Growth performance of Nalgene PETG flasks: a comparative analysis

Purpose

This paper demonstrates the potential of Thermo Scientific™ Nalgene™ Single-Use Polyethylene Terephthalate Glycol-Modified (PETG) Erlenmeyer Flasks to support the growth of Gibco™ ExpiCHO-S™ and Expi293F™ cells, in comparison with polycarbonate (PC) Erlenmeyer flasks from another supplier ("Supplier PC flasks").

Introduction

Nalgene Single-Use PETG Erlenmeyer Flasks serve as an ideal choice for the culture of suspension cells often used in expression systems for the production of recombinant proteins. These flasks are offered in a wide range of capacities ranging from 125 mL to 2,800 mL to facilitate cell culture from lab scale to commercial scale. To enable better growth of cells, it is recommended to use one of two different types of flask bottoms (plain or baffled) with vented-cap closures of 0.22 µm to allow better gas exchange. In this study, we compared the growth and viability of ExpiCHO-S and Expi293F cells in plain-bottom, vented Nalgene PETG flasks and in Supplier PC flasks.

Methods

Five different volumes (125 mL, 250 mL, 500 mL, 1,000 mL, and 2,000 mL) of Nalgene PETG and Supplier PC flasks were selected for comparison. Design and shape were similar between each flask volume. ExpiCHO-S cells (Cat. No. A29127) and Expi293F cells (Cat. No. A14527) were cultured using Gibco™ ExpiCHO™ Expression Medium (Cat. No. A2910002) and Gibco™ Expi293™ Expression Medium (Cat. No. A1435102), respectively. Cells were revived according to the manufacturer's recommended protocol and seeded at a density of 0.3 x 10^6 cells/mL in the 5 different volumes of flasks. A summary of flask volumes and culture conditions is shown in Table 1. Cells were harvested 3 days after seeding and subcultured for up to 3 passages. At each passage, cells were counted using the Invitrogen™ Countess™ II Automated Cell Counter to assess cell viability and viable cell density.

Table 1. Culture conditions for growth of ExpiCHO-S and Expi293F cells.

Flask volume	Volume of medium	Total cells seeded (x 10^6)	Culture conditions	Shaking speed, diameter
125 mL	25 mL	7.5	37°C, 8% CO_2	125 rpm, 19 mm
250 mL	50 mL	15	37°C, 8% CO_2	125 rpm, 19 mm
500 mL	100 mL	30	37°C, 8% CO_2	125 rpm, 19 mm
1,000 mL	200 mL	60	37°C, 8% CO_2	125 rpm, 19 mm
2,000 mL	400 mL	120	37°C, 8% CO_2	125 rpm, 19 mm

Results

Growth kinetics in PETG and PC flasks are similar

ExpiCHO-S and Expi293F cells were seeded individually at a density of 0.3 x 10^6 cells/mL in 5 different flask volumes with 3 replicates of each. Samples were taken at day 2, 3, 4, 5, and 6 after inoculation, and cell growth was measured. At lower volumes (125 mL, 250 mL, 500 mL, and 1,000 mL), Nalgene PETG and Supplier PC flasks produced nearly equivalent ExpiCHO-S cell densities at all time points tested. At the highest volume (2,000 mL), Nalgene PETG flasks produced higher cell densities than did Supplier PC flasks. This difference was most apparent on day 4, where Nalgene PETG flasks had 13.2 ± 0.6 x 10^6 cells/mL (mean ± SD), while Supplier PC flasks had 8.2 ± 0.7 x 10^6 cells/mL (Figure 1A).

With Expi293F cells, both flask types produced nearly equivalent cell densities in all 5 volumes and at all time points tested. At the highest volume (2,000 mL) on day 4, Nalgene PETG flasks had 5.0 ± 0.8 x 10^6 cells/mL, while Supplier PC flasks had 4.3 ± 0.1 x 10^6 cells/mL, which are comparable to each other (Figure 1B).

