A publication of
The Williamsburg BioProcessing Foundation

Fall 2006

BioProcessing™ JOURNAL

The Most Trusted Source of BioProcess Technology™

Vol. 5 No. 3

www.bioprocessingjournal.com

Evaluation of Filtration Products in the Production of Adenovirus Candidates Used in Vaccine Production: *Overview and Case Study*

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Adenoviral vectors (AAV's) offer a promising new approach to vaccine development. They have the ability to be rapidly manipulated for bearing transgenic coding for specific antigenic proteins, efficiently infect a variety of mammalian cell types (including antigen-presenting cells) and induce a broad immune response against the target antigen in vaccine recipients. Furthermore, AAV's offer an excellent safety profile, in that they can be engineered to be non-replicating in the vaccine recipient and they lack the molecular mechanism for integration into the host genome. AAV's are highly amenable to scalable manufacturing processes such as the use of stirred tank bioreactors, high capacity filtration methods, and chromatographic purification procedures.

GenVec and Millipore have collaborated to evaluate Millipore's technologies for potential use in AAV vaccine production. We have summarized the filter options evaluated on GenVec's AAV product candidates, along with the results and filter sizing estimates for the process steps of medium exchange, lysate clarification, post-clarification filtration, concentration/diafiltration, and post-hold sterile filtration prior to column chromatography.

Introduction

AAV's are non-enveloped DNA viruses (double stranded), 70-110 nm in size, that are very effective at infecting target cells and delivering their genetic payload. AAV's are replication-deficient in normal human cells due to the deletion of adenoviral sequences that are normally expressed early in viral infection and are required for replication (*e.g.,* E1, E3, E4). These genetic deletions provide ample space for insertion of a transgene cassette bearing vaccine antigen genes.[1,2]

Overview of Adenovector Production Steps

Cell Lines

Efficient manufacture of AAV's can be accomplished using genetically engineered human cell lines that complement the deleted adenoviral genes required for replication (*e.g.,* 293-ORF6 cells).[4,5] These cells can be adapted for growth in serum-free suspension and have a well-characterized safety profile.

Cell Culture Production– Virus Barrier for Medium Filtration

Production cells are grown in stirred tank bioreactors with serum-free culture medium. For large-scale production (≥100 L), culture medium is filtered upon addition to the bioreactor.

Cell Culture Production– Medium Exchange

During the AAV infection phase, the metabolic processes of the production cell line are significantly increased to support vector manufacture. In order to facilitate successful vector production, a medium exchange step must be performed to remove spent medium containing metabolites such as lactic acid, which can be detrimental to virus production. A 50–100% medium exchange is commonly performed at this stage.[5] In this step, cells are concentrated and followed by resuspension in fresh medium. A microfiltration cartridge is commonly employed to provide efficient medium exchange and control of cell concentration.

Lysate Clarification– Normal Flow Filtration

At AAV harvest, the cells are lysed either mechanically or by a chemical lysis agent (*e.g.,* non-ionic detergent). Depth filters are used at this step to remove cellular debris. Filter capacities depend upon cell density at harvest, the degree of lysis, and the particle size distribution.

Concentration/Diafiltration

A vector concentration step is performed to reduce overall volume, and then diafiltration is conducted to facilitate buffer exchange for fur-

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ther processing, such as enzymatic digestion of cellular DNA. At the end of this operation, clarified lysate is placed in an appropriate buffer by diafiltration in preparation for the downstream chromatographic step.

Post-Hold Filtration

An overnight hold step is commonly employed prior to downstream purification. Consequently, a filtration step is performed to reduce the risk of bioburden and to protect the downstream chromatography columns.

Chromatography

For small-scale production of clinical lots, CsCl gradient purification is typically performed. However, for large-scale production, column chromatography is employed because it is more conducive to process scale-up. Two or three-step column chromatography purification is normally done for adenovirus production. Purification methods commonly used are ion exchange and size exclusion chromatography.[6]

Sterile Filtration

This step ensures the sterility of the final formulated product. A filter pore size of 0.22 µm or less is required to eliminate microbial contaminants.

Materials and Experimental Design

We will describe the evaluation of several of Millipore's products for medium exchange, clarification, concentration/diafiltration, and bulk sterile filtration in GenVec's vector manufacturing process.

Cell Line

GenVec has developed a proprietary AAV/production cell platform that enables efficient production of replication-incompetent adenovirus vectors.[1] The GV11 AAV backbone contains deletions of two regions essential for adenovirus replication, E1 and E4, as well as a partial deletion of the E3 domain. These deletions essentially eliminate the generation of replication competent adenovirus (RCA) while also providing sufficient genomic space for insertion of transgenes of interest. The complementing production cell line, 293-ORF6, contains a full set of E1 genes as well as open reading frame #6 from the adenoviral E4 domain under the control of a zinc-inducible promoter (sheep metallothionein promoter). Together, these genetic insertions within the chromosomes of the cell line enable controlled complementation of the deleted adenovirus genes to facilitate potent vector production. This cell line has been adapted for growth in serum-free suspension culture and has been extensively characterized for safety.[2] For all work described herein, 293-SFMII medium (Gibco/Invitrogen) was used.

Medium Exchange Experiments

Test Method A: Millipore Pellicon® 2 Cassettes.

