

Cationic Polymer Transfections in Corning® HYPERFlask® Cell Culture Vessels



SnAPPShots

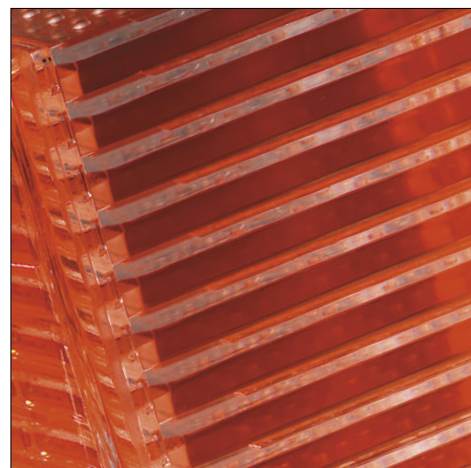
A brief report
from the Corning
Applications Group

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Introduction

One of the most useful tools in cell biology research is transfection, the introduction of foreign DNA into eukaryotic cells. Transient transfection of mammalian cell lines is a popular technique used to express genes of interest for a brief period of time, typically 2 to 3 days. In today's industry, there is an increasingly growing need to carry out these transfections in larger quantities of cells.

The Corning High Yielding Performance *Flask* (HYPERFlask) cell culture vessel utilizes a multilayered gas permeable growing surface for efficient gas exchange, can help researchers transfect larger amounts of cells. In this study, HeLa and CHO-K1 cells were transfected using Polyplus-transfection jetPEI™ transfection reagent along with two different DNA constructs: one designed to test transfection via secreted protein production and the other to determine transfection efficiency through GFP expression within cells.



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jetPEI™, a polyethylenimine (PEI) derivative, is a proprietary transfection reagent that binds to DNA through electrostatic forces for a cationic polymer transfection. The jetPEI/DNA complex gently enters the cell by endocytosis, where the DNA is then released into the cytoplasm. The jetPEI reagent is water-soluble and can be used in the presence of serum in culture media, eliminating the need for medium changes before or after transfection, making this method ideally suited for large scale transfections when using the Corning® HYPERFlask® vessel.

Materials and Methods

Transfections were carried out following the Corning HYPERFlask Cell Culture Vessel Polyplus jetPEI Transfection Protocol (http://catalog2.corning.com/Lifesciences/media/pdf/CLS_AN_111_HYPERFlask_jetPEI_Transfection_Protocol.pdf) as well as a time saving transfection method described in Corning HYPERFlask Cell Culture Vessel jetPEI Fast Transfection Protocol. For detailed instructions, please go to http://catalog2.corning.com/Lifesciences/media/pdf/CLS_AN_110_HYPERFlask_jetPEI_FastTransfection_Protocol.pdf.

Briefly, early passage cultures of exponentially growing HeLa (ATCC® CCL-2™) or CHO-K1 (ATCC CCL-61™) cells were harvested and plated at either 20,000 or 50,000 cell/cm² in 0.326 mL/cm² of growth medium (IMDM with 10% FBS, Mediatech) into 24 well microplates (Corning Cat. No. 3524) and HYPERFlask vessels (Corning Cat. No. 10024). The 24 well microplate was used to monitor the overall transfection efficiency as well as the transfection efficiency of the large-scale complex prepared to transfect the HYPERFlask vessel. The jetPEI/DNA complex was prepared using a jetPEI Transfection Kit (Polyplus Transfection, Cat. No.101-40N) at an N/P ratio of 5. For these studies, the manufacturer's suggested transfection protocol was used to transfect cells with DNA expressing a secretable form of alkaline phosphatase, and a modified "fast" transfection protocol was used to transfect cells with DNA encoding green fluorescent protein (GFP). Once transfected, cells were incubated in a humidified incubator at 5% CO₂ and 37°C for 48 hours and analyzed for transfection efficiency.

Transfection Efficiency Assessment

Transfection effectiveness was monitored using two different approaches: production of secreted embryonic placental alkaline phosphatase (SEAP) and expression of green fluorescent protein (GFP). The first approach measured the production of SEAP by cells transfected with a pSEAP DNA construct. Forty eight hours after transfection 110 µL of media from each condition was collected, spun at 15,000 xg for 2 minutes and frozen until analysis. Determination of SEAP production was done using Clontech's Great EscApe™ SEAP Chemiluminescence Detection Kit (Cat. No. 631701). Luminescent signal was captured using 96 well solid white plates (Corning Cat. No. 3912) and a LJL Analyst plate

reader (Molecular Devices). In the second approach the efficiency of transfection was determined through the expression of GFP by cells transfected with a gWIZGFP DNA construct. Forty eight hours after transfection cells were harvested. The percent of transfected cells was measured using a Guava EasyCyte Mini System (Guava Technologies) along with the Express plus software.

