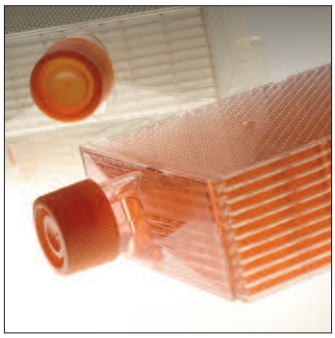
Large-Scale Suspension Cell Growth and Secreted Product Yields Using the Corning® HYPER*Flask*™ Cell Culture Vessel



SnAPPShots
A brief report from the Corning Applications Group

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Abstract

The Corning HYPER*Flask* cell culture vessel was developed to meet increased needs for larger amounts of cells and secreted products such as antibodies by the pharmaceutical industry. With the same overall dimensions as the standard T175 flask, the HYPER*Flask* vessel is comprised of ten individual gas permeable layers, which allow for approximately ten times the total growth surface of a T175. As previously shown (see *A Novel Flask Design for High Density Cell Culture*), this increased growth area translates to ten times the total cell yield for adherent cell lines. We show here that similar yields are also achieved with both serum-free and serum-dependent suspension cell lines. Growth comparisons of CHO-S and MH-677 cells in the HYPER*Flask* vessel relative to a standard T175 flask showed no difference in cell density or viability. Further, the production of monoclonal antibodies (mouse IgG2a) by hybridoma cells was equivalent, per cm^2 of growth surface, in both flasks, resulting in a 10-fold greater yield of cells from the HYPER*Flask* vessel.

Materials and Methods

CHO-S cells (Gibco Cat. No. 11619-012) cultured in serum-free CD-CHO medium (Gibco Cat. No. 10743029) supplemented with 20 mM L-Glutamine and 1X hypoxanthine thymidine solution (Mediatech Cat No. EF8104N) were seeded into T175 (Corning Cat. No. 431306) and HYPER*Flask* vessels (Corning Cat. No. 10010) at a density of $2.5x10^5$ cells/mL and volume of 0.326 mL/cm^2. The vessels were placed in a humidified incubator at 37°C with 5% CO_2 for 96 hours. Cell suspensions were collected and counted with a Z2 series particle counter (Beckman Coulter). Each experiment was performed in triplicate and replicated a total of three times.

For antibody production studies, hybridoma cells were seeded into T175 flasks and HYPER*Flask* vessels at a density of 5×10^4 cells/mL and volume of 0.326 mL/cm^2 in IMDM (Mediatech Cat. No. 10-016-CV) supplemented with 10% fetal bovine solution (FBS; Mediatech Cat. No. 35-010-CV). The vessels were incubated at 37°C and 5% CO_2 for 96 hours. One milliliter samples from each flask were collected and counted every 24 hours. Cell viability was also determined with a Nova BioProfile Flex via trypan blue exclusion. At the end of the 96-hour growth period, an additional sample was taken from each flask and centrifuged at 270 RCF for 7 minutes. The supernatant was collected and used to determine antibody production following the Alpha Diagnostic International ELISA kit protocol (Cat. No. 6340). The experiment was replicated a total of three times, each performed in triplicate.

Results

The CHO-S cell line is a commercially available serum-free adapted clone of CHO cells and was selected to demonstrate the performance of suspension cells in serum-free media in the HYPER*Flask* vessel. Results showed equivalent cell densities, resulting in a 10-fold greater yield of cells from the HYPER*Flask* vessel. (Figure 1, see reverse side). As a further example of suspension cell growth in the HYPER*Flask* vessel, hybridoma cells were seeded in T175 and HYPER*Flask* vessels, and cell density and viability were determined at 24-hour time points for 96 hours. Data showed equal growth rates for each vessel (Figure 2), with average viability greater than 95% for both (data not shown). As a hybridoma cell line engineered for the production of monoclonal antibodies, these cells serve as a model for relative secretory production. ELISA quantification of antibody production shows equal concentrations of mouse IgG2a per cm^2 generated in the HYPER*Flask* vessel compared to the T175 control (Figure 3). With ten times the total growth area of a T175, net IgG2a production in the HYPER*Flask* vessel was also tenfold (Figure 4).

