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ORIGINAL RESEARCH PAPER

Photocatalytic and antimicrobial properties of surgical implant coatings of titanium dioxide deposited though cathodic arc evaporation

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Abstract Nanostructured crystalline titanium dioxide coatings deposited by cathodic arc evaporated on titanium grade five medical implant substrates were demonstrated to exhibit UV-induced photocatalytic activity that can be utilized to provide bactericidal effects against *Staphylococcus epidermidis.* The photocatalytic activity of the coatings was confirmed via degradation of Rhodamine B under UV illumination. A 90 % reduction of viable bacteria was achieved in a clinically suitable time of only 2 min with a UV dose of 2.4 J delivered at 365 nm. These results are encouraging for the development of antimicrobial surfaces in orthopedics and dentistry in order to prevent or treat post-surgical infections.

Purpose of work To assess the possibility of employing photocatalysis for elimination of *S. epidermidis*, known to cause medical device related infections, under short enough times to be clinically useful on an implant surface produced with a technique that is suitable for mass production.

Keywords Antimicrobial activity · Coating · Infection prevention · Photocatalysis · Surgical implants · Titanium dioxide

Introduction

Implant related infections constitute a large and growing problem within orthopedics and dentistry (Pulido et al. 2008; Costerton et al. 2005). They are difficult to treat and often lead to patient suffering and substantial societal costs (Lavernia et al. 1995). The infections arise at the implant-tissue interface, referred to as an immune-incompetent fibro-inflammatory zone (Gristina 1994). At such interfaces a local depression of the immune system occurs (Zhao et al. 2009), which makes implant surfaces extra susceptible to bacterial colonization and biofilm formation (Costerton et al. 2005). Biofilm bacteria are challenging to eliminate with systemic antibiotic therapy (Shiraishi et al. 2008), hence, the only way to combat implant related infections is, often, to remove the implant and replace it with a new one. Skin penetrating implants are especially susceptible to bacteria colonization since they are in constant contact with skin bacteria

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(Temple et al. 2004). Examples of such implants are external fixation wires and pins, which often become colonized by *Staphylococcus aureus* and *S. epidermidis* (Collinge et al. 1994). On skin penetrating implants, one generally finds a higher prevalence of Gram-positive bacteria (90.6 % *S. epidermidis*) compared to Gram-negative ones (Mahan et al. 1991).

Several strategies have been employed to develop antibacterial modifications of implant surfaces in order to prevent microbial colonization and subsequent biofilm formation (Brunski et al. 2000). These include incorporation of releasable traditional antibiotics (Brohede et al. 2009a, b; Forsgren et al. 2011a, b) and metal ions (Heidenau et al. 2005; Ewald et al. 2006).

Titanium dioxide (TiO_2) is known for its biocompatibility, that becomes evident by rapid biomimetic hydroxyapatite formation upon immersion in biological fluids (Åberg et al. 2009; Piskounova et al. 2009; Mihranyan et al. 2009; Brohede et al. 2009c; Forsgren et al. 2011b; Lilja et al. 2011). It is a well-studied and clinically widely used biomaterial for bone applications with excellent chemical stability, no toxicity and good mechanical properties (Brudnik et al. 2008; Uzunova-Bujnova et al. 2008; Diebold 2003). Crystalline TiO_2 is not only a bioactive, non-resorbable (Kasuga et al. 2002; Crawford et al. 2007) material promoting tissue integration (Zhu et al. 2006), it also exhibits excellent photocatalytic properties, generating photo-induced reactive oxygen species (ROS), when irradiated with UV light (Sirghi et al. 2003; Carp et al. 2004; Hossain et al. 2008; Welch et al. 2010). These properties make crystalline TiO_2 very attractive as implant coatings.

