

Simplified Large Scale Production of Influenza A Virus Using the Corning® HYPERFlask® Cell Culture Vessel

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Customer Application Note

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Abstract

MDCK cells are commonly used in laboratories for the production of influenza A virus. In this study, we validated the usage of the Corning HYPERFlask cell culture vessel for the large-scale production of influenza A virus. The HYPERFlask vessel is a multilayered cell culture flask with approximately 10 times the cell growth area of a standard T-175 flask. Although the concentration or viral titer was equivalent in both the HYPERFlask vessel and a standard T-175 flask, the HYPERFlask allows increased efficiency in scale-up by using a single vessel compared to multiple flasks. Production of influenza A virus in the HYPERFlask vessel also allowed for an adsorption-free step protocol. Together, these results indicate that the HYPERFlask vessel offers considerable benefits for large-scale production of influenza A virus.

Introduction

Large-scale production of influenza A virus using cultured cells is routinely carried out in many laboratory settings. To increase virus production, numerous tissue culture treated vessels can be used at one time to achieve the desired quantity. Virus production in cultured cells follows a protocol with a series of steps that prepares the cells prior to

adsorption of the inoculated virus. These steps not only use many flasks, but also take a lot of time and effort. The HYPERFlask vessel is a revolutionary ten-tiered vessel that provides the growth surface of ten T-175 flasks with the same footprint. Therefore, it is presumed that the use of the HYPERFlask vessel would increase the efficiency of large-scale production of viruses. In this study, we compared the production of influenza A virus using a conventional T-175 flask and the HYPERFlask vessel.

Materials and Methods

Cells

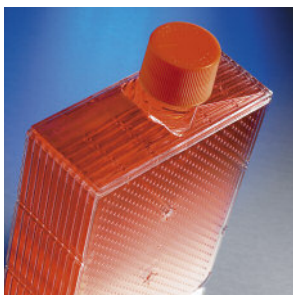
Madin-Darby Canine Kidney (MDCK) cells were grown in minimal essential medium (MEM) containing 5% newborn calf serum, penicillin and streptomycin. All cells from one T-175 flask were seeded into one HYPERFlask vessel and incubated at 37°C, 5% CO₂ for 3 days.

Viruses

Three different strains of influenza A virus were used in this study; the common laboratory strain, A/WSN/33 (WSN), H1N1, and H3N2. The H1N1 and H3N2 strains were previously isolated from humans in 2003 and 2002, respectively, and used as clinical strains.

Virus production

MDCK cells grown in the T-175 flask or HYPERFlask vessel were rinsed three times with MEM solution, and then infected with one of above viruses at a multiplicity of infection (MOI) of 0.01. Infected cells were cultured in MEM containing 0.3% bovine serum albumin (BSA) and 50 µg/mL of TPCK-Trypsin for 48 hours at 37°C. Culture volumes for the T-175 flask and HYPERFlask vessel were 50 mL and 560 mL, respectively. The amount of viral production was measured using plaque assays on MDCK cells. Each experiment was carried out in three independent replicates.



Results and Discussion

Virus Growth in the HYPERFlask® Vessel

A cell culture flask with multiple layers has an advantage for large-scale virus production. With its revolutionary and compact design, the HYPERFlask vessel was tested for the production of influenza A virus. MDCK cells were grown in either a T-175 flask or the HYPERFlask vessel and infected with influenza A virus. The amount of virus produced after a 48-hour incubation period in each vessel was compared. The amount of virus produced per mL in the HYPERFlask vessel was equivalent to that in the T-175 flask (Fig. 1). MDCK cells cultured in the HYPERFlask vessel expressed cytopathic effects (CPE) when observed with the inverted microscope (Fig. 2). This observation was consistent with all three viruses used in this study. These results indicated that the HYPERFlask vessel supported propagation of influenza A virus equivalent to the conventional T-175 flask.

In a standard protocol for the propagation of influenza A virus, cultured cells are incubated with a small amount of viral inoculum for one hour to allow adsorption to take place. The adsorption step is typically considered a necessary step to increase the infection efficiency of the virus. We evaluated the adsorption step of MDCK cells grown in the HYPERFlask vessel, as well as a simplified protocol that bypassed this step. For the standard protocol, 50 mL of viral inoculum was incubated in cells grown in the HYPERFlask vessel for 1 hour as the adsorption step, and replaced with 560 mL medium afterwards. To simplify the protocol, we bypassed the adsorption step by incubating MDCK cells

grown in the HYPERFlask vessel with 560 mL of medium containing the same viral inoculum. The appearance of CPE was similar in MDCK cells following the traditional protocol and the simplified bypass protocol (data not shown). The virus production was also similar between the two protocols (Fig. 3). These results indicated that bypassing the adsorption step did not affect virus production in the HYPERFlask vessel and thus allowed for a simplified procedure for virus production.

