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Fabrication and characterization of antimicrobial surface-modified stainless steel for bio-application

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ABSTRACT

We report a photofunctional stainless steel (PSS) that has antimicrobial property which is provided by reactive oxygen species (ROS) generated from the photosensitizer (PS). For the fabrication of the photofunctional stainless steel, the photosensitizer of hematoporphyrin (HP) was covalently bonded to the surface of 316L stainless steel (316LSS) through an esterification reaction. The PSS plate was investigated by x-ray photoelectron spectroscopy (XPS), reflectance UV–Vis absorption, and fluorescence spectroscopy. ROS generation from the PSS plate was studied by using the decomposition reaction of 1,3-diphenyl-isobenzofuran (DPBF). The results suggest that the immobilized photosensitizer molecules on the surface of the PSS plate still possess their intrinsic optical and functional properties including the ROS generation. The antimicrobial property of the PSS plate was successfully demonstrated with the decomposition of biofilm and the suppression of the biofilm formation on the surface of the PSS plate.

1. Introduction

Centers for Disease Control and Prevention (CDC) reported that Healthcare-Associated Infections (HAIs) occur while receiving medical treatment in a hospital facility [1]. HAIs such as pneumonia, surgical-site infections, urinary tract infection, and gastrointestinal infections were annually arisen up to 38.1% of 1.7 million hospitalized patients in the US [2,3]. Morbidity or mortality rate of HAIs has been increasing constantly [4] and, particularly, the medical device related infections such as central-catheter-associated bloodstream infection, catheter-associated urinary tract infection, and ventilator-associated pneumonia, etc., have been accounted for 25.6% in HAIs [2]. To prevent the infections, medical devices and materials were commonly sterilized by alcohol solutions, autoclaving, ultraviolet (UV) exposure, and gamma irradiation in most hospitals [5].

However, these methods have the limitations to be applied for the implanted medical materials such as surgical pin, implant-tooth in the body. Also, some medical devices including polymers and/or high sensitive medical instruments cannot be sterilized by alcohols and UV light due to the possible damages to the materials and/or malfunction of medical instruments. To prevent contamination of bacteria, many researches have been performed with the surface modification technologies such as drug coated catheter [6], super-hydrophobic catheter for inhibition of bacterial biofilm [7], enzyme-based antimicrobial materials [8], and metal coating stainless steel, etc., [9,10]. However, the drug-coated biomaterials work only for a limited time of period due to the fixed amount of drug and moreover these drugs may cause the occurrence of new drug-resistance bacteria [11]. Super hydrophobic catheter can be fabricated with various substrates by simple methods. Although they show the efficacy for decomposition of biofilm, it does not have suitability for use in the practical field due to the low cell adhesion efficiency [12]. In the case of the enzyme-based medical devices, they have no toxicity, long-term lifetime, and high antibacterial activity. Nevertheless they cannot be widely applied to the medical field due to the enzyme sensitivity that is easily affected by the environmental condition of pH, temperature, and existence of other enzymes [13]. Finally, in the case of the metal coating stainless steel, it can easily be fabricated by the simple method and low cost. Also, they have the high efficacy for decomposition of biofilm [14]. However, it also has a limitation for the application of the clinical surgery since the continuously activated metal coating stainless steel by liquid can influence not only to the bacteria but also other cells when it is implanted in the body. Recently, ROS are being reported to be very effective tool for the elimination of harmful bacteria including the multi-resistant bacteria [15], Gram-positive bacteria [16], and Gram-negative bacteria [17].

In this study, we demonstrate a simple fabrication of the photofunctional stainless steel plate that generates ROS from the surface of the stainless steel plate. 316LSS is one of the major materials that are widely used for medical materials due to good mechanical properties [18], corrosion resistance [19], low cost and biocompatibility [20]. The generation of ROS from the fabricated PSS plate is confirmed

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with the photocatalytic reactions. In order to check the antimicrobial effect, the decomposition of the formed biofilm and the suppression of the biofilm formation on the surface of the PSS plate are evaluated with the low photon energy of green light emitting diode (GLED, 3.5 mW/cm^2).

2. Experimental

2.1. Preparation of the photofunctional stainless steel plate

Overall process of manufacturing the photofunctional stainless plate is shown in Fig. 1. HP and 316LSS were purchased from Aldrich and Han-Yang Stainless co. Ltd. (Republic of Korea), respectively. The HP solution was prepared at a concentration of 2.1×10^{-3} M in ethanol. The 316LSS plate was cut to a circle form (d = 8 mm, thickness = 0.25 mm) and treated with acetone (Merck, HPLC grade) under ultrasonication for 10 min. The 316LSS plate was, then, washed with hexane (Merck, HPLC grade) and distilled water for 3 times. In order to enhance the hydroxyl groups on the surface of the 316LSS plate, they were immersed in the piranha solution (H_2O_2: H_2SO_4 = (1:3 vol.%), Showa chemical co. Ltd.) for 1 h at 100 °C and then dipped in HNO_3 solution (40%, Matsunoen chemicals Ltd.) for 30 min at 70 °C [21]. For stabilization of hydroxyl group on the surface of the 316LSS plate, they were immersed into boiling H_2O_2 for 30 min [21]. For fabrication of the PSS plates, the oxidized 316LSS plates were reacted with EDC/NHS (Sigma.%, 1:5, TCI/Sigma-Aldrich) for 6 h in the HP solution at room temperature [22]. Then the fabricated PSS plates were washed with ethanol under vigorous stirring condition and dried in the oven at 60 °C for 6 h. For the quantization of the HP molecules bonded to the surface of the oxidized 316LSS plate, the solution after the reaction and the washing solution were collected and O.D. of HP in the solution was measured with UV–Vis spectrophotometer (Hitachi, U-2900, Japan) [23]. Amount of HP bonded to the surface was estimated to be 1.1×10^{-8} mol/cm^2.

