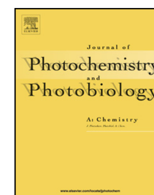
Journal of Photochemistry and Photobiology A: Chemistry 315 (2016) 52–58

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem



Bactericidal effect through non-uptake pathway with photofunctional silicon polymer that generates reactive oxygen species

Kang-Kyun Wang[a], Seung-Jin Jung[a], Jeong-Wook Hwang[a], Bong-Jin Kim[a], Da-Hee Kim[a], Il-Kwon Bae[b], Seok Hoon Jeong[c,**], Yong-Rok Kim[a,*]

[a] Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea
[b] Department of Dental Hygine, Silla University, College of Medical and Life Science, Busan 617-736, Republic of Korea
[c] Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University, College of Medicine, Seoul 120-752, Republic of Korea

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 24 August 2015
Accepted 4 September 2015
Available online 10 September 2015

Keywords:
Reactive oxygen species
Bactericidal effect
Photofunctional polymer
Non-uptake

ABSTRACT

We report bactericidal effect of photosensitizer (H_2TPP: 5,10,15,20-tetraphenyl-21H,23H-porphyrin) through non-uptake pathway and efficacy of the photofunctional silicon polymer to the decomposition of the formed biofilm and the suppression of the biofilm formation. The photofunctional silicon polymer (PSP), which is the silicon polymer embedded with a photosensitizer, is fabricated by a simple solvent swell-encapsulation-shrink method. Reactive oxygen generation from PSP was confirmed by using the decomposition reaction of 1,3-diphnyl-isobenzofuran (DPBF). Also, singlet oxygen generation which is one of the reactive oxygen species (ROS) from PSP is directly confirmed with time and wavelength resolved singlet oxygen phosphorescence spectroscopy. For the influence study of ROS under the non-uptake condition of photosensitizer (PS to bacteria), photodynamic inactivation (PDI) effect of PSP is evaluated for Gram-positive, Gram-negative bacteria, and fungi. Those microorganisms were inactivated by PSP within 1 h under the given power of laser light (63.7 mW/cm^2). Among the bacteria, especially, *Staphylococcus* aureus as the Gram-positive bacteria were completely disinfected under the given experimental condition. Furthermore, PSP successfully demonstrates the decomposition of the formed biofilm and the suppression of the biofilm formation with green light emitting diode (GLED, 3.5 mW/cm^2, λ_{max} = 517 nm, FWHM = 37 nm), which shows the practical application possibility of bactericidal material.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Reactive oxygen species that are commonly associated with photodynamic inactivation (PDI) have been applied to the sterilization of medical instrument and drinking water contaminated by bacteria, viruses, yeasts, and parasites [1–4]. The mechanism of PDI is now well understood: a photoactive material, referred as photosensitizer (PS), is delivered to the microorganism and then is irradiated with harmless visible light. The photosensitizer in its ground state absorbs light and undergoes intersystem crossing (ISC) with high efficiency to its triplet state. Then the reactive oxygen species (ROS) are generated from the triplet state of photosensitizer by energy transfer or charge transfer processes [5]. The generated ROS affect the integrity and the function of microbial cell walls, membranes, enzymes, and nucleic acids [6–8]. For the effective treatment of PDI, a photosensitizer should satisfy the factors of good water solubility, low cytotoxicity, high ROS quantum yield, photodynamic activation with long wavelength of visible light, and cell uptake efficiency [9–17]. Among the factors, the cell uptake efficiency of the photosensitizer has been considered to be a major component since the cell death by PDI is dominantly influenced by ROS generated from the inside of the cell. Therefore, various photosensitizers with the enhanced efficiency of cell uptake were synthesized by many researches groups [18–20]. However, Ivan P. Parkins recently reported a bactericidal effect of the polymer embedded with PS and gold nanoparticles. For efficacy evaluation of the photofunctional silicon polymer, Gram-negative and Gram-positive bacteria were dropped on to the surface of photofunctional silicon polymers and the laser light was irradiated to the samples for generation of ROS from the photofunctional silicon polymer. The results showed that both Gram negative and Gram positive bacteria were effectively inactivated by the photofunctional silicon polymer with the laser

* Corresponding author at: Department of Chemistry, Yonsei University, 50 Yonsero, Seodaemun-Gu, Seoul 120-749, Republic of Korea. Fax: +82 2 364 7050.
** Corresponding author at: Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 211 Eonjuro, Gangnam-Gu, Seoul 135-720, Republic of Korea. Fax: +82 2 2057 8926.
E-mail addresses: kscpjsh@yuhs.ac (S.H. Jeong), yrkim@yonsei.ac.kr (Y.-R. Kim).

http://dx.doi.org/10.1016/j.jphotochem.2015.09.005
1010-6030/© 2015 Elsevier B.V. All rights reserved.

light irradiation (250 mW). Also, it suggested that gold nanoparticles enhanced the PDI effect due to the enhanced yield of the generated ROS from PS [21–24].

In this study, we report the maximized bactericidal effect of the photosensitizer isolated in the silicon polymer through non-uptake pathway under mild experimental condition of low light energy and high amount of bacteria. For possibility of application, efficacy of the photofunctional silicon polymer tubes to the decomposition of the formed biofilm and the suppression of the biofilm formation were evaluated with low photon energy of green light emitting diode (GLED, 3.5 mW/cm^2).

