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Application Note

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Evaluating the Filterability of Chemically Defined Cell Culture Media with the Virosart® Media Filter

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Abstract

The contamination of bioreactors with adventitious agents such as bacteria, mycoplasma, and viruses is a potential risk to patient safety. Viruses have been the cause of multiple bioreactor contamination events in recent years. A number of biopharmaceutical companies have reported production-scale bioreactor contamination events by small non-enveloped viruses such as minute virus of mice (MVM) or vesivirus[1]. The consequences of such an event may be severe and result in GMP facility contaminations, along with drug shortages and financial losses. Therefore, several large biopharmaceutical operations are evaluating risk mitigation strategies for the minimization of contaminations by adventitious agents. Classical sterilizing-grade filters and even 0.1 µm-rated filter membranes cannot prevent contamination by small non-enveloped viruses[2].

Size exclusion-based filtration is the preferred technology for virus clearance, as it is robust and non-invasive. The Virosart® Media filter mitigates virus contamination risks which may arise from the addition of nutrients and other additives into the bioreactor system.

Introduction

The Virosart® Media filter has been developed specifically for chemically defined cell culture media. The filter is an asymmetric polyethersulfone hollow fiber membrane with 20 nm nominal pore size rating that exhibits high capacity (1000 L/m^2 at 2 bar in 4 hour filtration time) for filtration of chemically defined cell culture media while providing ≥ 4 LRV (log_{10} reduction value) for small non-enveloped viruses and ≥ 6 LRV for large enveloped viruses[3].

The purpose of this study was to evaluate how effectively the Virosart® Media filter can process several different chemically defined cell culture media (table 1). In total, three studies were conducted:

Study 1: Filtration Capacity

Study 2: Evaluation of Different Pre-filters

Study 3: Impact of a 0.1 µm Pre-filter on Virosart® Media Performance

Media	Supplier	Cat. No	Cell line	NAO*	Protein free	Peptide free	CD**	Gln containing	Polaxamer containing
Express Five™ SFM	Thermo Fischer	10486025	High Five	N/A	Yes	N/A	N/A	No	0.2%
CD CHO Medium		10743-029	CHO	Yes	Yes	Yes	Yes	No	Yes
VP-SFM AGT™		12559-027	MDCK, COS-7, BHK-21, VERO, HEp2	Yes	No	Yes	N/A	No	0%
PERMEXCIS	Sartorius	BE02-039Q	PER.C6® and related cell lines	Yes	Yes	No	Yes	No	Yes
Power CHO™ Advanced		12-929Q	CHO	Yes	Yes	No	Yes	No	No
Power CHO™ 2		BE12-771Q	CHO (DG44, CHO-S, CHO K1, DHFR-...)	Yes	Yes	No	Yes	No	Yes
Power CHO™ 3		12-772Q	CHO (DG44, CHO-S, CHO K1, DHFR-...)	Yes	Yes	No	Yes	No	Yes
Power Feed™ A		BE02-044Q	CHO	Yes	Yes	Yes	Yes	No	No
ProCHO™ 4		BE12-029Q	CHO (DG44, CHO-S, CHO K1, DHFR-...)	Yes	Yes	No	No	No	0.1%
ProCHO™ 5		WPW-045D		Yes	Yes	No	No	No	0.1%
ProPer™ 1		BE02-028Q	PER.C6® and related cell lines	Yes	Yes	No	Yes	No	0.2%
UltraMDCK™		BE12-749Q	MDCK	No	No	No	Yes	Yes	Yes
Ex-Cell® 325 PF CHO	Sigma-Aldrich	24340C-1L	CHO	N/A	Yes	N/A	N/A	No	N/A
Ex-Cell® EBx™ GRO-I		24530C		Yes	No	N/A	N/A	N/A	N/A
Ex-Cell® CD CHO-3		C1490-1L		Yes	N/A	N/A	Yes	No	0.2%
ExCell® MDCK		SAFS24581C-1L		N/A	Yes	N/A	Yes	No	N/A

Table 1: Chemically defined cell culture media used to evaluate the filterability characteristics with
Yes = contained in the cell culture media, No = not contained in the cell culture media, N/A = information not available[4-5]
* Non-animal origin; ** chemically defined

Material and Methods

Filtration trials were performed on the Virosart® Media with and without an inline pre-filter depending on the study. Virosart® Media lab modules (5 cm^2, part number: 3V2--28- BVGML--V) and Sartopore® 2 XLM SartoScale 25 0.1 µm-rated (4.5 cm^2, part number: 5445358MV--LX--C) were used.

