Process for production of oncolytic adenovirus

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

CY14107-04Jun20-AN

Process for production of oncolytic adenovirus

This application note describes a process for oncolytic adenovirus production, from upstream cell culture to downstream purification, using modern tools and technologies. Upstream virus production was performed in A549 cells using the single-use ReadyToProcess WAVE™ 25 rocking bioreactor system. Virus was released from the host cells by treatment with Tween™ 20 and clarified using ULTA™ filter capsules followed by concentration and buffer exchange using hollow fiber filters. Anion exchange (AEX) chromatography was used for virus capture and size exclusion chromatography (SEC) for polishing. The downstream process was optimized to meet regulatory demands on product purity and quality. Well-established analytical methods were used to ensure accurate monitoring of the processed material.

Introduction

Oncolytic viruses constitute a new promising therapeutic approach for treatment of cancer. These viruses selectively replicate in tumor cells and effectively kill these cells without harming normal cells. Selectively engineered, the virus does not only destruct the target tumor cell, but also stimulate the host's anti-tumor immune response.

Adenovirus is an extensively characterized and well-studied viral vector that infect both dividing and non-dividing cells without the risk of integration into the host genome. Also, generally causing a mild nature of disease, adenoviruses are considered as safe delivery vectors for gene therapy applications. Recently, oncolytic adenovirus has successfully been applied as cancer immunotherapies or tumor vaccines. As one of the most studied vectors for experimental and clinical use, adenovirus serotype 5 (AdV5) is a suitable system for development of a process for oncolytic adenovirus production.

Human lung carcinoma cells (A549) are commonly used for production of recombinant adenovirus for human gene therapy, including oncolytic adenovirus. A549 cells are traditionally propagated in adherent culture with serum-containing medium. As scaling up of anchorage-dependent cells can be a challenge, suspension cell cultures are therefore preferred as these are more easily scaled. However, adaptation of suspension cells can be time-consuming and difficult, and might affect virus titer negatively, making alternative solutions more feasible for laboratory- and clinical-scale production. One attractive alternative for scale-up using adherent cells is the use of microcarriers.

Previous work has demonstrated adenovirus production in HEK293 suspension cells using the ReadyToProcess WAVE 25 bioreactor system (1–4). In this work, performed by iBET, Oeiras, PT, anchorage-dependent A549 cells were grown in serum-containing medium using HYPERFlask™ cell culture vessels, and thereafter in suspension for production of oncolytic adenovirus in serum-free medium using the ReadyToProcess WAVE 25 bioreactor system.

In the harvest step, 0.5% Tween 20 was used for cell lysis to release adenovirus from the host cell instead of the commonly used Triton™ X-100, now on the authorization list (Annex XIV) of registration, evaluation, authorization, and restriction of chemicals (REACH) (5). For downstream purification, normal flow filtration (NFF) was used for clarification and tangential flow filtration (TFF) was used for concentration and buffer exchange. Two AEX resins were evaluated for the capture step. Following a second concentration step, polishing was conducted by SEC before sterile filtration of the final bulk product. An overview of the process is given in Figure 1.

Materials and methods

Cell expansion and bioreactor culture

A549 cell line (ATCC) was routinely propagated in tissue culture flasks at an inoculum density of 3×10^3 cells/cm^2 using Ham´s F12 medium, Kaighn´s modification supplemented with 10% fetal bovine serum (FBS). Prior bioreactor cultivation, adherent A549 cells were amplified in HYPERFlask vessels. At approximately 90% confluence, cells were harvested by trypsination and centrifuged at room temperature to remove serum-containing medium. Cells were resuspended in HyClone™ CDM4HEK293 medium supplemented with 4 mM L-glutamine for growth in suspension bioreactor culture using the ReadyToProcess WAVE 25 system equipped with a 10 L Cellbag™ culture vessel. Cells were inoculated at 0.5×10^6 cells/mL in a working volume of 5 L. Samples were taken every 24 h for determination of cell density and viability.

