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Visible light induced photocatalytic inactivation of bacteria by modified titanium dioxide films on organic polymers

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Commercially available polypropylene foil was pretreated with a low temperature oxygen plasma and covered with a thin film of nanocrystalline titanium dioxide by dip coating. The films were then photosensitized by titanium(IV) surface charge transfer complexes formed by impregnation with catechol. The photoactivity of the coatings up to 460 nm was confirmed by photoelectrochemical measurements. The photoinactivation of *Escherichia coli* and *Staphylococcus aureus* was evaluated by a glass adhesion test based on ISO 27447:2009(E) in the presence of visible light. The coating showed good antimicrobial activity induced by light from a light-emitting diode (405 nm), in particular towards *E. coli* ATCC 25922 strain. Adaptation of ISO 27447:2009(E) to assess bacterial photoinactivation by photocatalytic coatings will allow this procedure to be applied for the comparison of photoactivity under a range of irradiation conditions.

1. Introduction

During recent decades there has been a significant increase in the use of polymers in almost every field of human activity, the main reasons being their low cost and their flexibility of use. Polymers have found wide application in medicine, for example in single-use items such as syringes, catheters and vials and for these products safety in use is a vital issue. A polymer surface is exposed to the formation of biofilms, which can be an etiological factor in nosocomial infections. According to the World Health Organization (WHO) nosocomial infections, defined as: '*infections acquired in hospital by a patient who was admitted for a reason other than that infection*', are one of the major problems of modern medicine.[1] They contribute to morbidity and mortality, thereby increasing healthcare costs.

A new strategy in the fight against nosocomial infections is the use of photocatalytic coatings as antimicrobial agents. Among these, titanium dioxide (TiO_2) shows high activity in the photodynamic inactivation of bacteria, and has the benefits of physical and chemical stability, low toxicity, biocompatibility and low cost.[2] Unmodified TiO_2 can only be used as a photocatalyst under ultraviolet (UV) light, which frequently makes the use of artificial radiation necessary. Charges (electrons and holes) generated by TiO_2 photo-excitation can recombine or participate in redox reactions involving molecules adsorbed on the surface of a photocatalyst. The redox potential at the edges of conduction and valence bands determines the feasibility of oxidation and reduction.[3] When TiO_2 is used, the possibility of photogeneration of reactive oxygen species (ROS) is fundamental, in order to facilitate the inactivation of microorganisms such as bacteria, as reported by Matsunaga *et al.* in 1985.[4] Since that date, photoinactivation has become widely used for eliminating bacteria, fungi and viruses.[5]

A number of methods for the photosensitization of TiO_2 have been developed to achieve activity to visible light. The emphasis has been on methods encompassing doping with metals or non-metals, dye sensitization and semiconductor coupling, and surface modification with organic compounds has tended to be neglected because of the low photostability of such systems. According to Lewis theory, the chemisorption of organic compounds at the TiO_2 surface can be described as an acid–base reaction. Donor groups containing oxygen, nitrogen or sulphur are electron donors (Lewis bases) for the surface atoms of Ti(IV) (Lewis acids). Organic compounds containing hydroxyl or carboxyl groups can become coordinated to the TiO_2 surface, forming sustainable, often colourful, complexes. The most efficient binding is achieved by compounds containing two binding groups, assisted by a number of possible effects, including the formation of a stable ring, the possibility

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of stabilization by hydrogen bonding and the entropic effect of the formation of chelate complexes.[6] Derivatives of catechol, salicylic acid or phthalic acid appear to form ideal ligands,[6a] and the good photocatalytic activity of such materials under UV or visible light irradiation has been reported by a number of authors.[7]

The aim of the present study was to investigate the photoinactivation of *Escherichia coli* and *Staphylococcus aureus* using TiO_2 coatings on the surface of polymers photosensitized by visible light irradiation.

2. Experimental

2.1 Preparation of coatings on the polymer substrate

Transparent polypropylene (PP) films (Goodfellow, Huntingdon, UK), 0.1 mm thick were cleaned with pure water, then ethanol (Sigma-Aldrich, 99.5% purity), and used as substrates. A low pressure, low temperature plasma (Zepto, Diener Electronic Plasma Technology, Detroit, MI) was applied to remove impurities and generate oxygen-containing groups on the polymer surface. An oxygen flow (99.5% purity, Air Products) at a pressure below 0.3 mbar was introduced to the chamber. The plasma generator was used at 100 W power and a radio frequency of 40 Hz for 30 s.

