A scalable adenovirus production process, from cell culture to purified bulk

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Introduction

Adenovirus (AdV) vectors are commonly used in cancer gene therapy trials and evaluated for use in vaccines for various diseases. In this study, we have combined technical evaluation of process steps and process economy calculations, from AdV production in cell culture to purified bulk product in up to 10 L scale (1–8). Human AdV5, expressing the green fluorescent protein (GFP), was used for process development. The cells were lysed using Tween™ 20 as an alternative to Triton™ X-100 that is now on the authorization list (Annex XIV) of registration, evaluation, authorization, and restriction of chemicals (REACH). Analytical methods for determination of virus titer are challenging and depend on purity and quality of the sample. We used traditional analytics but also developed new sensitive and reproducible assays for virus titer. Based on analytical data, the novel downstream process was compared with a reference process regarding virus load capacity, recovery, and purity (Fig 1).

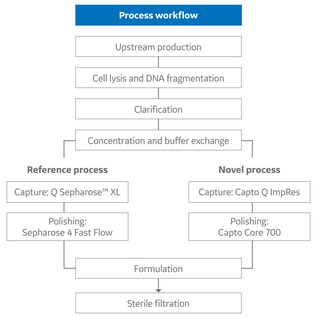

Fig 1. Process outline for the novel and reference processes for adenovirus production.

Materials and methods

Adenovirus production

HEK293 suspension cells (HEK-293.2sus, ATCC, grown in HyClone CDM4HEK293 using the Xcellerex™ XDR-10 or ReadyToProcess WAVE™ 25 bioreactor systems, were infected at cell density of approx. 1×10^6 cells/mL and a multiplicity of infection (MOI) of 10 with E1/E3-deleted recombinant AdV5 coding for the GFP reporter protein (1–3).

Harvest and filtration

42 h post infection, the cells were lysed with 0.5% Tween 20 and treated with 20 U/mL Benzonase™ + 1 mM $MgCl_2$ for 4 h in the bioreactor. The harvest was thereafter clarified by normal flow filtration (NFF) using a combination of 2 µm and 0.6 µm ULTA GF filters. Concentration and buffer exchange was performed by tangential flow filtration (TFF) on a ReadyToProcess™ hollow fiber filter with a nominal molecular weight cut-off (NMWC) of M_r 300 000 (10 × ultrafiltration [UF]/5 × diafiltration [DF] into 20 mM Tris, pH 8.0 + 300 mM NaCl, 2 mM $MgCl_2$) (4).

Chromatography steps

Columns were operated on an ÄKTA™ pure 150 system (5, 7).

Novel process

For capture, Capto™ Q ImpRes resin (HiScale™ 26 column, 88 mL [3 L scale] or HiScale 50 column, 294 mL [10 L scale]) was used. Elution was conducted with 20 mM Tris, pH 8.0 + 2 mM $MgCl_2$ using a linear gradient of 480–570 mM NaCl. Polishing was conducted on Capto Core 700 resin (HiScale 16, 10 mL [3 L scale] and 29 mL [10 L scale]).

Reference process

For capture, Q Sepharose™ XL resin (HiScale 50 column, 249 mL [3 L scale]). Elution was performed in two steps using 20 mM sodium phosphate, pH 7.3 + 2 mM $MgCl_2$ + 2% sucrose with 500 mM + 750 mM NaCl. Polishing was performed using Sepharose 4 Fast Flow resin (HiScale 50 column, 382 mL [3 L scale]).

Formulation and sterile filtration

Sample was concentrated and buffer exchanged (5 × UF/ 5 × DF) into 20 mM Tris, pH 8 + 25 mM NaCl, 2 mM $MgCl_2$, 2.5% glycerol. Process aliquots of the final purified bulk were sterile filtered using a syringe filter (PES, 0.2 µm).

