Serum-free Culture of Vero Cells in the Corning® CellCube® System

Application Note

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Introduction

The Vero cell line, derived from the kidney tissue of an African green monkey, is widely used for both viral vector and vaccine production. As a continuous cell line, Vero cells offer significant advantages for their relative ease of adherent or suspension-adapted culture, compared to traditional manufacturing methods (i.e., chicken eggs or primary cells). Their robust growth characteristics in combination with increased susceptibility to multiple viruses due to the lack of interferon expression—make Vero cells a stable growth substrate for several different viral vaccines.[1,2,3]

Moreover, Vero cells have proven to be adaptable to a variety of different culture platforms including microcarriers and fixed-bed bioreactors, from small-scale to large-scale production, in both serum-containing and serum-free media.[1,2,3] In fact, Vero culture in serum-free medium has become routine with multiple commercially available serum-free media formulations; some specifically optimized for Vero cells.[1] Serum-free culture delivers more consistent performance and lowers the downstream purification burden.[2] In addition, though the regulatory environment is already favorable for Vero cell use in vaccine production, serum-free culture can further ease regulatory hurdles.[1]

Even with the precedent for serum-free Vero cell culture for viral vaccines manufacturing, there continues to be development and optimization of both media formulation and culture platforms,[3] driving toward lower cost and higher yield. Hence, our work to establish a protocol for culturing Vero cells in serum-free medium on the Corning CellCube System, a compact and scalable method for mass culture of attachment-dependent cells.

Materials and Methods

Cell Adaptation and Scale up

Vero cells (ATCC® CCL-81™) were thawed in Dulbecco's Modified Eagle Medium (DMEM; Corning 10-013-CM) plus 10% fetal bovine serum (FBS; Corning 35-010-CV) according to manufacturer's instructions. The cells were plated onto Tissue Culture (TC)-treated 75 cm^2 U-shaped flasks (Corning 430641U) and cultured for several days to a target harvest density of 1 x 10^5 cells/cm^2. Cells were subcultured by releasing the cells using TrypLE™ Express enzyme (1X) (Thermo Fisher 12604) for an additional 2 passages before direct adaptation to serum-free media. At passage 3 post-thaw, cells were seeded at 4 x 10^4 cells/cm^2 on Corning CellBIND® surface-treated 75 cm^2 U-shaped flasks (Corning 3290) in serum-free medium (Gibco™ VP-SFM; Thermo Fisher 11681020) plus 1X Gibco GlutaMAX™ supplement (Thermo Fisher 35050061). Cells were expanded for 3 to 4 passages until cell growth rate stabilized before generating freeze stocks, each time seeding at 4 x 10^4 cells/cm^2 and allowing cells to reach full confluence before passaging.

To initiate the Corning CellCube module seed train, serum-free-adapted Vero cells were thawed and plated in serum-free medium on Corning CellBIND surface-treated 75 cm^2 U-shaped flasks. During subsequent passages, cells were subcultured on TC-treated culture vessels. Vero cells were scaled up through 4 to 5 passages before seeding into a Corning CellSTACK® 5-chamber culture vessel (Corning 3313, 3319), each time seeding at 2 x 10^4 cells/cm^2 to 4 x 10^4 cells/cm^2 and allowing cells to reach full confluence before passaging. The CellSTACK 5-chamber vessel was cultured to a final confluence of >5 x 10^4 cells/cm^2 with the desired yield of >1.7 x 10^8 cells.

Corning CellCube Closed System Expansion

In this study, Vero cells were expanded in serum-free medium using a Corning CellCube 10-layer module (Corning 3231) with the Eppendorf BioFlo® 120 controller (Eppendorf B120ACS000) and BioBLU® 3c single-use bioreactor (SUB; Eppendorf 1386000300) to adequately control medium conditioning. On the day prior to seeding (Day -1), the CellCube closed system was prepared for equilibration according to established protocols.[4,5] The inlet and outlet circulation loops (Corning 3235, 3234) were attached to the CellCube 10-layer module via AseptiQuik® G connectors. Terminal ends of the inlet and outlet circulation loops were connected to the bioreactor return and outlet of the SUB via AseptiQuik S connector-to-MPC adapters (Corning 3238). The CellCube closed system was placed next to the controller in a warm room (37°C). The system was filled with warm serum-free medium plus 1% penicillin-streptomycin (Corning 30-002-CI), and the SUB was readied for operation and probe calibration. Following calibration, the controller was set to 20% dissolved oxygen (DO), pH 7.2 with 4-gas (Air, O_2, CO_2, N_2) mixing, and sodium bicarbonate for base control. Circulation was initiated at 200 mL/minute. The entire system was allowed to equilibrate overnight.

