Efficient Expansion of Suspension CHO Cells in Corning® PETG Erlenmeyer Flasks

Application Note

CORNING

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Introduction

The adoption of single-use technologies for biopharmaceutical manufacturing is a rapidly growing trend due to a number of advantages including the reduced risk of contamination, reduced capital costs, and faster time to market. Disposable Erlenmeyer flasks are traditionally made of polycarbonate (PC) and are commonly used as seed train vessels to expand cells grown in suspension. Corning offers a wide range of PC Erlenmeyer flasks. To broaden our Erlenmeyer flask portfolio beyond polycarbonate material, we introduced 2L, 3L, and 5L Erlenmeyer flasks made of bisphenol A(BPA)-free polyethylene terephthalate glycol (PETG). The addition of PETG flasks allows scientists to choose the material (PC or PETG) and flask size (2L, 3L, or 5L) that work best with their specific cell lines and applications.

In this study, we assessed the growth of CHO-S cells in Corning 2L, 3L, and 5L PETG flasks with different fill volumes using a typical batch culture protocol. Our results demonstrate efficient expansion and high viability of CHO-S cells in all three flask sizes during 6 days in culture.

We also compared the performance of our Corning PETG flasks to Competitor-1 PETG 2L and 2.8L flasks and Competitor-2 polypropylene (PP) 5L flasks. Our results show significantly higher viable cell yield of CHO-S cells in Corning 3L and 5L flasks compared to Competitor flasks of the similar size, especially with the larger culture volumes. This advantage is due to the more optimal design of Corning flasks that provides higher liquid surface area to culture volume ratio resulting in a better culture aeration and mixing.

Materials and Methods

Culturing Conditions

For all routine cell culture and benchmarking experiments described below, the cells were maintained at 37°C with 8% CO_2 and 80% relative humidity. Celltron 25 mm (1") orbital shaker (Infors Cat. No. I69222) at an agitation rate of 130 rpm was used for 125 mL Erlenmeyer flasks. A Multitron Pro 25 mm (1") orbital shaker (Infors Cat. No. I10102P) at an agitation rate of 130 rpm was used for 2L and at 90 rpm for 2.8L, 3L, and 5L Erlenmeyer flasks.

FreeStyle™ CHO-S cells (Thermo Fisher Cat. No. R80007) were adapted from FreeStyle CHO Expression medium (Thermo Fisher Cat. No. 12651014) to 1X CD OptiCHO™ medium (Thermo Fisher Cat. No. 12681-011) supplemented with 8 mM Corning glutagro™ medium (Corning Cat No. 25-015-CI) and 1X Hypoxanthine, Thymidine (HT) (Corning Cat No. 25-047-CI) using a protocol recommended by the medium manufacturer. Adapted cells were cryopreserved at 1 x 10^7 cells/vial and subsequently used for these studies. One vial of cells was thawed directly into 40 mL of CD OptiCHO medium in a 125 mL Corning Erlenmeyer flask (Corning Cat. No. 431143). Cells were routinely passaged at 0.15 x 10^6 cells/mL and considered fully recovered from cryopreservation when they consistently reached >2 x 10^6 cells/mL on day 3 with cell viability >95% (after 3 to 4 passages).

Benchmarking

Corning 2L PETG flasks: To compare the growth of CHO-S cells in Corning vs. Competitor 2L PETG Erlenmeyer flasks, cells from the mid-logarithmic growth stage were seeded at 1 x 10^5 cells/mL in two conditions: Corning plain bottom 2L PETG Erlenmeyer flask (Corning Cat. No. 431280) and a Competitor plain bottom 2L PETG Erlenmeyer flask. Two different working culture volumes were tested for each flask type: 0.6L/flask and 1.2L/flask. The flasks were incubated in an Infors Multitron Pro 25 mm orbital shaker at an agitation rate of 130 rpm until the maximum viable cell density was achieved (6 days). The cells were enumerated and assessed for viability using the Trypan blue exclusion assay with a Vi-CELL™ Cell Viability Analyzer (Beckman Coulter).