Trials for medium exchange were performed on infected 293-ORF6 cell culture using Millipore's 0.45 µm rated Pellicon 2 mini V screen filter. Three experiments were performed using the Pellicon 2 cassettes.

Experiment 1: The goal of this experiment was to obtain preliminary data on the concentration of infected 293-ORF6 cells using the above-mentioned filter. The experimental setup is shown in Figure 1. The feed consisted of 293-ORF6 cells infected 17 hours prior to the trial with AAV. The loading was 30 L/m^2. The cross flow used was 5 L/min/m^2 with no permeate control. A 10X concentration was performed.

Experiment 2: The goal of this experiment was to evaluate whether the V screen in the Pellicon device affected cellular viability in experiment 1. The feed consisted of 293-ORF6 cells infected 48 hours prior to the trials, which is a worst-case scenario. A loading of 10 L/m^2 was used because of the material available. A total recirculation was performed with the retentate valve fully open and the permeate valve closed. The cross flow was 5 L/min/m^2.

Experiment 3: The goal of this experiment was to investigate whether a permeate-controlled process would ensure good product recovery. The feed consisted of infected 293-ORF6 cells used in experiment 2. The loading used was 10 L/m^2. The cross flow

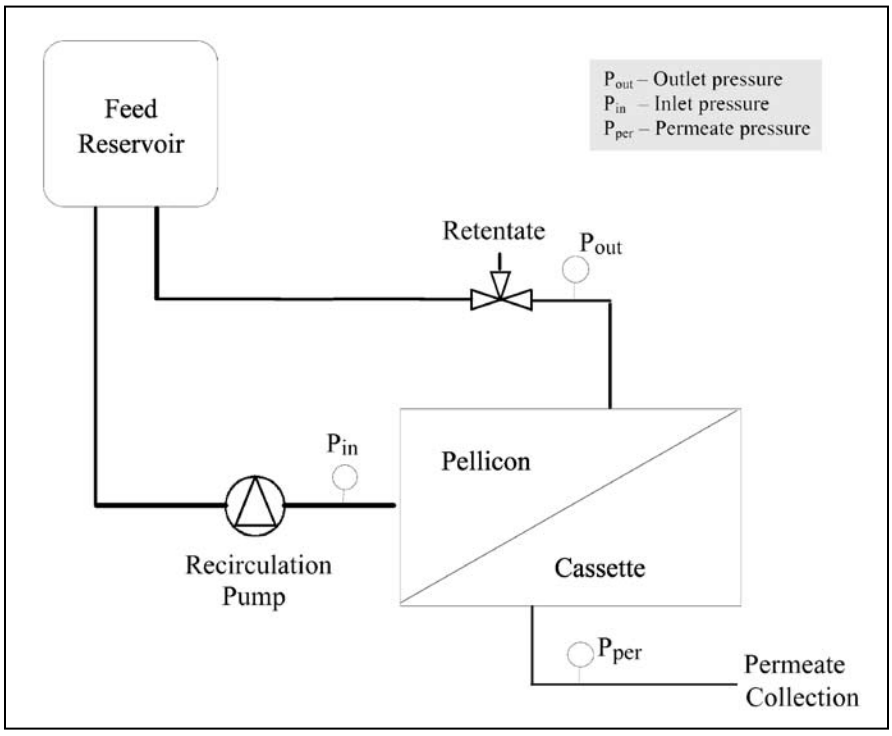

Figure 1. Pellicon V Screen Filter Concentration Setup.[3]

was 5 L/min/m^2 and the permeate was controlled at 60 L/m^2/hr (LMH). A 5X concentration was performed. A post-process polarization was also performed.

Test Method B: Millipore Prostak® Open-channel Modules.

Trials for medium exchange were performed on infected 293-ORF6 cell cultures using Millipore's Prostak Microfiltration Device. A single experiment was performed using the Prostak device.

Experiment: The goal of this experiment was to perform a 2–3X concentration using the Prostak device while measuring the cellular viability throughout the process. A two-pump system was set up to provide permeate flow rate control. Bags were used for both the feed vessel and permeate collection vessel to create a closed system. A single 2-stack Prostak device (0.17 m^2) was installed for the testing. Operating conditions were selected based on previous Pellicon testing and initial system pressures. The transmembrane pressure (TMP) was monitored and kept to a minimum (≤10 psi) to eliminate shear on the cells. The feed consisted of 293-ORF6 cells infected 24 hours prior to the trials. The permeate was controlled at 43 LMH. A 2.8X concentration was performed. Samples were collected along the way to measure the cell culture density and viability.

Lysate Clarification Experiments

Test Method A: Millipore Millistak+® Depth Filters.

Millistak+ filters are graded density depth type filters containing cellulose fiber, and may contain an inorganic or organic filter aid. They were chosen for the clarification trials due to their high capacity. The Millistak+ filters evaluated were C0HC, DE30, CE30 and B1HC.

Experiment: The goal of this experiment was to perform clarification for infected 293-ORF6 cell culture post-detergent lysis using the Millistak+ filters. The flow rate was approximately 8 mL/min for the 23 cm^2 Opticap® trial filters with Millistak+ media (208 LMH). 320 mL of cell lysate were processed through the DE30 filter, and 500 mL of cell lysate were processed through the C0HC filter. Differential pressure across the filter, filtrate turbidity, and total volume filtered data were recorded. Total volume filtered when the turbidity or pressure reached a certain value was scaled up directly.

