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FULL ARTICLE

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Antimicrobial activity of amphiphilic nanomicelles loaded with curcumin against *Pseudomonas aeruginosa* alone and activated by blue laser light

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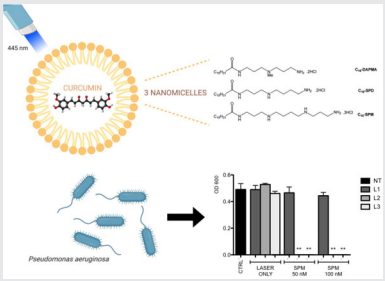
Abstract



The aim of this work was to assess the antimicrobial efficacy on *Pseudomonas aeruginosa* of nanomicelles loaded with curcumin (CUR) alone and activated by blue laser light in an antimicrobial photodynamic therapy (APDT) approach. First, free CUR in liquid suspension and loaded in three amphiphilic nanomicelles (CUR-DAPMA, CUR-SPD and CUR-SPM) were tested both on bacteria and keratinocytes. While free CUR exerted limited efficacy showing moderate cytotoxicity, a strong inhibition of bacterial growth was obtained using all three nanosystems without toxicity on eukaryotic cells. CUR-SPM emerged as the most effective, and was therefore employed in APDT experiments. Among the three sublethal blue laser (λ 445 nm) protocols tested, the ones characterized by a fluence of 18 and 30 J/cm^2 further decreased the antimicrobial concentration to 50 nM. The combination of blue laser APDT with CUR-SPM nanomicelles results in an effective synergistic activity that represents a promising novel therapeutic approach on resistant species.

KEYWORDS

antimicrobial, blue laser, curcumin, nanomicelles, photodynamic therapy

Katia Rupel and Luisa Zupin contributed equally to this study.

The graphical abstract and Figure 1 were created with Biorender.com.

1 | INTRODUCTION

Antibiotic resistance poses an important challenge in modern medicine worldwide. Indeed, this phenomenon has increased in recent years due to inappropriate overuse/misuse of common antibiotics and to the scarce development of novel drugs. This, in turn, reflected in the expansion of a broad range of resistant pathogens, including *Pseudomonas aeruginosa* [1, 2].

Among the *pseudomonas* genera, *P. aeruginosa*, ubiquitous in soil and aqueous environments, is one of the Gram-negative bacteria most frequently found in human infections [3]. It is considered an opportunistic pathogen, as it expressly infects immunocompromised subjects, damaged mucosae or proliferates in cases of antibiotic suppression of normal microbioma. Thus, the most common related pathologies are nosocomial urinary tract infections, skin burn wound superinfections, and airway infections in individuals afflicted by cystic fibrosis [4]. Moreover, in oncological patients *P. aeruginosa* activity was correlated with worsening of infections in both adults and pediatric subjects [5] suffering from oral mucositis [6] and with bacteremia [7]. Additionally, *P. aeruginosa* capacity of biofilm formation often leads to chronic infections of medical devices that, being 10- to 1000-fold more resistant than planktonic bacteria [2], are extremely difficult to eradicate via conventional antibacterial therapies. Therefore, alternative antimicrobial strategies are urgently needed.

Photodynamic therapy (PDT) is a treatment that requires a photosensitizing agent (PS), a low intensity light source (eg, laser, intense pulsed laser, light-emitting diodes) of suitable wavelength to match the PS absorption peak, and molecular oxygen [8]. Antimicrobial PDT (APDT) is emerging as a novel therapeutic strategy presenting several advantages, including: (a) potential inactivation of all known classes of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, protozoa and viruses; (b) activity against pathogen antibiotic-resistant strains [9]; (c) absence of treatment-related resistance phenomena; and (d) treatment/efficacy achieved in very short times (seconds) compared to the time scale of conventional antibiotics (hours or days), resulting in the extra-potential advantage against fast-spreading infections. In addition, thanks to the broad-spectrum activity, APDT can be performed before the identification of the infectious species [10]. Curcumin ([CUR] diferuloylmethane, $C_{21}H_{20}O_6$), is a polyphenolic compound isolated by *Curcuma longa*. It is a hydrophobic yellow powder—soluble in ethanol, dimethylsulfoxide (DMSO), and acetone—which has been used throughout centuries as a food pigment/spice, and as medication, for its antiinflammatory, antitumoral, and antimicrobial effects [11].

Interestingly CUR, showing an absorption peak within the range 300 to 500 nm with maximum at 430 nm, can be exploited as an effective PS in APDT. When excited by light at such wavelengths, CUR produces singlet oxygen ($^1O_2$) which, in turn, exerts phototoxic effects in micromolar amounts. Unfortunately, the successful adoption of CUR in this or other therapeutic applications is drastically limited by its hydrophobicity, which strongly correlates with drug poor bioavailability and, ultimately, low final biological effects [12]. However, this limitation could be overcome by adopting an appropriate drug delivery system and, in particular, by CUR encapsulation within amphiphilic nanostructures. Indeed, nanomicelles are colloidal structures (5-200 nm in size) generated upon self-assembly of amphiphilic monomers/molecules at a critical micellar concentration. While the molecular hydrophilic portion provides nanomicelles with water solubility, their hydrophobic core allows for the eventual entrapment of poorly water-soluble compounds [13].

