Maximizing Yield for Attachment-dependent Cells with the Corning® CellCube® System

Application Note

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Introduction

Scientific progress in the cell and gene therapy field will accelerate clinical cell therapy programs in the coming years[6,13]. As cell therapy trials advance from pre-clinical research to late-stage trials, cell culture platforms will need to meet increasing demands for cell yield[1,15]. To meet demand, traditional production methods that rely on planar cell culture vessels will require improvements in scale-out ouput[5,8], which can be quite tedious as a manual process[7-9]. The challenge, then, becomes establishing an adherent culture platform that can scale from relatively small needs of pre-clinical studies through Phase I/II trials and into Phase III. As a result, the ability to scale-out will ease the transition from process development to manufacturing[3,11].

The Corning CellCube system provides a simple, compact, and scalable method for mass culture of attachment-dependent cells (Figure 1). The CellCube module provides a large, stable surface area for the immobilization and growth of attachment-dependent cells. Each CellCube module consists of a series of parallel, polystyrene plates joined to create thin, sealed laminar flow spaces between adjacent plates. CellCube modules are available in three basic sizes (Table 1). The 10- and 25-layer modules are comprised of 10 and 25 culture plates, respectively, while the 100-layer module is made up of four 25-layer modules series. As gas-conditioned culture medium is circulated through the CellCube, design of the modules allows for reliable distribution of nutrients and oxygen with low differential gradients across all cells within the modules.

The Corning CellCube system, coupled with several different digital controllers for medium conditioning, has proven successful for expansion of multiple adherent cell lines, including BHK and CHO[4]. Furthermore, retroviral vectors have been produced by expansion of a variety of packaging cell lines, such as Phoenix Frape-1 and Frape-3[16] in the CellCube system, at levels comparable to other culture platforms[2,10,12,14,16]. In the current study, the CellCube system was used for expansion of two commonly used bioproduction cell lines – HEK293T and Vero. The basic closed system was configured with a peristaltic pump to drive continuous circulation through the CellCube 100-layer module (Figure 2; Corning 3264) and paired with the Eppendorf BioFlo® 120 (controller; Eppendorf B120ACS000) with a BioBLU® 3c single-use bioreactor (SUB; Eppendorf 1386000300) for medium conditioning. The controller simplified oxygenation and pH control of the circulating medium. Medium conditioning in the SUB was sufficient to sustain both HEK293T and Vero cell growth in the CellCube 100-layer module for 5- and 6-day expansions, respectively. Final harvest yields for both HEK293T and Vero cell lines greater than 10 billion cells demonstrate the utility of the CellCube system for mass culture of adherent cell lines with efficient medium usage.

Table 1. Corning CellCube System Scalability from Process Development to Production

Module	Surface Area (cm^2)	Module Volume (L)	Oxygenator and Circulation Loop (L)	Total Volume (L)
10-layer	8,500	0.6	2.0*	2.6
25-layer	21,250	1.5	2.0*	3.5
100-layer	85,000	6	2.0*	8.0

*Starting volume. Circulation volumes are up to discretion of user.

