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DIFFERENTIAL GLYCOSYLATION AND EXTRACELULLAR VESICLE BIOGENESIS IN HEK293 CELLS UPON TRANSIENT TRANSFECTION

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Vaccine therapies based on virus-like particles (VLPs) are currently increasing relevance due to the strong immune response they elicit and their manufacture advantages when compared to traditional biopharmaceuticals. During VLP production using mammalian cell-based platforms, different extracellular vesicles (EVs) are coproduced, leading to the need of a complex downstream purification process. Currently there is no effective and efficient method to separate VLPs from EVs which share very similar density and physicochemical properties[1,2]. Different methods to characterize the EV composition and their protein content and glycosylation signature are used in this work to further understand their biochemical nature. First, a sucrose cushion ultracentrifugation was carried out to isolate the VLP fraction, also containing co-purified EVs. Following, a multiplexed quantitative proteomic approach was used to characterize the VLP-copurified secretome. Three conditions were studied, a non-transfected condition, transiently transfected with an empty plasmid (mock) and with a plasmid for Gag VLP production. In addition to this, dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), cryogenic transmission electron microscopy (CryoTEM) and flow virometry were used to further characterize each of the samples. A shift from the generation of microvesicles (MV) to exosomes was noted upon transfection (Figure 1). This effect was reflected in MV and exosome quantification, where MV shifted from 53.2% in the non-transfected sample to 22.3% when producing VLPs[3]. Moreover, samples from the same three conditions were enzymatically treated to release *N*- and *O*-glycans that were then analyzed via LC-MS/MS. The differential glycan signature of VLPs and coproduced EVs was also characterized, identifying up and downregulated glycan species between the different conditions. These findings are useful to understand the extracellular environment surrounding the VLPs in order to better design separation processes. They can also be used to potentially design future engineered vesicles to work as therapy adjuvants, using glycosylations or immunomodulatory proteins like ENO3 or PRDX5 which are anti-inflammatory proteins.

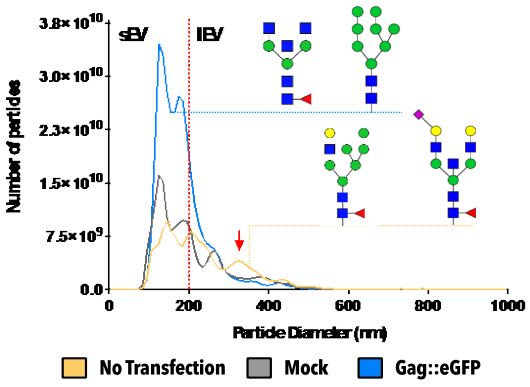

Figure 1 – Particle size distribution measured by nanoparticle tracking analysis (NTA) in the three studied conditions. sEV: small extracellular vesicles; lEV: large extracellular vesicles. Population of lEV pointed by red arrow.

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