Scale-Up Experiments: Scale-up runs at the 10 L scale were performed for the DE30 and the C0HC filters. For both the DE30 and C0HC, 10″ filters were used. The experiment was performed in triplicate. For the DE30 filter, a flux of 209 LMH, which is the same as the trial flux, was used. For the C0HC filter, the recommended flux of 154 LMH was used. Approximately 10 L of material was permeated through each of the 10″ filters. Pressure and permeate volume were recorded every few minutes.

Test Method B: Millipore Clarigard® and Polysep™ II Filters. Clarigard filters are graded-density polypropylene, while the Polysep II media consists of a borosilicate glass layer and a layer of mixed esters of cellulose membrane.

Experiment: The goal of this experiment was to evaluate a filter that, when scaled up, would accommodate GenVec's existing hardware (including two stainless steel filter housings available for use at 100 L scale). For the Clarigard filter trial, the flow rate was maintained at 50 mL/min for the 1″ Opticap XL trial filter. For the Polysep II CGW6 filter trial, the filtrate from the Clarigard filter trial was taken as the feed and run at 50 mL/min through an Opticap filter with an effective filtration area of 13.8 cm^2. For both filters, the differential pressure across the filter, filtrate turbidity (OD600), and total volume filtered were recorded. Total volume filtered when the turbidity or pressure reached a certain value was scaled up directly.

Scale-up Experiments: Scale-up runs at the 10 L scale were performed for the Clarigard and the Polysep II filters; specifically, the Clarigard XL10, Polysep II and Opticap XL2. The experiment was performed in triplicate and a recommended process time of 30 minutes was used. Approximately 10 L of material was permeated through each of the filters. Pressure and permeate volume were recorded every few minutes.

Post-Clarification Filtration Experiments

Test Method: Filters Containing Millipore Durapore® Media.

Durapore 0.45 µm hydrophilic filters are manufactured from polyvinylidene fluoride (PVDF) and polypropylene components, and are a good choice for post-clarification filtration.

Experiment: Constant flow trials were conducted using a 13.8 cm^2 OptiScale® filter at 20 mL/min. Pressure was recorded every few minutes.

Concentration/Diafiltration Experiments

Test Method: Millipore Pellicon 2 Ultrafiltration Cassettes.

This product line was designed with process scale-up in mind. Type C screens are recommended for concentration and diafiltration of moderately viscous solutions.

Experiment: A new Pellicon 2 mini module, with Biomax® 500,000 Da membrane and a C screen, was installed and flushed with water to remove the preservative solution. The module was integrity tested followed by pre-conditioning using buffer. Approximately 4 L of feed material was used to carry out the experiment. A five volume diafiltration was carried out with buffer after a 10X concentration at a transmembrane pressure TMP of 11–12 psi. The permeate flow rate was measured during both the concentration and the diafiltration steps. No flux or TMP excursion was performed during these trials because the system was run in an aseptic mode.

Post-Hold/Pre-Column Sterile Filtration Experiments

Test Method: Millipore Durapore 0.22 µm Hydrophilic Filter.

These sterilizing-grade, 0.22 µm hydrophilic PVDF membranes are low protein binding and provide steril-

ity assurance, high flow rates, and high throughputs.

Experiment: Trial loading was 764 mL for the 13.8 cm^2 OptiScale 0.22 µm device (550 L/m^2) with a Durapore membrane. This device was chosen because of limited feed material. Pressure was recorded every few minutes. Cell density and cell viability was measured during all the trials using the Cedex cell analyzer (innovatis AG) and the turbidity was measured using a UV spectrophotometer.

Results

Medium Exchange

Test Method A: Millipore Pellicon 2 Cassettes.

GenVec's current medium exchange process utilizes hollow fibers. For the purposes of reliable scale-up from 10–100 L, GenVec wanted to evaluate flat sheet technology. Three experiments were performed to evaluate the feasibility and to develop the use of the 0.45 µm V screen PVDF Pellicon mini TFF filter for medium exchange. The V screen was chosen due to the high suspended solids content in the feed material. In order to work with GenVec's current process, the filtration system used for medium exchange had to concentrate the cell culture by 3X while maintaining cell viability above 90%. In addition, the medium exchange required an aseptic mode of operation since cells are returned to the bioreactor post-medium exchange. Therefore, a steamable/autoclavable device was required. Cell culture concentration was carried out successfully using the 0.45 µm, V screen PVDF Pellicon mini TFF filter.

Experiment 1: Performed to obtain preliminary data on the concentration of infected 293-ORF6 cells using the Pellicon 2 cassette. During the experiment, high ΔP and TMP values were observed. The ΔP range was 3–6 psi and the (TMP) range was 4–8 psi. Filter polarization occurred which led to significant cell loss. The initial cellular viability was 96.5% while the final viability was 35.5%.

Experiment 2: Performed to investigate whether the V screen in the Pellicon 2 device affected cellular viability. The ΔP range was 1–1.5 psi and the TMP range was 3–4 psi. No significant loss of cellular viability was observed over the time period of the experiment. The initial cellular viability was 83.8% and final viability was 83.9%. Data clearly shows that the V screen in the Pellicon 2 device does not affect the viability of infected cells.

