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Microfluidics applied towards development of stealth liposomes functionalized with cyclic RGDfK peptide for targeted gene delivery

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Abstract

Targeted drug and gene delivery systems has rapidly gained importance, due to the offering of controlled delivery of biologically active agents at specific cells. In this context, microfluidics has been a valuable tool for the synthesis of nanocarriers in reproducible processes. Here we describe the microfluidic synthesis of positively charged stealth liposomes with low polydispersity, as a potential strategy to produce liposomes functionalized of cyclic RGDfK peptide.

Key words:

Microfluidics, stealth liposomes, targeted gene delivery.

Introduction

Gene therapy aims to insert therapeutic nucleic acids into target cells in order to modulate cellular expressions. For a successful delivery into the cells (transfection), nanocarriers have presented advantageous features. At this point, cationic liposomes (CL) are widely investigated as non-viral vectors for gene delivery applications due to the ability of condensing and protecting nucleic acids against enzymatic degradation, as well as efficiently interacting with cell membranes.¹ Modifying the CL surfaces with Poly(ethylene glycol) (PEG) and cRGDfK peptide provides an interesting strategy to improve gene delivery in targeted cells. In this framework, microfluidic systems based on hydrodynamic flow focusing have been widely explored for liposomes synthesis.² This work aims to investigate the synthesis of CL conjugated with PEG and cRGDfK peptide. For this purpose, two crucial steps were followed: (i) phospholipids were chemically modified with cRGDfK and (ii) stealth cationic liposomes were composed by the lipids (EPC/DOTAP/DOPE/DSPE-PEG), which exhibited convenient physicochemical properties.

Results and Discussion

Preceding the development of functionalized liposomes; the phospholipids 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) conjugated with PEG₂₀₀₀ containing a succinimidyl group were chemically modified with cRGDfK by a nucleophilic substitution. The efficiency of the chemical reaction was assessed by ESI(+)-TOF mass spectrometry (Image 1).

From the analysis of the mass spectrum (Image 1), it is possible to conclude that the product was formed and the reaction's efficiency is nearly 30%.

Using hydrodynamic focusing devices with side streams composed by phosphate-buffered saline (PBS 50 mM), it was possible to produce liposomes and stealth liposomes, both positively charged and with low polydispersity ($PDI \leq 0.2$). Taken together, these results suggest that this strategy has great potential for further investigations applying cRGDfK peptide in order to synthesize specific targeted liposomes.

Image 2. Intensity-weighted hydrodynamic diameter of cationic liposomes and stealth liposomes formed by flow rate ratio (FRR) of 7.3 in microfluidic devices, compared to stealth liposomes assembled via bulk process.

Conclusions

In this work, we have demonstrated the potential of using a microfluidic approach based on hydrodynamic flow focusing for the synthesis of stealth liposomes with interesting features, such as low polydispersity and positive charges. Additionally, for the first time, it was presented an interesting strategy to develop liposomes functionalized with cRGDfK peptide.

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Image 1. Mass spectrum of DSPE-PEG lipid derivatized with cRGDfK. Arrows indicate the product mass/charge ratio.