Laboratorio de Biología de Glioblastomas y Terapia Fotodinámica

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FCQ Facultad de Ciencias Químicas

CONICET UNC Universidad Nacional de Córdoba INFIQC

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IMPROVEMENT OF CELL-LIPOSOME INTERACTION FOR PHOTODYNAMIC THERAPY

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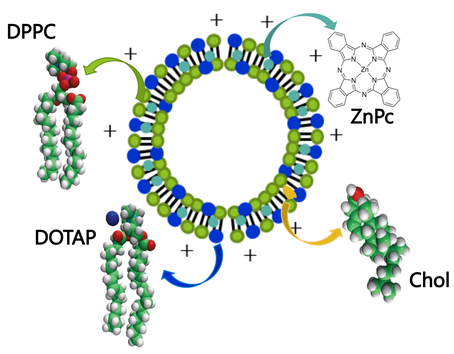
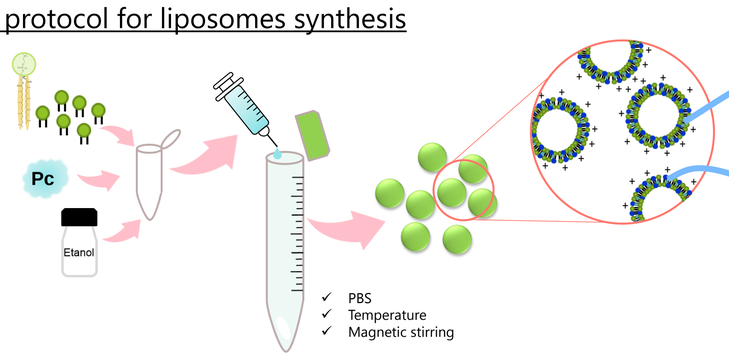
Introduction

Photodynamic therapy (PDT) is a therapeutic alternative for treating several pathologies, from microbial infections to cancer. PDT involves applying a photosensitizer (PS) that interacts with light in an appropriate wavelength, absorbing its energy and going from the basal to the excited singlet state. This excited state can experience intersystem crossing to the excited triplet state (^{3}PS*). ^{3}PS* can generate reactive oxygen species that induce injury and trigger cell death. Phthalocyanines (Pcs) are among the most studied PSs, and although they present interesting photophysical and photochemical properties, the main inconvenience is their hydrophobic nature, which limits their intravenous application. Using a transporter for the hydrophobic PSs becomes an exciting strategy with liposomes (LPs) being widely studied carriers composed of phospholipid bilayers. Positively charged carriers or cationic drugs increases interaction with surfaces that exhibit anionic charges, whether bacteria or eukaryotic cells, and therefore increases its delivery and therapeutic efficiency. The current research endeavors to investigate the benefits of enclosing a hydrophobic photosensitizer in cationic liposomes for application in a PDT protocol for glioblastoma cells, to optimize the protocol.

Methodology



Ethanol injection protocol for liposomes synthesis



Liposomes composition

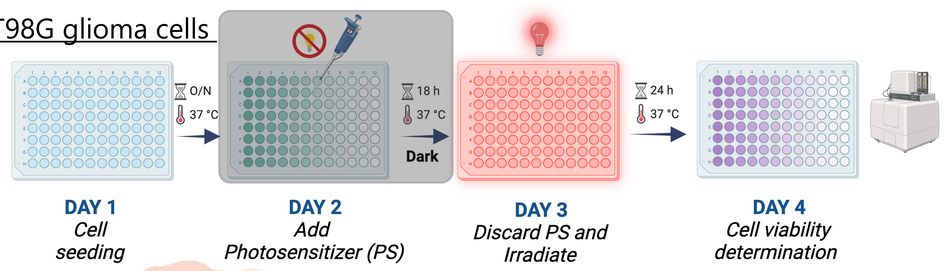
		ZnPc (mol/mol DPPC)	DPPC (mol%)	DOTAP (mol%)	Col (mol%)
Formulation	1a	0	90	10	0
	1b	0,01	90	10	0
	1c	0,05	90	10	0
	2a	0	75	10	15
	2b	0,01	75	10	15
	2c	0,05	75	10	15
	3a	0	75	25	0
	3b	0,01	75	25	0
	3c	0,05	75	25	0
	4a	0	60	25	15
	4b	0,01	60	25	15
	4c	0,05	60	25	15