Results

Effectiveness of jetPEI transfection of the Corning HYPERFlask vessel was measured through detection of secreted alkaline phosphatase into the medium of the transfected cultures. A comparison was made of the relative amount of activity for transfections in the HYPERFlask vessel compared to control wells of a 24 well plate (24 well control) for both CHO-K1 and HeLa cells. Both cell types show similar levels of production between control wells and the HYPERFlask vessel, demonstrated as milligrams of SEAP activity per cm² of surface area, Figure 1. This indicates that transfection using a polyethylenimine method such as jetPEI can be effectively applied to large quantities of cells in the HYPERFlask vessel with an efficiency of secreted product production equal to standard tissue culture ware.

In subsequent studies the transfection strategy was improved in two ways. First, the transfection procedure was streamlined and made more efficient for use in the HYPERFlask vessel, and second, a different DNA construct was utilized that allowed determination of the actual number of transfected cells in an easily quantifiable method.

To streamline the procedure, a "fast" transfection protocol was developed in which the transfection occurs in suspension with the cell inoculum. After incubation, the cells and transfection mix are plated together in the vessel of choice (see website link in Materials and Methods). In this study, the efficiency was visualized by the use of a GFP-encoding plasmid. This would allow enumeration of transfected cells by flow cytometry-based methods. Using the same N:P ratio optimized in the traditional protocol, both HeLa and CHO-K1 cell lines were transfected using the optimized "fast" protocol. As a control, a small aliquot of the HYPERFlask vessel transfection cocktail was plated in a well of a 24 well plate (HF-24 well control) prior to addition of the mix to the HYPERFlask vessel. The results showed that 47% of HeLa cells were transfected using the "fast" protocol for the control wells of the 24 well plate, Figure 2. Surprisingly, the amount of transfected cells in the HYPERFlask vessel was 60%. This represents approximately 28% more cells being transfected in the HYPERFlask vessel as compared to traditional vessels. Similar results were also evident with the CHO-K1 cell transfections, with the HYPERFlask vessel achieving 54% transfected cells compared to 47% for the control 24 well plate, Figure 3. Of note, GFP can be easily visualized in cells grown in the HYPERFlask vessels as shown in Figure 4.

At this time, the mechanism by which transfection in the HYPERFlask® vessel is greater than traditional cell culture vessels is unclear. One possible explanation is that the overall health of the cells and their recovery after transfection shock is greater in the HYPERFlask vessel as compared to traditional cell culture vessels by virtue of the cells growing on the gas permeable film. This may allow more cells to survive the transfection procedure. Other studies with HYPERFlask

vessels have indicated similar improved performance of cells grown in the HYPERFlask vessel versus standard tissue culture ware (http://www.corning.com/lifesciences/technical_information/TechDocs/snappshots_083_HYPERFlask.pdf; http://www.corning.com/Lifesciences/technical_information/techDocs/snappshots_097_HYPERFlask_largescale_adherent_cellprotein.pdf). Further studies are needed to determine the actual mechanism of this improvement. It is also not clear

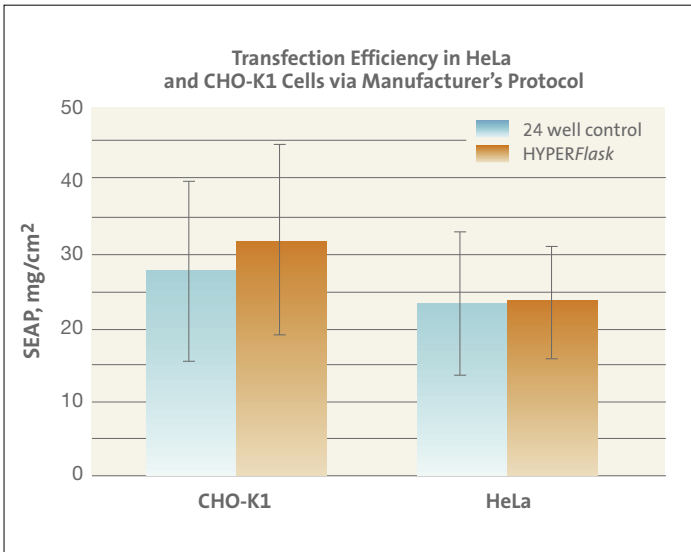


Figure 1. HeLa data is an average of four independent studies, CHO-K1 data is an average of three independent studies. All studies are ± standard deviation.