Analytics (6)

Infectious virus titer was determined using the 50% tissue culture infective dose ($TCID_{50}$) assay and by automated fluorescence microscopy (AFM) using the IN Cell Analyzer. Total virus titer was determined by hexon DNA qPCR and size exclusion chromatography (SEC)-HPLC using a Superose™ 6 Increase 10/300 GL column. The Biacore™ T200 system was used for determination of virus particles through binding of virus fiber or hexon protein to CAR or FX protein, respectively, immobilized on a Biacore Sensor Chip CM5. Host cell protein (HCP) was determined using an ELISA assay, total protein using a BCA assay kit, host cell DNA (hcDNA) was determined by qPCR and total DNA using Quant-iT™ PicoGreen™ dsDNA Reagent (Invitrogen). Analysis of AdV5 samples by transmission electron microscopy (TEM) was conducted in collaboration with Vironova AB using the MiniTEM™ system.

Results

Cell growth was similar before and after Adv5 infection between the two bioreactor cultures (Fig 2). To minimize co-elution with virus, Capto Q ImpRes with gradient elution was applied in the novel process (Fig 3A). Polishing with Capto Core 700 allowed binding of impurities inside the bead, while the virus eluted in the flowthrough (Fig 3C). The reference capture step, using Q Sepharose XL, was performed with step elution (Fig 3B) followed by polishing with SEC (Fig 3D). The final purified bulk meets the regulatory requirements for virus purity (Table 1).

Capto Core 700 allows up to 300-fold higher sample loads compared with SEC, but is not designed to remove full length DNA, giving the SEC approach a certain advantage. Comparable yields were achieved in both processes, but the novel process showed lower impurity levels. This was confirmed by TEM, showing lower levels of debris with the novel process (Fig 4). As seen by SEC-HPLC analysis of clarified feed and the final purified bulk, impurities were efficiently removed in the novel process (Fig 5). The Biacore assays for total virus titer were shown to be sensitive, reproducible, and robust (Fig 6), with the benefit of reduced assay and hands-on time while showing similar results to qPCR (Fig 7). AFM using the IN Cell analyzer was shown to give similar results as $TCID_{50}$ (Fig 7 and 8).

Process economic evaluations indicate that the novel process is favorable over the reference process at increasing titers and scales, mainly due to the choice of polishing resin (Fig 9). Additionally, single-use process setups proved more cost-efficient than their stainless-steel counterparts in all investigated scenarios (8).

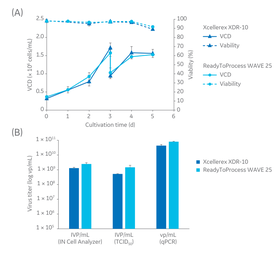

Fig 2. (A) Cell growth, viability, and (B) adenovirus titer in 10 L HEK293 cell cultures conducted using either the ReadyToProcess WAVE 25 rocking bioreactor system or the XDR-10 stirred tank bioreactor system.

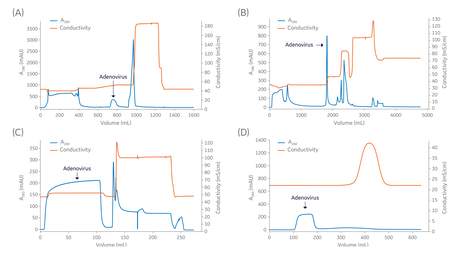

Fig 3. Chromatogram of adenovirus capture using (A) Capto Q ImpRes or (B) Q Sepharose XL, and polishing using (C) Capto Core 700 or (D) Sepharose Fast Flow. Adenovirus-containing fractions are indicated.

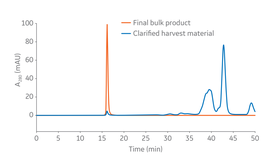

Fig 5. SEC-HPLC analysis of adenovirus purity of the novel process using a Superose 6 Increase column.

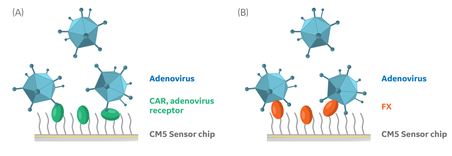

Fig 6. Principle of the two SPR assays for determination of adenovirus concentration. (A) CAR assay and (B) FX assay using the Biacore T200 system.

