Protection of Biomanufacturing Processes from Virus Contamination through Upstream Virus Filtration of Cell Culture Media

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Abstract

Medical progress as enabled by early plasma products has also revealed biological safety challenges. The combination of donor selection, donation testing and virus reduction processes has effectively addressed these concerns, and today medicinal plasma products feature significant safety margins. The safety tripod concept has since been adapted to biotechnology manufacturing platforms and has also ensured the safety of these products. However, cell-based manufacturing processes have occasionally been exposed to adventitious viruses, leading to manufacturing interruptions and unstable supply situations. The rapid progress of advanced therapy medicinal products (ATMPs) needs an innovative approach to ensure the learnings from more traditional biotechnology help to avoid any unwelcome reminder of the universal presence of viruses. The introduction of up-stream virus clearance steps has already been shown to be valuable for any products too complex for down-stream interventions in the sense of both assuring product safety and continuous supply. The gentlest method being virus filtration – the development of which is presented here. The experiments investigated the feasibility of implementing culture media virus filtration with respect to their virus clearance capacities under extreme conditions such as very high process feed loading (up to ~ 19,000 L/m ^{2}), very long duration (up to 31 days), and multiple process interruptions (up to 21, with cumulative interruptions of over 92 hours). Minute virus of mice (MMV) was used as a relevant target virus, and in general, as a model small non-enveloped virus, as these viruses are the main challenge for the investigated virus filters with a stipulated pore-size of about 20 nm. It was found that certain filters – especially of the newer 2 nd generation – are capable of effective virus clearance despite the harsh regimen they were subjected to. At the same time the investigation of biochemical parameters for un-spiked control runs showed the filters to have no measurable impact on the composition of the culture media. Based on these findings, this technology seems to be quite feasible for large volume pre-manufacturing process culture media preparations.

Introduction

The production processes of biopharmaceutical products based on eucaryotic expression systems carry an intrinsic risk for viral contaminations. An industry wide data collection of such contamination events revealed that contamination sources were traced primarily to raw materials or cell culture media including specific components thereof, like FBS (Barone, 2020).

Traditionally, to mitigate this risk, the safety tripod concept (Kreil, 2018) is applied; (a) raw materials used in manufacturing of biopharmaceuticals are selected for low risk, under consideration of viral safety aspects, (b) raw materials are tested for potential viral contaminants, and most importantly the main proportion of safety margins is achieved through (c) virus inactivation or removal steps integrated in the "down-stream" manufacturing process. These steps are either dedicated virus clearance steps, or steps with an intrinsic capacity to clear viruses. As required by current regulations (EMEA 1996; ICH, 1999, FDA, 1997), dedicated virus clearance steps are integrated into manufacturing processes to increase the safety margins of biopharmaceuticals, in a well-controlled manner.

However, for active ("live") biopharmaceuticals, such as live virus vaccines, or Advanced Therapy Medicinal Products (ATMPs) like cellular therapies and gene therapies, dedicated viral clearance procedures cannot always be applied to the down-stream processes as they would not only inactivate or remove potentially present viral contaminations but equally compromise activity of the biopharmaceutical ingredient.

This is especially true for virus filtration, one of the most robust and effective viral clearance procedures that can conceptually remove all pathogens larger than the stipulated pore-size of the filter. Unfortunately, for most live virus vaccines or ATMPs the therapeutic biomolecule is also larger, and thus effective virus clearance is not possible.

Furthermore, some large biomolecules, such as von Willebrand Factor (VWF) complexes cannot pass through the smallest type of virus filters which are suitable for the removal of e.g. Parvovirus without significant losses or severe clogging events due to the nature of the molecules (Parker, 2021). In these cases, the introduction of down-stream virus filtration as dedicated virus clearance step is technically impossible.

However, taking specifically the main source of virus risk for cell culture processes into account, the virus filtration procedure can be directly applied to mitigating that risk by filtration of culture media prior to its use in the manufacturing process.

The studies presented here investigated the feasibility of implementing culture media virus filtration with respect to its virus clearance capacity under extreme conditions, which have been shown to be worst-case for virus removal, such as high process feed loading, long duration of filtration, and multiple process interruptions.

