Microfluidic-generated lipid nanoparticles for *in vitro* delivery of plasmid DNA

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Abstract

Gene therapy holds great potential for treating almost any disease. This technique is based on the transfer of genetic material to a target tissue to prevent or treat a disease. To efficiently deliver the nucleic acid (NA) payload to its target tissue, the genetic material needs to be combined with a delivery system. Lipid nanoparticles (LNPs) have proven to be excellent delivery vectors for gene therapy and are increasingly entering into clinical practice [1]. Over the past two decades, the optimization of LNP formulations for NA delivery has led to a well-established body of knowledge culminating in the first-ever RNA interference therapeutic using LNP technology, i.e., Onpattro, and many more in clinical development to deliver various NA payloads. However, while some RNA-encapsulating LNP formulations passed clinical trials, DNA-loaded LNPs have been only marginally explored so far [2]. To fulfil this gap, herein we explored the effect of several factors influencing the manufacturing and transfection behavior of DNA-loaded LNPs. In fact, the LNPs synthesis process has been recently optimized to make their production more handling and reproducible by adopting the microfluidic mixing, a manufacturing strategy based on the rapid mixing of an organic phase containing lipids and an aqueous phase containing NAs, in a controlled environment [3]. Thus, we investigated how the microfluidic parameters (i.e., the total flow rate (TFR)), the surface PEGylation, the concentration and the particle density at the cell membrane can affect the biological behaviour of LNPs.We show that PEGylation and post-synthesis sample concentration facilitated formulation of homogeneous and small size LNPs with high transfection efficiency and minor, if any, cytotoxicity on human Embryonic Kidney293 (HEK-293), spontaneously immortalized human keratinocytes (HaCaT), and epidermoid cervical cancer (CaSki) cell lines. On the other side, increasing TFR had a detrimental effect both on the physicochemical properties and transfection properties of LNPs. Lastly, the effect of particle concentration at the cell surface on the transfection efficiency (TE) and cell viability was largely dependent on the cell line, suggesting that its case-by-case optimization would be necessary. Overall, we demonstrate that fine tuning formulation and microfluidic parameters is a vital step for the generation of highly efficient DNA-loaded LNPs.

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