Oncolytic adenovirus production

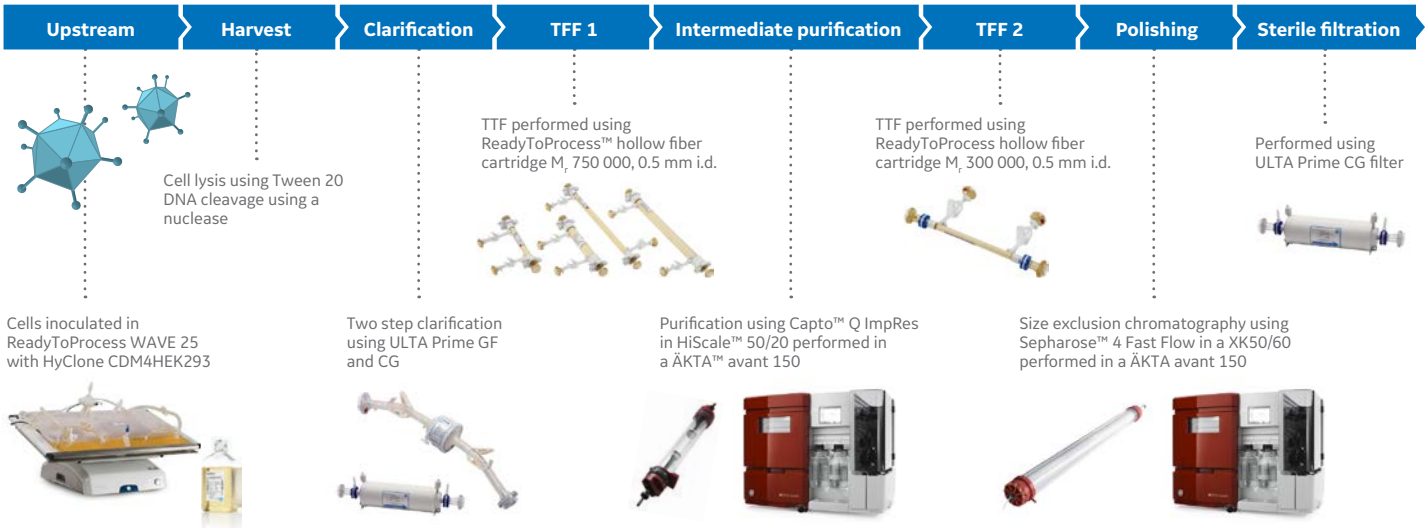

Fig 1. Process overview.

Virus propagation and harvest

The cells were infected with oncolytic AdV5 at a multiplicity of infection (MOI) of 10 at 72 h after inoculation. Cell lysis was performed 48 h post infection by the addition of Triton X-100 to a final concentration of 0.1% (evaluation experiments) or Tween 20 to a final concentration of 0.5% (scale-up experiments). Benzonase™ was added at 100 U/mL at the initiation of cell lysis for digestion of host cell DNA (hcDNA). Digestion was allowed to proceed for 4 h at 37°C and 16 rpm.

Clarification, concentration, and buffer exchange

Clarification of the harvest material was performed by NFF, using 47 cm^2 ULTA filter discs (2 µm GF and 0.2 µm CG) for the trials in 1 L scale and ULTA Prime capsules (5 µm GF 6 inch and 0.2 µm CG 4 inch) for 5 L scale up. These clarifications were performed with fixed pressure of 0.2 bar for 2 µm GF and 0.1 bar for 0.2 µm CG. For the 5 L scaled-up culture, the clarification was performed with constant flow of 600 L/m^2/h.

The clarified bulk was thereafter concentrated using hollow fibers with 26 and 50 cm^2 area for pore size trials NMWC M_r 300 000, M_r 500 000, and M_r 750 000 at a feed flow of 40 mL/min and a constant transmembrane pressure (TMP) of 1 bar. For the 5 L scaled-up culture, 290 cm^2 hollow fiber filter with NMWC M_r 750 000 at a feed flow of 300 mL/min and a constant TMP of 1 bar. The bulk was concentrated 2 times and diafiltrated 4 times, with 50 mM HEPES, pH 6.5 + 150 mM NaCl.

