Photochemical & Photobiological Sciences

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Cite this: *Photochem. Photobiol. Sci.*, 2013, **12**, 1622

Sub-lethal photodynamic inactivation renders *Staphylococcus aureus* susceptible to silver nanoparticles

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Received 6th February 2013,
Accepted 1st May 2013

DOI: 10.1039/c3pp50039j

www.rsc.org/pps

Staphylococcus aureus is a common etiological factor in infections of burns and other chronic wounds. The development of an effective and fast-acting treatment would be enormously beneficial and is highly desired. We focused on testing the bactericidal efficacy of photoinactivation using a known photosensitizer (protoporphyrin IX, PPIX) in sequential combination with silver nanoparticles against *S. aureus*. Using PPIX-based photoinactivation followed by silver nanoparticles we obtained a high bactericidal effect (7 $\log_{10}$ units reduction) with limited harmful effects on human epidermal keratinocytes. Moreover, we observed that the use of silver nanoparticles prevents bacterial re-growth 24 h post-PDI treatment. A sequential combination of photoinactivation and silver nanoparticles represents a potentially effective antibacterial approach.

1. Introduction

The rapid increase in the emergence of antibiotic-resistant strains among many species of pathogenic bacteria may bring to an end the "antibiotic era".[1] The growth of multi-drug resistant bacteria has encouraged research focused on alternative antibacterial therapeutic options to which bacteria will not easily be able to develop resistance.

One of the possible alternative therapies could be photodynamic inactivation (PDI) and photodynamic antimicrobial chemotherapy (PACT) utilizing the ability of photosensitizers (PS), in combination with visible light and molecular oxygen, to kill target cells.[2] In the presence of oxygen, the excited state PS transfers energy or electrons to ground state molecular oxygen-producing reactive oxygen species (ROS), such as singlet oxygen and hydroxyl radicals, which are able to kill cells.

A second possible alternative antibacterial approach could be nanobiotechnology combining biotechnology with nanotechnology (particles of 1–100 nm in size) to develop environmentally friendly technologies that have several applications, such as in food preservation, the decontamination of materials and, most interestingly, medicine. For microbial infections the most interesting are silver nanoparticles (AgNPs), which have been shown to be most effective against a broad spectrum of microorganisms, including *S. aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and several types of viruses.[3] The mechanism of action of silver nanoparticles has been extensively studied in the last few years on the model Gram-negative bacteria *E. coli*; this research has shown that the nanoparticles interact with the bacterial membrane and cause cell death. After attaching to the cell membrane, the silver nanoparticles also penetrate into the bacterial cell and release silver ions which preferably attack the respiratory chain, sulfur-containing proteins and phosphorus-containing compounds such as DNA.[4–6] *S. aureus*, including the multi-drug-resistant strains, was shown to be efficiently killed by silver colloid nanoparticle formulations.[7] On the basis of other interesting studies, concerning three forms of silver nanoparticles (dissolved, small – 10–20 nm, and large – 70–80 nm), we can assume that silver nanoparticles rapidly accumulate in eukaryotic cells, and further follow an efficient depuration process.[8] The area of research connected with silver nanoparticles remains controversial as from one side AgNPs are widely used in FDA regulated products, and on the other hand, some toxicity regarding biological systems is reported. We believe that special attention should be paid to adapting AgNPs in biological applications. However, there are several points which

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must be taken into account with respect to the matter, including the particular type of AgNP, and the method of AgNP application (*e.g.* local treatment of skin infections).

In this study, we present an innovative approach of sequentially combining photodynamic inactivation with the use of silver nanoparticles that could significantly increase the individual antibacterial activities of each of these previously described therapies. To exemplify the potential synergistic effect of sequential combination of AgNPs and photodynamic treatment, we used PPIX-based photoinactivation. The PPIX was chosen as it exerts a poor bactericidal effect when compared with other highly efficient photosensitizers *e.g.* phthalocyanines. The attainment of a significantly increased bactericidal effect of PPIX-mediated PDI in sequential combination with AgNP one could assume that even greater effects could be achieved with the use of more efficient photosensitizing agents.

