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Continuous Format ion of Cationic Liposomes and Lipoplexes for Gene Delivery Using Microfluidic Devices

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Extended Abstract

Microfluidics constitute an upcoming technology for continuous flow synthesis of nanostructured gene delivery systems. Due to the laminar flow, mixing of solutions in microfluidic devices occurs by molecular diffusion, what enables a more precise control of colloidal aggregation processes (Whitesides, 2005). Cationic liposomes (CLs) have been extensively employed as carriers to delivery plasmid DNA (pDNA) into diseased cells (Ma et al., 2007) Many processes used for liposome production require a post-processing step for the size control, and microfluidic systems emerge as an alternative to overcome these major drawbacks. Currently, the most common method of preparing pDNA/CLs complexes is the "bulk" process, in which the two solutions of CLs and pDNA are mixed by simple pipetting or brief vortexing. Such conditions can generate aggregates with uncontrolled physicochemical properties. In this study, we have designed a microfluidic device with two distinguish areas to perform the one-step liposome synthesis and the subsequent formation of pDNA/CLs complexes. We used polydimethylsiloxane (PDMS)/glass microfluidic devices fabricated using conventional UV photolithographic and soft-lithography methods (Balbino et al., 2013). All microchannels had an approximately rectangular cross section with a depth of 100 µm and a width of 140 µm. The first hydrodynamic flow-focusing area performed the CLs synthesis. In a second region, the stream containing the pre-formed CLs was subsequently focused by two side streams containing the pDNA. CLs were composed of egg phosphatidylcholine (EPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) (2:1:1 molar). We used a plasmid DNA that encodes the enhanced green fluorescent protein (EGFP) as vector model. The molar charge ratio between positive and negative charges from CLs and pDNA, respectively, was 6 and it was used in all experiments. This condition was previously identified as the best for *in vitro* transfection studies (Balbino et al., 2013). CLs liposomes were produced using a total volumetric flow-rate of 100 µL/min and varying flow rate ratios between side and central streams. To form lipoplexes in the second focusing area, the side streams containing the pDNA focused the CLs stream and the pDNA/CLs complexes, i.e. lipoplexes, formation occurred in the main mixing channel. CLs and lipoplexes presented hydrodynamic diameters weighted by number in the range of 40 nm and 150 nm. All lipoplexes presented positive zeta potential, what makes possible the electrostatic interaction with cell membranes. Thereby, we were able to produce nano-sized CLs and lipoplexes with suitable physicochemical characteristics using a microfluidic device with two distinguish regions, for gene delivery and vaccine therapy applications.

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