Experiment 3: Carried out to examine whether a permeate-controlled process would ensure good cell recovery. A loading of 10 L/m^2 was used, as compared to 30 L/m^2 for Experiment 1, due to the amount of material available. The aim was to concentrate the cell culture while maintaining the cellular viability. A 5X concentration was performed, and the permeate flux was maintained at a constant using a permeate pump at 60 LMH. (Please note that higher permeate fluxes could possibly be obtained). The TMP and ΔP remained very stable with a ΔP of 1–1.5 psi and a TMP of 3–4 psi. The cross flow used was 5 $L/min/m^2$. No loss in cellular viability was observed at the end of the process. Initial and final cellular viability was 83.9%.

Test Method B: Millipore Prostak Open-Channel Modules.

From the experiments performed, it was observed that post-process depolarization and permeate control is essential for maintaining high viability. However, since steamability is a requirement for GenVec, confirmatory experiments were performed using the steamable Prostak device. The data collected from the Pellicon device was used to perform Prostak trials. The starting volume was 10 L and a 2.8X concentration was performed.

Figure 2 shows the effect of loading on TMP. The graph shows that the TMP is stable and less than 1 psi from 0–20 L/m^2. The TMP rose above 5 psi after 29 L/m^2. At a loading of 35 L/m^2, the TMP climbed to 9 psi. At this point, the system was depolarized (recycle mode, permeate fully closed) to sweep the layer of debris that was trapped on the surface of the membrane. During processing, it is critical to monitor the TMP to make sure that it does not exceed 5 psi. This will prevent cell lysis, as well as a decrease in cell density and cellular viability.

Figure 3 shows the effect of TMP on cell density and viability. The viability slowly decreased as the TMP increased above 5 psi, although the viability remained above 90% for the entire concentration process. The cell density increased until the TMP reached approximately 7 psi and then slowly started to decline. The permeate pump was stopped once the TMP reached 9 psi. After the membrane was depolarized (the layer of plugging material was swept off the membrane surface), the TMP decreased to approximately 4.8 psi and the viability increased to 95%.

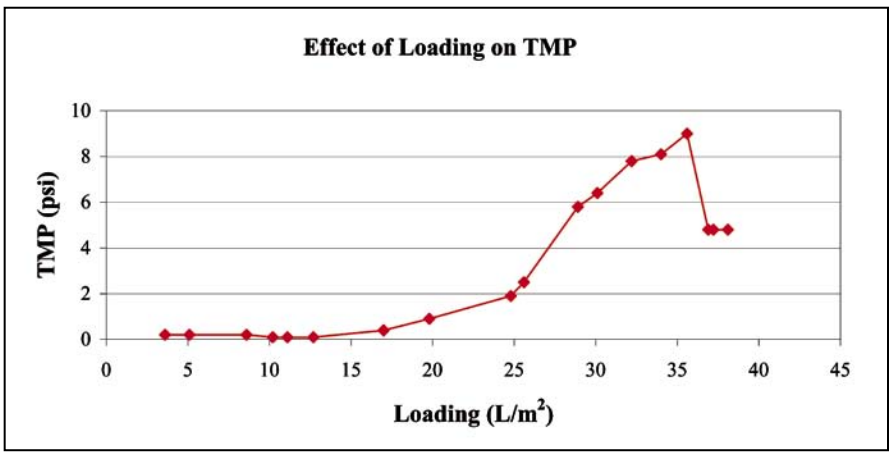

Figure 2. Effects of Loading on TMP for the Prostak Trial.

The data shows that the 0.45 µm Prostak device can successfully concentrate the adenovirus feed material. In addition, this device also meets GenVec's steam-sterilizable requirement. The TMP should remain at or below 5 psi for the viability to remain above 93%. At 5 psi and 93% viability, the feed can be concentrated 2X with a 2-stack Prostak device (0.17 m^2). At this point, the average flux was 43.2 LMH (40 minutes to process 4.9 L through permeate).

Adding filtration area will improve performance and allow the system to concentrate to 3X if necessary. It is suggested that a 4-stack Prostak device (0.33 m^2) be used to concentrate 10 L of adenovirus 2–3X. A 10 L concentration process will take approximately 0.5 hours if the average flux remains the same at 43.2 LMH. When adding area, it is possible to increase the average flux with proper optimization. A 20-stack Prostak filter device (1.7 m^2) would be the recommended filter area for a 100 L batch. Based on the experimental data, the following recommendations can be made for processing 10 L and 100 L batches.

Lysate Clarification and Post-Clarification Filtration

Test Method A: Millipore Millistak+ Depth Filters.

For the clarification step, the filter must be able to clear the cell culture lysate without turbidity breakthrough, resulting in low product loss. The objective of this trial was to evaluate Millistak+ C0HC, DE30, CE30, and B1HC depth filters which could result in process compression, since GenVec's current clarification process utilizes a three-filter-stage train. Process compression would save time and money, and minimize product loss. The filter sizing required for various batch sizes of 10 L, 100 L and 1,000 L is shown in Table 1.

Pressure data is shown in Figure 4, and as illustrated, the B1HC filter plugged prematurely. The OD600 reading was taken on the filtrate every ten minutes as a measure of turbidity clearance. The 30CE filter led to turbidity breakthrough, while the pool OD600, post C0HC and DE30 were below detection and comparable to the pool OD600 from the current three-filter-stage GenVec process (50 µm > 3 µm > 0.8 µm).

