PALL Life Sciences

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Industrialization of AdenoVirus Production and Purification with the iCELLis® 500 Single-Use Bioreactor

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INTRODUCTION

Diabetes is a major global health problem with the WHO reporting over 422 million affected persons and 3.7 million diabetes related deaths annually worldwide. Pall has generated adenovirus using a manufacturing large scale packed-bed iCELLis 500 single-use bioreactor. The conventional process used to purify adenovirus is not applicable to manufacturing, therefore Pall has developed a downstream purification industrialized process that has successfully generated the required amount of adenoviruses for pre-clinical study (Figure 1). In this study we developed and optimized an adenovirus purification manufacturing process including clarification, purification, concentration, buffer exchange and sterile filtration steps. Clarified harvest was processed over a Mustang® Q membrane in bind/elute mode. Under optimized conditions of pH and conductivity, negatively charged adenoviral vector was bound to the membrane. The Adenovirus 5 (Ad5) bind/elute strategy managed to reduce significantly impurities such as HCP and residual host cell DNA. The eluted Ad5 from Mustang Q membrane is immediately processed through the ultrafiltration/diafiltration (UF/DF) step for further concentration and buffer exchange to final virus formulation buffer. Final purified product was then sterile filtered and vialed for potency studies. Purified adenovirus containing the transcription factor, hPDX-1, was fully functional and comparable to the virus produced by the conventional cesium chloride non-industrial process.

PURPOSE

Develop an industrialized adenoviral vector production and purification process using iCELLis 500 single-use bioreactor

To go to clinical trials for trans-differentiation of liver cells, Orgenesis needs to produce purified Ad5 at an industrial scale. This goal was unachievable with the current cesium chloride (CsCl) gradient centrifugation process (Figure 1b) as it is time consuming and not scalable. Pall has developed an industrial scale purification process using Pall depth filters, low shear Mustang membrane chromatography (Figure 1a) and a Pall tangential flow filtration (TFF) membrane process which is both scalable and low shear, which minimizes damage to the virus. The process was then scaled up from less than 1 L to 10-200 L (Figure 2).

Figure 1

Illustrates the differences in purification methods between small scale and industrial scale purification of Adenovirus. A major discrepancy is the process time (1 day vs. 3 days)

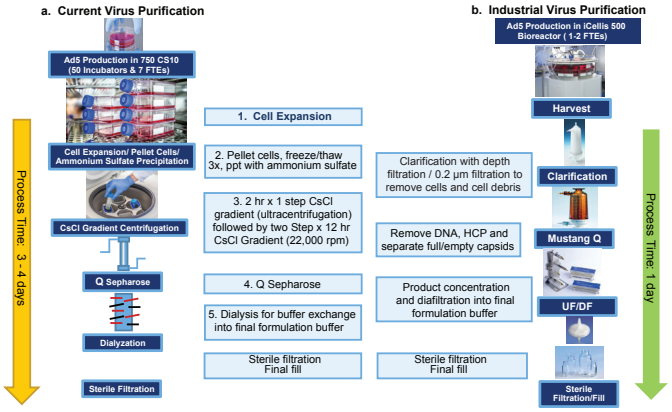


Figure 2

Adenovirus downstream purification process scalability. Required process consumables are shown as process was stepped up from 400 mL to 1-10 L then to 10-200 L

Downstream Process Purification Steps

Crude Harvest Volume (L)	Clarification	Mustang	UFDF	Sterile (0.2 µm) Filtration
0.400-0.800	Supracap™ 50 Capsules 	Mustang XT Acrodisc® unit 	Cadence™ SUTFF Module 	Acrodisc syringe filters 
1-10	Supracap 100 and Mini Kleenpak™ Capsules 	Mustang 5 mL Disc  Mustang 10 mL Capsule	Cadence SUTFF Module 	Mini Kleenpak 20 capsule 
Industrial Scale 10-200	Stax™ disposable depth filter and Kleenpak Nova capsule 	Mustang 60 mL Capsule 	Cadence SUTFF Module 	Kleenpak Nova Capsule 
	Allegro™ MVP system 	Mustang XT in holder 	Allegro SUTFF system 	Allegro filling system with capsule, biocontainers and needles 

