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ORIGINAL ARTICLE

Photodynamic inactivation of different pathogenic bacteria on human skin using a novel photosensitizer hydrogel

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Abstract

Background: The colonization of skin with pathogenic, partially antibiotic-resistant bacteria is frequently a severe problem in dermatological therapies. For instance, skin colonization with *Staphylococcus aureus* is even a disease-promoting factor in atopic dermatitis. The photodynamic inactivation (PDI) of bacteria could be a new antibacterial procedure. Upon irradiation with visible light, a special photosensitizer exclusively generates singlet oxygen. This reactive oxygen species kills bacteria via oxidation independent of species or strain and their antibiotic resistance profile causing no bacterial resistance on its part.

Objective: To investigate the antibacterial potential of a photosensitizer, formulated in a new hydrogel, on human skin ex vivo.

Methods: The photochemical stability of the photosensitizer and its ability to generate singlet oxygen in the hydrogel was studied. Antimicrobial efficacy of this hydrogel was tested step by step, firstly on inanimate surfaces and then on human skin ex vivo against *S. aureus* and *Pseudomonas aeruginosa* using standard colony counting. NBTC staining and TUNEL assays were performed on skin biopsies to investigate potential necrosis and apoptosis effects in skin cells possibly caused by PDI.

Results: None of the hydrogel components affected the photochemical stability and the life time of singlet oxygen. On inanimate surfaces as well as on the human skin, the number of viable bacteria was reduced by up to 4.8 $\log_{10}$ being more effective than most other antibacterial topical agents. Histology and assays showed that PDI against bacteria on the skin surface caused no harmful effects on the underlying skin cells.

Conclusion: Photodynamic inactivation hydrogel proved to be effective for decolonization of human skin including the potential to act against superficial skin infections. Being a water-based formulation, the hydrogel should be also suitable for the mucosa. The results of the present ex vivo study form a good basis for conducting clinical studies in vivo.

Daniel Bernhard Eckl and Anja Karen Hoffmann contributed equally to this study.

INTRODUCTION

In dermatology, antibiotics are the mainstay of therapy for common dermatoses, including acne and rosacea as well as for skin and soft tissue infections.[1] For example, in up to 90% of patients with atopic dermatitis, even normal-looking skin is extensively colonized by *Staphylococcus aureus*, a major trigger of this disease.[2] Surgical site infections significantly contribute to patient morbidity involving different pathogenic bacteria with *S. aureus* playing a dominant role.[3] Skin infections are the frequent clinical manifestation of *S. aureus* being the second most common cause of healthcare-associated infections in the United States.[4] Meanwhile, Gram-negative bacteria and mixed pathogens (Gram-negative, Gram-positive bacteria) also significantly contribute to skin infections and mixed infections are more likely to cause inappropriate antibiotic therapy.[5]

Various antibiotics or antiseptics like mupirocin, fusidic acid or chlorhexidine are used to prevent or cure colonization or skin infection. However, many skin pathogens like *S. aureus* show resistance against mupirocin in up to 13.5% of isolates tested, fusidic acid up to 57% and chlorhexidine up to 70%.[6] An overall prevalence of MDR *S. aureus* isolates of 30.2% was reported, with 8.7% resistant to at least four different classes of antibiotics.[7] Also, opportunistic pathogens like *Staphylococcus epidermidis* show resistance to rifampicin and reduced susceptibility to vancomycin.[8] In many countries, more than 50% of *Propionibacterium acnes* strains are resistant to topical macrolides.[9] Thus, there is an urgent need for further research on antimicrobial alternatives in order to prevent the appearance of even more drug-resistant bacteria.[10]

Such novel processes could be the use of bacteriophages[11] or antimicrobial peptides.[12] Another innovative approach is the photodynamic inactivation of bacteria (PDI), whose strength is to kill Gram-positive and Gram-negative bacteria regardless of their resistance profiles.[13–16]

Photodynamic inactivation uses three harmless components, a dye molecule (photosensitizer), visible light and molecular oxygen. Upon exposure to visible light, the photosensitizer transfers the absorbed light energy to adjacent molecular oxygen to generate biocidal reactive oxygen species (ROS) of which singlet oxygen is the most important species.[17,18] In case the photosensitizer is close enough to bacteria, singlet oxygen is able to reach the cell wall or outer membrane of bacteria by diffusion and destroying them via oxidative processes.

