

HyPerforma Single-Use Bioreactor for Vero cell culture on microcarriers

Scale-up study of a single-use bioreactor

Introduction to Vero cell culture

The manufacturing of vaccine- and virus-related products often utilizes kidney-derived, adherent cells during manufacturing. Microcarrier cultures can provide a large surface area for growth of attachment-dependent cells in a small overall footprint. Since microcarriers are maintained in suspension, they lend themselves to use in stir-tanks or other types of bioreactors. The Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.), designed as an alternative to conventional stainless-steel stirred-tank bioreactors used for animal cell culture, consists of an outer support container and single-use S.U.B. BioProcess Container (BPC). The BPC design incorporates an impeller system, gas sparger, and ports for integration of sensor probes similar to those of their stainless-steel counterparts. With the gamma-irradiated S.U.B. BPC, there is no need for cleaning or sterilizing between runs, providing for quick turnaround time with reduced capital and utility costs.

A current trend in the bioprocess industry is the increased interest in using non-animal origin (NAO) materials in its processes. In the case of adherent cell culture, microcarriers have often utilized collagen or other animal-derived material to promote cell attachment and spreading.



In this study, Vero cells were successfully cultured in a stirred-tank bioreactor on microcarriers using a serum-free medium in an NAO process. Through optimization of parameters such as bead concentration and cell seeding density, this system will be highly beneficial to virus production processes by alleviating many concerns regarding contamination, viral clearance, and safety while providing the capability to rapidly transition processes.

Materials

Vero cells (ATCC, CCL-81™ cells) were adapted to HyQ™ SFM4MegaVir™ medium, which was developed to support virus production in a variety of cell lines. Materials used in this study are described below.

Description	Material	Supplier*
S.U.B. (50 L)	Bioreactor hardware	Thermo Fisher Scientific
S.U.B. (50 L)	BPC	Thermo Fisher Scientific
HyQ SMF4MegaVir	Medium	GE
Plastic Plus HyQSphere™	Microcarrier beads	GE
Vero cells	Cell line	ATCC
DPBS (phosphate-buffered saline)	Saline solution	GE
HyQTase™ (non-mammalian)	Dissociation reagent	GE

*Suppliers of these products at the time of this study.

Methods

Bioreactor setup and microcarrier preparation

The S.U.B. system was set up according to the manufacturer’s guidelines (consult User Guide UG003) and equipped with a pH/DO control system (Applikon ADI 1010 Bio Controller and ADI 1025 Bio Console). Direct air flow to the sparger was set to 0.1 L/min and the overlay gas flow setting was 0.2 L/min. HyQ SFM4MegaVir medium (20 L) was added aseptically by sterile tubing welding of the media BPC to the addition line of the S.U.B. Plastic Plus HyQSphere microcarrier beads (250 g) were autoclaved in DPBS, washed twice, then resuspended in fresh culture medium. The medium was allowed to warm to 37°C at an agitation rate of 30 RPM before the microcarriers were pushed into the bioreactor via positive pressure (bead density may require process-specific optimization). Additional culture medium was used to “chase” the microcarriers into the bioreactor to flush any beads from the addition line into the vessel.

Parameter	S.U.B. setting
Final operating liquid volume	50 L
Seeding cell/volume ratio	2 x 10 ⁴ cells/mL
Agitation rate	50 RPM
Temperature	37±0.1°C
pH	6.8–7.1
Dissolved oxygen	40–60% air saturation
Air overlay sparge rate	0.2 L/min
Air direct sparge rate	0.1 L/min

Bioreactor seeding

The bioreactor was seeded (via positive pressure) with a viable cell density of 5.0 x 10⁸ cells in 25 L, or 2 x 10⁴ cells/mL. The remaining volume of culture medium was used to flush any remaining cells through the line and to bring the intermediate working volume to 25 L. Agitation speed was increased to 50 RPM, providing enough agitation to prevent settling of the microcarriers and promote homogeneity throughout the working volume.

Culture monitoring

Samples were collected aseptically using a luer-lock syringe connected to the sampling port of the S.U.B. Samples were taken every 24 hours after seeding to observe cell attachment on the microcarrier beads, determine cell density (stained nuclei counting), and monitor the culture biochemistry. On day 5 of the culture, the working volume was increased to 50 L to support the increasing cell density. This reduced the cell and microcarrier concentration by one-half. Other methods, such as halting agitation to allow settling of microcarriers and removing half of the working volume, can be used to feed the cells without affecting the concentration of cells and microcarriers.

