APPLICATION NOTE No. 463

Expansion of Vero Cells Grown on Microcarriers in Serum-free Medium using a BioFlo® 320 Bioreactor Control System

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Abstract

The Vero cell line is an anchorage-dependent line requiring bovine serum for optimal cell growth during cultivation. Because of the complexity of bovine serum and its animal origin, however, the manufacturing process of biological products based on Vero cells must resolve significant quality control issues. In this study, we used a stirred-tank bioreactor and microcarrier beads to investigate the process of Vero cell culture in serum-free medium. We equipped the bioreactor with a perfusion device to be able to perform a medium exchange and therefore maintain an appropriate glucose concentration in the culture medium. At the completion of the protocol, after 120 hours, the density of Vero cells in the bioreactor reached 7.2×10^6 cells /mL. These findings establish the strong potential of serum-free culture systems of Vero cells in bioreactors.

Introduction

The Vero cell line originates from the kidney of the African green monkey. The cell line has been shown to be highly susceptible to a wide range of viruses. It is recognized today as an important component of the cell technology recommended by WHO for the production of a wide range of viral vaccines [1, 2]. Because the Vero cell has the property of adherence, bovine serum for years has been a standard component of the growth medium. However, the production of bovine serum is labor intensive, batch-to-batch variation cannot be controlled, and consistency is a constant challenge. All those issues introduce difficulties into quality control of research experiments and production demands. At the same time, the use of serum also causes uncontrollable biosafety problems. Therefore, serum-free culture of Vero cells is currently the method of choice [3].

Stirred-tank bioreactors are commonly used for the culture of suspension cells. When adherent cells are cultured in such bioreactors, carriers are loaded into vessels to provide a growth surface for cells. Microcarriers represent an attachment matrix for cultivation, which can be stirred in a suspended state by agitation control. Currently, Vero cells culture on microcarriers is a well-established technology for the production of viruses, including rabies vaccine, EV71 vaccine, and COVID-19 vaccines [4].

One important parameter for achieving optimal cell growth and virus production is the concentration of nutrients in the culture medium. Cell cultures on microcarriers allow the setup of perfusion bioprocesses, in which used medium

is constantly harvested and fresh medium is added to the process, while retaining the cells in the bioreactor. Integration of a perfusion device therefore facilitates maintaining the appropriate nutritional requirements for the growth of Vero cells.

We evaluated the utility of serum-free culture medium for the cultivation of Vero cells grown on microcarriers in stirred-tank bioreactors. The process was controlled using a BioFlo 320 bioprocess control system. The system was connected to a 1.75 L glass bioreactor, which was equipped with a Cell-Lift impeller and decanter colunm for culturing in perfusion mode.

Material and Methods

Cells and reagents

The Vero cell line was purchased from the Chinese Academy of Sciences Cell bank and adapted for growth in serum-free medium until the 145th passage. Gibco® VP Serum Free Medium (Thermo Fisher Scientific®) was used in the cell expansion phase in T-flasks, multilayer flasks, and for cultivation in the bioreactor. Recombinant trypsin was used as the cell digestive solution, and a trypsin inhibitor was added for terminating digestion. Cytodex® 1 microcarriers (Cytiva®) were used. Glucose concentrations were measured by a commercial glucose kit (Nanjing Jiancheng Bioengineering Institute).

Bioprocess equipment

Cells were expanded in a BioFlo 320 bioreactor control system equipped with a glass bioreactor (Figure 1A). To run the bioprocess in perfusion mode, three accessories, Cell-Lift impeller (Figure 1B), foam elimination cage and decanter column were installed. The setup of the system is schematically illustrated in Figure 2.

Preparation of seed cells

The frozen Vero cells (5×10^6 cells) adapted to serum-free medium were introduced into T-175 flasks and placed in a CO_2 incubator at 37 °C and an atmosphere of 5 % CO_2. When Vero cells grew to complete confluence, they were detached with recombinant trypsin, then transferred to eight T-175 flasks at an inoculation density of 3.0×10^4 cells/cm^2. When the cells reached 100 % confluence, they were trypsinized, harvested and inoculated into a five-layer-flask at a density of 3.0×10^4 cells/cm^2. After the Vero cells in the multilayer flask reached confluence, they were detached and collected. As per the cell counts, the cells were diluted to a density of

Fig. 1: A. BioFlo 320 bioreactor control system with glass bioreactor. **B.** Cell-Lift impeller

2.1×10^6 cells/mL with a total of 250 mL of cell suspension, and the viability of the cells was assessed at 99 %. Those seed cells were transferred into 500 mL sterile bottles and prepared for inoculation.