Intermediate purification

The intermediate purification step was executed using 120 mL of Capto™ Q or Capto Q ImpRes resin packed in HiScale 50/20 columns. The column was packed according to manufacturer's recommendation on compression factor and thereafter qualified by determination of height equivalent to the theoretical plate (HETP) and asymmetry. The column was equilibrated with 50 mM HEPES, pH 6.5 + 150 mM NaCl and loaded with 1.5×10^{11} virus particles (VP)/mL resin. The runs were performed at 300 cm/h. The column was washed with 2 column volumes (CV) of 50 mM HEPES, pH 6.5 + 150 mM NaCl. A one-step elution was performed using 50 mM HEPES, pH 6.5 + 1 M NaCl. The pooled fractions for each run were diluted 1:4 with 20 mM Tris, pH 8 + 25 mM NaCl.

Concentration and polishing

The semi-purified samples were concentrated 9 times using a 290 cm^2 hollow fiber filter with a nominal molecular weight cut-off (NMWC) of M_r 300 000 (UFP-300-C-3X2MA) with a flow of 200 mL/min and a TMP of 1 bar. The samples were diafiltrated 2 times with 20 mM Tris, pH 8 + 25 mM NaCl. For evaluation of loading volumes, an XK16/20 column packed with 34.5 mL Sepharose 4 Fast Flow was used. Obtained samples were thereafter loaded onto 300 mL Sepharose 4 Fast Flow resin packed in an XK50/60 column pre-equilibrated with 20 mM Tris, pH 8 + 25 mM NaCl. The virus-containing fractions were collected, pooled, and formulated by the addition of a stock solution of 20 mM Tris-HCl, pH 8 + 25 mM NaCl + 25% glycerol to a final concentration of 2.5% of glycerol.

Sterile filtration

For the sterile filtration, an ULTA Prime CG disc was used. The membranes were pre-equilibrated with 20 mM Tris-HCl, 25 mM NaCl, 2.5% glycerol, pH 8. The filtration was performed at a flow of 600 L/m^2/h.

Analyses

Total protein quantification
Total protein concentration was determined by BCA protein assay kit (23225, Thermo Fisher).

Total DNA quantification
Total DNA was assessed using the Quant-iT™ Picogreen™ dsDNA Assay Kit (P7589, Invitrogen), according to the manufacturer 's instructions.

A549 host cell protein ELISA
A549 host cell protein (HCP) were measured using an ELISA method (F230, Cygnus Technologies).

Benzonase quantification
To ensure that the endonuclease added in the process was removed, the Benzonase concentration in the final sample was measured using the Benzonase ELISA kit II (1016810001, Merck).

Infectious particle quantification
Oncolytic AdV5 infectious titer was determined by an end-point dilution method ($TCID_{50}$). In brief, 100 µL A549 cells (0.5×10^6 cells/mL) were seeded in 96-well tissue culture plates and incubated overnight at 37°C in 5% CO_2. The next day, the supernatant was replaced by serial dilutions (10^{-1} to 10^{-11}) of virus. Cytopathic effect on cells was determined 10 days later using inverted microscope and the $TCID_{50}$ titer determined using Spearman-Karber statistical method.

Viral genome particle quantification
Oncolytic AdV5 quantification of viral genome copies was determined by quantitative real-time polymerase chain reaction (qPCR). Before DNA extraction, samples were treated with 10 U DNAse for 30 min at 37°C. The reaction was stopped by addition of 8 mM EDTA and incubation at 75°C for 10 min. Viral copies were thereafter extracted using High Pure Viral Nucleic Acid Kit (11858874001, Roche). The viral genome copies were quantified by qPCR using the LightCycler™ 480 Probe Master (0470749001, Roche) and the LightCycler 480 instrument (Roche).