Photodynamic inactivation was already successfully tested in dentistry,[19] food areas,[20] as antimicrobial surfaces,[21–23] and in the treatment of bacterial infections or colonisations.[24–29] Animal models already showed some good success of PDI on skin in vivo.[25,30] Ex vivo experiments on human skin yielded preliminary results against methicillin-resistant *S. aureus* (MRSA),[31] in which the photosensitizer SAPYR exclusively generated singlet oxygen.[17]

Nevertheless, there are still problems when performing PDI on skin due to the presence of some substances on the skin surface like divalent ions.[32–34] Moreover, the application of a photosensitizer dissolved in pure water is not only impractical but also hampers the important wetting of the bacteria with the photosensitizer solution. Such wetting is important for the maximum proximity of photosensitizers and bacteria, which ensures good accessibility of the bacteria by singlet oxygen.[17]

Therefore, the present study aimed at solving these problems by using the photosensitizer SAPYR in combination with a chelator of divalent ions in a novel hydrogel. Representatives of Gram-positive and Gram-negative pathogens were used to prove the efficacy of PDI, firstly on inanimate surfaces and then on skin ex vivo in the presence of different interfering substances. To check the safety of the PDI procedure for the underlying skin, possible damages were evaluated by performing histology and staining for necrosis and apoptosis in cells of the treated skin samples.

MATERIALS AND METHODS

Preparation of the hydrogel

The photodynamic hydrogel consisted of the water-soluble photosensitizer SAPYR (2-((4-pyridinyl)methyl)-1H-phenalen-1-one chloride) (TriOptoTec GmbH) at 100 µmol L^{-1}, ultrapure water, citrate and hydroxy ethyl cellulose.

Light source

A light source with an emission in the visible range was used for irradiation in all experiments using 18 mW cm^{-2} (802-L, equipped with blue_V lamps with a broadband emission spectrum from 385 to 480 nm, Waldmann GmbH). The radiant exposure was measured with a photosensor PD300-SH (Ophir Optronics GmbH) and as a display device the Nova-display (Ophir Optronics GmbH).

Spectroscopy, oxygen measurements and singlet oxygen detection

The hydrogel was investigated using absorption spectroscopy before and after irradiation (10.8 J cm^{-2}) with a photometer (Spectrocord 50 plus, Analytik Jena GmbH). Potential quenching effects of singlet oxygen by substances in the hydrogel were checked by measuring its luminescence at 1270 nm. The luminescence signal was fitted with a bi-exponential function and the accuracy of the fit was checked by calculating the studentized residuals.[35]

Cultivation of bacteria

Test organisms was *Staphylococcus aureus* DSM 13661 and *Pseudomonas aeruginosa* DSM 1117 (Leibniz Institute DSMZ).

Solvents

Bacteria were re-suspended in H_2O, synthetic sweat solution or synthetic sweat solution without histidine. The synthetic sweat was composed according to the literature[36] and is additionally described in the Appendix S1. The addition of histidine to synthetic sweat solution was added in order to simulate potential quenching substances, which may be present in human sweat.

Experiments on inanimate surfaces

Fifty microliter of the bacterial suspension were placed on glass slides, which were kept at 37°C for 60 min in complete darkness (droplet macroscopically dried), then covered with hydrogel using a sterile swab. After 10 min in darkness, the slide was irradiated (10.8 J cm^{-2}). After irradiation, bacteria were recovered with a sterile swab into sterile MH broth, a 10-fold dilution series was produced and plated with the drop-plate method. After 12 h, the colonies were counted and the decrease of viable bacteria was calculated as logarithmic reduction (log_{10}) by comparison to the untreated reference (bacterial samples without any photosensitizer or light treatment). The PDI references were dark control (photosensitizer without light) or light control (light without photosensitizer). The loss of bacteria (termed recovery loss) on the surfaces was calculated by determining the colony-forming units per mL of the initial bacterial suspension and the colony-forming units of the untreated reference.

Experiments on human skin ex vivo

Tissue samples were obtained according to the framework of the non-profit foundation (HTCR) including the informed patient's consent.[37] The skin samples came from the abdomen of female donors (Fitzpatrick skin type II) who underwent a surgical procedure for other reasons. The samples arrived after a maximum of 12 h after surgical excision and were kept on ice during shipment. The skin was thoroughly cleaned with H_2O and cut into 2 × 2 (thickness ~5 mm) cm large pieces with a surgical lancet. The cleaning procedure was applied to remove excess blood, and H_2O was chosen in order to avoid undesired ion contamination that could potentially impact the composition of the applied synthetic sweat solution. In each subset of experiments, a piece of skin was swabbed and checked for potential bacterial contamination. In none of the cases, contamination was observed. The other procedures were the same as described for inanimate surfaces.