Microcarrier Preparation

According to the instructions provided by the microcarrier supplier, a total of 17.5 g Cytodex 1 microcarriers were weighed, diluted with Dulbecco's Phosphate Buffered Saline solution (DPBS) and soaked for 12 hours. After that, DPBS was used to clean the microcarriers three times, then an autoclave sterilization step was performed.

After that, the serum-free medium was added to replace the DPBS solution with microcarriers by three washing and centrifugation steps. Finally, this bottle was placed in an incubator at 37 °C for 12 hours.

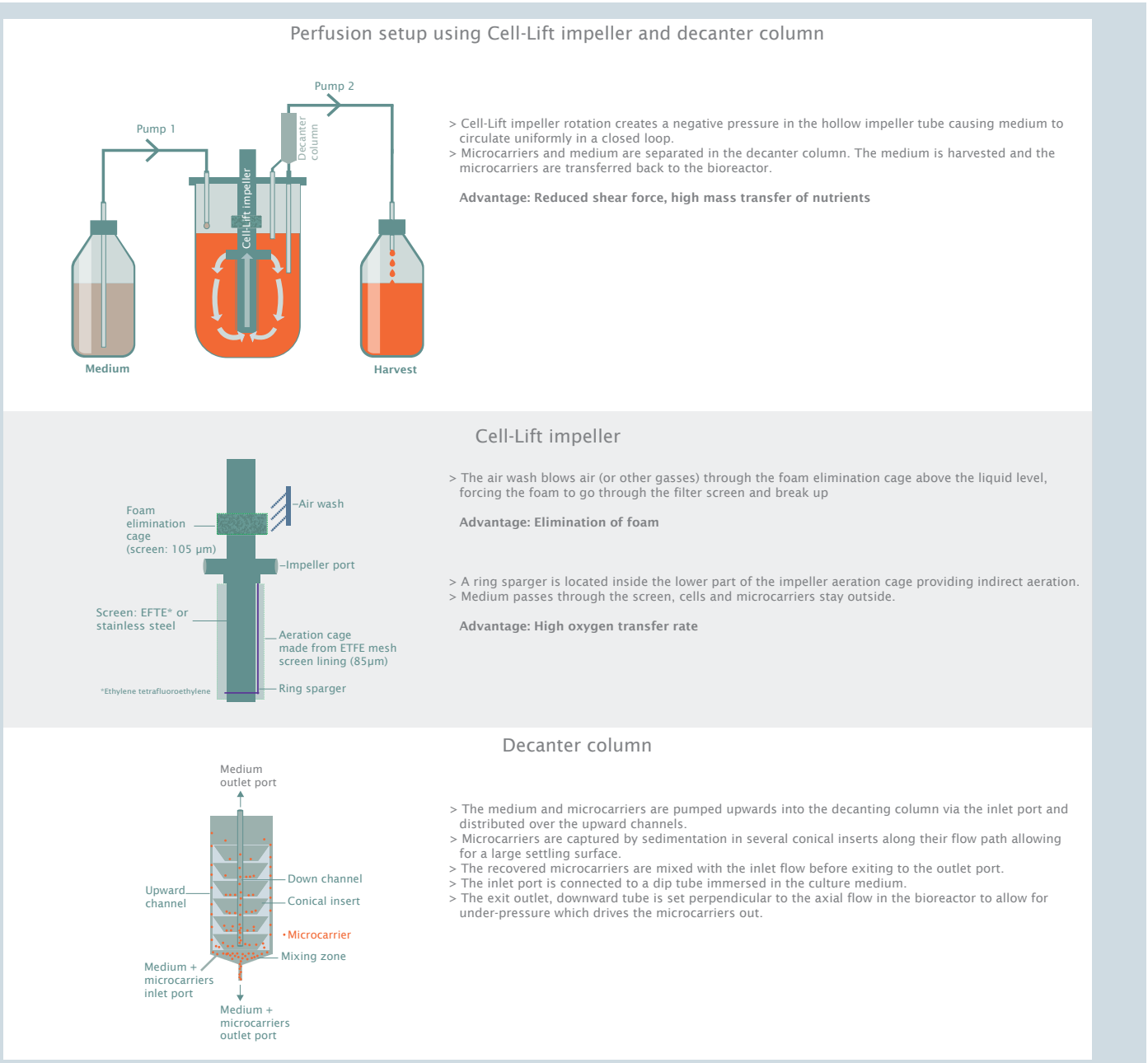

Fig. 2: Perfusion process using a Cell-Lift impeller and decanter column.

Bioprocess control and process parameters

A BioFlo 320 bioreactor control system equipped with a glass bioreactor with a working volume of 1.75 L was selected for this study. Two accessories, a Cell-Lift impeller with foam elimination cage and a decanter column, were installed after cleaning the bioreactor.

The pH sensor was calibrated at two points (pH 7 and pH 4), then DPBS solution was added to the vessel to a final volume of 1.75 L. Subsequently, the silicone tubing and DO sensor were installed in the bioreactor head plate. Next, the bioreactor was autoclaved. The DO sensor was calibrated after sterilization. Then the DPBS solution in the vessel was removed, followed by the introduction of the prepared

microcarrier suspension and the medium into the bioreactor to a final volume of 1.5 L. Next the microcarrier and medium were adjusted to the parameter setpoints. When the setpoints were stable, 250 mL Vero seed cells were added into the bioreactor with an inoculation density of 3.0×10^5 cells/mL. The control parameters of the bioreactor were set as described in Table 1.

Table 1: Process parameters

Parameter	Device/setpoint
Bioreactor system	BioFlo 320 bioreactor control system
