Expansion of DF-1 Chicken Fibroblast Cell Line in the Corning® CellCube® System

Application Note

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Introduction

Traditional methods of vaccine production in primary chicken embryo fibroblasts (CEF) are costly time- and labor-intensive processes.[1] Therefore, virus propagation in continuous cell lines has gained traction for the relative ease and scalability of cell culture-based vaccine production. The spontaneously immortalized chicken embryo fibroblast cell line, DF-1,[2,3] has been shown to have applications for production of a variety of avian virus vaccines for Marek's disease, avian influenza, and infectious bursal disease virus (IBDV).[4] DF-1 cells are a suitable alternative to CEF for avian virus propagation exhibiting faster growth rates, capacity for indefinite continuous culture, and lack of endogenous viruses.[1,4] Consequently, the following study establishes basic cell culture parameters for expansion of DF-1 cells in the Corning CellCube System—a compact and scalable method for mass culture of attachment-dependent cells.

Materials and Methods

Cell Scale-up

UMNSAH/DF-1 cells (ATCC® CRL-12203) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Corning 10-013-CM) plus 10% fetal bovine serum (FBS; Corning 35-010-CV), and 1% penicillin-streptomycin (Corning 30-002-CI) in a 39°C, 5% CO_2 humified incubator. To initiate the seed train, cells were thawed onto Corning CellBIND® surface-treated 75 cm^2 U-shaped flasks (Corning 3290), scaled up to Corning CellBIND CellSTACK® 2-chamber culture vessels (Corning 3310), and finally to 2 Corning Tissue culture (TC)-treated CellSTACK 10-chamber culture vessels (Corning 3270, 3271) before terminal seeding into Corning CellCube 100-layer modules (Corning 3264).

Corning CellCube Closed System Expansion

DF-1 cells were expanded using a Corning CellCube 100-layer module (Corning 3264) with the Eppendorf BioFlo® 120 controller (Eppendorf B120ACS000) and BioBLU® 3c single-use bioreactor (SUB; Eppendorf 1386000100) for medium conditioning. On the day prior to seeding (Day -1), the closed system was prepared for equilibration according to established protocols.[5,6] The controller was set to 50% dissolved oxygen (DO) with 4-gas (Air, O_2, CO_2, N_2) mixing, pH 7.35 with sodium bicarbonate for base control, and an initial media recirculation rate of 400 mL/min. at a system temperature set point of 39°C. The entire system was allowed to equilibrate overnight in a 39°C warm room.

On the day of seeding (Day 0), DF-1 cells were harvested from the CellSTACK 10-chamber vessels with 1X Dulbecco's Phosphate-Buffered Saline (DPBS; Corning 21-031-CM) wash followed by TrypLE™ Express Enzyme (Thermo Fisher 12604013) for 10 minutes at 37°C. The CellCube 100-layer module was seeded at a density of 5 x 10^3 to 7 x 10^3 cells/cm^2 using the single seeding protocol integrating rotational seedings with alternating 15-minute (front side) and 25-minute (back side) intervals for a total seeding duration of approximately 2 hours.[5] In addition, a TC-treated 75 cm^2 U-shaped flask (Corning 430641U) was seeded from the same cell suspension to use as a satellite vessel for additional culture monitoring. During the expansion period, confluence of the CellCube module was monitored with a handheld USB microscope (Bysameyee Microscope 1000X). Daily samples were drawn from the SUB for offline gas, electrolyte, and metabolite analysis.

The CellCube 100-layer module was harvested on Day 4 according to established protocols.[5,6] Harvest was performed after a 20- to 30-minute incubation with 6L (to fill the module) of prewarmed 0.05% Trypsin-EDTA (Corning 25-052-CV) plus 0.1% Poloxamer 188 (Corning 13-901-CI) in the vertical position. In lieu of recirculation, air pockets were introduced into the CellCube module during the harvest incubation, and the module was shaken as necessary to dislodge tightly adherent cells.[7] The harvested cell suspension was quenched with an equal volume of spent medium, mixed well, and enumerated. The satellite vessel was also harvested for comparison. The study was repeated 3 times.

