CONTINUOUS PRODUCTION OF LENTIVIRAL VECTORS USING A FIXED-BED BIOREACTOR

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Lentiviral vectors (LVs) have emerged as indispensable tools for mediating stable transfer of large transgenes in mammalian cells, which has resulted in the widespread application of LVs for the manufacture of gene-modified cell therapies, particularly chimeric antigen receptor T-cell (CAR-T-cell) therapies.

The typical manufacture of LVs through chemical transfection of adherent embryonic kidney 293T-cells with plasmid DNA is highly versatile and enables manufacturing to pivot to produce vectors with alternative transgenes and envelope proteins. However, transient transfection presents several challenges, including batch-to-batch variability, limited scalability and high costs attributed to the plasmid DNA and transfection reagent. Therefore, the transition towards continuous LV production using producer cell lines would allow for cost effective and scalable manufacturing along with enhanced safety and reproducibility. Specifically, continuous production using stable producer cell lines could extend vector expression by counteracting the limited post-induction expression time and eliminating the requirement for removal of the inducing agent during downstream processing.

In this study, a continuous LV manufacturing process was established using a stable producer cell line, which constitutively expresses third-generation LVs pseudotyped the RDpro envelope protein (WinPacRDpro). Production of LVs in typical 2D culture vessels was performed, however, these systems lack the control of culture parameters, such as pH and dissolved oxygen, and require laborious handling, which introduce risks of contamination. Therefore, the transition to LV production in bioreactors is a necessary step to achieve sufficient LV supply, e.g. for late-stage clinical trials.

This work demonstrates the establishment of a quasi-perfusion process using repetitive batch strategy with medium exchange, first, in 2D culture vessels and then, to an iCELLis fixed-bed bioreactor to continuously produce RDpro-pseudotyped LVs. This initial work using a continuous process-mimic in a fixed-bed bioreactor enables cell expansion in a controlled environment and the bioreactor characterization was used to gain insights for the development of a perfusion strategy. Growth, substrate utilization, lactate generation and LV production were evaluated under different medium exchange strategies. Finally, the established bioreactor process was then transformed to perfusion culture with studies aimed at understanding the impact of perfusion conditions on LV production.