Research Article

TiO$_2$ and TiO$_2$-Doped Films Able to Kill Bacteria by Contact: New Evidence for the Dynamics of Bacterial Inactivation in the Dark and under Light Irradiation

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This paper addresses recent developments in the design, evaluation, and characterization of flexible, uniform polyethylene-TiO$_2$, TiO$_2$-In$_2$O$_3$, and TiO$_2$-polyester able to inactivate bacteria under band gap irradiation and in the dark. The preparation of these bactericide films by sol-gel or by sputtering techniques is reported. The E. coli loss of viability kinetics under low intensity and actinic light is evaluated. Evidence for kinetics of the major steps leading to bacterial disinfection in the dark is presented by electron microscopy (TEM). The film surface properties were characterized by surface techniques like EM, DRS, XPS, ATR-IR, CA, AFM, XRD, and XRF. The surface characterization allows the correlation of the film surface morphology with the self-disinfection performance. The events taking place at the cell wall leading to bacterial inactivation when in contact with the TiO$_2$ films are presented and the steps related to the bond stretching preceding bond scission identified by ATR-IR.

1. Introduction

The ambient contamination by biofilms spreading bacteria for long times in hospitals, schools, and many public places requires the preparation of more effective adhesive antibacterial and antifungal films showing an improved kinetics/performance/stability. Antimicrobial nanoparticulate films preparation is a topic of increasing attention since they can reduce/eliminate the formation of infectious bacterial biofilms leading to hospital acquired infections (HAI) [1, 2]. These nosocomial infections are due to antibiotic resistant bacteria. They are becoming more frequent during the last decade and contribute to the increase in hospital care costs. Touching by hand a second person or touching surfaces and walls of hospital deposits bacteria that to a great extent disappear in about 5–8 hours since they do not find the surface humidity or residual C-compounds necessary to feed their metabolism. But there are many and resistant pathogenic bacteria that form stable and robust biofilms secreting proteins to cover/protect themselves and spread pathogenic bacteria like the Gram-positive Staphylococcus. Biofilms are formed from a complex mixture of proteins, saccharides, amino acids, and other extracellular polymeric substances.

The formation of these pathogenic biofilms may be avoided precluded by robust self-disinfecting films. Disinfecting surfaces have been shown to inhibit microbial growth, since bacterial concentrations found in hospital rooms are not high. Regrowth of bacteria was not observed on effective antibacterial films [1–3]. Therefore, the investigation of self-disinfecting surfaces as presented in this study is warranted [4–11].

The colloidal deposition of TiO$_2$ on textiles, polymers, glass, and steel plates is used to prepare self-disinfectant and self-cleaning surfaces showing a significant photocatalytic activity [12–14]. But the colloidal or sol-gel preparations deposited films are not mechanically stable, nor reproducible, and present low uniformity and little adhesion since they can be wiped off by a cloth or thumb [15]. This shortcoming of the colloidal depositions moved us to work on the sputtering
of antibacterial films to overcome the shortcomings of colloidal loaded films. The problem to fix colloids on surfaces encountered during CVD depositions is due to the heat needed for the film fixation on the substrate. The substrate has to be resistant to heat at temperatures that textiles, polymer films, polyethylene 3D, and polyurethane complex shaped objects do not withstand. Deposition by sputtering of metal/oxides/semiconductors on nonheat resistant substrates is possible since plastic/polymer thin films and textile fabrics can be heated for short times only up to 120–140 °C.

In a typical CVD process, the substrate is exposed to the volatile precursors. Due to the heat applied the precursor decomposes on the substrate surface depositing amorphous/polycrystalline coatings. The volatile species condense on the substrate having a lower temperature. The disadvantages of conventional CVD deposition are the high investment costs and the high temperatures needed besides the large amount of heat used requiring costly cooling systems. Recently, Foster et al. [16], Yates et al. [17], Dunlop et al. [18], and Brook et al. [19] have reported antibacterial TiO₂, Ag, and Cu coatings on glass and polymer films depositing the metal/oxides by CVD.