The different dehydrated media described in table 1 were reconstituted in deionized water according to the manufacturer's instructions. Before each run, the filters were flushed for 15 minutes with deionized water at 2.0 bar | 30 psi using compressed air and the water flux recorded. The deionized water in the reservoir was replaced with the cell culture media to be tested. The filtration was performed at room temperature (20 – 22°C | 68 – 71.5°F) at constant pressure of 2.0 bar | 30 psi. The filtration was stopped after 240 min. The weight of the collected filtrate was recorded at specific time points in order to calculate the flow rate, flux and flux decay.

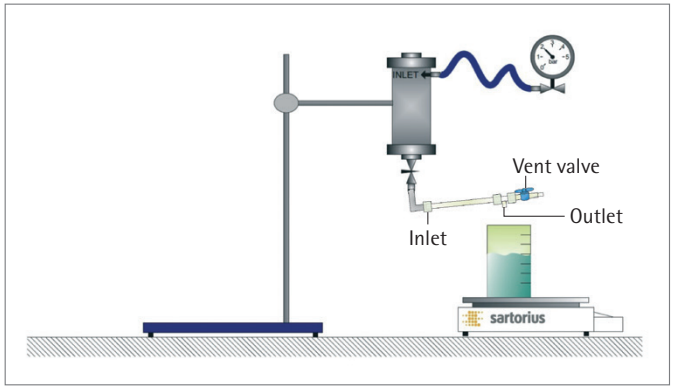

Figure 1: Experimental set-up of small-scale filtration using Virosart® Media lab module 5 cm^2

Results and Discussion

Study 1: Filtration Capacity

The capacity of the Virosart® Media membrane was determined with 16 different chemically defined cell culture media from three different suppliers. Figure 2 shows the capacity data [L/m^2] during a 4 hour filtration at constant pressure of 2.0 bar | 30 psi. The data shows that volumes of media that could be processed varied widely depending on the composition of the media. Some media tended to block the filter relatively quickly whereas other media did not appear to block the filter at all. The Virosart® Media filter showed highest capacity with VP SFM AGT™ media and ProPer™ 1 of over 1500 L/m^2 for a 4-hour duration.

For most media the targeted total capacity of approximately 800 – 1000 L/m^2 after 4 h filtration is reached with Virosart® Media. However Ex-Cell® MDCK, Express Five™ SFM and PowerCHO™ 2 tended to block the filter more rapidly. Media supplements such as polaxamer or soy hydrolysates are known to have an impact on filtration performance and can reduce the flux rate dramatically[6]. Reducing the polaxamer concentration or adding it to the media after filtration can increase the filter capacity significantly.

Figure 3 shows the total capacity with Virosart® Media for the different cell culture media after 4 hour filtration time at 2.0 bar | 30 psi. The developed 20 nm Virosart® Media exhibits a high total capacity of approximately 800 – 1000 L/m^2 within 4 hours filtration time for many commercially available cell culture media.

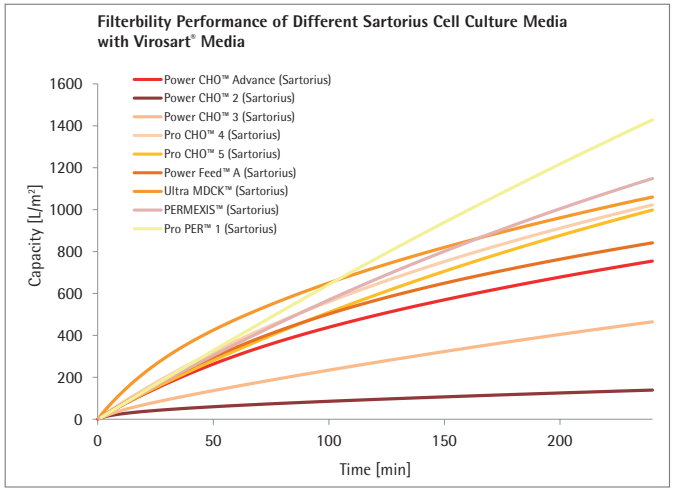

Figure 2a: Filterability performance for 9 different chemically defined cell culture media from Sartorius with Virosart® Media over 4 hour filtration time at 2.0 bar | 30 psi

