Increased Cell Yields in CO_2-Independent Conditions Using the Corning® HYPER*Flask*® Cell Culture Vessel

Sn*APP*Shots

A brief report from the Corning Applications Group

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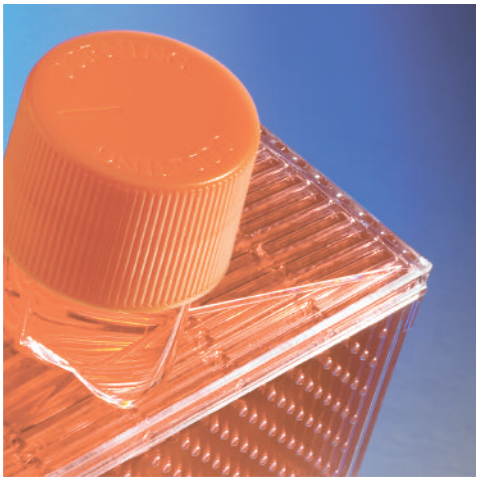
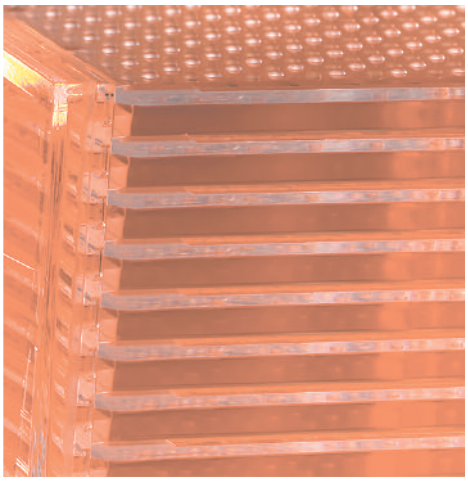
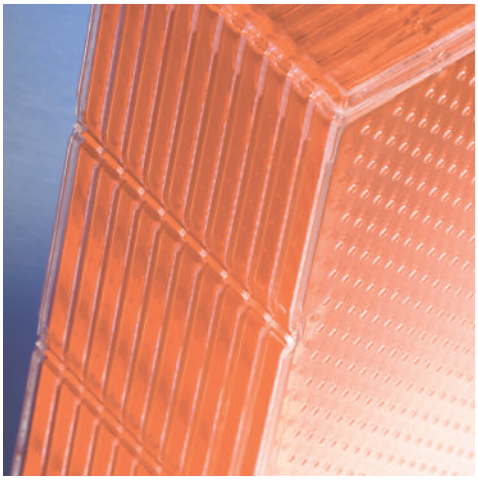
Abstract

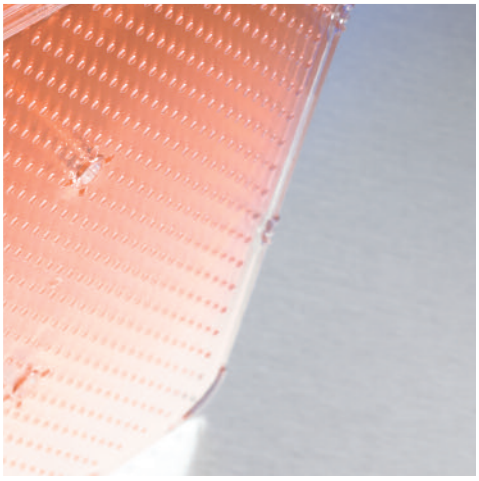
The drug discovery and biotechnology industries continually demand large numbers of cells and high quality biologicals with minimal investment. One by product of this is the movement of some cell culture growth being conducted in large warm rooms without atmospheric control. To achieve this, media designed with buffering systems suitable for atmospheric CO_2 levels must be used.

We show here that in addition to its use in more standard CO_2 dependent culture environments Corning's HYPER*Flask* cell culture vessel can be used in CO_2-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_2-independent media demonstrates that there is no difference in growth dynamics in the HYPER*Flask* vessel as compared to a traditional plugged T-flask. The versatility of the HYPER*Flask* cell culture vessel enables large-scale cell yields and biological production in a variety of atmospheric environments without the constraint of limited incubator space.









