Evaluating the Corning® CellCube® System using a 3L Corning Disposable Spinner Flask in a Cell Culture Incubator

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Application Note

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Introduction

The Corning CellCube system is a robust, scalable platform for adherent cell culture and is well suited to diverse applications ranging from cell and gene therapy to viral vector and vaccine production.[1-3] At the core of the system is the Corning CellCube module, consisting of 10, 25, or 100 parallel, polystyrene plates joined to create thin, sealed laminar flow spaces between adjacent plates for a large growth surface area in a compact footprint. The basic CellCube system also incorporates a peristaltic pump, which drives continuous recirculation of fluids through the CellCube module for efficient gas and nutrient delivery, using a bioreactor vessel to house excess media and a bioreactor controller for medium conditioning.

Linear scalability of the CellCube system enables the transition from research through process development to manufacturing. However, prior to process development in even the smaller CellCube 10- and 25-layer modules, evaluating the performance of the perfused parallel-plate growth technology for specific cell line(s) or applications will likely be beneficial. This may be especially important if choosing the CellCube system as the cell culture platform for your application requires capital investment in the form of a bioreactor controller. As an alternative, this study describes a "plug-and-play" setup for cell expansion in Corning CellCube 25-layer modules in the absence of a bioreactor controller. Instead, the process described utilizes a reach-in cell culture incubator, standard equipment in all labs, Corning CellCube closed system accessories, and a Corning 3L disposable spinner flask (DSF) as a substitute for a bioreactor controller.

Materials and Methods

Cell Scale-up

HEK293T Cells (ATCC® CRL-3216™) were cultured in Dulbecco's Modified Eagle's 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 37°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) and scaled up to Corning CellSTACK® 2-chamber culture vessels (Corning 3269), before terminal seeding into Corning CellCube 25-layer modules (Corning 3232).

Corning CellCube Closed System Expansion

The modified CellCube system was assembled with CellCube accessories to connect the CellCube 25-layer module to a 3L DSF (Corning 3559), which functioned as the medium conditioning vessel (Figure 1), in lieu of a traditional bioreactor.[4] Briefly, the 3L DSF was outfitted for circulation by replacing the solid cap with a disposable aseptic transfer cap (Corning 3558). To enable sterile connection to the CellCube circulation loops (Corning 3234 and 3235), CellCube aseptic connector-to-MPC adapters (Corning 3237) were connected to each of the aseptic transfer caps of the DSF. A programmable magnetic stir plate was utilized to drive DSF impeller agitation at a constant 55 rpm and a peristaltic pump drove circulation through the system. The entire system was positioned on the shelf of a reach-in 37°C, 5% CO_2 humified incubator for passive oxygenation, pH buffering, and medium aeration.

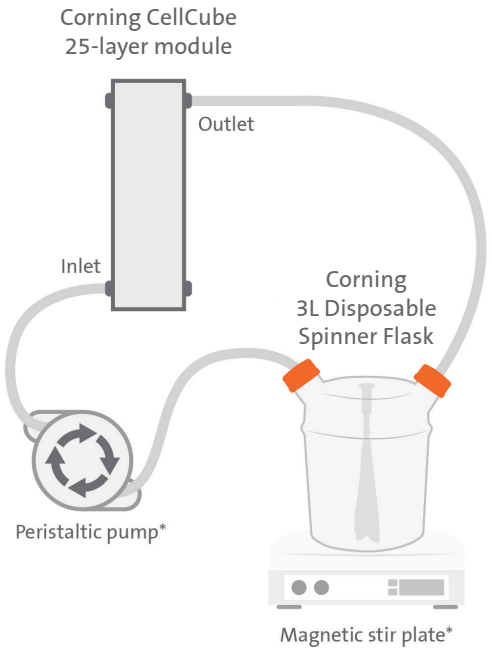

Figure 1. Schematic of the Corning CellCube closed system setup with DSF for use in the incubator. Culture medium within the system is pumped from the 3L DSF via a peristaltic pump through the inlet and distributed throughout the Corning CellCube 25-layer module. Medium then flows from the outlet of the CellCube module, back to the DSF for passive oxygenation, pH buffering, and medium aeration. Passive gas exchange through the 2 cap filters, silicone tubing and agitation of the DSF impeller—driven by a magnetic stir plate—aerates the medium. *Peristaltic pump and magnetic stir plate are sold separately.