For the many experiments reported here, Minute virus of mice (MMV) was chosen as small challenge virus. This is based on the fact that although culture media increasingly do not contain animal derived components (Grillberger (2009)) viral contaminations of cell derived bulk harvests have still occurred, e.g., with MMV (Garnick, (1998); Nims, (2006)). Therefore, MMV is a target virus. Furthermore, MMV represents small non-enveloped viruses which are the main challenge for virus filters with a stipulated pore-size of 20 nm and is thus also a suitable model virus (EMEA 1996).

Materials and Methods

Culture Media

For the small scale virus removal studies, including validation studies according to guidance (EMEA, 1996), fermenter feed media used for the manufacture of a biopharmaceutical product was used. The media consists of DMEM / HAMS F12 to which Ethanolamine, Poloxamer 188, Soy Peptone, L-Glutamine, $NaHCO_3$ and Copper were added. The pH of the media was initially adjusted to 7.2 to 7.6. Soy-peptone (as animal-derived material free nutrient) was added in different concentrations and from different manufacturers.

In order to avoid bacterial contamination during the virus filtration experiments, antibiotics (Gentamicin 0.1 g/L and optionally Penicillin 90 U/L with Streptomycin 0.1 g/L) were added to the un-spiked media.

Equipment

The virus removal filtration equipment consisted of an electronic recorder, two pairs of redundant pressure sensors (placed up- and down-stream of the pre-filter) and temperature sensors as generally described elsewhere (Wieser, (2015)).

A pressure vessel made of electropolished 316L steel with a capacity of 5 Liters that could be pressurized to 7 bar (Millipore Cat. No. XX6700P05) was used as a feed tank.

Pressure was applied to the tank via a manual controller for nitrogen gas pressure. The nitrogen gas was passed over a 0.2µm air filter in order to avoid introduction of bacterial contaminants.

The filtrate was collected in vessels placed on an electronic balance. The data collected by electronic recorder

was used to calculate process feed loads, feed flow rates, process duration and duration of pressure interruptions.

The virus removal filters used were:

- 1st generation filters:
- Asahi Planova (Asahi Kasei Medical Co., Ltd., Tokyo, Japan) 15N filters; 10 cm^2, rating: mean pore size 15 ± 2 nm (Asahi KASEI, (2018))
- Millipore (Merck KGaA, Darmstadt, Germany) NFP; 3.5 cm^2, rating: >4 log removal of small viruses (Merck Millipore, (2012))
- Pall DV20 (Pall Corp., Port Washington, USA); 10 cm^2, rating: > 3 log for 20 nm viruses (Pall Life Sciences, (2010))
- Pall Pegasus SV4; 9.6 cm^2, rating: 20 nm (nominal) (Pall Life Sciences, (2012))
- 2nd generation filters
- Asahi Planova BioEX filters; 3 cm^2, rating: designed to deliver > 4.0 logs of parvovirus removal (Asahi KASEI, (2018))
- Millipore Barrier; 3.1 cm^2, rating: [?] 3.0 log removal of parvovirus (Merck Millipore, (2021))
- Pall Pegasus Prime; 2.8 cm2, rating: 20 nm (nominal) (Pall Life Sciences, (2016))
- Sartorius CPV; 5 cm2, rating: 20 nm (nominal) (Sartorius Stedim Biotech GmbH, (2011))
- Sartorius HF; 1.7 cm2, rating: 20 nm (nominal) (Sartorius Stedim Biotech GmbH, (2014))

Each set-up included a Millipore Durapore pre-filter (rating: 0.1 µm (nominal), 13 cm^2) set-up in-line in a dead-end filtration configuration. The 0.1 µm pre-filter was flushed and conditioned with water for injection before it was autoclaved at 100°C for 30 minutes. Then it was re-introduced into the filtration equipment and conditioned with WFI again (together with the virus removal filter).

0.1µm Pall Acrodisc syringe filters were used to remove virus aggregates from the thawed virus stocks.

The virus-spiked fermenter feed media was passed over a 0.2 µm Pall Vacucap 90PF filter as needed in order to avoid the introduction of bacterial contaminants.

The pre-use suitability of each viral filter used was verified according to the vendors instructions:

Asahi filters were flushed with WFI, subjected to a pre-use air leakage test and then filled with WFI again. Only filters passing this pre-use test were used for individual experiments – none of the filters tested failed.