Figure 1. Corning CellCube 25- and 100-layer modules

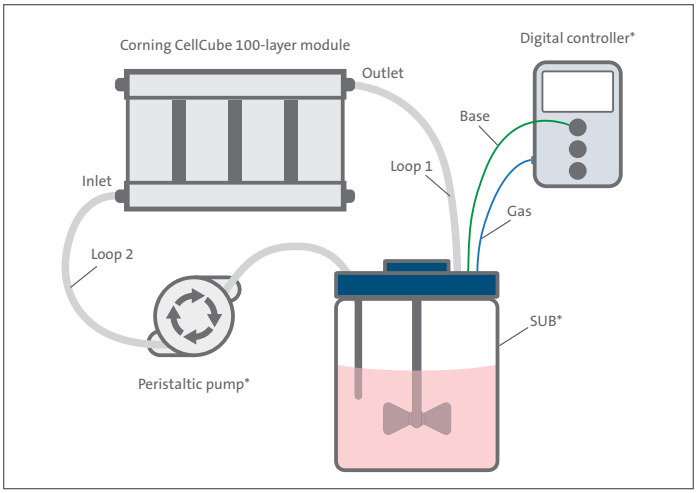


Figure 2. Schematic of the Corning CellCube closed system. The culture medium (approximately 2L) within the system is removed from the SUB by a peristaltic pump and is then pumped into and distributed throughout the Corning CellCube 100-layer module. Medium flows from the outlet of the CellCube module, back to the SUB for conditioning. The controller automatically controls pH via $NaHCO_3$ and CO_2. Dissolved oxygen (DO) in the medium is also maintained by the controller, which continuously refreshes the gas mixture supplied to the headspace of the SUB and sparged directly into the medium. Fluid flow and gas exchange within the SUB are carefully controlled to help eliminate turbulence, foaming, and to prevent protein degradation. Addition of antifoam in the culture medium aids in reducing medium foaming. *Peristaltic pump, controller, and SUB sold separately.

Materials and Methods

Cell Scale-up

HEK293T Cells (ATCC® CRL-3216™) were thawed in Dulbecco's Modified Eagle Medium (DMEM; Corning 10-013-CM) plus 10% fetal bovine serum (FBS; Corning 35-010-CV) and plated onto Corning® CellBIND® surface-treated U-shaped 75 cm^2 flasks (Corning 3290). Cells were subcultured for 2 passages on CellBIND surface-treated flasks before splitting onto Tissue Culture (TC)-treated culture flasks. Once plated on TC-treated flasks, cells were cultured an additional 2 to 3 passages before plating in Corning CellSTACK® 5-Stack culture chambers (Corning 3313 and 3319). The cells were cultured to 70% to 80% confluence with the final desired yield of 2.2 x 10^8 cells.

Vero Cells (ATCC® CCL-81™) were thawed in standard medium, plated onto TC-treated U-shaped 75 cm^2 flasks (Corning 430641U) and cultured for several days to a target harvest of 1 x 10^5 cells/cm^2 or approximately 7.5 x 10^6 cells total yield. Cells were scaled up an additional 2 to 3 passages before plating in two CellSTACK 10-Stack culture chambers (Corning 3270 and 3271). Each CellSTACK 10-Stack culture chamber was cultured to a final confluence of approximately 1 x 10^5 cells/cm^2 with the desired yield of 2.2 x 10^8 cells per vessel.

Table 2. Medium Conditioning Parameters

	HEK293T	Vero
Agitation	100 rpm	100 rpm
Temperature	37°C	37°C
pH level	7.40 ± 0.04	7.40 ± 0.05
pH control	$NaHCO_3$, CO_2	$NaHCO_3$, CO_2
DO level	30% ± 10%	60% ± 10%
DO control	Air, O_2, N_2 overlay and sparge	Air, O_2 overlay and sparge

Reagent Setup

For expansion in the Corning® CellCube® closed system, standard medium was supplemented with 1% penicillin-streptomycin (Corning 30-002-CI) and 0.1% EX-CELL® Antifoam (MilliporeSigma 59920C). On the day prior to seeding, a 10L collection bag (Corning 91-200-45) was filled with 8L of warm medium for system equilibration. On the day of harvest, 6L Trypsin EDTA (Corning 25-052-CV) was prepared with 0.1% Poloxamer 188 (Corning 13-901-CI), warmed to 37°C and transferred to a 10L collection bag. In addition, 315 mL of warm FBS was transferred to a clean 10L media bag as a quench for the harvest solution.

Equipment Setup

To adequately condition the medium during the expansion process, the CellCube system was paired with a bioreactor controller system. The Eppendorf BioFLO 120 monitored the medium through parameters measured inside the BioBLU 3c vessel. The entire closed system relied on 2 circulation loops. Loop 1 connected the CellCube return outlet to the BioBLU 3c vessel, while Loop 2 connected the BioBLU 3c to the CellCube inlet and threaded through an external peristaltic pump (Figure 2).

