Harvest of Corning® HYPERStack® 36-layer Vessels Using the Corning Automated Manipulator System

CORNING

Application Note

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Introduction

Adherent cell culture remains a mainstay of many bioprocess applications, including viral vector and vaccine manufacturing, as well as stem cell culture for cell and gene therapy.[1] However, manual handling of adherent cell culture vessels in a large scaled-out process presents challenges during production, especially as the sizes of the vessels increase. Transitioning from process development to manufacturing scale demands consistency and standardization, and thus, benefits from some form of automation.[1,2]

Corning's Automated Manipulator Platform is a programmable manipulator designed to automate the scale up of adherent cell culture processes in Corning CellSTACK® and HYPERStack vessels. By automating the handling of multiple vessels during critical liquid handling steps, the manipulator platform simplifies fill, empty, and harvest steps for a more reproducible process. The use of automated handling is especially important for final harvest steps because they require the most manipulations, and are therefore, subject to the most variability. For example, a single HYPERStack 36-layer vessel requires a detailed process of setting the vessel at specific angles to empty spent medium, fill, and equilibrate with harvest solution, agitate the vessel to release cells from the surface, and finally to collect the harvested cells.[3,4] Accordingly, this study utilized HEK293T cells cultured in Corning HYPERStack 36-layer vessels for the comparison of the automated 3-dimensional (3D) harvest—with integrated left/forward and right/reverse rotations to agitate vessels at compound angles—against a traditional manual harvest method.

Materials and Methods

HEK293T Cells (ATCC® CRL-3216™) were cultured in complete medium—Dulbecco's Modified Eagle Medium (DMEM; Corning 10-013-CM) plus 5% fetal bovine serum (FBS; Corning 35-010-CV) for 3 to 4 passages before scaling into a Corning CellBIND® surface-treated CellSTACK 10-stack culture chamber (Corning 3320). The cells were cultured to 70% to 80% confluence with a final targeted yield of 5.4 x 10^8 cells.

On the day prior to seeding, a 50L collection bag (Corning 91-200-48) was filled with 36L of complete medium. The medium and 6 Corning CellBIND surface-treated HYPERStack 36-layer vessels* were incubated at 37°C to warm overnight. On the day of seeding, cells were harvested from the CellSTACK 10-stack chamber with TrypLE™ Select enzyme (1X) (Thermo Fisher 12563029) plus 0.1% Poloxamer 188 (Corning 13-901-CI) for 10 to 15 minutes at room temperature following a 1X Dulbecco's Phosphate-Buffered Saline (DPBS; Corning 21-031-CM) wash.[5] Following cell enumeration, the collection bag was inoculated with cell suspension to seed the 6 HYPERStack 36-layer vessels (with a surface area of 1.8 x 10^4 cm^2 each) at 5 x 10^3 cells/cm^2 or 9 x 10^7 cells/vessel. The bag was mixed well by gentle massage. The HYPERStack 36-layer vessels were placed into the Corning Automated Manipulator Rack (Corning 6655) for HYPERStack 36-layer vessel that was locked onto the Corning Automated Manipulator Cart (Corning 6652) (Figure 1). The filled rack was then loaded into the manipulator and shifted into the horizontal seeding position, filled by gravity fill, and positioned upright to depressurize after filling and equilibration.[3,4] Seeded vessels were incubated at 37°C/5% CO_2 in a humidified incubator until 80% to 100% confluence was obtained (Figure 2).

**After the start of this study, Corning introduced design enhancements for HYPERStack vessels (Corning 20036, 20037), which improved performance but did not affect its use with the Corning Manipulator Platform as described in this application note.*

Basic Harvest

On the day of harvest, roller bottles (Corning 431644) were fitted with disposable tubing sets (Corning 10043), filled with 600 mL of complete medium, and warmed to 37°C. Next, 3 of the 6 HYPERStack vessels were loaded into the top right, top left, and bottom middle positions of the manipulator rack (Figure 1B, Positions 1, 3, and 5). The remaining positions were loaded with empty HYPERStack vessels as placeholders. The rack was readied for manipulator operation. The manipulator harvest program was run to completion to shift vessels in position for the following liquid-handling steps. Media from the 3 vessels was emptied into a 50L collection bag. Once emptied of spent medium, each of the 3 vessels was filled with 600 mL of warm TrypLE Select enzyme (1X). TrypLE Select enzyme (1X) was evenly distributed through the HYPERStack vessel layers by a series of rotations and rocking left-to-right consistent with manual manipulations.[3,4] Following a 5-minute incubation, the vessels were rocked several times left-to-right at 60°/sec. Then, the TrypLE Select enzyme (1X) was redistributed to each layer, and the vessels were incubated an additional 5 minutes. To dissociate cells from the vessel surface, the vessels were agitated aggressively by a series of 3D shaking motions at a compound angle (left and forward to right and reverse) at 60°/sec. At the completion of the program, the harvested cells and protease were collected manually into the 3 individual roller bottles containing complete medium.