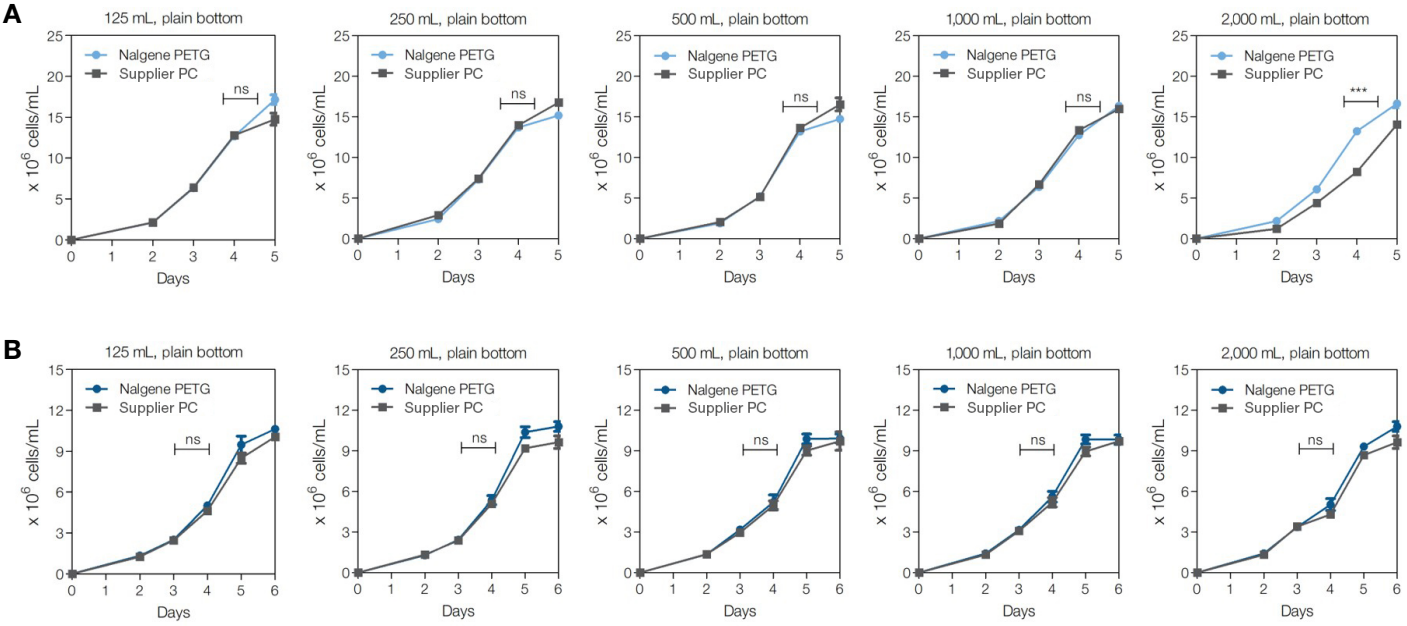

Figure 1. Comparison of growth kinetics of suspension cells in Nalgene PETG and Supplier PC flasks. (A) ExpiCHO-S cells were grown for 5 days and assessed for cell density on each day. All flask volumes performed similarly except 2,000 mL Nalgene PETG flasks, where cell growth was significantly higher (***P <0.001) than in Supplier PC flasks. **(B)** Expi293F cells were grown for 6 days and assessed for cell density on each day. All volumes of Nalgene PETG and Supplier PC flasks produced similar cell densities, and no significant difference was observed between flask types (n = 3; error bars represent SEM; ns: not significant).

Multi-passage study shows comparable performance between PETG and PC flasks

To elucidate the passage-to-passage variation between Nalgene PETG and Supplier PC flasks, ExpiCHO-S and Expi293F cells were cultured in 5 different flask volumes with 3 replicates of each. Both types of cells were seeded at a density of 0.3 x 10^6 cells/mL and subcultured on day 3. Cells were passaged up to 3 times. At the end of each passage, cell counts were taken to monitor the viable cell density for comparison between flasks. ExpiCHO-S cells grown in lower-volume flasks (125 mL, 250 mL, 500 mL, and 1,000 mL) produced nearly equivalent cell densities in all 3 passages in both Nalgene PETG and Supplier PC flasks. At the highest volume (2,000 mL), Nalgene PETG flasks produced higher cell densities than did Supplier PC flasks. Over the 3 passages, Nalgene PETG flasks had 6.0 ± 0.7 x 10^6 cells/mL, while Supplier PC flasks had 3.8 ± 0.8 x 10^6 cells/mL (Figure 2A). These results were consistent with the experiments measuring growth kinetics in Figure 1.

With Expi293F cells, the two flask types produced nearly equivalent cell densities in all 5 flask volumes. At every passage, cells grew to similar densities in both flask types. Over the 3 passages in 2,000 mL flasks, Nalgene PETG flasks had viable cell densities of 3.4 ± 0.2 x 10^6 cells/mL, while Supplier PC flasks had 3.3 ± 0.3 x 10^6 cells/mL, demonstrating equivalent performance (Figure 2B).