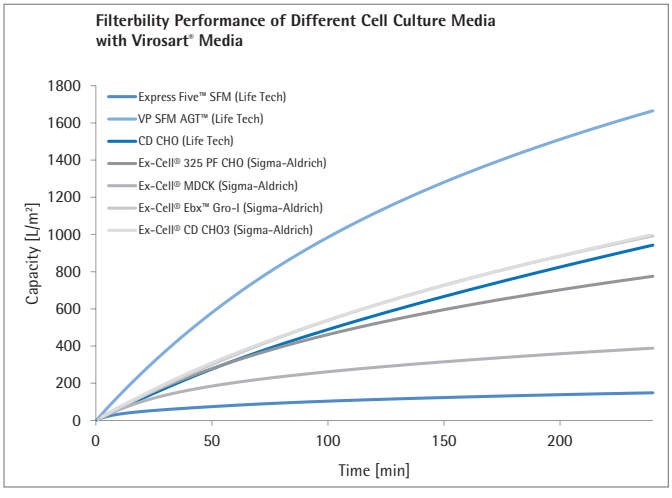

Figure 2b: Filterability performance for 7 different chemically defined cell culture media from Life Tech and Sigma-Aldrich with Virosart® Media over 4 hour filtration time at 2.0 bar | 30 psi

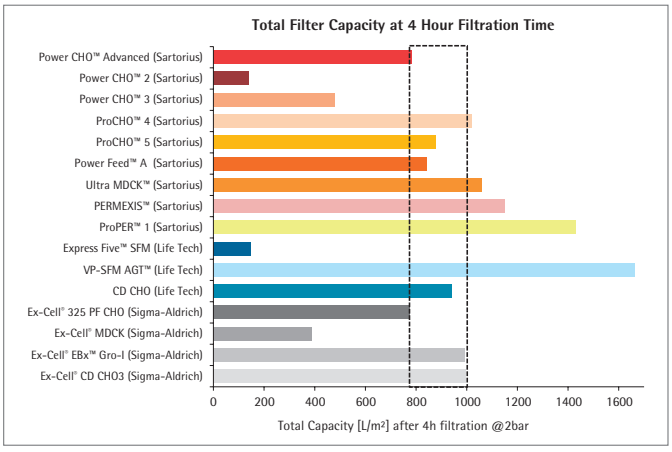

Figure 3: Total filter capacity for 16 different chemically defined cell culture media with Virosart® Media after 4 h filtration

Study 2: Evaluation of Different Pre-filters

The impact of different in-line pre-filters on the overall filterability characteristic of Virosart® Media was evaluated using four different pre-filters from three suppliers. The Sartopore® 2 XLG 0.2 µm and Sartopore® 2 XLM 0.1 µm asymmetric PES filters from Sartorius Stedim were tested along with a 0.1 µm rated PVDV | PES (PF I) and a 0.1 um rated PES (PF II) pre-filter.

Figure 4 shows the capacity data [L/m²] for the 4 different in-line pre-filters tested over four hour filtration at constant pressure of 2.0 bar | 30 psi with Ex-Cell® 325 media. The biggest difference could be seen between the 0.1 µm and a 0.2 µm pre-filter while little difference was observed between the three different 0.1 µm filters. The difference in performance between the 0.1 µm and a 0.2 µm pre-filters can be explained by size-exclusion. Therefore, we recommend the Sartopore® 2 XLM as a 0.1 µm in-line pre-filter.

In Figure 5 the total capacity [L/m²] of the different pre-filters are shown after 4 hours of filtration at 2.0 bar | 30 psi.

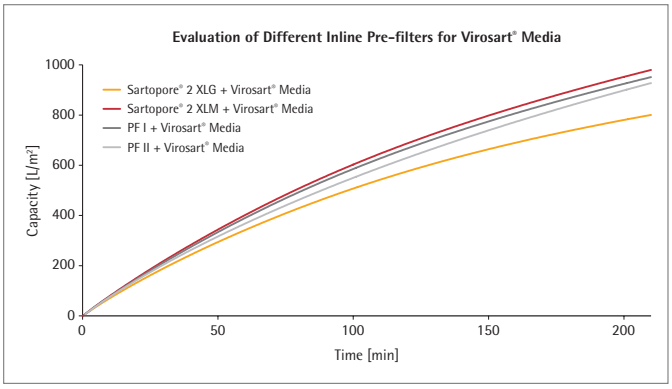

Figure 4: Evaluation of different in-line pre-filters for Virosart® Media in Ex-Cell® 325 media over 4 hour filtration time

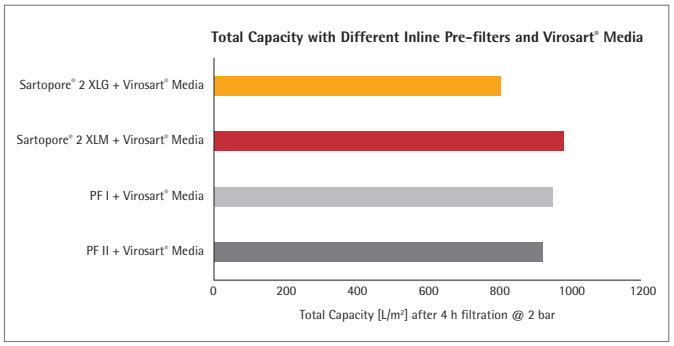

Figure 5: Impact of different pre-filters on the overall filterability characteristics of Virosart® Media in Ex-Cell® 325 media after 4 hours of filtration at 2.0 bar | 30 psi

Study 3: Impact of a 0.1 µm Pre-filter on Virosart® Media Performance

The impact of a 0.1 µm inline pre-filter on the overall filterability characteristics of Virosart® Media was evaluated. The filtration run was performed with five different cell culture media from three suppliers with and without the use of the Sartopore® 2 XLM as an inline pre-filter. The Sartopore® 2 XLM is a 0.2 | 0.1 µm pleated polyethersulfone filter.

Figure 6 shows the capacity data [L/m²] during a 4 hour filtration at constant pressure of 2.0 bar | 30 psi.

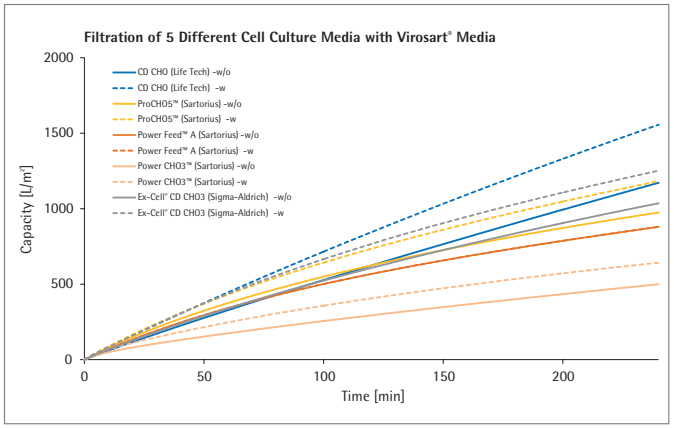

Figure 6: Impact of 0.1 µm inline pre-filter on the overall filterability characteristics of Virosart® Media for 5 different cell culture media

Figure 7 shows the total capacity of the different cell culture media with (w) and without (w/o) the use of a Sartopore® 2 XLM as an in-line pre-filter after 4 hour filtration time at 2.0 bar | 30 psi. The total capacity is increased by over 20% for most of the tested media (4 out of 5) by adding a 0.1 µm inline pre-filter. The improvement is based on size-exclusion and the use of a 0.1 µm inline pre-filter is recommended.

