Multifunctional lipid nanoparticles dedicated to RNAi screening

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Since its discovery fourteen years ago, investigations on RNA interference in biology and medicine are growing with applications ranging from molecular genetics to design of new therapeutic strategies. Indeed, synthetic siRNA (small interfering RNA) provide a simple and effective gene silencing means through a sequence specific down-regulation of the complementary messenger RNA.

However, naked siRNA are highly sensitive to the degradation enzymes (nucleases) and does not cross the cell membrane due to its large molecular weight (13kDa) and anionic nature. Thus, delivery systems are strongly required to facilitate its distribution to its intracellular sites of action.

Among the considered solutions, with their unique properties (targeting, monitoring, endosomal escape promoting …), nanoparticles are emerging as a promising approach in the specific delivery of siRNA. Lipid nanoparticles were specially brought to our attention for this application.

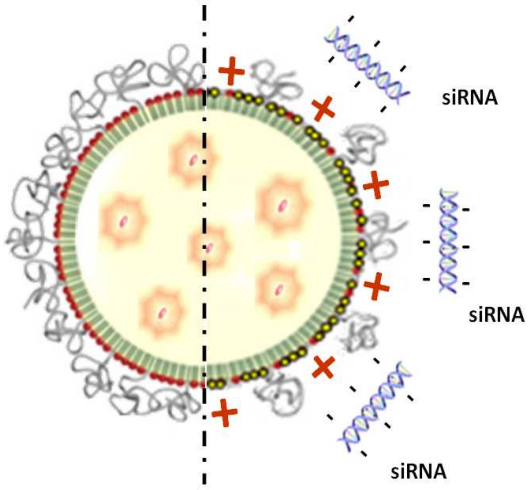
By incorporating some cationic compounds to lipid nanoemulsions, the electrostatic bonds establishment with negative charges of siRNA (Fig.1.) has been improved. The structure of cationic lipid nanoemulsions has been optimized with a design of experiment to isolate the most relevant formulation. Each design of particle has been studied (physical chemical properties, colloidal stability, especially in biological medium, toxicity) before checking the interaction with siRNA. Cationic lipid nanoparticles allow complexation with TAMRA-stained siRNA, as demonstrated by gel retardation assay.

Furthermore, *in vitro* transfection using a tumoral cell model overexpressing the green fluorescent protein (GFP) show a significant down-regulation of this targeted protein expression (Fig.2. and Fig.3.). These results show efficiency close to that obtained with commercial lipoplexes. Further investigations are ongoing to investigate the intracellular distribution of the “nanoparticle-siRNA” complex.

The association of synthetic siRNA with the benefits of nanotechnology should open the way to new biomedical applications in the near future.

Figures

A/



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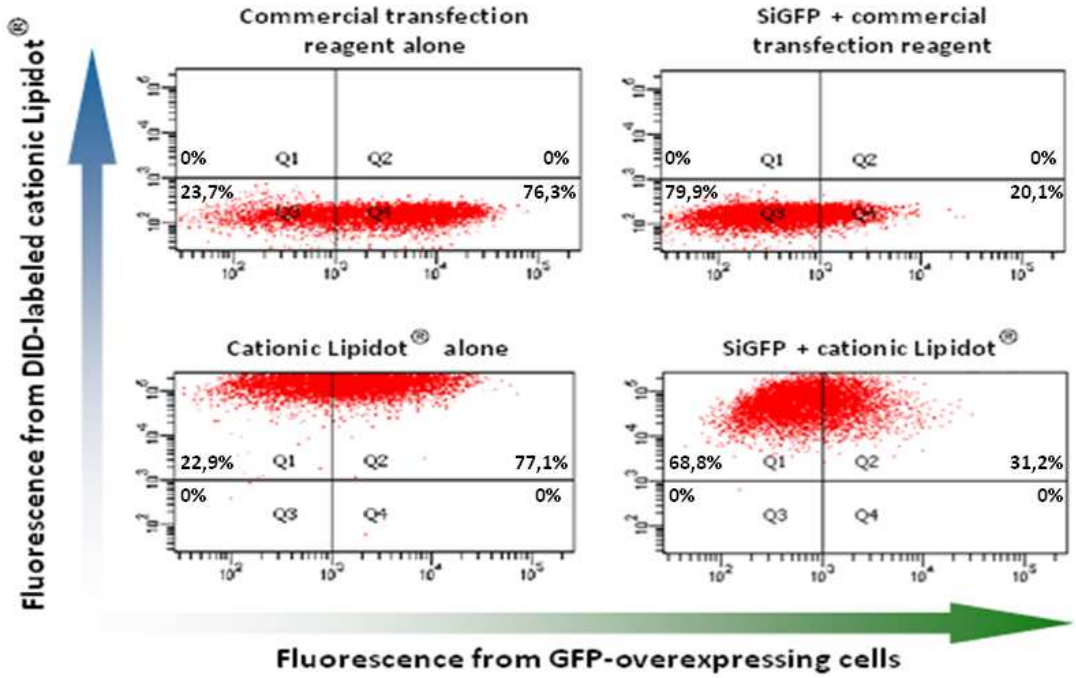

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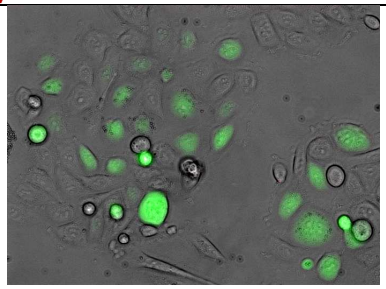
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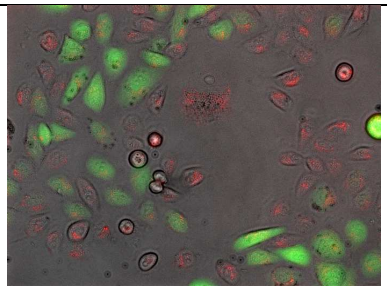
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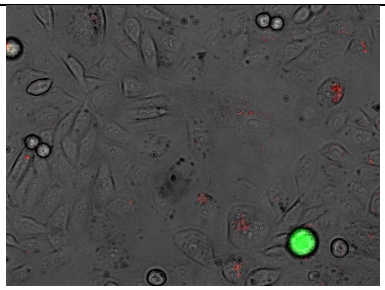
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Figures: A/ structure of lipid nanoemulsions, before (left) and after (right) incorporation of cationic compounds, B/ dot plots obtained by FACS displaying the down regulation of GFP expression and C/ microscopy imaging of down regulation of GFP expression with cationic lipid nanoparticles following times.