A Novel Tool for Efficient RNA Loading into Extracellular Vesicles: Advancing Gene Therapy

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Introduction

Extracellular vesicles (EVs) are emerging as innovative tools for intercellular communication and gene therapy due to their low immunogenicity and capability to deliver RNAs. However, their effectiveness as drug carriers is limited by low RNA-loading efficiency and potential structural and functional alterations from typical loading methods such as electroporation and sonication, which can compromise their natural targeting abilities.

This study presents an efficient and straightforward method for incorporating RNAs into EVs using HemiFect™ by EVis Bioscience. This innovative technology was demonstrated to successfully hemi-fuse with commercial purified EVs from various blood components by HansaBioMed at neutral pH. This process generates hemi-fused EVs (hfEVs) that incorporate both small and large RNAs while preserving the EVs biological functions. hfEVs have been characterized for size distribution, concentration, morphology, hemi-fusion potential, and RNA loading efficiency.

Materials and Methods

mRNA was entrapped into different populations of EVs provided by HansaBioMed: Platelet-derived EVs, red blood cell-derived EVs, and plasma-derived EVs. EVis' proprietary technology, HemiFect™ was exploited for hemi-fusion. HemiFect™ can be mixed with mRNA and successively with EVs at neutral pH, at 37 °C for 30 min and particle ratio 1:1. The produced hfEVs were investigated in terms of size distribution, hemi-fusion capability and mRNA loading with NTA, nanoFCM,and cryo-TEM.

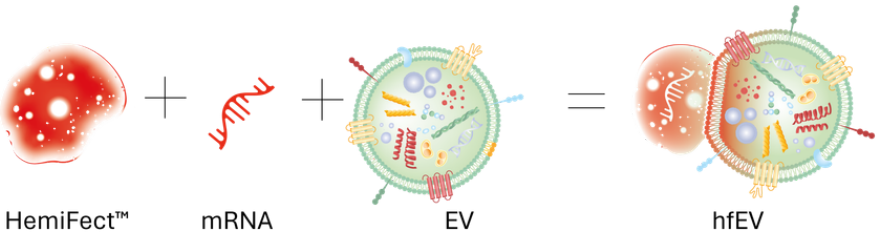

Figure 1: Preparation of hemi-fused EVs carrying mRNAs

Results

HemiFect™ - Fusion and mRNA loading efficiency in various EVs

HemiFect™ fusion efficiency with red blood cell-derived EVs (rbc-EVs), plasma-derived EVs (pm-EVs), and platelet-derived EVs (pt-EVs), and subsequently hfEV formation were reported in the second row of Figure 2. mRNA LE% into hfEVs was reported in the third row of the same figure. HemiFect™ and EVs were mixed at a particle ratio of 1:1. HemiFect™ and EVs alone were used as controls. Fusion efficiency and mRNA LE% were assessed through nanoFCM analysis (NanoFCM Co, Ltd, Nottingham, UK).