APDT is an emerging approach that still needs validation and optimization, especially considering its great potential against multi-resistant species. Although several combinations of light sources and PSs have been described in literature, there are no data about the antimicrobial effect of free CUR activated by blue laser light on *P. aeruginosa*, likely because the hydrophobic characteristics of CUR limit its clinical applicability.

In this research work, to address this issue and overcome the poor bioavailability of free CUR, the antimicrobial activity of three different types of amphiphilic nanomicelles loaded with CUR were evaluated, concurrently testing their toxicity on eukaryotic cells. After the selection of the most effective nanosystem, APDT experiments were performed using sublethal blue laser irradiation protocols to investigate any synergism of the combined treatment with CUR-loaded nanomicelles in their relevant therapeutic effectiveness. The study design and the procedures performed are represented in Figure 1.

2 | MATERIALS AND METHODS

2.1 | Nanomicelles

The three amphiphilic molecules selected in this study displayed a common C_{16}-long hydrocarbon chain and different amines as the hydrophilic portions: N,N-di-(3-aminopropyl)-N-methylamine (DAPMA), spermidine

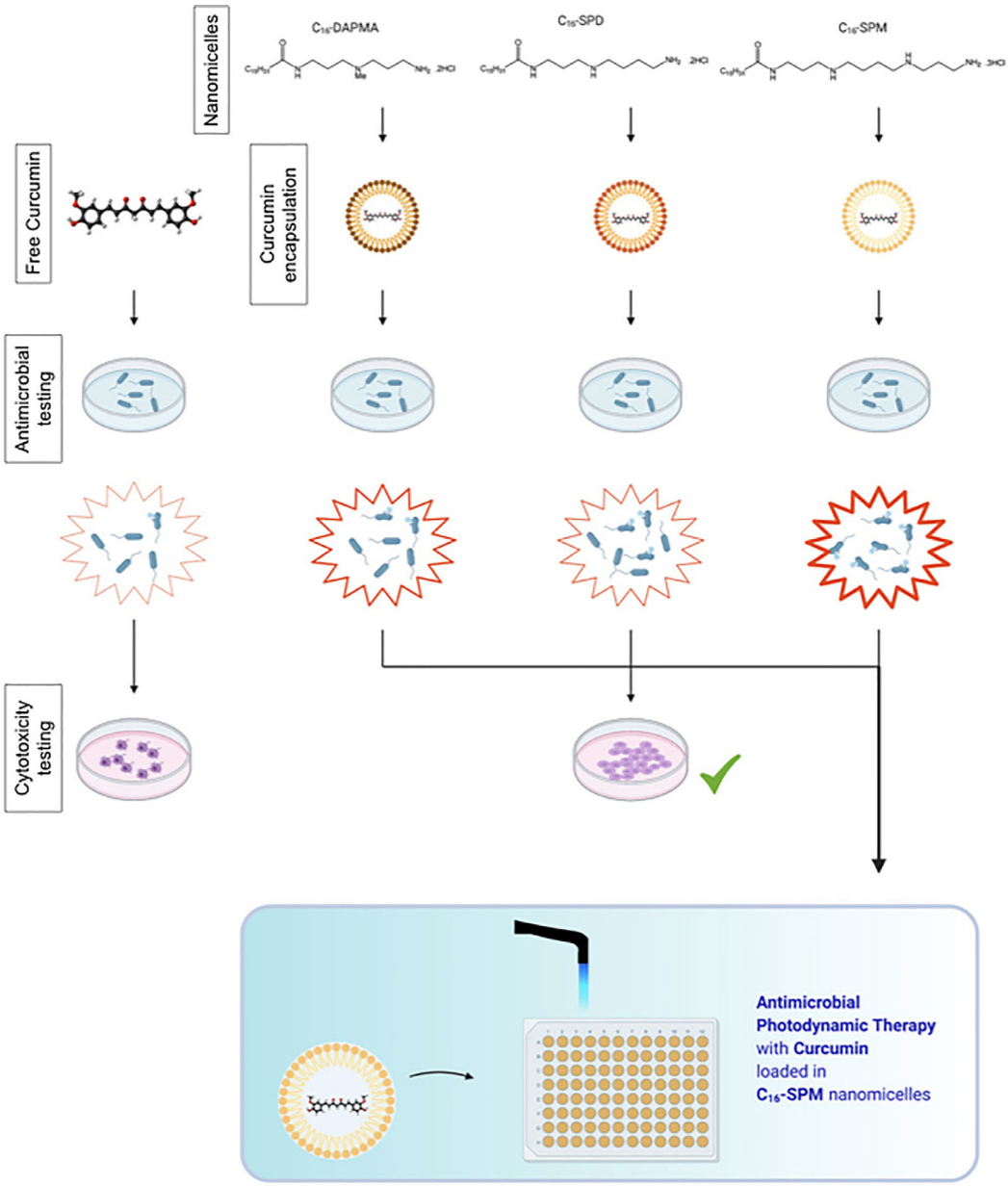


FIGURE 1 Graphical representation of the study design and the procedures performed

