

# NIOSOMES BASED ON A CATIONIC LIPID AND DIFFERENT HELPER LIPIDS FOR GENE THERAPY

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## INTRODUCTION

Gene therapy has become one of the main areas of interest for scientists because it focuses on the possibility of delivering a normal functioning gene into the cell to have a therapeutic effect (1-3). However, efficient delivery and expression of genes into the cells is not as easy as it seems. The entry of the DNA into the cells and the protection of the genetic material against enzymatic digestion before reaching the nucleus are two important factors that might, clearly, hamper this process (4-5). The comprehension of this process along with the mechanisms of their intracellular delivery will lead to the design of better adapted non-viral vectors for gene therapy applications. The addition of helper lipids in cationic lipids formulations is found to affect transfection efficiencies as it influences the intracellular trafficking.

## OBJECTIVE

The objective of this work was to evaluate the influence of the helper lipid in the physicochemical characteristics of the nioplexes and their effect on transfection efficiency and intracellular trafficking.

## MATERIAL & METHODS

We designed four different niosome formulations based on the same cationic lipid and only changing the helper lipid: squalene (Sque), cholesterol (Cho), squalane (Squa) and without helper lipid (None). The concentration for all the helper lipids in each formulation was 10 mM. The synthesized amino cationic lipid (Fig.1) was formulated with polysorbate 80 as non-ionic surfactant and the different helper lipids by oil in water emulsion (o/w). Resulted niosomes were characterized in terms of size and zeta potential. Upon the addition of the pCMS-EGFP (0.5 mg/ml) reporter plasmid, nioplexes (w/w ratio of 30:1 Lipid:DNA) were obtained and characterized in terms of size and zeta potential. The ability of the niosomes to bind, release and protect the DNA against enzymatic digestion was evaluated by agarose gel electrophoresis. *In vitro* experiments were performed to evaluate the transfection efficiency, cell viability (at 72 hours) and uptake (at 1 hour) in ARPE-19 cells by flow cytometry. For uptake analysis a reporter plasmid stained with cyanine dye (Cy3) was employed. Trafficking of the best formulation was analyzed by measuring colocalization between the nioplexes (elaborated with Cy3 stained plasmid) and the different entry pathways: Clathrin vias marked with AlexaFluor488-Transferrin; Caveolae vias marked with AlexaFluor488-Cholera Toxin B; Macropynocytosis vias marked with Alexa Fluor-488 Dextran and; Lysosome marked with Lyso Tracker-Green.