Bioreactor	Glass bioreactor with water jacket (working volume 1.75 L)
Microcarrier	Cytodex 1
Temperature	37 °C
pH	7.0 (deadband 0.05)
DO	50 %
Gas supply (submerged)	3-gas mix control (air, CO_2, O_2); 0.002-0.2 SLPM
Gas supply (overlay)	100 % air; 0.1 SLPM

Analytical methods and cell counting

Daily sampling from the bioreactor was performed to measure the glucose concentration and to calculate the glucose consumption per 24 hour period. At the same time, the morphology of the Vero cells on the microcarrier was observed by light microscopy. Cells attached to the microcarrier beads were trypsinized, then stained with Trypan blue. Cell counting and viability were assessed by a Countess-3 Automated Cell Counter (Thermo Fisher Scientific).

Feeding control

The continuous perfusion step was performed every day in order to maintain the glucose level between 1.0 to 2.0 g/L, according to the glucose concentration in the vessel. This step was necessary in order to ensure maintenance of the appropriate nutritional requirements for the growth of Vero cells. The perfusion step was carried out by a decanter column which blocks the microcarriers outflowing the vessel.

Results

Cell attachment and growth

Twenty four hours after inoculation the Vero cells were attached to the Cytodex 1 microcarriers, with an average of seven cells per microcarrier. 120 hours after inoculation, Vero cells reached full confluence on the microcarrier beads (Figure 3).

Cell density and glucose consumption

From the beginning of cell seeding to growth of 120 hours, the cell density increased from 3×10^5 cells/ml to 7.2×10^6 cells/mL, with 24-fold proliferation (Figure 4). Meanwhile, the cell viability detected by the Countess-3 cell counter was between 97.9 % and

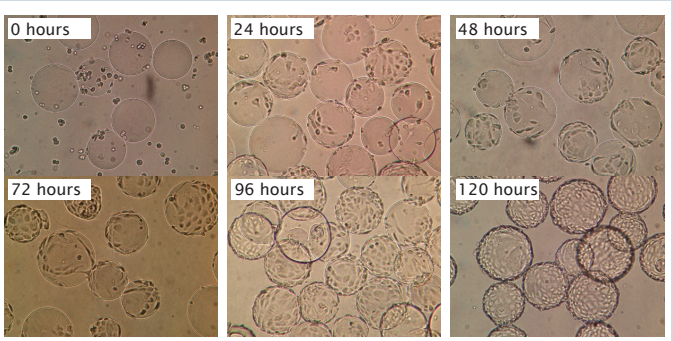

Fig. 3: Growth of Vero cells on microcarriers.

