Amplifying Adenoviral Particles in the Corning® HYPERStack® Cell Culture Vessel

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Application Note

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Introduction

Adenoviruses and other viral systems continue to be widely used in research and industrial applications. They are among the most commonly used vectors for the delivery of transgenes in both *in vitro* and *in vivo* studies.[1] Recently, adenovirus transductions have gained prominence at preclinical and clinical stages in gene therapy, vaccine development, and oncolytic virus therapy, driving increased demand for efficient virus production.[1,2] Additionally, adenoviruses are being explored for CRISPR/Cas9 delivery and in novel approaches to cancer immunotherapy,[1,2] further expanding their application in biomedical research and clinical treatments.

To allow researchers and vaccine manufacturers the ability to produce even higher yields in the same spatial footprint as stacked vessels, the Corning HYPERStack cell culture vessel features Corning's HYPER (**H**igh **Y**ield **PER**formance) technology which consists of a gas permeable film as the attachment surface, eliminating the air headspace requirement in traditional vessel types. This approach provides an increase in the number of layers and corresponding cell growth surface area compared to traditional rigid single or multi-layered culture vessels.

The focus of this study was to determine the efficacy of generating amplified virus using the unique Corning HYPER technology. Standard methodologies utilize traditional stacked vessels for virus generation. Utilizing the HYPERStack vessel, researchers can generate similar titers in a smaller spatial footprint saving both time and space. The results depicted here demonstrate that the experimental approach to generate adenovirus in the HYPERStack vessel led to similar titers but larger yields compared to a standard 2-layer stacked vessel.

Materials and Methods

Cell Culture

HEK-293AD cells (Cell BioLabs AD-100) were maintained in DMEM without sodium pyruvate (Corning 10-017-CM), 10% FBS, and 1X MEM nonessential amino acids (Corning 25-025-Cl).

Transduction of HEK-293AD Cells

Cells were seeded onto a Corning CellSTACK® 2-layer vessel with CellBIND® surface (Corning 3310) or Corning HYPERStack 12-layer vessel with Corning CellBIND surface (Corning 10012*) at 45,000 cells/cm^2 (0.217 mL/cm^2) and incubated overnight at 37°C, 5% CO_2, 98% relative humidity. The following day, the medium was removed and combined with adenovirus encoding Green Fluorescent Protein (GFP) (Multiplicity of Infection [MOI] 10). The medium was then added back to each vessel. The amount of virus (mL) added to each vessel was calculated using the following formula:

$$\frac{(\text{Cells/cm}^2)\ [\text{cm}^2 \text{ of Well}]) \ \text{x} \ (\text{MOI } 10 \ [\text{IFU/Cells}])}{(\text{IFU/mL})}$$

An MOI of 10 was selected to reach the desired cytotoxic effect (<50% cells remained) in 72 hours. The crude adenovirus added to each vessel was prepared as described in Generating Crude Adenoviral Particles in the Corning HYPERFlask Vessel.[3] GFP expression and cell morphology were monitored throughout the course of the experiment using the Olympus IMT-2 inverted fluorescence microscope.

Adenovirus Harvest

The cells and medium were collected 72 hours post-transduction (Figure 1). To collect the cells from the HYPERStack vessel, PBS (without Ca^{2+} and Mg^{2+}) (Corning 21-040-CM) was added to each vessel (0.033 mL/cm^2) and incubated at 37°C for 3 to 5 minutes. To collect cells from the stacked vessel, 2 to 3 PBS washes were necessary to remove all cells. To minimize volume during freeze-thaw cycles, the cells were pelleted in a centrifuge at 500 x g for 10 minutes at 4°C, and the cell pellet was resuspended in 10 mM Tris, pH 8.0, 100 mM NaCl (0.023 mL/cm^2). The medium was retained, aliquoted into 50 mL centrifuge tubes (Corning 430921), and stored

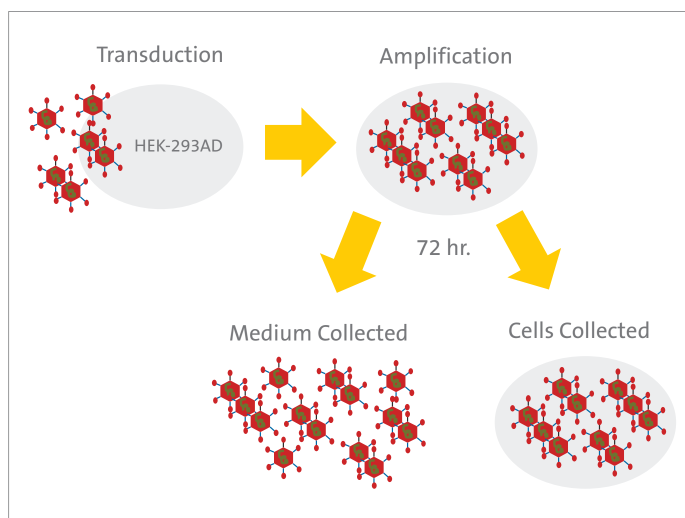

Figure 1. Schematic of adenovirus production.

