

# Increased Cell Yields in CO<sub>2</sub>-Independent Conditions Using the Corning® HYPERFlask® Cell Culture Vessel



## SnAPPShots

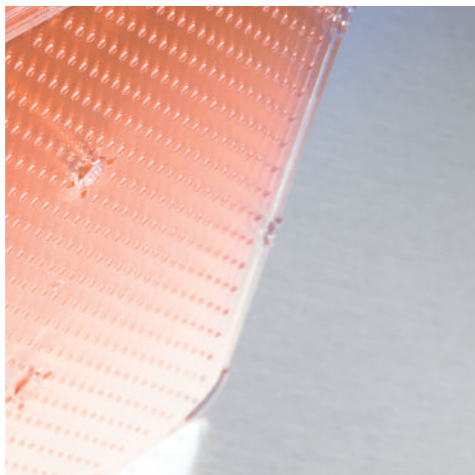
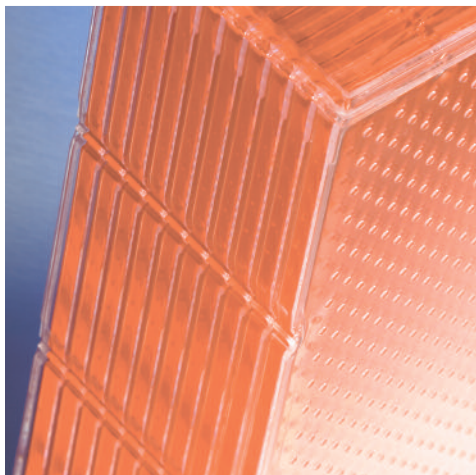
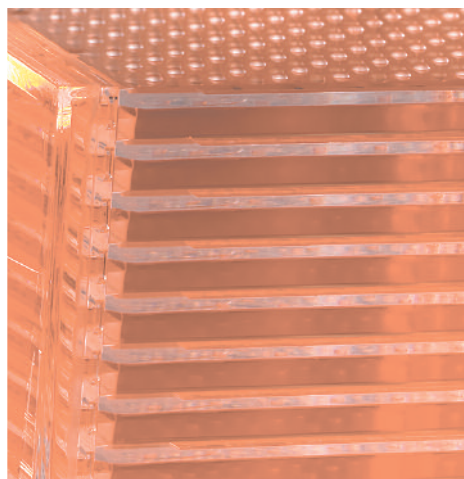
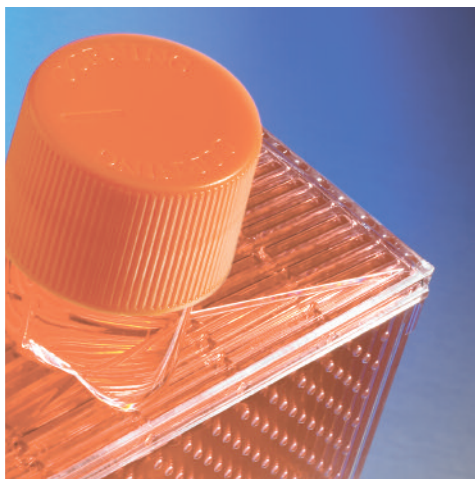
A brief report  
from the Corning  
Applications Group

*Hilary A. Sherman and Todd Upton, Ph.D., Corning Incorporated, Life Sciences*

### Abstract

The drug discovery and biotechnology industries continually demand large numbers of cells and high quality biologicals with minimal investment. One by product of this is the movement of some cell culture growth being conducted in large warm rooms without atmospheric control. To achieve this, media designed with buffering systems suitable for atmospheric CO<sub>2</sub> levels must be used.

We show here that in addition to its use in more standard CO<sub>2</sub> dependent culture environments Corning's HYPERFlask cell culture vessel can be used in CO<sub>2</sub>-independent environments. A comparison of cell yields and metabolite analyses from Vero, MDBK, and 5/9 alpha CHO cell lines in a variety of CO<sub>2</sub>-independent media demonstrates that there is no difference in growth dynamics in the HYPERFlask vessel as compared to a traditional plugged T-flask. The versatility of the HYPERFlask cell culture vessel enables large-scale cell yields and biological production in a variety of atmospheric environments without the constraint of limited incubator space.