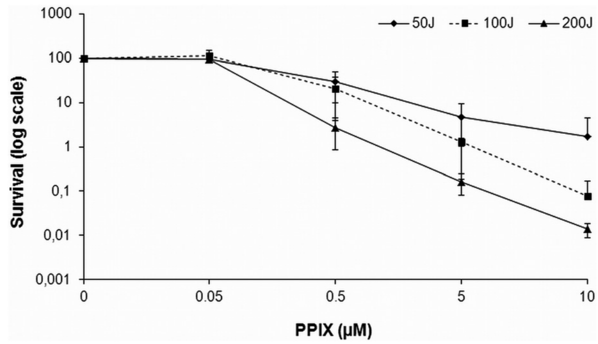

Fig. 1 PPIX-mediated PDI against *S. aureus* Newman strain in planktonic culture. The probes were illuminated after dark incubation for 30 min at 37 °C with different concentrations of PPIX (up to 10 μM). Bacteria were illuminated with different doses of blue light, and survival fractions were determined as described in Materials and Methods. Lines were only used for a better visualization. Values are means of three separate experiments, and bars are s.d.

2. Results

2.1. Minimal bactericidal concentrations of silver nanoparticles

MBC values of silver nanoparticles acting solely and preincubated with different PPIX concentrations are presented in Table 1. When analyzing silver nanoparticles acting solely, the MBC was determined as 6.74×10^{12} NP ml^{-1}. In the case of preincubation of silver nanoparticles with different PPIX concentrations, the MBC values increased accordingly to the PPIX concentration used, suggesting binding of PPIX to AgNP and preventing its effective interaction with bacteria (Table 1).

2.2. PPIX-based photodynamic inactivation

Photodynamic inactivation (PDI) using various doses of light was applied to an *S. aureus* Newman reference strain. The results from multiple experiments showed that the bactericidal effect ranged from a 0 to 4.0 $\log_{10}$ reduction in viable counts of bacteria, suggesting that the response of *S. aureus* to PDI changes in a light dose-dependent manner (Fig. 1).

The higher the dose of light applied, the higher the bactericidal effect obtained. In the presence of 10 μM PPIX, an eradication level of 4.0 $\log_{10}$ reduction was obtained using a light dose of 200 J cm^{-2}. Sub-lethal photoinactivation was obtained with the PPIX concentration of 0.05 μM (light dose up to 200 J cm^{-2}) and the PPIX concentration of 0.5 μM (light dose up to 100 J cm^{-2}). Higher sensitizer concentration resulted in lethal damage of *S. aureus*. Because light irradiation seemed not to induce any growth inhibition and the toxicity of the sensitizer itself did not exceed 0.2 $\log_{10}$ reduction in bacterial viability, these data are not included in Fig. 1.

Table 1 Changes in MBC values after preincubation of silver nanoparticles with different PPIX concentrations

MBC of AgNP (NPs ml^{-1}) after preincubation with PPIX[a]			
PPIX concentrations (μM):			
0	0.05	0.5	5
6.74×10^{12}	6.74×10^{12}	1.3×10^{13}	2.6×10^{13}

[a] Silver nanoparticles were preincubated with PPIX for 30 min at 37 °C prior to the addition of the bacteria suspension (10^5 CFUs).

