Disposable Spinner Flasks for Scale Up or Production of Proteins

Application Note

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Introduction

Suspension cell culture is increasingly being integrated into drug discovery campaigns, and suitable scale up vessels for suspension growth are evolving to keep pace. Historically, either disposable plastic shaker flasks or glass spinner vessels have been used for such culturing. However, shaker flasks have a mechanism of agitation that is different from spinner vessels which can negatively impact scale up efficiency, and glass spinner vessels require time-consuming and costly cleaning, assembly, sterilization, and validation. Corning 1L and 3L disposable spinner flasks are designed to be single-use with no impeller assembly required. We have tested disposable spinner vessels with a variety of cell lines including, SF9 (data not shown), CHO-S, and a hybridoma cell line used for the production of secreted antibodies. Cell densities as well as a variety of metabolic parameters were monitored daily. The data indicate equivalent performance between suspension cultures grown in disposable 1L and 3L vessels compared to standard glass vessels.

Materials and Methods

Cells were grown in 1L glass spinner flasks (Corning 4500-1L), 1L disposable spinner flasks (Corning 3561), and 3L disposable spinner flasks (Corning 3563). One liter vessels were filled with 500 mL of media and 3L vessels were filled with 1.5L of media and were placed on a slow speed stirrer at either 30 or 60 rpm. One mL samples were taken daily for metabolic and cell viability measurements using the BioProfile® FLEX analyzer (Nova Biomedical).

CHO-S cells were seeded at 2.5 x 10^5 cells/mL in serum-free CD CHO (Gibco) supplemented with 8 mM L-glutamine (Corning 25-005-CI) and 1X HT supplement (Corning 25-047-CI). Vessels containing CHO-S cells were incubated (humidified, 37°C and 5% CO_2) for 96 hours, spinning at 60 rpm.

The hybridoma cells (MH677), a proprietary cell line, were seeded at 5 x 10^4 cells/mL and also incubated (humidified, 37°C and 5% CO_2) for 96 hours, spinning at 30 rpm. These cells were cultured in IMDM (Corning 10-016-CM) and were supplemented with 10% fetal bovine serum (FBS, Corning 35-010-CF). On day 4, an additional 1 mL sample was taken from the hybridomas and was centrifuged at 1000 rpm for 5 minutes. The supernatants were collected and frozen for all three replicates in order to conduct an ELISA for antibody production.

An ELISA kit (Alpha Diagnostic International 6340) was used to quantify the production of mouse IgG2a in 1L and 3L disposable spinner flasks as compared to glass 1L vessels. Samples were thawed and diluted 500-fold with sample diluent from the ELISA kit. Samples were then processed following the manufacturer's recommended protocol and read on a SpectraMax® plate reader (Molecular Devices) at 450 nm and 630 nm to normalize for background.

Results and Discussion

To compare cell growth and vessel conditions CHO-S and hybridoma cells were grown in glass and disposable 1L and 3L vessels for 96 hours with samples taken daily. No significant differences were found in daily viable cell densities of CHO-S or hybridoma cells, regardless of culture vessel or size (Figures 1 and 2). The average yield for MH677 cells was slightly higher in disposable vessels vs. glass; however, this difference was not statistically significant. Using the Nova BioProfile FLEX analyzer we were able to analyze 13 different metabolic parameters of each culture vessel, including lactate, ammonia, pH, and dissolved carbon dioxide. Examples of the data are shown for air saturation and glucose consumption in Figure 3. As was the case with cell number, there was no significant difference in oxygen or glucose consumption. The same trend was observed for all 13 metabolic parameters measured.

To further investigate cell functionality in disposable spinner flasks, we measured the production of mouse IgG2a antibody, a secreted protein produced from our hybridoma cells. As shown in Figure 4, no significant difference in protein production was found in either 1L or 3L vessels. Further, this level of production was identical to the production of antibody in glass spinner vessels (data not shown).

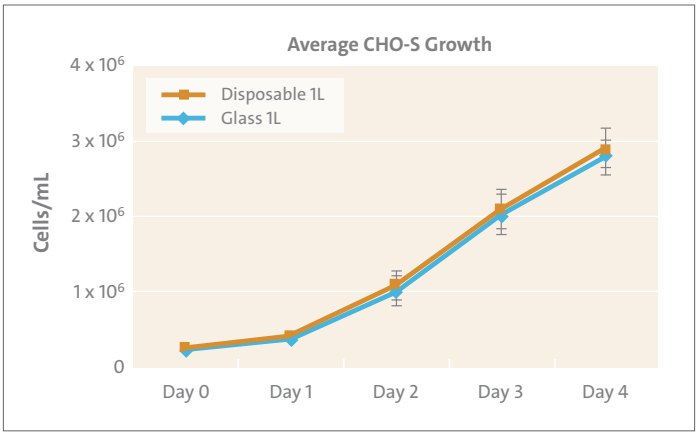

Figure 1. 96-hour growth of CHO-S cells in glass and disposable 1L spinner flasks from 3 studies at 60 rpm (n = 6). ± S.E. indicated by error bars; p>0.05.