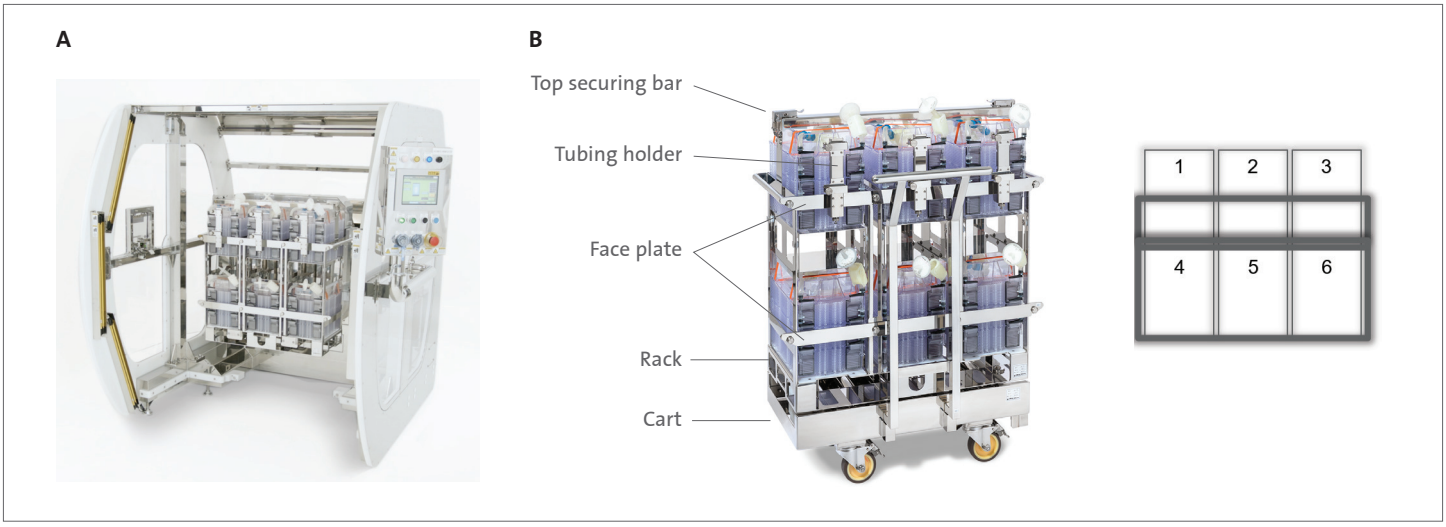

Figure 1. Corning Automated Manipulator rack for Corning HYPERStack 36-layer cell culture vessels. (A) Corning Automated Manipulator rack loaded with Corning HYPERStack 36-layer vessels raised to the home position in the Corning Automated Manipulator. (B) Loaded rack (Corning 6655) atop the cart (Corning 6652) with a schematic of vessel positioning inside of the rack (inset).

The 3 remaining vessels were processed manually one at a time according to established protocols[3,4] using 600 mL of warm TrypLE Select enzyme (1X) per vessel, which was diluted with 600 mL of warm complete medium after harvest. The study was repeated 3 times independently, with a different operator for each replicate.

Harvest Optimization

On the day of harvest, one of the HYPERStack vessels was loaded into the top right position of the manipulator rack (Figure 1B, Position 3). The remaining positions were loaded with empty HYPERStack vessels as placeholders. The rack was readied for manipulator operation. The manipulator harvest programs were run to completion to shift vessels in position for the following liquid-handling steps. Media from the HYPERStack vessel was emptied into a collection bag. Once emptied of spent medium, the HYPERStack vessel was filled with 600 mL of warm TrypLE Select enzyme (1X). At the completion of the program, the harvested cells and protease were diluted into a single roller bottle with 600 mL of complete medium. This was repeated for each of the remaining automated harvest protocols; each program differed in processing speeds ranging from 60°/sec. to 30°/sec. as outlined in the Basic Harvest section. The remaining vessel was processed manually according to established protocols[3,4] using 600 mL of warm TrypLE Select enzyme (1X), which was diluted with complete medium after harvest. All harvests were recirculated with a peristaltic pump at 750 mL/minute to dissociate large aggregates before samples were collected for enumeration. The study was repeated 4 times independently.