## RESULTS

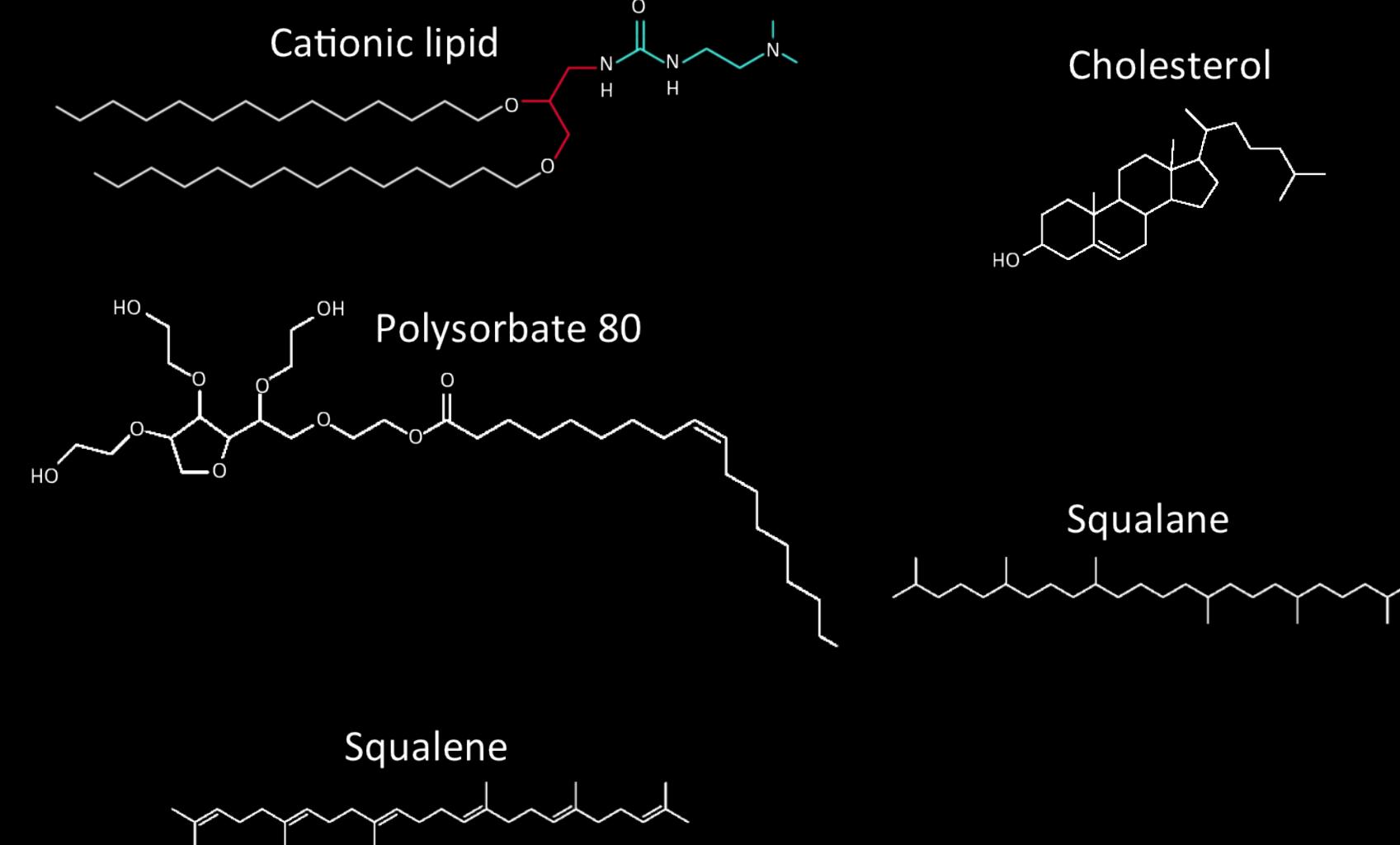


Figure 1. Chemical structure of cationic lipid: 3-[2,3-bis(tetradecyloxy)propyl]-1-[2-(dimethylamino)ethyl]urea, polysorbate 80, squalene, cholesterol and squalane