Photocatalysis on crystalline TiO_2 surfaces for disinfection purposes (Gamage et al. 2010; Chong et al. 2010) has been proven capable of eliminating a wide range of Gram-negative and Gram-positive bacteria. Nevertheless, there have been very few reports about its application to bio-implant-related infections (Cai et al. 1991; Choi et al. 2007, 2009; Riley et al. 2005; Welch et al. 2012), especially with focus on clinically relevant strains such as *S. aureus* or *S. epidermidis* (Gallardo-Moreno et al. 2010; Welch et al. 2010).

The photocatalytic activity (PCA) of a TiO_2 surface depends on a number of factors including surface area and crystallinity. Most studies investigating the PCA of TiO_2 surfaces have concluded that the anatase polymorph of TiO_2 is a more effective photocatalyst compared to rutile (Miyagi et al. 2004). However, some studies indicate that a certain mixture of anatase and rutile can produce a higher PCA, and consequently an enhanced bactericidal effect, than pure anatase (Miyagi et al. 2004; Sato et al. 2006). These studies demonstrate that the use of a photocatalytic implant coating may be a viable strategy for reducing implant related infections. However, the illumination time required in these studies in order to achieve a desired antibacterial effect (in excess of an hour of UV illumination) needs to be significantly reduced for the technique to be clinically relevant. Coatings on implant surfaces have high requirements on their physical properties (Brohede et al. 2009c). Hence, cathodic arc evaporation could be an attractive alternative for the deposition procedure of such coatings as it allows for rapid deposition of TiO_2 thin films in a mass production ready process with a high degree of control of the coating microstructure and excellent adhesion to the substrate (Kleiman et al. 2007).

The present study evaluates the antimicrobial effects on *S. epidermidis* under UV light illumination of a TiO_2 coating produced by cathodic arc deposition. The particular bacterium strain is chosen because it is a common cause of medical device-associated infections (Rodriguez-Martinez and Pascual 2006), especially on skin penetrating implants (Mahan et al. 1991; Collinge et al. 1994). The aim is to evaluate if it is possible to employ photocatalysis for bacteria elimination on implant surfaces under short enough times to be clinically practical on an implant surface produced with a technique that is suitable for mass production.

Materials and methods

Coating deposition

Cylindrical substrates (Ø = 9 mm, thickness 1 mm) of commercially available titanium grade 5 (Ti–6Al–4V) were used for the arc evaporation coating process (Lilja et al. 2012). Briefly, a crystalline TiO_2 coating with a pronounced anatase phase composition was deposited onto the substrates by cathodic vacuum arc evaporation during a deposition time of 30 min. For coating thickness measurements two notches of 3 mm in length were cut from both sides towards the center of the substrate prior to coating deposition.

Coating characterization

X-ray diffraction (XRD) measurements

The crystallinity of the coatings was examined using grazing incidence XRD (Siemens D5000 diffractometer). The measurements were recorded between 20 and 60° 2θ with an incidence angle of 1° using a step size of 0.1° and a scan step time of 4 s.

From the full width at half maximum (FWHM) of the anatase XRD peaks A(101), A(200) and A(004), the effective grain size of the as deposited TiO_2 films was calculated by using the Scherrer equation (Guinier et al. 1994):

$$\mathrm{FWHM}(2\theta) = \frac{0.9\lambda}{L\cos(\theta)}, \tag{1}$$

where L is the effective grain size and λ is the wavelength of the X-ray. Broadening caused by internal strain was not taken into account.

Scanning electron microscopy (SEM)

The TiO_2 coating topography and thickness was evaluated by using a scanning electron microscope (Zeiss Supra 40). In order to be able to view the cross section of the coating and measure the coating thickness, the notched coated samples were first frozen in liquid nitrogen and then broken into two halves by applying an abrupt mechanical force.

Photocatalytic activity testing

The PCA of the as deposited TiO_2 films was determined by measuring the degradation of Rhodamine B dye as a function of time as described in Lilja et al. (2012). Briefly, four coated samples were placed in a quartz cell containing 2.5 ml Rhodamine B solution (5 µM, pH ~ 6, Sigma) and irradiated with an average UV light intensity of 6.7 mW/cm^2 (UV LED NCSU033B, $\lambda = 365$ nm, Nichia, Japan) at 100 Hz and 10 % duty cycle. During the 250 min measurement, the solution was stirred with a magnetic bar and the degradation of the dye was evaluated by absorption measurements at 5 min intervals using a UV-photospectrometer (UV-1800, Shimadzu).