*After this study was completed, Corning introduced design enhancements for HYPERStack vessels (Corning 20036, 20037; previously Corning 10036, 10012), which improved performance but did not affect its use for viral vector production as described in this application note.

at -80°C to be titered later. The total volume of the medium was also recorded. The cell suspension was then subjected to three freeze-thaw cycles (-80°C/37°C), then centrifuged at 3,000 x g for 10 minutes at 4°C to pellet the cell debris and the supernatant containing the adenoviruses released from the cell suspension was collected. The recovered adenovirus encoding GFP was also aliquoted and stored at -80°C.

Adenovirus Titer

The QuickTiter™ Adenovirus titer ELISA kit was purchased from Cell BioLabs (VPK-110). The ELISA assay was performed as described previously[3] and the signal in the wells was measured utilizing a PerkinElmer EnVision® Multilabel Reader.

Functional Analysis of Adenovirus

MDBK and Vero cells were transduced with adenovirus obtained from both vessels and analyzed via flow cytometry as described previously.[3]

Results and Discussion

Cell Morphology and GFP Expression

To assess adenoviral production on a 2-layer stacked vessel compared to a HYPERStack vessel, HEK-293AD cells were transduced with adenovirus encoding GFP. GFP expression and cell morphology were monitored throughout the course of the experiment. The cells and medium were collected when less than 50% of the cell population remained attached to each vessel. Similar cell morphology and GFP expression were observed in both vessels (Figure 2) at the time of harvest.

Adenoviral Production

The viral particles obtained from either the cells or medium remained in two different fractions throughout the course of the study to (i) minimize processing and (ii) demonstrate the viral

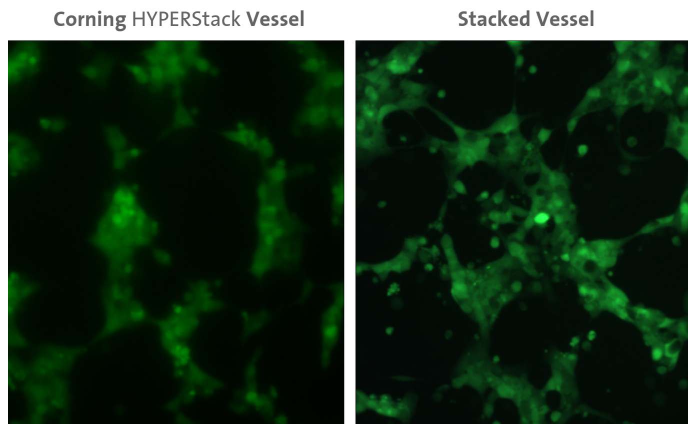

Figure 2. Similar cell morphology and GFP expression was observed between vessels. Representative images from the same experiment demonstrating morphology/GFP expression on the day of harvest of the HEK-293AD cells. Cells and medium were collected 72 hours post-transduction. Images obtained using an Olympus IMT-2 inverted fluorescence microscope. Magnification 100X.

yields obtained from both fractions. For large-scale production, the cells may be lysed by either lowering the ionic strength (hypotonic shock) or with the aid of mild pressure changes that can be induced by a Microfluidizer® (Microfluidics) or cross-flow filtration system.[4] Once collected, the titer of adenovirus encoding GFP from each vessel (either from cells or medium) was determined using the QuickTiter ELISA kit to quantitate infectious forming units (IFU)/mL. Similar titers were obtained from both vessels (Figure 3). The average titer obtained from the adenoviruses recovered from the cells were 2.4×10^9 IFU/mL and 2.2×10^9 IFU/mL from HYPERStack and stacked vessels, respectively (Figure 3A). The average titer obtained from the medium was 6.3×10^8 IFU/mL and 5.3×10^8 IFU/mL from HYPERStack and