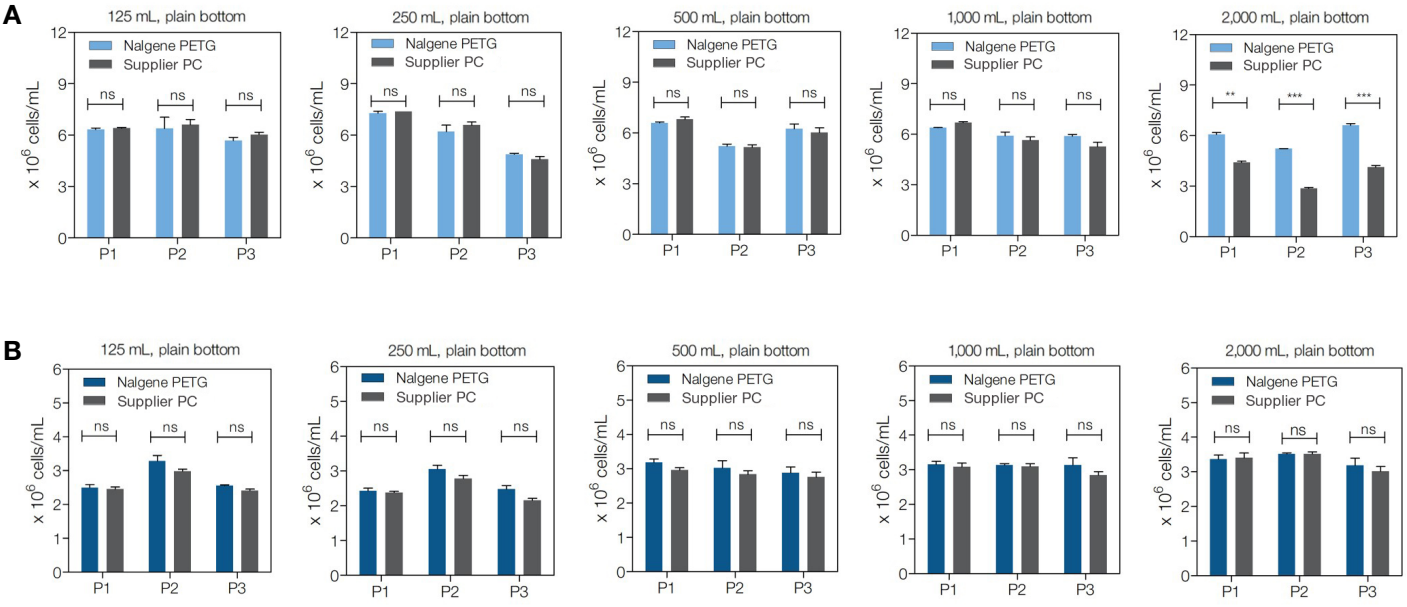

Figure 2. Comparison of viable cell densities of suspension cells in Nalgene PETG and Supplier PC flasks. ExpiCHO-S and Expi293 cells were subcultured for 3 passages (P1, P2, P3). Data were collected at the end of each 3-day culture period. **(A)** ExpiCHO-S cells showed comparable viable cell densities between Nalgene PETG and Supplier PC flasks at 125 mL, 250 mL, 500 mL, and 1,000 mL flask volumes. In 2,000 mL flasks at all 3 passages, Nalgene PETG flasks produced significantly higher cell densities (**P <0.01, ***P <0.001) than did Supplier PC flasks. **(B)** Expi293F cells showed nearly equivalent viable cell densities at every passage in both flask types, at all five volumes. No significant difference was observed between Nalgene PETG and Supplier PC flasks (n = 3; error bars represent SEM; ns: not significant).

A trypan blue exclusion assay showed that cell viability of ExpiCHO-S and Expi293F cells was comparable between Nalgene PETG and Supplier PC flasks across different volumes. The mean viability for all 3 passages in all flasks was >90%.

Adherent cell ring formation at the air–medium interface

Orbital shaking of a high-density cell suspension inside the incubator may result in formation of a ring-like structure, composed mainly of cells, at the air–medium interface of flasks (Figure 3). This ring is most apparent in cultures expanded for more than 4 days, and may increase in size over time. To investigate the impact to total cell growth, we collected and evaluated the cells found on the ring.

ExpiCHO-S and Expi293F cells were seeded in 5 different volumes of Nalgene PETG and Supplier PC flasks. ExpiCHO-S cells were expanded for 5 days, and Expi293F cells were expanded for 6 days. Cells that settled at the air–medium interface were then collected using Thermo Scientific™ Nunc™ Cell Scrapers, and samples were counted and assessed for viability by a trypan blue exclusion assay, using the Countess II Automated Cell Counter. Results showed that the mean viability of ExpiCHO-S and Expi293F cells deposited on the rings was significantly lower (<50%) than that of cells suspended in the medium (>90%). However, total counts of cells deposited on the rings were small, representing <1% of the total cells in each flask. Critically, no significant difference was observed in the amount of cells deposited on the rings, when comparing Nalgene PETG to Supplier PC flasks (Figure 4). Taken together, these findings indicate that the formation of the cell ring in long-term culture in Nalgene PETG flasks is unlikely to have a significant impact on peak cell growth or downstream assays, including protein expression yields.