TiO₂-films preparation and evaluation have gained much attention during the last decade since they have been reported to be effective in reducing hospital-acquired infections (HAI) [1–3, 20–23]. The particular interest in TiO₂-textiles is based on the fact that the porous hydrophilic structure as found in cotton textiles provides a suitable environment for bacterial growth. TiO₂-nanoparticles have shown recently to produce strong antibacterial effects in textiles designed for medical applications [3–18].

The improvement in the performance and utility of antibacterial TiO₂ and TiO₂-doped films is to produce a film microstructure inducing an accelerated bacterial inactivation concomitant with a cytotoxicity within the accepted standards accepted in medical applications [24–26]. The film uniformity, stable structure, and adhesive properties are currently investigated by academic institutions and by pharmaceutical companies for medical devices and implants covered with antibacterial coatings [27]. The improvement on the microstructure of TiO₂ films leading to thinner films inducing a faster bacterial inactivation kinetics (doped or not) compared with more traditional DC sputtering and DCP sputtering is being explored in our laboratory. The recent development of highly ionized pulsed plasma magnetron sputtering (HIPIMS) producing high-density plasma to deposit TiO₂ films on polyester is presented in this study [28]. The development of HIPIMS in the last decade is due to the growing demand for high quality anticorrosive uniform films [29]. In the case of HIPIMS, pulses from one microsecond up to milliseconds generate current densities able to induce \(10^{18} \text{e}^-/\text{m}^3\). This is \(10^2\)–\(10^4\) times higher than the electron density obtained by conventional DC-sputtering [30]. Until now, the deposition by direct current magnetron sputtering (DC) pulse sputtering (DCP) and HIPIMS of nanoparticles on surfaces has not been widely used to coat hospital textile clothing, glass, and metal-plates antibacterial surfaces.

This study will describe results obtained in our laboratory addressing TiO₂ self-disinfection due to (a) transparent non-scattering polyethylene (PE) sputtered TiO₂ films, (b) TiO₂-In₂O₃ polyester, and (c) nanoparticulate TiO₂ polyester.

2. Materials and Methods

2.1. Sputtering Details, Support Materials, and XRF Determination of the Eluted Species

2.2. Surface XPS Analysis and ICP-MS of the Eluted Species during Bacterial Inactivation. An AXIS NOVA photoelectron spectrometer (Kratos Analytical, Manchester, UK) equipped with monochromatic AlKα (hv = 1486.6 eV) anode was used.
Figure 2: *E. coli* inactivation on TiO$_2$ sputtered on PE for 8 min irradiated with simulated solar light (52 mW/cm$^2$).
Figure 3: (a) Photoinduced superhydrophilicity followed by water droplet contact angle during light irradiation at times: (A) $t = 0$ min, (B) 15 min, (C) 30 min, (D) 45 min, and (E) 60 min. (b) Restoration of the hydrophilicity in the dark after times (A) 6 h, (B) 12 h, (C) 18 h, and (D) 24 h.

Figure 4: (a) Kinetics of the hydrophobic-hydrophilic transformation under solar simulated light and (b) kinetics of the dark reverse reaction towards the initial state for PE-TiO$_2$ (8 min) RF air plasma pretreated for 15 min.

The absorption of the samples was plotted in Kubelka-Munk (KM) arbitrary unit versus wavelength. Irradiation of the samples was carried out in a tubular cavity of a Suntest Heraeus solar simulator, Hanau, Germany, or in the cavity of a reactor provided with indoor actinic light (white light).

Transmission electron microscopy (TEM) was carried out in a Philips CM-12 (field emission gun, 300 kV, 0.17 nm resolution) microscope at 120 kV and was used to measure grain size of the TiO$_2$. The textiles were embedded in epoxy resin 43539 Fluka and the fabrics were cross-sectioned with an ultramicrotome (Ultracut E) and at a knife angle of 35°. Crystal structures were characterized by X-ray diffraction (XRD) and recorded on an X’PertMPD PRO from PANalytical equipped with a secondary graphite (002) monochromator.
2.4. Contact Angle (CA) and ATR-IR Measurements. The hydrophilicity of the TiO$_2$ films was determined by the water droplet contact angle (CA). The CA of TiO$_2$ films on the substrate was determined by the sessile drop method on a DataPhysics OCA 35 unit. FTIR spectra were measured in a Portmann Instruments AG spectrophotometer equipped with a Specac attachment (the prim was a 45° one pass diamond crystal). Spectra were taken by 256 scans with a resolution of 2 cm$^{-1}$ in the range 900–4000 cm$^{-1}$. The position of the IR peaks was found by the second derivative of the spectra after Fourier deconvolution.