Liposomes characterization

Size, Zeta potential } Dynamic light scattering

UV-visible light absorption

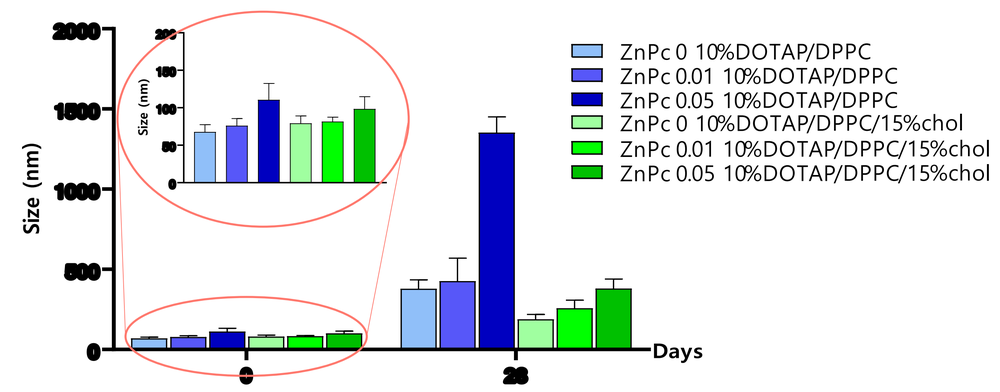
PDT protocol in T98G glioma cells

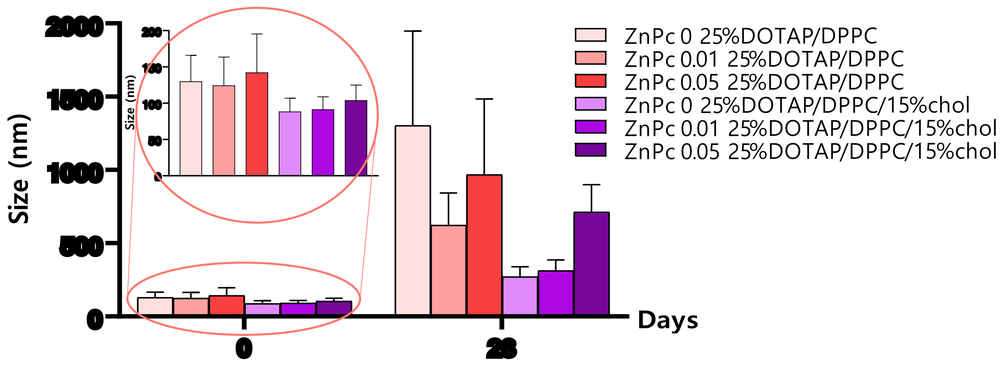


Results

1. Characterization

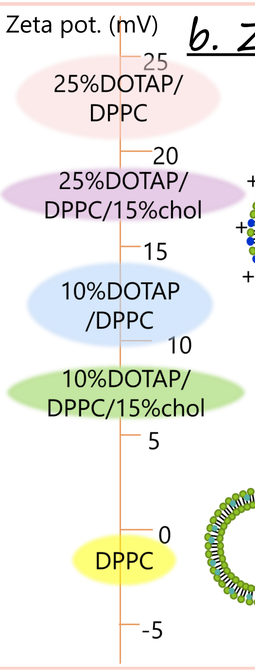
a. Size



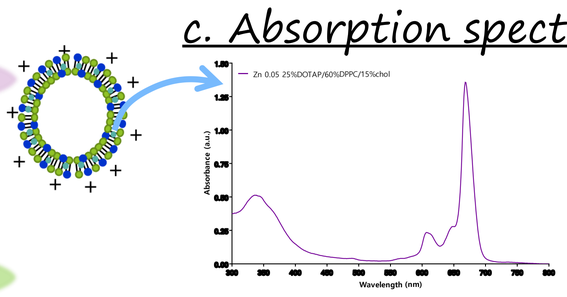


Size vs days, measured by dynamic light scattering. Up: liposomes composed of 10% DOTAP and DPPC with and without cholesterol. Down: liposomes composed of 25% DOTAP and DPPC with and without cholesterol. Insets: size measured at the day of preparation (day 0).
Mean ± SEM for three independent experiments performed in triplicate.

