Using the Corning® *HYPERStack*® Cell Culture Vessel and the Enhanced Attachment Microcarriers for Scale-Up and Production in the Vaccine Industry

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Introduction

Viral transductions are used at the preclinical and clinical stages in the vaccine industry leading to an increase in demand to produce more virus more efficiently. The Corning® HYPER technology and microcarrier beads offer the ability to increase efficiency by increasing surface area without increasing spatial footprint. The focus of this study was (i) to determine the efficacy of generating Adeno- and lentiviral particles using the unique Corning HYPER technology, and (ii) to evaluate the use of the Enhanced Attachment microcarrier beads on cell lines typically used in the vaccine-producing industry (e.g. Vero, and HEK-293).

To assess viral production on the HYPER technology HEK-293AD (adenovirus) or HEK-293LTV (lentivirus) were transduced (adeno) or transfected (lenti) on the HYPER*Stack*-12 to produce the viral particles. The results demonstrated that adeno- and lenti-viral particles can be generated in the Corning HYPER*Stack*® vessel at similar titers compared to traditional tissue culture vessels, while allowing for greater virus production in a smaller footprint.

To optimize cell scale-up on the Corning microcarrier beads various conditions were evaluated using multiple vaccine producing cell types. The data presented here demonstrate Vero and HEK-293AD cell expansion on Corning Enhanced Attachment microcarriers.

Methods and Results

Using the Corning HYPER*STACK* for lentiviral production

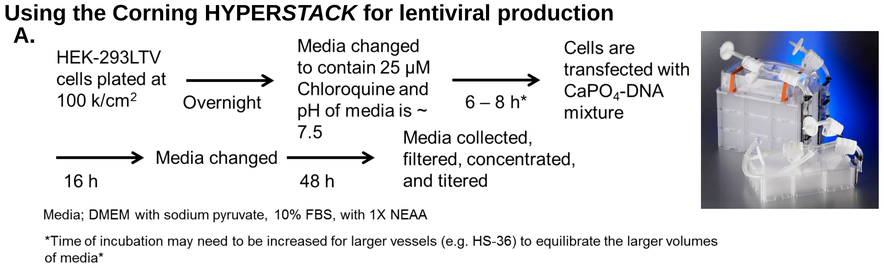

Figure 1. The Corning HYPER*Stack* -12 vessel supports comparable viral production, with a higher yield of total virus compared to a 2 layer stacked cell culture vessel. (A) Experimental outline. Media components purchased from Corning cellgro® and chloroquine from Sigma-Aldrich™. (B) Representative images demonstrating morphology/GFP expression on the day of harvest of the HEK-293LTV cells. Medium was collected 48 h post transfection. Images obtained using an Olympus® IMT-2 inverted fluorescence microscope. Magnification, 10X. (C - D) The Lenti-X™ qRT-PCR Titration Kit was purchased from Clontech (Cat. No. 631235), and the assay was performed according to manufacturer's instructions using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). The copies/mL were calculated based on the Cq values determined by the software. Similar titers were obtained between vessels (C) and when normalized on a per cm^2 basis the HYPER*Stack* vessel yields similar amount of lentiviral particles (D).