2.5. E. coli Loss of Viability Evaluation. The samples of *Escherichia coli* (*E. coli* K12) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) ATCC23716, Braunschweig, Germany, to test the antibacterial activity of the TiO$_2$-polyethylene and TiO$_2$-polyester films according to previous work reported by our laboratory [9]. Serial solutions were prepared in tryptone solution and the samples plated on agar Plate-Count-Agar (PCA, Merck, Germany). The bacterial counting data reported were obtained by the replicate of 3 experimental runs.
3. Results and Discussion

3.1. TiO$_2$-PE Bactericide Thin Films Obtained by Sputtering Increasing E. Coli LPS Cell Wall Fluidity and Leading to Cell Lysis

3.1.1. Support Choice for TiO$_2$ Deposition. This first study addresses the sputtering of TiO$_2$ transparent, uniform, non-scattering films on polyethylene (PE). Sol-gel commercial methods are used to prepare TiO$_2$ thin films on heat resistant substrates. But on nonthermal resistant substrates the thickness of the TiO$_2$ films is not reproducible and they are not mechanically stable [6–10, 15].

Polyethylene (PE) is a low cost and is widely available material. It is chemically inert, mechanically stable and flexible, and UV-resistant and does not oxidize in air under sunlight. In addition, the hydrophobic nature of PE allows the deposition of predominantly hydrophobic pathogens. Bacterial hydrophobic outer cell walls will adhere preferentially on hydrophobic surfaces promoting bacterial inactivation. PE-TiO$_2$ films intend to overcome the use of TiO$_2$ powders or suspensions for bacterial disinfection. Suspensions of TiO$_2$ need a long-settlement time after each disinfection cycle being applied with significant loss of catalyst mass [6, 7]. The PE-TiO$_2$ transparent films are designed to increase the quantum yield of the TiO$_2$ radical species in addition to the direct bactericide action of sunlight [11, 14]. TiO$_2$ sputtering does not lead to the deposition of enough TiO$_2$ on the PE due to the low binding capacity of the PE surface. RF-plasma pretreatment induces oxygen negatively charged functional groups, for example, carboxylic, percarboxylic, epoxide, and peroxide groups by the atomic O, excited O, and anionic groups, for example, carboxylic, percarboxylic, epoxide, and peroxide groups by the atomic O, excited O, and anionic generated in the RF-plasma chamber [12–14]. The PE functionalized negative sites bind the slightly positive sputtered Ti$^{4+}$ (TiO$_2$) through electrostatic attraction involving chelation/complexation [34, 35]. The TiO$_2$ was sputtered immediately after the RF-plasma pretreatment due to the short lifetimes of the surface polar hydrophilic surface sites.

3.1.2. PE Pretreatment, TiO$_2$ Surface Sputtered PE, Sample Absorption, and TiO$_2$ Crystalline Phases. The amount of TiO$_2$ on the pretreated PE fabrics was determined by X-ray fluorescence XRF using the nonpretreated fabric as the blank. For the sample pretreated in air for 15 min by RF plasma and sputtered for 8 min, a TiO$_2$ thickness of ~58 nm was attained on PE equivalent to 290 layers. Taking one layer with $10^{14}$ atoms/cm$^2$ and the thickness of one layer as 0.2 nm, the rate of TiO$_2$ deposition was of $6 \times 10^{14}$ atoms/cm$^2$. Self-cleaning TiO$_2$ films up to 10 microns thick have been reported [1–5], but in the case of antibacterial coatings TiO$_2$ thicknesses of 1 nm–20 nm have been reported to be effective [3, 6, 7]. TiO$_2$ films DC sputtered for 8 min consist
of nanoparticles 10–30 nm in size [36] and the films were reproducible.

The RF-plasma induced functional groups only on the PE topmost layers since no color changes occurred destroying the PE structure indicative of PE-degradation [37].