For post-clarification filtration, approximately 500 mL of C0HC filtrate was filtered at 20 mL/min through a 0.45 µm-rated OptiScale device (area 13.8 cm^2). No pressure increase was observed with this loading. The 0.45 µm filter was evaluated at this point because we do not claim sterility at this stage in the process. Table 2 summarizes the product recovery/yield for the Millistak+ filter and 0.45 µm experiments.

The results from the Millistak+ C0HC and DE30 filter trials, including filter sizing for a 10 L batch with a one-hour process time, are summarized in Table 3. The clarity post-Millistak+ filtration was comparable to what the current process yields following the three-stage filtration, 50 µm > 3 µm > 0.8 µm (*i.e.*, an OD reading of 0.023). Therefore, GenVec has two options for clarification filters.

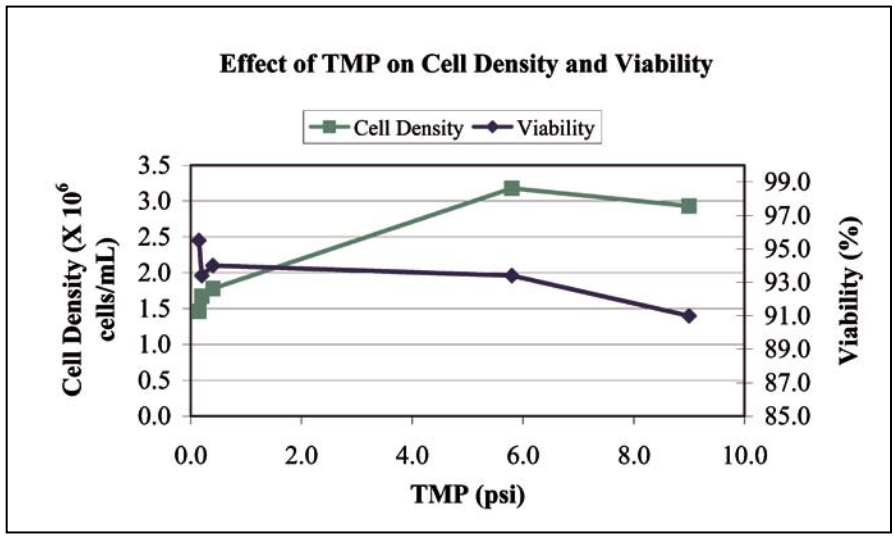

Figure 3. The Effects of TMP on Cell Density and Viability for the Prostak Trial.

Table 1. GenVec Filter Sizing Estimates.

Process Step	Device	10 Liters	100 Liters	1,000 Liters
Medium Exchange	Prostak	0.33 m^2 membrane 1 X 4 Stack Module	3.4 m^2 membrane 2 X 20 Stack Module	34.0 m^2 membrane 20 X 20 Stack Module
Lysate Clarification	Option 1/Step 1: Millistak+ DE30 or Millistak+ C0HC Option 2/Step 1: Clarigard (3 µm) Option 2/Step 2: Polysep II (1/0.5 µm)	 0.093 m^2 membrane 0.060 m^2 membrane 1 X 10" 1 X 2" Opticap XL	 0.7 m^2 membrane 0.47 m^2 membrane 4 X 30" 1 X 10" Opticap XL	 7.0 m^2 membrane 4.7 m^2 membrane 34 X 30" 4 X 30" Opticap XL
Post-Clarification Filtration	0.45 µm Durapore	0.025 m^2 membrane	0.25 m^2 membrane	2.5 m^2 membrane
Concentration/ Diafiltration	Pellicon 2 Module Biomax, 500 kD membrane C screen	0.3 m^2 membrane (3X)	2.5 m^2 membrane	25 m^2 membrane (10X)
Post-Hold / Pre-Column Filtration	0.22 µm Durapore	5 Liters Millipak 20 100 cm^2 membrane	50 Liters Millipak 200 1000 cm^2 membrane	500 Liters 1 X 20" Capsule 1.38 m^2 membrane

Note: The small-scale trials were performed using the Pellicon device, and future pilot-scale experiments will have to be performed prior to scale-up using the Prostak open-channel devices. Due to system limitations, a 10X concentration factor may not be feasible for a 10 L scale with the Prostak.