Results and discussion

Upstream production

After stepwise evaluation on individual process steps for oncolytic AdV5 production and purification, the complete process was performed. Adherent A549 cell expansion was conducted in HYPERFlask vessels using Ham´s F12 medium, Kaighn´s modification supplemented with 10% FBS. Harvested cells were centrifuged for removal of serum, resuspended in complete CDM4HEK293 medium, and inoculated (0.54×10^6 cells/mL) in the Cellbag culture vessel at 5 L working volume (Fig 2). Three days post inoculation, when grown to 0.8×10^6cells/mL in suspension, the cells were infected with oncolytic AdV5 at MOI 10. The culture was harvested by addition of Tween 20 to a concentration of 0.5% in 10 mM Tris, pH 8 to lyse the cells, 100 U/mL of Benzonase for DNA fragmentation, and incubated for 4 h at 37°C. Volumetric productivities of infectious virus particles (IP) and viral genomes (VG) were assessed by $TCID_{50}$ and determined to 1.17×10^{10} IP/mL and 1.12×10^{11} VG/mL, respectively (Fig 3). The VG/IP ratio after harvest was calculated to 9.5.

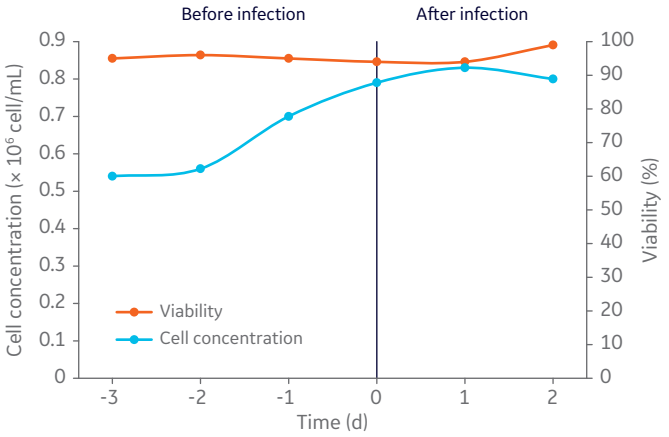

Fig 2. A549 cell concentration (× 10^6 cells/mL) and viability (%) in 5 L CDM4HEK293 medium, determined in bioreactor culture before and after infection for production of oncolytic adenovirus in suspension culture.

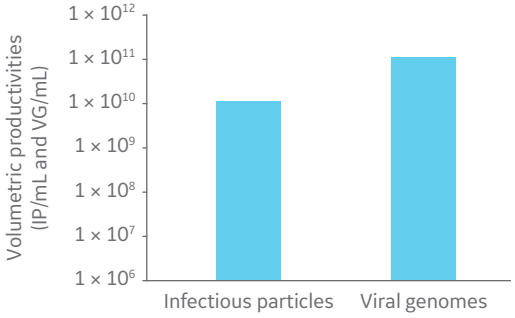

Fig 3. Volumetric productivities of infectious virus particles (IP) and viral genomes (VG).

Downstream purification

Clarification filters were evaluated in two steps, with initial trials performed with 1 L bioreactor harvest and scale-up with 5 L bioreactor harvest (Table 1). The two filters 5 µm GF followed by 0.2 µm SG filters were selected and resulted in removal of cell debris and initial reduction of proteins and DNA with high virus recovery, showing no significant reduction in total vp/mL. The turbidity level was also significantly reduced.

Table 1. Total protein, total DNA, turbidity reduction, and total virus recovery (%) before and after each clarification step with 2 µm GF and 0.2 µm CG (trials) and 5 µm and 0.2 µm CG (scale up) filters

	Sample	Turbidity	Protein	DNA	Viral particles
		Removal (%)			Recovery (%)
Trials	Initial	–	–	–	–
	2.0 µm GF	80	25	47	95
	0.2 µm CG	61	7	35	93
Scale up	Initial	–	–	–	9
	5.0 µm GF	65	5	7	90
	0.2 µm CG	78	6	32	92

Ultrafiltration/diafiltration (UF/DF) was evaluated on hollow fiber filters with three different membrane NMWCs of M_r 300 000, 500 000, and 750 000 according to Table 2. Samples were concentrated 4 times and diafiltrated 5 times. Results suggest that DNA and protein removal in all the hollow fiber devices, converges to the same removal percentage (Fig 4). Moreover, it was also observed that at M_r 750 000, the maximum impurity removal was achieved earlier, which can be justified by the larger pore size that facilitates impurity permeation.

