INTEGRATED SEMI-CONTINUOUS MANUFACTURING OF LENTIVIRAL VECTORS

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Lentiviral vectors (LVs) have become a prevailing gene delivery tool in cell and gene therapy in the past three decades. LVs are primarily used for *ex vivo* modifications, such as transduction of T-cells for expression of chimeric antigen receptor in CAR-T cell therapies, and they are gaining popularity in *in vivo* applications. To date, there are 6 approved *ex vivo* LV products, 222 LV clinical trials including 84 CAR-T cell trials, and considerable ongoing research activities that use LVs for treatments that are steadily advancing toward clinical applications. With this growing trend, it is even more crucial to address the persisting challenge of producing LVs at manufacturing scale to support treatments beyond early clinical trials. In addition to the challenge of generating a sufficient quantity of LVs, their innately labile nature poses an equally important obstacle in LV production. As LVs lose function over time and they are sensitive to environmental factors in each unit operation in the bioprocess workflow, integrated continuous manufacturing is an attractive strategy for process intensification. Implementing the upstream process in perfusion mode increases productivity and continuously harvesting LVs from the bioreactor reduces the residence time, thus preserving their quality attributes. Executing this at manufacturing scale has been a challenge due to limitations of currently commercialized cell retention devices. To circumvent this, we demonstrated a scalable technology that can be used as a cell retention device line that does not retain the product in perfusion mode for LV production using a stable producer cell. This upstream work was published in June 2022 and served as the first piece of an integrated semi-continuous manufacturing process of LVs. Building on our upstream work, we utilized the harvested LV material to implement the first several steps of the downstream process (nuclease treatment, clarification, and capture step) in a semi-continuous mode using 3 Mustang Q membranes with 2 cycles per membrane. There are two systems that operate concurrently in the semi-continuous downstream setup. In system 1, nuclease treated LV material was pumped through a depth filter before being directly loaded onto 2 membranes connected in series. While the clarification and loading processes occur in system 1, a third fully loaded membrane was subjected to wash, elution, and regeneration steps in system 2, the AKTA. Combining the clarification and loading steps as well as operating those steps in parallel to the purification steps expedite the processing time. With this implementation, 516 mL LV harvest was processed in a total of 2.25 h. In comparison, processing the same amount in batch mode, where each unit operation is completed before moving onto the next step, would take 8.75 h. Thus, in reducing the processing time by almost 4-fold, the semi-continuous operation shows a significant advantage. This semi-continuous operation also improves the recoveries of functional vector particles and total vector particles. Functional vector particles are reported as transducing units (TU), assessed by a cell-based assay that measure the GFP-transgene expression in transduced suspension HEK293SF cells, and total vector particles are reported as vector genome (Vg), assessed by a droplet digital PCR assay. Defining the starting LV harvest material to have 100% TU and Vg amounts, the recoveries for the Mustang Q elution for one single membrane operated in batch mode are 43% TU and 73% Vg, whereas the recoveries for the semi-continuous operation are 69% TU and 91% Vg. These results show an added benefit in loading the membranes in series, with recovery improvements of functional vectors by 26% and total vectors by 18% using the semi-continuous operation. In addition, in experiments for a single membrane in batch mode, we observed that processing LV material held at $4^{o}C$ for 6 days versus 1 day lowers the recoveries from 42% TU and 69% Vg to 33% TU and 59% Vg, respectively. These results support the importance of reducing process hold times. Although there is room for optimization of the downstream steps, overall, we demonstrated the integration of upstream and downstream processes in a semi-continuous manner and showed improvements in LV product recovery. The established semi-continuous downstream operation greatly reduces processing time. Furthermore, implementing this in conjunction with LV production in perfusion mode by processing the harvest every 12 h or 24 h would also reduce process hold times. In summary, an integrated semi-continuous process that has the capability to be implemented at manufacturing scale was demonstrated to address two notable challenges of LV production – generating a sufficient quantity for treatments and increasing the vector quality.