Assuming a pseudo first order reaction rate for the Rhodamine B degradation, the degradation rate, k, which is a measure of the PCA, was obtained by fitting the dye concentration vs. time curves to the following equation (Konstantinou et al. 2001):

$$C(t_{uv}) = C_0 e^{-kt_{uv}}, \tag{2}$$

where C_0 is the initial dye concentration and $C(t_{uv})$ is the concentration after UV light irradiation for a given time t_{uv}.

Antibacterial testing with UV irradiation

Gram-positive *S. epidermidis* (CCUG 18000A) was used as the model bacteria in the bactericidal tests. It constitutes an interesting test bed for investigating non-antibiotic bactericidal treatments since (i) it is part of the human skin flora, (ii) it has a low requirement for nutrition and forms biofilms characterized by a high degree of antibiotic resistance (Stewart et al. 2001) and (iii) it is a common cause of medical device-associated infections (Rodriguez-Martinez et al. 2006).

S. epidermidis was inoculated into 12 ml of Müller Hinton broth II (MH II) nutrition medium (Difco, Becton, Dickinson and Company, Sweden) in a 15 ml falcon tube. The tube was incubated at 37 °C overnight before the bacteria were collected by centrifugation at 1,000×g (Heraeus Primo, Thermo Scientific) and re-suspended in 300 µl of sterile distilled water. The bacteria suspension was diluted to an optical density of 1.0, corresponding to a cfu concentration of about 10^9/ml. Prior to antibacterial testing, the coated samples were ultrasonically cleaned for 5 min in first ethanol and then distilled water.

An 8 µl drop of bacteria suspension was spread over each sample disc before UV irradiation. The samples were irradiated with a 365 nm light source at an intensity of 20 mW/cm^2 between 0 and 13 min, corresponding to UV doses between 0 and 16 J. Tests were performed in triplicates for each irradiation time. Uncoated titanium grade 5 discs were used as reference.

After UV illumination, the samples were rinsed with 0.5 ml distilled water to remove loosely adhered bacteria from the surfaces and this water was collected in individual wells of a well plate. The sample disks were then placed face down in their corresponding well with the rinsing water and the well plate was placed in an ultrasonic bath for 30 s to ensure that all the bacteria were removed from the sample surfaces. Thereafter 100 µl of bacteria suspension from each

well was transferred to individual wells in a 96-well plate and diluted to 300 µl with MH II broth containing 2.5 vol% Resazurin for viability measurements. Resazurin functions as a viability indicator as it is reduced to Resorufin by the mitochondria in viable bacteria (Gonzalez et al. 2001). Since Resorufin is fluorescent, the amount of viable bacteria can be quantified by measuring the fluorescence of the medium containing the bacteria. To increase the sensitivity of the metabolic assay for low numbers of viable bacteria, the well plate was incubated for 14 h at 37 °C to allow the bacteria to multiply and produce a larger amount of Resorufin. The fluorescence measurements were performed with an Infinite M200 microplate-reader from Tecan, using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. In order to correlate the fluorescent signal to the number of viable bacteria, a standard curve was made from a dilution series of the original bacteria suspension with a known cfu concentration.

Results and discussion

The XRD diffraction pattern of the as deposited TiO_2 coating in Fig. 1a shows that the microstructure is dominated by the anatase phase with minor amounts of rutile phase present in the structure. It should be noted that the substrate material used in the present work is one of the most commonly used biomaterials in orthopedic and dental applications due to its favorable mechanical properties and good biocompatibility (Navarro et al. 2008). As well the anatase phase, which is the dominating phase of the deposited coatings, has in several studies been proven to be bioactive and non-toxic (Brunette et al. 2001).