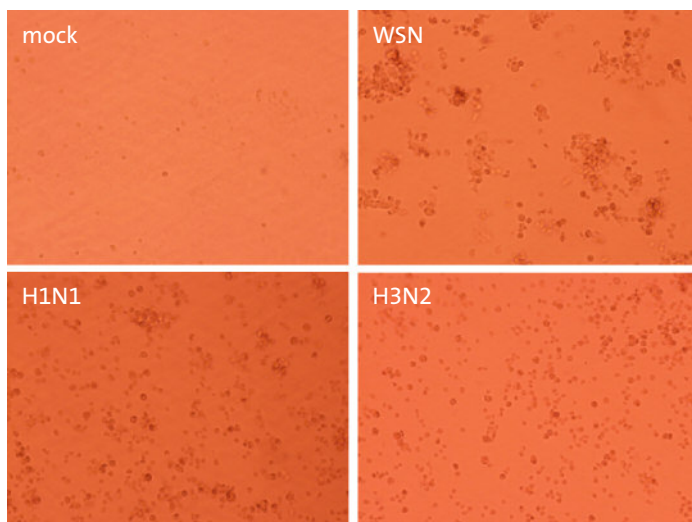


Figure 2. CPE on MDCK cells in the HYPERFlask vessel were visualized using an inverted microscope after a 48-hour incubation. Objective lens magnification, 10X.

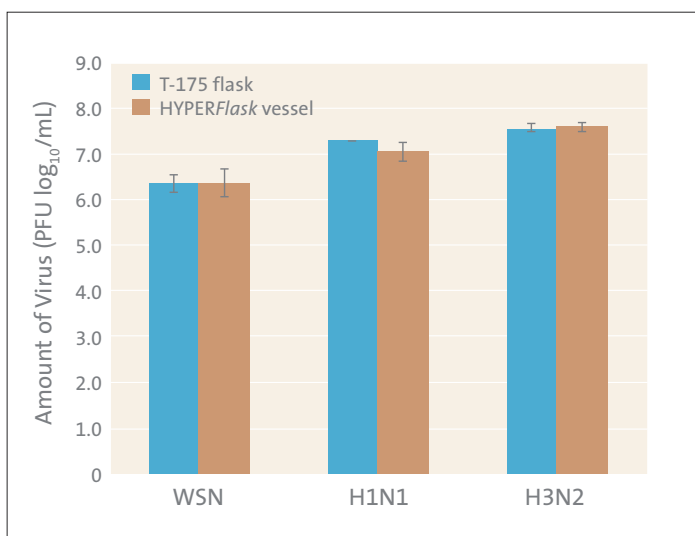


Figure 1. Virus production in the HYPERFlask vessel was similar to a conventional T-175 flask. Data represents an average from three independent experiments with standard deviations of $p > 0.1$.

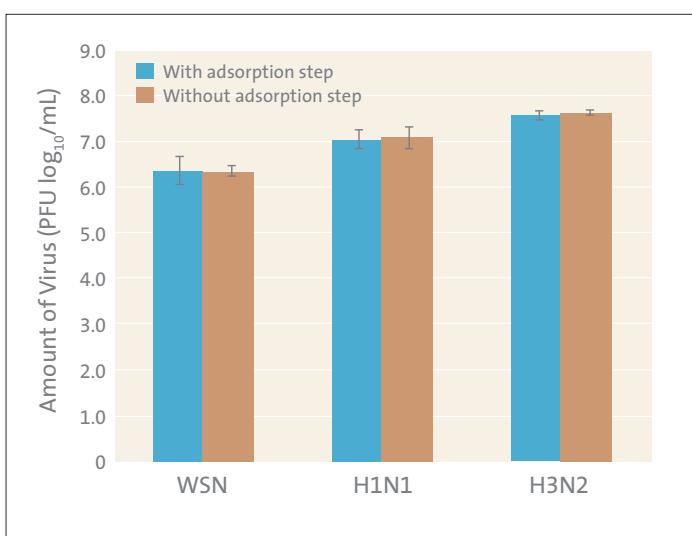


Figure 3. Bypass of the adsorption step did not affect virus production in all three viruses. Data represents an average from three independent experiments with standard deviations of $p > 0.3$.