TABLE 1 List of irradiation protocols

Protocol name	Output power (W)	Irradiation time (sec)	Irradiance at sample (W/cm^2)	Fluence (J/cm^2)
L1	0.2	60	0.1	6
L2	0.2	180	0.1	18
L3	0.2	300	0.1	30

(SPD), and spermine (SPM). The preparation and characterization of the corresponding self-assembled nanomicelles per se and loaded with CUR are reported in detail in two previous works [14, 15].

2.2 | Bacteria

P. aeruginosa strain ATCC 27853 was purchased from LGC Antimicrobial (LGC, Teddington, UK) and grown in

lysogeny broth (LB broth, Sigma–Aldrich, Saint Louis, Missouri, USA) overnight at 37°C under vigorous agitation the day before the experiments.

2.3 | Human spontaneously immortalized keratinocytes cell line

Human spontaneously immortalized keratinocytes (HaCaT) were maintained in DMEM supplemented with 10% (vol/vol) fetal bovine serum, 100 U/mL penicillin/streptomycin, 2 mM glutamine (Euroclone, Milan, Italy). Cells were seeded at passages 2 to 8 (10 000 cells/well in 96 multi-well plates) the day before testing.

2.4 | Light source

A class IV diode laser (K-Laser Blue series, K-laser d.o.o., Sežana, Slovenia) has been used to perform the APDT experiments, using the protocols described in Table 1. The laser is associated with a programmable scanner conveniently designed to delivery uniform irradiation to different multiwell plates, providing blue (445 nm) wavelength laser light in different combinations of power and energy densities. Plate covers were removed during irradiation and the emission tip was held perpendicular above the cells. Laser light was provided in continuous wave (CW), and the output power was adapted in order to provide the desired irradiance at sample level, which was measured using an optical power meter (LaserPoint Plus+, Milan, Italy). The spot size completely covered each well of the bacterial culture (0.32 cm^2). Details of each protocol are described in Table 1.

2.5 | Preparation of CUR nanomicelles

CUR (0.32 mg) (C1386, Sigma-Aldrich, Munich, Germany) was dissolved in 1.0 mL of mixed solvent (chloroform: methanol = 3:2, vol/vol) and then mixed with 3 mg of nanomicelles in 3.0 mL of mixed solvent. The solvent was then removed with a dry heated nitrogen evaporator (VLM GmbH, Bielefeld, Germany) to form a dry film, which was hydrated adding Hepes buffer (10 mM, pH 7.4) at 60°C for 30 minutes under stirring. Non-encapsulated CUR was separated by filtration through a 0.45 μm polycarbonate membrane (Millipore Co., USA) followed by 9-hour dialysis (changing water every hour) using a membrane with molecular weight cutoff of 2000 Da. The product in the dialysis tube was subsequently lyophilized (Eppendorf, Hamburg, Germany). The amount of CUR encapsulated in the nanomicelles was measured using a spectrophotometer (Ultrospec 3100pro, Amersham Bioscience, GE Healthcare, Buckinghamshire, UK) at a 420 nm wavelength. Blank nanomicelles were prepared using the same procedures without CUR addition. Drug-loading content was calculated using a standard curve obtained at specific CUR concentrations dissolved into the same mixed solvent.

2.6 | Assessment of antibacterial activity of CUR encapsulated nanomicelles

P. aeruginosa bacteria from an overnight culture were diluted to 0.5 McFarland's standard corresponding to 10^8 CFU/mL and then diluted 1:1000 in fresh LB broth growth medium. A volume of 100 μL of this bacterial suspension were then inoculated in 96-well plates. Planktonic bacterial suspensions were treated with free CUR at different concentrations (50, 20, 10 μM), and with three types of CUR nanomicelles (DAPMA, SPD, SPM) at different concentrations (1 μM, 500 nM, 250 nM, 100 nM, 50 nM). The optical density of the bacterial culture was measured at 600 nm using a spectrophotometer (OD_{600}) immediately after inoculation and after 24 hours to evaluate bacterial growth. The antimicrobial effect of free and encapsulated CUR was compared with non treated bacteria (CTRL), bacteria grown in growth medium with the addition of mixed solvent (DMSO) and bacteria treated with empty nanomicelles (blank). All experiments were performed in triplicates.

