Corning® CELLine™ Disposable Bioreactor and Corning hybrigro™ SF Medium Offer a Complete Solution for Serum-free Hybridoma Scale-up and Protein Production

Application Note

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Introduction

Monoclonal antibodies (mAb) are widely used for biochemistry, molecular biology, and as potential therapeutic candidates. The ability to generate large quantities of mAb to meet these basic research and other large-scale manufacturing needs can be challenging. The Corning CELLine disposable bioreactor enables the *in vitro* generation of high yields of mAb in an easy-to-use, disposable vessel. In addition to producing higher product yields when compared to traditional static vessels[1-2], the final product is also more concentrated. This is due to a two chamber design separated by a 10 kDa molecular weight cut-off membrane that allows nutrients and growth factors to diffuse into the production chamber, while retaining the mAb or cell product (e.g., recombinant protein) in a separate low volume compartment. This effectively reduces the downstream processing time that would be necessary to concentrate the final product. The reduced processing, in addition to higher overall production yields, can result in a 50% cost reduction per milligram of product produced[2]. To demonstrate increased protein production and the concentration of product generated using this system, hybridoma cells were cultured under serum-free conditions using Corning hybrigro SF medium in the Corning CELLine disposable bioreactor and traditional cell culture flasks. Corning hybrigro SF is a complete, animal component-free, defined medium specifically developed for serum-free growth and antibody production. This medium has been shown to support higher protein production compared to serum-containing culture medium[3].

Methods and Materials

MH677 cells (proprietary murine hybridoma cell line) were cultured in standard tissue culture flasks in hybrigro SF medium (Corning Cat. No. 40-215-CV). Cell densities were maintained between 2.5 x 10^4 and 1.5 x 10^6 cells/mL by passaging cells twice per week using the Vi-CELL® cell viability Analyzer (Beckman Coulter) for viable cell enumeration. MH677 cells were expanded to seed 15 mL at 2.5 x 10^4 cells/mL into two T-75 flasks (Corning Cat. No. 430641U) and two CELLine disposable bioreactor vessels (Corning Cat. No. 353137) following the protocol provided (Corning Literature Code CLS-DL-CELLine-13)[4], which includes filling the nutrient compartment with 1L of medium. T-75 vessels were harvested every 3 to 4 days, retaining a sample for viable cell enumeration, as well as 1 mL of medium to be frozen and stored for future analysis of IgG2a production. Cells collected from T-75 flasks were then re-seeded into new flasks with 15 mL of medium using 2.5 x 10^4 cells/mL. CELLine disposable bioreactors were sampled, and passaged every 7 days without changing the 1L of medium contained in the nutrient compartment. Cells were maintained in a humidified incubator at 37°C and 5% CO_2 for the duration of the 21-day culture period. To assess protein production, mouse IgG2a ELISA kits (Innovative Research Cat. No. IRKTAH1156) were

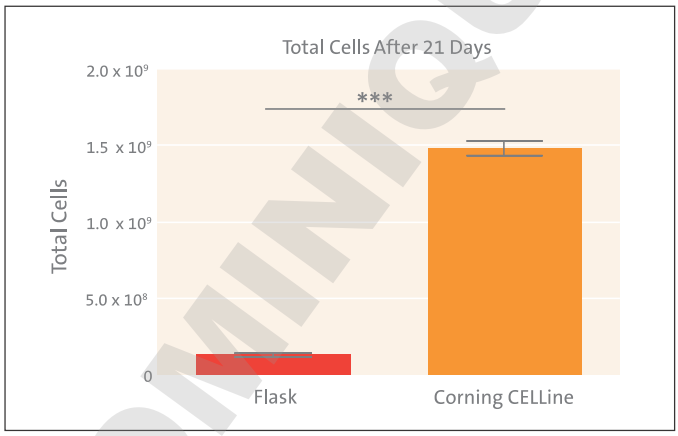

Figure 1. Total MH677 cell yields. Significantly more MH677 cells were recovered from Corning CELLine bioreactors after 21 days of culture compared to cells grown in T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