On the day prior to seeding, the closed system was assembled and prepared for equilibration. For base control, a 1L bottle filled with 7.5% $NaHCO_3$ solution (Corning 25-035-CI) fitted with a transfer cap connected to a length of pump-grade was connected to an accessory port of the SUB and routed through the controller pump. The CellCube module and SUB were filled with 8L complete medium via gravity-fill through a port in circulation Loop 2 connected to the prepared collection bag. Finally, the SUB and accompanying controller were readied for operation. Importantly, both pH and DO probes were calibrated according to the manufacturer's operation manual. Following calibration, the controller was set for the parameters listed in Table 3 and circulation was resumed. The entire system was allowed to equilibrate overnight.

Seeding and Expansion

After overnight equilibration, a medium sample was drawn from the SUB to check the pH. If the offline analysis indicated a difference in pH greater than the BioFlo controller deadband value the pH probe was recalibrated to the offline value. Following recalibration, the seeding process was initiated once vessel pH equilibrated.

HEK293T cells were harvested with 1X TrypLE™ Select Enzyme (Thermo Fisher 12563011) plus 0.1% Poloxamer 188 for 10 minutes at room temperature following 1X Dulbecco's Phosphate-Buffered Saline (DPBS; Corning 21-031-CM) wash. Vero cells were harvested from Corning® CellSTACK® 10-Stack vessel with Trypsin EDTA for 10 minutes at 37°C.

The closed system circulation was stopped, and then the medium was drained from the Corning® CellCube® module into the 10L collection bag. Following cell enumeration, the collection bag was inoculated with cell suspension to seed both sides of the CellCube 100-layer module (8.5 x 10^4 cm^2) at 5 x 10^3 cells/cm^2 via gravity-fill. The tubing was clamped at both the inlet and outlet, and the CellCube module was rotated vertically with the outlet facing up (Figure 3). Two more rotational seedings were performed to complete the seeding. After seeding, the vessel was returned to the resting position for 30 minutes before circulation was resumed to initiate cell expansion. During the expansion period, daily samples were drawn from the SUB for offline gas, electrolyte, and metabolite analysis with the Nova BioProfile® FLEX Analyzer.

Harvest

Control of the SUB was turned off and circulation through the system was stopped. The CellCube module inlet and outlet were disconnected from the SUB and connected to each other to create a loop through the CellCube module. Spent medium was emptied from the CellCube module into a collection bag. The CellCube module was filled with harvest solution via gravity fill. After 5 minutes, half the volume of the CellCube module was emptied in the quench solution, mixed and drained back into to the CellCube module. Circulation was resumed for an additional 5 minutes to ensure all cells were dissociated from the vessel. Finally, the circulation loops were reconfigured to recirculate the cell suspension through the pump to break up any remaining cell clumps. Samples were collected from the total harvest for enumeration.

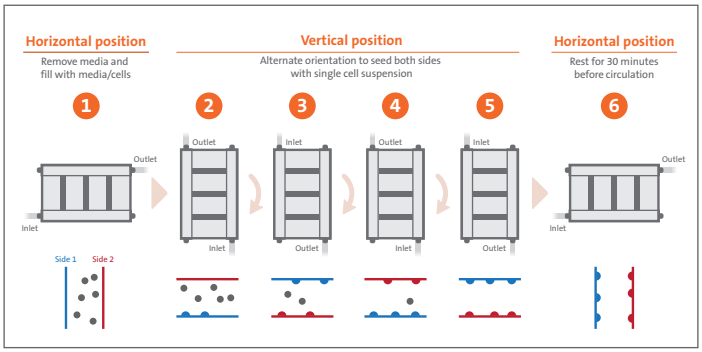


Figure 3. Corning CellCube module single seeding process. (1) Equilibrated medium is emptied from the Corning CellCube module, inoculated with cell suspension to seed both sides of the CellCube module (8.5 x 10^4 cm^2), and the CellCube module is refilled with cell suspension. Next (2-5), the tubing is clamped at both the inlet and outlet, and the Corning CellCube module is rotated vertically with the outlet facing up. Two more rotational seedings are performed to complete the seeding. (6) After seeding, the CellCube module is returned to the resting position for 30 minutes before resuming circulation.