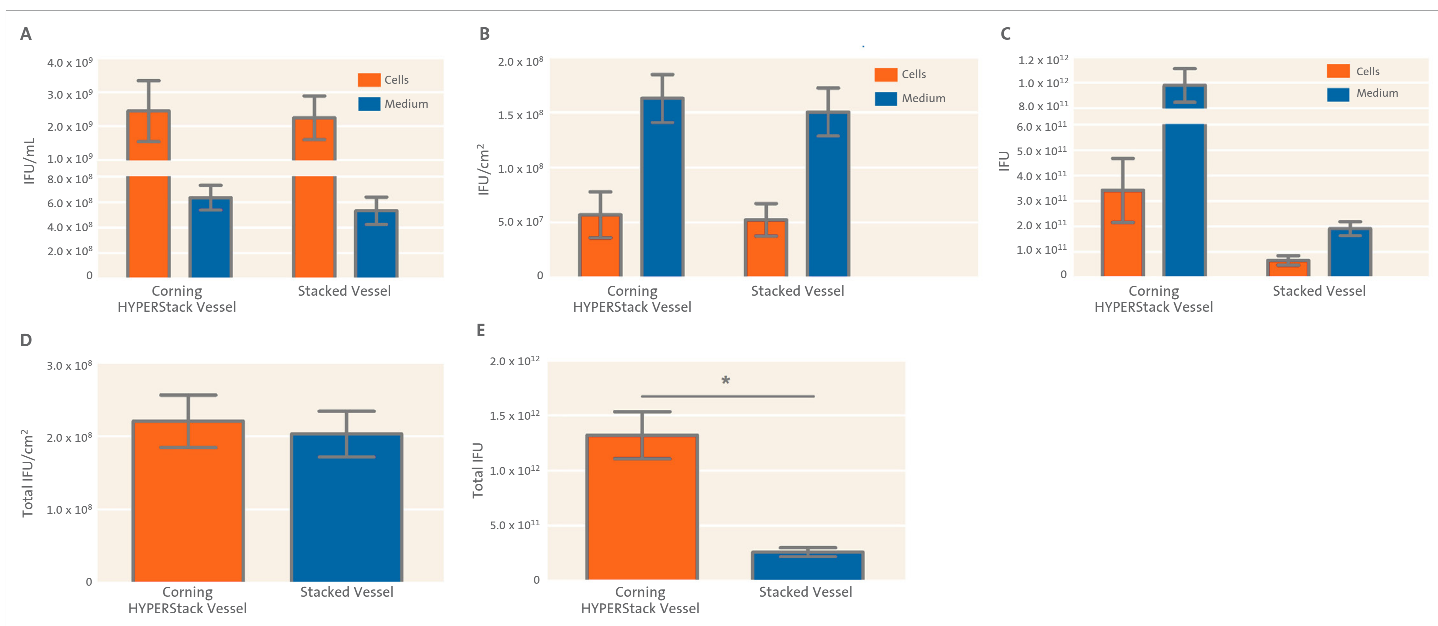

Figure 3. The Corning HYPERStack **vessel leads to equivalent viral production per cm² compared to a stacked vessel.** (A) Direct comparison between the HYPERStack vessel and stacked vessel titers obtained using the QuickTiter ELISA Adeno virus kit. (B, D) When normalized on a per cm² basis, the HYPERStack yielded similar infectious adenoviral particles. (C, E) The HYPERStack vessel generated a significantly higher amount of total infectious adenoviral particles. (D, E) Total infectious adenoviral particles were calculated based on titers and the volume of each fraction (cells and medium) Paired t-test, * $p < 0.05$, N=3.

stacked vessels, respectively (Figure 3A). When normalized based on surface area, similar IFU/cm^2 were observed with both vessels (Figures 3B and 3D). However, since the HYPERStack-12 vessel has a larger surface area compared to a 2-layer stacked vessel there was a significant increase in total viral yield (>5 times) (Figures 3C and 3E). These results indicate that adenovirus particles may be generated in the HYPERStack vessel with similar titers but larger yields compared to the standard 2-layer stacked vessel.

GFP Expression in Vero and MDBK Cells

To verify that the virus obtained from the HYPERStack vessel was as functional as virus obtained from the 2-layer stacked vessel, Vero and MDBK cells were transduced with amplified adenovirus encoding GFP. Each cell type was transduced with virus obtained from either the HYPERStack or stacked vessel at MOI of 100. After 72 hours, the cells were collected and analyzed via flow cytometry. After three independent experiments, the average GFP fluorescence in each cell line with each adenovirus was greater than 95% (Figure 4). Cells were transduced at MOI of 100 to ensure high expression. Previous results also demonstrated equal GFP expression regardless of the vessel when transduced at lower MOIs (10, 50, and 100) for shorter time periods (24 and 48 hours). GFP expression from each experiment varied between 30% to 95% depending on MOI and time (data not shown). These data indicate that the virus obtained from the HYPERStack 12-layer vessel is as infectious as virus obtained from a 2-layer stacked vessel.