2.7 | Toxicity of CUR nanomicelles on eukaryotic cells

To assess the safety of free CUR (50 μM, 20 μM, 10 μM, 1 μM, 500 nM, 250 nM, 100 nM, 50 nM) and CUR-loaded nanomicelles (1 μM, 500 nM, 250 nM, 100 nM, 50 nM) on eukaryotic cells, the 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay (Trevigen, Gaithersburg, Maryland, USA) was performed on cells (10 000 cells/well as above) seeded in 96 multi-well plates according to manufacturer's instructions. Cell viability was evaluated 24 hours after treatment and compared with non treated cells (CTRL), and cells grown in growth medium with the addition of mixed solvent (DMSO). All experiments were performed in triplicates.

2.8 | Antimicrobial photodynamic therapy

CUR-loaded SPM nanomicelles showed the most promising results in terms of antibacterial activity and

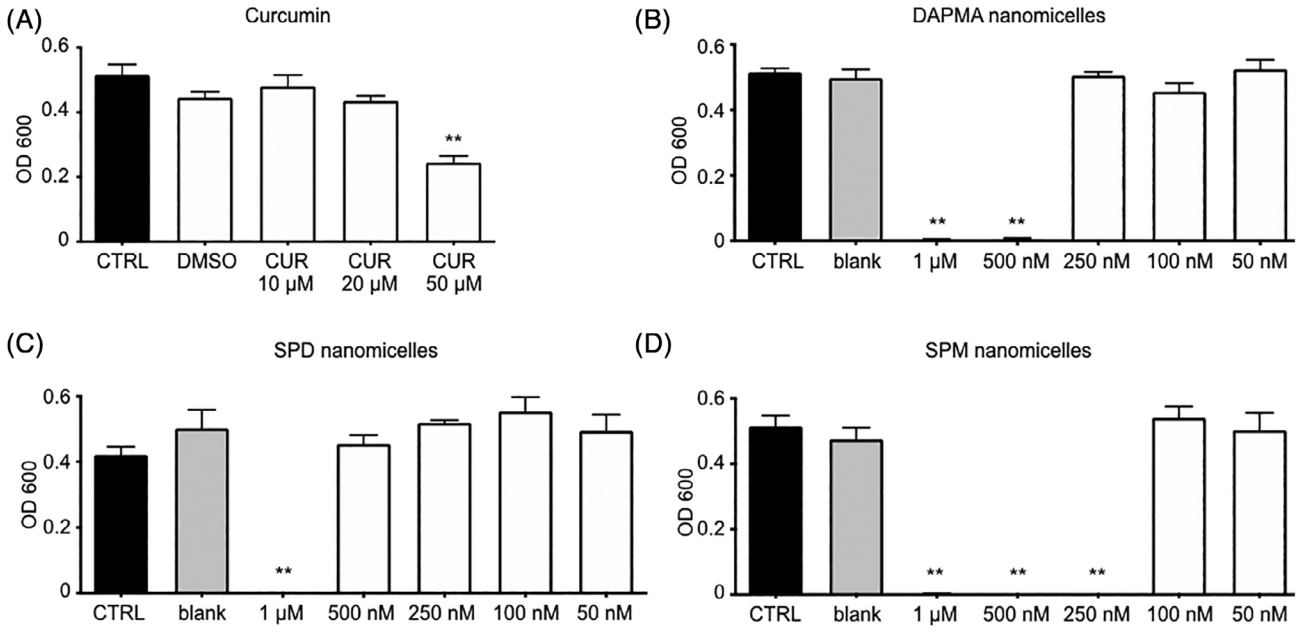


FIGURE 2 Effect of free curcumin and curcumin embedded in amphiphilic nanomicelles on planktonic *Pseudomonas aeruginosa*. A, Treatment of bacteria with free curcumin at 10, 20, and 50 μM. B, Treatment of bacteria with DAPMA nanomicelles loaded with curcumin at 1 μM, 500 nM, 250 nM, 100 nM, and 50 nM. C, Treatment of bacteria with SPD nanomicelles loaded with curcumin at 1 μM, 500 nM, 250 nM, 100 nM, and 50 nM. D, Treatment of bacteria with SPM nanomicelles loaded with curcumin at 1 μM, 500 nM, 250 nM, 100 nM, and 50 nM. Data are shown as mean ± SD of optical density (OD) at 600 nm (indicative of the bacterial growth). Blank, nanomicelles without curcumin; CTRL, control group; CUR, curcumin; DMSO, solvent only; OD, optical density. ** Mann–Whitney *U* test $P < .001$ (treatment vs control)