2.3. Combination of PPIX-based photodynamic inactivation and silver nanoparticles

To determine whether the addition of silver nanoparticles (AgNP) potentiates the efficacy of PPIX-based phototreatment, we sequentially combined these two reagents (AgNP and PPIX) in several unique ratios. These combinations are shown in a scheme in Fig. 2A. In this experiment, two identical plates were used; one of them was subjected to light treatment, and the second was kept in the dark. Under these experimental conditions, we were able to observe the effect of AgNPs alone (dark control) as well as the combined effects of AgNP and PDI treatment. It is important to remember, however, that in the sample recognized as the 'dark control', we also observed a possible effect of PPIX toxicity. The experiments were performed as described in the Materials and Methods section, and the results were analyzed 24 h post-PDI treatment. We observed that in the 'dark control' samples, AgNP concentrations equal to 1 MBC and above were effective in the total eradication of the *S. aureus* strain. However, higher concentrations of PPIX (5 μM and 10 μM) abrogated the antibacterial effect of AgNPs (Fig. 2A). This is presumably because the anionic PPIX can bind to the cationic AgNP and prevent the AgNP binding to the bacteria. This was tested by preincubating the AgNP with different concentrations of PPIX and then adding them to bacteria and measuring MBC. Increased MBC values of AgNP preincubated with different concentrations of PPIX (Table 1) suggest that PPIX interacts with the AgNP and prevents its binding to the bacteria. Thus, the PBS washing step preceded the addition of silver nanoparticles to limit the possible interactions of PPIX with the AgNP. When photodynamic treatment was applied (treating the cells with a combination of PPIX and 100 J cm^{-2} of

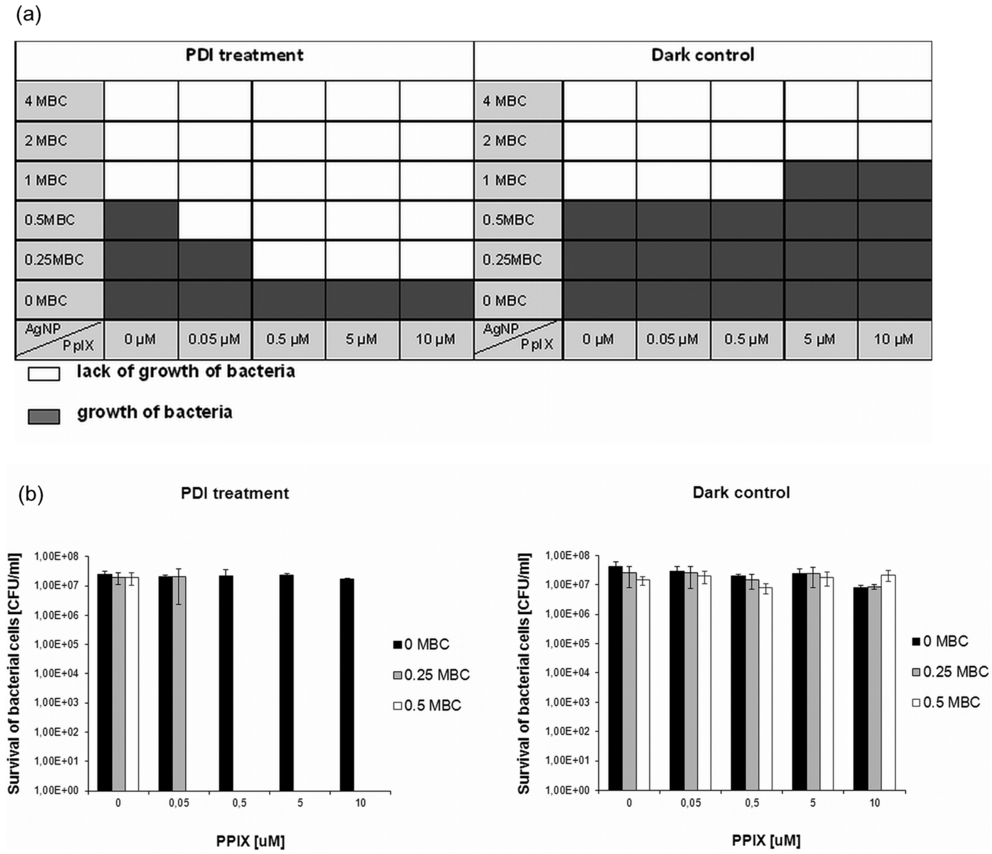

Fig. 2 Combined bactericidal effect of silver nanoparticles and photodynamic treatment against planktonic culture of *S. aureus*. (A) Schematic representation of the experimental design. AgNP MBC value – 6.74×10^{12}, PPIX concentration values are indicated in a grey panel (0–10 μM). (B) Survival fraction of the bacteria 24 h after PPIX treatment with 100 J cm^{-2} light and AgNP (left panel) and 24 h after PPIX treatment without illumination and AgNP (dark control) (right panel). MBC values of AgNPs are indicated in the legend.

