



TITOLO  
(maiuscolo)

LIPOPOLYPLEXES FOR pDNA DELIVERY: THE ROLE OF CATIONIC POLYMER ON CYTOTOXICITY AND TRANSFECTION EFFICIENCY

Autore (i)

C. Pozza; G. Anderluzzi, S. Franzé; F. Cilurzo

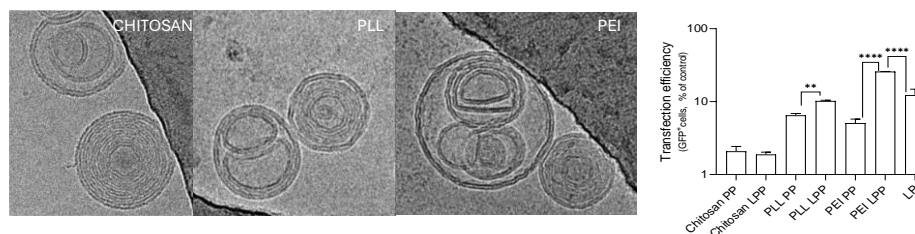
Ente  
di appartenenza

Department of Pharmaceutical Science – Università degli Studi di Milano – via G. Colombo, 71 - Milano

Riassunto

Carattere: ARIAL  
Corpo: 10  
Interlinea: 1

Lipopolyplexes (LPP), namely ternary complexes of biocompatible cationic polymers, nucleic acid and liposomes, represent an attractive though poorly investigated alternative to lipoplexes for gene delivery. Poly-L-lysine (PLL), polyethyleneimine (PEI), spermidine, spermine and protamine sulfate are the most used polymers for LPP preparation. Nevertheless, a systematic study of the polymer nature on nucleic acid complexation and transfection efficiency is missing. Here, we prepared LPP using three cationic polymers (Chitosan, PLL and PEI) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) based liposomes to complex a pDNA encoding the green fluorescence protein (EGFP). Briefly, pDNA encoding EGFP was complexed with chitosan, PLL or PEI in PBS 50mM pH 4.5 at different polymer/DNA mass ratios using a microfluidic chip to form polyplexes (PP). PP were further complexed with DPPC:DOPE:Chol liposomes (55:35:10 mol%, in PBS 50 mM pH 7.4) at different molar ratios by using microfluidics. All formulations were characterized in terms of hydrodynamic diameter, polydispersity index (PDI) and zeta potential by dynamic light scattering (Malvern, UK). Particle size distribution and concentration was also monitored by Nanoparticle Tracking Analysis (NTA) using a Nanosight NS300 (Malvern, UK). Cytotoxicity was assessed on HeLa human cervical adenocarcinoma cells by an MTT assay while transfection efficiency was quantified by GFP detection using flow cytometry. A pDNA/DOTAP lipoplex was used as positive control. The optimal polymer/DNA mass ratios were found to be 10 for chitosan and PLL polyplexes and 6 for PEI polyplexes. These polyplexes were around 100 nm in size, PDI<0.2 and positively charged, while final LPP had a mean diameter of about 180 nm, PDI<0.2 and they were slightly positive/neutral. The formation of the ternary complexes were confirmed by Fluorescence resonance energy transfer (FRET) and cryo-EM. In vitro, generally LPP were significantly less toxic than corresponding PP and showed higher transfection efficiency. While chitosan LPP resulted the most biocompatible complex, PEI LPP induced the highest GFP expression, both as percentage of positive cells and mean fluorescence intensity, resulting more efficient DNA carriers compared to DOTAP lipoplex also. In conclusion, in this work, the choice of the polymer for lipopolyplexes preparation was rationalized; generally, PEI resulted the most promising polymer for the design of LPP, offering a valid alternative platform for the delivery of pDNA.



**Fig.1- Cryo-EM images of LPP and transfection efficiency in HeLa cells**

**Acknowledgments:** This research was supported by EU - NextGenerationEU (PNRR M4C2-Investimento 1.4-CN0000041-23 PNRR\_CN3RNA\_SPOKE8).

**IMPORTANTE: inviare il testo in formato (word o pdf) editabile e NON in formato immagine.**

Autore di riferimento da contattare per ulteriori informazioni:

Nome e Cognome: Camilla Pozza

E-mail: