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Adhesion and inactivation of Gram-negative and Gram-positive bacteria on photoreactive TiO_2/polymer and Ag–TiO_2/polymer nanohybrid films

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ABSTRACT

The aim of this study was to develop photoreactive surface coatings, possessing antibacterial properties and can be activated under visible light illumination (λ_{max} = 405 nm) using LED-light source. The photocatalytically active titanium dioxide (TiO_2) was functionalized with silver nanoparticles (Ag NPs) and immobilized in polyacrylate based nanohybrid thin film in order to facilitate visible light activity ($\lambda_{Ag/TiO2,max}$ = 500 nm). First, the photocatalytic activity was modelled by following ethanol vapor degradation. The plasmonic functionalization resulted in 15% enhancement of the activity compared to pure TiO_2. The photoreactive antimicrobial (5 log reduction of cfu in 2 h) surface coatings are able to inactivate clinically relevant pathogen strains (methicillin resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) within short time (60–120 min) due to the formed and quantified reactive oxygen species (ROS). The existence of electrostatic interactions between the negatively charged bacteria (from −0.89 to −3.19 μeq/10^9 cfu) and positively charged photocatalyst particles (in the range of +0.38 and +12.3 meq/100 g) was also proven by charge titration measurements. The surface inactivation of the bacteria and the photocatalytic degradation of the cell wall component were also confirmed by fluorescence and transmission electron microscopic observations, respectively. According to the results an effective sterilizing system and prevention strategy can be developed and carried out against dangerous microorganisms in health care.

1. Introduction

Interdisciplinary researches of nanotechnology and microbiology have become great interest to develop environmental applications against bacteria causing nosocomial infections [1–5]. *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa (P. aeruginosa)* are common pathogenic bacteria with a possible multidrug resistance by mutation [6,7]. Immunosuppressed patients are compromised, these infections highlights the importance of prevention in the health care [8–11]. Methicillin resistant *S. aureus* and multidrug resistant *P. aeruginosa* can cause nosocomial infections and are responsible for postoperative infection. The number of infections caused by these bacteria is increasing in every year, so the prevention of the spread from human to human is the key to maximize the function of infection control. Antimicrobial applications are able to inactivate bacteria, viruses and fungi on different surfaces, in water or in the air, so they can be the main actor of prevention in the health care facilities [12–14]. There are various techniques to develop antimicrobial surfaces, but only a few optimize the structure and the chemical properties [15–20]. Surfaces with TiO_2 content can kill numerous microorganisms because of its photocatalytic properties [21–26]. Moreover, during photocatalysis under UV light illumination strong oxidizing power is generated and the surface containing TiO_2 shows antibacterial effect as well. In the course of photocatalysis different free radicals (*e.g.,* hydroxyl radicals) are also produced with different wavelength emitting light

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sources. [27,28]. Hydroxyl radicals can destroy the bacterial cell wall due to breaking covalent bonds in peptidoglycan layer, which is mainly responsible for the stability of the cell wall in Gram-positive (GR+) and Gram-negative (GR−) bacteria [29]. Due to the destruction of cell wall and DNA the reproduction and infectivity of the microorganisms can be inhibited in a short time period [30–34]. UV-active photocatalyst particles (*e.g.,* TiO_2, or ZnO) can be modified with different plasmonic nanometals, so the wavelength of the absorbing light can be tuned from the UV to the visible region. Due to this disinfection method a wide range of pathogen bacteria can be inactivated under visible light illumination [35–39]. The wavelength of the absorbing light on TiO_2 nanoparticles (NPs) can be extended to the visible region functionalizing with silver, without significant amount of silver ion release to the environment. The antibacterial activity and photochemical qualifications of TiO_2 photocatalysts and its modified forms have been reported in many publications, but the connection properties between the NPs and the bacterial cells are relatively unknown. Investigation of the bacterial adhesion on the surface of antibacterial NPs is very important [40,41]. It is already well-known, that the cell wall of bacteria are negatively charged because of the presence of teichoic acids for GR+ bacteria [42] while in case of GR− bacteria the presence of lipopolysaccharides and lipoproteins cause the negative charge and thus [43] they can be electrostatically attached to the positively charged surfaces of the applied photocatalysts. The knowledge of the surface charge values of the prepared antimicrobial surfaces may contributes to the better understanding of the successful inactivation of microorganisms.

In our work photoreactive surface coatings, possessing antibacterial properties have been developed and the adhesion properties of methicillin resistant *S. aureus* (GR+), *P. aeruginosa* (GR−) and *Escherichia coli (E. coli)* (GR−) were investigated on the surface of photoreactive nanohybrid films containing TiO_2 and plasmonic Ag–TiO_2 NPs in polymer matrix. In addition, the antibacterial activity of Ag–TiO_2 photocatalyst was also investigated. Our main goal was to highlight the connection between the bacterial adhesion and the antimicrobial properties of the developed nanohybrid films for possible application in health care.

2. Materials and methods

2.1. Preparation of polyacrylate hybrid films containing TiO_2 and Ag–TiO_2 photocatalyst

Degussa P25 TiO_2 (75% anatase, 25% rutile) was functionalized with Ag NPs. Different Ag NPs/TiO_2 ratios were tested, but according to the TiO_2 content 0.5 w/t% concentration of Ag NPs was applied [44]. During the synthesis the calculated amount of $AgNO_3$ aqueous solution (Molar, Hungary) was directly added to the TiO_2 aqueous suspension. On the second step the silver ions were reduced by one hour of irradiation with UV light (Hamamatsu L8251 lamp; $P = 150$ W; $\lambda_{max} \geq 300$ nm). The nanocomposites were washed four times, dried at 50 °C and pulverized [44]. For microbiological measurements the photocatalyst NPs were immobilized by using polyacrylate [poly(ethyl-acrylate *co* methyl-methacrylate) (p(EA-*co*-MMA)) binder material (obtained from Evonik Industries, Germany). Photocatalyst NPs in polymer matrix were prepared on the surface of glass plates (2.5 × 2.5 cm) with spray coating technique. The amount of the nanohybrid surface coating deposited on the glass plates was 1 ± 0.1 mg cm^{-2} in all cases. The concentration of the photocatalyst in the composite film was 0.6 mg cm^{-2} which corresponds to the photocatalyst/polymer mass ratios of 60:40 wt%. The calculated thickness of these films was 1.48 ± 0.1 μm [45]. Pure polymer matrix was also prepared in the same manner for reference.

2.2. Characterization of the nanohybrid surfaces

Diffuse reflectance spectra of the photocatalysts were recorded with CHEM 2000 UV–vis (Ocean Optics Inc.) spectrophotometer [44,45]. The photocatalytic activity of the polyacrylate based nanohybrid films and the pure photocatalysts thin layer (without polymer binder) were performed with the ethanol (as test molecules) degradation test under LED-light illumination (General Electric's, Hungary, $\lambda = 405$ nm) [44]. Photooxidation of ethanol vapor on catalyst films was performed in a circulation reactor (volume *c.a.* 165 mL) at 25.0 ± 0.1 °C. The light source was fixed at 50 mm distance from the 45 cm^2 films. After injection of ethanol and water vapor, the system was left to stand 30 min for the establishment of adsorption equilibrium on the surface of films. The composition of vapor phase was analyzed by gas chromatograph (Shimadzu GC-14B) equipped with a thermal conductivity (TCD) and a flame ionization detector (FID). The flow rate of the gas mixture in the photoreactor system was 375 mL × min^{-1}. The initial concentration of the ethanol was 0.36 ± 0.018 mmol L^{-1} at relative humidity of ~70%. During the long- term photocatalytic test experiments of the Ag–TiO_2 containing nanohybrid film the 45 cm^2 layer was illuminated continuously with $\lambda = 405$ nm LED light from a distance of 10 cm. At given time intervals the irradiated hybrid layer was put in the above described circulation reactor and the photooxidation rate of ethanol vapor was measured. After the measurement the specific amount of photodegraded ethanol (Δc_{EtOH}/mM 60 min^{-1} g^{-1}/) was calculated. The surface pH of the Ag–TiO_2 containing polymer based nanohybrid film was determined under UV-light illumination (GCL307T5VH/HO type low-pressure mercury lamp, LightTech, Hungary, $P = 35$ W). During the measurement the 5 × 5 cm^2 nanohybrid film was immersed in 40 mL of distilled water (initial pH 5.3 due to the dissolved CO_2). The nanohybrid films were illuminated and shaken continuously during the experiment with a magnetic stirrer. The distance of the light source from the nanohybrid films was 10 cm. The pH electrode (Metrohm 6.0228.010) was placed into the distilled water nearest to the hybrid film and the actual pH values were measured as a function of illumination time.

2.3. Surface charge measurements

Surface charge values of the photocatalyst and the bacterial suspensions were measured by means of a particle charge detector (PCD-04 Particle Charge Detector; Mütek Analytic GmbH, Germany) with manual titration. Under a titration process the surface charge of the bacteria and photocatalysts will be compensated with opposite charged surfactants like: sodium dodecyl sulfate (SDS) and hexadecylpyridinium chloride (HDPCl) with concomitant streaming potential measurements. 10 mL of 0.1% TiO_2 suspension was measured in the particle charge detector at pH 4.5, because the optimal pH of the three investigated bacteria is in the range of $4.2 \leq pH \leq 9$ and TiO_2 is positively charged below pH 6 (point of zero charge) [46]. Photocatalyst and bacterial suspensions were titrated with anionic 1% SDS and cationic 0.01% HDPCl solutions, respectively. In view of the amount of the added surfactant at the charge compensation point (streaming potential = 0 mV) the equimolar amount of surfactant was calculated and specified to the amount of photocatalyst (meq/100 g) or to the number of bacteria (μeq/10^9 cfu). All experiments were repeated in three times.

2.4. Determination of the produced hydroxyl radicals on the nanohybrid films

The amount of reactive hydroxyl radicals was determined from the hydrogen peroxide induced luminol dependent chemiluminescense reaction (Scheme 1.) The detailed measurements