Red blood cells-derived extracellular vesicles for the loading and delivery of RNA molecules

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The recent advent of biologics needs increasingly sophisticated drug delivery systems. Red blood cells (RBCs) have long been used as cell therapy thanks to their unique properties. Lately, extracellular vesicles (EVs) are attracting much more attention for the delivery of therapeutic cargoes. RBCs-derived EVs (RBCEVs) are one of the most promising. However, their production is still challenging for several reasons (e.g., yield of production, loading efficiency and translatability to the clinics). Here, we propose a new way for the generation of RBCEVs loaded with RNA molecules that overcomes most of these issues.
Our strategy is based on the preloading of the cargo into RBCs followed by vesiculation. The first is carried out by an already established technology, while the latter by "soft extrusion", a newly developed and patented physical vesiculation method.
We showed that RBCs could be efficiently preloaded with several kinds of molecules and further used to generate RBCEVs. Indeed, our method was able to produce a very high yield of cargo-loaded RBCEVs. Preloading of fluorescent dextran-conjugates enabled to demonstrate that the cargo was definitely retained in RBCEVs. Moreover, the obtained RBCEVs population has been deeply characterised by DLS, NTA, TEM and flow cytometry, showing great homogeneity in terms of size, biological features and cargo. In vitro results demonstrated that RBCEVs were abundantly internalised by cells. Finally, proof-of-concept studies proved that a miRNA could be efficiently loaded into RBCEVs, effectively delivered to HUVEC and able to exert its biological effect. Briefly, miR-210 was capable of inhibiting its target PTP1B and lowering mitochondrial metabolism. Studies with long mRNAs to treat metabolic diseases are ongoing.
Of note, the bench-scale process might be easily scaled-up and translated into the clinics. Hence, this investigation could lay the foundation for the development of a new biomimetic platform for RNA-based therapies.