Using the Corning HYPER*STACK* for adenoviral production

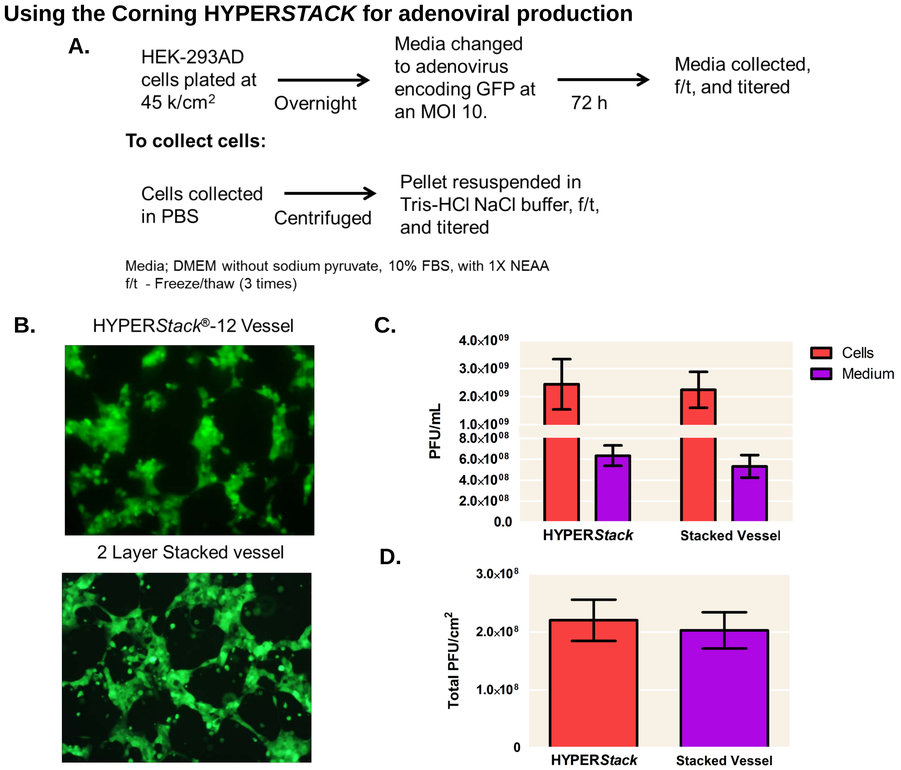

Figure 2. The Corning HYPER*Stack* -12 vessel supports comparable viral production, with a higher yield of total virus compared to a 2 layer stacked cell culture vessel. (A) Experimental outline. Media components purchased from Corning cellgro®. (B) Representative images demonstrating similar morphology/GFP expression on the day of harvest of the HEK-293AD cells. Medium was collected 72 h post transfection. Images obtained using an Olympus® IMT-2 inverted fluorescence microscope. Magnification, 10X. (C) Direct comparison between the HYPER*Stack* vessel and Stacked Vessel titers obtained using the QuickTiter Elisa Adeno kit (Cell Biolabs). (D) When normalized on a per cm^2 basis the HYPER*Stack* yielded similar infectious adenoviral particles.

Expansion of Vero cells with Corning microcarrier beads

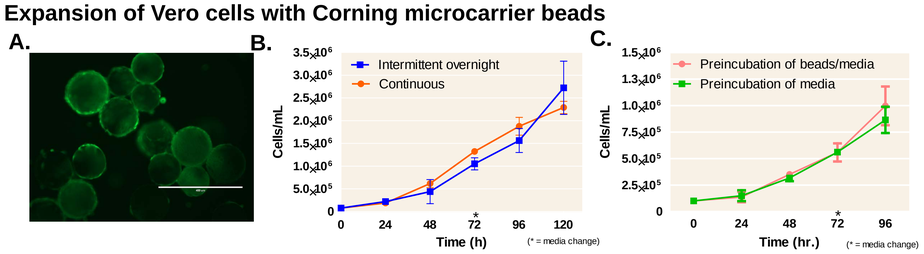

Figure 3. Vero cell expansion on Corning Enhanced Attachment microcarriers. (A) Vero cells on microcarriers and stained with Calcein AM. Image was captured using the AMG EVOS® Fl microscope. Scale bar represents 400 µm. (B – C) Vero cells were expanded in a 125 mL disposable spinner flask (DSF), 0.1 mLs/cm^2, 10,000 cells/cm^2 and agitated at 30 rpm. Cells were cultured in DMEM with 5% FBS, 1X NEAA (Corning cellgro). The data suggest similar cell growth in a 125 mL DSF. Current studies are ongoing evaluating cell growth in a 1 L DSF.

Expansion of 293-AD cells with Corning microcarrier beads

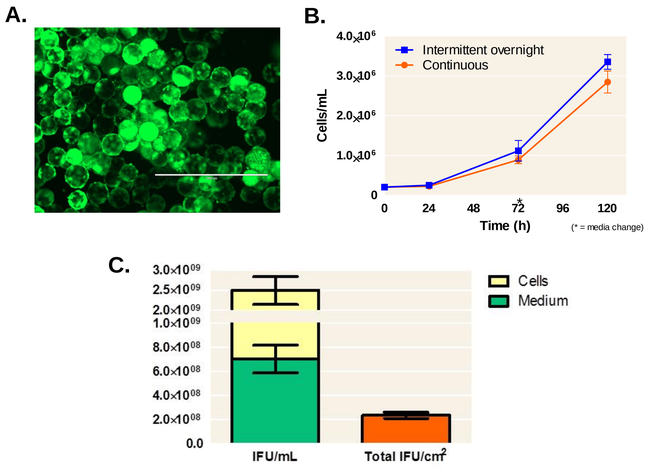

Figure 4. Preliminary results demonstrate HEK-293AD cell expansion and viral production on Corning Enhanced Attachment microcarrier beads. (A) HEK-293AD cells on microcarriers stained with Calcein AM. Image was captured using the AMG EVOS® Fl microscope. Scale bar represents 1000 µm. (B) Cells were expanded in a 125 mL DSF, 0.1 mLs/cm^2, 20,000 cells/cm^2 and agitated at 30 rpm. Cells were cultured in DMEM with 5% FBS, 1X NEAA (Corning cellgro) (N=2). (C) Cells were cultured in a 125 mL DSF, 0.2 mLs/cm^2, 50,000 cells/cm^2 with intermittent agitation overnight at 30 rpm. The following day cells were transduced at a MOI = 15 and changed to continuous agitation. Cells cultured in DMEM with 10% FBS, 1X NEAA (N=2, in duplicate).

Summary

- Lentiviral and adenoviral particles can be generated in the Corning HYPER*Stack* vessels at similar titers compared to normal tissue culture vessels, allowing for greater virus production in a smaller footprint. Additionally, the viral particles generated on the HYPER technology platforms also exhibit similar levels of infectivity as in a traditional vessel (data not shown).
- Corning microcarrier beads can be used to expand Vero and HEK-293AD cells. Additionally, preliminary studies demonstrate the use of these beads for adenovirus production.

Future Work

- Evaluate cell growth and viral production in 1L DSF and larger.
- Compare cell growth on Corning microcarriers to other commercially available microcarriers.