Titanium dioxide film was synthesized using a dip coating method (Dip Coater, MTI Corporation, Richmond, CA). The speed of withdrawal of the sample from an aqueous 1.5 wt% colloidal solution of nanocrystalline TiO_2 (Nanostructured and Amorphous Materials, Houston, TX; anatase, particle size 5–30 nm, original concentration 15%) was 1 cm min^{-1}. The coating process was carried out in air and the films were dried at room temperature. The films were additionally impregnated by immersion for 5 min in an alcoholic solution of catechol (10 mmol dm^{-3}), as organic modifier. Finally, the impregnated films were washed with distilled water and dried in air at room temperature.

Thin films of TiO_2 and surface-modified TiO_2 were similarly prepared on indium–tin oxide (ITO) foil (resistivity: 60 Ω per sq).

The UV-Vis diffuse reflectance spectra of the coatings were determined using a Shimadzu UV-3600 spectrophotometer equipped with an integrating sphere. The thickness and surface topography of the TiO_2 films were examined by scanning electron microscopy (SEM) using a Tescan Vega3 LMU at 15 kV.

2.2 Photoactivity

The photoactivity of TiO_2 and cat@TiO_2 were tested by photocurrent measurements, using a three-electrode cell with platinum wire and Ag/AgCl as counter and reference electrodes, respectively. The films prepared on ITO foil were used as working electrodes. A quartz cuvette filled with aqueous KNO_3 solution as electrolyte (0.1 mol dm^{-3}, pH = 6.0) was used as the electrochemical cell. Irradiation was by monochromatic light, every 10 nm over 330–550 nm, using a XBO 150 xenon lamp equipped with monochromator and shutter (Instytut Fotonowy, Kraków). The photogenerated current was measured in the range −0.2 to 1.0 V *vs.* Ag/AgCl and at an incident wavelength range of 330–550 nm using a Metrohm Autolab PGSTAT 302N electrochemical analyser. The solution was purged with argon both before (5 min) and during the measurement.

2.3 Cultivation of the test bacteria

Two bacterial strains, representing gram negative (G−) rods: *E. coli* (ATCC 25922) and gram positive (G+) coccus: *S. aureus* (ATCC 25923), were used to evaluate antibacterial activity. *E. coli* was cultured overnight on MacConkey Agar medium (McC, Difco, Franklin Lakes, NJ) for 24 h at 37 °C, and *S. aureus* on tryptic soy agar medium (TSA, Difco) under similar conditions. Aliquots of the cultures were inoculated in fresh fluid medium (tryptic soy broth, Difco) and incubated under aerobic conditions at 37 °C until the exponential growth phase was reached. A standard suspension of bacteria (approximately 10^5 colony forming units (CFU) mL^{-1}) was obtained by serial dilution of the cultures.

2.4 Antimicrobial activity – adhesion glass method

Photoinactivation was evaluated by a glass adhesion test based on ISO 27447:2009(E).[8] Because layer manufacturing might contaminate the test surface, all TiO_2-coated foils were exposed to UV radiation for 5 min prior to the microbiological tests. The bacterial suspension (0.1 mL; 10^5 CFU mL^{-1}) was pipetted onto the test surface (30 × 30 mm^2) and a cover glass applied (24 × 24 mm^2). The sample was then placed in a square Petri dish (100 × 100 × 20 mm^3) equipped with wet filter paper and a square metal plate for support (Fig. 1).

In this test a 405 nm 10 mW light-emitting diode (LED) light (Instytut Fotonowy, Kraków) with an intensity of 1.0 mW cm^{-2} (measured at the sample level using an Ophir Nova II Radiometer) was used. After irradiation, the samples on the cover glass were shaken out into 3 mL of saline solution, serially diluted and spread onto an appropriate agar media. In order to determine the number of viable cells (counted as CFU mL^{-1}), the samples were incubated at 37 °C for 24 h and the bacterial colonies were then counted. Control tests on bacteria, involving neat and modified films kept in the dark and then irradiated, were carried out for all experiments applying standard procedures.