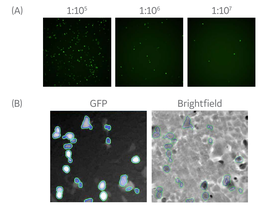

Fig 8. Determination of infectious virus titer by AFM using the IN Cell Analyzer. (A) AdV5-GFP dilutions at 42 h post infection. (B) Automated counting of GFP foci and infectious virus titer (IVP/mL).

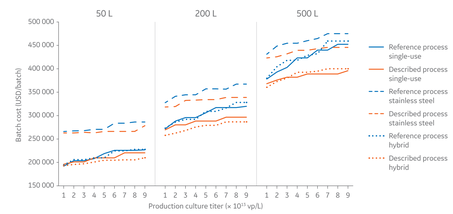

Fig 9. Batch cost comparison between the processes using primarily single-use or stainless steel equipment for different scales and virus production titers.

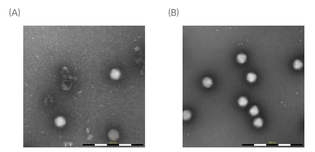

Fig 4. TEM images of final bulk samples from (A) reference process and (B) novel process. Analysis made in collaboration with Vironova AB using the MiniTEM system. The size bar corresponds to 500 nm.

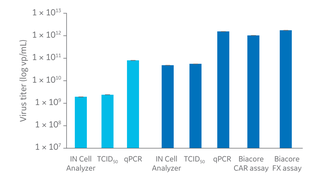

Fig 7. Comparison between methods for analysis of virus titer. IN Cell and $TCID_{50}$ were used for determination of infectious virus titer, and qPCR and Biacore assays were used for analysis of total virus titer. Results from harvest samples are shown in light blue, concentrated purified final bulk in dark blue.

Table 1. Overview of results from analysis of the final purified process samples (Run 1 and 2 were conducted at 3 L scale, with total yield in average 1.5×10^{13} and 1.7×10^{13} virus particles/L harvest for the reference process and novel process, respectively)

Process	Total virus titer (TVP/mL)	Infectious virus titer AFM IVP/mL	Recovery TVP (%)	Recovery IVP (%)	HCP (ng/mL)	Total protein (µg/dose)	gDNA (ng/dose)
Reference, run 1	6.7×10^{11}	4.0×10^{10}	31/38	36	17	11/13	< LOD
Reference, run 2	7.0×10^{11}	4.8×10^{10}	35/64	53	27	38/20	3
Reference, average	$\mathbf{6.8 \times 10^{11}}$	$\mathbf{4.4 \times 10^{10}}$	**42**	**45**	**22**	**20**	**< LOD - 3**
Novel,run 1	8.7×10^{11}	3.8×10^{10}	46/68	39	<LOD	13/11	< LOD
Novel , run 2	7.6×10^{11}	3.1×10^{10}	17	40	<LOD	10	< LOD
Novel, run 3 (10 L)	1.3×10^{12}	4.8×10^{10}	38/25	50	<LOD	4/10	< LOD
Novel, average	$\mathbf{1.0 \times 10^{12}}$	$\mathbf{3.9 \times 10^{10}}$	**39**	**43**	**<LOD**	**10**	**< LOD**

IVP = infectious virus particles, TVP= total virus particles, LOD= limit of detection, AFM = automated fluorescence microscopy. Disclaimer: analytical methods for IVP and TVP show variation between analysis occasions, which will affect the results for recovery and IVP/TVP ratio.
The reference process was run in duplicate at 3 L scale, whereas the novel process was run in duplicate at 3 L scale and once at 10 L scale.
Two numbers indicate that the same sample was analyzed twice (two separate occasions in triplicate for TVP (qPCR) and in duplicate for total protein (BCA)).
WHO guideline for protein impurity: max. 20 µg protein/dose
WHO guideline for DNA impurity: < 10 ng DNA/dose

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Conclusions

We propose a novel process for Adv5 production with technical and process economical advantages compared with an existing reference process. The novel process, using a combination of Capto Q ImpRes and Capto Core 700 in the downstream purification steps, produced pure final bulk product that meets regulatory requirements. New convenient and sensitive virus titer assays using Biacore T200 and IN Cell Analyzer were developed with comparable results to traditional methods.

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