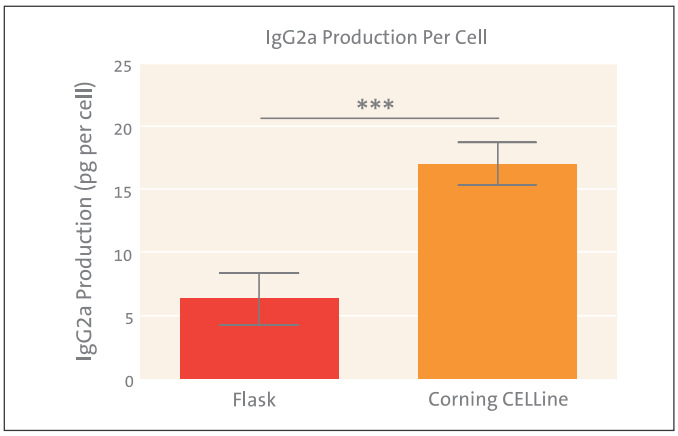

Figure 2. IgG2a production per cell. MH677 cells grown in the Corning CELLine bioreactors produced more IgG2a on a per cell basis compared to cells grown in T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

used according to the manufacturer's protocol. Cell counts and IgG2a production from each harvest (3 for Corning® CELLine™ disposable bioreactors, and 6 for T-75 vessels) were combined to achieve the total yield after 21 days in culture.

Results and Discussion

The unique design of the CELLine disposable bioreactor combined with Corning hybrigro™ SF medium is ideal for users that require high yields of concentrated mAb that are produced under defined, serum-free culture conditions. The total yield of MH677 cells from each CELLine disposable bioreactor was approximately 12-fold greater than the cell yield derived from the T-75 vessels after 21 days of culture (Figure 1). In addition to the generation of greater cell yields, MH677 cells produced approximately 3X greater IgG2a on a per cell basis when cultured in the CELLine disposable bioreactor compared to the T-75 vessels (Figure 2). By combining greater cell yields with higher mAb production efficiency, the cumulative production of mAb was 30-fold greater in the CELLine disposable bioreactor when compared to a standard T-75 flask (Figure 3). In addition to the benefit of attaining more protein with fewer vessels and reduced cell maintenance, there is also a time and cost savings associated with the reduced need for post-processing steps to concentrate mAb produced in the CELLine disposable bioreactor[2]. Based on the total volume of supernatant collected after 21 days in culture, the IgG2a collected from CELLine disposable bioreactors was 60 times more concentrated compared to the more dilute product collected from the T-75 vessels (Figure 4). Although a higher volume of cell culture medium is required in the CELLine disposable bioreactor, the mAb production efficiency per volume of medium is significantly higher when compared to the production per volume of medium used in a T-75 vessel (Figure 5).

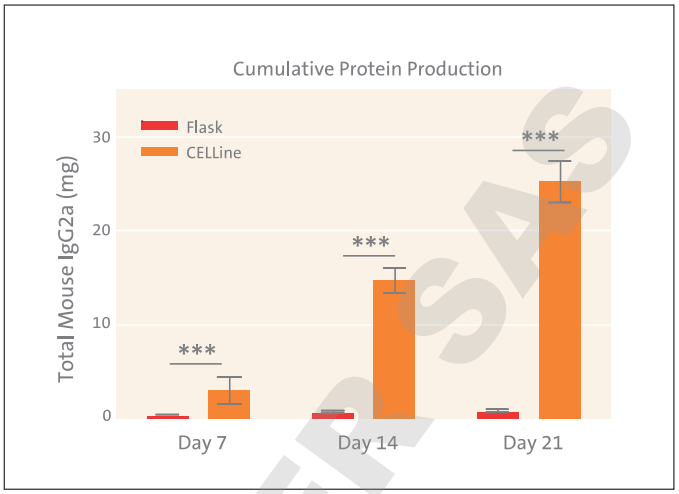

Figure 3. Cumulative IgG2a production. Significantly more IgG2a was produced in the Corning CELLine bioreactors when compared to production from T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

Conclusions

- The Corning CELLine disposable bioreactor sustained 21 days of hybridoma culture, resulting in greater viable cell yield than that achieved in T-75 flasks.
- Significantly more mAb was produced on a per cell basis in the CELLine disposable bioreactors than produced in T-75 flasks.
- Use of the CELLine disposable bioreactor results in higher concentrated protein product yields, which may reduce costs due to decreased handling required to passage cells, change medium, and concentrate the desired final product.

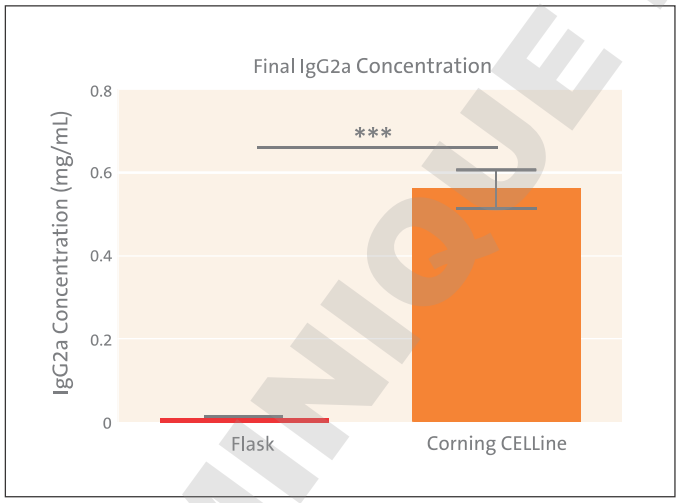

Figure 4. Final IgG2a concentration. When pooled, IgG2a collected from Corning CELLine bioreactors was 60 times more concentrated than IgG2a collected from T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

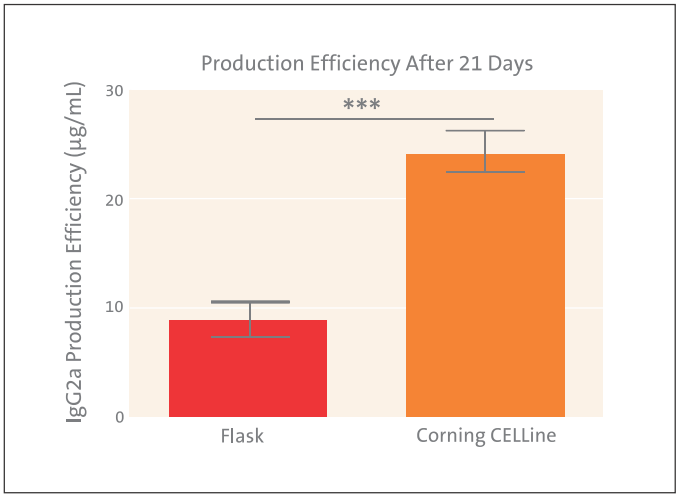

Figure 5. Production efficiency. Taking into account the higher volumes of medium required to fill the Corning CELLine bioreactor, the IgG2a production efficiency per volume of medium used was significantly greater than the T-75 flasks. Data shown with standard deviation. Unpaired T-test ***p<0.001. n=6 vessels from 3 independent studies.

References

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2. Mohamed Trebak, Jae Min Chong, Dorothee Herlyn, and David W Speicher. Efficient laboratory-scale production of monoclonal antibodies using membrane-based high-density cell culture technology. Journal of Immunological Methods (1999) 230:59-70.
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4. Hilary Sherman and Mark E Rothenberg. Quick guide for antibody production in a Corning CELLine disposable bioreactor (2014). Corning Literature Code CLS-DL-CELLine-13.

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