Histology and staining

Damage of skin cells were checked by measuring its mitochondrial activity using NBTC staining as described elsewhere with the exception that the cells were fixed with formaldehyde in our case.[38] APO-BrdU-TUNEL apoptosis assay was conducted with tissue sections on glass slides according to the manufacturer's instructions (Thermo Fisher Scientific GmbH).

Statistical methods

All results are shown as means of the log_{10} reduction of bacteria ± standard deviation using values of three independent experiments at least. Statistical analysis was performed using SPSS statistics software version 26.0.0 (IBM SPSS Software) using the non-parametric Mann–Whitney U test. *p* values

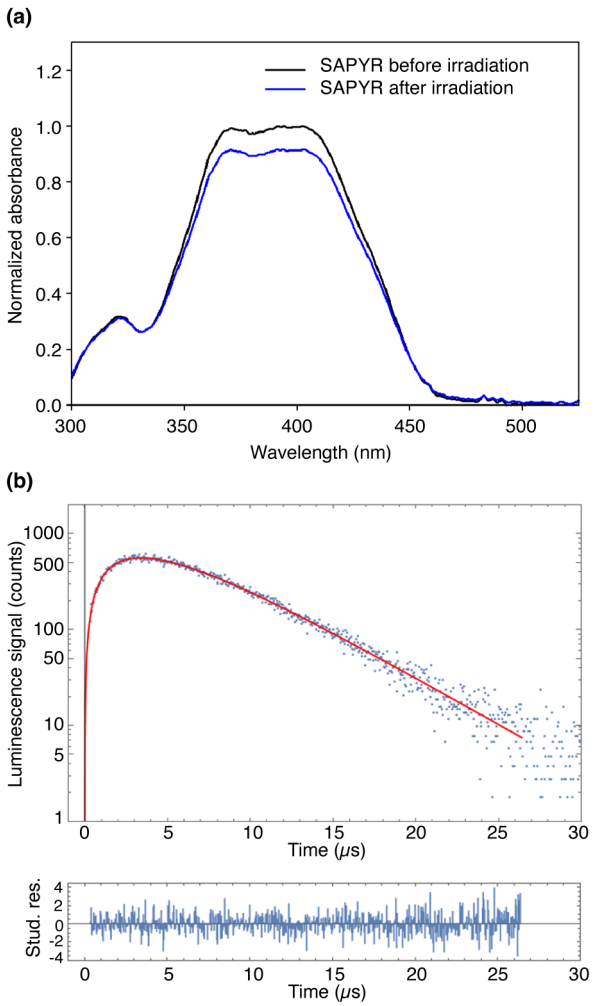

FIGURE 1 (a) Vis spectrum of the developed photodynamically active hydrogel with citrate before (black) and after (blue) light application of 10.8 J cm^{-2}. The *Y*-axis indicates the absorbance, and the wavelength is displayed by the *X*-axis in nm. (b) Time resolved luminescence signal of singlet oxygen at 1270 nm. The signal was fitted by two exponential functions (red line).[35] The fit algorithm revealed a rise time of the signal of 2.6 ± 0.2 μs and a decay time of 4.4 ± 0.2 μs with sufficient accuracy (studentized residuals, small chart below the luminescence signal). The latter time is equivalent to the life time of singlet oxygen generated by the photosensitizer SAPYR in the hydrogel.

were two-sided and considered statistically significant when <0.05.

For further experimental details, see Appendix S1.

RESULTS

Chemical and physical properties of the hydrogel

The absorption spectroscopy of the hydrogel with citrate revealed a certain photo-bleaching of the photosensitizer (Figure 1a). After irradiation (10.8 J cm^{-2}), the concentration of the PS declined by about 8.2%. The spectrum was not much altered with no spectral shifts indicating the chemical integrity of most of the photosensitizer molecules. The detection of luminescence additionally proved that singlet oxygen is generated in the complex environment like the present hydrogel. The luminescence signal showed a decay time of 4.4 ± 0.2 µs (Figure 1b).