## Methods & Materials

5/9 m alpha 3-18 CHO cells (ATCC® No. CRL-10154)<sup>TM</sup> were seeded into T175 Plug Seal Cap flasks (Corning Cat. No. 431079) and HYPERFlask® vessels (Corning Cat. No. 10010) at a density of  $5 \times 10^4$  cells/mL in 0.29 mL/cm<sup>2</sup> of L15 Media (Lonza Cat. No. 12-700 Q) supplemented with 4 mM L-glutamine (Mediatech Cat. No. 25-005-CI) and 10% FBS (Mediatech Cat. No. 35-010-CV). The vessels were placed in a 37°C warm room at atmospheric CO<sub>2</sub> and harvested after 96 hours with trypsin/EDTA (Mediatech Cat. No. 25-051-CI) plus 0.01% Pluronic (Pluronic F-68 Solution; Sigma-Aldrich P5556). Viable cells were counted using the Nova BioProfile Flex (Nova Biomedical, Waltham, MA) via trypan blue exclusion. The experiment was repeated four times, each in triplicate.

MDBK cells (ATCC No. CCL-22<sup>TM</sup>) were seeded in T175 flasks and HYPERFlask vessels at  $3 \times 10^4$  cells/mL in 0.29 mL/cm<sup>2</sup> in HMEM media (Gibco Cat. No. 11575-032) supplemented with 10% FBS. The flasks were placed in the warm room, harvested after 96 hours with trypsin and counted using the Nova BioProfile Flex. The experiment was performed in triplicate and repeated three times.

Vero cells (ATCC No. CCL-81<sup>TM</sup>), derived from kidney epithelial cells of a normal adult African green monkey, were seeded into T175 flasks and HYPERFlask vessels at  $3 \times 10^4$  cells/cm<sup>2</sup> in 0.29 mL/cm<sup>2</sup> in CO<sub>2</sub>-Independent Medium (Invitrogen Cat. No. 18045088) supplemented with 4 mM L-Glutamine and 10% FBS. Cells were harvested after 96 hours using trypsin and counted using the BioProfile Flex. In addition, daily media samples were collected from the vessels and

analyzed for gas, electrolyte and nutrient metabolite levels using the Nova Flex station for all cell lines tested.

## Results

To examine performance under CO<sub>2</sub>-independent conditions, a variety of cell lines were grown in the HYPERFlask cell culture vessel in a 37°C warm room under atmospheric CO<sub>2</sub>, and yields were compared to those from a traditional plug seal T-flask. In addition to a variety of cell lines 3 different media systems were employed to ensure universal applicability. It was previously demonstrated that tenfold cell yields are achieved in the HYPERFlask cell culture vessel as compared to conventional T-flasks under standard CO<sub>2</sub>-dependent conditions ([http://www.corning.com/lifesciences/technical\\_information/TechDocs/snappshots\\_083\\_HYPERFlask.pdf](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](http://www.corning.com/Lifesciences/technical_information/techDocs/snappshots_097_HYPERFlask_largescale_adherent_cellprotein.pdf)). Similarly in these studies, all cell lines attained equivalent or greater cell densities in the HYPERFlask vessel as compared to the closed T175 control. MDBK cells showed equivalent cell yields per cm<sup>2</sup> and 5/9 alpha and Vero cells showed approximately 10% greater cell yields per cm<sup>2</sup> (See Figure 1A-C).

To further evaluate the performance of the HYPERFlask® vessel under CO<sub>2</sub>-independent conditions the metabolite balance of the cultures in both flask types was compared. First, the pH profile of each culture vessel was examined. As shown in Figure 2, the pH profiles are nearly identical.

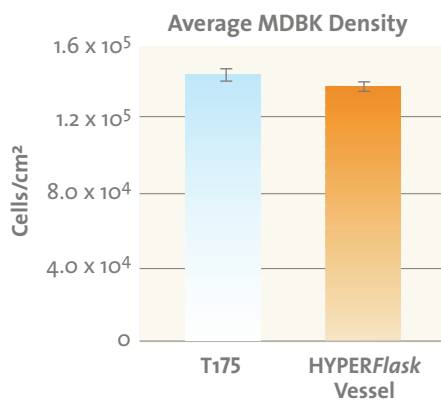


Figure 1A. Total MDBK density in HMEM after 96 hours in T175 and HYPERFlask cell culture vessels. Data represent an average of three independent studies, each done in triplicate. ( $\pm$ S.E. indicated by error bars;  $p > .05$ )