MATERIALS AND METHODS

Materials

- Clarification step using V100P Supracap 100 capsules followed by 0.2 µm filtration using Supor® EKV media in Mini Kleenpak capsule
- Anion exchange (AEX) chromatography with CL3MSTGQP1 (60 mL bed volume (BV)) Mustang Q capsule
- UF/DF step using Cadence 100 kD Molecular Weight Cut-Off (MWCO) Omega™ membrane
- Virus standard: The virus standard used in these experiments was purified using current CsCl density method

Methods

- Clarify harvest to remove course debris using depth/0.2 µm filtration as a single unit operation. PBS used as flush buffer. The V100P depth filtration media was used in conjunction with a 0.2 µm Mini Kleenpak filter to achieve the very low particulate sample required for chromatography performance
- AEX bind/elute step with phosphate buffer pH 7.2-7.4, with isocratic wash steps and elution using increasing sodium chloride
- UF/DF step: Concentrate 5X, diafilter 5X into 20 mM Tris, 25 mM NaCl, 2.5% glycerol pH 8.0
- Sterile filtration and final fill, freeze at -80 °C
- Analytics: Adeno X- infectivity titer from Clonetech
- ELISA: HEK293 Host Cell Protein (HCP) kit from Cygnus, used according to manufacturer directions
- PicoGreen◆ assays: Residual DNA (double stranded DNA)
- SDS-PAGE: BioRad consumables/hardware
- Transmission electron microscopy: Empty vs full capsid ratio, carbon-stabilized Formvar support films on 200 mesh copper grids

RESULTS

Purification Steps

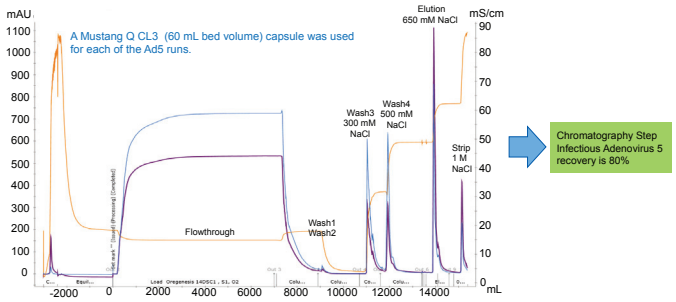
Step 1. Clarification (depth filtration)

Adenovirus crude harvest from the iCELLis bioreactor was clarified and sterile filtered.Depth filter (V100P) and 0.2 µm Mini Kleenpak filter steps were run in tandem to prepare crude harvest for the Mustang chromatography step. The V100P depth filter in a Supracap 100 capsule 5 in. (0.05 m^2) device performed well for each harvest in terms of high flow rates and low back pressure. This step had a high yield of recovery (>90%).

Step 2. Mustang Q bind/elute

Figure 3

Mustang Q (MQ) membrane chromatogram shows separation of protein species in the clarified harvest providing excellent clearance of impurities from the Ad5. Clarified adenovirus was loaded onto the Mustang Q membrane and the flowthrough/



wash/elution profile is shown below. The greatest process clearance for both HCP and dsDNA is provided by the Mustang Q membrane bind/elute step (>500X for HCP, >30X for dsDNA).

Adenovirus is known to bind to anion exchange chromatography media. The harvest contains ~8% serum, which is a huge purification challenge. Thus, most of the proteins seen in the harvest are from FBS. To purify Ad5, a Quaternary Amine (MQ) anion exchange membrane was used in bind/elute mode to separate adenovirus from host cell protein, host cell DNA, and protein contaminants. Due to their open accessible structure, membranes have specific advantages for virus purification. Once virus is eluted from the membrane an ultrafiltration/diafiltration (UF/DF) step is started immediately to concentrate and exchange adenovirus out of high salt buffer into final formulation buffer.

