

Fluorescence-based assessment of PEG-assisted fusion between cationic liposomes and bovine milk extracellular vesicles

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Extracellular vesicles (EVs) are explored as biomimetic platforms for drug delivery, yet their heterogeneity and limited membrane engineering remain constraints. Hybridization with liposomes may tune vesicle composition, surface properties and interactions with membranes. In this work, a fluorescence assay was developed to investigate the fusion between DOTAP:DOPE cationic liposomes and bovine milk EVs (mEVs). DOTAP:DOPE liposomes were produced using microfluidics (size: 123,3 nm; PDI: 0,291; ζ -potential: +29 mV), co-labeled with the FRET pair DiO (donor)/DiI (acceptor), while mEVs were obtained starting from raw tank milk treated with EDTA and subjected through differential centrifugations/ultracentrifugations. Unlabeled mEVs and (FRET)-labeled liposomes were incubated at different liposome:mEV ratios by particle number counts (NTA), adding PEG8000 at increasing concentrations to promote membrane contact and potential lipid mixing. Fluorescence kinetics were acquired at 37 °C for 50 minutes using donor, acceptor and FRET channels. Absolute FRET signal, apparent FRET ratio and apparent FRET efficiency were calculated to distinguish fluorescence changes from relative donor-acceptor transfer. The most interesting response was observed in samples containing a liposome:mEV ratio of 2:1 with 8% PEG8000. A decrease in absolute FRET over time was observed, accompanied by a corresponding decrease in apparent FRET ratio and apparent FRET efficiency. This profile suggests membrane reorganization and possible lipid mixing, compatible with partial dilution of DiO/DiI probes after interaction with unlabeled mEVs membranes. Although further studies will be required to confirm complete fusion and hybrid systems formation.