Results and Discussion

Virus propagation in anchorage-dependent cells for vaccine production requires a scalable adherent platform. The Corning CellCube System—a circulation/perfusion system for mass culture of adherent cells—is well-suited for such applications.[8] Consequently, the goal of the current proof-of-concept study was to establish basic cell culture parameters for expansion of DF-1 cells in CellCube 100-layer modules to demonstrate the utility of the CellCube system for avian vaccine production applications.

Initial attachment and spreading of DF-1 cells onto the CellCube culture surface was similar to many bioproduction cell lines.[6] DF-1 cells exhibited the characteristic spindle-shaped fibroblastic morphology (Figure 1). However, initial expansion was slow, evidenced by the low confluence for the first few days of culture. Importantly, nutrient and metabolite profiles mirrored the lag observed in cell confluence; glucose utilization, and lactate accumulation increased only after Day 3 of culture (Figure 2) and had not peaked by the day of harvest. Taken together, these data indicate that nutrient/metabolite profiles can signal the harvest date for this cell line and suggests that the circulating medium could have sustained cell expansion for additional day(s).

Conversely, the reserve of nutrients in the medium would support the use of a lower circulating medium volume per surface area in lieu of extending the culture period. The initial CellCube system volume in this study was 0.09 mL/cm^2, which is significantly less than the 0.2 mL/cm^2 used during seed train. Cells harvested from the CellCube 100-layer modules exhibited similar viability (93% ± 2%) to the N-1 harvest from CellSTACK 10-chamber vessels (97% ± 1%) and presented a faster average doubling time in CellCube modules (27 ± 3 hours vs. 34 ± 3 hours). This translated to a greater overall cell yield (7 x 10^4 ± 1 x 10^4 cells/cm^2) in the CellCube modules than in CellSTACK 10-chamber vessels (5 x 10^4 ± 1 x 10^4 cells/cm^2; Figure 3). Considering DF-1 cells have a low proliferative capacity, their culture in a perfusion system can be sustained by lower medium volumes than can be afforded in static 2D culture format. The efficient delivery of gas and nutrients in circulation culture, as provided in the CellCube system, drives DF-1 medium requirement to lower volumes with the additional benefit of boosting cell yield.

Further optimization of medium formulation and medium conditioning parameters could improve DF-1 cell expansion even further. The current study used a classical serum-supplemented medium formulation with generic medium conditioning parameters (i.e., 50% DO and pH 7.35). However, Lin, et al. hypothesized that medium optimization could increase DF-1 cell growth to a rate suitable for industrial production and established an optimization strategy based upon metabolomics and Design of Experiment (DOE) methodology.[4] The same or similar approach discussed in that publication could be employed to tailor medium for large-scale DF-1 culture and subsequent virus propagation in the CellCube system. Optimizations can occur in the smaller sized vessels (i.e., CellCube 10-layer module; Corning 3200) and scaled up as needed. Likewise, medium pH and DO levels could be tailored specifically to DF-1 metabolic requirements, with dynamic DO setpoints that shift with cell expansion, vaccine production and/or even perfusion of fresh medium. Yet, in the absence of process optimization, the conditions studied here were sufficient to support high-density growth of DF-1 cells in the CellCube system.