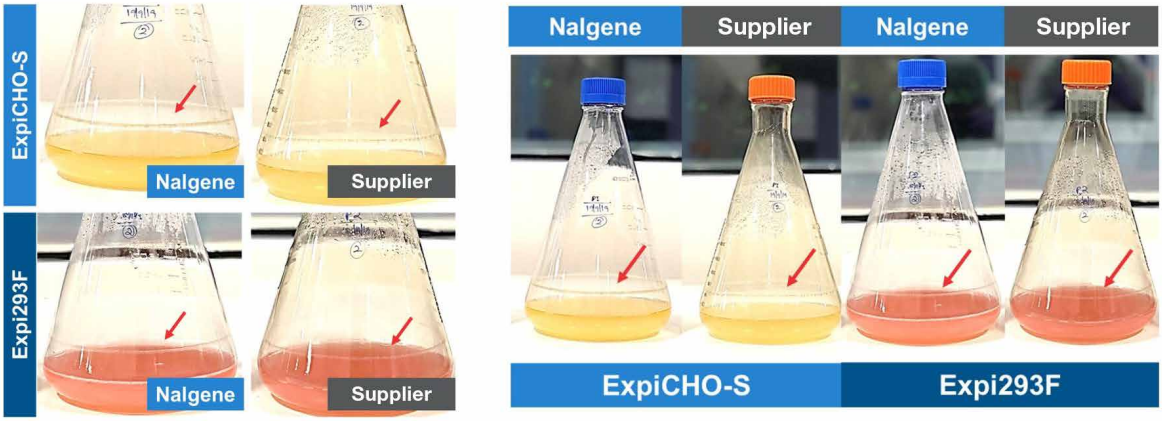

Figure 3. Adherent cell ring formation at the air–medium interface in Nalgene PETG and Supplier PC flasks. Each flask formed a similar ring on its interior surface, with no visual difference evident.

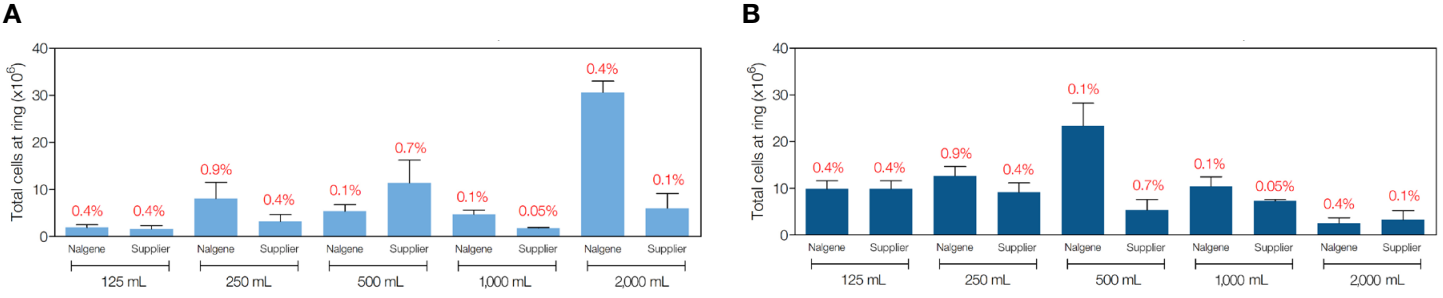

Figure 4. Assessment of cells settled at the air–medium interface in Nalgene PETG and Supplier PC flasks. Graphs show the number of adherent cells at the ring and percentage of adherent cells in the total cell population (numbers above bars in red). **(A)** ExpiCHO-S cells settled at the ring, compared between Nalgene PETG and Supplier PC flasks. **(B)** Expi293F cells settled at the ring, compared between Nalgene PETG and Supplier PC flasks. With both cell lines, the percentages of adherent cells deposited at the ring were minimal (<1%).

Conclusion

Nalgene Single-Use PETG Erlenmeyer Flasks provide performance comparable to that of Supplier PC flasks in the growth of CHO- and HEK293-based cultures. This suite of products allows for rapid expansion of high-quality suspension cells across a wide range of volumes. Altogether, Nalgene PETG flasks offer a great choice for suspension mammalian cell culture at an affordable price.

Ordering information

Product	Size	Cat. No.
Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom	125 mL	4115-0125
	250 mL	4115-0250
	500 mL	4115-0500
	1,000 mL	4115-1000
	2,000 mL	4115-2000
ExpiCHO-S Cells		A29127
Expi293F Cells		A14527
ExpiCHO Expression Medium		A2910002
Expi293 Expression Medium		A1435102
Expi293 Expression System Kit		A14635
ExpiCHO Expression System Kit		A29133
Trypan Blue Solution, 0.4%		15250061
Nunc Cell Scrapers		179707
Countess II Automated Cell Counter		AMQAX1000

Find out more at **thermofisher.com/erlenmeyerflask**

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