On the day of seeding (Day 0), the closed system was assembled, positioned in the incubator, and the DSF was filled with 3.2L of warm complete medium plus 25 mM HEPES (Corning 25-060-CI). Circulation was initiated at 250 mL/minute, and the entire system was allowed to equilibrate for 3 to 4 hours. After system equilibration, cells were harvested from the CellSTACK 2-chamber vessels, and the CellCube 25-layer module was seeded at a density of 5 x 10^3 cells/cm^2 using the single seeding protocol integrating rotational seedings with alternating 20 minutes (front side) and 30 minutes (back side) intervals for a total seeding duration of approx. 2 hours.[4] In addition, a Tissue Culture (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 media samples were drawn from the DSF for offline gas, electrolyte, and metabolite analysis.

The CellCube 25-layer module was harvested on Day 4 according to established protocols.[1,4] Harvest was performed after a 20-minute incubation with 1.5L (to fill the module) of pre-warmed 0.05% Trypsin-EDTA (Corning 25-052-CV) plus 0.1% Poloxamer 188 (Corning 13-901-CI) in the vertical position. Instead 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.[5] Harvested cell suspension was quenched with an equal volume of spent medium, mixed well, and enumerated. The satellite vessel was also harvested for comparison. This study was repeated a total of 3 times.

Results and Discussion

The traditional Corning CellCube system combines the simplicity of planar polystyrene cell culture vessels, the advantages of a closed circulating system, and integrated process characterization via the bioreactor controller. Yet, there can be a learning curve to dynamic cell culture and bioreactor methodology when transitioning from a static adherent platform or roller bottles, not to mention the necessary process development. An additional consideration is the capital investment required for a bioreactor controller for automated medium conditioning. Accordingly, the "plug-and-play" setup for a cell culture incubator that is presented in this study provides a simple means to determine if the CellCube system is the right platform for a specific application. The "plug-and-play" setup was developed with HEK293T cells for which there are well-defined CellCube system protocols for seeding and harvest, as wells as historical data for expected medium consumption and cell yield.[1,4]

As expected, HEK293T cell confluence was comparable to the control U-shaped flask, covering approx. 50% to 70% of the growth surface area by the day of harvest (Figure 2). Glucose levels in the circulating medium showed the characteristic depletion concurrent with accumulation in lactate (Figure 3A). However, there was glucose reserve (approx. 2 g/L) in the medium and lactate production had not yet reached maximum levels (approx. 3 g/L) at the time of harvest, suggesting that the cells might have tolerated a lower medium volume-to-surface area ratio or culture duration could have been extended. The ratio chosen for this study (0.15 mL/cm^2) is at the higher end of the available range of system volumes that the 3L DSF can accommodate (Table 1).

Because the CellCube incubator setup described herein relies upon passive oxygenation, pH buffering and medium aeration, it is important that the reserve bioreactor and circulation loop volume fall within the working volume range of the 3L DSF (300 to 3000 mL) for adequate medium mixing. In fact, the 3L DSF can support medium volume-to-surface area ratio as low as 0.09 mL/cm^2 for the CellCube 25-layer module. The 3L DSF working volume range does span the typical medium volume-to-surface area ratios used in many cell culture applications and would work for both the CellCube 10- and 25-layer modules.

