THE ROLE OF MEMBRANE CHEMISTRY IN LENTIVIRAL VECTOR CLARIFICATION RECOVERY FOR CELL AND GENE THERAPIES

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Many Cell and Gene Therapies (CGT) that utilise lentiviral vectors (LV) to carry therapeutic genetic material to host cells are advancing from early development which has led to a demand for scalable and robust manufacturing process that can overcome the current bottleneck of high cost and low recovery [1]. The manufacture of lentiviral vectors is broadly subdivided into upstream (generation of the vector) and downstream (designed to purify and produce a concentrated high-quality functional vector in a stable and sterile formulation). Membrane processing is commonly employed in the downstream steps, from normal flow filtration (NFF) during clarification and sterile filtration to tangential flow filtration during vector concentration or formulation [2]. In this presentation, we will look at the clarification of crude harvest through a NFF membrane of different materials. Different membrane chemistries demonstrate distinctive properties which can influence the rate and extent of fouling. One mechanism of fouling is through adsorption which can occur when the material in the feed is attracted to the membrane surface through hydrophobic interactions or ionic charges [3]. In our study, we produced transient transfected VSV-G pseudotyped third-generation LV using adherent HEK 293T cells and clarified the crude harvest through 0.45μm filters with different membrane chemistries. This highlighted that the choice of membrane material can improve LV recovery as PES recovered 93% as compared to Nylon 67% of functional vector. We then applied novel techniques such as surface zeta potential to predict interactions with surfaces and crude harvest feed. This showed that Nylon had a positive surface charge as compared to the negative LV crude feed which could result in higher rate of adsorption on, and interaction with, the membrane surface resulting in the loss of functional vector particles. Finally, we visualized the fouling and LV on the surface of the membrane using confocal (CLSM) and scanning electron microscopy (SEM). Further studies have been conducted to understand how harvest feed variability such as suspension culture or stable cell line material can alter these interactions and to what extent can pre-treatment or membrane preparation steps help in reducing these losses. With an industry aim to move towards closed single-use systems that can be operated in smaller and varied facilities (Grade C or D), material such as filtration membranes need to be carefully chosen for process compatibility and optimal recovery [4].

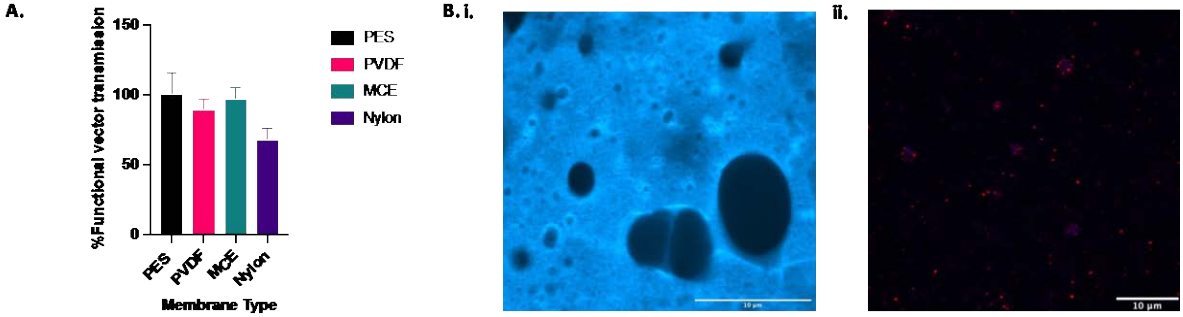

Figure 1: Tracking LV loss during clarification (A) %functional vector transmission through 0.45μm filter of different membrane chemistries. Crude harvest functional titre 8.41E+06 TU/mL measured using GFP expression in flowcytometry. (B) Confocal images of (i) clean PES membrane surface (ii) used PES membrane showing localisation of LV using antibodies against surface VSV-G (blue) and core capsid protein p24 (red). Images taken using Zeiss airyscan at 63x oil immersion and displayed with 10μm scale bar.

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