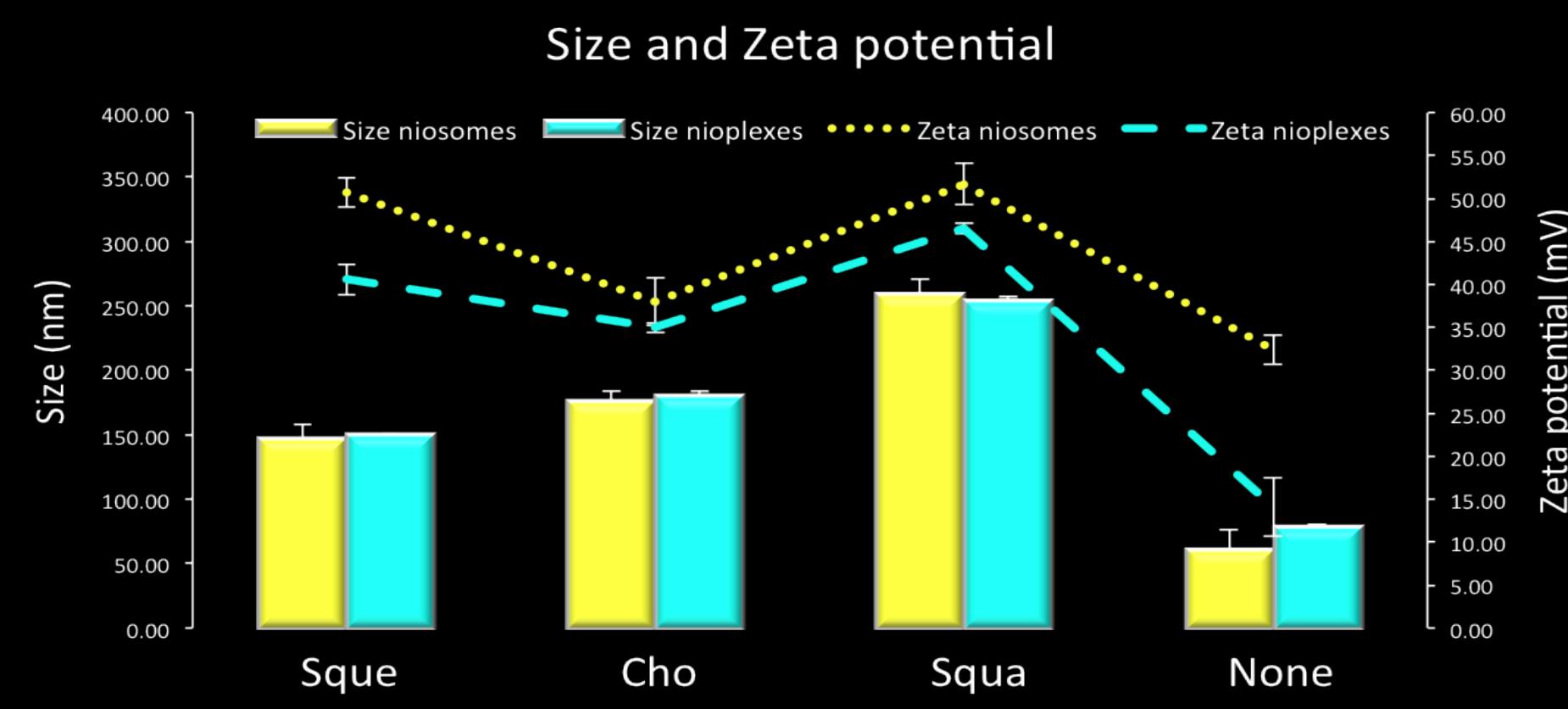


Figure 2. Physicochemical characterization of niosomes and nioplexes prepared with different helper lipids. Yellow color: Size and zeta potential of niosomes. Blue color: Size and zeta potential of nioplexes (niosome/pCMS-EGFP w/w ratio 30/1). Each value represents the mean  $\pm$  standard deviation of three measurements.

