AUTOMATED FILTRATION SCREENING OF LENTIVIRAL VECTORS WITH MULTIPLE ENVELOPE PROTEINS

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Lentiviral Vectors (LV) have been shown to successfully transfer therapeutic genes into dividing and non-dividing cells in laboratory and clinical environments for the benefit of cell and gene therapies. Current LV production features an initial clarification stage to remove cellular debris in addition to viral and serum protein aggregates prior to further downstream processing. Such filtration tasks have illustrated decreases in titer of vectors potentially via damage to external envelope proteins or the unwanted retention of particles [1]. LV production is generally characterized by its fragility and careful downstream processing design is required to ensure high recovery and purity of vectors. Evidence suggests that the selection of salt concentration and pH affects the aggregation propensity of proteins and the binding of vectors and contaminants to filters such as that seen with adeno-associated virus processing [2] whilst also negatively impacting the infectivity of the vector [3]. Such conditions need to be evaluated to ensure effective processing if vector development is to proceed to meet future demands.

A design of experiment definitive scree model was implemented in a Tecan liquid handling platform to rapidly screen various filters under different salt concentrations and pH ranges. Vectors containing the viral envelope proteins VSV-G, Cocal-G and RDPro was filtered across four membrane filter types. The vector transmission was measured by reverse transcriptase activity as a % of unfiltered product, and total protein transmission by Bradford assay. Data has shown vector and total protein transmission is not strongly affected by salt concentration, whereas pH 9 shows improved LV transmission across all envelopes and filters tested. RDPro enveloped LV report strongest filter transmission, whilst Cocal-G and VSV-G remain similar in efficiency. The highest reported LV transmission was found in filters with PVDF chemistry, whereas the best performer for protein removal was PES microwells. Positive correlation between LV and protein transmission was also seen. The work increases our understanding of how filtration affects initial clarification of vectors of differing envelope proteins harvested from cell culture and attempts to characterize the impact of salt concentration and pH value. In identifying the impact of such conditions on vectors, work can continue to improve LV processing, leading to ideal and scalable solutions to address demand for vectors in cell and gene therapies.

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