PDI on inanimate surfaces

After irradiation of *S. aureus* in H_2O and sweat without histidine (Table 1), the mean reduction of the treated sample was 5.2 $\log_{10}$. Experiments with artificial sweat solution with histidine showed a somewhat smaller logarithmic reduction of 3.9 $\log_{10}$ (Table 1). PDI against *P. aeruginosa* cells achieved mean reduction values, roughly comparable to experiments with *S. aureus* ranging from ~4.4 to 5.1 $\log_{10}$ (Table 1). In none of the dark or light controls, the reduction exceeded mean values of 0.6 $\log_{10}$. The differences in PDI efficacy against the Gram-positive and the Gram-negative bacteria were statistically not significant ($p = 0.863$). PDI yielded no significant differences when comparing results without and with histidine for *S. aureus* ($p = 0.050$) and for *P. aeruginosa* ($p = 0.127$). The mean recovery loss over all experiments on inanimate surfaces was 0.6 ± 0.5 $\log_{10}$ for *S. aureus* and 0.5 ± 0.5 $\log_{10}$ for *P. aeruginosa*. The antibacterial efficacy of the hydrogel without the addition of citrate was tested showing less performance (Table S1).

Photodynamic efficacy on the human skin

Photodynamic inactivation inactivated *S. aureus* on human skin with a mean reduction in the range of about 4.2–4.8 $\log_{10}$, which indicates a somewhat smaller reduction compared to inanimate surfaces (Table 2). The application of synthetic sweat without histidine led to a similar efficacy compared to H_2O (Table 2). PDI inactivated *P. aeruginosa* on human skin with a mean reduction in the range of ~3.8 to 4.4 $\log_{10}$ (Table 2). The reduction of all PDI controls was less than 0.8 $\log_{10}$. The difference of results against the Gram-positive and the Gram-negative bacteria was statistically significant ($p = 0.040$). PDI yielded no significant differences when comparing results without and with histidine for *S. aureus* ($p = 0.050$) and for *P. aeruginosa* ($p = 0.200$). The mean recovery loss over all experiments on human skin was 0.2 ± 0.1 $\log_{10}$ for *S. aureus* and 0.5 ± 0.2 $\log_{10}$ for *P. aeruginosa*.

TABLE 1 Efficacy of PDI on inanimate surfaces.

		Log reduction		
Bacteria	**Modality**	**Dark control**	**Light control**	**PDI**
S. aureus	Pure water	0.5 ± 0.2	0.5 ± 0.2	5.2 ± 0.4
	Sweat solution	0.5 ± 0.2	0.5 ± 0.2	5.2 ± 0.5
	Sweat solution + histidine	0.6 ± 0.2	0.5 ± 0.2	3.9 ± 0.3
P. aeruginosa	Pure water	0.3 ± 0.2	0.5 ± 0.2	4.6 ± 0.4
	Sweat solution	0.2 ± 0.3	0.1 ± 0.2	5.1 ± 0.3
	Sweat solution + histidine	0.3 ± 0.2	0.4 ± 0.2	4.4 ± 0.5

TABLE 2 Efficacy of PDI on skin – ex vivo.

		Log reduction		
Bacteria	**Modality**	**Dark control**	**Light control**	**PDI**
S. aureus	Pure water	0.2 ± 0.2	0.2 ± 0.4	4.8 ± 0.3
	Sweat solution	0.2 ± 0.8	0.8 ± 0.5	4.8 ± 0.5
	Sweat solution + histidine	0.2 ± 0.1	0.3 ± 0.1	4.2 ± 0.2
P. aeruginosa	Pure water	0.0 ± 0.7	0.1 ± 0.5	4.3 ± 0.1
	Sweat solution	0.0 ± 0.2	0.3 ± 0.2	4.4 ± 0.2
	Sweat solution + histidine	0.0 ± 0.3	0.3 ± 0.6	3.8 ± 0.5

Histology

The NBTC staining indicated the viability of mitochondria by the formation of an intracellular, deep-blue precipitate. The intentionally damaged skin samples (induced necrosis) showed no blue staining and therefore no mitochondrial activity (Figure 2a). The untreated reference (positive control) (Figure 2b) as well as the dark control (Figure 2c), light control (Figure 2d) and the treated sample (Figure 2e) clearly showed viable mitochondria in the skin cells. Some differences in the staining intensity might be due to small differences in staining efficacy and/or variations in sample thickness.

The results obtained for the TUNEL assay showed that intentionally induced apoptosis leads to a clear, green staining of the nuclei in the histological sample that correlates with the DNA staining that was applied (Figure 3). All other samples, the untreated reference, dark and light controls and the treated samples showed no specific staining of the nuclei but exhibited certain unspecific staining of the cytoplasm and membrane components. An unspecific staining of cell cytoplasm in the *Stratum spinosum* showed no indication of apoptosis.

