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Clarification of Adenovirus serotype 5: Robust protection of downstream purification steps

Application Note

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turning science into solutions

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Introduction

Viral vectors are widely used for various applications such as gene therapy, vaccines or treating cancer with oncolytic viruses. Recombinant Adenovirus serotype 5 vectors commonly serve as vaccine platforms. They can be efficiently produced using mammalian cells grown in suspension cell culture. The production is usually terminated by lysing the cells and releasing the viruses. Many contaminants such as HCP and DNA are also released during cell lysis and, therefore, the first challenging downstream step of the Adenovirus purification is clarification for cell debris removal.

An efficient clarification step needs to combine a high capacity for solids particle removal with high product yield, ease of scale-up and protection of the later downstream operation units. Altogether these features result in a robust and cost-effective process.

Sartopure® PP3 is the latest generation of a pleated synthetic filter made of advanced graduated polypropylene fleeces (Figure 1). It combines highly effective particle retention and exceptional total throughput [1]. This filter is often used at early clarification steps in viral vaccine production production. The structure of the filter is suitable for clarification of virus cell culture supernatant, for microcarrier removal and low cell density suspension cell cultures. It is available in a self-contained capsule format, assuring the highest operator safety.

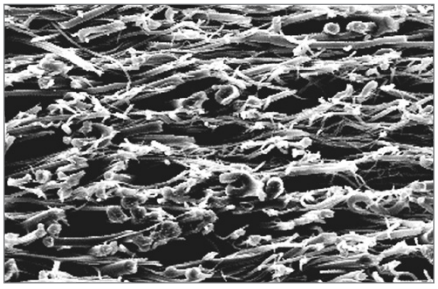

Figure 1: One of the Polypropylene fleeces used in Sartopure® PP3 prefilter.

Sartopore® 2 XLG is a sterilizing grade membrane filter made of two layers of polyethersulfone membranes [2]. The filter features a heterogeneous double layer construction made of a 0.8 µm rated prefilter followed by a 0.2 µm rated sterilizing-grade filter. The relatively coarse pore size of the prefilter effectively retains larger contaminants such as the remaining cell debris after initial harvest and protein aggregates, and prevents premature blocking of the final filter. Therefore, Sartopore® 2 XLG is perfectly suited for protecting early downstream processing steps after clarification such as crossflow filtration or chromatography.
Sartopore® 2 XLG filters are available in a range of sizes and formats suitable for small scale testing (Figure 2) up to those used in large scale production. Sartopore® 2 XLG is available as gamma irridiatable filter capsules and for that reason ideally suited for single-use filtration processes using pre-sterilized filter assemblies.

Figure 2: SartoScale 25 unit for small scale testing.

Objectives
The objective of this experiment was to optimize the clarification of an Adenovirus serotype 5 (Ad5) suspension by comparing different filters. The clarification is currently done using two membrane filters in line. In the following these filters are named "benchmark 1" and "benchmark 2".

Materials

1. Filters tested (Table 1)

Filtration Step	Filters tested
First step	Sartopure® PP3 (0.45 µm)
	Sartopore® 2 XLG (0.8/0.2 µm)
	Benchmark 1 membrane filter
Second step	Sartopore® 2 XLG (0.8/0.2 µm)
	Benchmark 2 membrane filter

Table 1: Different filters used in this study.

2. Adenovirus type 5 suspension:
The production of Ad5 was based on the infection of HEK 293 cells, cultured in a commercial serum-free media with 4 mM Glutamax in a 20 L working volume bioreactor. The bioreactor inoculum was 0.5×10^6 cells/mL, the cell concentration at infection was 1.0×10^6 cells/mL, and a MOI (Multiplicity of Infection) of five was used. The bioreactor was harvested 72 hours after infection. At the end of the culture but before Ad5 harvesting, the cells were lysed inside the bioreactor by adding Triton X-100 (X100, SIGMA-ALDRICH, Switzerland) to a final concentration of 0.1% (w/w) and increasing stirring to 1000 rpm for 1 minute. At the same time a nuclease treatment was performed in the bioreactor. The bulk was incubated for 240 minutes at 37°C with a nuclease final concentration of 50 U/mL and with intermittent stirring. The turbidity of the cell culture broth before filtration was 24.6 NTU.

