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A case study for understanding impact of nuclease elimination on bioprocess cost and downstream AAV5 quality using 3M™ Harvest RC Chromatographic Clarifier

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Highlights

- 3M™ Harvest RC Chromatographic Clarifier was used as a single-stage device to clarify a model HEK AAV detergent lysate without nuclease digestion, and achieved >90% AAV recovery, >95% turbidity reduction, and >2 logs HEK host cell DNA reduction.
- Process cost modeling with BioSolve Process (https://www.biopharmservices.com/biosolve-software/biosolve-process/) highlights the opportunity for substantial cost reduction if the expensive nuclease raw material is eliminated, even though a lower throughput is achieved for the primary clarification stage.
- AAV5 detergent lysate generated in the presence or absence of nuclease, and clarified with 3M™ Harvest RC Chromatographic Clarifier, demonstrated comparable behavior in downstream affinity purification and exhibited similar quality as measured by residual HEK host cell DNA and the absence of AAV5 aggregation.

Key words

- Gene therapy
- AAV manufacturing
- Cost modeling
- Nuclease elimination
- 3M™ Harvest RC Chromatographic Clarifier

Introduction

Adeno-associated virus (AAV) vectors are the primary gene delivery platform for treating various human diseases, with successful preclinical and clinical applications in gene replacement, silencing, and editing. By March 2024, five AAV-based therapies have FDA approval, and over 200 clinical trials are ongoing. The primary approach for AAV production is transient triple transfection in host cells like HEK293, complemented by other production methods such as baculovirus-based expression systems and helper virus-free approaches like the herpes simplex virus (HSV) system.[1]

AAV bioprocessing poses a unique challenge in that the cells often must be lysed in order to extract and harvest the AAV particles from the host cell culture. The lysis of the cells concurrently releases host cell DNA which must then be removed during the purification process. The presence of hcDNA in the final product poses substantial safety concerns for treated patients, given its potential infectivity and oncogenicity. Consequently, the FDA enforces stringent regulations regarding residual hcDNA concentration and fragment sizes.[2–3] Typically, expensive nucleases are deployed to degrade the host cell DNA prior to clarification.[4]

In this study, we investigate the performance of the charge-based 3M™ Harvest RC Chromatographic Clarifier in the AAV5 purification process. HEK AAV5 lysates were generated in the presence or absence of nuclease during detergent lysis, and 3M™ Harvest RC Chromatographic Clarifier performance was assessed by quantifying turbidity reduction, residual host cell DNA, and AAV5 recovery yield. The downstream purification of these clarified fluids was further evaluated, and essentially equivalent AAV quality and purity were measured in both fluids following AAVX affinity chromatography. BioSolve Process (https://www.biopharmservices.com/biosolve-software/biosolve-process/), an industry standard cost modeling program, was utilized to quantify the cost impact of nuclease elimination as a function of: (i) throughput at the primary clarification stage and (ii) nuclease raw material cost.

Method and materials

AAV production by triple transfection in HEK cells

AAV2, AAV5, and AAV6 were produced by transient triple plasmid transfection (AAVpro packaging plasmid sets, Takara; Rep/Cap Plasmids, Aldevron) of HEK293 cells (Viral Production Cells 2.0., Thermo Fisher Scientific) with viral production media (Thermo Fisher Scientific) in a 10 L bioreactor (Eppendorf, BF320). The transfection was performed when the cell density reached 2E+6 cells/mL and the cell culture was harvested post-72 h of the transfection.

Cell culture detergent lysis and nuclease treatment at harvest

At harvest, Tween20 (Sigma-Aldrich) with 0.5% w/v final concentration, was added to the bioreactor to lyse the HEK cells. Where indicated, 40 U/mL final concentration of DENARASE® endonuclease (c-LEcta GmbH) were added to the bioreactor to degrade host cell DNA. The cell culture was incubated in the bioreactor for 2 hours, under 37°C and 145 rpm agitation. When the 2 hours incubation was done, 5 M NaCl solution (Teknova) was added to adjust the lysate conductivity to 25 mS/cm.

Lysate clarification by 3M Harvest RC chromatographic clarifier

Before clarification, 20 mL of salt adjusted cell lysate was pulled out from the bioreactor and centrifuged at 3200 xg for 10 min. The supernatant was aliquoted and stored immediately at -20°C; this aliquoted fluid served as the baseline for determining AAV recovery and DNA removal.

