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

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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.

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

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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,