Results and Discussion

The CellCube 100-layer module was used as the culture platform for expansion of HEK293T and Vero cell lines with medium conditioning in a SUB (Figure 2). Cells were seeded onto the CellCube module via a single seeding. For the single seeding, pre-equilibrated medium was removed from the CellCube module, inoculated with cells to seed the entire 8.5 x 10^4 cm^2 surface area and returned to the CellCube module. The vessel was rotated into a vertical position to seed the first side of the polystyrene culture plates (Figure 3). Historical data indicated 20- to 30-minute attachment time for HEK293T and Vero cell lines on TC-treated vessels so the initial seeding was chosen as 20 minutes for the first side of the vessel. Each subsequent rotation was incubated for 20 to 30 minutes for a total of two incubations per side. Some cell lines, such as mesenchymal stem cells (MSCs), may need an extended seeding period.

Both HEK293T and Vero cultures expanded in the Corning® CellCube® system with minimal intervention (Figure 4). The controller provided tight control of temperature, pH, and dissolved oxygen which adequately conditioned the circulating medium in the SUB. Daily offline medium analysis was used to monitor pH and dissolved gasses. As necessary, the system pH was recalibrated according to the offline values. In addition, glucose depletion and lactate accumulation were tracked via medium analysis to determine the day of harvest (Figure 5). For the HEK293T cultures, medium glucose levels began to decrease between Day 3 and Day 4 of culture as lactate levels began to rise (Figure 5A). Vero expansion progressed more slowly, with a steady glucose depletion coincident with lactate accumulation from Day 3 through 5 (Figure 5B).

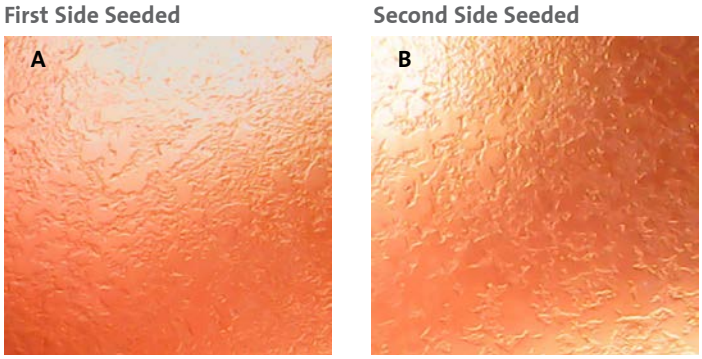
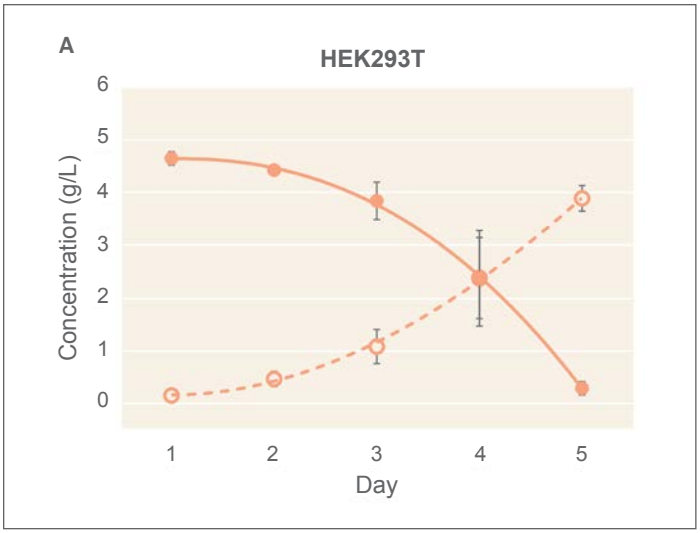


Figure 4. Confluent HEK293T monolayer culture. Representative images of confluent HEK293T cultures (approximately 5 x 10^4 to 6 x 10^4 cells/cm^2) from the first side seeded (back layer) of the Corning CellCube module (A) and the second side seeded (front layer) of the CellCube module (B). Images were acquired with a handheld USB microscope.