Clarifications of crude AAV lysates were carried out using 3M™ Harvest RC Chromatographic Clarifier (Solventum). Before clarification, the 3M™ Harvest RC Chromatographic Clarifier capsules were flushed with 1X PBS at a 100 LMH flow rate. For clarification, the 3M™ Harvest RC Chromatographic Clarifier capsule was connected to the bioreactor harvest line, and the crude lysate was processed by 3M™ Harvest RC Chromatographic Clarifier at a 100 LMH flow rate with a peristaltic pump. The clarification was terminated when the capsule inlet pressure reached 5 psi. Each filtrate was aliquoted and stored immediately under -20°C; these fluids served as the samples for AAV recovery and DNA removal. Turbidities were measured for the crude lysate and clarified fluid using a turbidity meter (Fisher Scientific).

AAV and HEK hcDNA quantification

AAV2, AAV5, and AAV6 capsid titers were determined using the AAV Xpress ELISA kits (Progen).

HEK host cell DNA was extracted using resDNASEQ™ HEK293 DNA Sample Preparation Kits (Applied Biosystems) and quantified by qPCR using resDNASEQ™ Quantitative HEK293 DNA Kits (Bio-Rad), following the manufacturer's instruction.

HEK host cell DNA fragment sizes were charactered by ddPCR using Vericheck ddPCR HEK293 Residual DNA Size Kits (Thermo Fisher Scientific) and Bio-Rad ddPCR system, with extracted DNA by resDNASEQ™ HEK293 DNA Sample Preparation Kits (Applied Biosystems), following the manufacture's instruction.

E1A fragment sizes were extracted and quantified by resDNASEQ™ Quantitative E1A DNA Fragment Length Kits (Thermo Fisher Scientific).

Cost modeling

Cost models were evaluated using BioSolve Process (https://www.biopharmservices.com/biosolve-software/biosolve-process/) Version 9.0 using parameters determined from internal AAV experiments and/or estimated from external discussions with AAV manufacturing experts. Assumptions include: 450 L and 1800 L working volume for 500 L and 2000 L reactors, respectively; 2-stage depth filtration (1:1 ratio) throughput of 140–225 L/m^2; nuclease cost of $6,700/0.02 L; nuclease concentration of 40 U/mL; Detergent used is Tween 20 at 0.05% w/v; AAV product titer of 1E+15 particles/L.

Affinity purification

Prior to affinity purification, the frozen, clarified lysates were thawed and subjected to 0.2 µm filtration. 1 mL POROS™ GoPure™ AAVX Pre-packed Column (Thermo Fisher Scientific). Loading: approximately 1E+14 viral particles/mL; Flow rate: 1 mL/min; Equilibration Buffer: 20 mM Tris, 200 mM NaCl, pH 7.8–10 CV; Wash Buffer #1: 20 mM Tris, 200 mM NaCl, pH 9.0–10 CV; Wash Buffer #2: 20 mM Tris, 200 mM NaCl, pH 9.0–10 CV ; Elution Buffer: 20 mM Tris, pH 2.5, 10 CV; Strip Buffer #1: 0.1 M Citric Acid, pH 2.1–15 CV; Strip Buffer #2: 6 M Guanidine HCl–15 CV. Elution fraction was immediately neutralized with 1 M Tris pH 8.9.

SEC

Affinity purified samples were stored at -80°C until analysis. After thawing, the samples were screened via STUNNER for large aggregates prior to injection.

A Waters™ XBridge Protein BEH SEC column (450 Å, 3.5 µm, 7.8 mm x 300 mm) was used for analysis on an Agilent 1260 Infinity II HPLC system. A phosphate buffer was used (10 mM Sodium Phosphate pH 7.4, 200 mm Sodium Chloride) as the mobile phase at a flow rate of 0.8 mL/min. Sodium azide was added to the mobile phase (0.2% w/v) to prevent microbial growth. All buffers were 0.2 µm sterile filtered and degassed prior to use. Sample injection volume was 50 µL for all samples.

The detection system consisted of the Agilent 1260 Infinity II UV-Vis detector (λ = 280 nm), a Wyatt DAWN® MALS detector, and a Wyatt Optilab® dRI detector. Data were analyzed using Wyatts ASTRA® software.