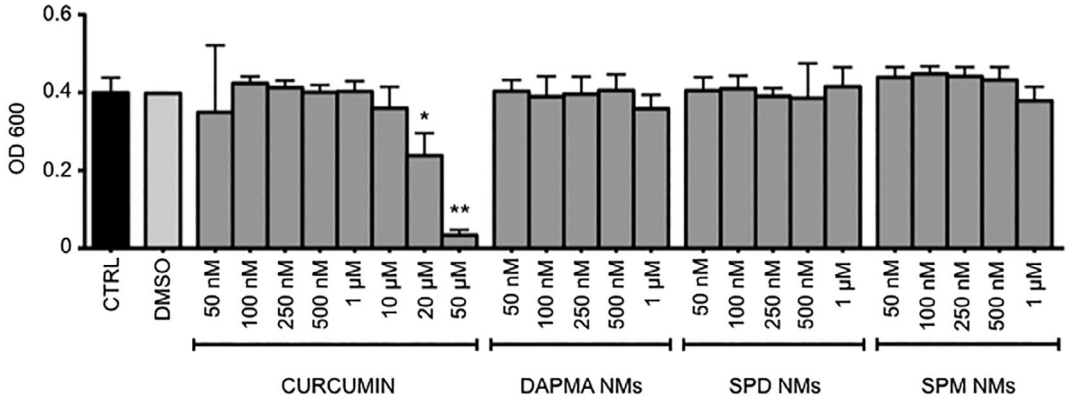


FIGURE 3 Cytotoxicity of curcumin-loaded nanomicelles at antimicrobial concentrations on HaCat (Human spontaneously immortalized keratinocytes) cell line by employing the MTT test. The cells were treated with DMSO (solvent only), free curcumin (50 nM, 100 nM, 250 nM, 500 nM, 1 μM, 10 μM, 20 μM and 50 μM), DAPMA nanomicelles (1 μM, 500, 250 nM, 100 nM and 50 nM) SPD nanomicelles (1 μM, 500, 250 nM, 100 nM and 50 nM), SPM nanomicelles (1 μM, 500, 250 nM, 100 nM and 50 nM). Data are shown as mean ± SD optical density (OD) at 600 nm (indicative of the viability of the cells) of three independent experiments. CTRL, control, CUR, curcumin; DMSO, solvent only; NMs, nanomicelles. * Mann–Whitney *U* test $P < .05$ (treatment vs control). ** Mann–Whitney *U* test $P < .001$ (treatment vs control

were therefore selected for the APDT experiments. *P. aeruginosa* bacteria from an overnight culture were diluted to 0.5 McFarland's standard corresponding to 10^8 CFU/mL and then diluted 1:1000 in fresh LB broth growth medium. A volume of 100 μL of this bacterial suspension were then inoculated in 96-well plates and treated as follows. Concentrations of 50 and 100 nM CUR-SPM were employed, alongside with increasing concentrations of free CUR (5 μM, 10 μM, 20 μM, 50 μM, 100 μM, 200 μM). For every condition, the wells

were irradiated with three sublethal blue laser protocols (L1, L2 and L3 as described in Table 1), based on our previous experience with antimicrobial blue laser irradiation [16]. Bacterial growth after 24 hours was evaluated by measuring the OD_{600} and compared with nontreated bacteria (CTRL). All experiments were performed in biological triplicates.

2.9 | Statistical analysis

Statistical analysis was performed using Graphpad Prism version 7.0a (GraphPad Software, La Jolla, California). Bacterial growth for every treatment protocol was compared to nontreated bacteria (CTRL) by using Mann–Whitney' *U* test. The safety of the nanomicelles in eukaryotic cell cultures was assessed by using Kruskal Wallis test followed by Dunnett's post-hoc test, considering the not treated wells as controls (CTRL).

3 | RESULTS

3.1 | CUR-loaded SPM nanomicelles are endowed with the highest antibacterial activity against *P. aeruginosa*

In order to assess any antimicrobial efficacy of CUR-loaded nanomicelles, a reference strain of *P. aeruginosa* was exposed to the nanocarriers under different experimental conditions. The results of *P. aeruginosa* growth inhibition after 24 hours of incubation with free CUR and CUR-loaded nanomicelles at different concentrations are shown in Figure 2. As seen from Figure 2A, CUR alone was not able to completely inhibit the bacterial growth in the suspension at any of the concentrations employed; specifically, CUR administered at its highest concentration (50 μM) resulted in a reduction of bacterial growth of approximately 50%. On the contrary, in the case of nanomicelle-assisted delivery, CUR-DAPMA nanomicelles were able to inhibit *P. aeruginosa* at 500 nM, while CUR-SPD nanomicelles completely blocked *P. aeruginosa* already at a concentration of 1 μM (Figure 2B). Finally, CUR-SPM nanomicelles were found to be the most effective, exerting their antimicrobial effects already at 250 nM (Figure 2C). Importantly, CUR-free nanomicelles did not inhibit bacterial growth per se (Figure 2D), thus confirming that the antimicrobial effect was provided by the natural drug which exerted its toxicity only once penetrated into microbial cells.