2. Material and methods

2.1. Materials

The photosensitizer, 5,10,15,20-tetraphenyl-21H,23H-porphyrin (H_2TPP), and the silicon polymer were purchased from Aldrich and Yusin medical, respectively. The H_2TPP solution was prepared at concentration of 9.7×10^{-4} M in dichloromethane (Merck, HPLC grade). The photofunctional silicon polymers (PSP) were fabricated into two types. One is the cube form for the enhanced surface area to bacteria in solution and the other is the tube type for the application purpose in many cases. For experiment with the cube type of the photofunctional silicon polymer (PSPC), the silicon polymer cubes (area of 1 cm^2 and thickness of 1 mm) of six equal pieces were placed into the H_2TPP solution (1 mL), and then it was magnetically stirred in the dark condition. After 2 h, the silicon cubes were washed with ethanol for 5 times and then kept in the oven at 60 °C for 30 min under dark condition. On the other hand, for the circular tube type of the photofunctional silicon polymer tube (PSPT), the inner pore of the silicon polymer tube (inner circular diameter of 3 mm, length of 30 mm) was filled with H_2TPP solution (1 mL). The sample was kept in the dark for 2 h and then the solution was removed from the silicon polymer tube. The silicon polymer tube was also washed and dried as the same process with the cube type silicon polymer.

2.2. Spectroscopy measurements

Steady-state absorption and emission spectra of 5,10,15,20-tetraphenyl-21H,23H-porphyrin (H_2TPP, Aldrich, <99%) in dichloromethane (Merck, <99.9%) were obtained by using a UV–vis spectrophotometer (Hitachi, U-2800) and a spectrofluorimeter (Hitachi, F-4500), respectively. For the PSP samples, the diffuse reflectance spectra were recorded by a UV–vis spectrophotometer (Jasco, V-550) equipped with an integrating sphere (Jasco, ISV-469).

2.3. Detection of reactive oxygen generation

Degradation of 1,3-diphenyl-isobenzofuran (DPBF), a reactive oxygen quencher, was studied with PSPC [25,26]. 1.5 mL of EtOH solution containing PSPC and DPBF (3.9×10^{-5} M) were introduced into a quartz cuvette cell in the dark condition. The light source for the irradiation to PSPC was the green light emitting diode (GLED, 3.5 mW/cm^2). At every 5 min of irradiation, the absorption spectra of DPBF were monitored with a UV–vis spectrophotometer (Hitachi, U-2800). Singlet oxygen generation which is one of the reactive oxygen species was directly measured with the phosphorescence signal from the deexcitation of singlet oxygen. The Nd-YAG (Continuum surelite II-10, 10 Hz, 7 ns FWHM pulse) pumped optical parametric oscillator (OPO) laser (Continuum OPO plus, 5 ns FWHM pulse) was utilized as an excitation source for detection of the time and wavelength-resolved singlet oxygen phosphorescence. Phosphorescence signals were collected perpendicular to the excitation beam and detected with a monochromator (Optometrics LLC, mini-chrom04) and a NIR-PMT (Hamamatsu, H10330A). The signals were acquired by a 500 MHz digital oscilloscope (Agilent technology, DS07052A) and transferred to a computer for data analysis [27].

2.4. Leaching test

In order to check the *release* of H_2TPP from the fabricated PSPC due to the physical collisions and other factors, PSPCs were placed in 1 mL of phosphate buffer saline solution (PBS) with the magnetic stirring for 24 h at 37°C, which is the same condition to the PDI experiments. Then the collected PBS was mixed with dichloromethane (1 mL) to extract H_2TPP in PBS. The dichloromethane solution was measured by a UV–vis spectrophotometer. Also, the release test of H_2TPP from PSPC in EtOH solution was performed with the same procedure as above. In order to evaluate the leaching of H_2TPP from PSPC, PSPCs were placed in the PBS with the laser irradiation condition (510 nm, 63.7 mW/cm^2, beam spot (r) = 0.5 mm) for 1 h. Then the collected PBS was used to immerse *Staphylococcus aureus* (*S. aureus*, ATCC 25923, 2.0×10^5 cfu/mL) and then it was treated with the laser light (510 nm, 63.7 mW/cm^2) in order to test the PDI effect with the possible photosensitizer which might be eluted in PBS. 100 μL of the suspension was inoculated into blood agar plate which was then incubated aerobically at 37 °C for 24 h to determine the viability. Also, the effluent test of H_2TPP in PSPT was performed with the same procedure as above. In order to evaluate the extraction of H_2TPP from the surface of PSPT by *S. aureus* biofilm (ATCC 25923), the formed biofilm was detached from the surface of PSPTs and the collected biofilm was homogeneously dispersed in PBS solution (2 mL) under the rigorous shaking condition. For the PS extraction test, GLED (3.5 mW/cm^2) was irradiated to the bacteria solution (1 mL) and the other 1 mL of bacterial solution was placed in a dark condition for the reference. After 2 h, 10 μL of each suspension was inoculated into blood agar plate which was then incubated aerobically at 37 °C for 24 h to determine the viability.

