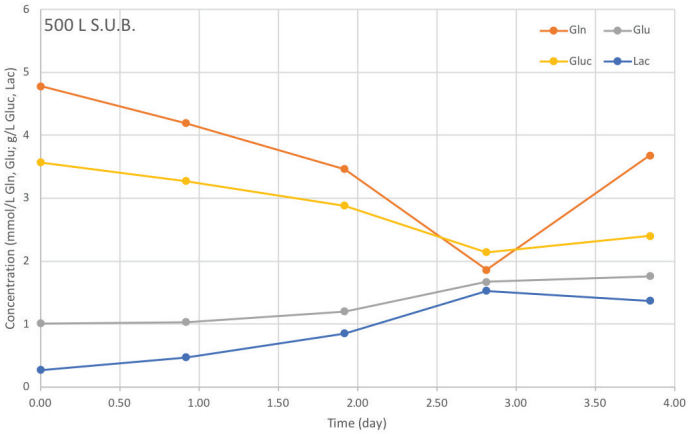

Figure 4. Media analysis data showing metabolic markers for Vero cell culture on microcarriers in an enhanced 500 L S.U.B.

thermo scientific

Conclusion

Vero cells can be successfully cultivated and scaled through bead-to-bead transfer by utilizing the enhanced HyPerforma S.U.B. systems. Key factors for successful microcarrier cultures include:

- The ability of the cells to attach and spread on the microcarrier surface during initial attachment. This can be accomplished via slower continuous mixing or via intermittent mixing strategies. In both cases, it is important to disable gassing during that time.
- 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 utilized.
- The ability to perform bead-to-bead transfer of cells within the reactor. This was done by adding additional Cytodex 1 beads to the system containing confluent microcarriers, followed by an intermittent mixing step, which lasted 8 hours.
- Decreasing the shear effects of the sparging air by the addition of 1 g/L of Pluronic F-68 surfactant.
- The ability to perform decanting and dissociation steps within the S.U.B. itself. This was done by modifying the BPC to have an additional decant line directly above the bead pack level, allowing for decanting and rinsing steps prior to dissociation.
- The ability to translate the process and design across scales. This process of intermittent mixing was replicated from 5 to 500 L vessels for a 100x volume increase, and cultures in S.U.B.s were successful through a 10x volume increase.

Authors

Tony Hsiao, Staff Engineer, Research and Development, Thermo Fisher Scientific

Paula Decaria, Senior Engineer, Research and Development, Thermo Fisher Scientific

Nephi Jones, Senior Manager, Research and Development, Thermo Fisher Scientific

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

ThermoFisher SCIENTIFIC