3.2 | CUR-DAPMA, CUR-SPD, and CUR-SPM nanomicelles are devoid of toxicity on human keratinocytes

The cytotoxicity of increasing concentrations of CUR and all CUR-loaded nanomicelles was tested on HaCaT keratinocytes. As shown in Figure 3, free CUR negatively affected cell survival at 20 and 50 μM concentrations (Mann–Whitney *U* test $P < .001$). When cells were treated with CUR-loaded nanomicelles, none of the nanosystems impacted cell survival at all concentrations considered, including those at which the most effective antimicrobial effects were observed (Figures 2B-D).

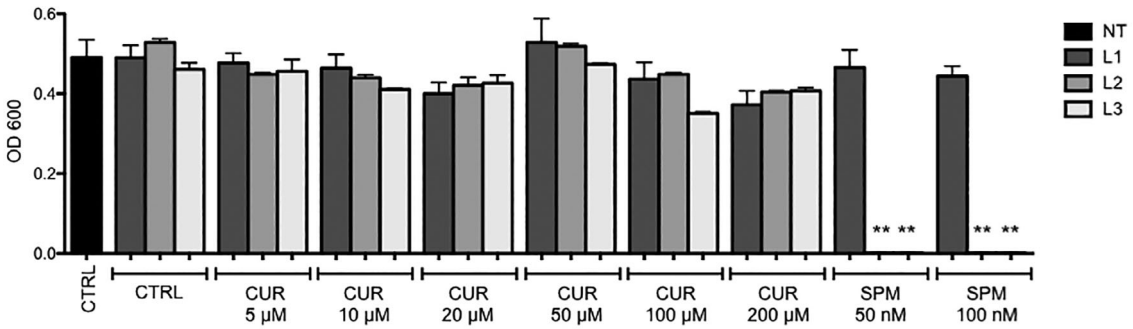


FIGURE 4 Antimicrobial photodynamic therapy (APDT) by employing blue laser irradiation on free curcumin and curcumin embedded in SPM nanomicelles against *Pseudomonas aeruginosa*. The bacteria were treated with free curcumin (5 μM, 10 μM, 20 μM, 50 μM,100 μM, 200 μM) and curcumin-SPM nanomicelles (50 nM and 100 nM) irradiated with blue laser light (λ 445 nm, continuous wave, L1 = 0.1 W/cm^2 6 J/cm^2, L2 = 0.1 W/cm^2 18 J/cm^2, L3 = 0.1 W/cm^2 30 J/cm^2). Data are shown as mean ± SD optical density (OD) at 600 nm (indicative of the bacterial growth) of three independent experiments. CTRL, control; CUR, curcumin; NMs, nanomicelles. ** Mann–Whitney *U* test $P < .001$ (treatment vs control)

3.3 | APDT increases the effectiveness of CUR-SPM nanomicelles against *P. aeruginosa*

The results presented above highlighted the CUR-SPM nanosystem as the most effective in quantitatively inhibiting the growth of *P. aeruginosa*. Accordingly, APDT and drug/APDT combined experiments were performed including both free CUR at increasing concentrations (5-200 μM) and CUR-SPM nanomicelles at the two concentration values at which they did not significant exert antimicrobial effect (50 and 100 nM, Figure 2D). As it can be seen from Figure 4, the application of all three laser protocols alone (L1, L2 and L3) did not affect bacterial growth (Figure 4). On the same line, when CUR was irradiated none of the protocols was able to effectively activate CUR in order to achieve a photodynamic antimicrobial effect. When CUR-SPM nanomicelles were irradiated, both laser protocols L2 and L3 were able to stimulate curcumin more efficiently, resulting in a complete inhibition of bacterial growth even at lowest concentration of 50 nM (Figure 4, $P < .001$ for L2 and L3 at both 50 and 100 nM).

4 | DISCUSSION

It is well known that blue light can be used to excite photosensitizing molecules in PDT. As happened for a lot of scientific discoveries over centuries, also PDT was accidentally discovered more than 100 years ago by Oskar Raab and Hermann von Tappiener, when they noticed that *Paramecium spp* protozoans stained with acridine orange died upon exposure to bright light [17]. Since then, PDT has entered the arena of anticancer therapies but recently, due to the increasing antibiotic resistance crisis, it is also being reconsidered for the treatment of localized bacterial infections. PDT is deemed relatively selective because the photosensitizer uptake is higher in rapidly proliferating biological entities, such as bacteria or neoplastic cells.

