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 viated 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
To go to clinical trials for trans-differentiation of liver cells, Oregenesis needs to produce purified Ad5 at an industrial scale. This goal was unachievable with the current cesium chloride density 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 1-200 L (Figure 2).

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 were used to prepare crude harvest for the Mustang chromatography step. The V100P depth filter was used in conjunction with a 0.2 µm Mini Kleenpak filter to achieve the very low particulate sample required for chromatography performance.

Step 2. Mustang Q bind/elute

Figure 3 Mustang Q (MQ) membrane chromatography shows separation of protein species in the clarified harvest providing excellent clearance of impurities of 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 (>500k for HCP, >30k for dsDNA).

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% recovery) between the UF/DF step.

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 (>500k for HCP, >30k 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%

REFERENCES
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