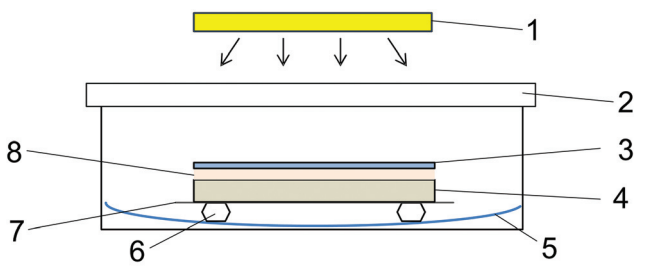

Fig. 1 Schematic diagram showing the irradiation set-up: (1) light source, (2) plastic lid, (3) cover slide glass, (4) TiO_2-coated polymer, (5) wet filter paper, (6) metal nut, (7) metal plate, and (8) bacterial suspension.

Each experiment was performed in quadruplicate and repeated at least twice. Mean, standard deviation and *T*-tests were calculated.

3. Results and discussion

3.1 Characterization of coatings

Nanocrystalline TiO_2 was successfully used for the preparation of thin coatings on a PP substrate. Coatings obtained by dip coating were opaque and homogeneous. Those produced at a withdrawal rate of 1 cm min^{-1} yielded films approximately 100–300 nm in thickness, as determined by SEM measurement.

The scanning electron micrographs of TiO_2 coatings of native (a), (b) and damaged (c), (d) samples are shown in Fig. 2. Micrographs (c) and (d) were obtained for mechanically damaged film, to allow thickness measurements. The native films (a) were smooth, with some narrow cracks up to 150–300 nm wide. These cracks were mainly close to the film edges and are a feature of mineral coatings on flexible polymeric foils.

UV-Vis diffuse reflectance spectra of the unmodified and modified TiO_2 coatings are shown in Fig. 3. The modified coating exhibits a clear absorption of visible light up to 550 nm. This results from the formation of surface charge-transfer complexes.[6a]

3.2 Photocatalytic activity

To check the photoactivity of the films, unmodified and sensitized thin layers of TiO_2 were produced on the surface of conductive ITO foil. Cyclic voltammetry measurements revealed a

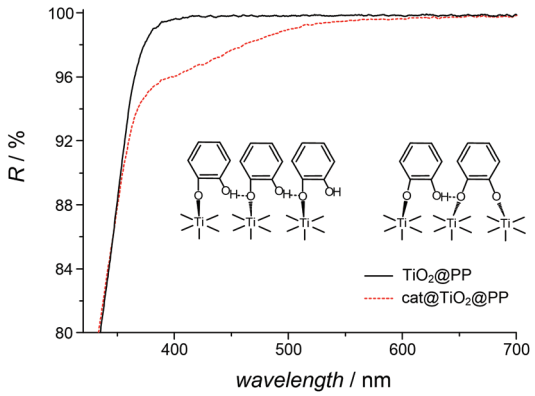

Fig. 3 UV-Vis diffuse reflectance spectra of films of neat TiO_2 (solid line) and TiO_2 modified with catechol (dotted line) deposited on PP foil. The structures of Ti(IV)–catechol surface complexes are also shown.[6a–c]