On the day of seeding (Day 0), Vero cells were harvested from the CellSTACK 5-chamber with TrypLE Express enzyme (1X) for 10 minutes at 37°C following 1X Dulbecco's Phosphate-Buffered Saline (DPBS; Corning 21-031-CM) wash. The CellCube 10-layer module was seeded at 2 x 10^4 cells/cm^2 following a single seeding protocol with rotational seedings at alternating 7-minute (front side) and 10 minute (back side) intervals for a total seeding duration of approx. 2 hours.[4] For detailed protocol, see Corning CellCube Culture System Cell Expansion Protocol Guidelines for Use (CLS-AN-626DOC). During the expansion period, confluence of the CellCube module was monitored with a handheld USB microscope (Bysameyee Microscope 1000X). In addition, daily samples were drawn from the SUB for offline gas, electrolyte, and metabolite analysis.

The CellCube 10-layer module was harvested on Day 7 according to established protocols[4,5] with the following modifications. First, the Vero cells were harvested in 2 batches; the initial harvest was performed after a 20-minute incubation with 600 mL (to fill the module) of cell dissociation solution, consisting of TrypLE Express enzyme (1X) plus 0.1% Poloxamer 188 (Corning 13-901-CI) pre-warmed to 37°C, in the vertical position, followed by a second harvest after a 1-hour incubation with a fresh 600 mL cell dissociation solution. Second, in lieu of recirculation, air pockets were introduced into the CellCube module during each harvest incubation, and the module was shaken as necessary to dislodge tightly adherent cells. Both harvests were diluted with equal volumes of spent medium. After mixing well, 4 samples each were drawn off the 2 harvest batches for enumeration.

Results and Discussion

Previous work established the utility of the Corning® CellCube® system for mass culture of attachment-dependent Vero cells.[5] The present study was intended as an extension of that work on a smaller scale to demonstrate performance of the CellCube system for Vero cell culture under serum-free conditions. The general process for set up and use of the CellCube system was the same as has been published,[4,5] with optimization for specific cell growth requirements of Vero cells in serum-free culture in the CellCube system.

Preliminary work revealed accelerated attachment kinetics relative to Vero cell seeding in serum-containing medium. Therefore, the CellCube 10-layer module was rotated more frequently during the rotational seeding process to adjust for faster cell attachment. The total duration (approx. 2 hours) of seeding was the same as prior CellCube 100-layer studies,[5] but with shortened rotational seeding intervals (7 minutes and 10 minutes) for Vero cells in serum-free medium. Cells had attached by 2 hours, and the observed seeding was even when the outermost culture surfaces were inspected by a handheld microscope at the start of circulation (Figure 1). Cell morphology and growth patterns in the CellCube system during the expansion period mirrored the initial characterization and maintenance of the Vero cell line grown in standard 2D flatware in serum-free medium. However, maintenance cultures were seeded at higher densities (3 x 10^4 cells/cm^2 to 4 x 10^4 cells/cm^2) and subcultured every 3 to 4 days when they reached confluence but before the medium was noticeably acidic (i.e., yellowed). By comparison, medium conditioning enabled extension of the culture period in the CellCube system to 7 days to maximize cell yield in this study.