2.5. Biological assay

A light source for the irradiation to PSP was a nanosecond Nd-YAG-pumped OPO laser (OPOTEK, opolett 355II, 20 Hz, 5 ns FWHM pulse). The total power output for the irradiation was measured with a laser power meter (Ophir-opironics Ltd., Nova, Israel). For the photodynamic bactericidal efficiency of PSP, *S. aureus* (*S. aureus*, ATCC 25923), *Escherichia coli* (*E. coli*, ATCC 25922), and *Candida albicans* (*C. albicans*, ATCC 90228) were used. The bacteria were grown aerobically in Brain Heart Infusion (BHI) broth (Oxoid) at 37 °C for 24 h. These overnight cultured bacteria were diluted in PBS and the resulting bacterial suspensions contained approximately 2.0×10^5 cfu/mL. The number of bacteria was determined with turbidity meter (Biomerieux, DensiCHEK plus). PSPCs were immersed in the PBS (1 mL) that included *S. aureus* or *E. coli* or *C. albicans*, and then it was magnetically stirred in the dark condition. After 30 min, the laser light (510 nm, 63.7 mW/cm^2) was irradiated to the samples for 1 h and then 100 μL of the suspension was inoculated into the blood agar plates in the case of *S. aureus* and *C. albicans*, and MacConkey agar plates were utilized for *E. coli*. After aerobic incubation at 37 °C for 24 h, the bacteria colonies were counted.

2.6. Decomposition effect of the formed biofilm and suppression effect of the biofilm formation

In order to evaluate the decomposition effect of the formed biofilm and the suppression effect of the biofilm formation,

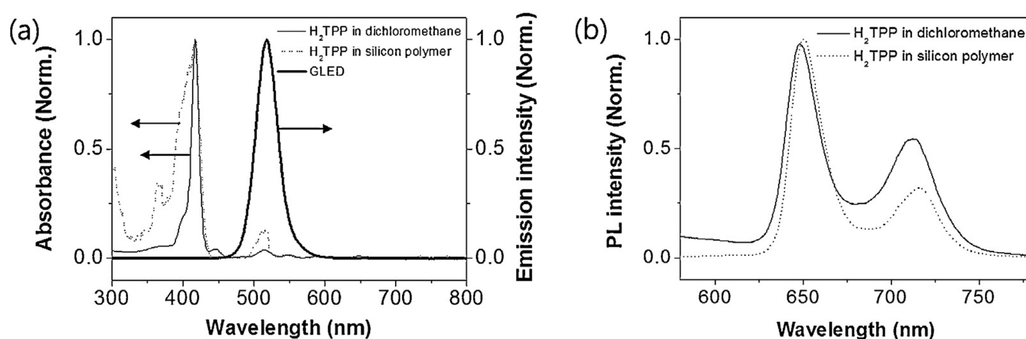


Fig. 1. (a) Steady-state absorption and (b) emission spectra of H_2TPP in dichloromethane (solid line) and H_2TPP in silicon polymer (dashed line).

Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) were used. For decomposition of the formed biofilm on the inner surface PSPT, PSPTs and the green light emitting diode (GLED) were utilized. To generates ROS, the GLED light was irradiated to PSPTs through cut-off filter in order to block the residual UV light of the GLED (<400 nm, CVI). Power density of the GLED light was also measured at the sample position with the same power meter as above. PSPTs were included in the solution of *S. aureus* or *E. coli*. For the formation of the biofilm on the inner surface of PSPT, PSPTs were filled with the bacterial solution, and then placed in a dark condition at 37 °C for 24 h [28,29]. After 24 h, PSPTs were cut (d = 1 cm) and opened. The biofilm formation on the inside surface of PSPT was confirmed by the existence of the extracellular polymeric substance (EPS) stained with a Texas Red-conjugated concanavalin A which was observed by fluorescence microscopy (Carl Zeiss, Axio imager Z2m) [30]. The GLED light (3.5 mW/cm^2) was irradiated to the bacterial solution filled inside of PSPT from the side direction for 2 h and then the bacterial solution within PSPT was removed. And then, PSPTs were further washed with PBS to remove non-adhesive residual bacteria on the inner surface of PSPT except the biofilm of PSPT. It was divided into three equal parts. Each part of PSPT was immersed to 3 mL of PBS with strong agitation for 10 min [31,32]. 100 μL of the suspension was inoculated on the blood agar plates (*S. aureus*)/MacConkey agar (*E. coli*) plates. After aerobically incubation at 37 °C for 24 h, bacteria colonies were counted. For the suppression effect of the biofilm formation on the inner surface of PSPT, the GLED light was irradiated to PSPT filled with the bacterial solution without the pre-incubation process.

3. Results and discussion

The photosensitizer, 5,10,15,20-tetraphenyl-21H,23H-porphyrin (H_2TPP), was embedded in a silicon polymer by swell-encapsulation-shrink method. Photophysical properties of the fabricated PSPs were characterized by steady-state absorption and emission spectroscopies. Fig. 1a shows the characteristics of the H_2TPP absorption bands: the soret band at 417 nm and the Q bands at 510, 545, 588, and 642 nm are nearly identical for both H_2TPP in CH_2Cl_2 solution and the silicon polymer. The fluorescence emission bands of H_2TPP in the silicon polymers at 650 nm and 720 nm are also similar to those of H_2TPP in CH_2Cl_2 (Fig. 1b). The broadened feature of the soret band and the Q bands of H_2TPP within the silicon polymer can be ascribed to the inhomogeneity of the inter- and/or intra-molecular potentials and the red shifted emission peaks can be explained by the stabilization effect of the matrix and/or the aggregation of porphyrin molecules [33,34].