All other filters were subjected to normalized water flow tests during which water was passed through the filter at specified pressures and the flow rate measured. Only filters with water flow rates within the ranges specified by the manufacturer were used for individual experiments – none of the filters failed.

After each experiment the integrity of each viral filter used was verified according to the vendors instructions. For the Asahi filters this was identical to the pre-use leakage test. For all other filters the test was a pressure hold test. Only filters for which no air was seen exiting the filter at the specified testing pressure within a given time period were seen to be integral.

Viruses and virus assays

MMV (ATCC VR-1346, strain Prototype; Family *Parvoviridae* ; non-enveloped ssDNA virus; Ø = 18 - 26 nm) was propagated and titrated on A9 cells (ATCC CCL-1.4), as previously reported Berting, (2010).

Titers from the end-point dilution assays were expressed as $\log_{10}$ 50% tissue culture infectious dose/mL ($TCID_{50}$/mL) and any influences of the process material upon the virus infectivity assay characteristics, cell toxicity and interference were considered.

Analysis of the virus removal capacity of the process was carried out as described in or according to regulatory guidance (EMEA, (1996)). The virus load before and after treatment were used to calculate the logarithmic virus reduction factor (R). It is noted that the "after treatment" virus load is the sum of the virus loads of

each filtrate fraction drawn during an experiment. Whenever virus was undetectable, the limit of detection was taken as the virus titer for calculation.

General Procedure

The laboratory scale experiments were designed to simulate the feeding of a perfusion fermenter with a fermenter feed media solution containing MMV as a contaminant. The intention of each laboratory scale experiment was to investigate the maximum process feed load capacity of an investigated filter when loading it with fermenter feed media spiked with MMV at a ratio of 1:250 (i.e., 0.4% spike load). The MMV-spiked, 0.2 µm filtered fermenter feed media solution ("spiked starting material") was passed over a filter train consisting of one 0.1 µm prefilter followed by a virus removal filter (each with a stipulated pore size of approximately 20 nm, see Materials and Methods). During each investigation the impact of multiple interruptions during the loading process on virus clearance of a given filter was also investigated. This was done when the filtrate collecting flask was replaced by a fresh one (samples for virus titration were drawn from the filtrate and stored at [?]-60°C until the end of the experiment when all filtrate samples were titrated together) – during this activity filtration was interrupted intentionally for at least one hour. One of these intentional interruptions lasted at least 72 hours to represent the pause between the first filling of the fermenter and the build-up of the inoculum before the actual perfusion fermentation started. Filtrate collection flask replacement – and thus process interruption – took place approximately once a day. The overall number of interruptions per experiment ranged from 1 to 21 depending on filter performance. Filtration was continued until a flow decay of around 90% was reached or the duration of the experiment had exceeded 30 days (duration ranged from one to 34 days). Virus removal filtration was performed at constant transmembrane pressures (TMP, i.e., the pressure difference between feed and filtrate – which was at ambient pressure and, therefore, taken as 0.0 bar for calculations) over the virus removal filter. The TMP was set to 1.0 to 2.6 bar for each experiment (the maximum TMP set-point was determined either by the maximum value suggested by the filter manufacturer or by the maximum pressure allowed by the tubing used) and to 0.0 bar during the intentional interruptions. Filtration was performed under ambient temperature for all experiments. pH was measured for the spiked starting material and for each filtrate fraction.

Controls for aggregates (EMEA, (2010)) were performed for (a) the virus stock and (b) the spiked starting material matrix. For (a) the titers of the thawed virus stock and of the 0.1 µm filtered virus stock (which then was used for spiking) were compared – a titer reduction of less than 1 $\log_{10}$ is not considered to be significant according to regulatory guidance (EMEA, (1996)). For (b) the titer of the spiked starting material was compared to the titer of the pre-filtrate drawn during the first interruption.

In order to evaluate the influence of the freeze/thaw cycle, to which the filtrate fractions were subjected, on the virus titer of these samples, freeze controls were performed (EMEA, (1996)). Two freeze control levels were investigated: (c) "high titer" and (d) "low titer". For (c) the titer of the spiked starting material (titrated immediately) was compared to the titer of the same matrix which had been stored at [?]-60°C throughout the experiment and titrated together with all other samples which had been stored frozen. For (d) the titer of the spiked starting material diluted with starting material to a theoretical MMV titer of 2.0 $\log_{10}$ [$TCID_{50}$/mL] (titrated immediately) was compared to the titer of the same matrix which had been stored at [?]-60degC throughout the experiment and titrated together with all other samples which had been stored frozen.

