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Microfluidic Assembly of Nanoscale Lipoplexes for Gene Delivery

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Extended Abstract

Gene delivery is a promising therapeutic method for treating various genetic and acquired diseases. The efficient delivery into cells needs the foreign DNA to be protected. Cationic liposomes (CLs) have shown to be powerful nanocarriers systems of nucleic acids. Currently, the bulk mixing (BM) is the most common method of preparing nonviral complexes, which involves the mixing of two solutions by pipetting (Hsieh et al., 2009). However, at low positive/negative molar charge ratios (R±), lipoplexes tend to present large particle size and high polydispersity values, which may impair transfection efficiency and colloidal stability. Furthermore, under certain conditions, the conventional BM method may yield inconsistent and poorly reproducible transfection efficiencies. In this fashion, microfluidic devices have become a technological alternative to overcome these drawbacks (Koh et al., 2011). Microfluidics is a multidisciplinary technology that is mainly dedicated to miniaturized systems and manipulating small volumes of fluid. Microfluidic devices have particular fluid-flow characteristics and are able to control concentrations of molecules in space and time (Whitesides, 2006). Thus, the goal of the present work was to investigate the microfluidic formation of DNA/CL lipoplexes at low R± conditions. We used a hydrodynamic flow-focusing microfluidic device to produce lipoplexes at varying R±, and bulk mixing method as comparison. The device had microchannels with depth of 100 μm and width of 120 μm, constructed by soft lithography (polydimethylsiloxane (PDMS) bounded to glass). CL were prepared by thin film method and 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 model encoding enhanced green fluorescent protein (EGFP) that was purified using the PureLink HiPure Plasmid DNA Purification Kit-Maxiprep (Invitrogen, MD). All lipoplex preparations were carried out with temperature control of 4°C. Dynamic light scattering results of both assembly methods showed that the lipoplexes size was increased when the R± values were decreased. Nevertheless, the microfluidic process produced lipoplexes with smaller hydrodynamic diameter and polydispersity index values when compared with BM lipoplexes. Lipoplexes prepared by the BM method had lower zeta potential values, which indicates that BM and microfluidic complexes had different isoelectric points. Gel retardation assay showed that the DNA was completely retained in the liposomal structures of complexes obtained by both preparation methods for all R± values studied. The biological evaluation of *in vitro* transfection in human epithelial carcinoma (HeLa) cells pointed that lipoplexes prepared by the microfluidic method achieved higher transfection levels then BM lipoplexes. Therefore, we were able to show the potential use of hydrodynamic flow-focusing microfluidic device to provide better control of particle size at low R± conditions.

References

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