The degradation of DPBF as the ROS quencher was studied for the proof of ROS generation from PSPC. DPBF reacts irreversibly with ROS generated by photo-excited PSPC and the reaction can easily be followed by measuring the decrease in optical density of the DPBF absorption at 415 nm. Fig. 2 indicates the photo-degradation rate of DPBF as a function of irradiation time in the presence of PSPC. The result shows that the light only and the PSPC with light induce the degradation of DPBF whereas DPBF does not show any degradation without the light irradiation. The release of H_2TPP from PSPC was tested in the EtOH solution. The UV–vis absorption measurements for 1 h in the EtOH solution indicate that H_2TPP is not released from the fabricated PSPC within the given

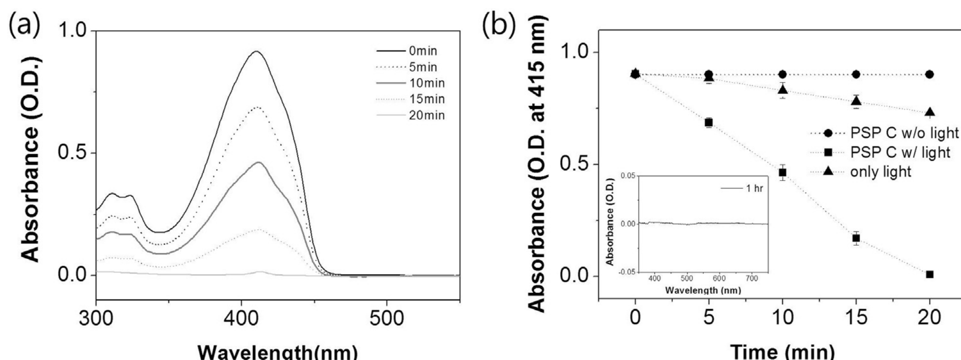


Fig. 2. (a) Reaction time dependent UV–vis spectra of DPBF in the presence of PSPC in EtOH solution with the light. (b) The decay curves of DPBF absorption O.D. at 415 nm as a function of irradiation time in the presence of PSPC without GLED light (circle symbol, dot line) and in the presence of PSPC with GLED light (square symbol, dot line), and only GLED light irradiation (triangle symbol, dot line). The inset presents the absorption O.D.

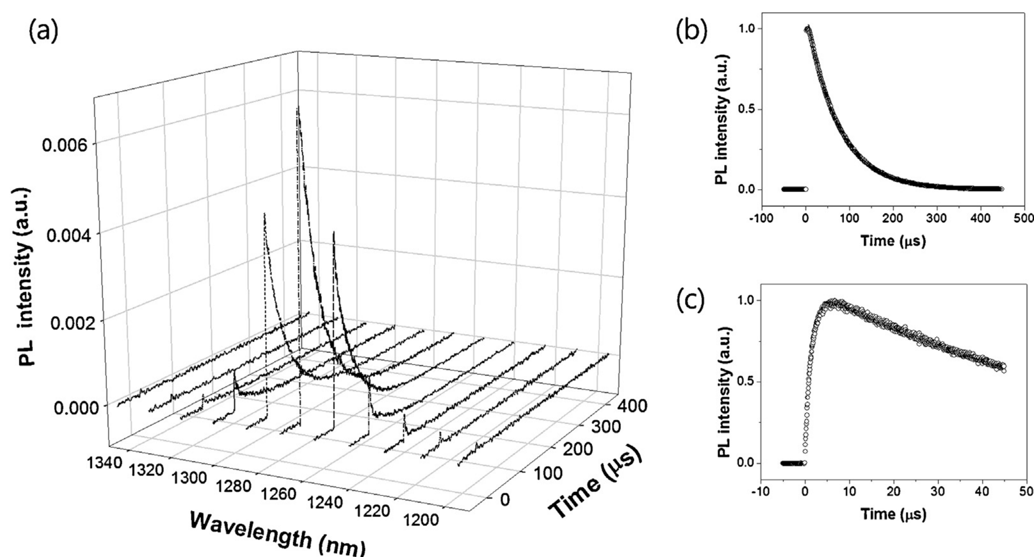


Fig. 3. (a) Time and wavelength-resolved singlet oxygen phosphorescence from PSPC in water. (b) The singlet oxygen decay detected at 1270 nm includes a single exponential fitted line (solid line). (c) The singlet oxygen rise detected at 1270 nm.

experimental condition as shown in Fig. 2(b). The most direct measurement method of singlet oxygen that is one of the ROS generated from the PSP is the detection of the phosphorescence from the deactivation of singlet oxygen molecules induced by the photo-excited H_2TPP within the silicon polymer. As shown in Fig. 3, the singlet oxygen phosphorescence signal from PSP was measured in water at various detection wavelengths between 1195 and 1345 nm. The phosphorescence decay signals were fitted to a single exponential function that resulted 42 μs of decay time in the silicon polymer matrix. The singlet oxygen lifetime of 42 μs is due to the polymer environment condition that is the OH-free condition as reported in the references [35,36].

Effluent of H_2TPP from PSP was checked in the PBS. The UV–vis absorption measurements for 24 h in the PBS (pH 7.0) were utilized to verify that H_2TPP did not leach out of PSP as shown in Fig. 4a. The absorption peaks of H_2TPP were not observed during the checking period of 24 h although the extinction coefficient of H_2TPP in dichloromethane was 542,000 at 417 nm [37]. Such high extinction coefficient results that the optical density (O.D.) of 0.01 for H_2TPP was equal to 0.1 ppm. Therefore, it was expected that the