In order to evaluate the influence of ambient conditions during an experiment on the virus titer, process controls were performed. To this purpose an aliquot of the spiked starting material was kept at ambient temperature in the biosafety cabinet where the virus filtration was performed, until the end of the NF procedure. At evenly spaced intervals samples were drawn and stored at [?]-60degC throughout the experiment and titrated together with all other samples which had been stored frozen. The virus titers of these samples were compared to that of the spiked starting material (titrated immediately).

Un-spiked control runs were performed for the virus clearance validation studies. All settings were identical to those for the virus spiked runs with the exception that no intentional pressure interruptions were performed.

Samples were drawn for the analysis of glucose concentration and osmolality. Furthermore, a cell proliferation use-test was performed with the pooled filtrate in order to evaluate if the composition of the fermenter feed media was significantly altered during filtration with respect to supporting cell growth. The use test consisted of the analysis of the cell density, cell viability and cell productivity after a 3-day incubation period with the filtered media.

Results

The aim of this investigation was to evaluate which virus filters were suitable for culture media virus filtration. This was done by investigating the MMV removal capacity of different virus filters under the conditions which can be expected when filtering culture media. The intention of each laboratory scale experiment was to investigate the maximum process feed load capacity of an investigated filter when loading it with MMV-spiked fermenter feed media whilst demonstrating an effective virus clearance capacity with no to minimal virus breakthrough.

The culture media used for all experiments contained a base media with an anti-foaming agent and a wide variety of nutrients typical for high performance process. Soy-peptone (as animal-derived component free nutrient) was added in different concentrations and from different manufacturers – these variations were, however, not seen to have any impact on the virus clearance capacity of the investigated filters and are, therefore, not discussed in further detail here.

MMV was chosen as – on the one side – it is a target virus as a contaminant for some cell cultures – such as CHO (Barone, (2020)), and – on the other hand – it represents small non-enveloped viruses which are the main challenge for the investigated virus filters with a stipulated pore-size of about 20 nm.

The worst-case conditions investigated in this study were transmembrane pressure (TMP) – including pressure interruptions, duration of filtration, and process feed load (PDA, (2022)).

The TMP set-point (which is reflected in the mean TMP measured during the filtration phases) was determined either by the maximum value suggested by the filter manufacturer or by the maximum pressure allowed by the tubing used (i.e., 2.6 bar). The complete TMP range was bracketed by intentionally introducing multiple process interruptions. These were typically one [?] 72 hour pause (representing e.g., the duration of inoculum build-up in the fermenter), followed by daily [?] 1 hour pauses. During some experiments a lower mean TMP (i.e., 1 bar) was also investigated in order to broaden the data base.

Duration was a decision point, as the goal was to continue filtration for at least 30 days or until flow decay reached or exceeded 90% (sometimes earlier if a decision had to be made whether the feed tank should be refilled). As a result of these decision points the maximum load and duration possible whilst maintaining effective virus clearance could be determined.

The filters investigated are grouped into first- and second-generation filters (Miesegaes, (2010)). First generation filters are designed for down-stream manufacturing processes. Typically, these processes need virus filters designed to handle up to 1,000 L/m2 of an aqueous protein solution as rapidly as possible. Furthermore, first-generation filters were not typically designed to tolerate the impact of pressure interruptions or low flow phases on their virus retention capacities. Second generation filters do generally have improved virus retention capabilities even if pressure interruptions occur.

Table 1. Filtration conditions and results for MMV retention using first generation (FG) filters.