light followed by administration of AgNP), we observed augmented bactericidal effects. The 24 h post-PDI treatment, the *S. aureus* eradication and re-growth prevention were obtained using the following conditions: ≥0.05 μM PPIX and AgNP ≥0.5 MBC; ≥0.5 μM PPIX and AgNP ≥0.25 MBC (Fig. 2A and 2B). It is important to underline that upon PDI treatment without the addition of AgNPs the re-growth effect was observed (Fig. 2A and 2B – 0 MBC). Although, directly after PDI treatment we observed decrease in viable cell counts by 4 $\log_{10}$ (Fig. 1). Only the sequential combination of AgNP and PDI treatment allowed for total eradication of bacteria after 24 h incubation. Moreover, this high bactericidal effect was reached with significantly lower concentrations of AgNP and PPIX when compared to AgNP and PPIX used separately. Protoporphyrin IX-mediated photoinactivation rarely exceeds 4 $\log_{10}$ reduction in the survival fraction. Current studies indicate that PPIX used in combination with the appropriate AgNP concentration leads to reduction in the survival fraction as high as 7 logs CFU ml^{-1} and prevents bacterial re-growth. Interestingly, and similar to the 'dark control', we observed a lack of bactericidal effect of sequentially combined PDI and AgNP treatment when 10 μM PPIX was used without a PBS washing step (data not shown). As indicated above, higher concentrations of PPIX could abrogate the bactericidal effect of AgNP leading to increased MBC values (Table 1). This effect was probably caused by the interactions between PPIX and AgNP that prevent AgNP binding to bacteria and lead to re-growth of bacterial cells within the 24 h incubation. Successfully, bacterial re-growth could be effectively eliminated by washing the cells prior to administration of AgNPs.

2.4. Cyto- and phototoxic effects of antimicrobial agents against HaCaT cells

The viability of mammalian cells treated with the sensitizer and silver nanoparticles was assessed using an MTT assay. The toxicity of different concentrations of protoporphyrin IX and AgNP on human skin cells was evaluated using epidermal keratinocytes. As shown in Fig. 3, incubation of keratinocytes with PPIX reduced cell viability only upon illumination. Incubation of keratinocytes with silver nanoparticles at concentrations up to 6.74×10^{12} NPs ml^{-1} (1 MBC) with and without illumination did not significantly influence cell viability (Fig. 3). The effects of PPIX upon illumination showed concentration-dependent differences in bacterial and eukaryotic cells. Protoporphyrin IX at concentrations of 0.05 and 0.5 μM in the presence of 0.5 MBC of silver nanoparticles resulted in a 7 $\log_{10}$ reduction in bacterial viability after illumination, whereas keratinocytes were still viable (80%) with or without illumination (Fig. 3).

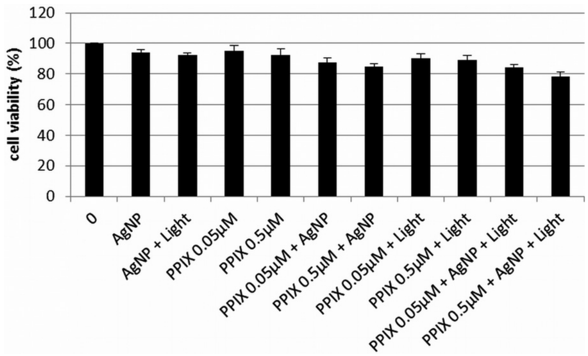

Fig. 3 Cyto- and phototoxic effects of AgNP and PPIX against human epidermal keratinocytes. Illumination parameters: blue light, 100 J cm^{-2}. Toxicity of PPIX and silver nanoparticles was assessed after 24 h incubation at 37 °C in the dark. Each point is the mean of three experiments ± s.d.