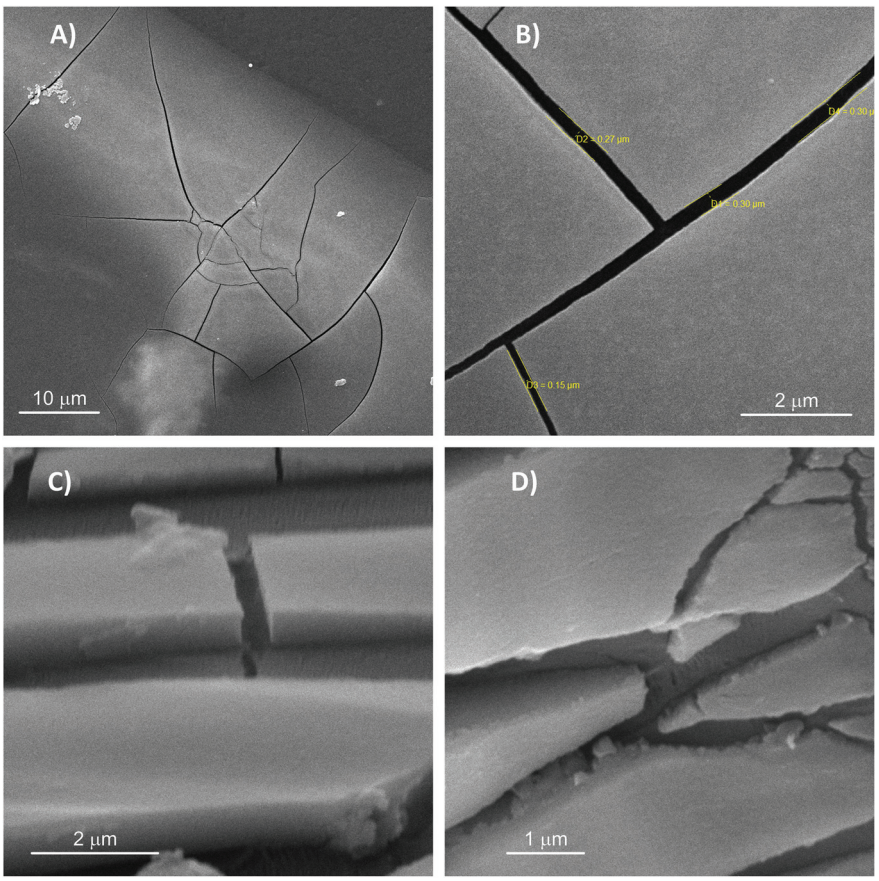

Fig. 2 SEM micrographs of TiO_2 coating on a PP foil (a, b). The edge of the TiO_2 film can be seen in (a), in the upper right corner. The mechanically damaged TiO_2 films (c, d) were used for the estimation of the film thickness.

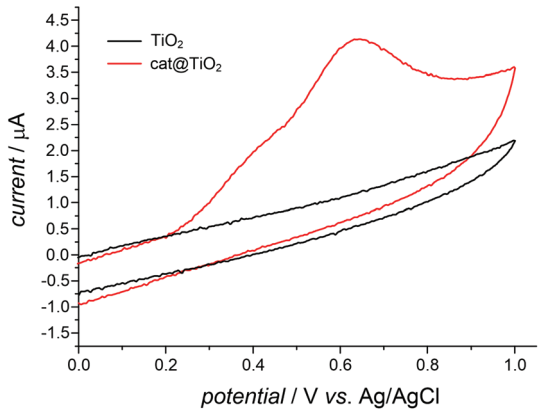

Fig. 4 Cyclic voltammograms for films of TiO_2 and cat@TiO_2 deposited on ITO foil.

peak related to the oxidation of catechol, observed with the cat@TiO_2 material, but not for unmodified TiO_2 film (Fig. 4). The oxidation of catechol at approximately 0.6 V *vs.* a Ag/AgCl electrode is in agreement with measurements reported previously.[9]

Photocurrent measurements made for ITO electrodes with deposited materials were conducted for a wide range of electrode potentials and wavelengths of incident light. Generation of anodic photocurrents was observed for the unmodified TiO_2 film when UV light (λ < 390–400 nm) was applied (Fig. 5a). At positive potentials anodic photocurrents were also recorded for the electrode covered with cat@TiO_2, but in this case the spectral range of activity was broadened to approximately 520 nm (Fig. 5b). This demonstrated the photosensitization of TiO_2 by surface charge transfer complexes of titanium(IV), with catechol as the ligand.

The modified materials also induced cathodic photocurrents when the electrode was biased at negative potentials. This effect can be explained by the change in the Fermi level on modification of TiO_2 with catechol. It should be noted that the values of photocurrents measured for the modified material induced by UV radiation were lower than those for unmodified TiO_2. This difference originates from the less efficient electron–hole recombination when the excited state is reached by direct excitation of TiO_2, compared to the ligand to metal charge transfer excitation of the surface complex.