Filter	FG I	FG II	FG III	FG IV
Mean TMP [bar]	2.1	2.1	2.1	1.1
Process pauses	10 *	7	9 *	6*
flow decay [%]	> 90	> 90	> 90	˜ 80%
Duration [days]	11	14	14	7
Media feed load [L/m^2]	**4,611**	**4,913**	**4,511**	**4,841**

Filter	FG I	FG II	FG III	FG IV
Virus reduction factor (R) [$\log_{10}$]	**1.0**	**1.1**	**2.6**	**4.4**
Virus breakthrough?	yes	yes	yes	yes

* Short pauses (under 1 hour each)

Table 1 shows the results for the investigated first generation filters. For all filters the decision point flow decay was reached rather than duration. The MMV reduction factors (R) for the filters FG I to FG III indicate significant virus breakthrough. These filters were, therefore, unsuitable for high-volume long duration applications. Only filter FG IV showed effective virus clearance (4.4 $\log_{10}$) at a higher than 1,000 L/m^2 load. However, the experimental run was stopped after 6 days as the flow decay had already reached 80%. The short duration of the experiment indicated that the filter is not suitable for a high-volume long duration application.

Table 2. Filtration conditions and results for MMV retention – 2nd generation filter I (SG I) – Sartorius Sartopore CPV.

Mean TMP [bar]	1.0	1.0	2.2	2.3	2.6
Process pauses	20	17	16	21	19
flow decay [%]	78	90	76	80	78
Duration [days]	30	32	23	33	26
Media feed load [L/m^2]	**10,242**	**9,273**	**19,617**	**19,531**	**19,440**
R [$\log_{10}$]	**> 6.1**	**5.0**	**> 6.3**	**> 6.2**	**> 6.5**
Virus breakthrough?	no	minor	no	minimal	no

Per TMP set-point a maximum of two representative experiments are presented here.

A ">" preceding the logarithmic reduction factor (R) combined with a "no" result for the parameter "virus breakthrough" indicates removal below the limit of detection for all of the filtrate fractions.

If minimal virus infectivity was found in a minority of the filtrate fractions (i.e., the titers were at the limit of detection) then the result for virus-breakthrough is "minimal".

A "minor" virus breakthrough indicates that minimal infectivity was found in the majority of the filtrate fractions.

Table 3. Filtration conditions and results for MMV retention – 2nd generation filter II (SG II) – ASAHI Planova BioEX.

Mean TMP [bar]	1.0	1.0	2.4	2.4
Process pauses	18	19	14	14
flow decay [%]	87	> 90	> 90	> 90
Duration [days]	30	34	27	27
Media feed load [L/m^2]	**9,043**	**7,845**	**15,504**	**14,098**
R [$\log_{10}$]	**> 6.5**	**> 5.6**	**> 6.1**	**> 5.8**
Virus breakthrough?	no	minimal	minimal	minimal

A ">" preceding the logarithmic reduction factor (R) combined with a "no" result for the parameter "virus breakthrough" indicates removal below the limit of detection for all of the filtrate fractions.

If minimal virus infectivity was found in a minority of the filtrate fractions (i.e., the titers were at the limit

of detection) then the result for virus-breakthrough is "minimal".

The early 2nd generation down-stream filters investigated (filters SG I and SG II – see tables 2 and 3), however, were found to have robust virus reduction capacities even under worst case load and duration conditions. Virus reduction was found to be effective for both filters (R ranging from 5.0 $\log_{10}$ to > 6.5 $\log_{10}$) although some minimal virus break-through was found in several fractions. Variation of the TMP had no impact on the virus clearance capacity of either of the filters – although it did impact the process feed loads possible before reaching any of the decision points.

Both filters are deemed to be equally suitable for the high-volume long duration filtration which would be needed for a perfusion reactor – and thus also for any smaller volume set-up, including batch filtrations.

It is noted that biochemical parameters, i.e., glucose concentration and osmolality pre and post filtration, confirmed that virus filtration had no significant impact on the composition of the fermenter feed media. Furthermore, the use test regarding cell culture performance with the filtered media showed cell density, cell viability and cell productivity (rate of expression of the target protein) to be within specifications, and fully equivalent to that of unfiltered media (data not shown).

Table 4. Filtration conditions and results for MMV retention using improved second generation filters SG III to SG VI.

