A Comparative Study of Nanostructured Photolyase Delivery Systems for Counteracting UV-Induced DNA Photodamage

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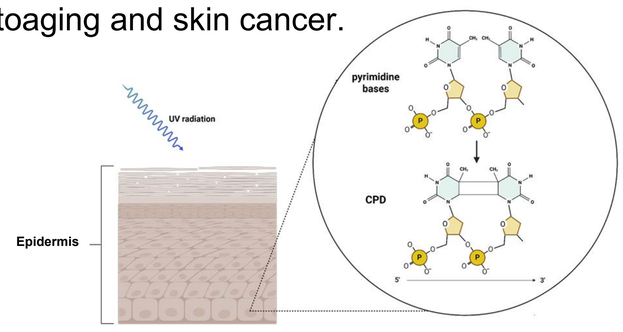
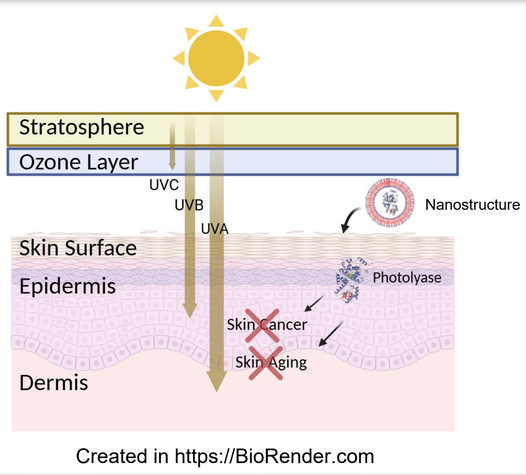
INTRODUCTION AND OBJECTIVE

Ultraviolet radiation (UVR) exposition induces DNA lesions that when accumulated leads to premature photoaging and skin cancer.





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Photolyase is an enzyme capable of directly repairing those DNA lesions, but topical use might be limited by its instability and poor skin penetration.

This study aimed to comparatively evaluate three nanostructured delivery systems, namely polymersomes, liposomes and polymeric nanoparticles, to improve photolyase delivery for active photoprotection.

MATERIALS AND METHODS

Photolyase Production and Purification

Culture in LB broth with carbenicillin, at 250 rpm, 37 °C for 16 hours.

Affinity Chromatography (IMAC)

Photolyase from *Thermus thermophilus was* recombinantly expressed in *E. coli* and then purified by affinity chromatography.

Production of the Nanostructures

Liposomes (LPs)

PC, ethanol

Produced via thin-film hydration method.

Polymersomes (PLs)

1 h at 4 °C
12 h at 25 °C

Produced via self-aggregation using direct dissolution with temperature switch.

Nanocapsules of Polycaprolactone (PNPs)

W1: Phosphate buffer, sucrose, photolyase, PVA (0.5%)

PCL, ethyl acetate 60 °C

W2: Saturated ethyl acetate with 1% of PVA (w/w)

Produced using the double emulsion (water/oil/water) solvent evaporation method.

Nanostructures Characterization

DLS and Zeta Potential

Formulation optimization

***IN VITRO* Release**

24 h at 25 °C

12, 000×g, 30min, 4°C

IN VITRO Cytotoxicity

HaCaT 5×10^4 cells/well

Nanostructures

MTS

IN VITRO Performance

PLs or LPs

UVC 33 J/m²

HaCaT 4×10^5 cells/well

UVB 1.035 J/cm²

ELISA CPD

HaCaT 4×10^5 cells/well

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RESULTS

NANOPARTICLES PRODUCTION (DoE)

Table 1: Experimental design 3^2 applied to the preparation of photolyase-loaded polymersomes. In red: optimal condition.

Photolyase (mg/mL)	Sucrose (mM)	hD (nm)	EE (%)	PDI
1	0	162 ± 9	2 ± 0.2	0.346 ± 0.05
1	50	150 ± 20	13 ± 0.3	0.386 ± 0.04
1	100	115 ± 11	16 ± 2	0.423 ± 0.11
3	0	165 ± 18	2 ± 0.6	0.330 ± 0.09
3	50	181 ± 18	21 ± 2	0.299 ± 0.07
3	100	126 ± 12	23 ± 11	0.551 ± 0.02
5	0	136 ± 20	3 ± 2	0.413 ± 0.21
5	50	143 ± 15	6 ± 4	0.408 ± 0.02
5	100	126 ± 11	6 ± 2	0.573 ± 0.04

Sucrose (⇑) was the main effect, influencig the hD (⇓)and EE (⇑).

Table 2: Experimental design 2^2 applied to the preparation of photolyase-loaded liposomes. In red: optimal condition.

Photolyase (mg/mL)	Phosphatidylcholine (mg/mL)	hD (nm)	EE (%)	PDI
1.5	5	99 ± 30	23 ± 8	0.291 ± 0.02
1.5	20	89 ± 6	10 ± 3	0.514 ± 0.11
3.0	5	66 ± 11	23 ± 8	0.250 ± 0.01
3.0	20	60 ± 14	9 ± 3	0.392 ± 0.05

Photolyase (⇑) was the main effect, influencig the hD (⇓) and PC (⇓) influenced EE (⇑).

Table 3: Experimental design 2^3 applied to the preparation of photolyase-loaded polymeric nanoparticle. In red: optimal condition.

Photolyase (mg/mL)	PCL (mass)(mg)	Emulsion condition (rpm/min)	hD (nm)	EE (%)
1.5	100	3000/2	678 ± 25	94 ± 0.1
1.5	100	7000/6	86 ± 4	88 ± 1.6
1.5	1000	3000/2	1230 ± 26	89 ± 0.8
1.5	1000	7000/6	184 ± 8	85 ± 1.2
3.0	100	3000/2	642 ± 29	96 ± 0.02
3.0	100	7000/6	99 ± 3	93 ± 0.04
3.0	1000	3000/2	1310 ± 28	96 ± 0.4
3.0	1000	7000/6	179 ± 7	89 ± 0.04

Emulsion conditions was the main effect, with rotation (⇑) and time (⇑) influencig the hD (⇓).