Results and discussion

3M™ Harvest RC Chromatographic Clarifier capsules have been found to be effective in clarifying AAV lysate and recovering AAV

3M™ Harvest RC Chromatographic Clarifier, a charge-based clarification solution offered by Solventum for biopharmaceutical manufacturing, has demonstrated efficacy in increasing product yields and streamlining recombinant protein processes by replacing centrifugation and/or depth filtration steps.[5] Furthermore, 3M™ Harvest RC Chromatographic Clarifier has been shown through industry standard platform modeling to reduce the cost of goods sold (COGS)[6]. We sought to explore if 3M™ Harvest RC Chromatographic Clarifier, while designed for simplifying mAb bioprocessing, could provide similar process simplification and cost benefits to AAV bioprocessing. In particular, AAV bioprocessing faces significant challenges in harvesting AAV particles due to concurrent hcDNA release during cell lysis, with expensive nucleases often being utilized for pre-clarification removal of the hcDNA.

3M™ Harvest RC Chromatographic Clarifier was used to clarify AAV5 detergent lysed material and achieved >90% average recovery yield (Fig. 1B), low filtrate turbidity (<10 NTU) (Fig. 1C), and substantial DNA reduction in the filtrate (Fig. 1D). Of note, similar performance was obtained in the presence (40 U/mL) or absence (0 U/mL) of DENARASE® endonuclease in the crude lysate (Fig. 1B, C, and D). Further data on the evaluation of 3M™ Harvest RC Chromatographic Clarifier for the clarification of AAV2, AAV5, and AAV6 serotypes, along with deeper characterization of the DNA removal observed can be found in the corresponding case study (An investigative case study of the removal of host cell DNA from HEK detergent lysates using 3M™ Harvest RC Chromatographic Clarifier Application Note, Solventum, 2024). Observing these performance capabilities and 3M™ Harvest RC Chromatographic Clarifier's consistent AAV recovery from bench to commercial scale products (Fig. 1E), we wanted to further evaluate the downstream purification behavior of AAV5 lysate clarified with 3M™ Harvest RC Chromatographic Clarifier. Hence this study aimed to further investigate the process impacts of eliminating pre-filtration nucleases on 1) the theoretical cost reduction of the primary clarification step (Fig. 2), 2) affinity purification step performance (Fig. 3), and 3) AAV quality and residual hcDNA level (Fig. 4).