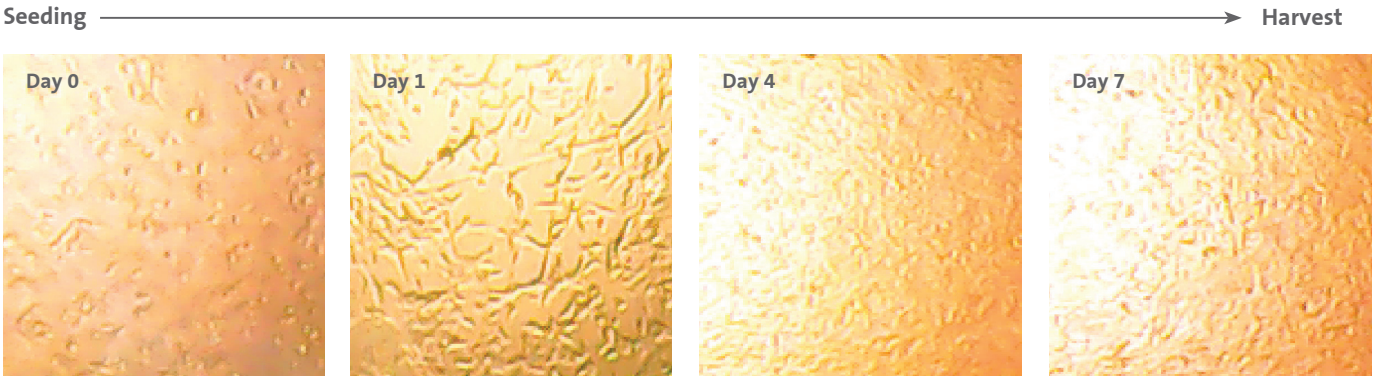

Figure 1. Vero cells grow to high confluence during the course of a 7-day expansion. Representative images from a Corning CellCube 10-layer module throughout the 7-day expansion period: Day 0 post-seeding, days 1, 4, and 7 prior to harvest. Images were acquired with a handheld USB microscope.

To accommodate the longer culture period with yet unknown requirements for growth, a surplus of serum-free medium (0.4 mL/cm^2) was used in the system to feed the culture. The system medium volume proved to be an excess for cell expansion, as the glucose was barely consumed by harvest day and lactate accumulation had not outpaced glucose consumption (Figure 2). Yet, an increase in lactate accumulation did correlate to glucose consumption toward the end of the culture period, suggesting that metabolite concentration is an indicator of cell confluence. Thus, glucose and lactate concentrations in the spent medium could be used as an indicator for harvest-timing of Vero cells in serum-free medium, given the appropriate medium volume-to-culture area ratio is used. One of the benefits of using the Corning® CellCube® system is its flexibility in allowing for variable medium usage, perfusion, and/or bolus nutrient addition to feed cultures during cell expansion.

System medium volume is only one example of experimental parameters that can be tailored to the cell type and medium formulation. Of note, the initial Vero cell expansion studies in the CellCube system in serum-free medium—using the proven parameters for Vero cell expansion in serum-containing medium (i.e., 60% DO and pH 7.2)—exhibited poor yield relative to control U-shaped flask cultures. This poor yield was attributed to the cells being stripped off of the culture surface from high media recirculation flow rate as well as higher cell doubling times. In order to address the first issue, the system medium circulation rate was slowed to a maximum of 200 mL/minute. This adjusted flow rate was adequate for medium exchange through the CellCube 10-layer module used in these experiments but will likely need to be increased for the larger CellCube 25- and 100-layer modules. Medium conditioning parameters were adjusted to improve cell doubling times. Based on customer feedback and the peer-reviewed literature,[2,6] DO was reduced to 20% and pH set to 7.2. These simple adjustments were sufficient to drive cell expansion and underscore the importance of optimizing parameters for each application.

Not surprisingly, two harvests were necessary to collect all cells from the CellCube module on Day 7 of the cell expansion. Strong attachment of Vero cells in serum-free medium was observed in maintenance cultures, necessitating extended incubation with harvest enzyme. Back-to-back harvests with TrypLE Express enzyme, plus 0.1% Poloxamer 188, enabled harvest of all cells from the CellCube 10-layer module without sacrificing cell viability. The average cell yield from the first TrypLE Express enzyme incubation was 2.5 x 10^9 ± 0.8 x 10^9 cells with >98% cell viability (Figure 3). The average cell yield from the second harvest was 8.5 x 10^8 ± 7.7 x 10^8 cells with >96% cell viability or approx. 5% addition to the cell yield. The sum of both the first and second harvests averaged 3.3 x 10^9 ± 1.8 x 10^9 cells which corresponds to a yield of 3.9 x 10^5 cells/cm^2. The harvest process could be further optimized by changing the timing and/or harvesting reagent, for a single-step process.

Strong attachment of Vero cells in serum-free medium to the CellCube module would be beneficial for transfection or viral transduction processes since the final product is collected from medium alone or by using an *in-situ* lysis protocol to directly lyse the cells on the surface of the module. Regardless of application, this study provides the framework for process optimization and protocol development for Vero cell expansion in serum-free medium at a small scale that can ultimately be scaled to production in the larger CellCube systems.