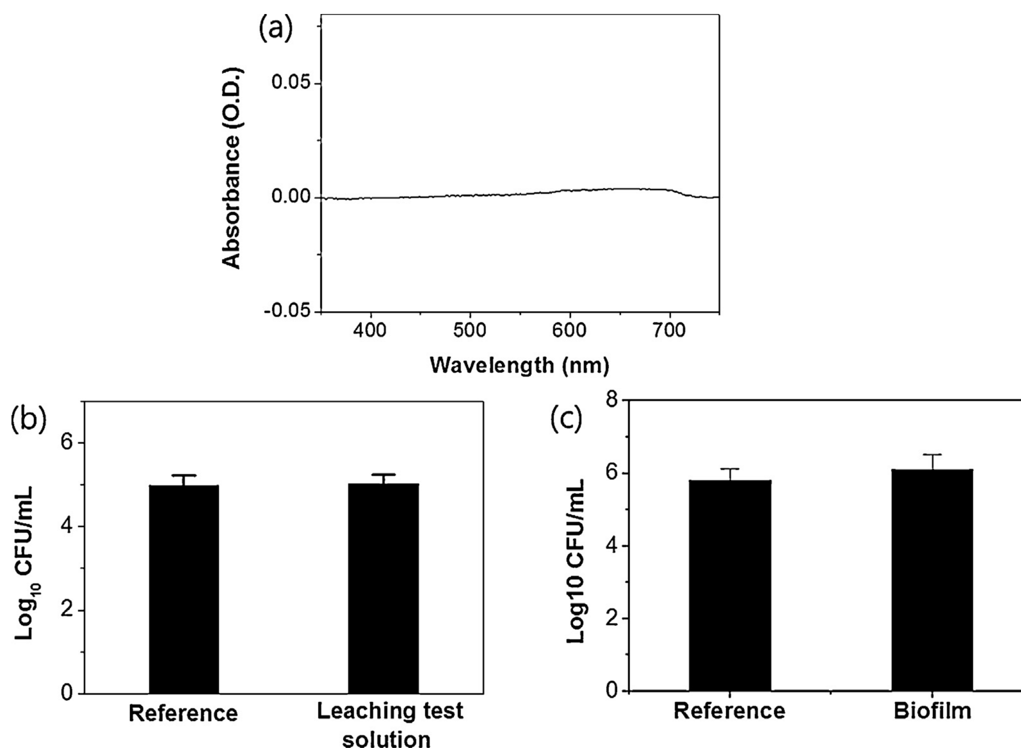


Fig. 4. (a) Absorption spectra of the leaching test solution, (b) the survival of *S. aureus* in the leaching test solution, and (c) the survival of *S. aureus* in the extraction test of PS by the biofilm.

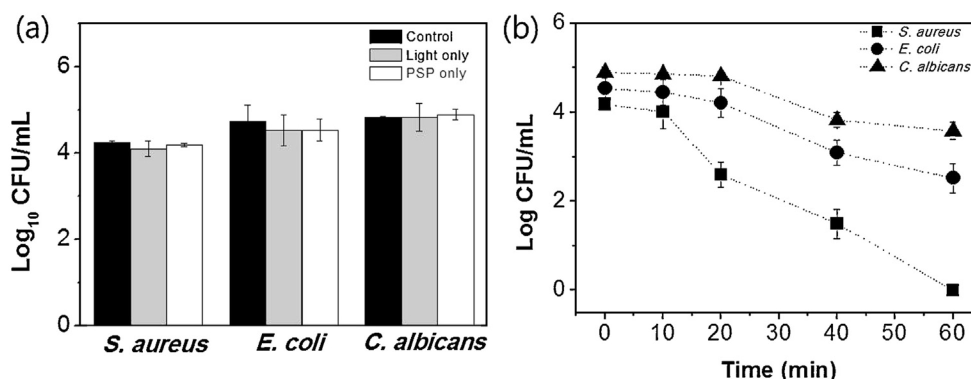


Fig. 5. (a) Bactericidal effect only by irradiation light or PSP material itself: control (black bar), light only for 1 h w/o PSP (grey bar), PSP only for 1 h w/o light (white bar), (b) survival curves of *S. Aureus* (square symbol, dot line), *E. coli* (circle symbol, dot line), and *C. albicans* (triangle symbol, dot line) in solution with PSPC and 63.7 mW/cm^2 of light irradiation at 510 nm. The values represent the mean ± standard deviation from three separate experiments.

photosensitizers were not leached from the silicon polymers within our concentration resolution of 0.1 ppm. The leaching of H_2TPP from PSP under the light irradiation condition was also confirmed by the biological assay. PSPCs were immersed in the PBS and irradiated by laser light (510 nm, 63.7 mW/cm^2) for 1 h. Then the collected PBS was used to immerse *S. aureus*. The PBS including *S. aureus* was treated with the laser light (510 nm, 63.7 mW/cm^2) for PDI. The CFU (clot forming unit) value of the PDI treated bacterial solution did not decrease compared with the control as shown in Fig. 4b. No extraction of H_2TPP from the surface of PSPT by the biofilm was confirmed by the biological assay. The detached biofilm was immersed in the PBS(1 mL) and irradiated by GLED light (3.5 mW/cm^2) for 2 h. The CFU value of the bacterial solution for the extraction test did not decrease compared with the reference as shown in Fig. 4c.

