On the effect of cationic polymer on the structure and pDNA transfection efficiency of lipopolyplexes

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Lipopolyplexes (LPPs), i.e. hybrid ternary complexes of cationic polymers, nucleic acids and liposomes, represent a second-generation non-viral vector aiming to overcome the limitations of the first-generation polyplexes (PPs) and lipoplexes (LPs), i.e. poor cell uptake and cytotoxicity. Although effective both in vitro and in vivo, [1,2] a systematic comparison of the polymer nature on nucleic acid complexation, ternary complex formation and transfection efficiency has not been conducted yet. Herein, we tested a panel of LPPs containing either the cationic polymer chitosan, poly-L-lysine (PLL) or polyethyleneimine (PEI) for the delivery of a plasmid DNA encoding the green fluorescence protein as model (pDNA-GFP) to provided evidence of the effect of particles composition on their physicochemical features and biological activity. LPPs were prepared by a two-step microfluidic process consisting of i) the formation of PPs by complexing pDNA-GFP with cationic polymers, followed by ii) the formation of the ternary complex by mixing PPs with neutral liposomes (Fig.1). The optimal polymer/DNA/lipid charge ratios and microfluidic operating parameters were tuned to obtain particles with desired properties. All LPPs had a mean diameter of around 180 nm, a PDI<0.2 and a slightly positive/neutral z-potential. FRET, SAXS and Cryo-EM analyses demonstrated the formation of a ternary complex in which chitosan LPPs assume a globular core-shell structure of an external lipid layer and a dense core, likely composed of chitosan PPs, conversely, PLL and PEI LPPs showed a rearrangement among the three components to form a packed complex, which strongly differed from the original PPs (Fig.1). In vitro, LPPs were more biocompatible and induced higher protein expression than corresponding PPs and control LPs. Moreover, despite the three ternary complexes displaying similar uptake kinetics, PEI LPPs showed the highest endosomolytic activity and promoted the most effective DNA transfection in both tumor and non-tumor/primary cells (Fig.1). This study demonstrates that lipopolyplexes are a valid and promising platform for pDNA delivery and underscores the importance of cationic polymer selection in influencing both toxicity and transfection efficiency.

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References

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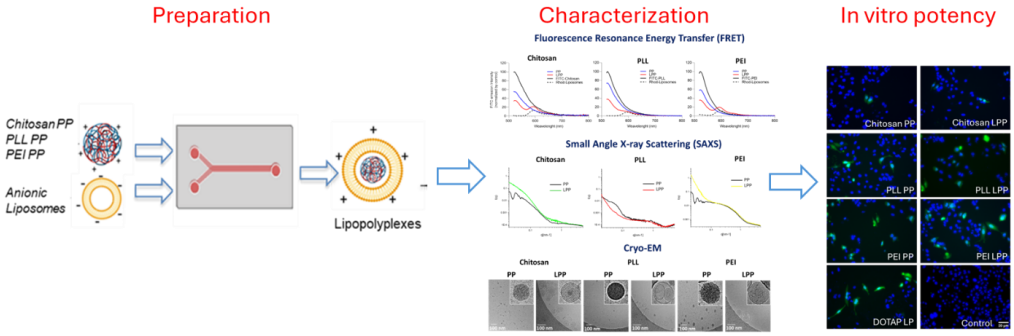

Figure 1: Schematic representation of the workflow. PPs and LPPs containing Chitosan, PLL or PEI were prepared by microfluidics (left panel) and characterized. FRET, SAXS and Cryo-EM results were reported (central panel). In vitro, LPPs were significantly more potent than corresponding PPs and control LPs in promoting cell transfection, among which PEI LPPs induced the highest protein expression (right panel).