Corning 3L PETG flasks: To compare the growth of CHO-S cells in Corning 3L PETG vs. Competitor 2.8L PETG Erlenmeyer flasks, cells from the mid-logarithmic growth stage were seeded at 1 x 10^5 cells/mL in two conditions: Corning plain bottom 3L PETG Erlenmeyer flask (Corning Cat. No. 431282) and a Competitor plain bottom 2.8L PETG Erlenmeyer flask. Two different working culture volumes were tested for each flask type: 0.8L/flask and 2L/flask. The flasks were incubated in an Infors Multitron Pro 25 mm orbital shaker at an agitation rate of 90 rpm until the maximum viable cell density was achieved (6 days). The cells were enumerated and assessed for viability using the Trypan blue exclusion assay with a Vi-CELL Cell Viability Analyzer.

Corning 5L PETG flasks: Because Corning is the only vendor to provide 5L Erlenmeyer flasks made of PC or PETG, we compared the performance of our 5L PETG flask to a Competitor 5L PP shaker flask. CHO-S cells from the mid-logarithmic growth stage were seeded at 1 x 10^5 cells/mL in two flask conditions: Corning baffled bottom 5L PETG Erlenmeyer flasks (Corning Cat. No. 431285) and Competitor baffled bottom 5L PP shaker flask. Two different working culture volumes were tested for each flask type:

2.5L/flask and 3L/flask. The flasks were incubated in an Infors Multitron Pro 25 mm orbital shaker at 90 rpm agitation rate for 6 days. The cells were enumerated and assessed for viability using the Trypan blue exclusion assay with a Vi-CELL Cell Viability Analyzer.

Results

Figure 1 shows viable cell density (panel A) and viability (panel B) of CHO-S cells cultured in Corning® 2L PETG vs. Competitor 2L PETG Erlenmeyer flasks using 0.6L and 1.2L culture volumes. Efficient CHO-S cell growth with high viability (>95%) was observed in all flasks regardless of the culture volume used. Similar results were demonstrated with CHO-5/9 alpha and CHO-DG44 cell lines (data not shown).

Figure 2 shows viable cell density (panel A) and viability (panel B) of CHO-S cells cultured in Corning 3L PETG vs. Competitor 2.8L PETG Erlenmeyer flasks using 0.8L and 2L culture volumes. Comparable CHO-S cell growth and viability was observed for both Corning and Competitor flasks at 0.8L culture volume. However, 4-fold higher viable cell density was observed with Corning PETG flasks (11E6 cells/mL on day 6) relative to Competitor PETG flasks (2.63E6 cells/mL on day 6) with 2L culture volume. The observed difference in performance is likely due to the Corning 3L flask's higher liquid surface area/culture volume ratio that enables better gas exchange and culture mixing (see Table 2 for liquid SA/volume ratio data).

Figure 3 shows viable cell density (panel A) and viability (panel B) of CHO-S cells cultured in Corning 5L PETG flasks vs. Competitor 5L PP flasks using 2.5L and 3L culture volumes. Our results show significantly higher viable cell density (2-fold for 2.5L culture volume and 6-fold for 3L culture volume) of CHO-S cells in Corning flasks compared to the Competitor flasks. Table 1 shows total viable cell number/flask that was achieved with 3L culture volume for Corning 5L PETG and Competitor 5L PP Erlenmeyer flask on day 6. Over 6-fold higher total viable cell number was achieved with Corning flasks (32.7 billion cells) relative to Competitor flasks (5.13 billion cells). The observed difference in performance is likely due to the Corning 5L flask's higher liquid surface area/culture volume ratio that enables better gas exchange and culture mixing (see Table 2 for liquid surface area/volume ratio data). Collectively, these results suggest that the Corning 5L PETG flask enables significantly higher total viable CHO-S cell yield compared to the Competitor 5L PP shaker flask.

Table 2 compares liquid surface area (SA in cm^2) to culture volume (in L) ratio for different Corning and Competitor flasks at different culture volumes. Surface aeration is a commonly used aeration method for shaker flasks. At a given agitation rate, the higher the liquid surface area/culture volume ratio is the better gas exchange is. The data in the Table 2 show comparable SA/volume ratio for Corning and Competitor 2L flasks at both 0.6L and 1.2L volumes, which is consistent with comparable CHO-S viable cell densities in Figure 1. For Corning 3L and 5L flasks the SA/volume ratio was significantly higher compared to Competitor flasks and correlated with significantly higher CHO-S viable cell densities in Figures 2 and 3, respectively.

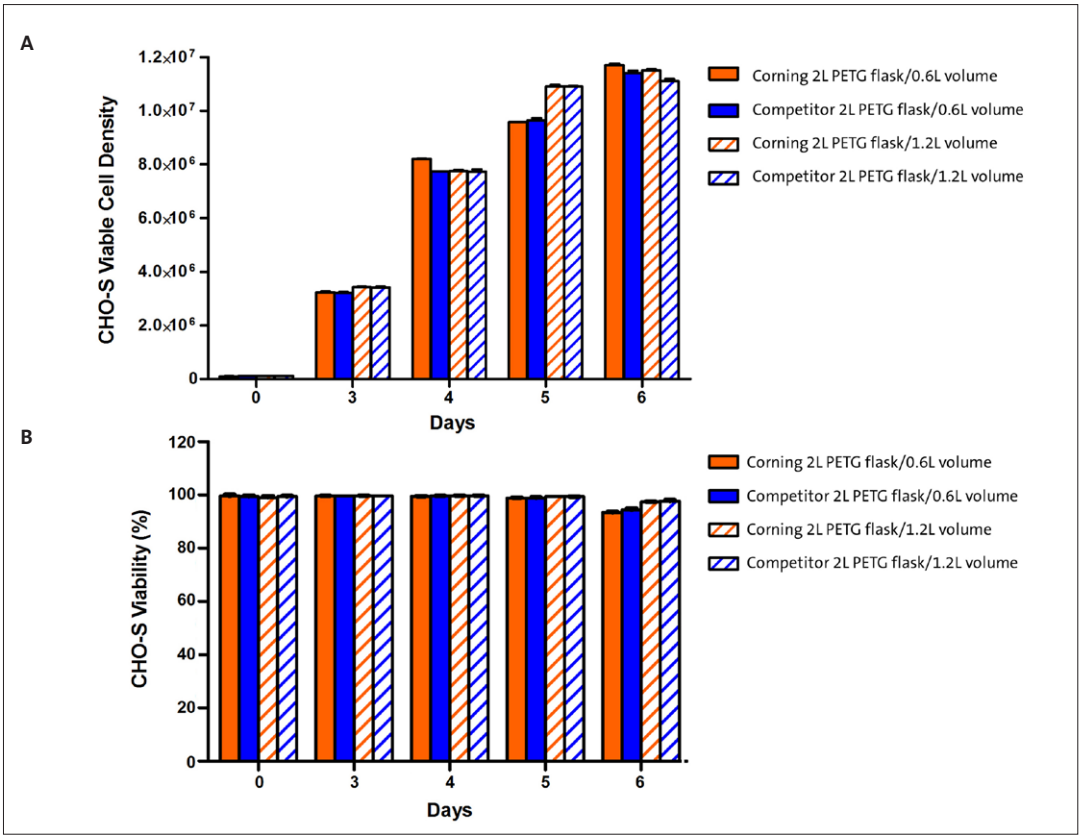

Figure 1. Viable cell density (A) and viability (B) of CHO-S cells cultured in Corning and Competitor 2L PETG Erlenmeyer flasks with 0.6L and 1.2L fill volumes. For each condition the data represent the average ± STDEV of 3 flasks.