Table 2. Characteristics and conditions for evaluation of hollow fiber filters

Hollow fiber	Area (cm^2)	ID (mm)	Feed flow (mL/min)	Feed volume (mL)	Particles (VG/mL)	TMP (bar)
750-C	26	0.5	30	200	1.43×10^9	1
500-C	26	0.5	50	250	1.43×10^9	1
300-C	50	0.5	30	347	1.43×10^9	1

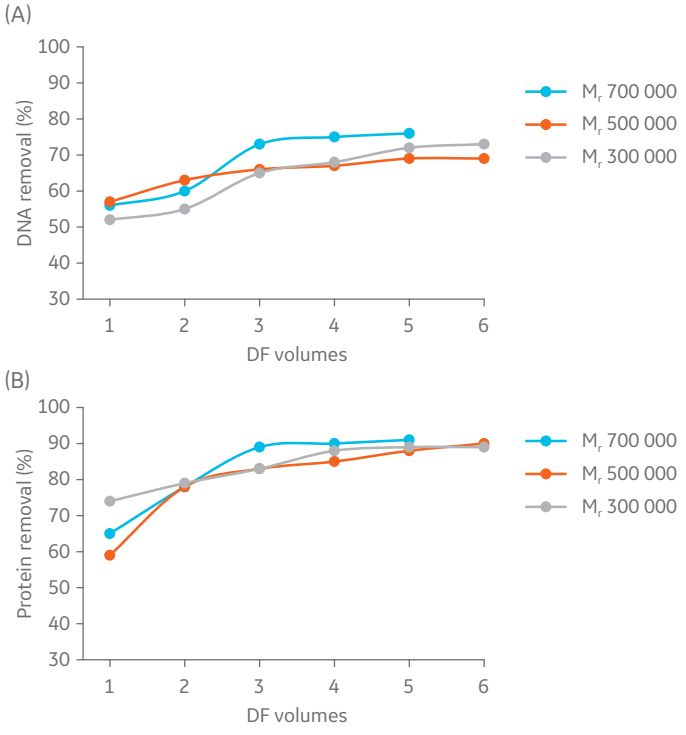

Fig 4. Removal of A) DNA and B) protein content in the retentate in each step of UF/DF using hollow fiber filters with NMWC of M_r 300 000, 500 000, and 750 000.

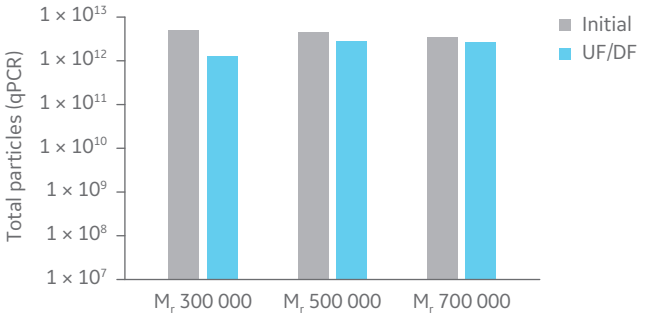

Fig 5. Total particles (qPCR) for UF/DF using hollow fiber filters with NMWC of M_r 300 000, 500 000, and 750 000.

Total particle comparison between the different hollow fiber filters displayed highest recovery with M_r 500 000 and 750 000 (Fig 5). Considering impurity removal and virus recovery, the M_r 750 000 filter presented the best performance of the filters evaluated and was therefore selected for process evaluation.

Traditional intermediate purification protocols commonly use AEX chromatography. Two resins, Capto Q and Capto Q ImpRes, were evaluated. Capto Q ImpRes showed 3.6 times higher dynamic binding capacity (DBC) and higher virus recovery at similar impurity removal (Table 3). The higher DBC seen with the Capto Q ImpRes resin can be explained by the smaller bead size, offering a larger surface area for virus binding. Capto Q ImpRes was therefore selected for the capture step.

The removal of hcDNA is facilitated by fragmentation using nuclease treatment (Benzonase). Shorter DNA fragments will be removed in the permeate of the UF step, and remaining DNA is removed in the wash fractions of the AEX step, as shorter fragments bind less strong and elute at lower salt concentration than longer DNA fragments. Any remaining long DNA fragments after the nuclease treatment can be removed in the final SEC polishing step.