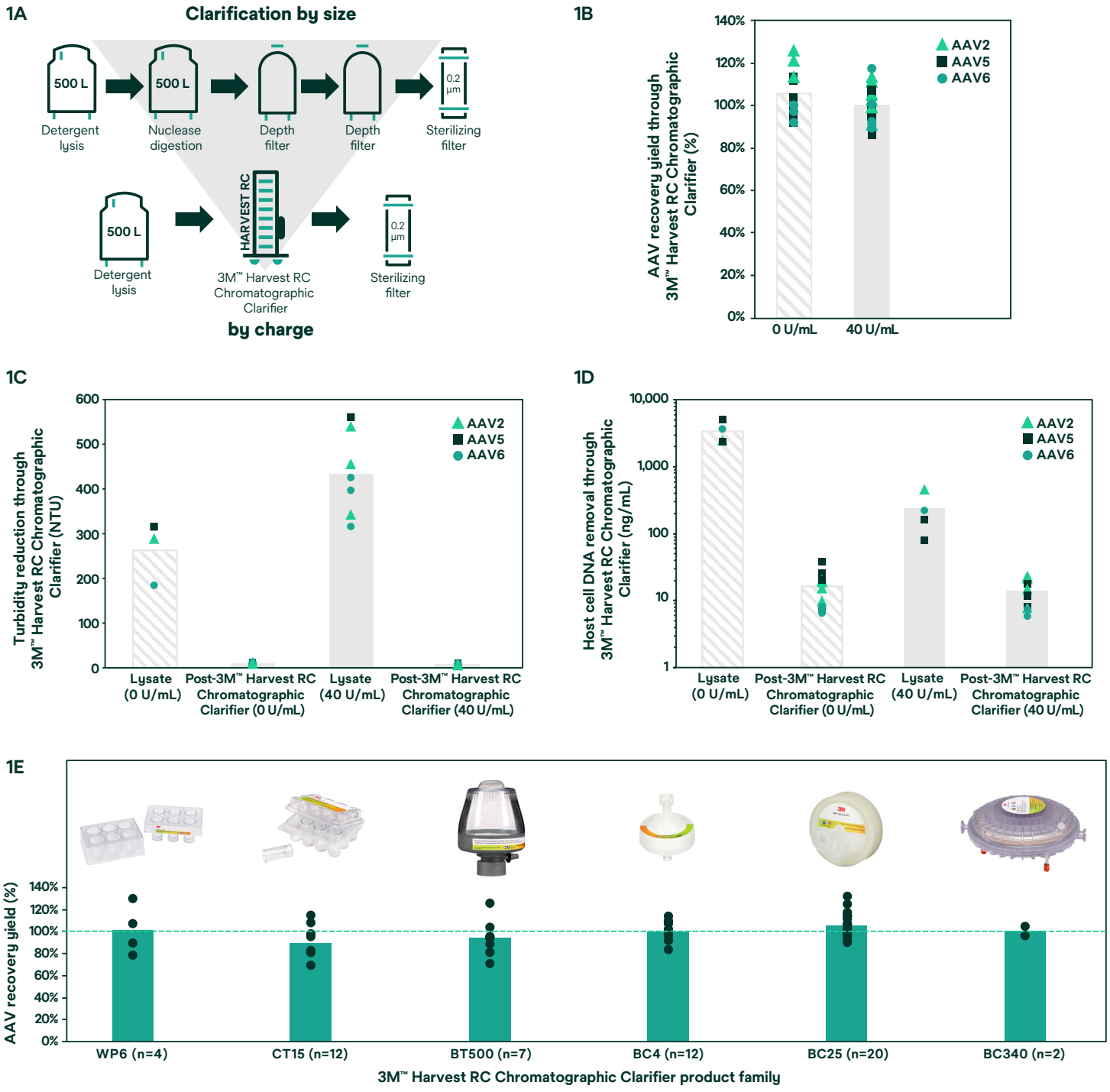

Figure 1. A single-stage and nuclease-free AAV clarification approach using 3M™ Harvest RC Chromatographic Clarifier. (A) 3M™ Harvest RC Chromatographic Clarifier simplified the AAV clarification step by replacing the nuclease digestion and 2-stage depth filtration. (B) AAV2, AAV5, and AAV6 recovery yields through 3M™ Harvest RC Chromatographic Clarifier clarification. (C) AAV2, AAV5, and AAV6 crude lysate turbidity reductions through 3M™ Harvest RC Chromatographic Clarifier clarification. (D) HEK host cell DNA removal by 3M™ Harvest RC Chromatographic Clarifier from lysates with 0 U/mL DENARASE® endonuclease or 40 U/mL DENARASE® endonuclease. Bars represent mean values; dots represent individual clarification runs by 3M™ Harvest RC Chromatographic Clarifier. (E) AAV recovery yields through 3M™ Harvest RC Chromatographic Clarifier clarification at various product scales. Bars indicate mean values; dots indicate individual clarification runs using 3M™ Harvest RC Chromatographic Clarifier.

Cost modeling identifies the opportunity for cost reduction by implementing 3M™ Harvest RC Chromatographic Clarifier

The industry standard platform BioSolve Process (https://www.biopharmservices.com/biosolve-software/biosolve-process/) was used to model the impact of implementing a nuclease-free 3M™ Harvest RC Chromatographic Clarifier step on the cost of the primary clarification stage of AAV bioprocessing. The cost model calculated up to a 38% reduction in the cost of the primary clarification stage (when using average throughputs obtained experimentally in Fig. 2A and Nuclease B raw material costs). This calculated cost reduction was driven by the massive reduction in the nuclease raw material cost (Fig. 2C). This net cost reduction is substantial and is achieved despite the lower relative throughput achieved when nuclease is removed (Fig. 2A). It is known that nuclease disrupts DNA-associated complexes that can contribute to membrane fouling.[7] While every process is unique, potential cost reduction can be evaluated as a balance of throughput achieved and the nuclease raw material cost (Fig. 2B). Nuclease unit costs are likely to be higher early in development when manufacturers are producing fewer, smaller batches per year or when the drug target population is small. The model predicts that high nuclease unit cost scenarios (Nuclease A) could experience higher cost savings per batch by implementing a nuclease-free 3M™ Harvest RC Chromatographic Clarifier clarification process. However, cost savings per batch are also observed in the models using a lower unit cost nuclease (Nuclease B), which could be more realistic for discounted raw material costs when purchased at large volumes or using a lower unit cost nuclease alternative.