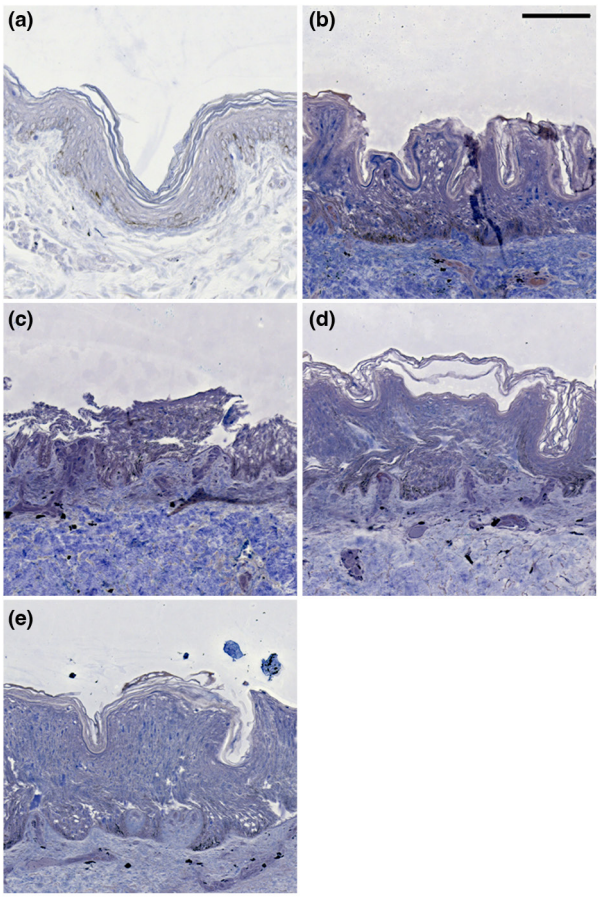

FIGURE 2 Micrographs of histological sections of the NBTC staining. (a) Depicts the skin sample in which necrosis was induced, (b) shows a piece of untreated skin, (c) was skin treated with photosensitizer hydrogel but without illumination (dark control) and (d) displays a sample where the skin was treated with light and hydrogel without photosensitizer (light control) and (e) shows a sample treated with light (10.8 J cm^{-2}) and the photodynamically active hydrogel. The scale bar in the upper right corner equals 100 µm.

The sample with induced apoptosis yielded the mean percentage of co-localized pixel of the nucleus staining versus the BrdU-TUNEL staining a high value of 15.8 ± 4.2. The values were 0.1 ± 0.1 for the dark control, 1.9 ± 1.3 for the light control, 0.3 ± 0.1 for the untreated reference and 2.3 ± 1.8 for the treated sample. Statistical analysis revealed that only the intentionally induced apoptosis differed significantly from PDI controls and PDI samples ($p < 0.05$).

DISCUSSION

The World Health Organization (WHO) assigns ESKAPE pathogens like *S. aureus* and *P. aeruginosa* to a group, which poses the greatest threat to human health and has a high or critical priority for research and development of new antibacterial strategies.[39] The results of the present study impressively showed that PDI with the novel hydrogel clearly inactivated two of these ESKAPE pathogens on human skin yielding a reduction of up to 4.8 $\log_{10}$. However, before this result on skin was successfully achieved, the photodynamic process was investigated step by step to identify possible obstacles.

Since PDI requires sufficient contact of photosensitizer molecules to the surface of bacteria cells, a hydrogel was created, which contained water, the water-soluble photosensitizer SAPYR, hydroxyethyl cellulose and citrate. In such a complex solution, the photochemical stability of the photosensitizer SAPYR and its ability to generate singlet oxygen was unknown. Firstly, the shape of the photosensitizer absorption spectrum after irradiation was almost not affected, only the photosensitizer concentration decreased by less than 10% (Figure 1a). Secondly, a photosensitizer like SAPYR exclusively generates biocidal singlet oxygen. When detecting its luminescence, none of the hydrogel ingredients caused quenching, because the detected lifetime of singlet oxygen (4.4 µs) was comparable to values in pure water (3.5–3.7 µs)[35,40] (Figure 1b). Thus, both experiments showed that singlet oxygen can take care of its main task of oxidatively destroying the bacteria.