3.3 Photocatalytic antibacterial activity – adaptation of the ISO standard

To narrow down the wide variety of methods available for testing the antibacterial activity of photocatalytic coatings, the ISO 27447:2009(E) standard was chosen.[8] However, this procedure is mainly designed for photocatalytic coatings deposited on inorganic supports and irradiated with UV light. Because of differences in the support (polymer) used for the TiO_2 coatings and the irradiation light source (visible light), the procedure was therefore adapted to our own requirements. The differences between the modified and ISO procedures,

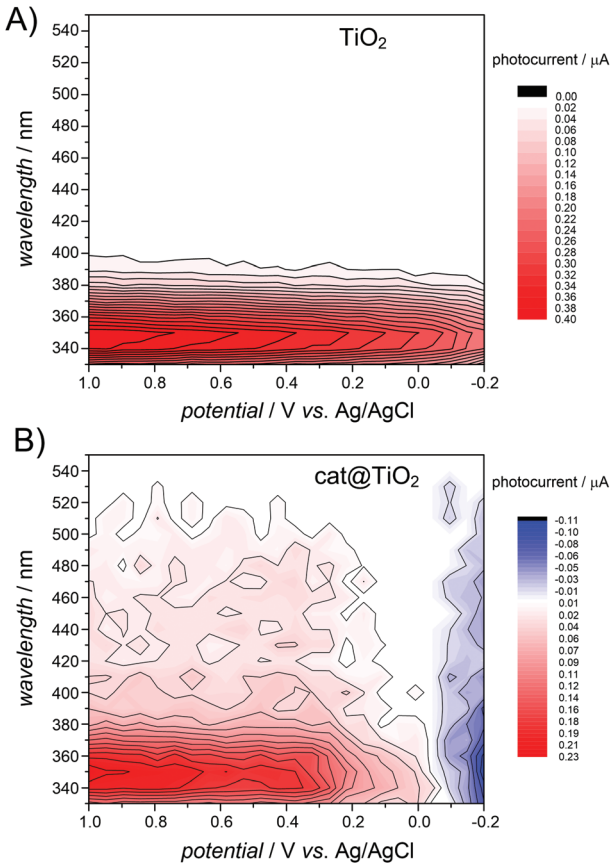

Fig. 5 Photocurrent measurements for thin layers of materials deposited on ITO foil recorded for incident light in the range 330–550 nm at a potential of −0.2 to 1.0 V: (A) TiO_2, (B) cat@TiO_2.

with a description of the experimental set-up, are listed in Table 1 (compare also with Fig. 1).

After 4 h irradiation with visible light (LED source, λ_{max} = 405 nm) the modified TiO_2 films were found to be most effective for bacterial inactivation at the starting concentration of 10^5–10^6 CFU mL^{-1}. Because of the fact that bacteria are able to absorb light at wavelengths lower than 340 nm, the applied light could be absorbed efficiently only by the Ti(IV)–catechol

Table 1 Comparison of the major changes in the parameters of the ISO 27447:2009(E) procedure with the glass adhesion method used in the present study

Parameter	ISO 27447:2009(E)	Adapted glass adhesion test
Bacteria cultivation	Nutrient agar	McC medium (*E. coli*), TSA (*S. aureus*)
Sample size	50 × 50 ± 2 mm^2	30 × 30 ± 2 mm^2
Sample size covered by an adhesive glass	400–1600 mm^2	576 mm^2
Volume of test bacterial suspension	0.15 mL	0.10 mL
Light source	Fluorescent backlight blue lamp 300–400 nm	405 nm LED
Light intensity	0.001–0.25 mW cm^{-2}	1.0 mW cm^{-2}

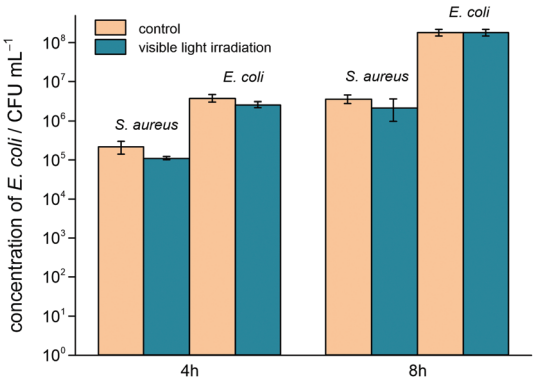

Fig. 6 The concentration of *S. aureus* and *E. coli* after 4 h and 8 h of visible light irradiation (405 nm, 1.0 mW cm^{-2}) for unmodified TiO_2 coatings.