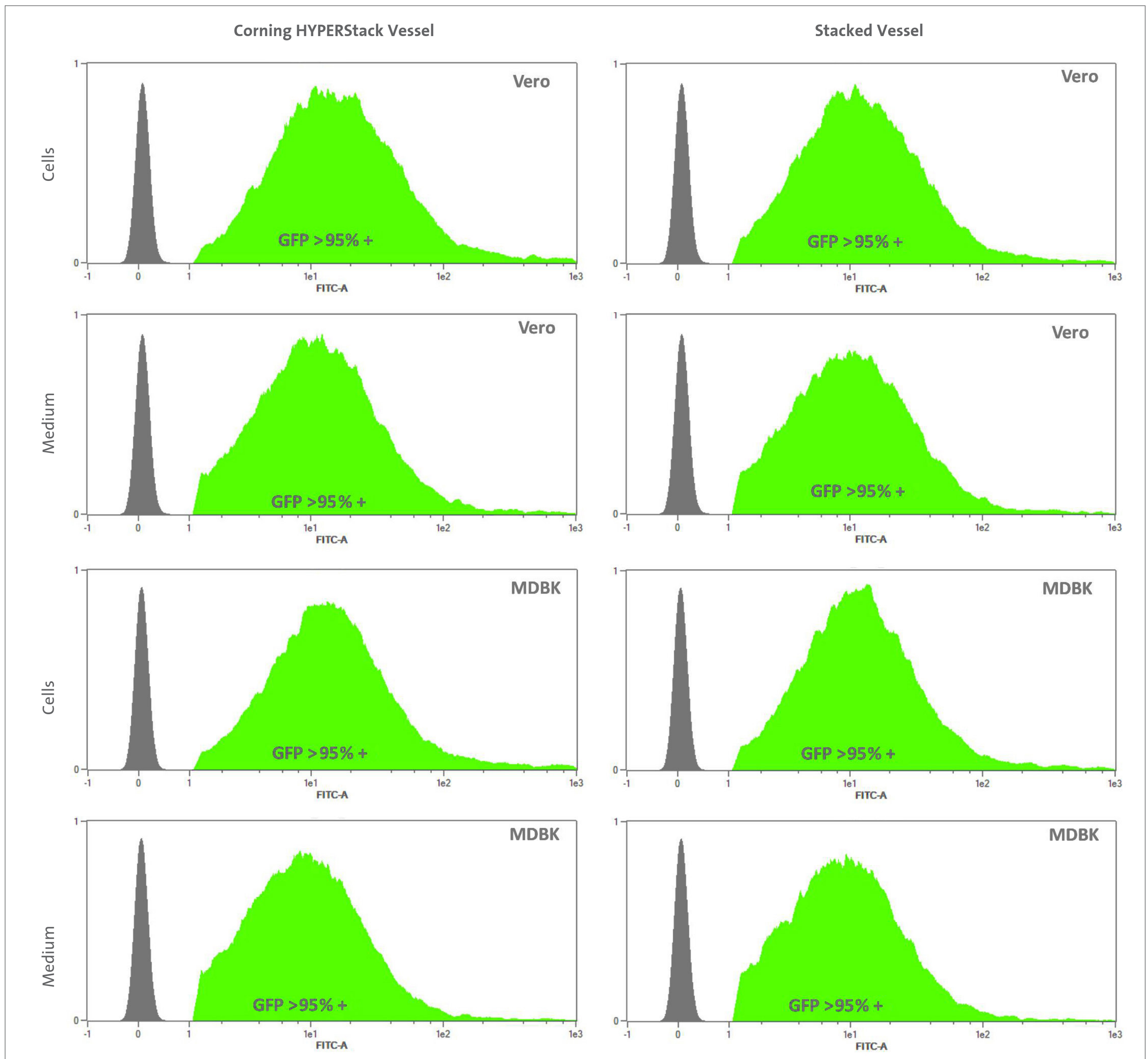

Figure 4. Vero and MDBK cells transduced with adenovirus exhibit similar GFP expression levels. Representative flow cytometry data shows the expression of GFP (green) compared to a negative control of non-transduced cells (black). After three independent experiments, the GFP expression in both the Vero or MDBK cells was greater than 95% regardless of which vessel or fraction the virus was generated in.

Conclusions

- This study demonstrates the utility of the HYPER technology in adenovirus production.
- Adenoviral particles can be amplified in the Corning HYPERStack vessel at similar titers compared to traditional tissue culture vessels, allowing for greater virus production in a smaller footprint.
- Adenoviral particles generated on the HYPER technology platforms also exhibit similar levels of infectivity as in a traditional vessel.

References

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3. Generating Crude Adenoviral Particles in the Corning HYPERFlask Cell Culture Vessel. Corning Application Note (CLS-AN-213).
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