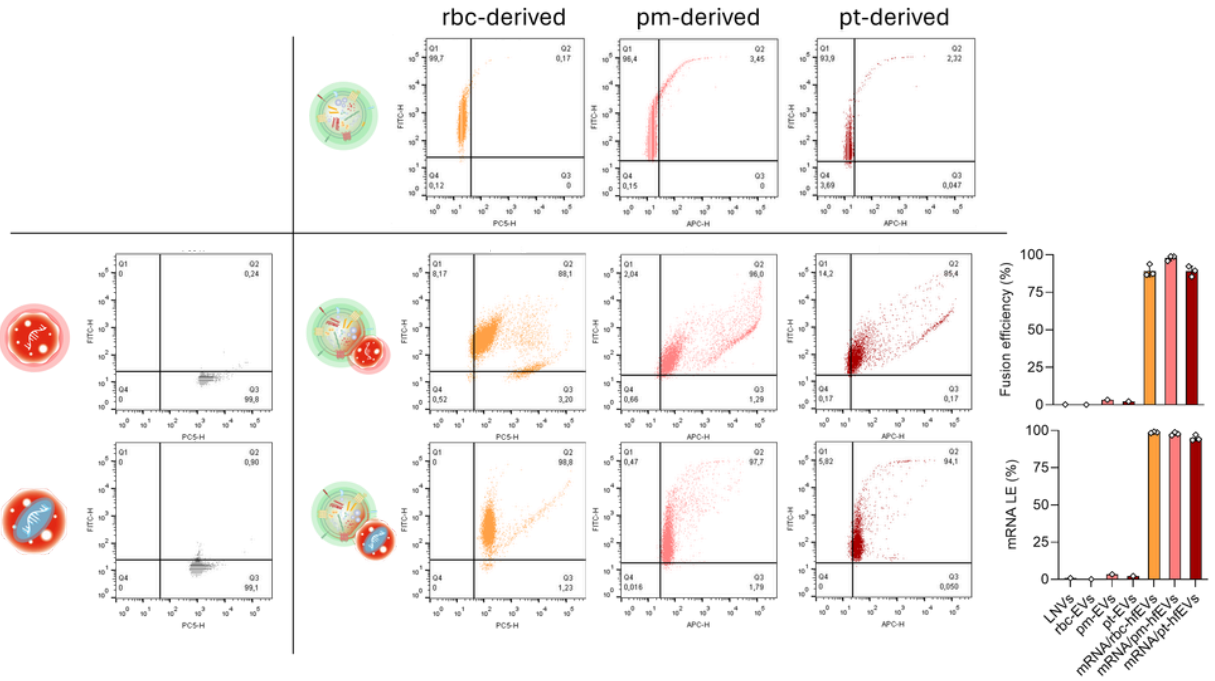

Figure 2: Fusion efficiency comparison between rbc-derived, pm-derived, and pt-derived EVs (Graphs on the right side show the mean ± s.d. (n = 3) of three independent batches.)

CryoTEM - Morphological characterization

Morphology of HemiFect™, mRNA-loaded HemiFect™, native EVs (first row) and mRNA-loaded hfEVs (second row) were evaluated by Cryo-TEM imaging.

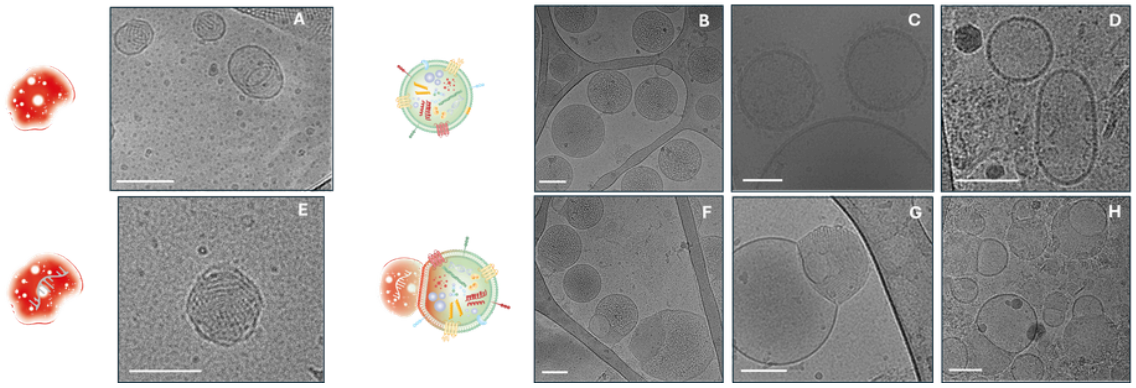

Figure 3: (A) HemiFect™, (B) rbc-EVs; (C) pm-EVs; (D) pt-EVs; (E) mRNA-loaded HemiFect™; (F) mRNA-loaded rbc-hfEVs; (G) mRNA-loaded pm-hfEVs; (H) mRNA-loaded pm-hfEVs. HemiFect™ and EVswere mixed at a particle ratio of 1:1. The scale bars correspond to 100 nm.

Conclusion

HemiFect™ allows loading mRNA into up to 90% of all produced hfEVs and purified EVs available from HansaBioMed prove useful reference materials for EV loading studies, providing a useful toolset for therapeutics research. HemiFect™ complete fusion with EVs enables the use of mRNA-loaded hfEVs without any further purification. The morphology of native EVs is preserved after mRNA loading, ensuring that their biological properties remain intact.

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