The SEM image of the coating in cross section, Fig. 1b, displays that the TiO_2 film has a total thickness of about 700 nm and consists of columnar grains throughout the entire coating. The SEM image of the TiO_2 coating topography, Fig. 1c, shows that the coating surface consists of distinctive grains. The diameter of the grains is approximately 40–50 nm, which is consistent with the average calculated grain size of 45 nm using Eq. (1). The surface roughness of the underlying titanium substrate and the impact of the deposition process are reflected by the contoured surface topography of the coating.

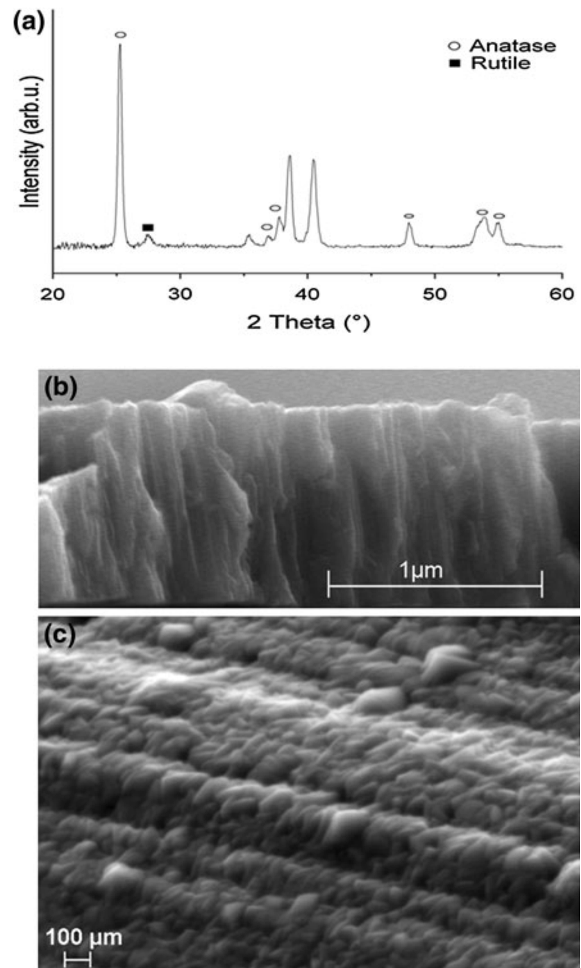

Fig. 1 XRD pattern of the TiO_2 coating deposited by cathodic arc evaporation. Anatase and rutile peaks are indicated **(a)**. Coating cross section **(b)** and topography **(c)** of the as deposited TiO_2 sample. The topography image is taken at a 50° angle from the normal

Figure 2 displays the PCA of the TiO_2 surface, where the Rhodamine B concentration is displayed as a function of time in a solution containing the samples under UV illumination. Compared to the Ti grade 5 reference surfaces, a clear degradation of the Rhodamine dye is observed with the coated Ti surfaces. From curve fits to Eq. (2) it is found that the TiO_2 coating induce a degradation rate of $k = 5.63 \times 10^{-4}$/min, which is more than 20 times larger than the rate of 0.25×10^{-4}/min induced by the reference surface. Since the reference surface is not expected to be photocatalytic, it is likely that the observed degradation rate in tests performed on

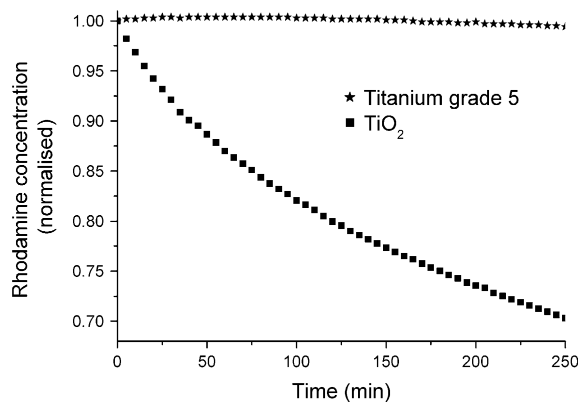

Fig. 2 Photocatalytic activity of the as deposited TiO_2 coating and the titanium grade 5 reference surfaces measured as Rhodamine B concentration versus time under UV illumination