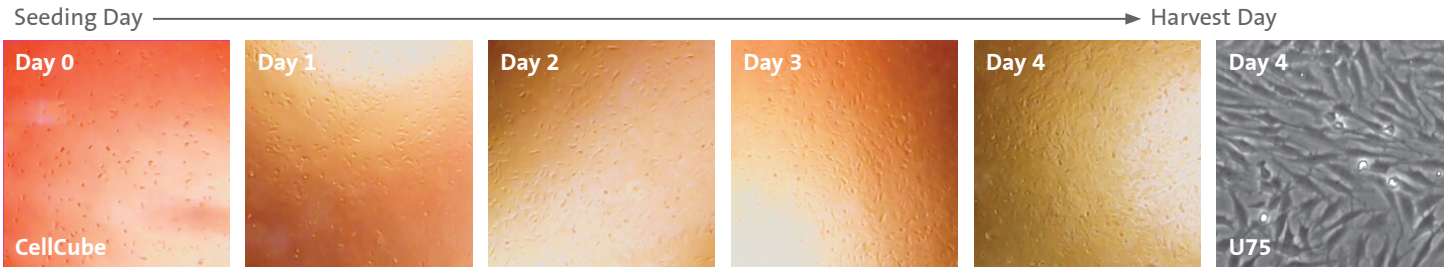

Figure 1. Confluent monolayer of DF-1 cells. Representative images of DF-1 cells during a 4-day expansion: Day 0 post-seeding through Day 4 pre-harvest on a Corning CellCube 100-layer module and Day 4 pre-harvest on a Corning 75 cm^2 U-shaped flask. CellCube module images were acquired with a handheld USB microscope. Flask image (75 cm^2 U-shaped) was acquired with digital inverted microscope.

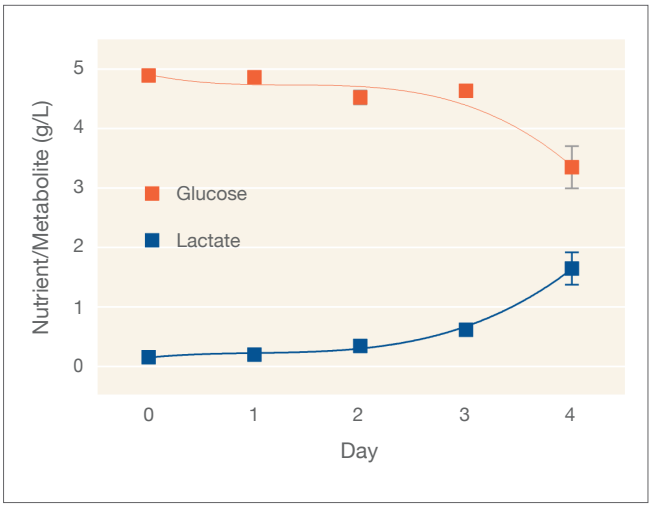

Figure 2. Glucose utilization tracks with initial cell expansion lag. Daily samples of system medium were drawn from the SUB during DF-1 cell expansion in the Corning CellCube 100-layer module. Glucose (orange) and lactate (blue) were monitored to track the progress of cell expansion. Third order polynomials (solid lines) were fit to data points (mean ± SD) to show the trends of glucose depletion and lactate accumulation. N = 3.

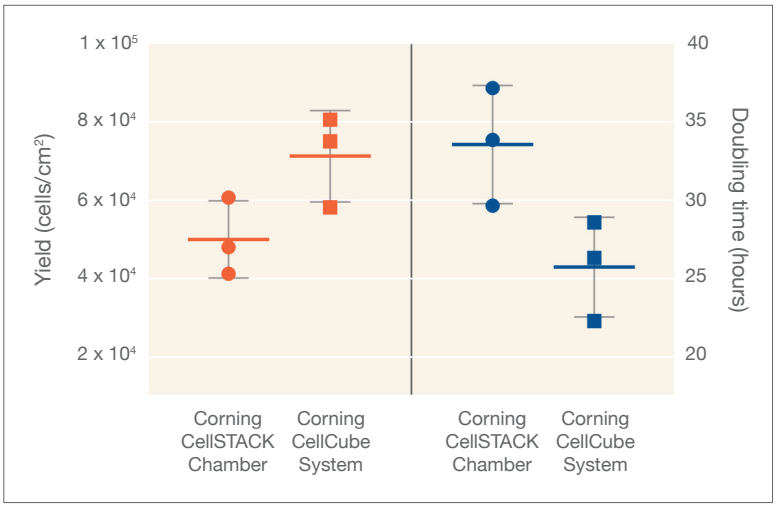

Figure 3. Improved harvest yield in the Corning CellCube System. Scatter plots of harvest yield (orange, left axis) and doubling time (blue, right axis) for N-1 Corning CellSTACK 10-chambers (circle) and CellCube 100-layer modules (square). Solid line marks mean values with whiskers representing SD. N = 3.

Conclusions

- Recirculation of system medium in the Corning® CellCube® system sustained DF-1 cultures at a low volume of medium per surface area.
- The Corning CellCube 100-layer module enables high-density growth of DF-1 cells.
- Medium and process optimization can be tailored for DF-1 cell expansion in the Corning CellCube System for avian vaccine production.

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