99.0 % (Figure 4). Perfusion based on glucose consumption was activated with the initial flow rate at 0.5 L/24 h from the 24th hour. At the time of 96 to 120 hours, the perfusion rate increased to 3.0 L/24 h (Figure 5). With the cell density rising, the glucose consumption also increased rapidly after 48 hours. Then the consumption rate slowed down until 96 hours when the cells grew approximately to full confluence. During this period, the maximum glucose consumption reached 8.5 g/24 h (Figure 5).

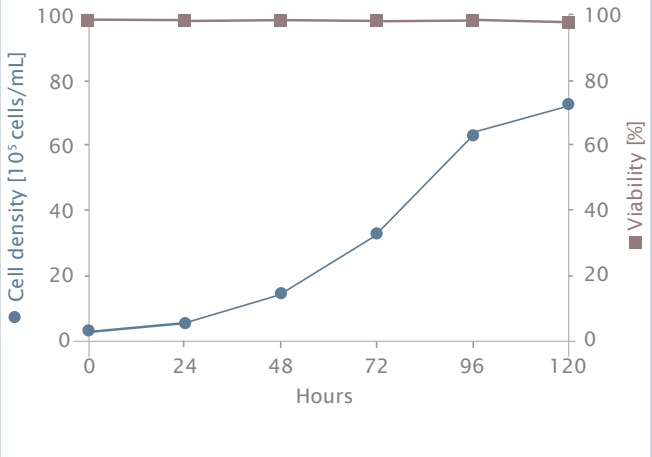

Fig. 4: Proliferation curve and viability of Vero cells on microcarriers.

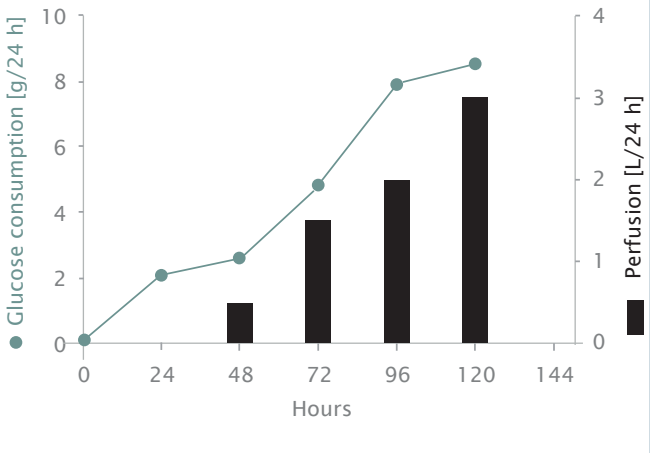

Fig. 5: Glucose consumption of Vero cells in bioreactor and perfusion rate.

Conclusions

These studies demonstrate the feasibility of Vero cell culture on microcarriers in serum-free medium in a stirred-tank bioreactor. We used the BioFlo 320 bioreactor control system equipped with a Cell-Lift impeller and decanter column to culture Vero cells at high density. The final Vero cell density reached 7.2 × 10^6 cells/mL, with a microcarrier density of 10 g/L.

Successful serum-free culture technology for high-density Vero cells has two basic requirements:

1. A low shear force stirring system which reduces the damage to cells in serum-free culture;

2. The continuous perfusion process which provides the nutrients for high-density growth and cell maintenance.

In these experiments, the low shear force of the Cell-Lift impeller and the decanter column for perfusion were combined to realize the microcarrier culture process in serum-free medium, which is more in accordance with the current trend in animal cell culture. Additionally, it is compatible with a production process and quality control. Our findings demonstrate the significant potential for vaccine production using Vero cells in a serum-free culture system.

References

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Ordering information

Description	Order no.
BioFlo® 320, base control station, with water connection	1379963011
Vessel Bundle, for BioFlo 320, water jacket, magnetic drive, 1 L	M1379-0310
1 L Cell-Lift Impeller Kit, 80 µm	M1379-1110
1 L Decanter Kit	M1287-1190

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