Recominant EVs for spike-in references

Recombinant EV (rEV) are currently produced by transfection of HEK 293T cells using a plasmid containing the GAG-EGFP fusion protein. GAG is a polyprotein present in retroviruses, such as HIV-1, and is necessary for the formation of virions. This protein consists of a matrix (MA) domain, a capsid (CA) domain, a nucleocapsid (NC) domain flanked with two spacer peptides, and a p6 domain. The N-terminal MA domain undergoes post-translational myristoylation causing to be located to the inner side of the plasmamembrane while the CA domain causes virion formation, together with the SP1 (spacer1) domain. The NC domain is required for incorporation of nucleic acids into the virion, and the p6 domain interacts with host- 18 cell ESCRT machinery, the same machinery involved in the secretion of endogenous EV, causing release of the virions from the cell [51]. The fusion of GAG with the 'enhanced green fluorescent protein' (EGFP) causes the virus like particles (VLP) released from the cell to be fluorescent, when excited with a light source of 488 nm. It is important to note that these rEV contain about 2500 to 5000 gag-EGFP proteins [52], causing them to be highly fluorescent (paper E. Geeurickx in prep). Previous research has defined the biochemical and physical characteristics, trackability and stability of rEV, proving that they share the size distribution, zeta potential, buoyant density and morphology of endogenous EV. The commutability of rEV has also been confirmed, meaning that rEV perform equally when undergoing different procedures compared to the representative sample [53].

Use of rEV

Once rEV are separated from the culture medium of the transfected cells, they can be spiked in biofluids and can be used for normalization of the amount of endogenous EV in a sample. From previous research it is known that an unknown amount of EV get lost during the separation process. This problem makes it difficult to draw the right conclusion in case EV would be used for diagnosis or therapy monitoring, because the EV concentration in a sample is an important parameter. In order to solve this problem, a known amount of rEV can be spiked in a sample. After the separation process, the amount of rEV left in a sample can be distinguished from endogenous EV based on their fluorescence or EGFP proteins and RNA, which allows to calculate the recovery of rEV for a sample. Because rEV have similar characteristics compared to endogenous EV and they are commutable in multiple biofluids during separation, the rEV recovery can be used as a measure to normalize the amount of endogenous EV. This normalization method enables that the EV concentration can be used as a biomarker in multiple biomedical applications, including for diagnosis, disease progression and therapy monitoring of cancer (paper E. Geeurickx in prep).

rEV in plasma

Previous research on the behavior of rEV in plasma has shown that, when spiked in plasma, rEV shift in densities. This causes them to shift to a higher density, (1,141-1,215 g/mL instead of 1,086-1,103 g/mL) when Opti-prep density gradient (ODG) is executed. This shift in density is caused by corona formation, which is the binding of proteins to the rEV surface, including IgG and IgM. Luckily, this shift is reversible. Reversing the shift in density can be done by proteinase K (PK) treatment before executing a density gradient or by post-insertion PEGylation using DMPE-PEG (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine conjugated to polyethyleneglycol). This PEG-phospholipid conjugate is incorporated in the lipid bilayer of the EV causing the attachment of the PEG molecule. The PEG molecule works as a protective layer, surrounding the rEV, which prevents corona formation. Once PEGylated the rEV float at the same density as the endogenous EV. This leads to a higher persistency in human plasma while the rEV maintain their activity as the PEGylation does not alter the fluorescence and the size distribution of the rEV (paper E. Geeurickx in prep).