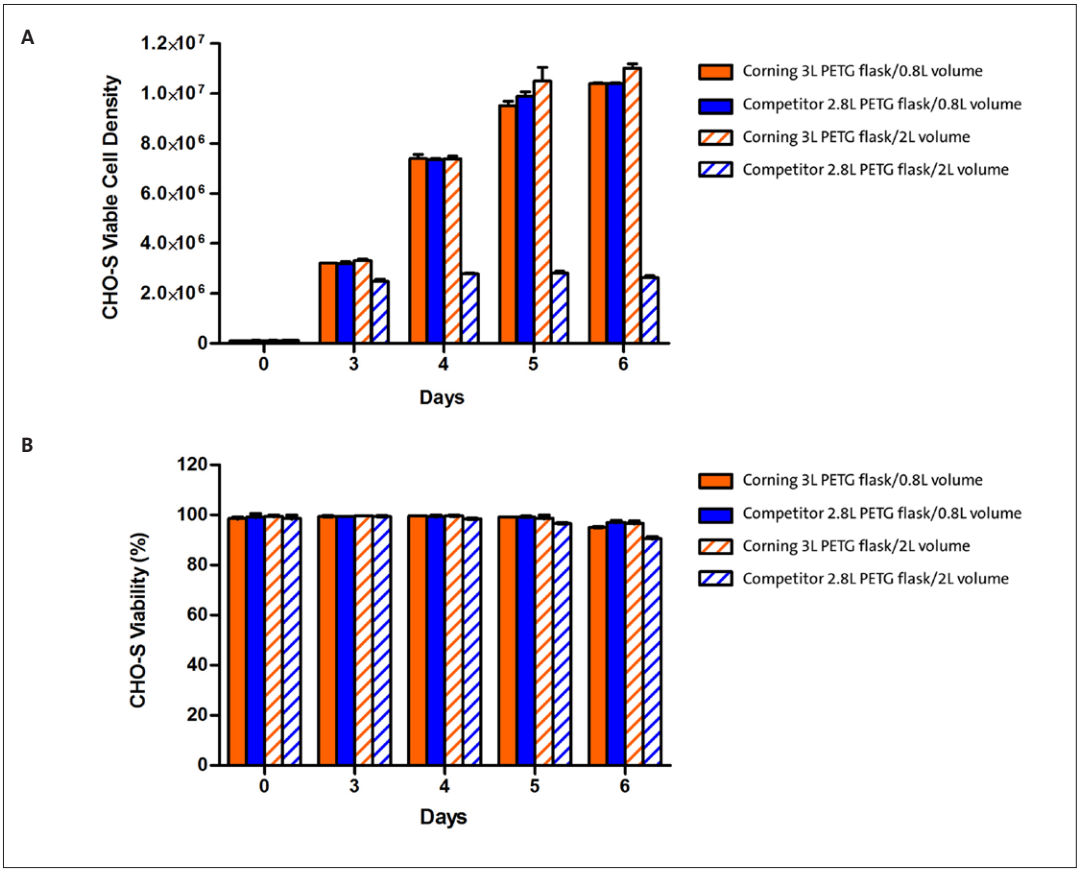

Figure 2. Viable cell density (A) and viability (B) of CHO-S cells cultured in Corning® 3L PETG and Competitor 2.8L PETG Erlenmeyer flasks with 0.8L and 2L fill volumes. For each condition, the data represent the average ± STDEV of 3 flasks.