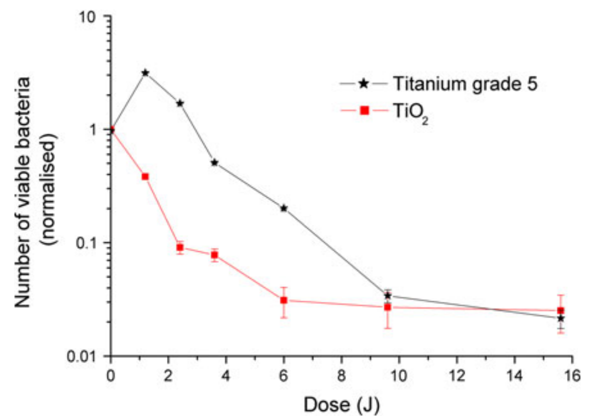

Fig. 3 Number of viable bacteria on TiO_2 coated and Ti grade 5 irradiated surfaces as a function of UV light irradiation dose, normalized with respect to bacteria concentration of tested suspensions. *Error bars* show the standard deviation of 3 measurements

this surface is due to the effect of the UV light itself on the Rhodamine B solution. The degradation rate measured for the TiO_2 coating is comparable to previous results obtained with similar PVD coatings (Kleiman and Marquez 2007; Lilja et al. 2012). As suggested in other studies (Fox et al. 1993; Miyagi et al. 2004), the degree of PCA is likely governed by the anatase dominated phase composition of the coating in association with the uneven and coarse surface morphology that results in an increased surface area.

Figure 3 shows the bactericidal effects of the TiO_2 coated and Ti reference surfaces under UV illumination. The coated surfaces exhibit a larger bactericidal effect than that seen with the reference surfaces when comparing the UV dose range from 1.2 J (corresponding to 1 min of illumination) up to approximately 10 J. Whereas the number of viable bacteria decreases monotonically with increasing UV dose for the coated samples, an increased bacterial viability is observed for UV doses $\leq$3 J for the reference samples. For higher UV doses, the number of viable bacteria is decreasing also on the reference samples. The latter can be related to the inherent bactericidal effect of UV light alone (Shiraishi et al. 2008). The greater antibacterial effect seen with the coated disks, particularly at clinically relevant UV doses less than 3 J, is attributed to the photo-induced ROS formed at the TiO_2 surface, which in turn react with the *S. epidermidis* bacteria present on the surface, rendering them non-viable.

The observed increase in number of viable bacteria on the reference samples at low UV doses has also been reported in previous studies (Shiraishi et al. 2008) and may be ascribed to an increased, and more favorable, temperature for bacterial proliferation (Ratkowsky et al. 1981; 1983; Cooper et al. 2001).

Summary and conclusion

Cathodic arc evaporated, nano-structured anatase thin films on titanium grade 5 substrates were demonstrated to exhibit a UV-induced photocatalytic activity that can be utilized to provide a bactericidal effect against *S. epidermidis.* A 90 % reduction of viable bacteria was achieved in only 2 min with a UV dose of 2.4 J.

Photocatalytic elimination of bacteria has been presented in previous studies with TiO_2 coatings (Panda et al. 2010; Gallardo-Moreno et al. 2010; Shiraishi et al. 2008) but the treatment time required to achieve a bactericidal effect in previous studies was on the order of one hour or more.

Post-surgical infections and infections around metallic implants associated with biofilm formation are major concerns in clinical research. Obtaining the desired antimicrobial effect in as little as 2 min should make the presented technique more clinically relevant than previously presented approaches and, thus, open up for new on-demand antimicrobial surface treatment methods for biomedical implants in order to combat infections or reduce infection risks.

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