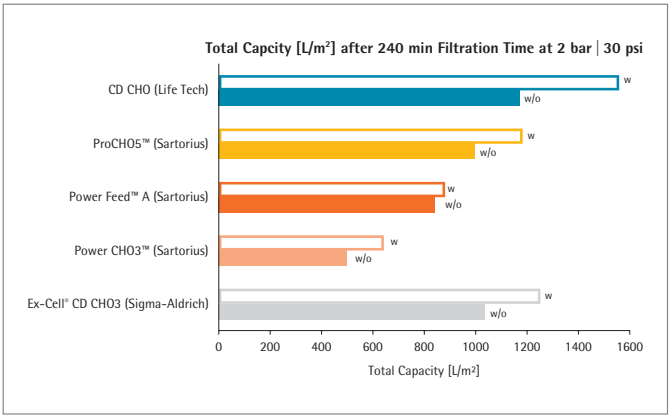

Figure 7: Impact of 0.1 µm inline pre-filter on the overall filterability characteristics of Virosart® Media for 5 different cell culture media after 4 hour filtration time at 2.0 bar | 30 psi

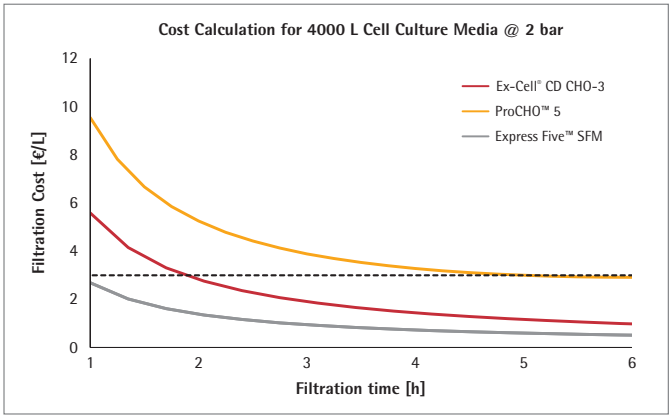

Figure 8: Cost Calculation for three cell culture media of 4000 L with Virosart® Media at 2 bar | 30 psi over 6 hour filtration time

Filter Sizing

The pre-filter final filter ratio in the trial above was 1:1 with respect to the filtration area. Further studies were performed in order to optimize the ratio of pre-filter to final filter area. The optimal ratio we recommend based on overall flow rate is 1:5 – 1:10 mainly depending on a minimum of filter devices to avoid connections and line splits. During scale-up, we recommend a 35 % safety margin based on the actual data of the filtration trials in small scale.

Economic Analysis

The cost of performing the filtration of 4000 L of three different cell culture media was calculated. The three media were the Ex-Cell® CD CHO-3, ProCHO™ 5 and Express Five™ SFM media over a filtration time of 6 hours at 2.0 bar | 30 psi (figure 8). The filter costs are in the range of 1 – 3 €/L media depending on the filterability of the cell culture media. The costs of filtration are lower with decreasing polaxamer concentration e.g. ProCHO5™ contains 0.2% whereas VP-SFM AGT™ contains none. Also, a longer processing time of over 4 hours reduces the filtration cost further. The cost effective filtration of cell culture media is feasible with the Virosart® Media and mitigates the risk of viral contaminations of cell cultures.

Summary and Conclusions

The data demonstrates that the Virosart® Media is the filter of choice for upstream applications where high capacities and process economics are desired. The newly developed 20 nm Virosart® Media has a high total capacity of approximately 800 – 1000 L/m^2 for commercially available cell culture medias during a 4-hour filtration. Different pre-filters were tested to improve the overall capacity of Virosart® Media. Sartopore® 2 XLM, a 0.1 µm pre-filter was found to have the largest impact. Furthermore, different cell culture media were tested with Sartopore® 2 XLM as a pre-filter. The total capacity can be increased by over 20% for most of the media tested by using a 0.1 µm inline pre-filter. This makes Virosart® Media an economically feasible method for the batch preparation of chemically defined cell culture media while reducing the risk of viral contaminations.

References

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2) A. Manzke and B. Kleindienst, Virus Risk Mitigation in Cell Culture Media, BioPharm International, October 2016

3) Application note: Retention Characteristics of Virosart® Media when filtering Chemically Defined Cell Culture Media, Publication No.: SPK4116-e, Order No.: 85037-560-92

4) Link: https://www.thermofisher.com/

5) Link: http://www.sigmaaldrich.com/

6) Application note: Influence of Cell Culture Media Components on the Filtration Characteristics of Virosart® Media, Publication No.: SPK4118-e, Order No.: 85037-560-94

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