Step 3. Ultrafiltration/diafiltration (UF/DF) step to concentrate sample and exchange adenovirus. The Ad5 has a diameter of approximately 80-100 nm. The 100 kDa Omega ultrafiltration membrane has relatively large pores allowing for rapid exchange of small molecules and liquid resulting in faster process times and the passage of small contaminating proteins during this step. The elution fraction from the Mustang Q membrane step is concentrated 5X and then exchanged from high salt buffer into final formulation buffer over 5 diafiltration volumes (>98% buffer exchange) during this step. Step yield was ~80%.

Product Quality Analysis

Downstream Process Purification from Crude Harvest to Final Sterile Filtrate Illustrated by SDS-PAGE

Figure 4

Process steps for Ad5 purification (SDS-PAGE)

This gel shows the purification process from the crude harvest through final sterile filtration of adenovirus. Notice the improvement between lane 3 and lane 8 in purity and comparability with the CsCl purified adenovirus standard (lane 2). This figure shows comparability between the Pall industrial scale purified standard and the CsCl purified standard.

Final Purified Product Analyzed by Transmission Electron Microscopy, (TEM)

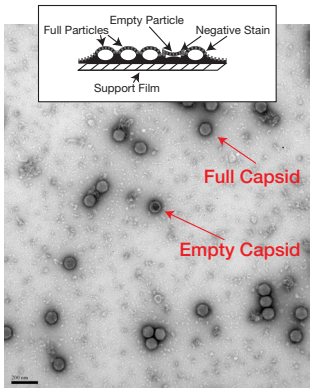


Figure 5

Empty vs full capsid as shown by transmission electron microscopy of negative stained adenovirus particles. Scale is shown in lower left had corner (200 nm)

As shown in the image above, empty capsids have a donut shaped appearance with a light boundary and dark center. Full capsids have a light center with no distinguishable boundary. There is only one empty capsid in the field of view shown above.

Table 1

Full vs empty adenovirus 5 by transmission electron microscopy. The ratio of full to empty capsid for three runs is >90%

Insert	Adeno X (Orgenesis), IU/mL	EM Full, %	EM Empty + Broken, %
Ad5.CMV.PDX-1	1.42E+11	97%	3%
Ad5.CMV.NeuroD	1.32E+11	97%	3%
Ad5.CMV.MafA	6.60E+10	93%	7%

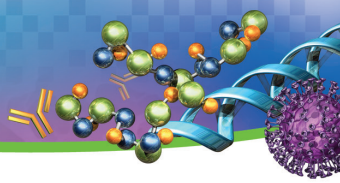
CONCLUSIONS

- In this study we have demonstrated the successful development of industrial scale purification of adenovirus with a 1 day process time, compared to 3 days for the conventional CsCl gradient centrifugation process
- Infective virus particles were efficiently purified from clarified lysate using a single membrane chromatography step in less than 2 hours
- The Mustang Q membrane chromatography step provides a processing time of less than 3 hours. The greatest process clearance for both HCP and dsDNA is provided by the Mustang Q bind/elute step (>500X for HCP, >30X for dsDNA)
- For the clarification step, crude harvest from iCELLis bioreactor was clarified and sterile filtered with a high yield of recovery (>90%)
- For the chromatography step, a Mustang Q (MQ) quaternary amine anion exchange membrane was used in bind/elute mode to purify adenovirus from host cell protein and host cell DNA
- Ultrafiltration/diafiltration steps concentrated adenovirus material 5X and diafiltered out of the high salt MQ elution buffer and into final formulation buffer in less than 2 hours
- For three runs overall process recovery was greater than 65%

Table 2

Process recovery for Ad5 downstream process steps

Downstream Process Step	Recovery (%)
Clarification step	90%
Mustang Q membrane step	~80%
UFDF step (5X concentration, 5X diafiltration)	80%
Overall process recovery for three runs	65%



- The purity of the adenovirus purified by the industrial scale process is comparable to the purity of the product purified by the conventional CsCl method
- Full to empty capsid ratio is >90% for adenovirus purified by the industrial process

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