INVESTIGATING THE KEY MANUFACTURING PARAMETERS FOR THE MICROFLUIDIC PRODUCTION OF LIPID NANOPARTICLES

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Background: In order for genetic drugs to be used clinically, sophisticated delivery systems are required. Lipid nanoparticle systems (LNP) for delivery of small molecule drugs have led to rigorous design criteria. These criteria include a size range of 100 nm or less, highly efficient encapsulation processes, robust, scalable manufacturing processes, and product stability of at least 1 year at 4°C. Here, we assessed the effect of the formulation and production parameters for the formulation of LNP for gene delivery.

Methods: The NanoAssemblr® Benchtop from Precision Nanosystems Inc. was used for the preparation of LNP. Neutral (HSPC, cholesterol, DOPE), cationic (DOTAP and DDAB) and polyethyleneglycol lipids (DMG-PEG2000 and DSPE-PEG2000) were dissolved in ethanol or methanol, and used in different lipid combinations. As aqueous phase, Tris buffer (pH 7.4 10 mM) was used. Both aqueous and solvent phases were then injected into the system and the microfluidic production parameters were evaluated. In order to reduce the final solvent content to acceptable levels, samples were diluted post-production in Tris buffer. Polyadenylic acid (PolyA) and salmon ssDNA were used for method optimization due to their relatively low cost and were loaded in-line (in the aqueous phase) within the microfluidics system.

Results: In general, all formulations tested showed low sensitivity to the total flow rate and lipid concentration and high sensitivity to the flow rate ratio. Solvent selection impacted on the characteristics of the produced particles and the data shown is highly reproducible between laboratories. Regardless of the choice of cationic lipid or PEGylated lipid, the manufactured vesicles showed comparable physicochemical characteristics. Loading of Poly(A) and ssDNA resulted in high loading efficiencies.

Conclusions: Here, we have shown a comprehensive study of the effect of the lipid choice and microfluidic process parameters for the production of LNP loading Poly(A) and ssDNA as surrogates for RNA. This method optimisation and formulation screening could be used as a model for the preparation of LNP for gene delivery.