Sepharose Fast Flow 4 was used for the polishing step. Sepharose Fast Flow allows to inject between 0.1 to 0.3 CV of sample for optimal remaining impurity removal. Different ratios of sample volume (SV) to CV were evaluated for the polishing step (Fig 6).

As an alternative for the polishing step, Capto Core 700 with the advantage of a 100–300-fold higher load capacity can be used instead of SEC (6) provided that the majority of any longer DNA fragments has been removed in the prior steps. Longer DNA fragments may not be efficiently reduced by Capto Core 700 due to the cut-off of this resin.

Table 3. Capacity screening for two different chromatographic resins in terms of DBC at 10% (Q_{B10}) breakthrough (vp/mL), virus recovery, and protein/DNA removal percentage (%) after an elution step with 2 M NaCl at a flow velocity of 300 cm/h

Chromatographic resin	Column volume (mL)	Q_{B10} (TP/mL)	2 M NaCl elution, recovery (%)	Total protein and DNA removal (%)
Capto Q	4.7	1.7×10^{11}	74	94/95
Capto Q ImpRes	4.7	6.2×10^{11}	85	90/90

(A)

V_{sample}/V_{column}	0.05
Virus recovery (%)	69
DNA removal (%)	96
Total protein removal (%)	67
Ratio areas (virus peak/total area)	37

(B)

V_{sample}/V_{column}	0.1
Virus recovery (%)	65
DNA removal (%)	94
Total protein removal (%)	80
Ratio areas (virus peak/total area)	34

(C)

V_{sample}/V_{column}	0.2
Virus recovery (%)	70
DNA removal (%)	97
Total protein removal (%)	72
Ratio areas (virus peak/total area)	42

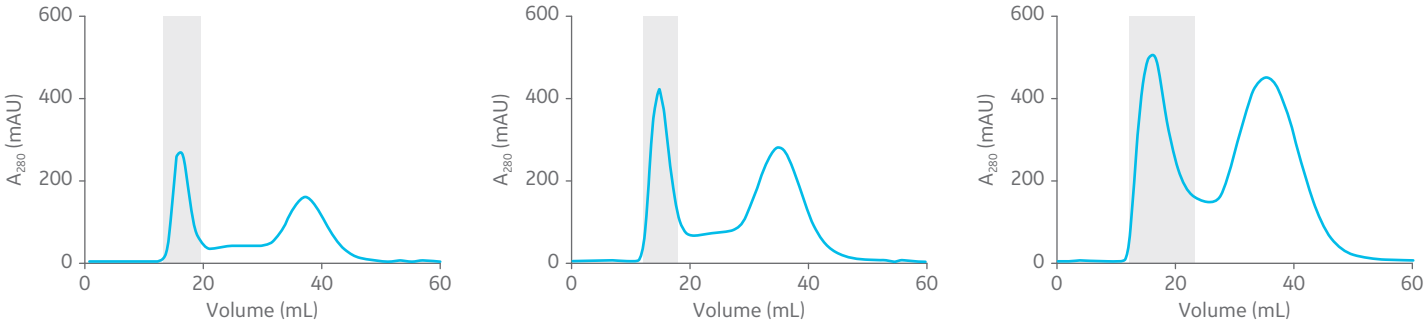

Fig 6. Summary of virus recovery and impurity removal by SEC on Sepharose 4 Fast Flow using SV-to-CV ratios of (A) 0.05, (B) 0.1, and (C) 0.2. The shadowed area represents the collected fractions. The recovery and impurity removal results are presented in the table on top.

Oncolytic adenovirus process runs

Two complete processes of 2 L oncolytic adenovirus production, from a 5 L bioreactor culture were used for process verification. First, the 2 L productions were harvested by adding Tween 20 to a concentration 0.5% (v/v), using a stock solution, containing 5% Tween 20 in 10 mM Tris, pH 8 and 100 U/mL of Benzonase, to the bulk previously diluted in 50 mL of medium and incubated for 4 h at 37°C.

