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Application Note

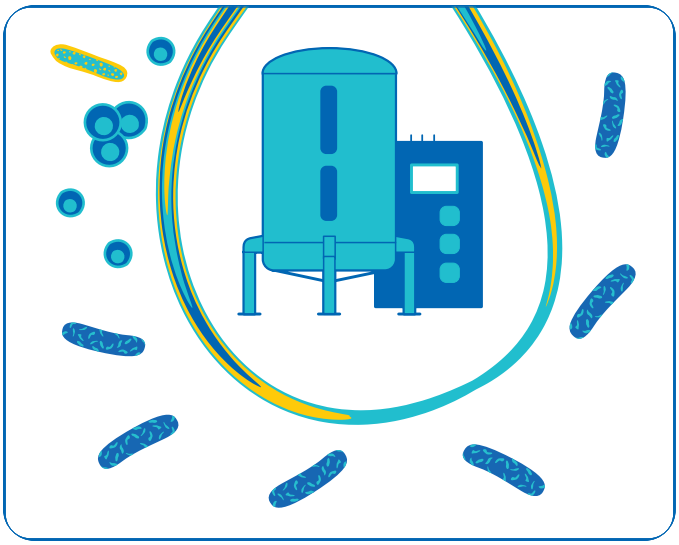
Cell Culture Media Filtration

Evaluating Cell Culture Performance

Summary

Selecting a membrane filter for processing cell culture media is highly dependent on the specific process needs. An important consideration is cell culture performance in media that has been processed through the selected filter. This application note describes an evaluation of CHOZN® GS antibody-producing CHO cell line performance in EX-CELL® Advanced CHO Fed-batch medium filtered through different sterilizing-grade filters.

Results showed that both growth and cell viability profiles were consistent, irrespective of which membrane filter was used for cell culture media processing. For this cell line and cell culture medium combination, equivalent cell culture performance was observed following media filtration through 0.1 µm and 0.2 µm polyethersulfone (PES) membrane filters. Importantly, performance was equivalent to that of 0.1 µm Durapore® polyvinylidene fluoride (PVDF) membrane filters.



Introduction

Optimization of upstream cell culture processes is key to developing a robust, efficient manufacturing template for biopharmaceuticals. Selecting the right cell culture medium is an important first step. Cell culture media are available in a pre-sterilized liquid format or as dry powders. Dry powder media is generally reconstituted at point-of-use, and the bulk aqueous solution is then sterilized before use in bioreactors[1]. Some manufacturers treat reconstituted cell culture media with high temperature short time (HTST) or other physical treatments before use[2]. More commonly, reconstituted media is filtered through sterilizing-grade membrane filters before transfer to bioreactors.

When selecting a membrane filter for processing cell culture media, users should consider microbial retention objectives, filter sizing and membrane area requirements based on throughput testing, as well as the planned process conditions[3]. Perhaps the most important consideration before finalizing selection is confirming acceptable cell culture performance in the filtered media to assure process objectives can be met.

This application note describes an approach for assessing cell culture performance in filtered cell culture media. The results of this type of assessment should be considered with bioburden risk reduction objectives and filter sizing information before finalizing selection of a filter for processing cell culture media.

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Materials and Methods

Medium Preparation and Filtration

A 3 L batch of EX-CELL® Advanced CHO Fed-batch powdered medium (Sigma-Aldrich Cat. No. 24366C) was prepared. Aliquots of media were filtered through Millipore Express® polyethersulfone (PES) membrane filters and through 0.1 µm Durapore® polyvinylidene fluoride (PVDF) filters, which acted as a control.

All membranes were tested in 47 mm stainless steel filter holders with polypropylene support material downstream of the membranes. Four replicate holders were assembled for each membrane: three replicates for the 20 L/m^2 test condition representing 'worst-case', (20 L/m^2) and a single replicate for the low throughput condition (300 L/m^2). All assembled filter holders were autoclaved before use.

After autoclaving, media was processed through the sterilized filter holders at 50 mL/min flowrate and collected in sterile glass bottle assemblies. Filtered media was then used for cell growth studies.

Cell Growth Study in Spin Tube Bioreactors

Spin tube bioreactors (three per test condition) were inoculated at 0.3 x 10^6 cells/mL with CHOZN® GS antibody-producing CHO cells in 25 mL filtered cell culture medium. The spin tubes were cultured in an incubator at: 37.0 °C, 80% relative humidity, 5.0% CO_2, 300 RPM agitation speed. Samples were collected daily from each bioreactor and analyzed for Viable Cell Density (VCD) and viability using the Beckman Coulter Vi-CELL™ XR Cell Viability Analyzer. Sampling was stopped when peak VCD was reached.

Results and Discussion

As with any bioprocess operation, there is a risk of unexpected interactions between plastic components and the process fluid. Before selecting a filter for processing cell culture media, it is recommended to confirm acceptable cell culture performance in the filtered media. In order to more easily detect any negative impact of leachables or adsorbed components from the filter or filtration process on cell growth, the ratio of membrane area to medium volume should be maximized. This represents a relatively low filtration throughput, corresponding to 'worst-case' conditions.

This study evaluated cell viability and cell growth of CHOZN® GS cells in EX-CELL® Advanced CHO Fed-batch medium filtered with Millipore Express® membranes. The cell growth assessments were performed using spin tube bioreactors which offer a small volume, low cost, easy to use, experimental format. Results of VCD and viability profiles are shown in **Figure 1**.