3. Discussion

The light-mediated killing of many pathogens *in vitro* has been extensively investigated. A wide range of organisms, ranging from the Gram-positive *S. aureus* to the Gram-negative *P. aeruginosa*, has proved to be susceptible to PDI with a number of different photosensitizers *in vitro*. It is well known that multidrug-resistant strains are just as susceptible as their naive counterparts[9] and moreover, it has not yet been possible to artificially induce resistance to PDI in any microbes on which it has been tested.[10] It has been shown that human cells (keratinocytes and fibroblasts) can survive PDI conditions that are lethal to microorganisms.[11] Zeina *et al.*[11] have demonstrated that PDI conditions yielding a reduction in bacterial load of 7 $\log_{10}$ units have no effect on mammalian keratinocytes. The low toxicity of PDI on human cells at doses that are lethal to microorganisms indicates that PDI has potential for *in vivo* use. Our study indicates that with the use of PPIX-mediated photoinactivation we can achieve 4 $\log_{10}$ units reduction in viable counts with no harmful effect towards human keratinocytes.

Although silver-based materials have been used for centuries to treat burns in humans, their role decreased substantially after the introduction of penicillin. It was demonstrated by Leaper[12] that silver nanoparticles promote healing of burn wounds and improve the cosmetic appearance, while Tian *et al.*[13] demonstrated the effective therapeutic use of silver for scarless wound healing. The same group also demonstrated *in vivo*, using a mouse model of burn-related skin infections, that in addition to their antibacterial properties, silver nanoparticles modulate the inflammatory state in the organism by influencing IL-10 and IL-6 cytokines. This modulation significantly shortens the healing process. In most studies, silver nanoparticles have been shown to be non-toxic to humans when used in low concentrations. However, after exposure of rat liver cell line BRL 3A to increasing concentrations of silver nanoparticles, abnormally sized and irregularly shaped cells were observed.[14] We observed no cytotoxic effects of the silver nanoparticles used in our studies.

Next, we aimed at combining the action of highly effective silver nanoparticles with PPIX to enhance their bactericidal effects against the most common pathogen. Although there is already some evidence indicating that PS activity is substantially increased when conjugated with nanoparticles, the idea of a sequential combining effect of PS and nanoparticles is quite new. Our study indicates that sequential combination of PPIX-mediated photoinactivation with silver nanoparticles results in high bactericidal effects. Only the combination of PDI treatment followed by addition of AgNP allowed for total eradication of bacteria after 24 h incubation. In addition, this high bactericidal effect was obtained with significantly lower concentrations of AgNP and PPIX when compared to AgNP and PPIX used separately. The low water solubility of protoporphyrin IX promotes its usage at low concentrations. It is worth emphasizing that with the use of nontoxic silver nanoparticles, very low PPIX concentrations (0.05 and 0.5 μM) could be applied to reach high bactericidal activity (7 $\log_{10}$ reduction in bacterial viability). It indicates that photoinactivation renders *S. aureus* more susceptible to the action of silver nanoparticles and is indeed synergistic with AgNPs. It also indicates the possible usage of sequentially combined PDI and AgNP as a therapeutic approach for superficial infections treatment. Once irradiated, an infected skin lesion could be administered with a solution of silver nanoparticles to enhance the bactericidal effect of photoinactivation and prevent bacterial regrowth. As supported by the current research, such an approach could be safe for human epidermal cells.

4. Experimental

4.1. Strains and culture conditions

The *S. aureus* NEWMAN strain was used in all experiments.[15] The strain is stored in the Faculty of Biotechnology, UG & MUG, Poland. *S. aureus* was grown overnight in TSB medium (Oxoid, UK) at 37 °C.