3. Zero-T is a filtration setup with software for filterability trials and scale-up calculation [3]. The system comprises flexible hardware with a balance and up to 4 pressure transducers in combination with a sophisticated, priority data logging and analyzing software (Figure 3). The system is used to quickly identify the best filter material and sizing combinations using only a small volume of product.

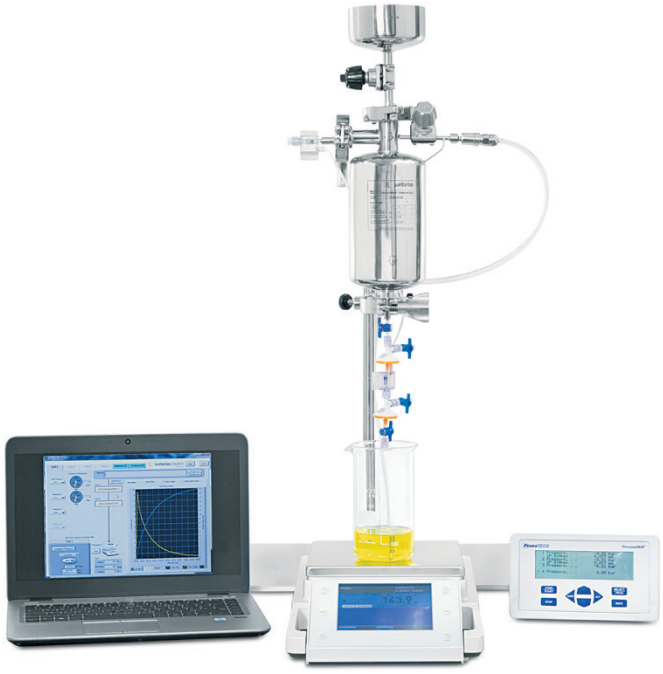

Figure 3: Zero-T set-up for constant pressure filterability trials.

Methods

In a first experiment Ad5 suspension was filtered with three different filters: (i) Sartopure® PP3 0.45 µm, (ii) Sartopore® 2 XLG and (iii) benchmark 1 membrane filter. In a subsequent second experiment the filtrate from Sartopure® PP3 0.45 µm was processed with two filters: (i) Sartopore® 2 XLG and (ii) benchmark 2 membrane filter.

The filterability trial was performed under constant pressure conditions using a pressure vessel. For each filtration step the amount of filtrate measured with a balance and the inlet pressure in relation to time are recorded and transmitted to a computer. The filtrate was collected in a glass container.

The scale-up model used by the Zero-T software, derives from the combination of DARCY's law and the gradual pore plugging model and is defined by following Equation (1):

V = Jo*Ao P*t/1 + Jo*KS*P*t (1)

V = filtered volume (mL)
Jo = flow per pressure unit (ml/min*cm²*bar)
P = pressure (bar)
t = filtration time (min)
KS = blocking constant for the product filtered (cm²/ml)
Ao = filtration area (cm²)

Equation (1) can be re-arranged as:

t/V = (KS/Ao)*t + 1/Ao*P*Jo (2)

In addition, the total filtration capacity **(V_{final})** is obtained by using equation **(2)**: For high t-values, 1/Ao*P*Jo becomes negligible, and the equation **(2)** can then be summarized as:

t/V = (KS/Ao)*t,

so

V_{max} = Ao/KS

Total Adenovirus particles were quantified by real-time PCR and the level of DNA and HCP impurities were determined using Pico Green and HEK 293 HCP assays respectively. The turbidity was measured using a turbidity meter with scattered light detection.

Results

Using a constant pressure of 1 bar, the following filters were compared for the first filtration step: Sartopure® PP3 0.45 µm, Benchmark 1 filter (0.8/0.45 µm) and Sartopore® 2 XLG. The normalized flow rate and total throughput results are shown in Figures 4 and 5, respectively.