The downstream process steps were conducted as described in Table 4. Product recovery for all steps of the process was assessed by quantification of viral genomes (VG) by qPCR, and infectious particles (IP) by $TCID_{50}$ assay. Removal of dsDNA, total protein, and HCP throughout the process was also evaluated, as was the residual Benzonase concentration in the final sample.

The VG and IP recoveries are listed in Table 5. The recovery of each step in the process will affect the overall process recovery and optimization of each step is therefore important. Average overall recovery was 52% and 61% for VG and IP, respectively.

However, overall recoveries varied, ranging from 34% to 70% in the two successful proof-of-concept runs. The lower recovery in the first run can be explained by the difference of almost 1 log in virus concentration throughout the process. This might indicate that the process favors higher virus concentrations (~ 10^{11}).

Average impurity removal, total protein, dsDNA, and HCP for both runs are shown in Table 6. In the first run, more that 99% of all impurities were removed. As expected, DNA was the most difficult impurity to remove. In the second run, only 94.5% of the dsDNA was removed. This can be due to the presence of higher concentration of virus in the sample. As the virus can form virus-DNA complexes, the amount of Benzonase added in the beginning of the process might not have been enough to break such complexes and the DNA removal was thus less effective throughout the process. The VG/IP ratio was similar in the final sample as obtained after harvest, which suggests that the process steps did not affect infectivity of the virus negatively. The purity of the final samples meets the regulatory requirements (Table 7).

Table 4. Materials used in each step of the downstream process train

Step	Material	Parameters/conditions
Clarification	ULTA 5 µm GF 6 inch ULTA 0.2 µm CG 4 inch	600 L/m²/h
Concentration and buffer exchange TFF 1	Hollow fiber 750-C	2× UF/4× DF 50 mM Hepes, pH 6.5 + 150 mM NaCl
Intermediate purification	200 mL Capto Q ImpRes in HiScale 50/20	Flow velocity: 300 cm/h Load: 1.5 × 10^{11} vp/mL resin Equilibration: 50 mM Hepes, pH 6.5 + 150 mM NaCl Step elution: 50 mM Hepes, pH 6.5 + 1 M NaCl followed by dilution 1:4 with 20 mM Tris, pH 8 + 25 mM NaCl
Concentration and buffer exchange TFF 2	Hollow fiber 300-C	9× UF/2× DF 20 mM Tris, pH 8 + 25 mM NaCl
Polishing	300 mL Sepharose 4 Fast Flow in XK50/60	0.2 CV load, 20 mM Tris, pH 8 + 25 mM NaCl, flow rate 4 mL/min
Formulation	Addition of glycerol to final conc. of 2.5%	20 mM Tris, pH 8 + 25 mM NaCl, 2.5 glycerol
Sterile filtration	ULTA 0.2 µm CG 47 cm² disc	600 L/m²/h

Table 5. Average recoveries (%) for all process steps for two separate runs

Sample	Genome particles	Infectious particles
	Recovery (%)	Recovery (%)
Harvest	-	-
Clarification	96.5	98
UF1	93.5	93.5
AEX	82	86.5
UF2	95.5	92.5
SEC	62	72.5
Final	100	100
Total	52	61

Table 6. Average total protein, dsDNA, and HCP removal (%) for each process steps for two separate runs

Sample	Total protein	dsDNA	HCP
	Removal (%)	Removal (%)	Removal (%)
Harvest	-	-	-
Clarification	13.5	29.5	36
UF1	81	49	36.5
AEX	69.5	37.5	94.5
UF2	40.5	46	17.5
SEC	83.5	68	99.5
Final	17.5	16.5	34.5
Total	99.52	97.05	99.99

Table 7. Summary of average impurities and virus particles observed in the final samples of the two runs and the purity targets requested for the sterile filtrated bulk

	Purity results	Purity target
DNA (ng/dose*)	2.5	10
HCP (µg/mL)	2.8	1–100
Benzonase (ng/mL)	BDL**	< 5
Viral genomes qPCR (VG/mL)	8.2×10^{11}	-
Infectious particles $TCID_{50}$ (IP/mL)	1.3×10^{11}	-
Ratio TP/IP	6.3	< 30