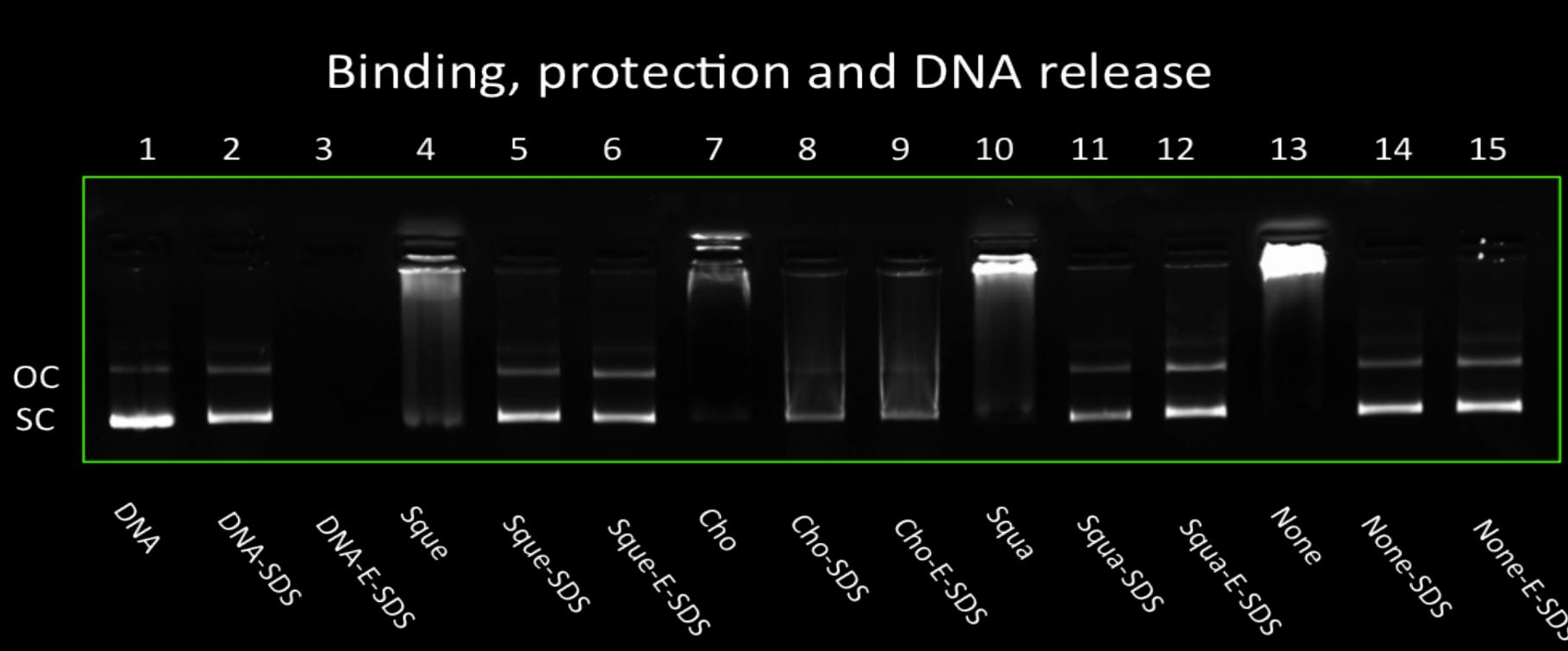


Figure 3. Binding, SDS-induced release of DNA and DNA protection against DNase I degradation with niosomes based on different helper lipids. OC: open circular form, SC: supercoiled form. Lanes 1-3 correspond to free DNA; lanes 4-6, niosomes based on aqualene; lanes 7-9, niosomes prepared with cholesterol; lanes 10-12, niosomes based on squalane; lanes 13-15, niosomes prepared without helper lipid. Naked DNA and niosomes were treated with SDS (lanes 2, 5, 8, 11 and 14) and DNase I+SDS (lanes 3, 6, 9, 12 and 15).