To demonstrate the influence of ROS to the bacteria without the uptake of the photosensitizer, Gram-positive bacteria of *S. aureus* (ATCC 25923), Gram-negative bacteria of *E. coli* (ATCC 25922), and fungi of *C. albicans* (ATCC 90228) were used [38–41]. In order to check the bactericidal effect of the irradiation light and PSP material itself, the light was irradiated to bacteria without PSP and PSP was also stirred with bacteria under dark condition for 1 h. As shown in Fig. 5a, the viabilities of all bacteria were not changed by the irradiation light or PSP itself. The number of *S. aureus* in the PBS containing PSPC effectively decreased from 10^4 CFU to 10^0 CFU, whereas those of *E. coli* and *C. albicans* were decreased from 10^5 CFU to 10^4–10^3 CFU within 1 h and the laser irradiation condition of 63.7 mW/cm^2 as shown in Fig. 5b. The result showed that Gram-negative bacteria of *E. coli* and fungi of *C. albicans* were less influenced by ROS than Gram-positive bacteria of *S. aureus*. It is

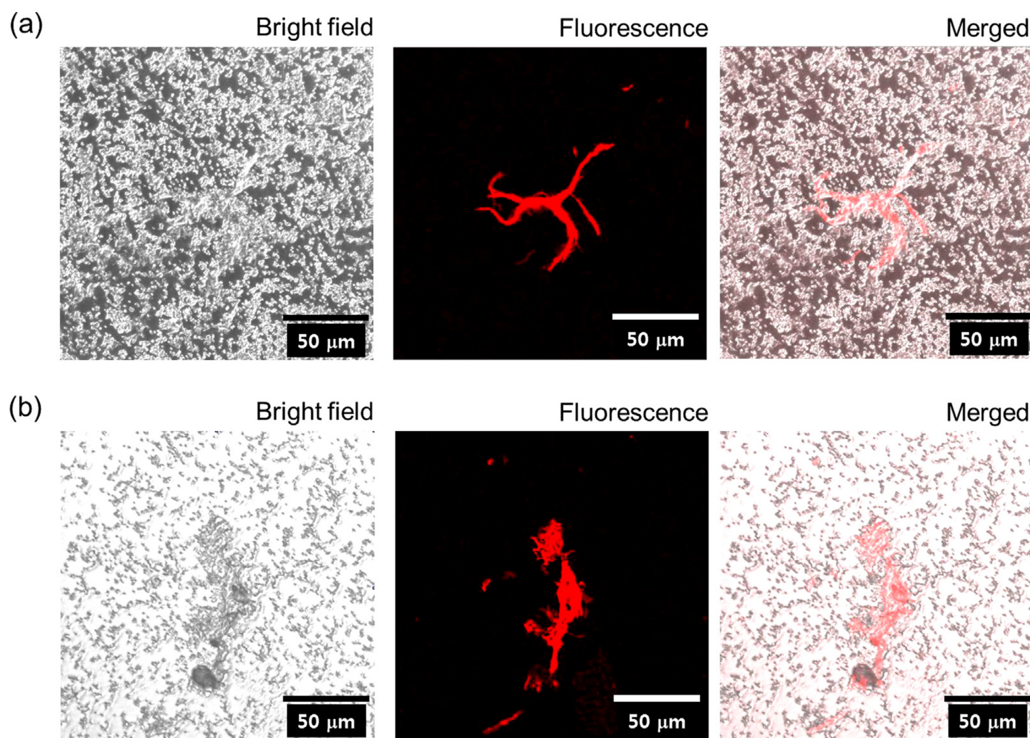


Fig. 6. Bright field, fluorescence, and merged microscope imges of (a) *S. aureus* biofilm and (b) *E. coli* biofilm on the inner surface of PSPT after pre-incubation for 24 h: EPS was stained with Texas Red-conjugated concanavalin A (λ_{ex} = 545 nm).