* Dose 1×10^{10}, **below detection limit (BDL)

Conclusion

The described oncolytic AdV5 production process ranges from cell expansion to purified virus bulk product. Here, adherent A549 cells were amplified in serum-containing medium, after which virus was produced in suspension bioreactor cultures using serum-free medium in working volumes ranging from 1 to 5 L. CDM4HEK293 medium was selected to support A549 cell growth and productivity in suspension culture using the single-use ReadyToProcess WAVE 25 rocking bioreactor system. This system was used as it supports smooth rocking motion that lowers shear force for sensitive cells. The system is also favorable for its simplicity when performing laboratory-scale production cultivations.

Two complete downstream process runs, using Capto Q ImpRes for the capture step and Sepharose 4 Fast Flow for the polishing step, were executed, both runs confirming impurity removal of above 94% and virus recovery of up to 70%. It was shown that a higher virus particle concentration in the bulk after harvest favors process recovery. The downstream process provided high virus purity, with impurity concentrations below target in the final sample for both processes.

Acknowledgment

We thank iBET, Oeiras, Portugal for producing and sharing data and for good collaboration.

References

1. Application note: Evaluation of HEK293 cell growth and adenovirus productivity in HyClone CDM4HEK293 medium. GE Healthcare, 29264715, Edition AA (2017).
2. Application note: Adenovirus production in single-use Xcellerex™ XDR-10 bioreactor system. GE Healthcare, KA874021017AN (2017).
3. Application note: Adenovirus production in single-use ReadyToProcess WAVE 25 bioreactor system. GE Healthcare, KA879160418AN (2018).
4. Application note: Scalable process for adenovirus production. GE Healthcare, KA877080618AN (2018).
5. Application note: Optimization of midstream cell lysis and virus filtration steps in an adenovirus purification process, GE Healthcare, KA875220218AN (2018).
6. Application note: Scalable process for adenovirus production. GE Healthcare, KA877080618AN (2018).

Ordering information

Product	Description	Product code
ReadyToProcess WAVE 25	Rocker	28988000
ReadyToProcess CBCU Full	Control unit	29044081
CDM4HEK293	1 L	SH30858.02
Cellbag Bioclear 11	Cellbag, 10 L, BC11, pHOPT, DOOPT II	CB0010L11-31
L-glutamine 200 mM	100 mL	SH30034.01
Ham's F12 medium, Kaighn's modification	500 mL	SH30526.01
Fetal Bovine Serum	500 mL	SH30071.03
ULTA Disc GF: 2.0 µm	47 cm^2	DGF-A-02-470
ULTA Disc CG: 0.2 µm	47 cm^2	DMP-CG92-470
ULTA Prime GF: 5 µm	Capsule 6 inch, 0.19 m^2	KGF-A-0506GG
ULTA Prime CG: 0.2 µm	Capsule 4 inch, 0.10 m^2	KMP-CG9204GG
Start AXH Ultrafiltration Cartridge, UFP-750-C-MM01A	750 000 NMWC, 26 cm^2	29014304
Start AXH Ultrafiltration Cartridge, UFP-500-C-MM06A	500 000 NMWC, 26 cm^2	56410053
Start AXH Ultrafiltration Cartridge, UFP-300-C-2U	300 000 NMWC, 50 cm^2	11000547
Xampler ultrafiltration cartiridge, UFP-750-C-3X2MA	750 000 NMWC, 290 cm^2	29011051
Xampler ultrafiltration cartiridge, UFP-300-C-3X2MA	300 000 NMWC, 290 cm^2	56410151
Capto Q	100 mL	17531602
Capto Q ImpRes	100 mL	17547002
Sepharose 4 Fast Flow	1 L	17014901
ÄKTA avant 150	Chromatography system	28976337
HiScale 50/20	50 mm i.d., 200 mm tube height	28964445
XK16/20 column	16 mm i.d., 200 mm tube height	28988937
XK50/60 column	50 mm i.d., 600 mm tube height	28988964

gelifesciences.com/bioprocess

TR 29314587

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752, USA
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan
GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
For local office contact information, visit gelifesciences.com/contact.

KA3940170818AN