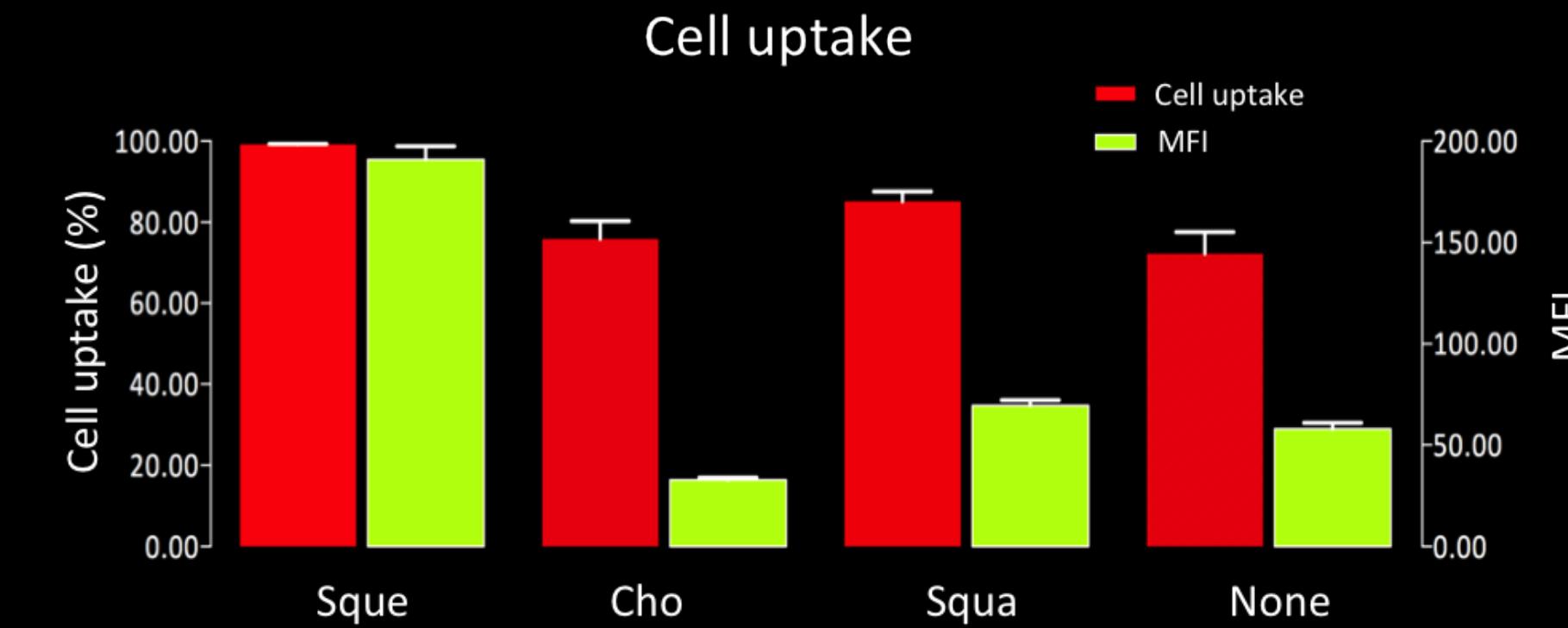


Figure 4. Cell uptake at 1 hour after the addition of nioplexes prepared with different helper lipids in ARPE-19 cells. Red: Percentage of cell uptake. Green: Mean fluorescence intensity (MFI) of cells. Each value represents the mean  $\pm$  standard deviation of three measurements.