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Methods & Materials

5/9 m alpha 3-18 CHO cells (ATCC® No. CRL-10154)™ were seeded into T175 Plug Seal Cap flasks (Corning Cat. No. 431079) and HYPER*Flask*® vessels (Corning Cat. No. 10010) at a density of $5x10^4$ cells/mL in 0.29 mL/cm^2 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_2 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™) were seeded in T175 flasks and HYPER*Flask* vessels at $3x10^4$ cells/mL in 0.29 mL/cm^2 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™), derived from kidney epithelial cells of a normal adult African green monkey, were seeded into T175 flasks and HYPER*Flask* vessels at $3x10^4$ cells/cm^2 in 0.29 mL/cm^2 in CO_2-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_2-independent conditions, a variety of cell lines were grown in the HYPER*Flask* cell culture vessel in a 37°C warm room under atmospheric CO_2, 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 HYPER*Flask* cell culture vessel as compared to conventional T-flasks under standard CO_2-dependent conditions (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). Similarly in these studies, all cell lines attained equivalent or greater cell densities in the HYPER*Flask* vessel as compared to the closed T175 control. MDBK cells showed equivalent cell yields per cm^2 and 5/9 alpha and Vero cells showed approximately 10% greater cell yields per cm^2 (See Figure 1A-C).

To further evaluate the performance of the HYPER*Flask*® vessel under CO_2-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.

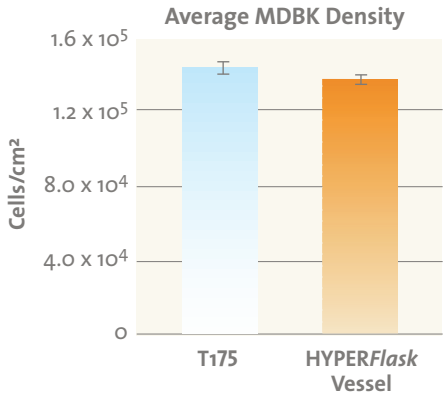

Figure 1A. Total MDBK density in HMEM after 96 hours in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies, each done in triplicate. (±S.E. indicated by error bars; p > .05)

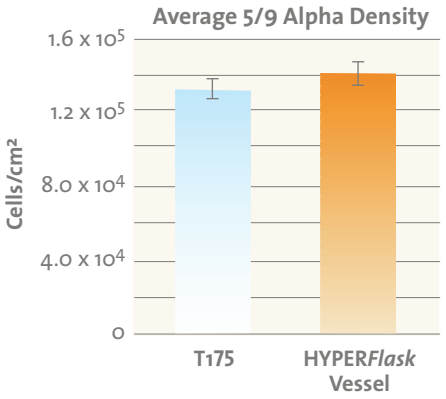

Figure 1B. Total 5/9 alpha cell density in L-15 media after 96 hours in T175 and HYPER*Flask* cell culture vessels. Data represent an average of four independent studies, each done in triplicate. (±S.E. indicated by error bars; p > .05)

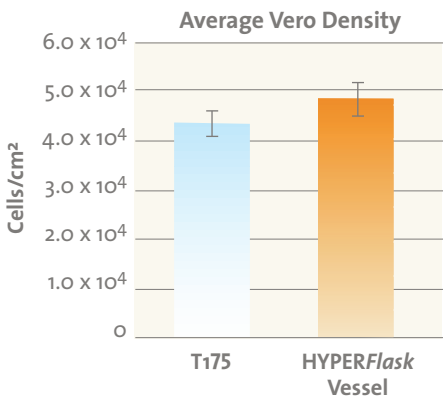

Figure 1C. Total Vero density in CO_2-Independent Medium (Gibco) after 96 hours in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies, each done in triplicate. (±S.E. indicated by error bars; p > .05)

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_2 or lactate in the HYPER*Flask* 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 HYPER*Flask* 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 HYPER*Flask* 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 HYPER*Flask* cell culture vessel and the closed T175 flask, indicating similar metabolic activity.