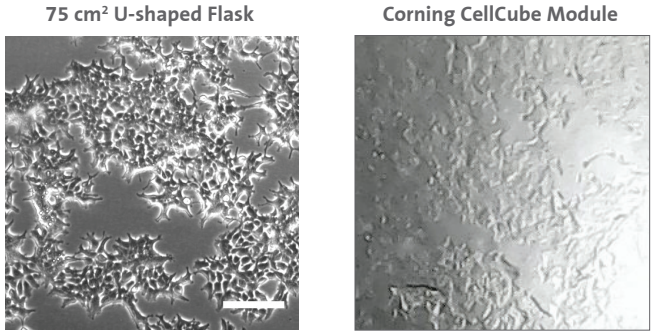

Figure 2. HEK293T cell monolayer confluence. Representative images of HEK293T cells in Corning 75 cm^2 U-shaped flask (left) and Corning CellCube 25-layer module (right) on the day of harvest. CellCube module images were acquired with a handheld USB microscope. Flask image was acquired with digital inverted microscope (scale bar = 200 µm).

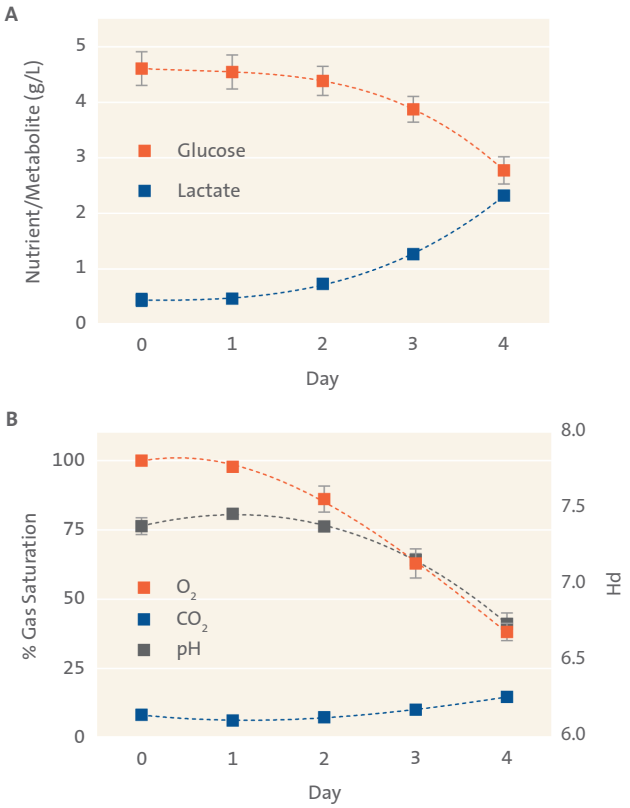

Figure 3. Medium analysis to assess Corning CellCube system health. Daily samples of system medium were drawn from the DSF during HEK293T cell expansion in the CellCube 25-layer module. (A) Glucose (orange) and lactate (blue) were monitored to track the progress of cell expansion. (B) %O_2 saturation (orange, left axis), %CO_2 saturation (blue, left axis), and pH (grey, right axis) were monitored to assess gassing efficiency of the circulating medium. Third order polynomials (solid lines) were fit to data points (mean ± SD) to show data trends. N = 3.

Table 1. Medium-to-surface area ratio.

	Corning CellCube 10-layer Module		Corning CellCube 25-layer Module	
Module area	8,500 cm^2		21,250 cm^2	
Module volume	600 mL		1,500 mL	
Medium-to-surface area ratio	Minimum	Maximum	Minimum	Maximum
	0.15 mL/cm^2	0.4 mL/cm^2	0.09 mL/cm^2	0.2 mL/cm^2
Bioreactor and circulation loop volume	675 mL	2,800 mL	413 mL	2750 mL
Total system volume*	1,275 mL	3,400 mL	1,913 mL	4,250 mL

*Includes associated tubing volumes.

Adjusting system medium volume provides a point for passive medium conditioning optimization without additional intervention, as does changing the impeller speed. However, the CellCube incubator setup with a 3L DSF is amenable to medium addition or exchange to support cell growth and extend the culture period as necessary. For this study, the setup was intended to run with minimal interventions, that is, no pH correction or medium exchange. Passive gas exchange was dependent upon the spinner flask vent filters, passive diffusion through the silicone tubing and aided by medium aeration by the spinner flask impeller. HEPES (25 mM) was added to the medium prior to system batching to help buffer the medium, which helped to keep pH stable at approx. 7.4 to 7.5 until Day 2 into Day 3. At that time, the natural acidification of the medium occurred with the increase in $\%CO_2$ saturation. Not surprisingly, these changes follow closely with glucose consumption, lactate production and decrease in $\%O_2$ saturation, which are all indicative of an acceleration of cell growth (Figures 3A and B).