Among the variety of possible photosensitizers, CUR is on the edge of interest due to its multiple antimicrobial, anti-inflammatory and anti-cancer effects. Poor water solubility is yet seriously limiting the clinical applications of this natural molecule as a consequence of the related bioavailability and instability. Specifically, curcumin acts against both Gram+ and Gram− bacteria (including *P. aeruginosa*) by causing membrane damage. However, Gram- pathogens are less CUR-sensitive due to the outer bacterial cell membrane restricting drug permeation [18], and at least 100 μM CUR concentrations are necessary to significantly decrease *P. aeruginosa* viability [19]. This result is consistent with our current data, since 50 μM CUR concentrations significantly decreased bacterial growth (50%, Figure 2A) but are not active enough to inhibit the growth of planktonic bacteria.

In a recent work Abdulrahman et al [20] showed that a concentration of 6.75 mM of CUR combined with irradiation at 405 nm (300 mW of output power, 5 or 10 J/cm^2) was necessary to decrease *P. aeruginosa* growth, without totally inhibiting its viability (2.12 log10 and 4.26 log10 reduction with 5 and 10 J/cm^2, respectively). However, the potential toxicity of this condition was not tested on eukaryotic cell lines.

Remarkably, when CUR was encapsulated in silica nanoparticles, its efficacy against *P. aeruginosa* increased but the minimum inhibitory concentration was 1 mg/mL [21]. In our study, we evaluated the antimicrobial efficacy of curcumin delivered by three types of amphiphilic self-assembling nanomicelles featuring a common C_{16}-long hydrocarbon chain as the hydrophobic part and three different cationic moieties—namely DAPMA, SPD and SPM—bearing a nominal charge of +2, +2 and +3, respectively, at physiological pH (7.4).

When challenged in decreasing concentrations against *P. aeruginosa* planktonic cells, all three types of CUR-loaded nanomicelles showed antimicrobial efficacy, inhibiting the growth of *P. aeruginosa* at 500 nM (DAPMA), 1 μM (SPD) and 250 nM (SPM), respectively (Figure 2B-D). Accordingly, the CUR-SPM was assessed as the most effective antibacterial nanosystem. We speculated that one possible reason for the remarkable CUR-SPM efficacy at such low concentrations (to our knowledge one of the lowest among those reported in literature so far) could be related to the antimicrobial activity of spermine itself; however, spermine MIC is 16 mM [22], a concentration value substantially higher than those employed in the present study. In addition, we evaluated the cytotoxicity effects of the blank (ie, drug-unloaded) nanomicelles themselves, and all three nanomicelle types were equally devoid of any antimicrobial effect (Figure 3). This led us to definitively discard the hypothesis of an intrinsic antimicrobial activity of SPM micelles in the concentration range adopted in our experiments (50-100 nM). We therefore reasoned that their efficacy could be related to the high positive charge on each SPM amphiphile (+3), which increases significantly the capacity of the corresponding self-assembled micelles to bind polyanions, as tested experimentally during their characterization [14]. Like all Gram-negative bacteria, *P. aeruginosa* also has an outer membrane located above a thin peptidoglycan layer which, together with periplasm, constitutes the bacterial envelope. The outer membrane is populated by proteins, phospholipids, and lipopolysaccharides (LPS), which are asymmetrically distributed so

that the outer face is constituted only by LPS. LPS contains more charge per unit of surface area than any phospholipid, and most of this charge is anionic at neutral pH because of exposed phosphoryl and carboxyl groups that can be readily ionized. For this reason, the outer face of the outer membrane in these pathogens is highly charged; as such, it is expected to strongly interact with any cationic species present in the external milieu [23], including positively-charged nanomicelles. As SPM is the most positively charged nanosystem among those included in our study (+3), it is reasonable to assume that its interaction with *P. aeruginosa* outer membrane is enhanced with respect to the other two nanomicelles (+2).

To assess the potential toxicity of the nanoparticles on eukaryotic cells, namely, human keratinocytes, the same concentrations employed for the antimicrobial tests were next analyzed: no effect on the viability of the cells was observed (Figure 3), proving that the results obtained were specific for prokaryotic cells.

When CUR-SPM micelles at 100 and 50 nM were supplemented to bacterial suspensions and irradiated with blue laser protocol L1, we did not observe differences in bacterial survival compared to not irradiated samples (Figures 2D and 4). However, when laser fluences were higher (as per protocols L2 and L3), the minimum inhibitory concentrations of CUR-SPM nanomicelles further decreased to 50 nM (Figure 4). Since all protocols were characterized by the same wavelength and irradiance, the higher fluences were obtained prolongating irradiation time. Interestingly, protocol L3 employs the same fluence (30 J/cm^2) used in a recent APDT research work involving *P. aeruginosa* treated with LED-activated photosensitizer TONS 504 [24].