Filter	SG III	SG III	SG IV	SG IV	SG V	SG V	SG VI	SG VI
Mean TMP [bar]	2.0	2.1	2.1	2.1	2.1	2.1	2.2	2.1
Process pauses	3	4	2	1	4	2	8	8
flow decay [%]	> 90	> 90	> 90	> 90	90%	> 90	> 90	> 90
Duration [days]	6	6	2	1	9	6	13	13
Media feed load [L/m^2]	**10,500**	**8,700**	**3,893**	**3,582**	**15,700**	**11,400**	**11,500**	**11,000**
R [$\log_{10}$]	**> 5.3**	**> 5.8**	**4.4**	**3.7**	**4.3**	**4.4**	**> 6.3**	**> 6.2**
Virus break-through?	no	no	yes	yes	yes	yes	minimal	minimal

A ">" preceding the logarithmic reduction factor (R) combined with a "no" result for the parameter "virus breakthrough" indicates removal below the limit of detection for all of the filtrate fractions.

If minimal virus infectivity was found in a minority of the filtrate fractions (i.e., the titers were at the limit of detection) then the result for virus-breakthrough is "minimal".

In the meanwhile, new filters are continuously being developed – some of which focus on increasing throughput whilst maintaining virus clearance capacity. These improved 2nd generation filters reduce the filter area demand for large volume virus filtration, and thus reduce facility floor footprint for large-volume filtration, as well as filter cost.

All investigated filters showed a significantly higher initial feed flow rate than the until then commercially available filters (see Table 4). This led to higher initial throughput, but filter fouling occurred relatively early for all filters.

For filter SG IV this is probably the reason for the termination of the process due to significant flow decay within 1 to 2 days after up to 3,900 L/m^2 had been filtered. For this filter virus reduction was effective – with mean R values of 4.4 and 3.7 $\log_{10}$ but virus breakthrough was observed in most filtrate fractions. Nonetheless, this filter is a significant improvement to filter FG III from the same manufacturer, with which only 2.6 $\log_{10}$ MMV reduction was achieved. This filter could be suitable for batch filtration prior to the actual fermenter process.

Filter SG V showed an increased initial flow rate when compared to filter SG I of the same manufacturer, but all other parameters such as throughput and duration were not as good. Furthermore, although virus

reduction was effective (mean R value: 4.4 $\log_{10}$) it was significantly lower than what was achieved for filter SG I (5.0 to >6.5 $\log_{10}$).

Filter SG III shows a significant improvement of the filter characteristics with respect to high volume long duration filtration when compared to filters FG I and FG II from the same vendor. The same can be said about developmental filter SG VI when compared to filter FG IV of the same manufacturer (however, when compared to filter SG II – which is an early 2nd generation filter from the same vendor – no significant improvements can be seen).

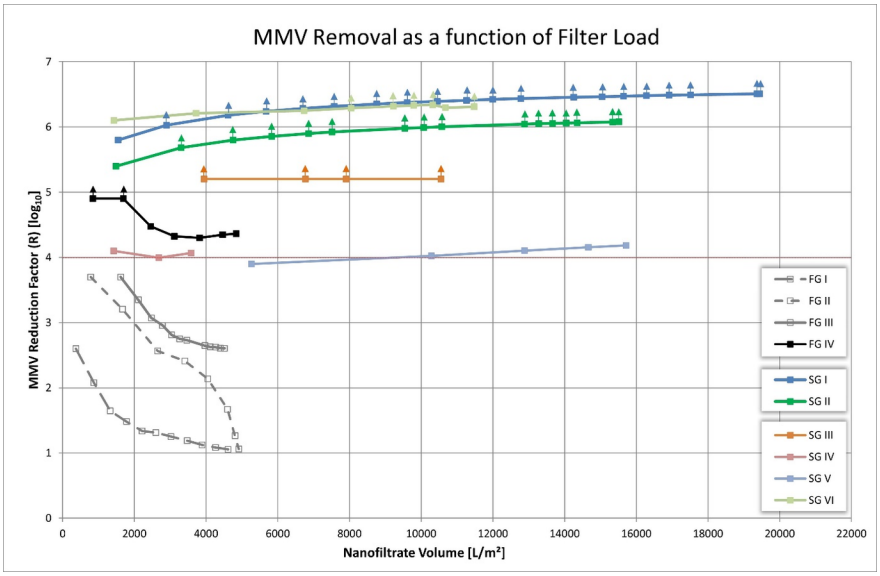

Figure 1. Overview of the development of the logarithmic reduction factor (R) during representative runs for each filter investigated. Arrows pointing up indicate the corresponding filtrate fraction titer was below the limit of detection.