Discussion

A priori, it would not be expected that the HYPER*Flask* vessel would work equally well in both CO_2-dependent and -independent conditions, given that the gas permeable film serves the function of the vent filter or cracked cap of CO_2-dependent cultures. CO_2-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_2-independent medium. The unique properties of the film enable the HYPER*Flask* vessel to be used in both the open systems of CO_2-dependent culture systems and the closed systems of CO_2-independent culture systems. The film allows passage of air molecules, including O_2 and CO_2, 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 HYPER*Flask* vessel, the rate at which CO_2 escapes the culture vessel is fast enough to allow the pH of the media to be properly maintained in CO_2-dependent cultures. Also, given that the cells are growing directly on the gas permeable film, some of the CO_2 likely escapes directly through the film without entering the medium and thereby not overwhelming the culture with excessive amounts of CO_2. This thereby maintains the pH balance of the culture system (Figure 2).

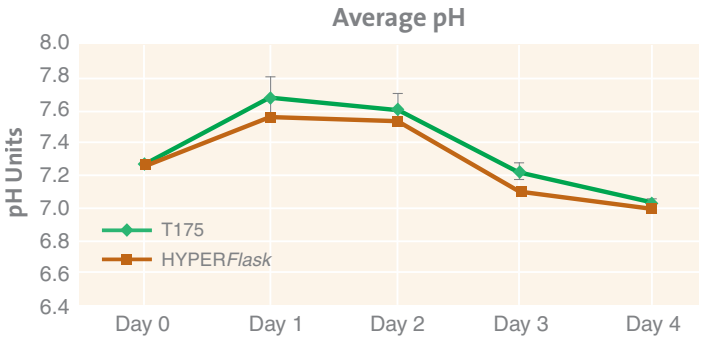

Figure 2. pH profile of MDBK cells in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies, each done in triplicate. (±S.E. indicated by error bars; p> .05)

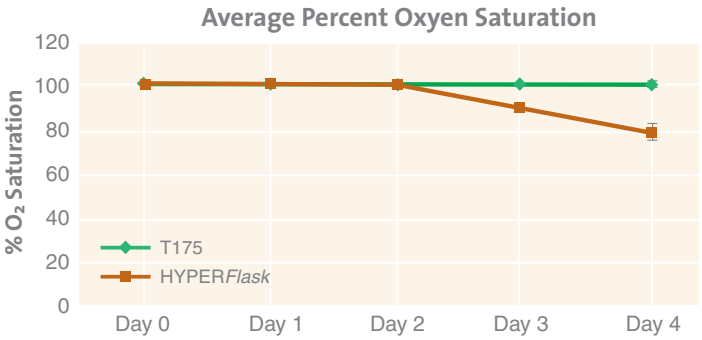

Figure 3. Oxygen consumption profile of MDBK cells in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies, each done in triplicate. (±S.E. indicated by error bars)

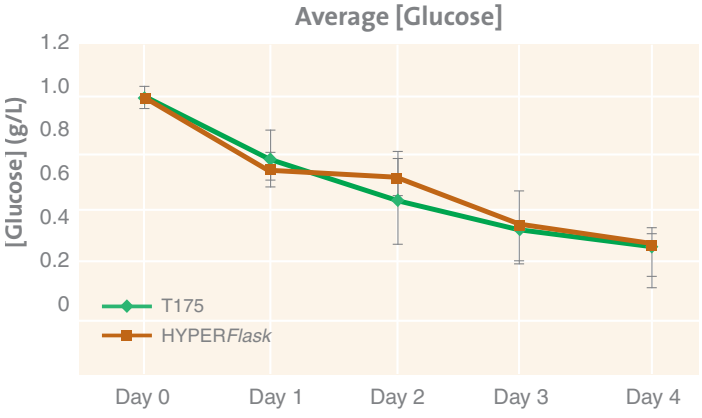

Figure 4. Average MDBK glucose consumption (g/L) over 96 hours in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies, each in triplicate.

The same principle applies to re-oxygentation 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 HYPER*Flask*® vessel and traditional T-flasks.

Conclusions

- Cell yields in the HYPER*Flask* cell culture vessel are equal to or greater than those achieved in traditional T175 plug seal vessels under CO_2-independent conditions.
- Media analyses show equivalent metabolic activity in the HYPER*Flask* cell culture vessel and the plugged T-flask control.
- The ability of the HYPER*Flask* vessel to be used alternatively in CO_2-independent environments allows versatility in large-scale cell and biological production without the constraint of limited incubator space.

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