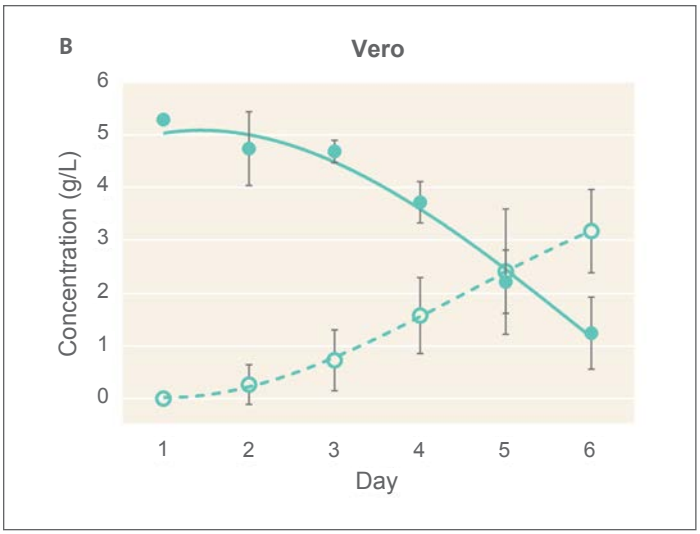
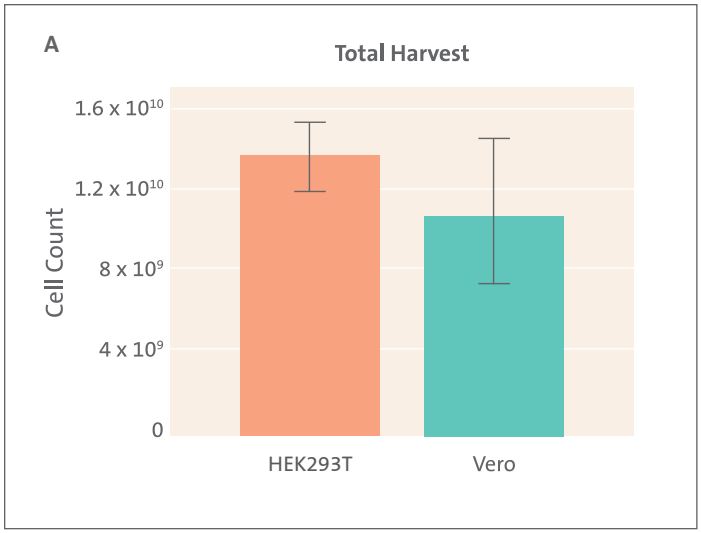


Figure 5. Glucose depletion and lactate accumulation determine time of harvest. Daily samples of medium were drawn from the SUB during HEK293T (A) and Vero (B) cell expansion. Glucose (closed circle) and lactate (open circle) were monitored to determine the time of harvest. Third degree polynomials were fit to data points (mean ± SD) to show trends in glucose depletion (solid line) and lactate accumulation (dotted line). N = 4 (A), N = 3 (B).

Metabolite concentration proved to be a predictable determinant of cell confluence, as final harvest yields were comparable between different expansion runs for the same cell type. Total HEK293T harvest averaged 1.4 x 10^{10} ± 1.4 x 10^{9} cells with >97% viability (Figure 6A), which corresponds to 1.6 x 10^{5} ± 1.9 x 10^{4} cells/cm^2 or 33 ± 4X expansion (Figure 6B). Total Vero harvest averaged 1.1 x 10^{10} ± 3.6 x 10^{9} cells with >97% viability (Figure 6A), which corresponds to 1.3 x 10^{5} ± 4.3 x 10^{4} cells/cm^2 or 26 ± 9X expansion (Figure 6B).