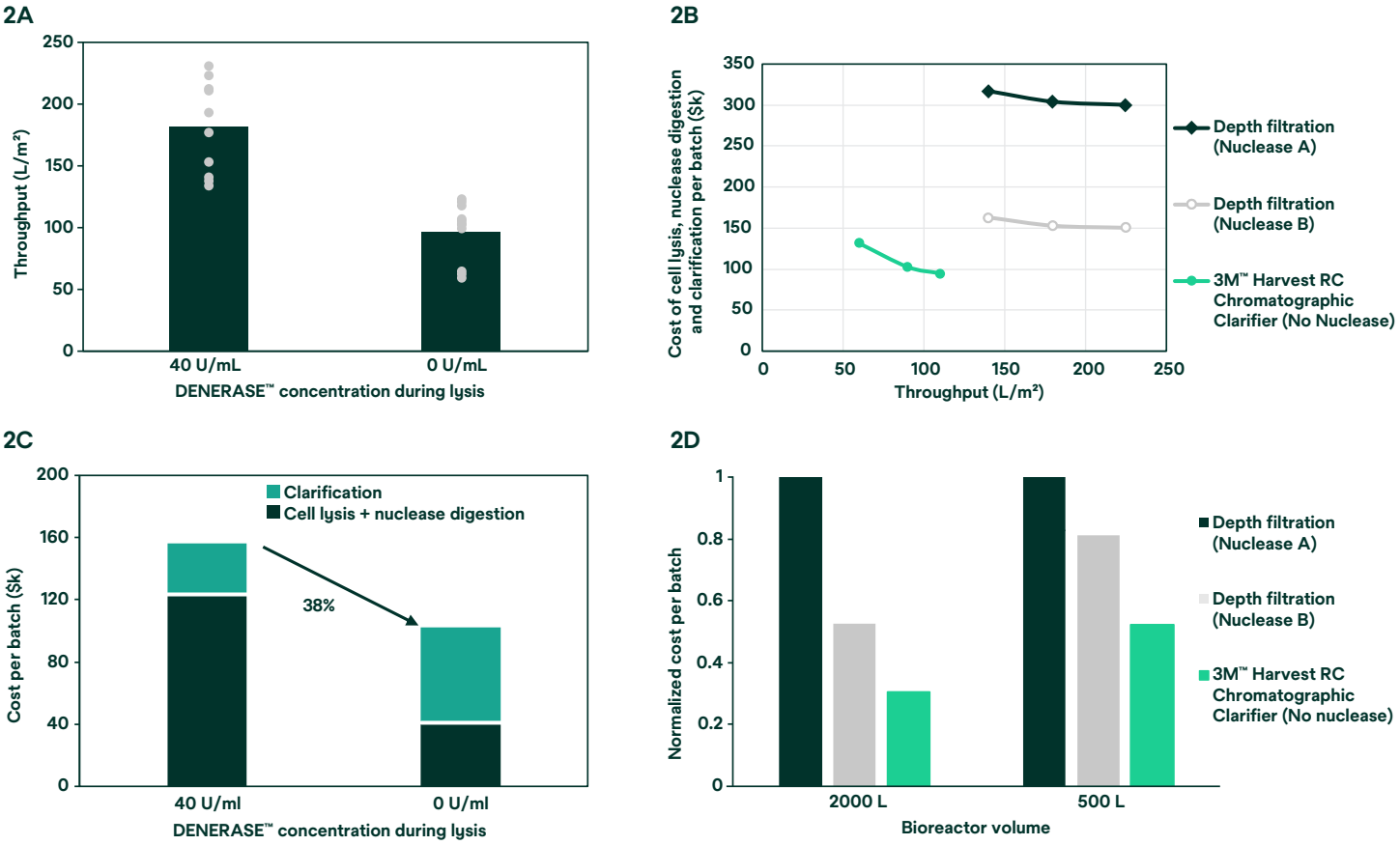

Figure 2. Impact of nuclease elimination on the primary clarification step. (A) 3M™ Harvest RC Chromatographic Clarifier throughput achieved from internal experiments clarifying lysates generated with 0 U/mL DENARASE® endonuclease or 40 U/mL DENARASE® endonuclease (B) Cost model dependence on throughput and nuclease raw material cost. Nuclease A — $18,700/0.02 L; Nuclease B — $6,700/0.02 L. (C) Relative cost of primary clarification of AAV5 lysates by 3M™ Harvest RC Chromatographic Clarifier with 0 U/mL DENARASE® endonuclease or 40 U/mL DENARASE® endonuclease using average throughput from (A) and an endonuclease cost of $6,700/0.02 L. (D) Cost model — Normalized cost per batch of cell disruption, DNA digestion, and clarification steps for 500 L and 2000 L bioreactors. Nuclease A — $18,700/0.02 L; Nuclease B — $9,300/0.02 L. BioSolve Process (https://www.biopharmservices.com/biosolve-software/biosolve-process/) Version 9.0 used for all cost modeling.