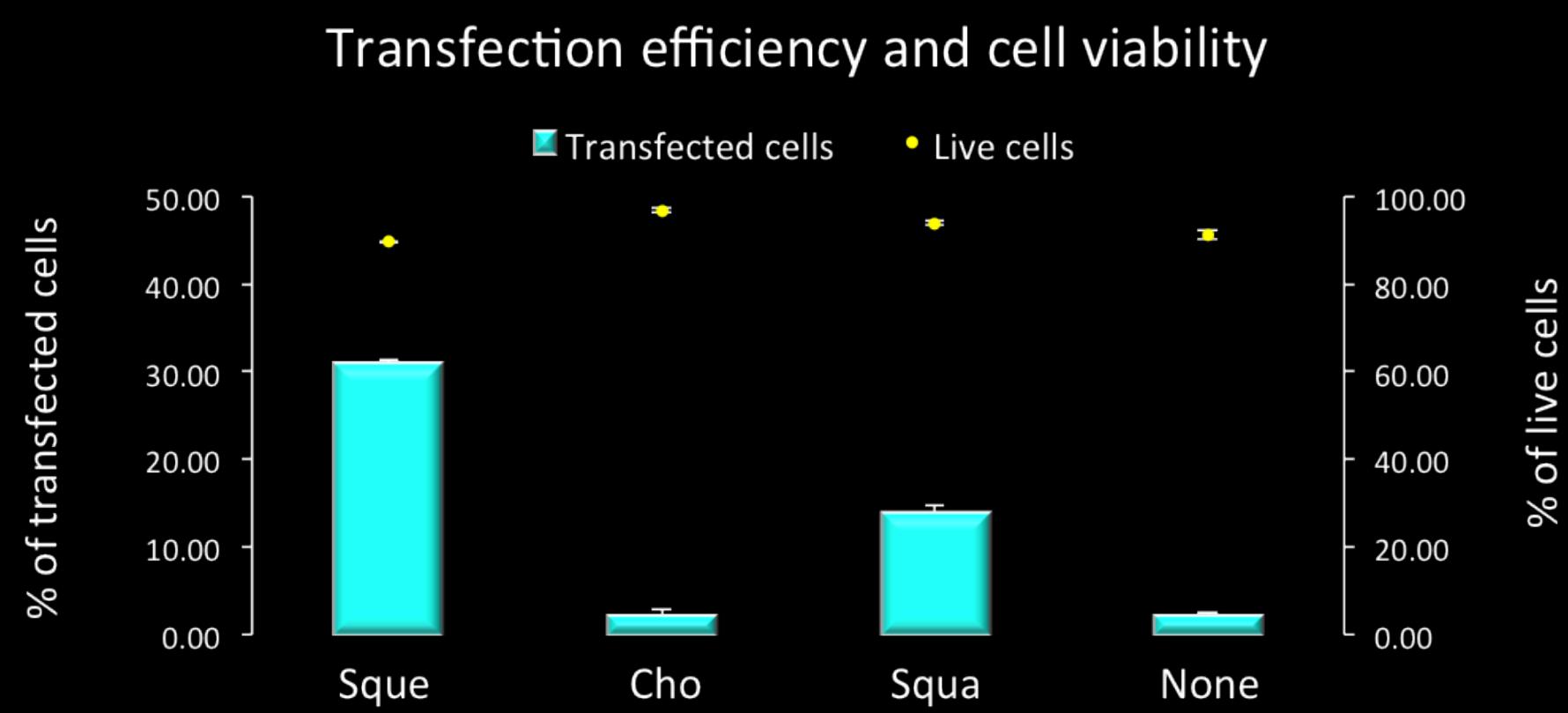


Figure 5. Transfection efficiencies at 72 h post-addition of nioplexes prepared with different helper lipids in ARPE-19 cells. Percentage of transfection (blue bars) and viability (yellow dots) of nioplexes. Each value represents the mean  $\pm$  standard deviation of three measurements.

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## CONCLUSION

Our results showed that the change of the helper lipids in the formulations modified the charge and size of the niosomes and nioplexes. We could observe that such modifications resulted in the alterations of the release and protection of DNA in each formulation and consequently modifying the uptake and transfection efficiencies of the nioplexes in the ARPE-19 cells. It also has to be pointed out that the helper lipids can clearly affect the entry via of the nioplexes into the cells, resulting in low or high transfection efficiencies. This is the case of the formulation prepared with squalene where high transfection efficiency was observed. Such finding can be explained by the entry pathways used by this formulation where macropinocytosis pathway and lysosomal elution played an important role. Overall, these studies bring new insights into the role of helper lipids towards the development of highly efficient niosome formulations as non-viral gene delivery vectors for the treatment of inherited retinal diseases.