The VCD profiles for all test conditions are similar. For each test membrane, differences between the ‘worst-case’ and ‘low throughput’ filtration conditions were minimal. Moreover, cell density profiles were consistent irrespective of which membrane filter was used demonstrating that the membrane filter did not impact growth of the CHOZN® GS cells in EX-CELL® Advanced CHO Fed-batch medium.

Table 1: Test conditions for evaluating impact of filtration membranes on cell culture medium and subsequent cell growth. All membranes were tested in 47 mm disc format (17.7 cm^2).

Membrane	Membrane Pore Size and Material	Replicates	Filtration Throughput (L/m^2)*	Volume of medium required per filter (mL)
Millipore Express® SHR	0.1 µm PES	3	20	30
		1	300	415
Millipore Express® SHR with Prefilter (SHRP)	0.5/0.1 µm PES	3	20	30
		1	300	415
Millipore Express® SHC	0.5/0.2 µm PES	3	20	30
		1	300	415
Durapore® 0.1 µm Control Membrane	0.1 µm, PVDF	3	20	30
		1	300	415

* Two low-throughput conditions were tested: 20 L/m^2 (worst-case condition) and 300 L/m^2 (low throughput condition).

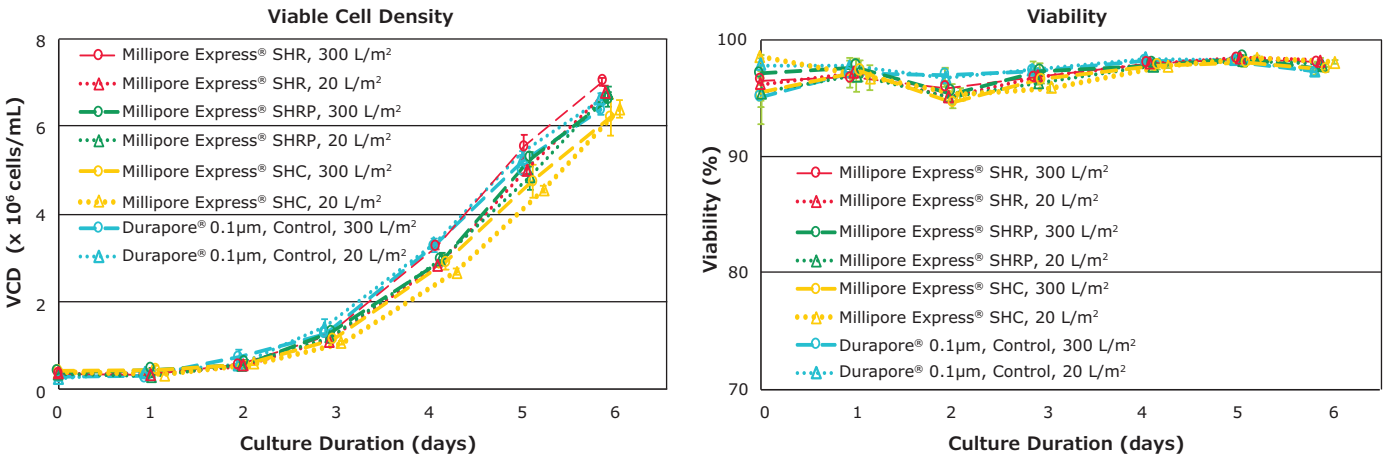

Figure 1: Viable cell density and viability of CHOZN® GS cells in filtered EX-CELL® Advanced CHO Fed-batch Medium. Each data point represents the average of triplicate runs, with error bars representing one standard deviation.

Similarly, there were minimal differences in cell viability between the tests with different membrane filters or filtration volumes. Cell viability remained high throughout the duration of culture for all test conditions. These results confirm no negative interactions between the filter and cell culture medium that might impact cell viability.

This data set shows that filtering cell culture media through either 0.1 µm or 0.2 µm Millipore Express® PES membrane filters, yields comparable cell culture performance to that achieved with traditional PVDF membrane filters.

Even though an extremely low filtration throughput of 20 L/m² may not be used practically, for process understanding it is helpful to assess performance under these 'worst-case' test conditions. However, if a user is considering these types of evaluations, it is recommended that scale-down cell culture studies should be representative of processing conditions (throughput, flux, time) that will be used at large scale. For these type of studies, OptiScale® 25 capsules are useful tools for filtering smaller volumes of cell culture media.

Conclusion

Cell culture performance is a key metric for consideration in sterilizing filter selection. In this study, we describe an experimental approach for assessing cell culture performance. Cell growth was evaluated in spin tube bioreactors and a 'worst-case' low throughput filtration test condition was included to maximize the likelihood of detecting any negative impact of filtration on cell growth or viability.

Results showed that for the CHOZN® GS cells with EX-CELL® Advanced CHO Fed-batch medium:

- No differences in cell viability and cell density were observed following growth in media processed through PES or PVDF membranes filters
- Filtration over membrane filters with 0.1 µm or 0.2 µm pore size did not affect cell culture performance.

For a comprehensive assessment of all components, these types of studies could be expanded to include single-use consumables and components for mixing, fluid handling and storage[1]. It is always recommended that any assessment is conducted with the cell line and cell culture medium, under the same processing conditions that will be used in scale-up.

References

1. White Paper "Mitigating Risks Associated with Cell Culture Media Preparation and Handling", Lit. No. WP3648EN, Merck KGaA, Darmstadt, Germany
2. Barone, P. et al (2020) Viral Contamination in biologic manufacture and implications for emerging therapies. Nature Biotechnology https://doi.org/10.1038/s41587-020-0507-2
3. Application Note “Cell Culture Media Filtration: Filter Selection and Sizing”, Lit. No. AN5144EN, Merck KGaA, Darmstadt, Germany

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