Development and scale up of next generation lentiviral vector batch process demonstrating increased productivity and enhanced purity

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Introduction

For 30 years, Oxford Biomedica (OXB) has been a pioneer in the development of products and innovative technologies based on lentiviral vectors (LVs). To meet the forecast on vector demand for gene and cell therapies (1), OXB has recently introduced a next-generation transient lentiviral manufacturing process that incorporates innovative process modifications which simultaneously enhance lentiviral process yields and improve vector quality attributes. This new process takes advantage of advances in anion exchange (AIEX) chromatography specifically designed for Lentiviral Vector production (Sartobind Convec®D by Sartorius) and developments in salt-activated nuclease formulation that significantly reduces residual DNA levels.

Importantly, this process adopts a plug-and-play approach facilitating incorporation of small molecule enhancers. The AIEX for LVs has been the biggest bottleneck in the process where up 80% of the vector can be lost over this unit operation (2).

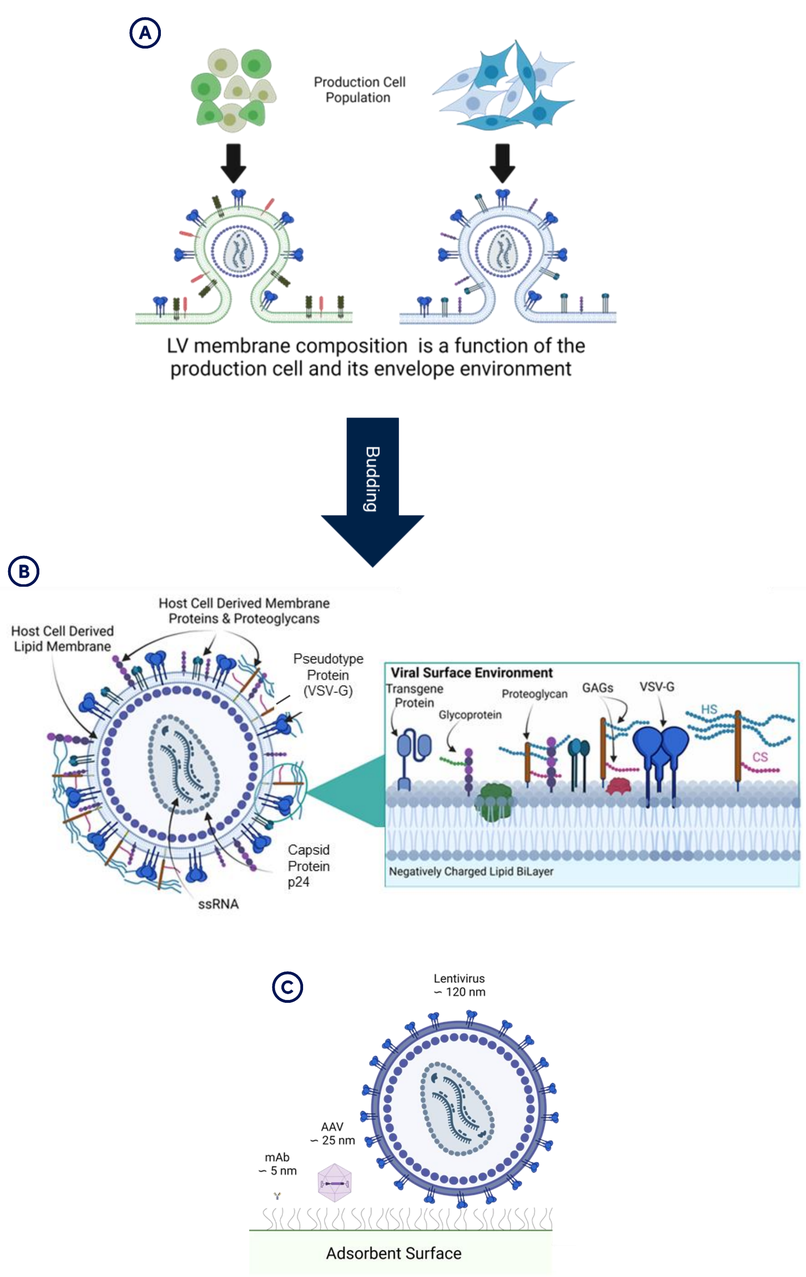
The Sartobind Convec®D membranes have improved process recovery by up to 3-fold and the combination of this with the enhanced salt activated nuclease leads to 3-fold increase in titre and a decrease in residual DNA and protein. Furthermore, this new process has been evaluated with five different therapeutic lentiviral vectors and has been successfully scaled up to 50 L. This new manufacturing platform offers significant benefits for clinical production of lentiviral vectors and will further enable OXB to support the continuing global demand for high quality gene and cell therapy products.