2.2. Charcterization of the photofunctional stainless steel plate

X-ray photoelectron spectroscopy (XPS, Thermo VG, Escalab 220i-XL, United Kingdom) was performed in order to confirm the covalent bonding nature between the hydroxyl groups of HP and the hydroxyl groups on the surface of the oxidized 316LSS plate. X-ray source of a monochromated Al X-ray (Al Kα line: 1486.6 eV) and 12 kV were utilized, and the compositional survey and detailed scans were acquired using pass energy of 100 eV with 1 eV stepwise and 50 eV with 0.1 eV stepwise [23,24]. The binding energies were corrected by referencing the C 1s binding energy of 285 ezV. In order to evaluate thickness of the covalently bonded HP molecular layer on surface of the stainless steel plate, an atomic force microscope (AFM, Nanowizard-I, JPK instrument, Germany) topography image was obtained in non-contact mode with the HP molecular layer partially bonded to the surface of PSS (scan range: XY: 0–100 μm; Z: 0–15 μm, noise level: XY < 0.02 nm RMS, Z < 0.035 nm RMS). The hardness of PSS was measured with the nano-indentation technique (MTS XP, MTS Systems Co., USA) [25]. The indentation was made to a maximum depth of 100 nm (with a maximum load of about 500 mN) and under a strain rate of 0.05 s^{-1}. Steady state absorption spectra of the HP solution and the PSS plate were obtained with a UV–Vis spectrophotometer (Hitachi, U-2900, Japan) and a diffuse reflectance UV–Vis spectrophotometer (Jasco, V-550, Japan) equipped with an integrating sphere to avoid the scattering of the 316LSS plate, respectively [23]. Steady state emission spectra of the HP solution and the PSS plate were obtained with a spectrofluorimeter (Hitachi, F-4500, Japan).

2.3. Reactive oxygen generation from the photofunctional stainless steel plate

For the confirmation of ROS generation from the PSS plate, degradation of 1,3-diphenylisobenzofuran (DPBF, Sigma-Aldrich) of a reactive oxygen quencher was studied with the PSS plate [23]. The PSS plate was introduced into DPBF solution (2 mL, 1.0×10^{-5} M, ethanol) in the dark condition. The light source for irradiation to the PSS plate was green light emitting diode (GLED, λ_{max} = 520 nm, FWHM = 40 nm, 3.5 mW/cm^2, Itswell Co. Ltd., Republic of Korea). The total output power for the irradiation was measured with a laser power meter (Ophir-opironics Ltd., Nova, Israel). At every 10 min of irradiation, the absorption spectra of DPBF were monitored with a UV–Vis spectrophotometer (Hitachi, U-2900, Japan) [23,24].

2.4. Antimicrobial effect of the photofunctional stainless steel plate

In order to check the photodynamic antimicrobial effect of the PSS plate, the decomposition efficiency of the formed biofilm and the suppression efficiency of the biofilm formation on the surface of the PSS plate were evaluated [24]. The ROS generation was done by irradiation of the GLED (λ_{max} = 520 nm, FWHM = 40 nm, 3.5 mW/cm^2) light onto the PSS plate through a cut-off filter (<400 nm, CVI, USA) which blocked the residual UV light from GLED. Power density of the GLED light was measured at the sample position by a light power meter (Ophir-opironics Ltd., Nova, Israel). For the photodynamic antimicrobial study of the PSS plate, *Staphylococcus aureus* (*S. aureus*, ATCC 25923)

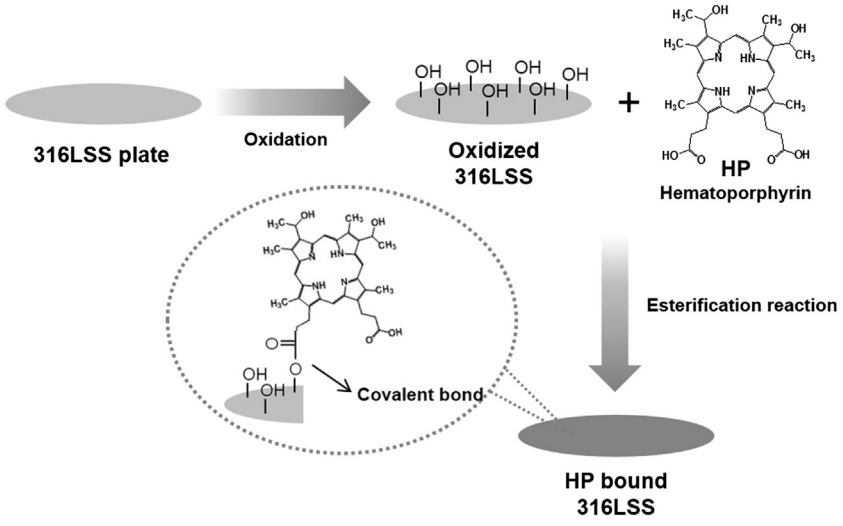

Fig. 1. Fabrication of the PSS plate.