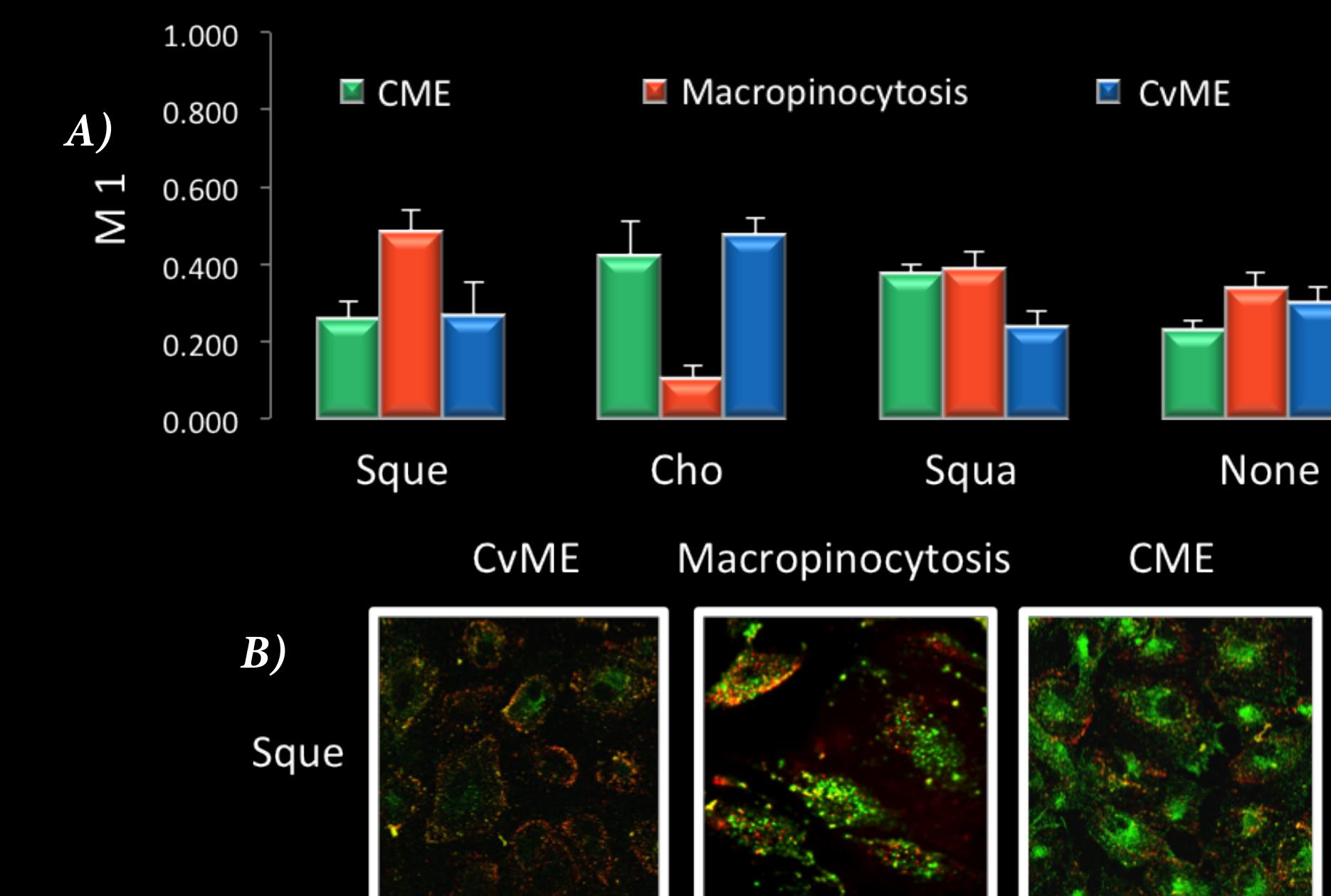


Figure 6. Cell trafficking results in ARPE-19 cells co-incubated with nioplexes based on different helper lipids. A) Colocalization values were given as the fraction of cell-associated nanoparticles colocalizing with fluorescently labeled endocytic structures. M1 is equal to Mander's overlap coefficient between the red signal from the stained DNA and the green fluorescence of the stained entry pathway. Each value represents the mean  $\pm$  standard deviation of three measurements. B) Confocal microscopy merged images showing the cells co-incubated with niosomes based on squalene helper lipid. Each image displayed in the optical section was representative of the cell population. Green coloring shows cells stained with Transferrine Alexa Fluor 488 for CME, Dextran Alexa Fluor 488 for Macropinocytosis and Cholera Toxin B Alexa Fluor 488 for CvME. Red coloring shows the DNA stained with Cy3.