The virus reduction factor values (R) in relation to filtered volumes are summarized in Figure 1. Filters FG I to FG III exhibited significant MMV breakthrough resulting in a rapidly decreasing R values – even though initial values (when volumes were near to the volumes the filters were designed for) were not unpromising. Filter FG IV shows effective virus clearance, however, with some significant virus breakthrough after a loading of more than 2000 L/m^2 or due to process interruptions. All other filters show effective virus clearance, whereby filters SG I and SG II show the best virus clearance capacities at the highest process feed loads and were thus best suited for the intended purpose.

Discussion

Raw materials or cell culture media including specific components thereof, like FBS, have been identified as source of viral contaminants for biopharmaceutical products based on eucaryotic expression systems. It has been reported that such contaminations have led to virus contamination and the subsequent collapse of the cell population in these fermenters. As a result, severe measures to clean up the upstream, and should any intermediate have been forward-processed already also the down-stream equipment, to minimize the recurrence of such an event had to be taken. The downtime can lead to unmet patient needs, dramatic economic consequences, and severe impact on the reputation of an affected company (Bethencourt, (2009)).

The introduction of a contaminating virus might not necessarily become obvious by degenerative effects presented by the production cells, as “silent infections” have also occurred (Moody, (2011)). The specific adventitious agent testing (AAT) program on fermenter harvest material would be the point where a virus contaminant would be detected. However not necessarily: although AAT is designed to detect as many

potential viral contaminants as possible, there might be viruses that would not be picked up (Gombold, (2014)). Similarly, the testing of raw materials does not necessarily reveal a viral contaminant, especially when the concentration of the contaminant in the raw material is lower than the detection limit of the testing procedure, or the contaminant is unevenly distributed across the large volumes involved versus the small volumes actually tested.

Therefore, to make sure that a potential contaminant would never reach the patient, a series of effective virus clearance steps is implemented in down-stream biomanufacturing operations, where feasible. Some biopharmaceutical products can, however, not be subjected to dedicated viral clearance steps in the down-stream processes as they would not only inactivate or remove potentially present viral contaminations but have the same clearance effect on the biopharmaceutical components. This is true for live virus vaccines, ATMPs (e.g., cellular or gene therapies) or particularly complex biotechnology products which are simply too large to be subjected to filtration with virus filters with a nominal pore size of 20 nm, e.g.: vWF (Parker, (2021)).

A potential solution is to introduce a method which ensures a significant reduction of theoretically present viruses in the culture media before it even enters a manufacturing process, i.e., an upstream virus barrier.

Different methods capable of this feat have been investigated: high temperature short time treatment (HTST), ultraviolet light irradiation (UV-C) and finally virus filtration. The implementation of HTST and UV-C have, however, been faced with complications as in both cases media components are susceptible to degeneration (Cao, (2013)), (Meunier, (2016)).

Virus filtration, however, is one of the most robust and effective virus clearance procedures, as it conceptually removes all pathogens as long as their size is larger than the stipulated pore-size of the filter, while at the same time being neutral to the biological activity of the biopharmaceutical compound(s) smaller than the pore size. It is, therefore, best suited to reduce the risk of the introduction of adventitious viruses through culture media. For this process to be economically feasible the filter area must ideally be kept small while at the same time large volumes of culture media are processed for a potentially prolonged times of filtration, and the impact of low or no flow incidents on the virus clearance capacity needs to be understood also (Wieser, (2016)).

The studies presented here investigated the feasibility of implementing culture media virus filtration with respect to their virus clearance capacities under extreme conditions such as very high process feed loading (up to ˜ 19,000 L/m^2), very long duration (up to 31 days), and multiple process interruptions (up to 21, with cumulative interruptions of over 92 hours). MMV was used as a relevant target virus as a contaminant for some cell cultures, and in general as a small non-enveloped viruses which are the main challenge for the investigated virus filters with a stipulated pore-size of about 20 nm.

It was found that a number of filters – especially of the newer 2nd generation – are capable of effective virus clearance despite the harsh regimen they were subjected to. At the same time the investigation of biochemical parameters for un-spiked control runs showed the filters to have no measurable impact on the composition of the culture media. Again, indicting this technology to be especially suitable for large volume pre-manufacturing process culture media preparation.

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