Antimicrobial Photodynamic Therapy Using Encapsulated Protoporphyrin IX for the Treatment of Bacterial Pathogens

Natalia Izquierdo Brusau[1], Enrique Gamez[1,2,3], Teresa Alejo[2,3], Gracia Mendoza[1], Manuel Arruebo[1,2,3]

[1]Aragon Health Research Institute (IIS Aragon), 50009-Zaragoza, Spain.
[2]Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza 50009, Spain.
[3]Department of Chemical Engineering. University of Zaragoza, Campus Río Ebro-Edificio I+D, C/ Poeta Mariano Esquillor S/N, 50018 Zaragoza, Spain.

nizquierdo@iisaragon.es

In Antimicrobial Photodynamic Therapy (APDT), photosensitizers induce light-driven local photochemical reactions that generate reactive oxygen species (ROS) responsible for pathogen inactivation [1]. APDT offers precise spatio-temporal control and clinical utility against a wide range of microbial infections. However, challenges include limited light tissue penetration and potential photosensitizer photobleaching [2] [3].To address these issues, photosensitizer encapsulation within nanoparticles enhances antimicrobial efficacy. In this study, we investigate the photodynamic antimicrobial efficacy of protoporphyrin IX, a clinically approved photosensitizer, both in its free form and loaded into PLGA nanoparticles, against Staphylococcus aureus. We also assess the reduction in its cytotoxicity when encapsulated compared to equivalent doses of its free form in mammalian cell cultures.

For the selection of the photosensitizer, DHR123 probe quantified ROS production upon photodynamic activation of ICG (indocyanine green) and PpIX (Protoporphyrin IX). For ICG ROS analysis, samples were measured before and after 3-minute irradiation with an 808 nm diode laser (1 or 0,5 W/cm²). For PpIX ROS evaluation, PpIX in DMSO mixed with MilliQ water was irradiated with a 532 nm diode laser at 0,5 W/cm². Control measurements ensured ROS generation only when irradiating light, and control experiments ruled out temperature-induced ROS. Photosensitizer photobleaching was examined by recording UV-Vis spectra before and after 5 minutes of irradiation (808 nm for ICG, 532 nm for PpIX) at 0,5 W/cm² irradiance. PpIX-NPs were synthesized using the emulsification-solvent evaporation method, with protoporphyrin IX in the core. Transmission electron microscopy (TEM) assessed particle morphology and size. Nanoparticle tracking analysis (NTA) determined NPs size and distribution and zeta potential of the nanoparticles was measured by Dynamic Light Scattering (DLS). UV-Vis spectrophotometry measured drug loading by quantifying the encapsulated photosensitizer in the NPs. Release from PpIX-NPs was evaluated in PBS with 2% Tween® 20 (v/v) and quantified by UV-Vis spectrophotometry. Free PpIX antibacterial activity against S. aureus was tested at concentrations (0,5-10 ppm) in 2% DMSO. Positive controls included untreated S. aureus and 2% DMSO. After a 1-hour incubation, samples were exposed to a 532 nm laser (0,5 W/cm2, 5 minutes). Viable bacteria were quantified via serial dilution. To assess the antimicrobial efficacy of PpIX-NPs, the previous doses of the free photosensitizer were tested considering the drug loading (0,5-10 ppm). Positive controls included untreated bacteria and samples with empty (PpIX-free) nanoparticles. NPs cytotoxicity in fibroblasts was determined using the Blue Cell Viability Assay after 24 hours of incubation with different concentrations (0,5 - 2 ppm) of PpIX released from PpIX-NPs or the free drug at the same concentrations.

ICG and PpIX were assessed for antimicrobial photodynamic therapy based on ROS production and photobleaching. PpIX displayed superior ROS production, and photostability, making it the preferred choice for further antimicrobial studies. Then, PpIX was encapsulated obtaining PpIX-NPs. They had a spherical morphology, with a slightly larger size (33,6 ± 9 nm) than empty NPs. Empty and PpIX loaded NPs showed electrokinetic potentials of -11,9 ± 0.6 and -12,2 ± 1 mV, respectively at neutral pH. PpIX release reached 54 wt.% in 1 hour for a potential rapid application. Encapsulation efficiency was 13,7 ± 1,7 wt.%, and PpIX loading was 0,14 ± 0,09 wt.%.

Protoporphyrin IX-loaded PLGA nanoparticles demonstrate high aqueous solubility, photostability, and retained antimicrobial activity upon light irradiation when compared to equivalent doses of the free photosensitizer.

References

[1] Leonardo do Prado-Silva, Guilherme T.P. Brancini, Gilberto Ú.L. Braga, Xinyu Liao, Tian Ding and Anderson S. Sant'Ana, Food Control, 132 (2022).Authors, Journal, Issue (Year) page (Arial Narrow 11)

[2] Eun Hye Kim, Sangwoo Park, Yun Kyu Kim, Minwoo Moon, Jeongwon Park, Kyung Jin Lee, Seongsoo Lee and Young-Pil Kim, Science Advances, 37 (2020).

[3] Qingyan Jia, Qing Song and Peng Li and Wei Huang, Advance Helathcare Materials, 14 (2019).

Figures

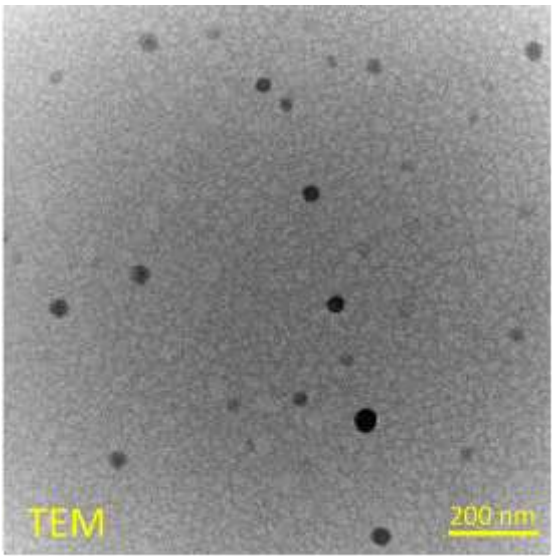

Figure 1. TEM image of PpIX-NPs.