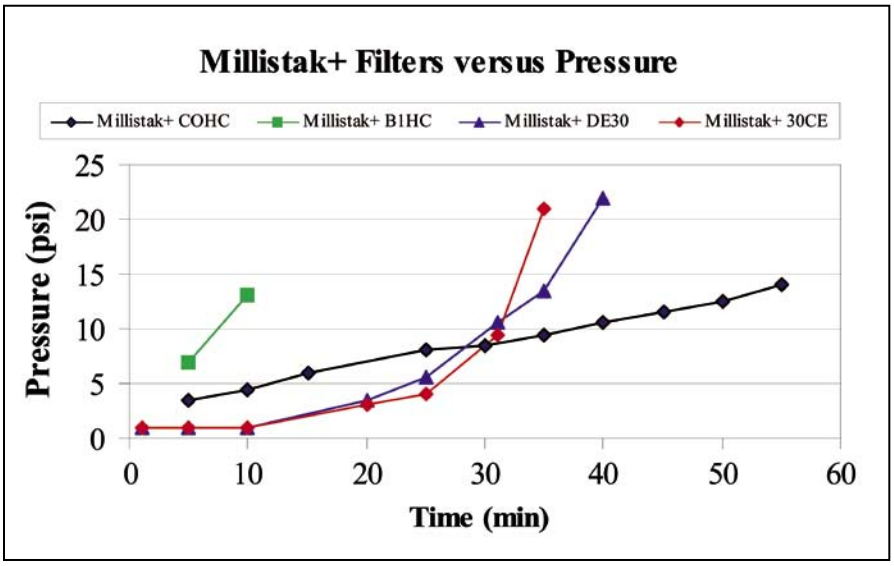

Figure 4. Millistak+ Filter Results.

Table 2. Product Recovery Data.

Sample Description	Titer (PU/mL)	% Yield
Before lysis	2.36e10	100
Post lysis	2.67e10	113
Current clarification filter train	2.17e10	92
B1HC filtrate	6.75e9	29
C0HC filtrate	1.98e10	84
30CE filtrate	2.28e10	97
30DE filtrate	2.07e10	88
0.45 micron filtrate (post C0HC)	1.91e10	96

Table 3. Millistak+ Sizing for 10L with a One-Hour Process Time.

	C0HC (2 over 0.2 µm nominal rated)	DE30 (2 µm nominal rated)
Minimum Required Area, Amin (m^2)	0.047	0.07
Filter Configuration	1 X 10"	1 X 10"
Overall Safety Factor	1.38	1.4
Process Loading (l/m^2)	154	100
Trial Loading (l/m^2)	217	139
Process Flux (LMH)	154	100
Trial Flux (LMH)	203	208.7

Option I: Millistak+ DE30 filter (2 µm nominally rated). Option II: Millistak+ C0HC filter (double layered, 2 over 0.2 µm, nominally rated). For both options, sizing for a process time of one hour is shown in Table 3.

Included in Table 1 are recommendations and sizing for: 1) a scale of up to 1,000 L for Options I and II; and 2) post-clarification filtration (0.45 µm-rated Durapore filter post-Millistak+ filter clarification). It is important to note that the 0.45 µm rated filter can be connected in series to the Millistak+ filter for running the process. If Option I is chosen, small-scale trials to evaluate 0.45 µm filtration post-Millistak+ DE30 filter should be performed.

Test Method A: Scale-Up Data. The processing parameters for the scale-up data matched well with the small-scale trial data for both the DE30 and C0HC filters, as shown in Figures 5 and 6, respectively. The differences in the curves are due to the different cell densities in the bioreactor at the time of harvest.

Test Method B: Millipore Clarigard and Polysep II Filters. For the Clarigard filter experiment, the flow rate was maintained at 50 mL/min for the 1″ Opticap XL trial filter. The OD reading after the Clarigard filter was 0.059. Note that a buffer was used as a blank for the OD600 readings. For the Polysep II CGW6 filter trial, the filtrate from the Clarigard filter trial was taken as the feed, then run at 50 mL/min through an OptiScale filter with an effective filtration area of 13.8 cm^2. The OD reading after the Polysep II filter was below the limit of detection. The titer recovery after both filters was 97%. This demonstrates that the Clarigard filters can be connected in series to the downstream Polysep II filters, with a processing time of approximately 30 minutes for this train. Therefore, the recommended filtration train for the lysate clarification step would be the Clarigard filter (3.0 µm nominal) followed by the Polysep II CGW6 filter (1.0 over 0.5 µm nominal). Step 1: Clarigard filter (3 µm nominally rated). Sizing and recommendations for a process time of 30 min are shown in Table 1. Step 2: Polysep II CGW6 filter (double layered, 1.0 over 0.5 µm nominally rated). Refer to Table 1 for sizing and recommendations.

Test Method B: Scale-up Data. The processing parameters for the

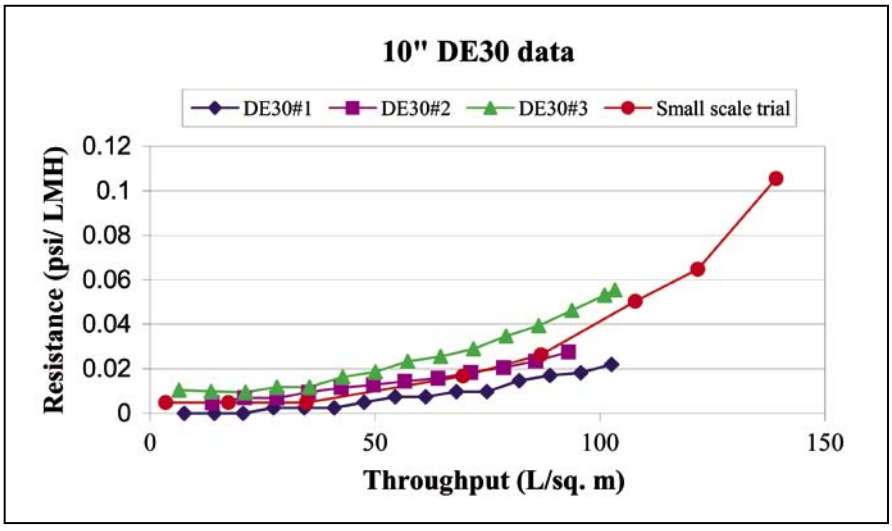

Figure 5. DE30 Scale-Up Test Method A Data.

scale-up data matched well with the small-scale trial data for both the Clarigard and Polysep II filters as shown in Figure 7. The differences in the curves are due to the different cell densities in the bioreactor at the time of harvest. Both filters showed good filtrate clarity and recovery.