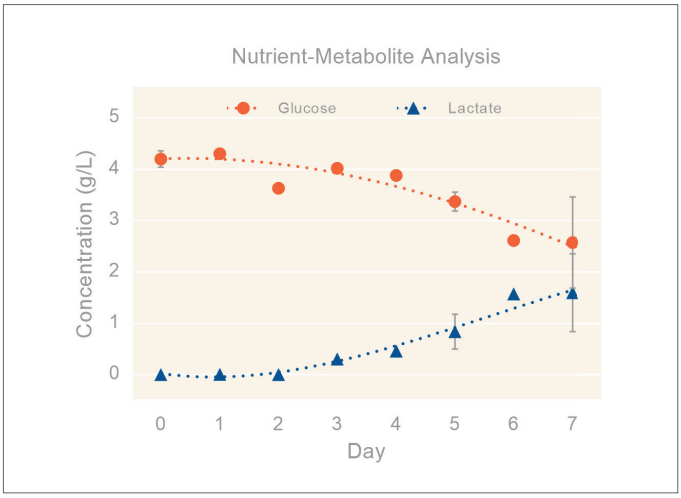

Figure 2. Lactate accumulation follows increase in glucose utilization for Vero cell expansion in serum-free medium. Daily samples of medium were drawn from the SUB during Vero cell expansion. Glucose (orange circle) and lactate (blue triangle) were monitored during cell expansion. Third degree polynomials were fit to data points (mean ± SD) to show trends in glucose depletion (orange dotted line) and lactate accumulation (blue dotted line). N = 2.

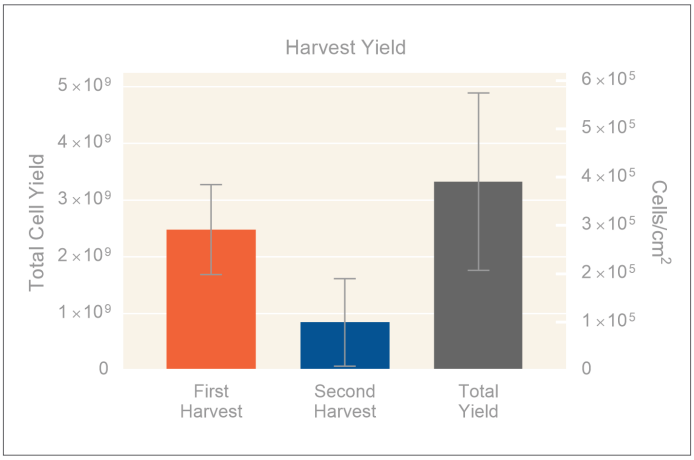

Figure 3. The Corning CellCube system supports 20-fold expansion of Vero cells in serum-free medium. Total harvest yield (left axis) and cell yield/cm^2 (right axis) after the first (orange) and second harvests (blue), and the sum total yield (grey) following a 7-day expansion from an initial seeding of 2 x 10^4 cells/cm^2. Bars represent mean ± SD. N = 2.

Conclusions

- Vero cell attachment kinetics differ in serum-containing and serum-free medium, with the high density seeding in serum-free medium requiring shorter rotational seedings.
- Low 20% DO and pH 7.2 are favorable for Vero cell expansion in serum-free medium.
- System medium volume must be optimized to utilize glucose consumption and lactate accumulation as a marker of cell expansion and harvest timing.
- The Corning® CellCube® system supports high-density Vero cell culture greater than 3.9 x 10^5 cells/cm^2 in serum-free medium.

References

1. Alfano R, Pennybaker A, Halfmann P, Huang CYH. Formulation and production of a blood-free and chemically defined virus production media for VERO cells. Biotechnol Bioeng 2020;117:3277–3285. doi: 10.1002/bit.27486.
2. Frazzati-Gallina M, Paoli RL, Mourao-Fuches RM, Jorge SAC, Pereira CA. Higher production of rabies virus in serum-free medium cell cultures on microcarriers. J Biotechnol 2020;92:67–72. doi: 10.1016/s0168-1656(01)00362-5.
3. Kiesslich S, Kamen AA. Vero cell upstream bioprocess development for the production of viral vectors and vaccines. Biotechnol Adv 2020;44:107608. doi: 10.1016/j.biotechadv.2020.107608.
4. Corning CellCube Culture System Cell Expansion Protocol Guidelines for Use (CLS-AN-626DOC).
5. Maximizing Yield for Attachment-dependent Cells with the Corning CellCube System Application Note (CLS-AN-568).
6. Jiang Y, van der Welle JE, Rubingh O, van Eikenhorst G, Bakker WAM, Thomassen YE. Kinetic model for adherent Vero cell growth and poliovirus production in batch bioreactors. Processs Biochem 2019;81:156–164. doi: 10.1016/j.procbio.2019.03.010.

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