The DRS spectra of the PE-TiO₂ films are shown in Figure 1 for samples pretreated by RF and UVC light. Sputtering for 1 to 3 min leads to transparent TiO₂ films with no significant absorbance and very low antibacterial activity. A decrease of ~5% or more in optical transmittance has been reported for RF-plasma treated PE [38]. The decrease in transmittance for the sputtered films in Figure 1 was due to the inherent high refractive index of TiO₂.

The TiO₂ crystal phases on PE show by XRD a high anatase (A) peak at 2θ = 25.5° [34] for a PE-TiO₂ with and without RF-plasma pretreatment. But XRD peaks of rutile (R) at temperatures ≤130°C found in the DC-magnetron chamber were also observed. The generation of rutile at low temperatures is due to the structure forming function of the PE film on the TiO₂ as reported for polyamide and other sputtered TiO₂ textiles [12].

3.1.3. Antibacterial Kinetics Evaluation and Sample Recycling. Figure 2 shows E. coli inactivation on PE-TiO₂ films under simulated solar light with an integrated light dose of 52 mW/cm². The fastest bacterial inactivation was found for pretreated PE samples TiO₂ sputtered for 8 min in Figure 2(a). Bacterial inactivation on PE-TiO₂ samples is presented in Figure 2 for RF-pretreated PE for different times. No significant bacterial inactivation was observed for bacteria on uncoated PE (Figure 2(a), trace (4)). Only trace (1) was subjected to statistical analyses since it describes the results for the most favorable kinetics and presents the highest amount of TiO₂ sites in exposed positions to interact with bacteria [39]. PE-TiO₂ sputtered samples for 1 up to 5 min were not loaded with sufficient TiO₂ to drive a fast bacterial inactivation. The Ti-loading on PE-TiO₂ was determined by XRF for sputtering times of 1, 3, and 5 min and the values found were, respectively, 0.009, 0.019, and 0.031 TiO₂ wt%/wt PE.

Samples sputtered for times >8 min in Figure 2(b) led to layer thickness >45 nm. This thickness leads to charge bulk inward diffusion decreasing the charge transfer between the PE-TiO₂ and bacteria [40]. TiO₂ sputtering for 8 min led to a TiO₂ loading with the most suitable thickness for the charge diffusion able to reach bacteria. Figure 2(c) shows the fastest bacterial inactivation in PE-TiO₂ films. No significant bacterial inactivation was observed for bacteria on PE in the dark.

To verify that no regrowth of E. coli occurs after the first bacterial inactivation cycle, the PE-TiO₂ film was incubated...
on an agar Petri dish for 24 hours at 37°C. No bacterial regrowth was observed meaning that there was no bacteria adhered to the surface after the inactivation cycle.

The bacterial inactivation by PE-TiO₂ samples sputtered for 8 min and pretreated with RF-plasma in air for 15 min was investigated up to the 6th cycle. The recycling of the samples showed stable inactivation kinetics up to the 5th cycle; then the recycling kinetics became slower by ~20%. After each cycle, the samples were washed with distilled water and dried. Then, the samples were kept in an oven at 60°C to avoid bacterial contamination. After washing PE-TiO₂ the samples were left standing for 24 h before to regain the initial sample hydrophobicity. This aspect will be discussed below in Section 3.1.4 when discussing the hydrophilic-hydrophobic transformation on PE-TiO₂ in the dark and shown in Figures 3(a) and 3(b).

### 3.1.4. Contact Angle (CA) and Hydrophobic Reversible Photoswitching

Figure 3(a) presents the hydrophobic to hydrophilic transformation occurring on the PE-TiO₂ sample induced by simulated solar light. A decrease of the CA from 121° to less than 5° within 60 min is shown in Figure 3(a) (traces (A)–(E)) concomitant with the time of bacterial inactivation. The recovery from the PE-TiO₂ superhydrophilic surface to a hydrophobic initial surface proceeded within 24 hours in the dark as shown in Figure 3(b).

The transformation of the initial hydrophobic TiO₂ under light irradiation involves the dissociative chemisorption of water on the PE-TiO₂ generating Ti-OH groups [41, 42]. Under light irradiation, the PE-TiO₂ generates electrons (e⁻) and holes (h⁺) producing OH⁻ and O₂⁻ radicals. The photogenerated h⁺ are the precursors of OH⁻. The structural changes in the TiO₂ surface due to hydrophilicity are associated with the generation of these radicals. This process does not require high quantum efficiency in comparison to the E. coli photocatalytic oxidation. A second mechanism has been suggested for the hydrophobic to hydrophilic transformation and would be due to TiO₂ generated charges leading to oxygen vacancies reducing Ti³⁺ to Ti⁴⁺ [43].