Concentration/Diafiltration

For the concentration step, the initial feed stock must be concentrated 10X followed by a 5X diafiltration. Pellicon 2 ultrafiltration flat-sheet TFF cassettes were tested. Concentration experiments were carried out at a starting TMP of 8 psi and a ΔP of ~ 7 psi. The permeate flux dropped from a range of 100–130 LMH to 70 LMH as the product was concentrated 10X. This is not a large drop in flux and therefore, shows good filter performance. From the permeability versus volumetric concentration factor (VCF), VCFdf was determined as 12X for the buffers, as shown in Figure 8. Therefore, theoretically we can use a slightly higher concentration factor, then perform the diafiltration after confirming that the VCFdf in the other buffers is less than 12X. After completion of the concentration, a 5X diafiltration was performed using a buffer. The TMP remained stable, around 11–12 psi and the ΔP was 8–9 psi. The permeate flux dropped from 79 to 59 LMH during the diafiltration. This drop in flux was not large, and therefore showed good filter performance. From these results, the recommendations made for sizing are shown in Table 1.

Post-Hold/Pre-Column Sterile Filtration

The purpose of this step was to reduce any potential bioburden and protect the downstream chromatography column. Trial loading was 764 mL for the 13.8 cm^2 OptiScale 0.22 µm device (550 L/m^2) with Durapore membrane. No significant pressure increase was observed at the end of the filtration. Also, no significant loss in the viral titer was observed after the 0.22 µm filtration. The titer,

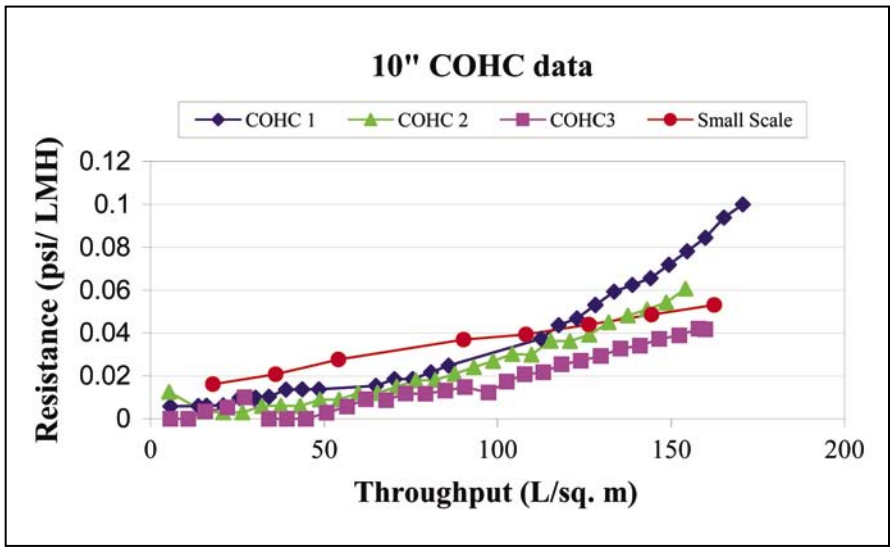

Figure 6. C0HC Scale-up Test Method A Data.

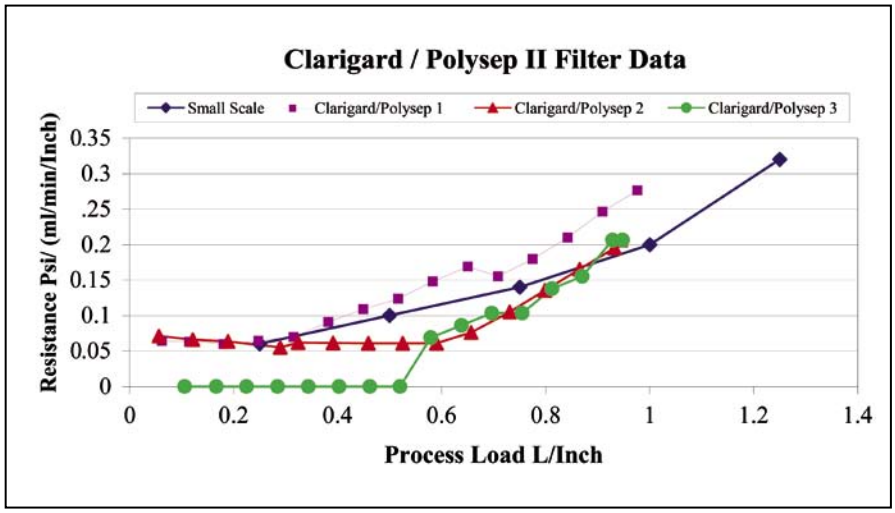

Figure 7. Clarigard and Polysep II Filter Scale-up Test Method B Data.