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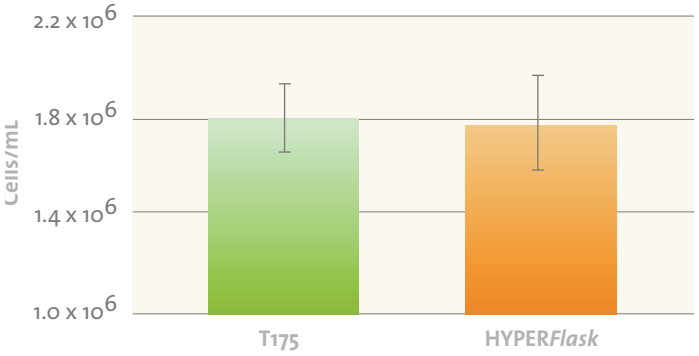

Figure 1. Total CHO-S cell concentration after 96 hours in T175 and HYPER*Flask*™ cell culture vessels. Data represent an average of three independent studies each done in triplicate (±S.E. indicated by error bars; p>.05).

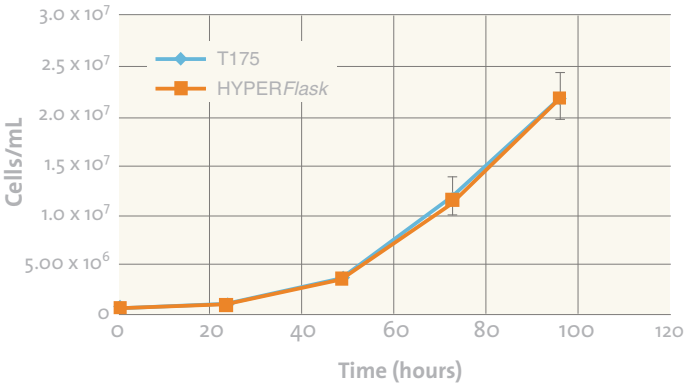

Figure 2. Average MH-677 cell density at 24-hour time points for 96 hours in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies each done in triplicate (±S.E. indicated by error bars; p>.05).

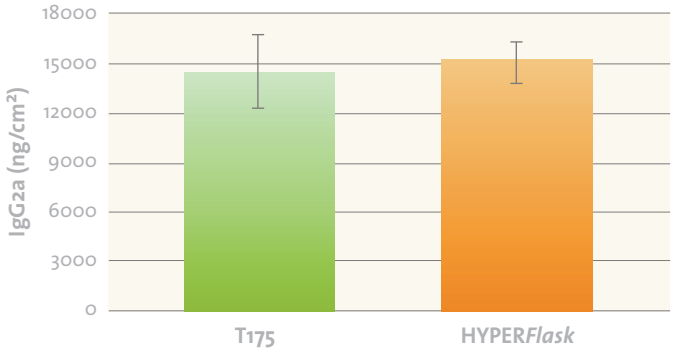

Figure 3. Average mouse IgG2a production/cm² after 96 hours. Data represent an average of three independent studies each done in triplicate (±S.E. indicated by error bars; p > .05).

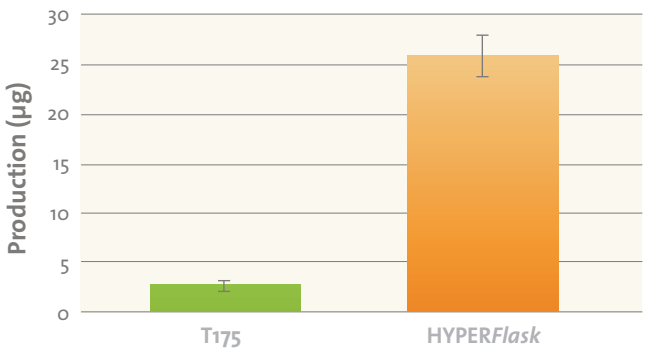

Figure 4. Total mouse IgG2a production after 96 hours in T175 and HYPER*Flask* cell culture vessels. Data represent an average of three independent studies each done in triplicate (±S.E. indicated by error bars).

Conclusions

- A single HYPER*Flask* vessel produces the same amount of suspension cells as 10 T175 flasks.
- A single HYPER*Flask* vessel produces the same amount of secreted product, i.e., antibody, as 10 T175 flasks.
- Suspension cells grown in HYPER*Flask*, with and without serum, have equivalent cell densities and viabilities as traditional T-flasks.
- Use of HYPER*Flask* vessels reduces handling ten fold to generate equivalent outputs of cells or secreted product as traditional T-flasks.

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