Figure 3(b) shows the hydrophilic to hydrophobic reversible transformation in the dark reinstating the initial TiO₂ hydrophobicity within 24 hours. The hydrophilic samples were kept in the dark and the contact angles (CA) were measured at preselected times to follow the back reaction to the initial hydrophobic state. This second process involves the destruction of airborne bacteria or hydrocarbons adsorbed on the PE-TiO₂ surface along TiO₂ surface dehydration and the back conversion of Ti⁴⁺/Ti³⁺ [42].

Figure 4(a) illustrates the rate of photoinduced hydrophilicity and Figure 4(b) the restoration rate of the hydrophobicity in the dark as a function of “cos θ”. According to Young’s theory the “cos θ” of a liquid droplet on a solid surface is a function of the interfacial energy between the solid and the liquid. The rate of the hydrophobic to hydrophilic conversion and for the reverse reaction in Figures 4(a) and 4(b) was 0.277 min⁻¹ and was 8.71 × 10⁻² min⁻¹, respectively. These rates were calculated by integrating “cos θ” in Young’s equation (1).

The contact angle (CA) conventionally measures the angle where the liquid meets the solid quantifying the wettability of a solid surface via the Young equation. The Young equation (1) involves solid-vapor, liquid-vapor, and solid-liquid interfacial energies. The solid-vapor interfacial energy is denoted by γ_{SG}, the solid-liquid interfacial energy by γ_{SL}, and the liquid-vapor interfacial energy (i.e., the surface tension) by γ_{LG}; then the equilibrium contact angle θₐ is determined from these quantities by Young’s equation:

\[ \cos \theta = \frac{\gamma_{SL} - \gamma_{SG}}{\gamma_{LG}} \]  

(1)

Upon illumination the surface TiO₂ energy increases since the TiO₂ surface is transformed into a metastable state as shown in (2) decreasing the initial contact angle of 121° (Figure 3(a)) to a value of <5° after 60 min irradiation (Figure 3(b)). Equation (2) shows the TiOH metastable hydrophilic intermediate induced under light:

\[ \cos \theta = \frac{\gamma_{SL} - \gamma_{SG}}{\gamma_{LG}} \]  

(2)
The hydrophobic properties of the PE-TiO$_2$ surface are important in antibacterial films. *E. coli* and *Staphylococcus aureus* present a preferential adhesion to hydrophilic surfaces [44]. Bacteria with hydrophilic surface properties like *S. epidermidis* adhere preferentially to hydrophobic surfaces [45]. Hydrophobic bacteria adhere to a variety of surfaces forming biofilms to a greater extent than hydrophilic bacteria [46]. Recently, R. Amal recently has reported the reversible photocurrent behavior under light by TiO$_2$/Ag nanoparticles [47, 48]. This study shows that UV-A irradiation of brownish Ag/TiO$_2$ changed the surface Ag$_2$O to violet black, the characteristic color of Ag-plasmon. Nano-Ag particles are responsive to visible light due to the enhanced surface plasmon resonance (SPR) absorption band at ~550 nm. As a result of visible light excitation a reverse electron flow from Ag$^{+}$ to the TiO$_2$ occurs along the oxidation of metallic Ag$^{+}$ back to Ag$_2$O. A reversible antimicrobial photocurrent of nano-Ag/TiO$_2$ particles was reported in this study when irradiating the Ag-nanoparticles in two different wavelength regions [48].

### Figure 11: XRD Diffraction Data for (1) TiO$_2$ sputtered for 10 min on polyester and (2) TiO$_2$, 10 min /In$_2$O$_3$10 s sputtered on polyester: (a) diffraction patterns of anatase (b), rutile (c), and (d) cubic In$_2$O$_3$.