Background

Complex absorption for a complex target

- Current anion exchange chromatography technologies are not suited for purifying Lentiviral vectors.
- Lentiviruses are enveloped viruses meaning they are enrobed in the envelope of the production cell.
- Lentiviruses are labile in nature and contain hundreds of charged species on their surface (3&4).

Figure 1 – complex absorption of Lentiviral vectors

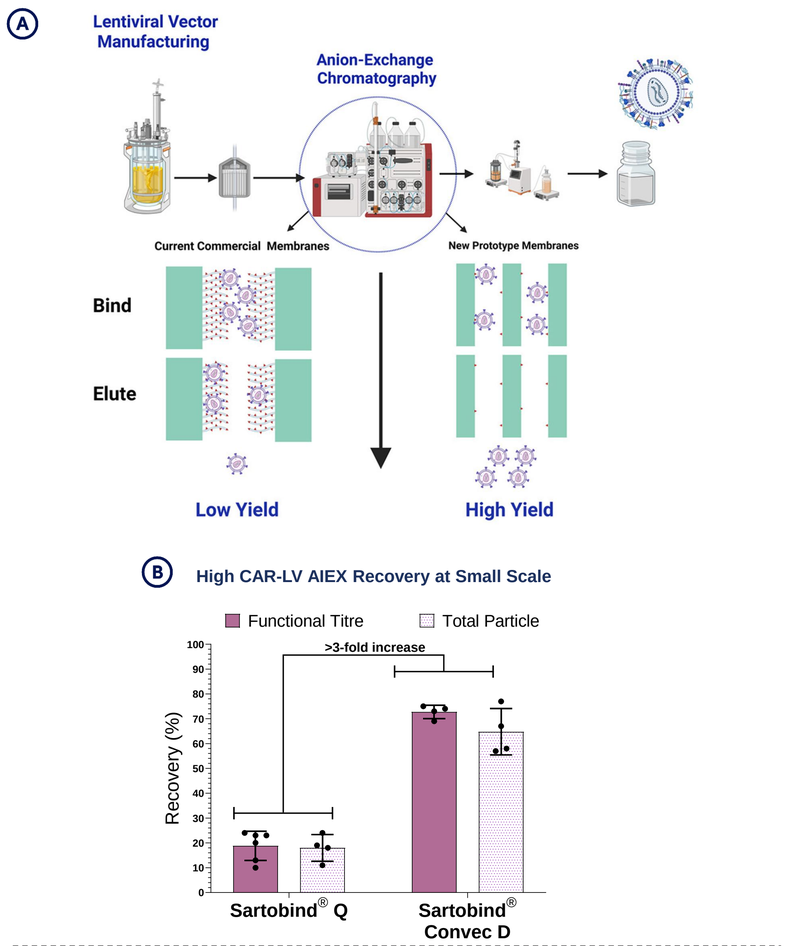


- High level of complexity at the adsorption interface
- As a result, typical viral vector recoveries over the down stream = 10-25%

Results

The Sartobind Convec®D, developed by Sartorius, is a specialised weak anion exchange chromatography solution designed for the efficient downstream processing of lentiviral vectors. Using convective mass transfer and weak anion exchange chemistry, the device facilitates the gentle elution of viral particles, ensuring high infectious recovery during purification.

Figure 2 – Convec®D AIEX membranes improve recovery over AIEX by 3-fold compared to Sartobind® Q at small scale



Developments in salt activated nuclease formulation

- The physiological conditions in the production bioreactor at the end of the process are not optimal for the current endonuclease used by OXB
- Medium Salt Active Nuclease **(M-SAN)** is an alternative non-specific endonuclease from ArcticZymes that is an optimal solution for removal of nucleic acids **near physiological conditions**

Figure 3 – Optimum pH and salt for current endonuclease activity is not in-line with physiological conditions at the end of production.