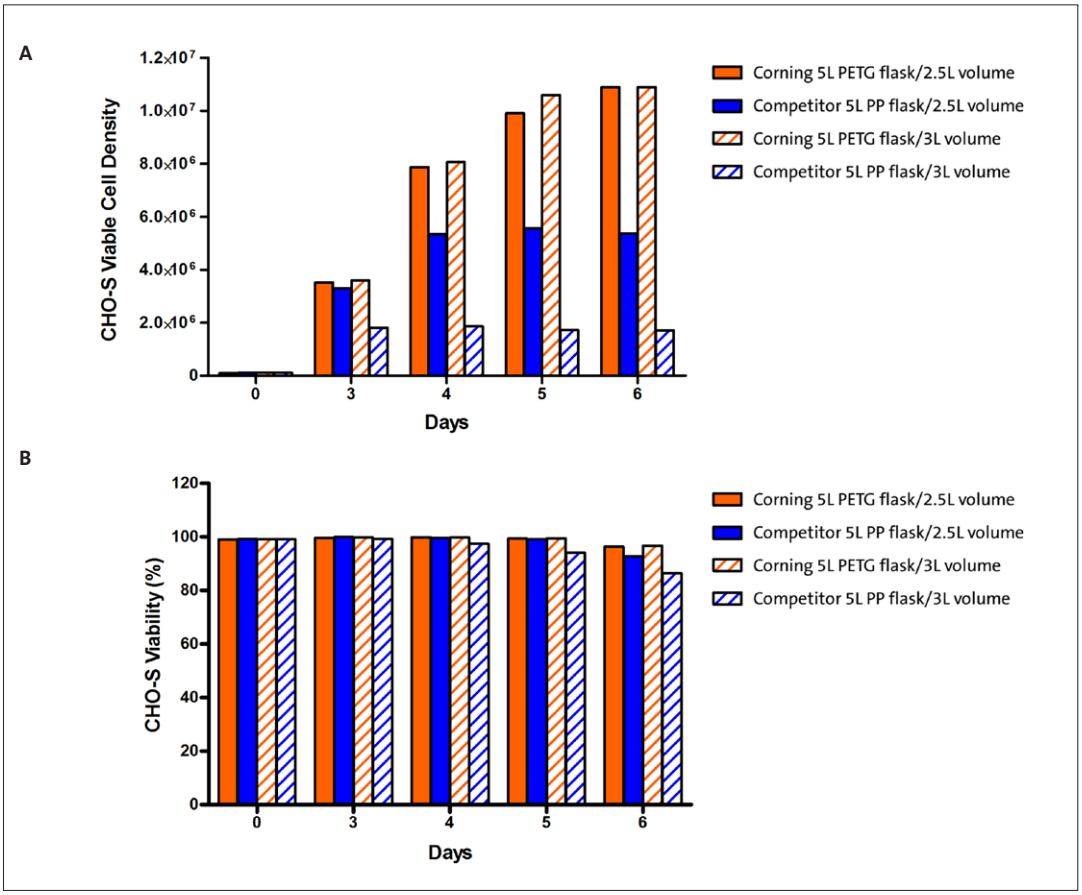

Figure 3. Viable cell density (A) and viability (B) of CHO-S cells in Corning 5L PETG and Competitor 5L PP flasks with 2.5L and 3L fill volumes.

Table 1. CHO-S total viable cell number/flask in Corning® 5L PETG flask vs. Competitor 5L PP flask on day 6. The numbers were calculated by multiplying day 6 viable cell density (cells/mL) for each condition by 3,000 mL of total culture volume per flask.

	Viable Cell Number/mL	Viable Cell Number/Flask/3,000 mL
Corning 5L PETG flask	10.9 million	32.7 billion
Competitor-2 5L PP flask	1.71 million	5.13 billion

Table 2. Liquid surface area (cm^2) to culture volume (L) ratio for different Corning and Competitor flasks at different fill volumes.

	Flask Fill Volume					
Flask Description	**0.6L**	**0.8L**	**1.2L**	**2L**	**2.5L**	**3L**
2L Corning PETG flask	322.5	–	117.3	–	–	–
2L Competitor-1 PETG flask	307.9	–	116.2	–	–	–
3L Corning PETG Flask	–	514.2	–	151.4	–	–
2.8L Competitor-1 PETG flask	–	397.3	–	96.8	–	–
5L Corning PETG flask	–	–	–	–	152.2	123.3
5L Competitor-2 PP flask	–	–	–	–	109.5	70.0

Summary and Conclusions

- The Corning 2L, 3L, and 5L PETG Erlenmeyer flasks support efficient growth and high viability of CHO-S cells.
- Significantly higher CHO-S viable cell density was demonstrated for Corning 3L PETG flask compared to Competitor-1 2.8L PETG flask with 2L culture volume.
- Significantly higher CHO-S viable cell density was demonstrated for Corning 5L PETG flask compared to Competitor-2 5L PP flasks for both 2.5L and 3L culture volumes.
- Total viable cell number/flask was 32.7 billion for Corning 5L PETG flask vs. 5.13 billion for Competitor-2 5L PP flask with 3L culture volumes.
- The higher viable cell densities for Corning 3L PETG and 5L PETG flasks relative to Competitor flasks is likely due to the more optimal design of Corning flasks that provides higher liquid surface area/volume ratio resulting in a better culture aeration and mixing.
- The Corning 2L, 3L, and 5L PETG Erlenmeyer flasks are made of PETG material that is free of antioxidants, including Tris(2,4-di-tert-butylphenyl)phosphite, commonly known under the trade name Irgafos 168®, that was associated with the formation of cytotoxic degradation product bis(2,4-di-tert-butylphenyl) or bDtBPP in certain single-use bioprocess bags[1].
- The Corning Erlenmeyer flask family provides scientists with the flexibility to choose the material (PC or PETG) and flask size (2L, 3L, or 5L) that work best for their specific cell lines and applications.

Reference

1. Matthew Hammond, Liliana Marghitoiu, Hans Lee, Lourdes Perez, Gary Rogers, and Yasser Nashed-Samuel. A Cytotoxic Leachable Compound from Single-use Bioprocess Equipment that Causes Poor Cell Growth Performance. Biotechnol. Prog., 2014, Vol. 30, No. 2.

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