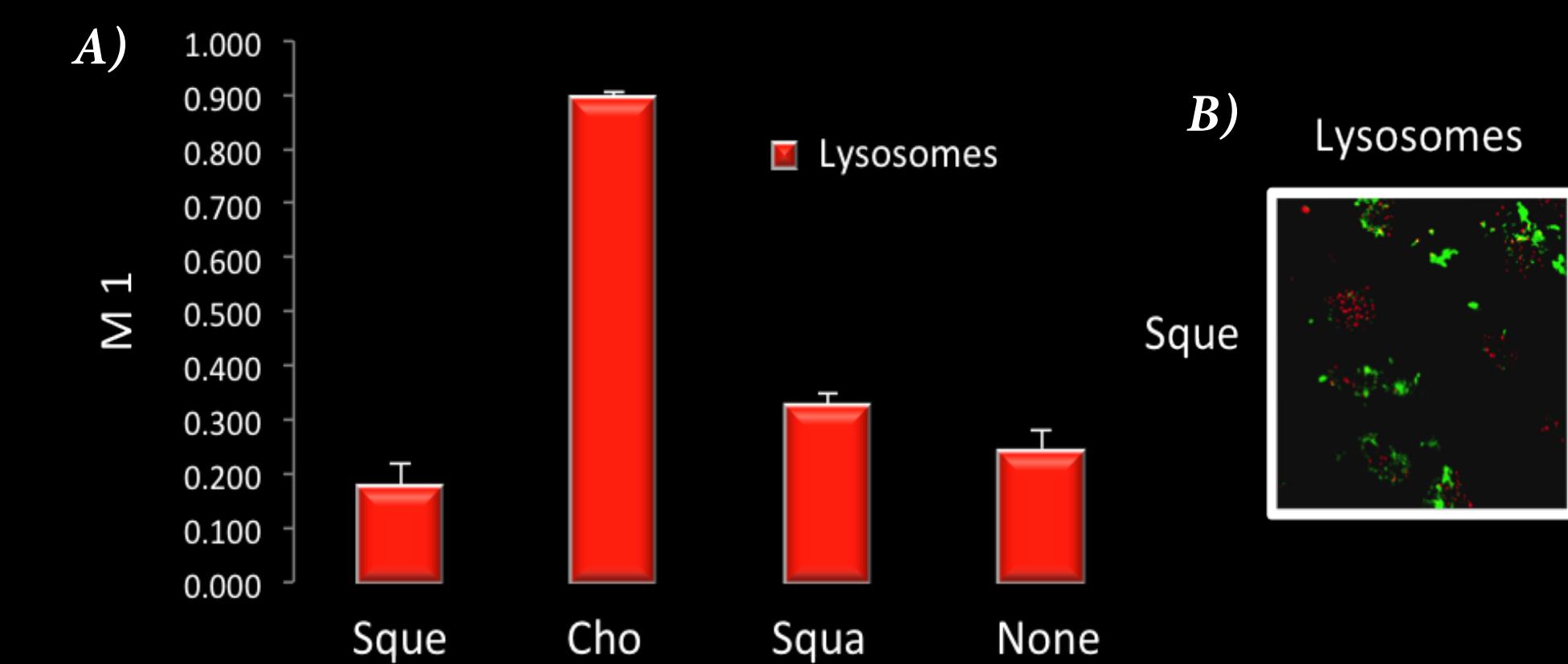


Figure 6. Lysosome colocalization results in ARPE-19 cells co-incubated with nioplexes based on different helper lipids. Niosomes were complex with Cy3 stained DNA. A) Colocalization values were given as the fraction of cell-associated nanoparticles colocalizing with fluorescently labeled lysosomes. M1 is equal to Mander's overlap coefficient between the red signal from the stained DNA and the green fluorescence of the stained lysosomes. Each value represents the mean  $\pm$  standard deviation of three measurements. B) Confocal microscopy merged image showing the cells co-incubated with nioplexes based on squalene helper lipid. This image displayed in the optical section was representative of the cell population. Green coloring shows cells stained with Lysotracker Green and red coloring shows the DNA stained with Cy3.

## ACKNOWLEDGMENT

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