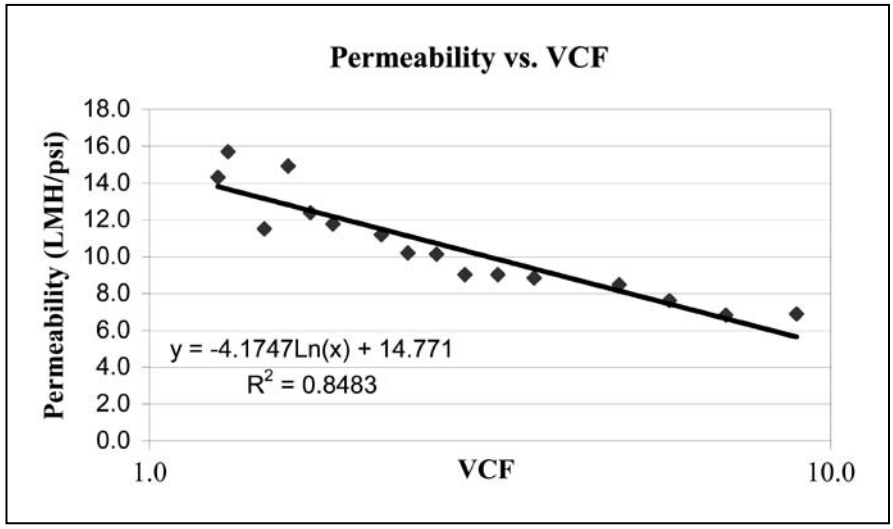

Figure 8. Volumetric Concentration Factor for Diafiltration

before the 0.22 µm filtration, was 7.15^{10} PU/mL. After filtration, the titer was 7.16^{10} PU/mL. Based on this data, a variety of filters were recommended for the various batch sizes, as listed in Table 1. Prior to scale-up, pilot-scale trials will be performed.

Conclusions

This article describes the product evaluation at GenVec, in collaboration with Millipore, in identifying alternatives to the filters currently being used in the medium exchange, clarification, and concentration/diafiltration process steps. In addition, filters were evaluated for post-clarification filtration and post-hold sterile filtration. Scale-up filter sizing was estimated based on small-scale data. For most of the process steps, pilot-scale trials will have to be performed prior to scale-up.

Medium Exchange

Millipore's Pellicon 2 device was able to perform medium exchange successfully when permeate flow control was used. The small-scale Prostak device trial performed while using the same 0.45 µm membrane showed equivalent performance to the Pellicon 2 device and has the advantage of being steamable. Therefore, 0.45 µm-rated Prostak flat sheet filters are recommended for this process.

Clarification

Option I: The Millistak+ 30DE and C0HC filters showed good filtrate clarity, recovery, and will provide results in the desired process compression. The scale-up data agreed acceptably with the small-scale data. The best option is the C0HC filter, available in Millipore's pod format. 30DE is also a good option but requires the purchase of a new stainless steel housing at 100 L scale and above.

Option II: Post-filtration clarity and product recovery utilizing the Clarigard and Polysep was comparable to the existing filtration process. The Clarigard filter, followed by a CGW6 Polysep II filter, can successfully clarify the lysate and fit within the existing stainless steel housing at the 100 L scale.

Post-Clarification Filtration

Millipore's Durapore 0.45 µm PVDF filter showed no loss of titer and is a good option for this step.

Concentration/Diafiltration

A 10X concentration for a 10 L batch of material, followed by a 5X diafiltration, can be successfully performed using Millipore's flat sheet 500KD Biomax with a C screen in a Pellicon 2 device.

Post-Hold/Pre-Column Filtration

The purpose of this step was to reduce any potential bioburden and protect the downstream chromatography column. The Durapore 0.22 µm PVDF filter showed no loss of titer and therefore, is a good option for this step.

ACKNOWLEDGEMENTS

The authors would like to thank Perry Newton of GenVec, Inc. for the technical discussions, and Ranti Ogunsola of GenVec, Inc. for her assistance with the scale-up experiments. We would also like to thankKara Fothergill of Millipore Corp. for her assistance with the Prostak experiments, and John Stefanyk and Gisela Schimke of Millipore Corp. for their comments and suggestions.

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Terminology

Cross Flow Rate:	Flow rate of the feed solution across the membrane surface
Delta P:	Pressure drop along the length of the membrane surface on the feed side due to energy losses (due to friction and turbulence and permeate); $\Delta P = P_{feed} - P_{retentate}$
Permeate:	Product that passes through the membrane
Retentate:	Material retained by the membrane
Permeate Flux:	Permeate Flow Rate/Area, typically expressed as Liters per m^2 per hour or LMH
TFF:	Tangential Flow Filtration, denotes a mode of filtration where the feed flows parallel to membrane surface and the resultant sweeping actions prevents build-up of the retained material on the membrane surface
TMP:	Trans-membrane pressure, is the average pressure acting across the membrane surface; $TMP = (P_{feed} + P_{retentate}) \div 2 - P_{permeate}$
VCF:	Volumetric Concentration Factor at time t = Initial feed volume ÷ Retentate volume at time t
VCF_{gel}:	Volumetric Concentration Factor when permeate flux is zero
VCF_{df}:	Volumetric Concentration Factor for diafiltration minimized for process time. VCF_{df} = VCFgel ÷ 2.718