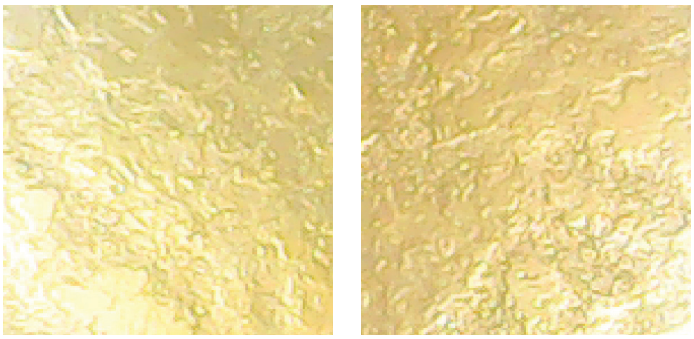

Figure 2. Confluent HEK293T monolayer culture. Representative images of confluent HEK293T monolayers on the day of harvest. The manipulator harvest yields from the corresponding Corning HYPERStack 36-layer vessels were 1.4 x 10^5 cells/cm^2 (left) and 1.6 x 10^5 cells/cm^2 (right). Images were acquired with a handheld USB microscope (Bysameyee Microscope 1000X).

Results and Discussion

The unique design of Corning HYPERStack vessels enables high density 2D cell culture but requires specific handling steps to realize high yields. The Corning Automated Manipulator makes simple work of this process. With precise movement control, the manipulator shifts 6 empty vessels into seeding position in one motion, allowing the vessels to be filled by a single large volume of cell seeding suspension. The operator needs only to open and close the vent filter clamps on each vessel before directing the fluid flow. After seeding, the vessels are rotated for medium equilibration and depressurization before continuing with incubation for cell expansion. Automation of the seeding process streamlines the labor of this manual process and removes much of the in-process human variation for both seeding and more importantly, for harvest.

The value of automation is realized most during cell harvest which presents a much greater ergonomic challenge and can pose a greater concern for operator's safety. During harvest, each HYPERStack vessel requires multiple empty and fill steps with agitation to distribute the harvest reagent and to dissociate cells from the vessel surface. This degree of agitation varies greatly and largely depends on how tightly adherent the cells are to the culture surface and is, thus, dependent upon the cell type and the application. The manipulator is programmable for a range of agitation, from a very slow rocking back-and-forth to an aggressive shaking motion in all 3 axes. In contrast, there are limits to the ability of an operator to safely manipulate the vessel manually to dissociate and harvest cells. Additionally, consistency during manual processing relies heavily upon adequate training of operators and adherence to a strict standard operating procedure (SOP). SOPs for manual processes are no guarantee that variations will not occur from day to day. Whereas the automated manipulator, once optimized for use with different cell types, will follow the exact set of movements set forth by the program selected thus increasing consistency of harvest regardless of the operator.