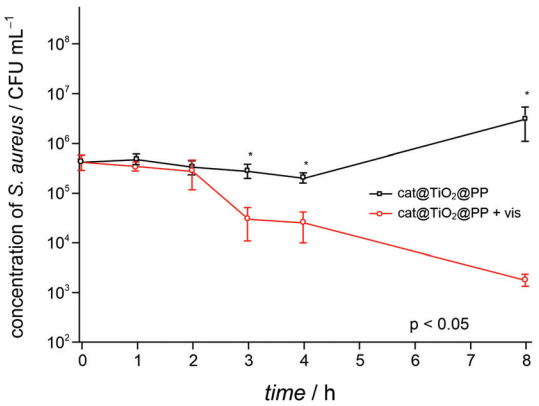

Fig. 8 Concentration of *S. aureus* under visible light irradiation (405 nm, 1.0 mW cm^{-2}) measured for TiO_2 coatings modified with catechol (dark: □, light: ○).

surface complex.[10] The irradiation conditions enabled neither direct titanium excitation nor direct bacterial inactivation, thus, no photoinactivation of neat TiO_2 coatings was observed under visible light irradiation (Fig. 6).

However, after 8 h of irradiation in the presence of cat@TiO_2, the concentration of *E. coli* decreased by a factor of 1000 from its initial concentration of approximately 10^6 CFU mL^{-1} (Fig. 7). In tests carried out on the modified coating (cat@TiO_2) in the dark, a non-toxic effect was observed. These results are consistent with the reported non-toxicity of catechol itself towards *E. coli*.[9]

In the case of gram positive coccus (*S. aureus*), the photoinactivation curve (Fig. 8) was similar to that for *E. coli*. The ratio between the initial and final concentrations of viable bacteria was again close to 1000.

The mechanism of the photoinactivation process can be deduced from the shape of the curves in Fig. 7 and 8. For both bacterial strains a two hour incubation step is observed in the first phase. This phase is characterised by balancing the amounts of ROS generated with their elimination by the bacterial self-defence mechanism, which involves superoxide dismutase (SOD) and catalase. These enzymes protect cells from oxidative stress, according to the following mechanism:

$$2O_2^- + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2 \quad (1)$$

$$2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O \quad (2)$$

The increasing concentration of ROS initiates oxidation of the cell wall and membrane components, contributing to bacterial death.[11] The photocatalytic activity of cat@TiO_2 film towards inactivation of *S. aureus* appeared to be slightly lower than its activity against *E. coli*. Inspection of Fig. 7 and 8 confirms the essential influence of visible light on bacterial photoinactivation.

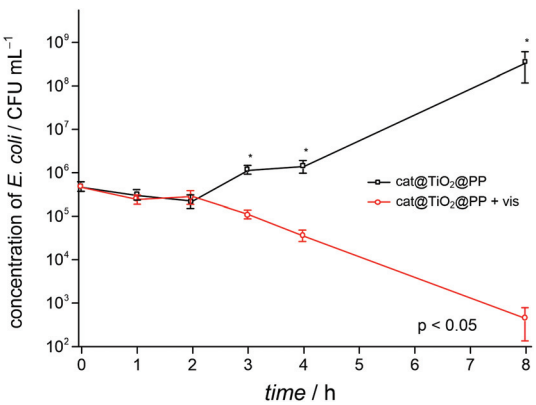

Fig. 7 Concentration of *E. coli* under visible light irradiation (405 nm, 1.0 mW cm^{-2}) measured for TiO_2 coatings modified with catechol (dark: □, light: ○).

4. Conclusions

We have demonstrated that catechol-photosensitized TiO_2 films on polymeric foils can potentially be used as antimicrobial coatings. The test bacteria *E. coli* and *S. aureus* were inactivated by the photocatalyst (cat@TiO_2). The ratio of population reduction decreased by approximately three orders of magnitude after 8 h of visible light (405 nm) irradiation. In the presence of non-sensitized TiO_2 films the irradiation conditions used did not provide bacterial inactivation. The adaptation applied to the ISO 27447:2009(E) standard procedure could in future be used for evaluating the antibacterial activity of films and coatings under a variety of irradiation conditions, including irradiation with visible light.

The photocatalytic coatings synthesized may provide a new strategy for reducing the risk of nosocomial infections by applying them to plastic items used in medical practice.

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