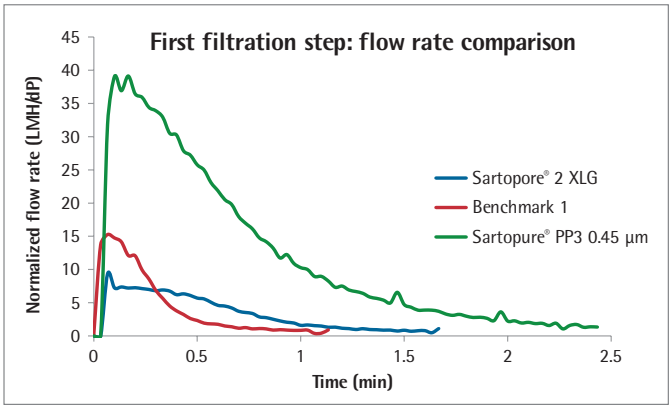

Figure 4: Normalized flow rate with Sartopure® PP3 0.45 µm (green), Benchmark 1 filter (red) and Sartopore® 2 XLG (blue).

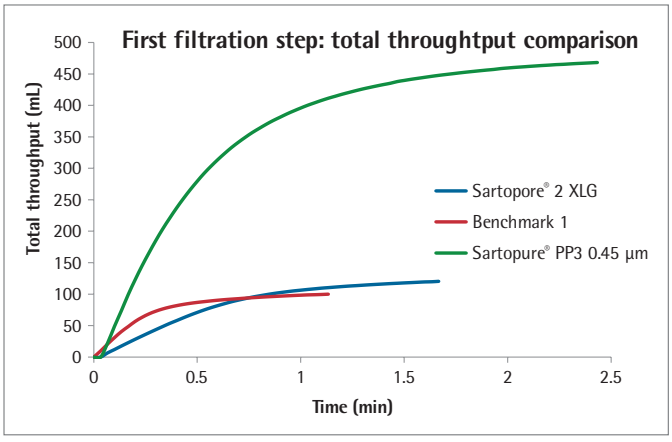

Figure 5: Total throughput with Sartopure® PP3 0.45 µm (green), Benchmark 1 filter (red) and Sartopore® 2 XLG (blue).

The filtrate from the Sartopure® PP3 0.45 µm was then tested with two different sterilizing-grade filters: the current benchmark 2 filter (0.45/0.2 µm) and Sartopore® 2 XLG 0.8/0.2 µm. The normalized flow rate and total throughput results are shown in Figures 6 and 7, respectively.

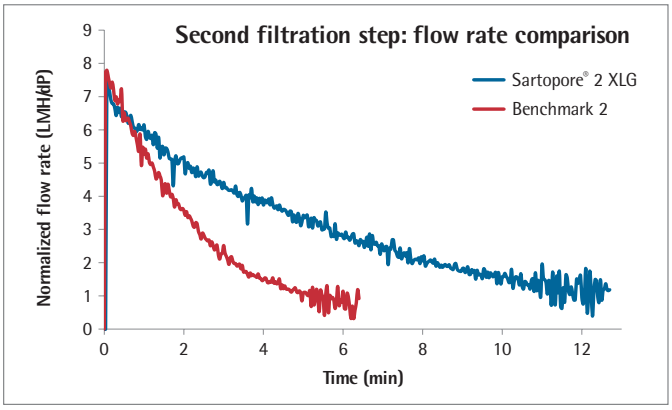

Figure 6: Normalized flow rate with Benchmark 2 filter (red) and Sartopore® 2 XLG (blue).