b. Zeta potential

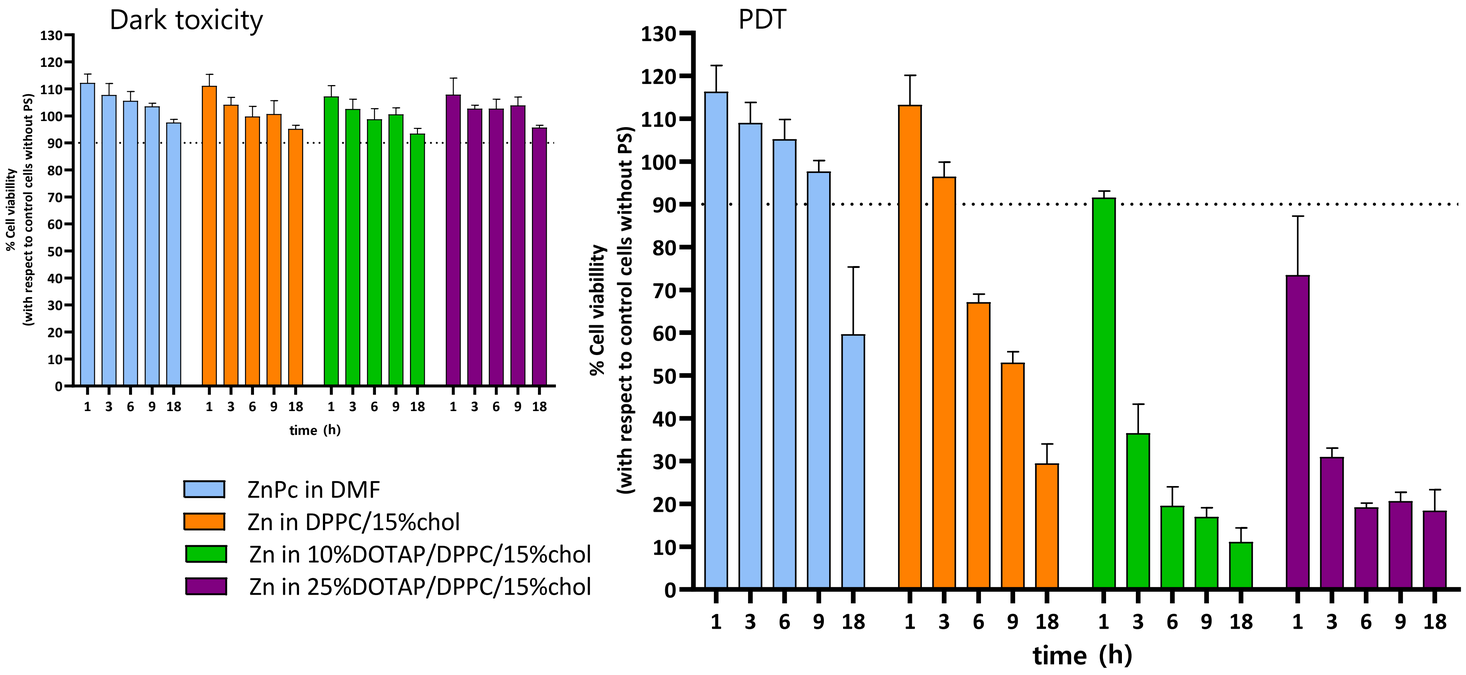


c. Absorption spectra



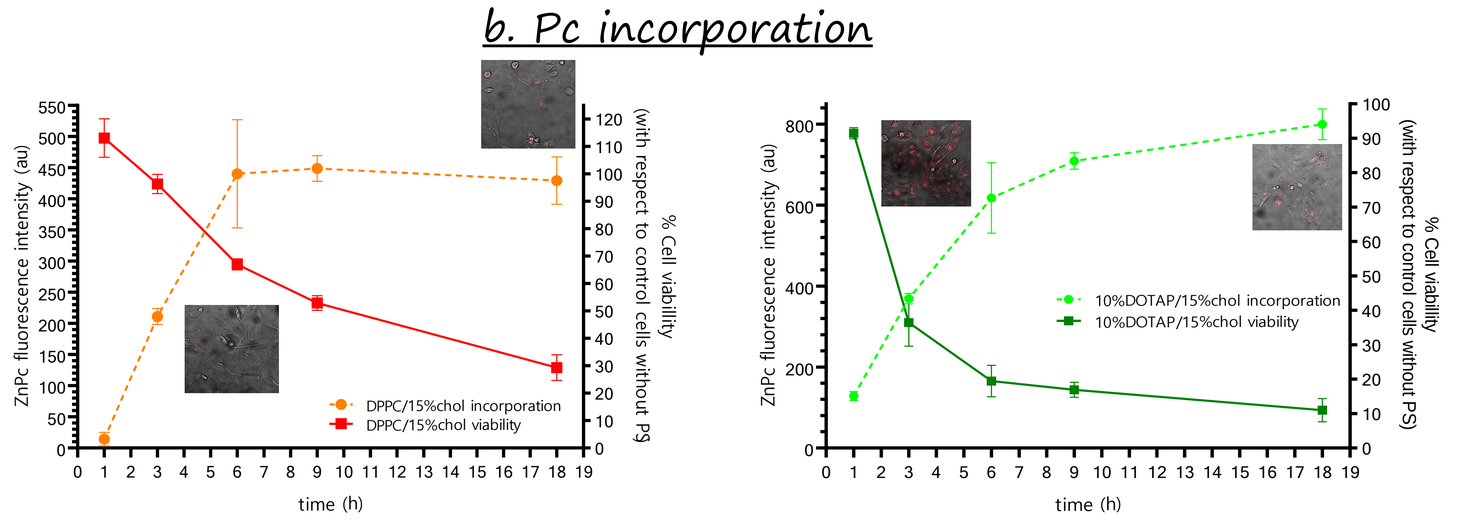
Zeta potential measured for each formulation by dynamic light scattering, performed in triplicate.
UV-visible absorption spectra shown for ZnPc 0.05 in 25%DOTAP/DPPC/15%chol liposome shows that the phthalocyanine maintains its absorption capacity despite being inside the vesicle.

2. In vitro assays



Dark toxicity and photodynamic therapy protocol in T98G cells. Both graphs show the percentage of cell viability vs different times of incubation between cells and photosensitizers. Final ZnPc concentration in each well = 0.05 µM.
Light dose: 10 J/cm^2. Mean ± SEM for three independent experiments performed in triplicate.

b. Pc incorporation



ZnPc fluorescence intensity (as an indicative of ZnPc incorporation in cells) and percentage of cell viability vs different times of incubation. Final ZnPc concentration in each well = 0.05 µM. Mean ± SEM for three independent experiments performed in triplicate.
Fluorescence was determine through direct measurement. Microphotographs were obtained by epifluorescence microscopy, showing red ZnPc fluorescence for each formulation at 3 h and 18 h of incubation. Incorporation was performed as one assay in triplicate, shown as mean ± SD.

Conclusions

- Sizes of all formulations increase over time, but cholesterol "slows down" that size increase.
- DOTAP increases zeta potential, cholesterol decreases it.
- DOTAP containing liposomes reduce incubation times, reaching the same cell viability as DPPC liposomes at shorter times.
- Epifluorescence microscopy evidences differences in ZnPc incorporation depending on incubation time and formulation.
- The results show a direct relationship between the amount of ZnPc incorporated and cell death.

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