Results and discussion

Bioreactor control and operation

Culture temperature was controlled during the run at 37°C. The pH of the culture was maintained by addition of CO₂ only, and remained within allowable limits for the length of the culture. Additionally, the dissolved oxygen (DO) level was maintained at the desired level of 50% by addition of air through the integrated gas sparging system of the S.U.B. Agitation speed was easily controlled during the run with the built-in mixing control. The bioreactor system was microscopically evaluated and remained free of contamination for the duration of the experiment.

Microcarrier culture evaluation

Seeding of the S.U.B. is best accomplished through gravity feeding or positive pressure, as passing the seed culture through a peristaltic pump decreases cell viability and can cause an initial lag in cell growth. During the attachment phase, it is important to slow agitation to allow cells to attach and spread on the growth surface, especially under serum-free conditions. The lowest acceptable agitation rate capable of maintaining the microcarriers in suspension while allowing cell attachment was determined to be 50 RPM. Microscopic observation of the microcarriers at 18 hours post-seeding revealed that cells had attached and spread on the surface of the beads and nearly half of the culture had undergone mitosis. Figure 1 shows the daily Vero cell population density daily over a 15-day period.

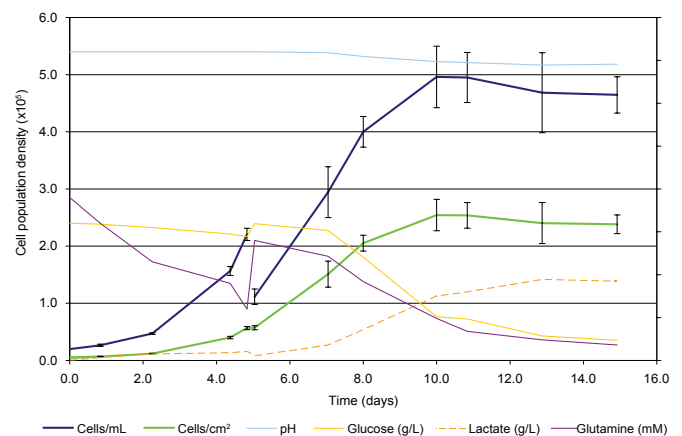
The decrease in cell population seen on day 5 occurred when the working volume was increased to 50 L. Cells reached the peak density of 5.0×10^5 cells/mL on day 10 of the culture and were observed to be confluent. The final microcarrier concentration of 5 g/L appeared to not be optimized in this evaluation and, as a result, the surface area available to cell growth was limited. (Note: Microcarrier concentrations of up to 20 g/L have been used successfully with the same combination of microcarriers, cells, and culture medium.) However, by calculating the number of cells per cm², the peak population density of 2.5×10^5 cells/cm² is consistent with the density of a confluent Vero cell culture in traditional vessels.

The average rate of growth over the entire culture period was approximately one doubling per 36 hours a rate slightly slower than Vero cells cultured in HyQ SFM4MegaVir medium in static culture (one doubling per 28–30 hours). However, this may be attributed to the low cell seeding density, a longer attachment phase with microcarrier cultures, or the fact that these parameters were not optimized for this experiment.

The culture biochemistry (Figure 1) was comparable to other cultures of Vero cells in this culture medium. Lactate reached nearly 2 g/L, with a peak concentration at approximately day 12. This is consistent with the utilization of glucose during the logarithmic growth phase, predominantly between days 4 and 10. Inversely, glutamine was consumed rapidly during early culture through day 5. These utilization/production rates are indicative of a normal metabolic pattern with this culture combination.

The culture was maintained for approximately 15 days, exhibiting normal cell metabolism and growth kinetics within acceptable limits. A peak cell density of approximately 2.5×10^5 cells/cm² was achieved by day 10, typical of a confluent culture. Throughout this culture, the pH and DO were adequately maintained by sparging of CO₂ and air only.

Figure 1. Vero cell culture growth using Plastic Plus HyQSphere microcarriers and SFM4MegaVir culture medium over a 15-day culture period in the 50 L stirred-tank S.U.B. Culture substrate utilization and biochemical production rates were indicative of a normal metabolic pattern with this culture.



Conclusion

Key factors in a successful microcarrier culture include:

1. The ability of the cells to attach to and spread on the microcarrier surface.
2. The ability to provide adequate dissolved oxygen (DO) to the complete culture.
3. The ability to maintain a generally homogeneous suspension throughout the working volume while minimizing the shear forces acting upon the cells. The S.U.B. in combination with HyQ SFM4MegaVir medium and Plastic Plus HyQSphere microcarriers supported these required conditions to provide a successful microcarrier culture.

Find out more at thermofisher.com/sut

ThermoFisher
S C I E N T I F I C