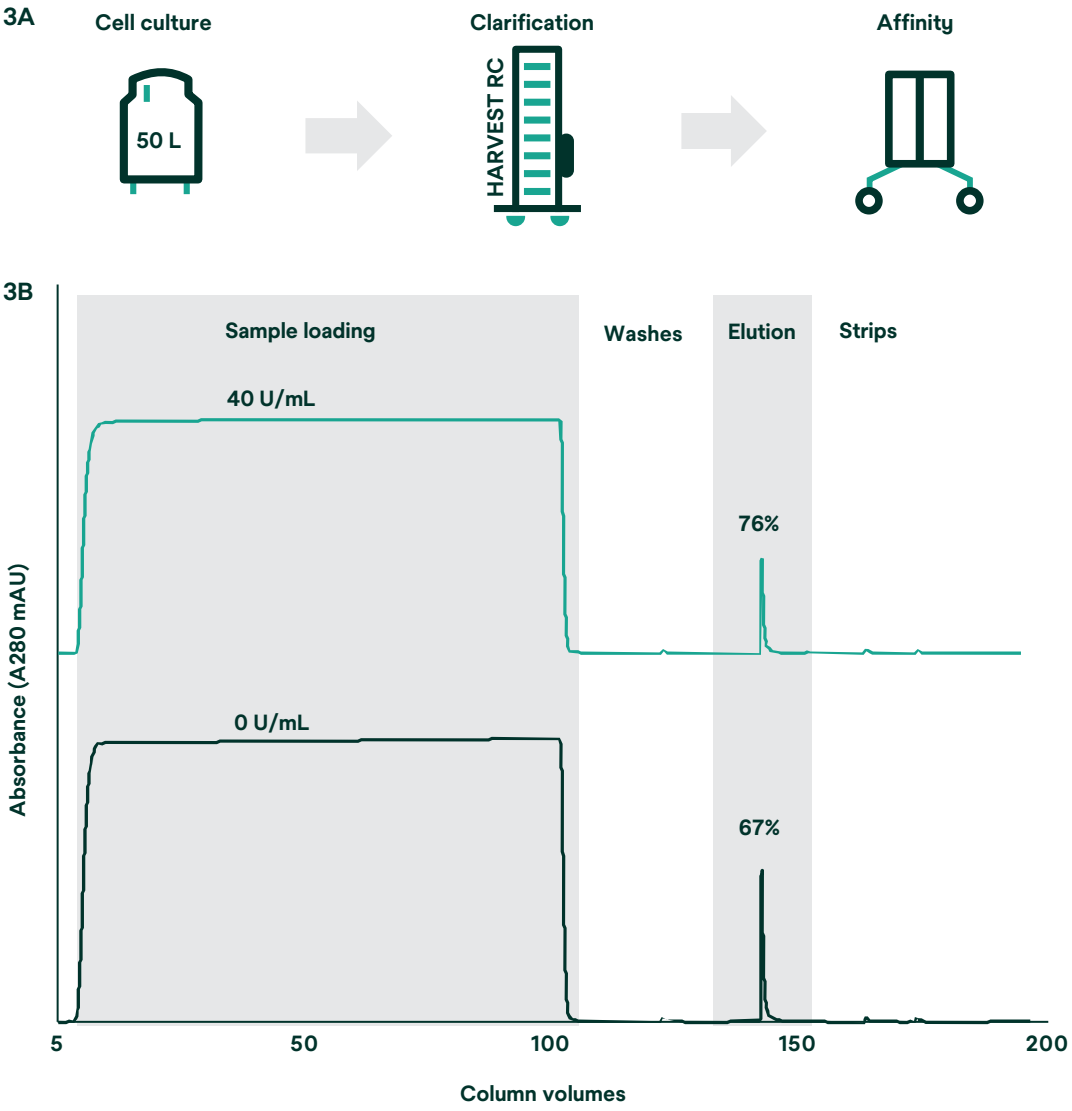

Figure 3: Effect of nuclease elimination on the downstream affinity purification step following 3M™ Harvest RC Chromatographic Clarifier clarification. (A) Process workflow schematic. (B) AKTA Chromatograms from affinity purification (n=1) of AAV5 clarified lysates. Percent recoveries are annotated above the elution step.