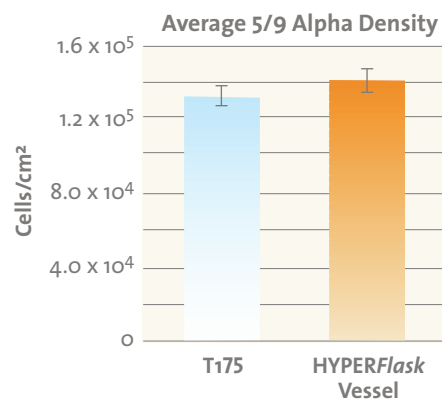


Figure 1B. Total 5/9 alpha cell density in L-15 media after 96 hours in T175 and HYPERFlask cell culture vessels. Data represent an average of four independent studies, each done in triplicate. ( $\pm$ S.E. indicated by error bars;  $p > .05$ )

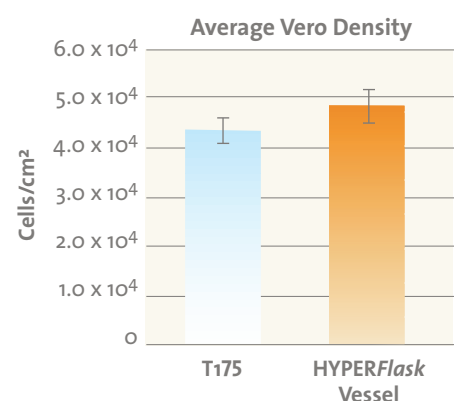


Figure 1C. Total Vero density in CO<sub>2</sub>-Independent Medium (Gibco) after 96 hours in T175 and HYPERFlask cell culture vessels. Data represent an average of three independent studies, each done in triplicate. ( $\pm$ S.E. indicated by error bars;  $p > .05$ )

Importantly, the kinetics of pH change and the final pH of the culture systems are equivalent. This indicates that there is no buildup of CO<sub>2</sub> or lactate in the HYPERFlask vessel, indicating that these cultures are equivalent to the T-flask control.

We also examined the oxygen consumption profile of each culture vessel. Figure 3 shows that for the first 2 days of culture the oxygen content of both systems is identical and at the maximum of 100%. There is a 10% reduction in oxygen content in the HYPERFlask vessel on days 3 and 4. This is due to the reduced rate of exchange of oxygen through the film: liquid interface vs. the air: liquid interface of the T-flask. Importantly, the reduction in oxygen saturation of the HYPERFlask vessel does not reduce cell yields or cellular performance (Figure 1 and below). This occurs because even though the rate at which oxygen is replaced in the media is reduced relative to the T-flask control, cells still have adequate exposure to oxygen as it diffuses through the film, the surface on which they are proliferating.

To further demonstrate the cellular and metabolic equivalence of the two culture systems we examined the glucose consumption profiles of each culture system. The graph in Figure 4 shows identical consumption rates in both the HYPERFlask cell culture vessel and the closed T175 flask, indicating similar metabolic activity.

### Discussion

A priori, it would not be expected that the HYPERFlask vessel would work equally well in both CO<sub>2</sub>-dependent and -independent conditions, given that the gas permeable film serves the function of the vent filter or cracked cap of CO<sub>2</sub>-dependent cultures. CO<sub>2</sub>-dependent cultures typically use solid caps to prevent either gas exchange between the culture flask and the external atmosphere, such as with HMEM, or to prevent evaporation, such as with L-15 or CO<sub>2</sub>-independent medium. The unique properties of the film enable the HYPERFlask vessel to be used in both the open systems of CO<sub>2</sub>-dependent culture systems and the closed systems of CO<sub>2</sub>-independent culture systems. The film allows passage of air molecules, including O<sub>2</sub> and CO<sub>2</sub>, in both directions at a rate that is approximately 10 times slower than the exchange rate between an air liquid interface. Additionally, the film prevents the passage of liquid and other small molecules, such as protein and virus, thus ensuring a sterile environment.

In the case of the HYPERFlask vessel, the rate at which CO<sub>2</sub> escapes the culture vessel is fast enough to allow the pH of the media to be properly maintained in CO<sub>2</sub>-dependent cultures. Also, given that the cells are growing directly on the gas permeable film, some of the CO<sub>2</sub> likely escapes directly through the film without entering the medium and thereby not overwhelming the culture with excessive amounts of CO<sub>2</sub>. This thereby maintains the pH balance of the culture system (Figure 2).