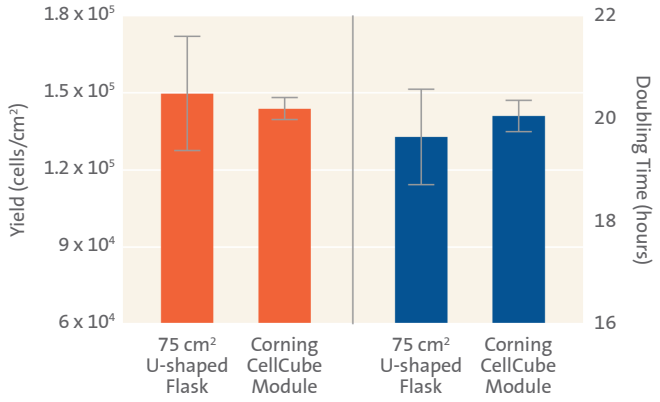

Figure 4. Passive medium conditioning in disposable spinner flask supports cell expansion in Corning CellCube modules. Bar graph comparing harvest yield (orange, left axis) and doubling time (blue, right axis) for the Corning 75 cm^2 U-shaped flask and CellCube 25-layer module for a 4-day expansion. Mean ± SD. N = 3.

By the time of harvest, $\%O_2$ saturation had fallen to 38 ± 3%, pH to 6.7 ± 0.1, and $\%CO_2$ saturation had risen to 15 ± 1%. Although the conditions may have been limiting if the culture period was extended past Day 4, cell viability was comparable to U-shaped flask control (96% ± 4% and 99 ± 1% for flask and CellCube 25-layer module, respectively). More importantly, cell expansion in the incubator CellCube module setup was consistent with both the control U-shaped flask (Figure 4) as well as reported HEK293T growth in CellCube 100-layer module using a bioreactor with automated medium conditioning.[1] Total harvest yields were $1.5 \times 10^5 \pm 2.2 \times 10^4$ cells/cm^2 and $1.4 \times 10^5 \pm 4.2 \times 10^3$ cells/cm^2 for flask and CellCube 25-layer module, respectively, while doubling times were 19.6 ± 0.9 hours and 20.1 ± 0.3 hours for flask and CellCube 25-layer module, respectively.[4]

In this study, cell growth characteristics in the CellCube incubator setup for the short duration expansion period were comparable to the traditional bioreactor-controlled CellCube system. Thus, for HEK293T and potentially other cells type, the CellCube incubator setup could serve as part of seed train for scale-up or a cost-effective method to test the CellCube system prior to purchasing a bioreactor controller. The baseline level of productivity delivered by the incubator setup described would be sufficient to evaluate how well a particular cell line will grow in CellCube modules and whether the CellCube system is suitable for the application.

Conclusions

- The Corning CellCube 25-layer modules with aseptic connectors easily connect to Corning 3L disposable spinner flasks via closed system accessories for a "plug-and-play" incubator setup to evaluate cell growth.
- Passive oxygenation, pH buffering, and medium aeration in the Corning 3L disposable spinner flask provides sufficient medium conditioning in the absence of a bioreactor and controller, to support cell expansion.
- The modified Corning CellCube system utilizing the Corning 3L disposable spinner flask, in lieu of a bioreactor and associated controller, supports HEK293T cell expansion in the Corning CellCube 25-layer module inside of a standard cell culture incubator.

References

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3. Expansion of DF-1 Chicken Fibroblast Cell Line in the Corning CellCube System Application Note (CLS-AN-734).
4. Corning CellCube Culture System Cell Expansion Protocol Guidelines for Use (CLS-AN-626DOC).
5. Bone Marrow-derived Mesenchymal Stem Cell Culture in the Corning CellCube System Guidelines for Use (CLS-AN-648DOC).

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