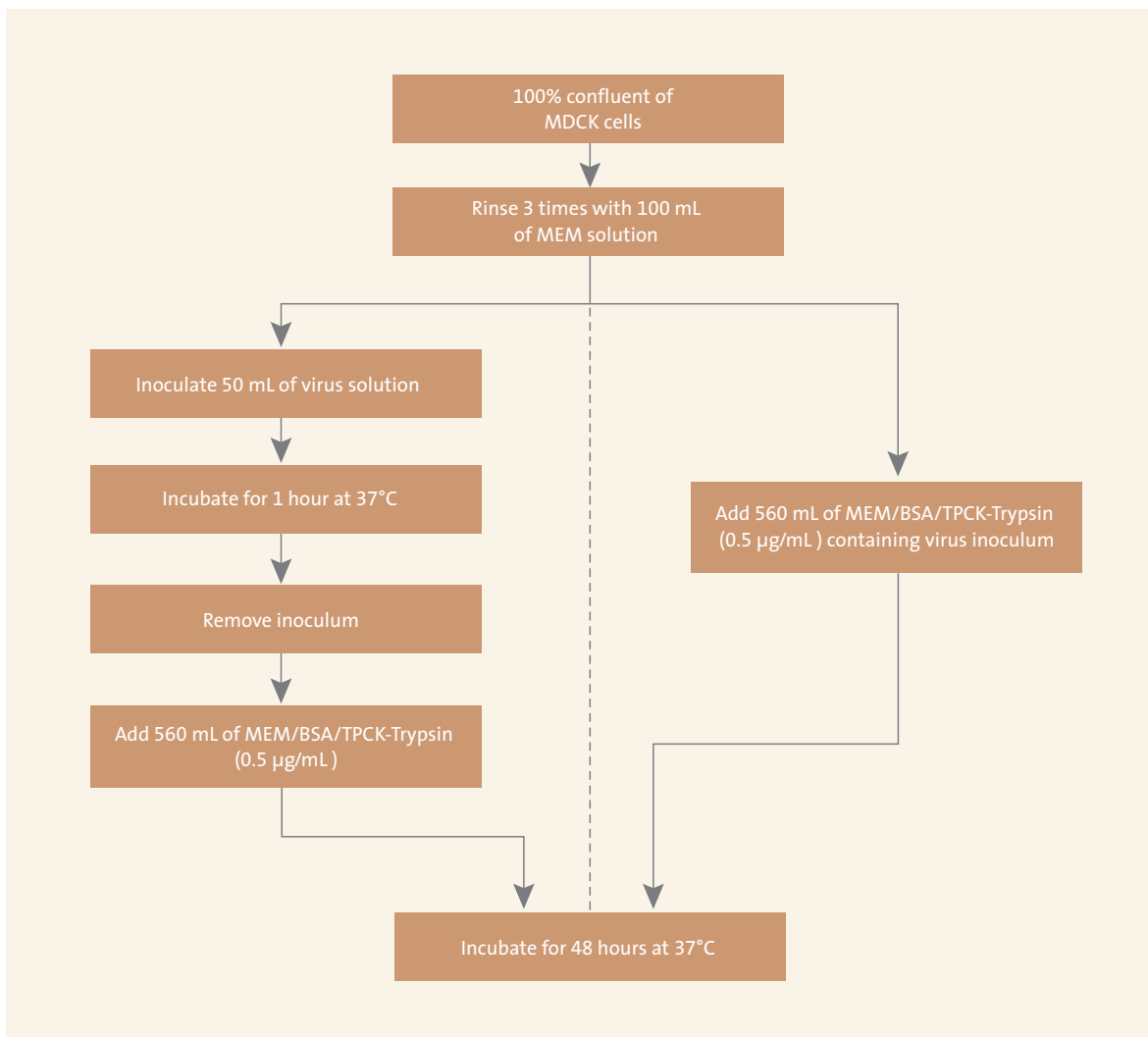


Figure 4. Schematic procedure for virus infection using standard protocol and bypass protocol. The left side indicates a standard procedure, and the right side indicates a simplified procedure by bypassing the adsorption step.

Conclusion

The performance of the Corning® HYPERFlask® vessel for the production of influenza A virus was similar to that of a conventional T-175 flask. In light of its size and capacity, the use of the HYPERFlask vessel, with a simplified procedure, is of considerable benefit for large-scale production of influenza A virus (Fig. 4).

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