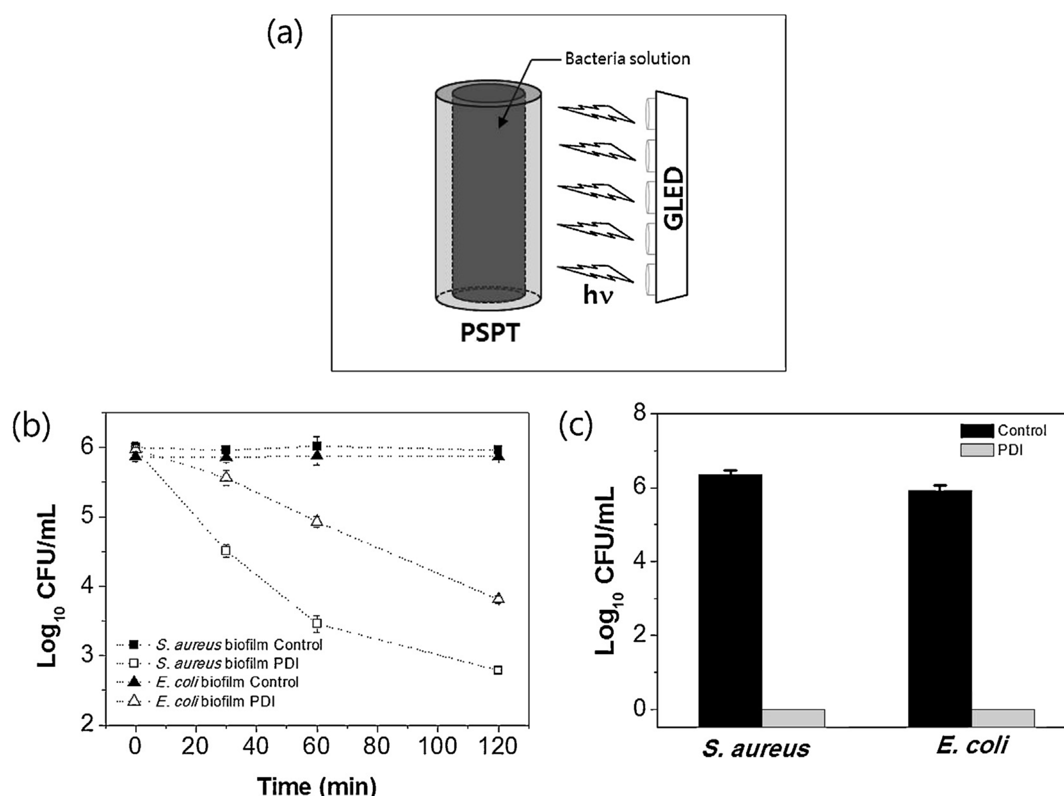


Fig. 7. (a) Experimental scheme for the PDI effect of PSPT. (b) Survival curves of *S. aureus* (square symbol, dot line) and *E. coli* (triangle symbol, dot line) on the inner surface of PSPT after pre-incubation for 24 h: control (w/o light), PDI (w/ light, GLED 3.5 mW/cm^2), (c) viabilities of *S. aureus* and *E. coli* on the inner surface of PSPT without pre-incubation: control (w/o light, 24 h incubation), PDI (w/ light, 3.5 mW/cm^2 of GLED light, 24 h incubation). The experiments were repeated by 3 times.

possibly due to the lipopolysaccharide (LPS) on the surface of *E. coli* and β-glucans on surface of *C. albicans* that are the efficient quenching site to ROS generated from PSPC [42–45]. Both cases of Gram-positive and Gram-negative bacteria were influenced by PSPC with the low dose of light under the non-uptake condition although the efficiency of bactericidal effect depended on the type of bacteria.

Formation of the biofilm on the inner surface of PSPT was observed by fluorescence microscope as shown in Fig. 6. EPS was visualized by staining with a Texas Red-conjugated concanavalin A. As shown in Fig. 7a, the number of *E. coli* and *S. aureus* were decreased from 10^6 CFU to 10^4 and 10^3 CFU within 2 h at 3.5 mW/cm^2 of GLED light, respectively. The survival rate of *E. coli* biofilm was slower than that of the *S. aureus* biofilm due to the reason described above [43–45]. In order to check the suppression effect of the biofilm formation on the inner surface of PSPT, PSPT that contained *E. coli* or *S. aureus* solution were irradiated by 3.5 mW/cm^2 of GLED light since the initial condition of the inclusion of the bacterial solution. After 24 h of incubation with the light on and the bacterial solution, the inner surface of PSPTs were analysed to check the existence of the bacteria. As shown in Fig. 7b, both *E. coli* and *S. aureus* solution did not induce any of the biofilm formations on the inner surface of PSPT for this suppression experiment.

4. Conclusion

We have demonstrated that the fabricated PSPs successfully generate ROS including singlet oxygen in the phosphate buffer solution. ROS generated from PSPs shows the strong bactericidal effect to *S. aureus*, *E. coli*, and *C. albicans* under the non-uptake condition. The photo-induced ROS is continuously generated when the light is irradiated, and the generated ROS consistently influences bacteria. And, also, the ROS constantly collide with bacteria with magnetic stirring. Therefore, the resulting bactericidal effect eventually leads the decomposition and the suppression of the biofilms on PSPTs. This study may provide important information on the practical aspects of the applications in the fields of bactericidal films and coatings.

Acknowledgment

This work was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A121133).

References

[1] S.C. de Souza, J.C. Junqueira, I. Balducci, C.Y. Koga-Ito, E. Munin, A.O.C. Jorge, J. Photochem. Photobiol. B: Biol. 83 (2006) 34–38.
[2] T. Maisch, S. Hackbarth, J. Regensburger, A. Felgenträger, W. Bäumler, M. Landthaler, B. Röder, J. Dtsch. Dermatol. 9 (2011) 360–366.
[3] T.W. Wong, H.J. Huang, Y.F. Wang, Y.P. Lee, C.C. Huang, C.K. Yu, J. Antimicrob. Chemother. 65 (2010) 2176–2182.
[4] M. Wainwright, Int. J. Antimicrob. Agents 21 (2003) 510–520.
[5] C. Schweitzer, R. Schmidt, Chem. Rev. 103 (2003) 1687–1757.
[6] N. Li, T.D. Oberley, J. Cell. Physiol. 177 (1998) 148–160.
[7] Y. Chen, E. McMillan-Ward, J. Kong, S.J. Israels, S.B. Gibson, J. Cell Sci. 120 (2003) 4155–4166.
[8] T. Saetre, H. Kaehler, S.J. Foster, T. Lyberg, Scand. J. Clin. Lab. Investig. 60 (2000) 311–322.
[9] K.E. Borbas, V. Chandrashaker, C. Muthiah, H.L. Kee, D. Holten, J.S. Lindsey, J. Org. Chem. 73 (2008) 3145–3158.
[10] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, J. Natl. Cancer Inst. 90 (1998) 889–905.
[11] J. Shi, T.W.B. Liu, J. Chen, D. Green, D. Jaffray, B.C. Wilson, F. Wang, G. Zheng, Theranostics 1 (2011) 363–370.
[12] E. Reddi, J. Photochem. Photobiol. B: Biol. 37 (1997) 189–195.
[13] J. Zhao, W. Wu, S. Guo, Chem. Soc. Rev. 42 (2013) 5323–5351.
[14] P. Majumdar, R. Jomula, J. Zhao, J. Mater. Chem. C 2 (2014) 5982–5997.