4.2. Sensitizer

Protoporphyrin IX (PPIX) (MP Biomedicals) was used in this study. PPIX was dissolved in 100% dimethyl sulfoxide to give a 1 mM stock solution, filtered-sterilized and stored at −20 °C in the dark until use. The concentration of the photosensitizer was determined spectrophotometrically (extinction coefficient 1.6×10^5 M^{-1} cm^{-1}, wavelength 408 nm).

4.3. Light source

The illumination was performed with a Q.Light® PDT Lamp (b & p® Shweiz AG, Switzerland) (ISO 9001 & EN 46001 – CE 1275). The light energy delivered was approximately 7.62 J cm^{-2} per minute. The Q.Light PDT Lamp emits polarized (98% level of polarization) light in the range of 385 to 480 nm.

4.4. Nanoparticles

Silver nanoparticles were provided by ProChimia Surfaces Co. (Poland). Positively charged particles of Ag coated with

$HS–(CH_2)_{11}–N(CH_3)_3^+$ were used. Average size: 5.5 nm, dispersity: 15%, concentration: 2.4×10^{14} NPs ml^{-1} (SPR maximum, λ_{max}: 420–424 nm). Soluble in water. Trypcase-soy broth (Oxoid, UK) was used for the dilutions.

4.5. Cell line

HaCaT (human epidermal keratinocytes) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. The cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C.

4.6. Minimal bactericidal concentrations of silver nanoparticles

By using the Broth Microdilutions Method[16] we determined the Minimal Bactericidal Concentrations (MBC) of AgNPs. We prepared solutions with the following ranges of concentrations: 8.4×10^{11}–9.5×10^{13} NPs ml^{-1}. To each well of the microtiter plate containing 100 μl of a studied antibacterial agent we added 100 μl of bacterial suspension containing 10^5 CFUs. The plates were incubated for 24 h at 37 °C. In order to determine the MBC values based on the wells in which no visible bacterial growth was found, we took 100 μl from each of those wells and placed it on dishes with TSB medium fixed with agar. The dishes were then incubated for 24 h at 37 °C. MBC was defined as the lowest concentration of an antibacterial factor that, during a defined period of time (24 h), reduces the bacterial inoculum by 99.9%, which is a 3 $\log_{10}$ reduction.[16] To establish the possible interaction between AgNP and PPIX, the MBC was also determined for silver nanoparticles preincubated with different PPIX concentrations (in the range of 0.05 to 5 μM). Solutions of AgNP with PPIX were incubated for 30 min at 37 °C. Next, to each well of the microtiter plate containing 100 μl of a mixture of AgNP and PPIX we added 100 μl of bacterial suspension containing 10^5 CFUs. The plates were incubated for 24 h at 37 °C in order to determine the MBC.

4.7. PDI studies and determination of lethal and sub-lethal conditions

Lethal and sub-lethal conditions were defined according to the definition by Dodd *et al.*[17] as conditions where the direct damage is sufficient or insufficient to destroy the majority of the bacterial population. Thus, lethal and sub-lethal conditions were defined as the combination of light and photosensitizer concentrations which resulted in 2 $\log_{10}$ and 0.5–1 $\log_{10}$ unit reduction in viable count, respectively. The bacterial cultures were grown overnight (16 h) at 37 °C in TSB broth (Oxoid, UK), diluted (10^7 CFU ml^{-1} bacteria) and then incubated with the sensitizer (in the range of 0–10 μM) at 37 °C in the dark for 30 min. Afterwards, the cells were transferred into a 96-well microtiter plate (100 μl per well) and illuminated with an appropriate dose of blue light (385–480 nm) (50, 100 or 200 J cm^{-2}). After the illumination, aliquots were removed to determine the cell number in colony forming units (CFUs) as described by Jett *et al.*[18] Three types of control conditions were used: illumination in the absence of PS, incubation with PS in the dark and treatment with neither photosensitizer nor light. Each experiment was performed at least three times. Survival fractions were expressed as the ratio of CFU of bacteria treated with PDI to CFU of untreated bacteria.[19]