3M™ Harvest RC Chromatographic Clarifier clarified AAV5 lysates evaluated for performance in downstream purification process

We previously observed similar AAV product quality after 3M™ Harvest RC Chromatographic Clarifier clarification, irrespective of whether nuclease was present or not in the cell lysis step (Fig. 1). This 3M™ Harvest RC Chromatographic Clarifier clarified material was further processed through affinity purification to explore the impact on downstream AAV product quality as analyzed by residual HEK hcDNA (Fig. 4A), oncogenic E1A concentration and fragment size (Fig. 4B), and AAV aggregation state (Fig. 4C). Behavior of nuclease-treated and untreated lysates through affinity purification was comparable, with consistent recovery yields (Fig. 3). Both processes produced highly monomeric AAV5 product post-affinity, indicative of high AAV5 product quality (Fig. 4C).

Overall, the presence or absence of DENARASE® endonuclease in the cell disruption step resulted in similar post-affinity residual DNA levels (Fig. 4A, Post-Affinity). These comparable post-affinity DNA levels are obtained even though the residual HEK hcDNA levels in the lysates are significantly different (Fig. 4A, Lysate), with 40 U/mL DENARASE® endonuclease-treated samples characterized by >1 log lower quantities than nuclease-free lysates. Interestingly, the two different lysates clarified with 3M™ Harvest RC chromatographic clarifier contained extremely similar quantities of residual HEK hcDNA, and these hcDNA amounts were not further meaningfully reduced after affinity purification in these model experiments (Fig. 4A, Post-3M™ Harvest RC Chromatographic Clarifier). A similar trend was observed

when quantifying the presence of oncogenic E1A, a gene found in engineered AAV production cell lines like HEK293 to facilitate recombinant virus replication within host cells; studies have posited that E1A could pose safety risks to patients.[8] At the lysate generation step, 40 U/mL of DENARASE® endonuclease effectively removed >95% of E1A through DNA digestion (Fig. 4B, Lysate bars). The quantifiable levels of the E1A gene in the fluids clarified with 3M™ Harvest RC Chromatographic Clarifier were similar irrespective of the presence or absence of nuclease in the previous lysis generation step, and these E1A levels remained consistent after affinity purification (Fig. 4B, 3M™ Harvest RC Chromatographic Clarifier and Post Affinity bars). It is hypothesized that the remaining quantifiable DNA in the post-3M™ Harvest RC Chromatographic Clarifier and post-affinity samples are residual DNA actually encapsulated within the AAV capsid, as these levels were unaffected by analytical pre-treatment with DNAse (data not shown),[9] indicating that these contaminants were likely encapsulated and only able to be mitigated through cell culture/transfection optimization.