[15] S.G. Awuah, Y. You, RSC Adv. 2 (2012) 11169–11183.
[16] O.J. Stacey, S.J.A. Pope, RSC Adv. 3 (2013) 25550–25564.
[17] Y. You, W. Nam, Chem. Soc. Rev. 41 (2012) 7061–7084.
[18] C. Pavani, A.F. Uchoa, C.S. Oliveira, Y. Iamamoto, M.S. Baptista, Photochem. Photobiol. Sci. 8 (2009) 233–240.
[19] I. Laville, S. Pigaglio, J. Blais, F. Doz, B. Loock, P. Maillard, D.S. Grierson, J. Blais, J. Med. Chem. 49 (2006) 2558–2567.
[20] R. Schneider, F. Schmitt, C. Frochot, Y. Fort, N. Lourette, F. Guillemin, J. Muller, M. Barberi-Heyob, Bioorg. Med. Chem. 13 (2005) 2799–2808.
[21] S. Perni, C. Piccirillo, J. Pratten, P. Prokopovich, W. Chrzanowski, I.P. Parkin, M. Wilson, Biomaterials 30 (2009) 89–93.
[22] J. Mosinger, O. Jirsak, P. Kubat, K. Lang, B. Mosinger Jr., J. Mater. Chem. 17 (2007) 164–166.
[23] A. PreuB, L. Zeugner, S. Hackbarth, M.A.F. Faustino, M.G.P.M.S. Neves, J.A.S. Cavaleiro, B. Roeder, J. Appl. Microbiol. 114 (2012) 36–43.
[24] A. Felgentrager, T. Masish, A. Spath, J.A. Schroder, W. Baumler, Phys. Chem. Chem. Phys. 16 (2014) 20598–20607.
[25] T. Ohyashiki, M. Nunomura, T. Katoh, Biochim. Biophys. Acta 1421 (1999) 131–139.
[26] A. Gomes, E. Fernandes, J.L.F.C. Lima, J. Biochem. Biophys. Methods 65 (2005) 45–80.
[27] K.K. Wang, K.H. Choi, H.W. Shin, B.J. Kim, J.E. Im, S.L. Oh, N.S. Park, M. Jung, J.B. Oh, M.J. Lee, H.K. Kim, Y.R. Kim, Chem. Phys. Lett. 482 (2009) 81–86.
[28] K.M. Kruszewski, L. Nistico, M.J. Longwell, M.J. Hynes, J.A. Maurer, L. Hall-Stoodley, E.S. Gawalt, Mater. Sci. Eng. C 33 (2013) 2059–2069.
[29] S. Furukawa, Y. Akiyoshi, M. Komoriya, H. Ogihara, Y. Morinaga, Food Control 21 (2010) 669–672.
[30] F. Cieplik, A. Späth, J. Regensburger, A. Gollmer, L. Tabenski, K.A. Hiller, W. Bäumler, T. Maisch, G. Schmalz, Free Radic. Biol. Med. 65 (2013) 477–487.
[31] J. Ma, Z. Tong, J. Ling, H. Liu, X. Wei, Arch. Oral Biol. 60 (2015) 1075–1081.
[32] D. Field, N. Gaudin, F. Lyons, P.M. O'Connor, P.D. Cotter, C. Hill, R.P. Ross, PLoS One 10 (2015) 1–17.
[33] R.F. Khairutdinov, N. Serpone, J. Photochem. Photobiol. B: Biol. 103 (1999) 761–769.
[34] H.M. Sung-Suh, Z. Luan, L. Kevan, J. Photochem. Photobiol. B: Biol. 101 (1997) 10455–10463.
[35] F. Manj'on, M.S. Mag̃ana, D.G. Fresnadillo, G. Orellana, Photochem. Photobiol. Sci. 9 (2010) 838–845.
[36] F. Manj'on, L. Villen, D.G. Fresnadillo, G. Orellana, Environ. Sci. Technol. 42 (2008) 301–307.
[37] S.A. Wolff, E.A. Alema'n, D. Banerjee, P.L. Rinaldi, D.A. Modarelli, J. Org. Chem. 69 (2004) 4571–4576.
[38] S. George, M.R. Hamblin, A. Kishen, Photochem. Photobiol. Sci. 8 (2009) 788–795.
[39] M.R. Hamblin, D.A. O'Donnell, N. Murthy, K. Rajagopalan, N. Michaud, M.E. Sherwood, T. Hasan, J. Antimicrob. Chemother. 49 (2002) 941–951.
[40] A. Minnock, D.I. Vernon, J. Schofield, J. Griffiths, J.H. Parish, S.B. Brown, J. Photochem. Photobiol. B: Biol. 32 (1996) 159–164.
[41] F. Gad, T. Zahra, T. Hasan, M.R. Hamblin, J. Antimicrob. Chemother. 48 (2004) 2173–2178.
[42] F. Shaki, J. Pourahmad, J. Pharm. Res. 12 (2013) 131–140.
[43] K.H. Choi, H.J. Lee, B.J. Park, K.K. Wang, E.P. Shin, J.C. Park, Y.K. Kim, M.K. Oh, Y.R. Kim, Chem. Commun. 48 (2012) 4591–4593.
[44] T. Maisch, R.M. Szeimies, G. Jori, C. Abels, Photochem. Photobiol. Sci. 3 (2004) 907–917.
[45] T.A. Dahl, W.R. Midden, P.E. Hartman, J. Bacteriol. 171 (1989) 2188–2194.