However, when generated on the skin, singlet oxygen can encounter various other quenching substances including sebum, sweat, corneocyte debris and natural moisturizing factors and sweat substances.[41] Sebum contains free fatty acids, wax esters, diglycerides and triglycerides.[42] Sweat contains different monovalent and divalent ions, amino acids like histidine, and lactate.[43] Among these substances, amino acids like histidine are important because they show high rate constants for singlet oxygen quenching, while the values for fatty acids are negligibly smaller.[44] Also, divalent ions like magnesium and calcium in sweat already proved to disturb PDI. They bind to bacterial cells and thereby interfere with the attachment of photosensitizers to the bacterial membranes,[34] but this can be overcome by using a chelator

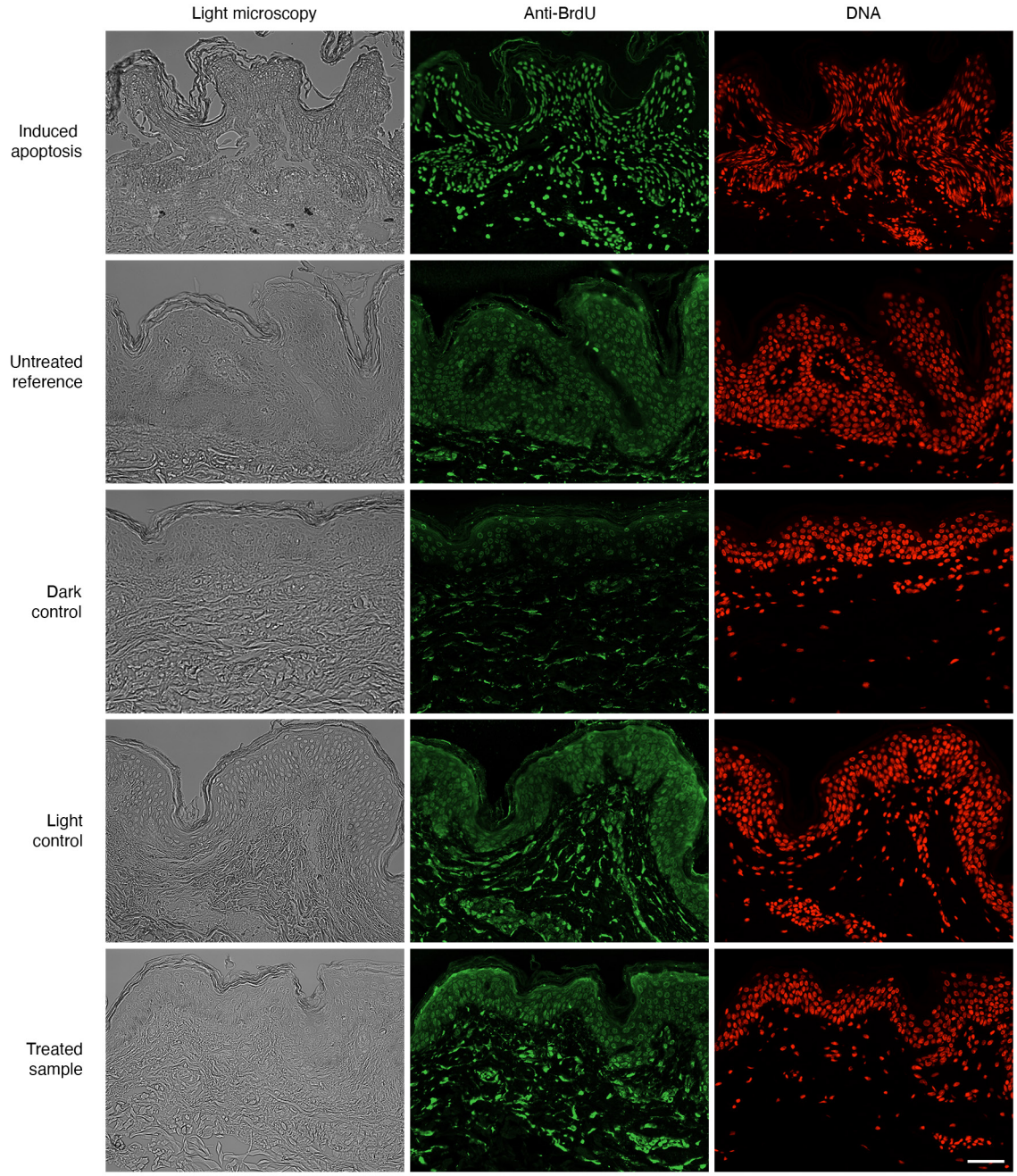

FIGURE 3 Results of the TUNEL assay. The left column displays the histological sample in the light microscope, the middle column indicates the anti-BrdU staining which detects apoptosis when clearly present in the nucleus, and the right column displays the DNA-specific staining corresponding to the nuclei of the skin cells. The first line was a sample where apoptosis was induced as described earlier, the second line displays an untreated control, the dark control sample (hydrogel with a photosensitizer, without light) is shown in the third line, the fourth line shows a sample that was subjected to light and a hydrogel without photosensitizer, and the last line depicts the results obtained for a treated sample (with light, with photosensitizer). The white scale bar in the lower right corner equals 25 µm.

like citrate.[45] In summary, the next experiments particularly focused on the influence of such sweat ions and histidine as exemplary amino acid.