Values represent mean ± SD. All error bars correspond to standard deviation between 3 replicates. For statistical significant diferences $p < 0.05$.

IN VITRO RELEASE

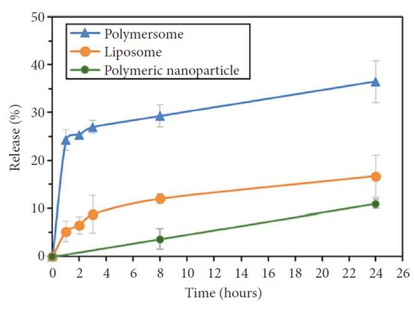

Figure 1: Photolyase release (PBS buffer, pH 7.4) over time from polymersomes (PLs), liposomes (LPs) and polymeric nanoparticles (PNPs).

NANOPARTICLES CYTOTOXICITY

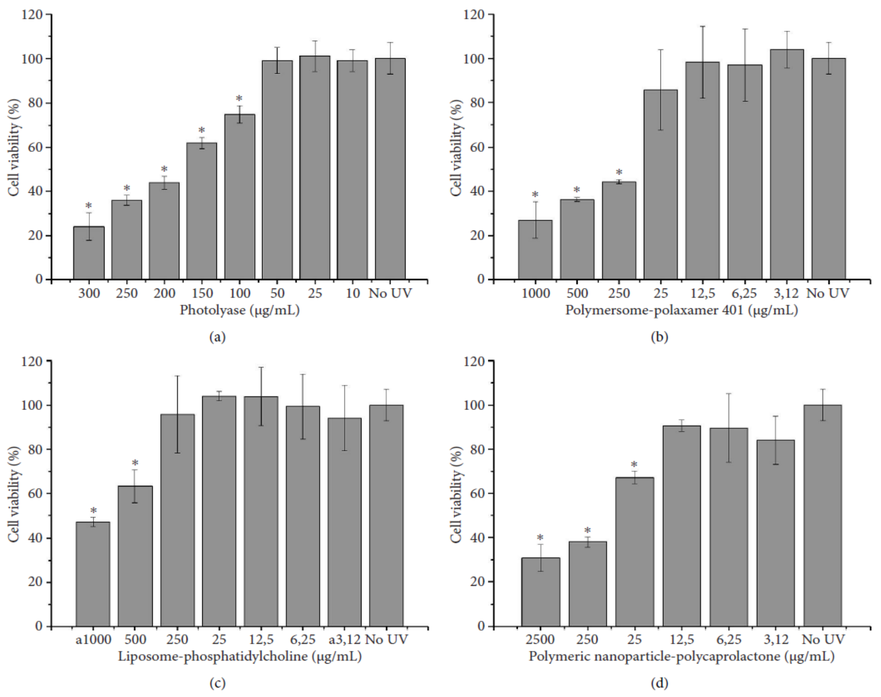

Figure 2: Cellular viability of HaCaT cells after 24h of exposure to (a) free photolyase, (b) polymersome, (c) liposome and (d) polymeric nanoparticle at diferente concentrations.

IN VITRO PERFORMANCE

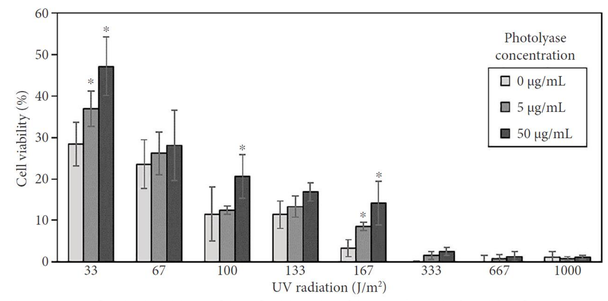

Figure 3: Cell viability of HaCaT cells (4 ×10^4 cells/well) after exposure to UVC irradiation at diferente intensities in the presence of different concentrations of pure photolyase.

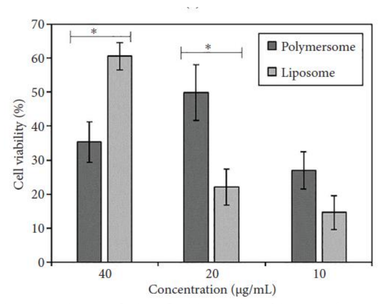

Figure 4: Cell viability of HaCaT cells (4 ×10^4 cells/well) after exposure to UVC irradiation at 33J/m² and in the presence of photolyase-loaded polymersomes or iposomes at different concentrations.

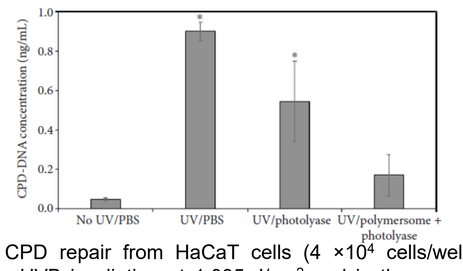

Figure 5: CPD repair from HaCaT cells (4 ×10^4 cells/well) after exposure to UVB irradiation at 1.035 J/cm² and in the presence of photolyase-loaded polymersomes (unpurified).

CONCLUSIONS

With these results we demonstrated that nanostructured carriers were able to improve the performance of photolyase *in vitro*, with polymersomes and liposomes emerging as the most promising systems. These findings support their potential application in photoprotection strategies and provide a foundation for future dermatological products.

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