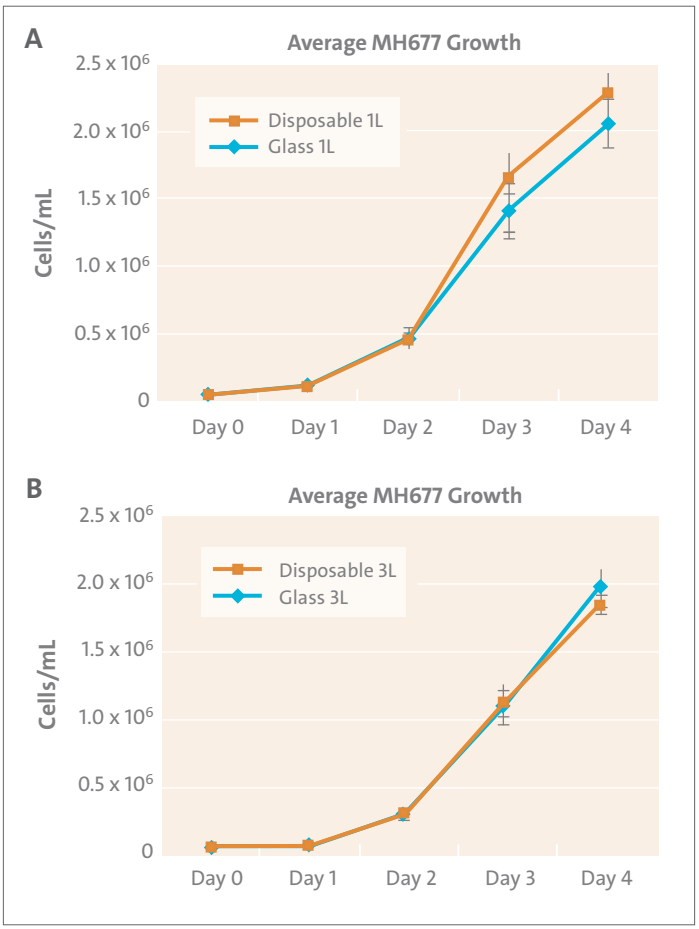

Figure 2. 96-hour growth of MH677 cells in glass and disposable 1L (A) and 3L (B) spinner flasks from 3 studies at 30 rpm (n = 6). ± S.E. indicated by error bars; p>0.05.

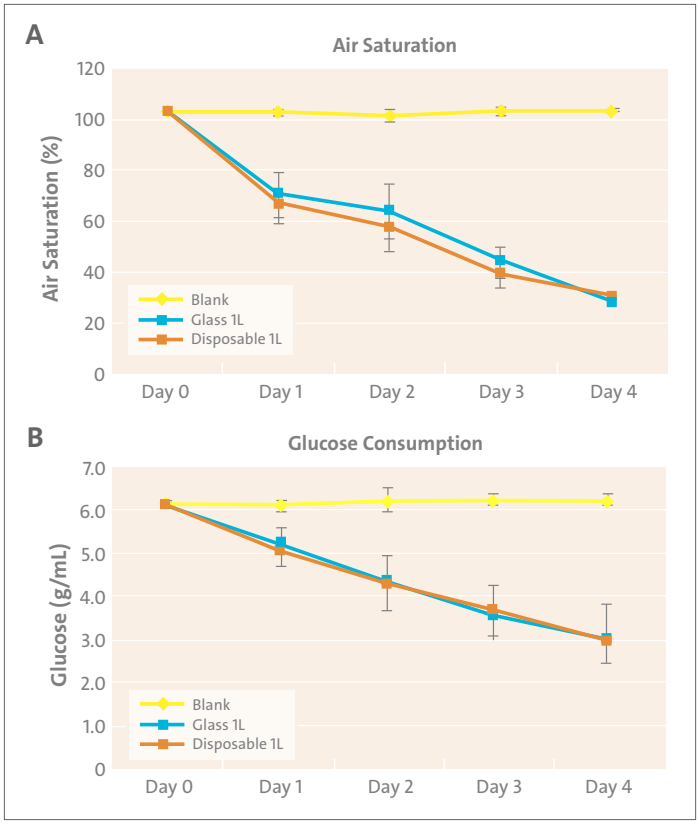

Figure 3. (A) Air saturation of CHO-S cultures in glass and disposable 1L spinner flasks taken over a 96-hour period. (B) Glucose consumption of CHO-S cultures sampled over a 96-hour period. ± S.E. indicated by error bars; p>0.05.

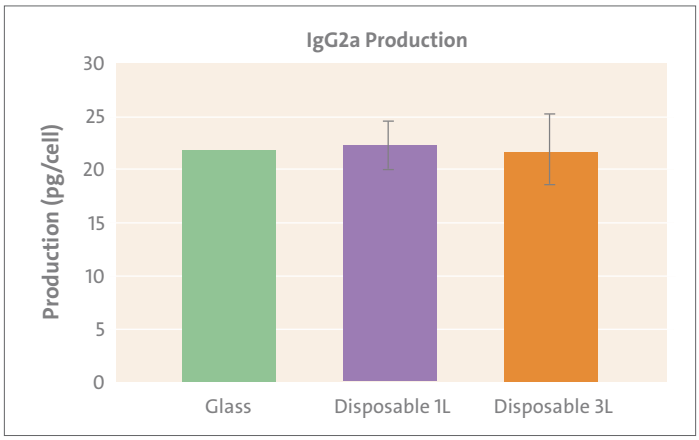

Figure 4. Mouse IgG2a production after 96-hour growth of MH677 cells in glass (1L) and disposable (1L and 3L) spinner flasks from 3 studies at 30 rpm (n = 6). For 1L glass n = 2; n = 4 for disposables. ± S.E. indicated by error bars; p>0.05.

Conclusions

- Disposable large volume spinner flasks are equivalent to conventional glass spinner flasks for cell growth and viability.
- A variety of cell types, including SF9 (data not shown), CHO-S, and a hybridoma cell line (MH677), can be cultured in large volume disposable spinner flasks.
- Disposable spinner flasks attain equal aeration compared to standard glass spinner flasks.
- Cells from glass and disposable spinner flasks are metabolically equivalent.
- Cells in disposable spinner flasks produce equal amounts of secreted proteins, such as Mouse IgG2a, as cells grown in traditional glass spinner flasks.

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