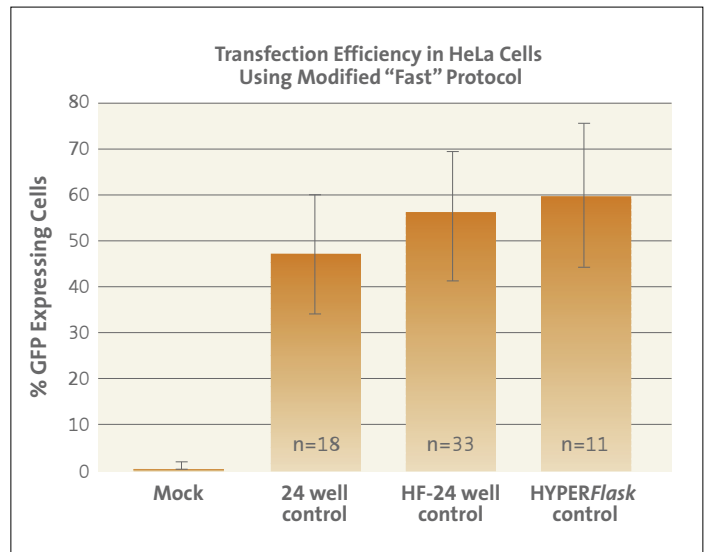


Figure 2. Sample number is given for each condition. HF-24 well control is for a small aliquot of the large HYPERFlask vessel transfection mix removed prior to addition to the HYPERFlask vessel and placed in a well of a 24 well plate. Data represent the average ± standard deviation of at least 3 independent studies.

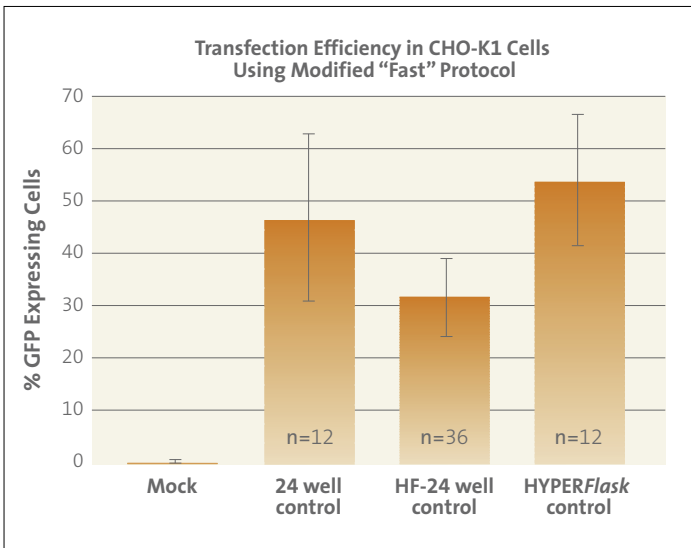


Figure 3. Sample number is given for each condition. Samples as in Figure 2. Data represent the average ± standard deviation of at least 3 independent studies.

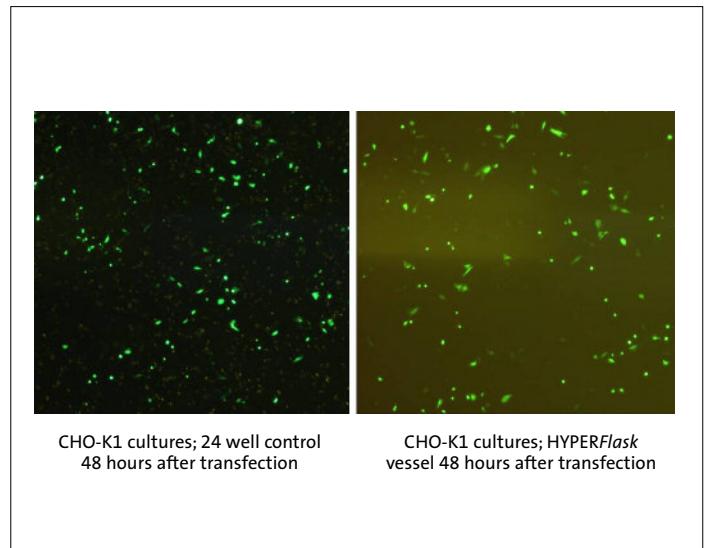


Figure 4. Fluorescent Image of control 24 well plate and the bottom layer of the HYPERFlask vessel. Cells can be easily viewed via fluorescence microscopy in the HYPERFlask vessel.

what role a larger transfection mix may play in this improvement as, based on the results of the 24 well control of the large scale transfection mix, it appears to benefit HeLa transfections yet inhibit CHO-K1 transfections. Regardless of this result, it is clear that transfections in the Corning® HYPERFlask® vessel are improved over traditional plastic vessels for this methodology.

Conclusions

- ▶ Transfection efficiency in the Corning HYPERFlask vessel was improved over control vessels for both HeLa and CHO cells.
- ▶ Large amounts of cells can be rapidly transfected in the Corning HYPERFlask vessel using a cationic polymeric transfection reagent.
- ▶ Transfection time and cost can be minimized through the use of a modified jetPEI™ “fast” transfection protocol without compromising results.

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