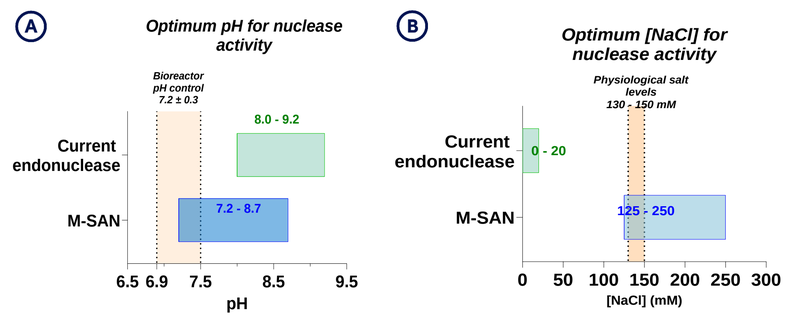
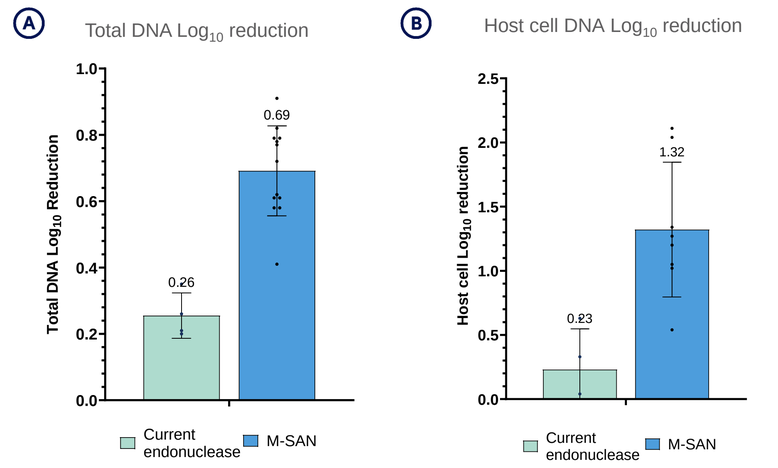
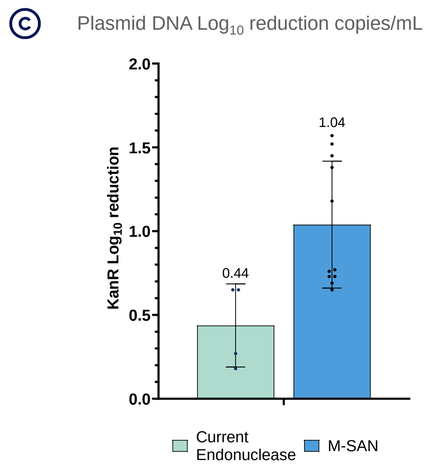


Figure 4 – Impact of MSAN on residual clearance compared to currently used endonuclease

The addition of M-SAN as an alternative to current endonuclease increased the Log_{10} reduction in Total DNA (A), host cell DNA (B) and plasmid DNA (C) by >2-fold.





Development and scale-up of next generation batch process demonstrating increased productivity and enhanced purity

Figure 5A – Schematic of next generation batch process

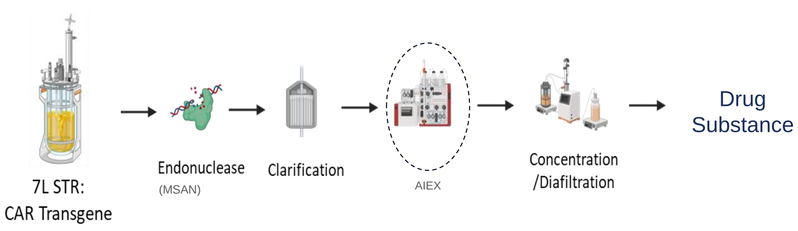
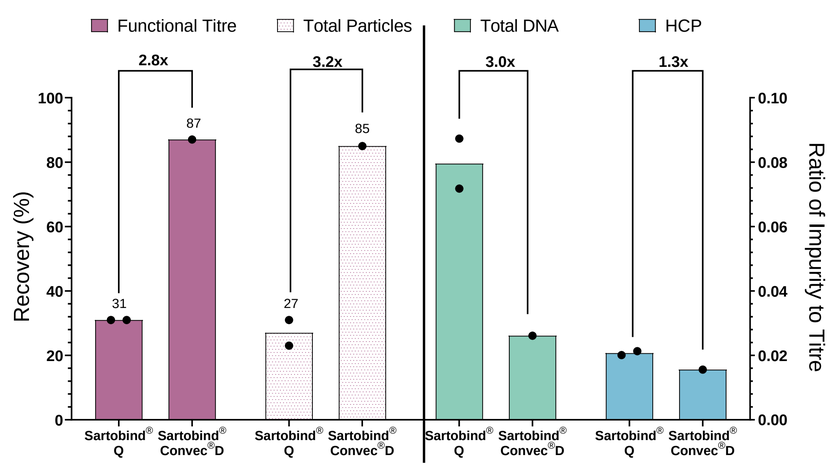


Figure 5B – Impact of combining Convec®D with M-SAN



Processing of OXBs CAR-LV to drug substance using the Sartobind® Covec®D membranes leads to a 3-fold improvement in process recovery and combining the process with the alternative endonuclease leads to a 3-fold reduction in Total DNA.

Conclusion

The combination of Convec®D and M-SAN has led to an overall process recovery increase of 3-fold for both functional and total lentiviral vector particles.

- 3-fold relative reduction in residual DNA levels
- Improved quality and safety profile of Drug Substance

References

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3. Lentiviral vector determinants of anion-exchange chromatography elution heterogeneity, Pamenter et al. 2024.
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Figure 5A was created with Biorender.com

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