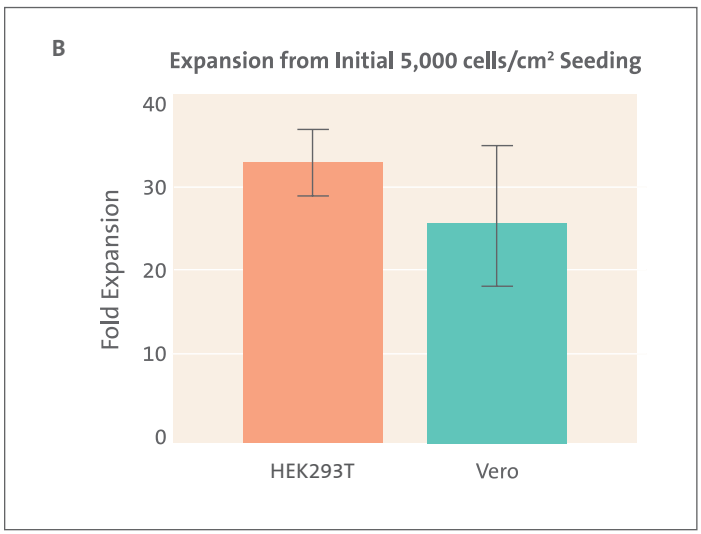


Figure 6. Large-scale harvest from cell expansion in the Corning CellCube 100-layer module. Total harvest yield (A) and fold expansion (B) of HEK293T (orange) and Vero (blue) cells after expansion from an initial seeding of 5 x 10^{3} cells/cm^2. Bars represent mean ± SD. N = 4 (A), N = 3 (B).

This yield from such a compact footprint is notable, given the number of standard planar culture vessels necessary for production level scale-out to the same surface area (Table 3). For example, the total surface area of four Corning® CellCube® 100-layer modules is equivalent to 20 Corning HYPER*Stack*® 36-layer vessels, up to 54 Corning CellSTACK® vessels, or 400 roller bottles. Using many stacked vessels would require significant incubator space or warm rooms and racks for roller bottle culture. Not to mention, the ergonomic challenge of handling so many culture vessels versus the compact CellCube system. The entire closed system with peristaltic pump, including the controller and SUB required minimal space, such as a 3-foot x 5-foot area in the warm room. Certainly, the total area would vary depending upon the size of the digital controller with SUB chosen for medium conditioning and the peristaltic pump.

Table 3. Corning Scale-up Platform Comparison

Platform	No. of Vessels	Total Surface Area (cm^2)	Media Volume (L)	Media-to-Surface Area Ratio (mL/cm^2)	Required Equipment
Corning CellCube 100-layer module	4	340,000	32*	0.09	Controller, oxygenator, warm room
Corning HYPER*Stack* 36-layer vessel	20	360,000	78	0.22	Incubators
Corning CellSTACK 40-chamber	14	356,160	38 to 45	0.11 to 0.13	Incubators or warm room, manipulator
Corning CellSTACK 10-chamber	54	343,440	38 to 45	0.11 to 0.13	Incubators or warm room
Roller bottle	400	340,000	51	0.15	Racks, warm room

*Starting volume. Circulation volumes are up to the discretion of user.

Furthermore, the circulation-based Corning CellCube system with medium conditioning uses media more efficiently (Table 3). Based on the 2L circulation volume utilized in this study, four CellCube 100-layer modules would require 32L of medium versus the 78L required for cell expansion in 20 HYPER*Stack* 36-layer vessels. CellSTACK vessels – cultured with 0.1 mL medium/cm^2 as were the CellCube system expansions presented here – require a similar absolute medium volume. Yet, continuous circulation in the CellCube system ensures adequate gas and nutrient delivery for high-density cultures. In the current study, <0.1 mL medium/cm^2 was sufficient to sustain both HEK293T and Vero cells for a typical culture period. However, the volume of the source medium could easily be adjusted to suit specific cell requirements. Similarly, the medium could be supplemented or exchanged in the SUB to prolong cell expansion. Our studies show that optimization of media formulations and culture parameters in the smaller CellCube 10-layer module can easily be scaled to the CellCube 100-layer module. Thus, the linear scalability of the CellCube system enables the transition from research through process development to manufacturing.

Conclusions

- Pairing the Corning CellCube 100-layer module with the BioFlo® 120 controller to condition the medium and regulate pH and dissolved oxygen resulted in optimal conditions for HEK293T and Vero cells during 5- or 6-day expansion, respectively.
- Recirculating 2L of medium in the BioBLU® 3c single-use bioreactor sustained both HEK293T and Vero cell growth for expansion without the need for perfusion exchange.
- Offline sampling to monitor glucose and lactate aided expansion and harvest timing.
- Incorporating Poloxamer 188 into the harvest solution enhanced cell recovery and reduced cell clumping.
- The Corning CellCube 100-layer module provides high density yields of HEK293T and Vero cells, for a total harvest greater than 10^{10} cells.

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