

## NANOFIBER BASED LENTIVIRAL VECTOR PRODUCTION

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Viral vectors are an indispensable part of gene therapy clinical trials and lentiviral vectors (LVs) are becoming significant tools in the field. Unlike other retroviral vectors, they can transduce non-dividing cells thus providing for a wider range of potential applications. Current cultivation methods produce titers of  $10^5$  to  $10^7$  TU/mL of cell culture supernatant, which is not convenient for clinical trial requirements of  $10^{11}$ - $10^{12}$  TU per patient [1], [2].

Therefore, it is necessary to concentrate the LV preparations and to remove process related impurities (e.g. serum proteins) and product related impurities, importantly including non-infective virus, as they can cause unwanted inflammation in patients. Small-scale purification can be achieved by ultracentrifugation but there are several disadvantages to this approach: the method is time consuming, there are limited scale-up possibilities, some impurities can be co-purified, and the success of the process is strongly dependent on well trained operator's skills. Alternative methods that can provide for scalable production include tangential flow filtration (TFF) and chromatography. Currently, chromatography is dominated by porous bead stationary phases, which are optimized for purification of small proteins such as mAbs. This is not adequate for LV purification since binding sites located within particle pores are typically not accessible to macromolecular complexes such as viral vectors therefore alternative stationary phases are necessary. One such material is Puridify's FibroSelect cellulose nanofibers. Due to its structural properties, this new purification platform provides high surface area and high capacity for viral vectors. High working flow rates are also possible due to excellent mass transfer properties based on convection, not diffusion that is typically seen in bead-based resins. [3].

In order to circumvent problems associated with transient plasmid transfection and the consequent removal of the plasmid material, we used a continuous producer cell line WinPac-RD [4] and HYPERFlask system for production of LV material. This vector has an RD-pro envelope protein and GFP reporter gene. The recovery through the purification process was monitored by several different methods: infectivity assay utilized GFP expression determined by flow cytometry, LV RNA genome was quantified via RT-qPCR using primers specific for GFP gene, LV particles were detected with p24 ELISA and SYBR Green I-based product-enhanced reverse transcriptase (SG-PERT) assays. By using TFF we were able to remove more than 99% of cell culture proteins, but LV recovery was less than 20%. While losses caused by diafiltration could be mitigated by adding stabilizing agents to the diafiltration buffer, the biggest loss occurred in the concentration step and the overall infectivity recovery remained low. This led us to investigate the implementation of a TFF-free nanofiber step based on ion-exchange chromatography to concentrate LV and eliminate a significant amount of impurities while maintaining high yield of a functional vector.

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