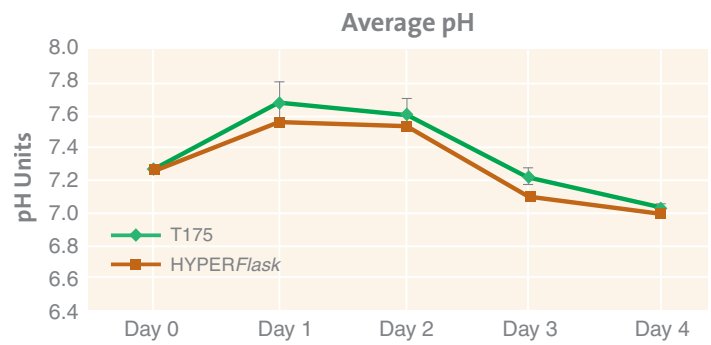


Figure 2. pH profile of MDBK cells in T175 and HYPERFlask cell culture vessels. Data represent an average of three independent studies, each done in triplicate. (±S.E. indicated by error bars; p > .05)

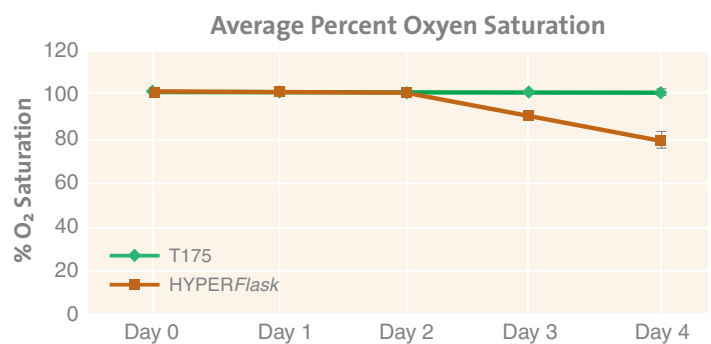


Figure 3. Oxygen consumption profile of MDBK cells in T175 and HYPERFlask cell culture vessels. Data represent an average of three independent studies, each done in triplicate. (±S.E. indicated by error bars)

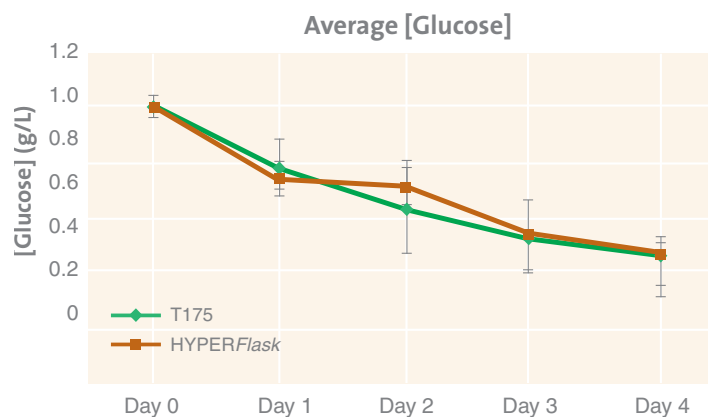


Figure 4. Average MDBK glucose consumption (g/L) over 96 hours in T175 and HYPERFlask cell culture vessels. Data represent an average of three independent studies, each in triplicate.

The same principle applies to re-oxygenation in that even though the rate of oxygen transfer through the film is reduced relative to a vented flask, exchange is still rapid enough to allow for equivalent cell yields and metabolic activity. Further, although the pace of medium re-oxygenation appears to lag behind cellular respiration in the later stages of the culture period (Figure 3), cells are likely able to obtain oxygen directly through the film surface without it first passing into the medium. This results in equal cell yields and metabolic profiles between the HYPERFlask® vessel and traditional T-flasks.

## Conclusions

- ▶ Cell yields in the HYPERFlask cell culture vessel are equal to or greater than those achieved in traditional T175 plug seal vessels under CO<sub>2</sub>-independent conditions.

- ▶ Media analyses show equivalent metabolic activity in the HYPERFlask cell culture vessel and the plugged T-flask control.
- ▶ The ability of the HYPERFlask vessel to be used alternatively in CO<sub>2</sub>-independent environments allows versatility in large-scale cell and biological production without the constraint of limited incubator space.

# CORNING

## Corning Incorporated Life Sciences

Tower 2, 4th Floor  
900 Chelmsford St.  
Lowell, MA 01851  
t 800.492.1110  
t 978.442.2200  
f 978.442.2476

[www.corning.com/lifesciences](http://www.corning.com/lifesciences)

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