5 | CONCLUSION

In the current work, among the CUR-loaded nanomicelles tested, the system based on CUR-loaded SPM nanomicelles showed the strongest antimicrobial effect against *P. aeruginosa* without cytotoxicity when tested on eukaryotic cells. Moreover, when the CUR-SPM nanomicelles were combined with laser irradiation, the photochemical effectiveness of the natural compound was greatly enhanced. These promising antibacterial results indicate that APDT can be considered as a potential approach to counteract *P. aeruginosa* infections, although further studies are necessary to unravel the APDT impact at molecular and cellular level. Furthermore, APDT should be tested on other resistant bacterial species and eventually in vivo, to validate its properties in a more complex system, and therefore to foster the potential translational application of this nanotechnology-based combined strategy with the final aim of adopting APDT in clinical practice.

CONFLICTS OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

All the data used to support the findings in this study are included in the article.

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REFERENCES

[1] G. M. Rossolini, F. Arena, P. Pecile, S. Pollini, *Curr. Opin. Pharmacol.* **2014**, *18*, 56.
[2] P. K. Taylor, A. T. Yeung, R. E. Hancock, *J. Biotechnol.* **2014**, *191*, 121.
[3] K. Streeter, C. Neuman, J. Thompson, E. Hatje, M. Katouli, *Can. J. Microbiol.* **2016**, *62*, 233.
[4] R. Ramphal, Infections due to pseudomonas species and related organisms. in *Harrison's Infectious Diseases* (Eds: D. Kasper, A. Fauci), New York: McGraw-Hill Companies, **2010**, p. 512.
[5] M. A. Yang, J. Lee, E. H. Choi, H. J. Lee, *J. Korean Med. Sci.* **2011**, *26*(5), 612.
[6] H. M. Wong, *ScientificWorldJournal* **2014**, *2014*, 581795.
[7] J. P. Donnelly, L. A. Bellm, J. B. Epstein, S. T. Sonis, R. P. Symonds, *Lancet Infect. Dis.* **2003**, *3*, 405.
[8] M. H. Gold, *J. Clin. Aesthet. Dermatol.* **2009**, *2*(2), 44.
[9] M. R. Hamblin, T. Hasan, *Photochem. Photobiol. Sci.* **2004**, *3*(5), 436.
[10] D. D. Ferreyra, E. Reynoso, P. Cordero, M. B. Spesia, M. G. Alvarez, M. E. Milanesio, E. N. Durantini, *J. Photochem. Photobiol. B* **2016**, *158*, 243.
[11] A. Goel, A. B. Kunnumakkara, B. B. Aggarwal, *Biochem. Pharmacol.* **2008**, *75*(4), 787.
[12] N. C. Araújo, C. R. Fontana, V. S. Bagnato, M. E. Gerbi, *Photomed. Laser Surg.* **2012**, *30*(7), 393.
[13] K. A. Mitra, *Drug Delivery*, Jones and Bartlett learning, **2015**; Hoboken, NJ: chapter 10, p. 244.
[14] L. E. Fechner, B. Albanyan, V. M. P. Vieira, E. Laurini, P. Posocco, S. Pricl, D. K. Smith, *Chem. Sci.* **2016**, *7*, 4653.
[15] G. Dagrada, K. Rupel, S. Zacchigna, E. Tamborini, S. Pilotti, A. Cavalleri, L. E. Fechner, E. Laurini, D. K. Smith, S. Brich, S. Pricl, *Mol. Pharm.* **2018**, *15*(10), 4689.
[16] K. Rupel, L. Zupin, G. Ottaviani, I. Bertani, V. Martinelli, D. Porrelli, S. Vodret, R. Vuerich, D. Passos da Silva, R. Bussani, S. Crovella, M. Parsek, V. Venturi, R. Di Lenarda, M. Biasotto, S. Zacchigna, *NPJ Biofilms Microbiomes* **2019**, *5*, 29.
[17] O. Raab, *Z. Biol.* **1900**, *39*, 524.
[18] S. George, M. R. Hamblin, A. Kishen, *Photochem. Photobiol. Sci.* **2009**, *8*(6), 788.
[19] P. Tyagi, M. Singh, H. Kumari, A. Kumari, K. Mukhopadhyay, *PLoS One* **2015**, *10*(3), e0121313.

[20] H. Abdulrahman, L. Misba, S. Ahmad, A. U. Khan, *Photodiagn. Photodyn. Ther.* **2019**, *30*, 101645.
[21] M. Mirzahosseinipour, K. Khorsandi, R. Hosseinzadeh, M. Ghazaeian, F. K. Shahidi, *Photodiagn. Photodyn. Ther.* **2020**, *29*, 101639.
[22] D. H. Kwon, C. D. Lu, *Antimicrob. Agents Chemother.* **2007**, *51* (6), 2070.
[23] T. J. Beveridge, *J. Bacteriol.* **1999**, *181*(16), 4725.
[24] K. Sueoka, T. Chikama, M. A. Latief, J. A. Ko, Y. Kiuchi, T. Sakaguchi, A. Obana, *Lasers Med. Sci.* **2018**, *33*, 1455.

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