As a first step, the antibacterial efficacy of the hydrogel was investigated on a neutral, inanimate surface (glass slide) to test the influence of these inhibitory substances on PDI. Without any quencher substance (only hydrogel components and H_2O), the irradiation of the inoculated surface showed a high reduction of both pathogens in the range from 4.6 to 5.2 $\log_{10}$ (Table 1). The addition of sweat components without histidine to bacteria suspension left the PDI efficacy nearly unchanged (Table 1). The chelator citrate also here prevented the inhibitory effect of the divalent ions, whereas the impact of the other substances was negligible.[45] This is confirmed when comparing the results shown in Table 1 (with citrate) and Table S1 (without citrate).

When using the sweat components with histidine, the PDI efficacy somewhat declined (Table 1). Obviously, the quencher rate constant of histidine reduced the number of singlet oxygen molecules, which should actually inactivate the bacteria via oxidation.[44] However, these differences in PDI efficacies were statistically not significant for *S. aureus* ($p = 0.050$) and *P. aeruginosa* ($p = 0.127$). Amino acids like histidine are usually very potent quenchers of singlet oxygen and may impair PDI efficacy in bacterial suspensions to a large extent.[45,46] This might be different for bacteria on surfaces. In bacterial suspensions, histidine and bacteria (loaded with singlet oxygen-generating SAPYR) can move freely towards each other and interact, which is not the case when dried on surfaces.

On the skin surface, PDI is also able to successfully inactivate both pathogens with ~4 to 5 $\log_{10}$. The efficacies are somewhat smaller compared to inanimate surfaces (Tables 1 and 2), whereat the differences are statistically significant for *P. aeruginosa* ($p = 0.011$) but not for *S. aureus* ($p = 0.730$). PDI efficacy is slightly better against Gram-positive compared to Gram-negative bacteria ($p = 0.04$) which is usually explained by the different structures of their cell envelopes.[47]

Importantly, the integrity of the human skin cells is not affected by PDI on top of the skin. The NBTC stain is a reliable and sensitive method to detect damage inside the skin.[48,49] No visible cell damage was detected as the NBTC staining showed intact mitochondria in the cells of epidermis and the upper dermis (Figure 2). Besides cell necrosis, also apoptosis of cells could be triggered by the PDI process on the skin surface or even already in the presence of bacteria.[50,51] However, the images of the TUNEL assay in the present study revealed no visible apoptosis in the skin cells (Figure 3). Comparable results were achieved when performing PDI on the cornea in an animal model.[52]

With regard to skin decolonization, PDI should be compared with other antibacterial agents, whose efficacy was studied on inoculated human skin.[53] However, such studies mainly used the uncritical K12 *Escherichia coli*, only a few studies used pathogens.[53] The reduction of bacteria like *S. aureus* or *P. aeruginosa* was about 0.5–3 $\log_{10}$ when using plain soap, triclosan and chlorhexidine, whereas about 3.5–4.4 $\log_{10}$ for povidone-iodine, benzalkonium chloride, and 2-propanol (Table 3). 60% isopropanol achieved a reduction of up to 6.8 $\log_{10}$ for *S. aureus* and *P. aeruginosa* (Table 3),[54] but disregarding a potential loss of bacteria on the skin, which was about 0.85 $\log_{10}$ for *S. aureus* and *P. aeruginosa* in another study.[55]

Noteworthy, the PDI efficacy in the present setting was achieved with a rather low concentration of the photosensitizer SAPYR at rather low radiant exposure. It is a major advantage of PDI that the concentration of the in situ generated biocidal singlet oxygen can be easily changed over a wide range by changing the photosensitizer concentration and the radiant exposure of light ($J\,cm^{-2}$). These correlations have already been successfully shown using SAPYR in PDI against other pathogens.[20,56]

Regarding adverse skin reactions, the use of soap, chlorhexidine or triclosan reduces skin hydration and impair the skin barrier. Chlorhexidine may be irritant to the skin possibly causing allergic reactions and can cause IgE-mediated urticaria/anaphylaxis.[53,57] Triclosan may cause local exacerbation of disease by eliciting irritative secondary reactions, especially in high concentrations.[58] The photodynamic hydrogel is water-based without any components that potentially irritate skin or mucosa of humans. Thus, PDI with hydrogel should be suitable for the safe decolonization of skin or mucosa. Further investigations on the penetration of photosensitizer into the epidermis and its effect on skin cells would need to be performed if the approach is to be employed in situations where the skin barrier is compromised.

