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

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Summary

For over 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 LV manufacturing process incorporating innovative process modifications which simultaneously enhance LV process yields and improve vector quality attributes.

This new process takes advantage of advances in anion exchange (AIEX) chromatography specifically designed for LV production (Sartobind Convec®D by Sartorius) and developments in salt-activated nuclease formulation that significantly reduces residual DNA levels (M-SAN by ArcticZymes).

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 M-SAN's enhanced activity 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 LV drug product candidates and has been successfully scaled to a 200 L GMP setting.

This new manufacturing platform offers significant benefits for clinical production of LVs and will further enable OXB to support the continuing global demand for high quality gene and cell therapy products.

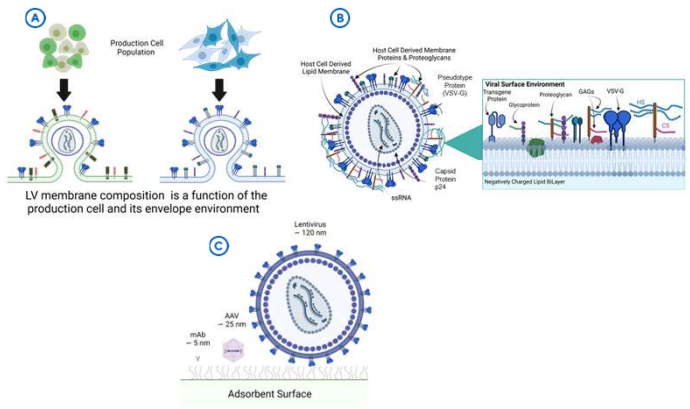
Background

COMPLEX ADSORPTION FOR A COMPLEX TARGET

Current AIEX technologies are not optimal for purifying LVs. LVs are enveloped viral vectors meaning they are enrobed in the envelope of the production cell (Fig. 1A). LVs are labile in nature and contain hundreds of charged species on their surface[3,4] (Fig. 1B). As such, there is a high level of complexity at the adsorption interface (Fig. 1C).

Resultantly, typical viral vector recoveries during downstream processing are only 10-25%.

Figure 1 – Complex absorption of lentiviral vectors

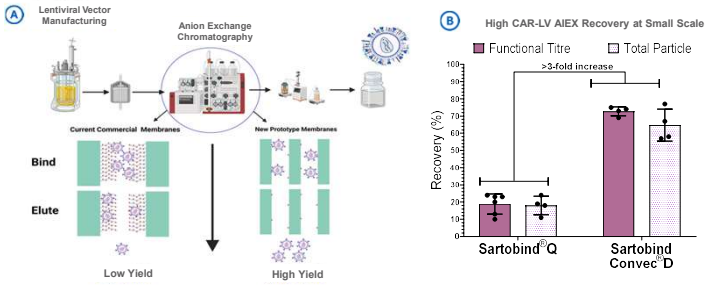


Results

1. DEVELOPMENTS IN AIEX TECHNOLOGIES FOR LV PURIFICATION

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

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



2. DEVELOPMENTS IN SALT-ACTIVE 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 a non-specific endonuclease from ArcticZymes that has been engineered for optimal activity near physiological salt concentrations.

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

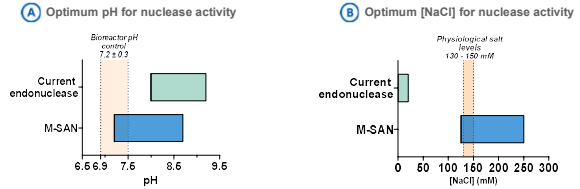
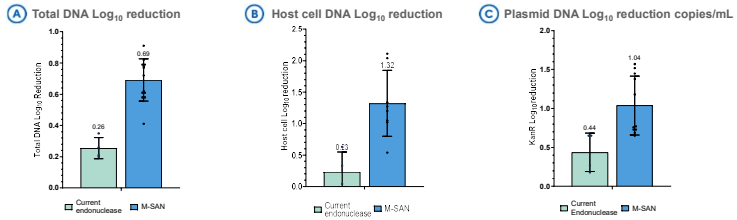


Figure 4 – Impact of MSAN on residual clearance compared to current endonuclease



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.

3. TECHNOLOGY INTEGRATION TO DEVELOP A SCALABLE NEXT GENERATION PROCESS WITH INCREASED RECOVERY AND ENHANCED PURITY

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

Figure 5 – Schematic of OXB's next generation batch process

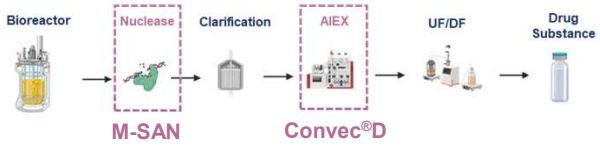
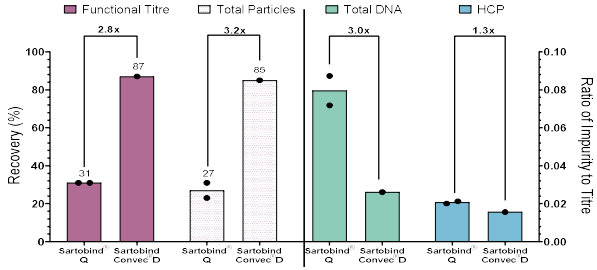


Figure 6 – Impact of combining Convec®D with M-SAN



Conclusion

This combination of both Convec®D and M-SAN resulted in a next generation LV production process exhibiting several key advantages:

- 3-fold overall process recovery increase for both functional and total LV particles
- 3-fold reduction in residual DNA levels
- Improved quality and safety profile of Drug Substance
- Demonstrated with multiple therapeutic LV products
- Successfully scaled to 200 L in a GMP setting

As such, this platform offers significant benefits for clinical supply of LVs and will further enable OXB to support the continuing global demand for high quality gene and cell therapy products.

References

1. Alliance for Regenerative Medicine. (2023, December). Cell and Gene Therapy Sector Data: Clinical Trials. https://alliancerm.org/data/
2. Viral vector manufacturing: how to address current and future demands? Ansorge & Cheeseman, 2019.
3. Lentiviral vector determinants of anion-exchange chromatography elution heterogeneity, Pamenter et al. 2024.
4. Time-dependent sorption behaviour of lentiviral vectors during anion-exchange chromatography, Pamenter et al. 2023.

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