Integrated downstream processing of therapeutic adenoviruses: optimizing enzymatic DNA digestion and membrane chromatography

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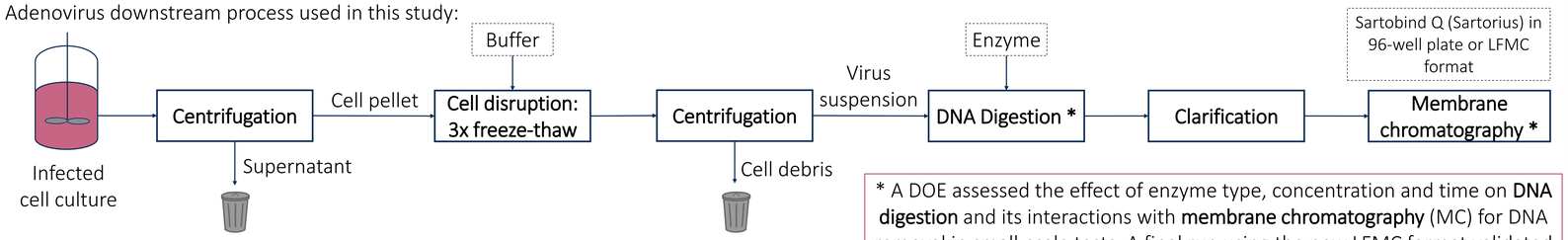
Introduction

- Residual host-cell DNA is one of the most concerning impurities in biotherapeutics.
- The FDA establishes a limit of 10 ng of residual DNA/dose[1].
- Benzonase is commonly used during virus manufacturing to digest DNA and this enzyme alone can account for approximately 5,000 USD for a 10 L batch[2].
- This study aims to **develop an integrated adenovirus downstream process comprised of enzymatic DNA digestion and removal via laterally-fed membrane chromatography (LFMC)**[3] to:
  - ✓ Minimize DNA levels in the final product while maintaining high virus recoveries;
  - ✓ Minimize costs through a head-to-head comparison between Benzonase (Millipore) and Denarase (c-LEcta), using a Design-of-Experiments (DOE);
  - ✓ Assess the effects of distinct DNA digestion conditions on membrane chromatography.

1. FDA, Guidance for Industry: Characterization and qualification of cell substrates and other biological materials used in the production of viral vaccines for infectious disease indications, (2010).
2. J. Vellinga, et al. Challenges in manufacturing adenoviral vectors for global vaccine product deployment, Hum. Gene Ther. 25 (2014) 318–327.
3. K. Kawka, et al. Purification of therapeutic adenoviruses using laterally-fed membrane chromatography, J. Memb. Sci. 579 (2019) 351–358.

Methods

Adenovirus downstream process used in this study:

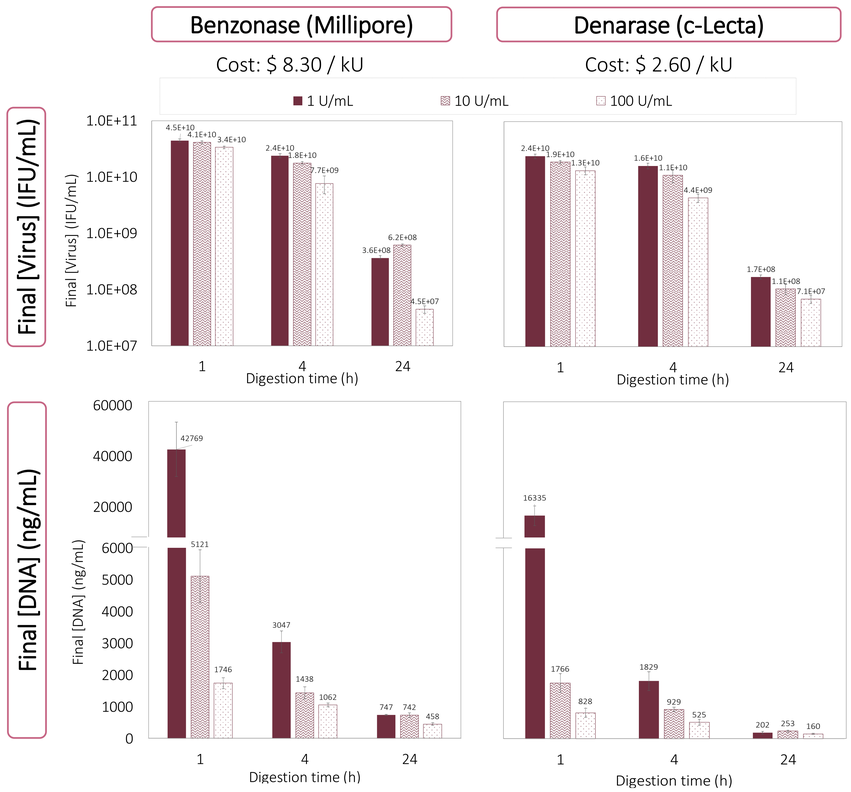


Assays and experiment conditions: Adenovirus was produced in FH-293 suspension cells using serum-free media; Virus titer was measured via Hexon Staining, DNA and protein were measured via PicoGreen and micro-BCA assays, respectively; DNA digestion was carried out at 37°C; Enzyme buffers contained 10 mM Tris, 2 mM $MgCl_2$, pH 8; MC buffers contained 10 mM HEPES, 4% sucrose and 0.36-1 M NaCl, pH 7.4.

* A DOE assessed the effect of enzyme type, concentration and time on **DNA digestion** and its interactions with **membrane chromatography** (MC) for DNA removal in small-scale tests. A final run using the new LFMC format validated the findings for the purification of lysates from 50 mL of culture.

Results

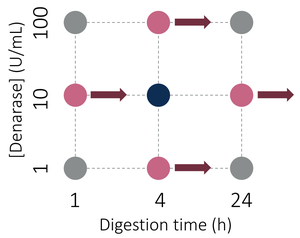
1. DNA Digestion – Design of Experiments



- **Denarase presented superior overall performance in terms of reducing DNA concentration**
- Long digestion times (24 h) cause significant losses in virus titer for both enzymes
- There is a slight reduction in virus titer with increasing enzyme concentration
- The shorter the digestion time, the larger is the effect of enzyme concentration on DNA degradation
- Similar final DNA concentrations were obtained when 100 U/mL of Benzonase or 10 U/mL of Denarase were used for 1 or 4 hours. Therefore, **Denarase enables up to 30 times reduction in costs with enzyme!**

2. Small-scale Membrane Chromatography

DOE conditions used for MC experiments:

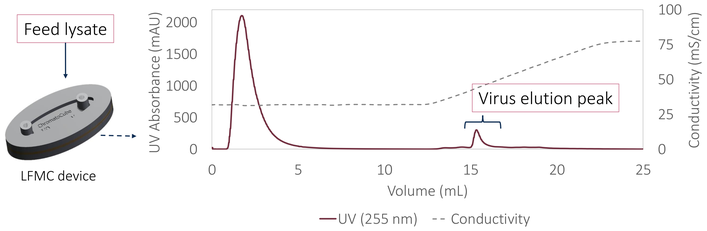


DNA Digestion Conditions		DNA Amount		Virus Amount	
[Denarase] (U/mL)	Time (h)	Before MC (ng/10^{10} IFU)	After MC (ng/10^{10} IFU)	Before MC (total IFU)	After MC (total IFU)
1	4	591	231 (39%)	3.2×10^{8}	1.5×10^{8} (45%)
10	1	357	183 (51%)	5.7×10^{8}	3.3×10^{8} (58%)
10	24	16,185	NA	2.7×10^{6}	3.9×10^{5} (14%)
100	4	701	387 (55%)	1.2×10^{8}	7.3×10^{7} (59%)
0	0	9,099	1,386 (15%)	6.5×10^{8}	4.1×10^{8} (64%)

NA: DNA concentration below detection limit.

- % DNA removal is reduced by increasing the initial amount of DNA.
- Lower % Virus recovery was obtained when 24 h digestion was used.

3. Laterally-fed Membrane Chromatography (LFMC)



Chromatography Performance		
Component	Feed lysate	Virus elution peak
Total Virus (IFU)	1.6×10^{10}	5.8×10^{9}
DNA (ng/10^{10} IFU)	774	139
Protein (µg/10^{10} IFU)	1,514	144

NA: Protein concentration below detection limit.

- Purification of adenovirus lysate (digested with 10 U/mL Denarase for 4 hours) using the Sartobind Q membrane in LFMC format.
- Virus was eluted using a gradient increase in NaCl concentration up to 1 M.

Conclusions

- Denarase was shown to be a more economic alternative to Benzonase for DNA digestion due to its lower cost and better performance on reducing DNA concentration.
- Small-scale MC in conjuction with DOE methods are a powefull tool for process development as it allows the evaluation of multiple conditions in parallel.
- Benzonase concentrations of 100 U/mL are commonly reported[2]. Using LFMC, we successfully purified lysates prepared with relatively low amounts of nuclease (10 U/mL).
- Future work will focus on scaling-up the LFMC devices and processes for the purification of pilot-scale batches of adenovirus.

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