4.8. Effects of PDI and AgNP used in combination

Overnight planktonic cultures of *S. aureus* cells were diluted in a fresh TSB medium to a concentration of 10^7 CFU ml^{-1}, and 100 μl aliquots were placed in 96-well plates. An appropriate concentration of PPIX was added to each well in the range of 0–10 μM (as indicated in the scheme, Fig. 2A, horizontal panel). Each plate was set up in duplicate, one for the treatment with light, the other for the unilluminated control. The plates were incubated for 30 min at 37 °C. Subsequently, the samples were illuminated with blue light (385–480 nm) at a dose of 100 J cm^{-2}. Next, the PBS washing step was performed. The cell suspensions were centrifuged (9000*g*, 1 min), the PS solution was aspirated, and cells were washed once with 100 μl of sterile PBS and centrifuged as described above. Finally, the pellet was diluted in 100 μl of fresh broth, and 100 μl of AgNP diluted in TSB medium were added to each well. The final AgNP concentration values ranged from 4 MBC to 0.25 MBC (as indicated in the scheme, Fig. 2A, vertical panel). Thus, each well in the plate contained a unique combination of PPIX and AgNP amounts. The cells were incubated at 37 °C, and bacterial growth was measured 24 h post PDI treatment. The contents of each well were pipetted to mix, serially diluted 10 times with sterile PBS and plated on TSA plates. After overnight incubation at 37 °C, colonies were counted and CFU ml^{-1} values were calculated.

4.9. Cytotoxicity assay with eukaryotic cells

Cells (5×10^4) were seeded in 96-well plates and allowed to adhere overnight. The chemicals of interest (photosensitizers, nanoparticles) were added to the medium in the range of concentration determined experimentally (8.4×10^{11}–6.74×10^{12} AgNPs ml^{-1}; 0.05–0.5 μM PPIX). In the case of photoinactivation, the cells were incubated for 30 min at 37 °C in the dark prior to irradiation. Cell survival was determined 24 h after the illumination step by a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in which MTT was an indicator of metabolically active cells and a mitochondrial enzyme activity results in a color reaction that can be measured spectrometrically. Following the treatment, MTT (0.5 mg ml^{-1}) was added, and the cells were incubated for 2 h at 37 °C. The cells were subsequently lysed in DMSO, and the optical density of the formazan solution was measured at 550 nm with a plate reader (Victor, 1420 multilabel counter).

5. Conclusions

We describe a potentially effective antibacterial therapeutic approach involving photoinactivation and silver nanoparticles. By combining these two elements, we can obtain significantly increased bactericidal effects against an *S. aureus* strain, indicating the potential synergistic effects between these agents. Moreover, applying the combined treatment, we can prevent bacterial re-growth and reach complete *S. aureus* eradication. In addition, the treatment regimens tested so far have acceptable levels of toxicity on human epidermal keratinocytes, suggesting that this approach is safe for use on human tissues.

Competing interests

Non-financial competing interests.

Authors' contributions

JN made substantial contributions to conception and design and carried out the nanoparticles-based experiments and has been involved in drafting the manuscript or revising it critically for important intellectual content.

AR carried out the PDI-related experiments.

AK carried out the eukaryotic cell-related analysis.

JK helped drafting manuscript.

KPB helped to draft the manuscript.

MG made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data and have been involved in drafting the manuscript and revising it critically for important intellectual content.

Acknowledgements

This work was supported by the grant no. IP2010 010970 from the Ministry of Science and Higher Education (MNiSW) and LIDER/32/36/L-2/10/NCRiR/2011 from the National Centre for Research and Development. The publication is financed from European Social Fund as a part of the project "Educators for the elite – integrated training program for PhD students, post-docs and professors as academic teachers at University of Gdansk" within the framework of Human Capital Operational Programme, Action 4.1.1, improving the quality of educational offer of tertiary education institutions. This publication reflects the views only of the author, and the funder cannot be held responsible for any use which may be made of the information contained therein.