Another important aspect is that antibiotics in dermatology are often used for prolonged courses, with significant

TABLE 3 Efficacy of PDI in comparison to other biocidal products on human skin.

Biocide/intervention	Test model	Test bacteria	Mean $\log_{10}$ reduction	Reference
Water and plain soap	Intact skin surface	*S. aureus*	0.52–3	[53]
Triclosan	Intact skin surface	*E. coli*	2.8	[70]
Chlorhexidine gluconate	Intact skin surface	*E. coli*	3.1	[70]
Povidone-iodine	Intact skin surface	MRSA	3.73	[71]
Benzalkonium chloride	Intact skin surface	*S. aureus*	4.12	[72]
Isopropanol 60%	Intact skin surface	*S. aureus*	6.36	[54]
Isopropanol 60%	Intact skin surface	*P. aeruginosa*	6.81	[54]
Ethanol 70%	Intact skin surface	*S. aureus*	3.81	[55]
Ethanol 70%	Intact skin surface	*P. aeruginosa*	4.28	[55]
Bacteriophages	Intact skin surface	*A. baumanii*	3	[73]
Molecular iodine	Nasal cavity, mouse model	MRSA	2.4	[74]
Bacteriotherapy for atopic dermatitis (AD)	Skin of AD patients	*S. aureus*	2	[75]
Cold atmospheric plasma	Intact skin surface	*P. aeruginosa*	2.9	[76]
PDI hydrogel	Intact skin surface	*S. aureus*	4.8	This work
PDI hydrogel	Intact skin surface	*P. aeruginosa*	4.4	This work

potential for microbiome alteration and antibiotic-related adverse effects.[1,9,10] Biocidal substances frequently used for the decolonization of human skin increasingly encounter the development of resistant bacteria, sometimes accompanied by cross-resistance to antibiotics.[6,59] Clones of *Escherichia coli* were recently detected, which were even less sensitive to povidone-iodine. These clones additionally showed cross-resistance with different antibiotics.[60] Pidot and co-authors for example found hospital isolates of *Enterococcus faecium* that were 10-fold more tolerant to killing by alcohol than other isolates.[61] In PDI, the oxidative action of singlet oxygen at the bacterial cell wall is highly unlikely to provoke resistance in bacteria as shown by several researchers.[16,62,63]

The use of the photodynamic hydrogel might be evaluated for skin and mucosa decolonization and treatment of superficial skin infections. PDI efficacy could be also studied against *Propionibacterium acnes* in acne vulgaris,[64] for infected burn wounds[65] or the treatment of atopic eczema in which the skin is extensively colonized by *S. aureus* up to 10^4 CFU cm^{-2}.[2,66] PDI might be also studied for the treatment of Hidradenitis suppurativa in which the skin is colonized by many different pathogens with a high level of resistance to several antibiotics.[67]

Photodynamic inactivation required about 20 min to achieve a substantial inactivation of pathogens on the skin. This PDI procedure is quite comparable to the well-known PDT of skin malignancies and should thus achieve acceptance in dermatology.[68] Noteworthy, an increase in light intensity can shorten the irradiation time to less than 1 min by keeping the antibacterial efficacy.[20]

Regarding the safety aspects of the hydrogel, hydroxyethyl cellulose is frequently used in the cosmetics and pharmaceutical industry.[69] Sodium citrate (E331) is an approved food additive in the EU. The photosensitizer SAPYR is not mutagenic (OECD 471), is no skin (OECD 431) and no eye (OECD 492) irritant, and local lymph node assay showed no skin sensitization (OECD 429).

CONCLUSIONS

The ongoing attempts of bacteria to escape the antiseptic and antibiotic measures on the skin require new and additional procedures that can effectively destroy such pathogens. The application of a present hydrogel might represent such an additional approach to fight against different pathogens on human skin. PDI is able to inactivate pathogenic bacteria regardless of their type and antibiotic resistance. The investigated PDI hydrogel achieved up to about 5 $\log_{10}$ reduction of important human pathogens on ex vivo skin samples. The current scientific knowledge about the efficacy and safety of PDI hydrogel has reached a level that allows the realization of studies on human skin under in vivo conditions.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors elect to not share data.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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