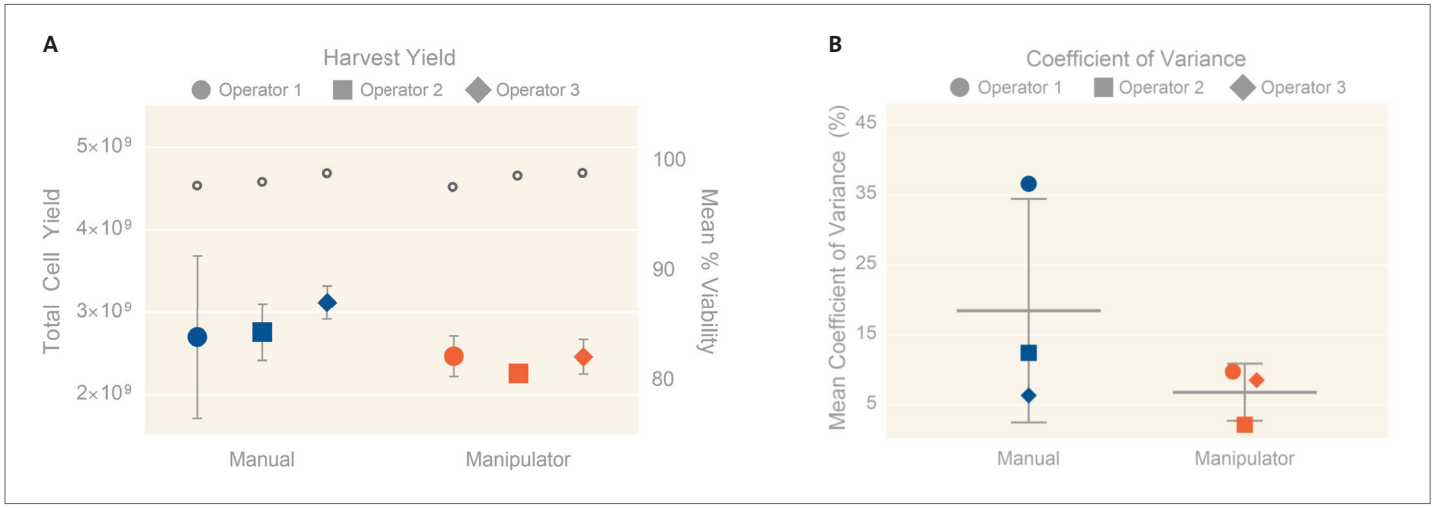

Figure 3. Automated harvests decrease the variability associated with manual Corning HYPERStack 36-layer vessel handling. (A) Total cell yield of manual (dark blue) and manipulator (orange) harvests by 3 different operators. Each data point represents the mean ± SD of 3 vessels. Mean cell viability (open circle) for each operator is plotted on the right axis. (B) Coefficient of variance calculated for each operator's 3 manual (dark blue) and 3 manipulator (orange) harvest yields. N = 3 vessels per operator per harvest mode.

Fatigue and training contribute to human error in manufacturing.[6] Therefore, harvests were performed with the automated manipulator and compared with harvests by 3 different operators in the current study to capture the inherent variability associated with manual processing of multiple HYPERStack vessels. As might be the case in a production setting, the 3 operators in this study had varying levels of training and experience with manual HYPERStack vessel harvests: basic, intermediate, and expert. Total harvest yields for both manual and manipulator averaged between 2 x 10^9 cells to 3 x 10^9 cells, with the manipulator harvests showing smaller deviation from the mean (Figure 3A). Analysis of the coefficient of variance confirms the result that the automated manipulator harvests achieved more consistent yields (Figure 3B). This level of variance is for a processing of 3 vessels on a laboratory scale. It would be reasonable to expect that the variance would be amplified with manual processing in a large-scale production run greater than 3 to 6 vessels.

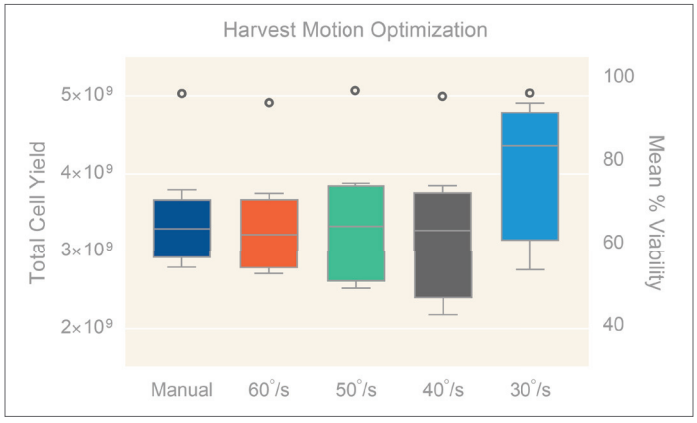

Figure 4. Optimization of manipulator speed of movement can improve harvest yields. Box plots comparing manual harvest (dark blue) with manipulator harvests at varying speeds of agitation: 60°/sec. (orange), 50°/sec. (green), 40°/sec. (gray), and 30°/sec. (light blue). Whiskers mark the minimum and maximum values, box boundaries mark the 25th and 75th percentiles, and the line marks the median. Mean cell viability (open circle) for each speed is plotted on the right axis. N = 4 vessels per condition.

Even with consistent automated manipulator harvests, it is possible to optimize parameters to boost harvest. The automated manipulator can precisely control movement of the HYPERStack vessels in increments of 0.5° with speeds as low as 1°/sec. For the second part of the study, the speed of motion was varied from aggressive (60°/sec.) to gentle (30°/sec.) in an effort to maximize harvests. Slowing the speed of 3D agitation to 30°/sec. during harvest produced the largest mean yield (Figure 4), albeit with the greatest range of harvest values among replicates. Importantly, cell viability was >95% regardless of processing speed. The range of harvest values showed an inverse relationship with harvest speed, increasing at slower agitation speeds. Conceivably, the different speed of agitation during automated manipulator harvests mirrors day-to-day and operator-to-operator variability and could explain some of the variation associated with manual processing. For applications requiring culture of HEK293T cells, like those utilized in this study, defining the optimal manipulator harvest parameters could require balancing yield vs. consistency. Regardless, the automated manipulator allows for tuning of parameters (i.e., angles, rotation, agitation speed, and timing) to suit a specific cell type and application to achieve optimal harvests. The ability to refine the HYPERStack vessel harvest process with that level of precision, in a reproducible manner, is not possible with manual manipulations.

Conclusions

- Programming enables precise control of angles, rotations, and speed to simplify manipulation of Corning® HYPERStack® 36-layer vessels for filling, depressurizing, emptying and harvesting.
- Automated manipulation reduces the labor and strain associated with manual handling of Corning HYPERStack 36-layer vessels for seeding and harvest processes.
- The Corning Automated Manipulator System reduces operator-to-operator and day-to-day variability of harvest for consistent cell yields.

References

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