References

1 T. Dai, Y. Y. Huang and M. R. Hamblin, Photodynamic therapy for localized infections – state of the art, *Photodiagn. Photodyn. Ther.*, 2009, **6**, 170–188.

2 M. Wainwright, Photodynamic antimicrobial chemotherapy (PACT), *J. Antimicrob. Chemother.*, 1998, **42**, 13–28.

3 M. Rai, A. Yadav and A. Gade, Silver nanoparticles as a new generation of antimicrobials, *Biotechnol. Adv.*, 2009, **27**, 76–83.

4 Q. L. Feng, J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim and J. O. Kim, A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*, *J. Biomed. Mater. Res.*, 2000, **52**, 662–668.

5 I. Sondi and B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, *J. Colloid. Interface. Sci*, 2007, **275**, 177–182.

6 H. Y. Song, K. K. Ko, L. H. Oh and B. T. Lee, Fabrication of silver nanoparticles and their antimicrobial mechanisms, *Eur. Cell Mater.*, 2006, **11**, 58.

7 A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V. K. Sharma, T. Nevecna and R. Zboril, Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity, *J. Phys. Chem. B*, 2006, **110**, 16248–16253.

8 M. Al-Sid-Cheikh, C. Rouleau and E. Pelletier, Tissue distribution and kinetics of dissolved and nanoparticulate silver in Iceland scallop (*Chlamys islandica*), *Mar. Environ. Res.*, 2013, **86**, 21–28.

9 H. M. Tang, M. R. Hamblin and C. M. Yow, A comparative *in vitro* photoinactivation study of clinical isolates of multidrug-resistant pathogens, *J. Infect. Chemother.*, 2007, **13**, 87–91.

10 F. M. Lauro, P. Pretto, L. Covolo, G. Jori and G. Bertoloni, Photoinactivation of bacterial strains involved in periodontal diseases sensitized by porphycene-polylysine conjugates, *Photochem. Photobiol. Sci.*, 2002, **1**, 468–470.

11 B. Zeina, J. Greenman, D. Corry and W. M. Purcell, Antimicrobial photodynamic therapy: assessment of genotoxic effects on keratinocytes *in vitro*, *Br. J. Dermatol.*, 2003, **148**, 229–232.

12 D. J. Leaper, Silver dressings: their role in wound management, *Int. Wound J.*, 2006, **3**, 282–294.

13 J. Tian, K. K. Wong, C. M. Ho, C. N. Lok, W. Y. Yu, C. M. Che, J. F. Chiu and P. K. Tam, Topical delivery of silver nanoparticles promotes wound healing, *ChemMedChem*, 2007, **2**, 129–136.

14 S. M. Hussain, K. L. Hess, J. M. Gearhart, K. T. Geiss and J. J. Schlager, *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells, *Toxicol. In Vitro*, 2005, **19**, 975–983.

15 E. S. Duthie and L. L. Lorenz, Staphylococcal coagulase; mode of action and antigenicity, *J. Gen. Microbiol.*, 1952, **6**, 95–107.

16 C. Thornsberry, Antimicrobial susceptibility testing of anaerobic bacteria: review, comments, and opinions, *Ann. Otol. Rhinol. Laryngol. Suppl.*, 1991, **154**, 7–10.

17 C. E. R. Dodd, R. L. Sharman, S. F. Bloomfield, I. R. Booth and G. S. A. B. Stewart, Inimical processes: bacterial self-destruction and sub-lethal injury, *Trends Food Sci. Technol.*, 1997, **8**, 238–241.

18 B. D. Jett, K. L. Hatter, M. M. Huycke and M. S. Gilmore, Simplified agar plate method for quantifying viable bacteria, *Biotechniques*, 1997, **23**, 648–650.

19 M. Grinholc, B. Szramka, J. Kurlenda, A. Graczyk and K. P. Bielawski, Bactericidal effect of photodynamic inactivation against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* is strain-dependent, *J. Photochem. Photobiol.,B*, 2008, **90**, 57–63.