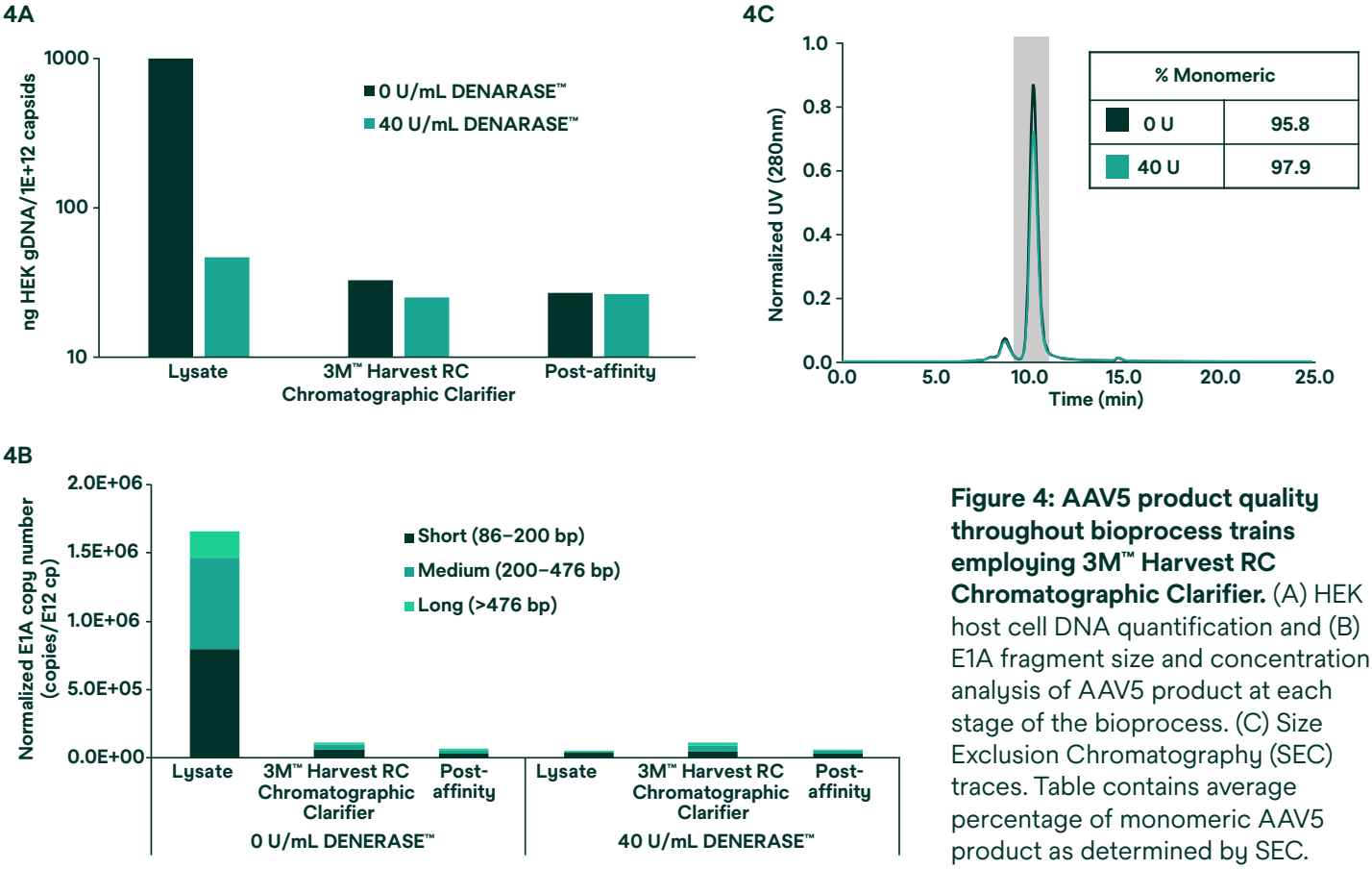

Figure 4: AAV5 product quality throughout bioprocess trains employing 3M™ Harvest RC Chromatographic Clarifier. (A) HEK host cell DNA quantification and (B) E1A fragment size and concentration analysis of AAV5 product at each stage of the bioprocess. (C) Size Exclusion Chromatography (SEC) traces. Table contains average percentage of monomeric AAV5 product as determined by SEC.

Summary

3M™ Harvest RC Chromatographic Clarifier was used in a single-stage operation to clarify HEK AAV detergent lysates. This approach provided >90% AAV recovery and >95% turbidity reduction for three serotypes (AAV2, AAV5, and AAV6). Our findings demonstrate that 3M™ Harvest RC Chromatographic Clarifier-clarified AAV5 lysates, treated with or without DENARASE® endonuclease, exhibited comparable AAV5 recoveries, turbidities, and HEK hcDNA contaminant profiles. A model was used to compare the costs of different approaches to harvest and hcDNA removal. A process that utilizes only 3M™ Harvest RC Chromatographic Clarifier, and no nuclease, is estimated to provide up to a 38% cost savings compared to traditional processes that utilize depth filters and nuclease. Researchers at Regeneron reported an even larger reduction in the cost-of-goods for the harvest step when 3M™ Harvest RC Chromatographic Clarifier was used in the manufacturing process for AAV8 and AAV9.[10] Contaminant profiles in this

AAV5 study were measured through the affinity purification step for processes with and without endonuclease. Both processes produced highly monomeric AAV products with comparable DNA contaminant profiles at the post-clarification and post-affinity steps.

The data described here demonstrate the potential for 3M™ Harvest RC Chromatographic Clarifier to be implemented in an AAV5 clarification strategy to achieve bioprocess simplification and cost reduction while still retaining high AAV5 product quality. While here we characterize only two nuclease conditions (40 U/mL and 0 U/mL), an intermediate nuclease condition that is customized for a specific process to balance throughput objectives with nuclease raw material cost savings could be envisioned. Though this case study used AAV5 as the representative serotype, some differences in results may be anticipated for other serotypes like AAV2 which are known to have more pronounced capsid: DNA interaction.[11]

Abbreviations

AAV: adeno-associated virus
HEK293: human embryonic kidney 293 cell line
hcDNA: host cell DNA
ELISA: enzyme-linked immunosorbent assay
qPCR: quantitative polymerase chain reaction
ddPCR: droplet digital polymerase chain reaction
NTU: nephelometric turbidity units

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