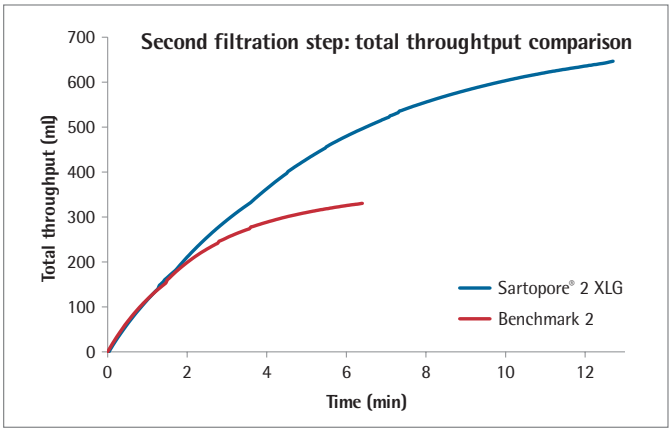

Figure 7: Total throughput with Benchmark 2 filter (red) and Sartopore® 2 XLG (blue).

Analytical results of the different filtrates can be compared in Table 2 and filterability parameters including scale-up to 100 L are shown in Table 3.

Sample	Turbidity (NTU)	Recovery[2] (%)	Host cell DNA (µg/mL)	Host cell protein (µg/mL)
Initial	24.6	-	0.91	16.21
Sartopure® PP3 0.45 µm	10.4	100	0.89	11.50
Sartopore® 2 XLG[1]	6.8	100	0.79	13.74

[1] After filtration with Sartopure® PP3 0.45 µm
[2] Based on total Adenovirus particles

Table 2: Analytical tests results from filtrates collected from Sartopure® PP3 0.45 µm and Sartopore® 2 XLG.

Filtration step	Filter	V_{final} (mL)	Area (m^2)[3]	R^2	Capsule size
First	Sartopure® PP3 0.45 µm	470	0.35	0.99	10" MaxiCaps® (0.4 m^2)
	Sartopore® 2 XLG	128	1.27	0.96	20" MaxiCaps® (1.6 m^2)
	Benchmark 1	122	1.7	0.99	30" Cartridge (1.8 m^2)
Second	Sartopore® 2 XLG	1165	0.26	0.99	Size 9 MidiCaps® (0.26 m^2)
	Benchmark 2	545	0.49	0.99	10" Cartridge (0.6 m^2)

[3] Filtration time (t) is 30 minutes

Table 3: Filterability parameters and scale-up data to 100 L for different filters tested.

Conclusion

The analytical assays reveal a product recovery of 100% and some contaminants removal. When using Sartopure® PP3 0.45 µm as a prefilter to protect a Sartopore® 2 XLG downstream filter allow a 5-fold reduction in pre-filter area and a 2-fold reduction in sterilizing-grade filter area when compared to the benchmark filters. The recommended filter train for 100 L production is a 10" MaxiCaps® Sartopure® PP3 0.45 µm with 0.4 m^2 (Figure 8) followed by size 9 MidiCaps® Sartopore® 2 XLG with 0.26 m^2 (Figure 9).

The advantages on the proposed filtration technology are: (i) self-contained single-use capsules to assure operator safety, (ii) no need for a centrifuge reducing the capital investment, (iii) high product yield, (iv) high total throughput resulting in less filter area requirement and (v) high flow rates resulting in a faster process.

Sartopure® PP3 and Sartopore® 2 XLG filters are part of our single-use downstream platform for adenovirus type 5 purification [4-6].

Figure 8: Sartopure® PP3 0.45 µm T-style MaxiCaps®.

References

[1] Brochure "Sartopure® PP3", Order No.: 85037-547-68
[2] Datasheet "Sartopore® 2 XLG", Order No.: 85032-535-11
[3] Datasheet "Zero-T Automated Filterability and Scale up System", Order No.: 85032-542-79
[4] Application note" Ultrafiltration and Diafiltration of Adenovirus Serotype 5 with Sartocon® Slice cassettes installed within a Sartoflow® Smart benchtop crossflow system, Order No.: 85037-560-49
[5] Application note "Optimizing Adenovirus Purification Processes", Order No.: 85037-558-81
[6] Enabling viral vaccine production, Amélie Boulais, Dr. Nick Hutchinson, Dr. Fritjof Linz, GEN, November 2016

Figure 9: Sartopore® 2 XLG MidiCaps®.

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