3.1.6. TiO$_2$ Films Sputtered by HIPIMS Leading to Accelerated *E. Coli*. Figure 6 shows the bacterial inactivation kinetics by the HIPIMS TiO$_2$ sputtered samples [28]. As shown in Figure 6, no bacterial inactivation takes place in the dark, but the bacterial inactivation becomes faster for HIPIMS sputtering times between 1 min (trace (5)) and 4 min (trace (2)). Longer deposition times between 10 and 30 min did not accelerate the loss of viability kinetics due to the fact that an increased TiO$_2$ thickness > 12 nm sputtered within 4 min leads to (a) bulk inward diffusion of the charge carriers generated on TiO$_2$ under light leading to highly oxidative radicals [6–10] and (b) longer sputtering times facilitate the TiO$_2$ interparticle growth decreasing the TiO$_2$ contact surface with bacteria.

The bacterial inactivation time shown in Figure 6 for polyester surface sputtered by HIPIMS is faster compared to DC and DCP TiO$_2$–polyester sputtered surfaces [36].

3.1.7. DCP and HIPIMS Sputtering of Samples, Electronic Density, and Voltage Considerations. Figure 7, left hand side presents a scheme for the DC sputtering and in the middle section Figure 7 shows the sputtering of DCP proceeding...
A synergic interaction of TiO$_2$ is required to lead to fast bacterial inactivation in Figure 8(a), trace (1). Figure 8(b), trace (4) shows that no bacterial inactivation was possible under light on bare polyester. Figure 8(a) reports the effect of the light intensity on the amount of charges in the coupled semiconductors at 30 mW/cm$^2$ and 50 mW/cm$^2$. A higher light intensity increases the amount of semiconductor charges interacting with bacteria leading to a faster bacterial inactivation. The accelerated bacterial inactivation by the TiO$_2$-In$_2$O$_3$ photocatalysts compared to bare TiO$_2$ samples is favoured by the electrostatic attraction existing between the positive charged Ti and the negative E. coli cell wall at pH 6-7. The E. coli is negatively charged between pH 3-9 due to the excess of carboxylic groups compared to the amide I and amide II cell wall positively charged groups [3, 58]. The TiO$_2$ bacterial inactivation under light has been widely reported and will not be discussed in this study [6-10].

Figure 9 shows repetitive disinfection cycles by a TiO$_2$-In$_2$O$_3$ sputtered for 10 min from a TiO$_2$ target and 10 s from an In target in a reactive O$_2$ atmosphere, up to the 5th cycle. The 8th cycle shows a loss of bacterial inactivation kinetics when actinic light was used in Figure 9(a) and when a solar simulator with a dose of 50 mW/cm$^2$ was applied. The slower kinetics shown in Figure 9(b) in the last cycle was due possibly to the leaching of In and Ti-nanoparticles detected by ICP-Ms (data not shown).

3.2. Uniform TiO$_2$-Doped In$_2$O$_3$ Films Increasing the Bacterial Inactivation Kinetics with respect to TiO$_2$-Films under Low Intensity Solar Simulated Light

3.2.1. TiO$_2$ Sputtered on Pretreated PE. This study shows that the surface modification of TiO$_2$ by doping with In$_2$O$_3$ is an effective route to increase the TiO$_2$ absorption into the visible region. A photoinduced interfacial charge transfer (IFCT) between TiO$_2$ and In$_2$O$_3$ takes place and couples the charge generation/separation between these two oxides. In$_2$O$_3$ has an absorption band in the visible between 400 nm and 500 nm [57].

The TiO$_2$ and TiO$_2$-In$_2$O$_3$ thin films were deposited onto polyester in the magnetron chamber by sputtering Ti by DC in a reactive O$_2$ atmosphere followed by DCP sputtering of In, in the presence of mixture of Ar and O$_2$ gases. The total working pressure $P = (P_{Ar} + P_{O_2})$ was fixed at 0.5 Pa and the ratio $P_{O_2}/P_{total} = 4.5\%$. The sputtering current on the Ti target was 280 mA providing a power of 120 W (U = −450 V) and a current density of 12.7 mA/cm$^2$. Pulsed magnetron sputtering (DCP) was used to sputter the In$_2$O$_3$ and was operated at 50 kHz with 15% reversed voltage. The sputtering power was fixed at 50 W providing a negative voltage of −500 V and a power of 140 W.

3.2.2. Evaluation of the Bacterial Inactivation of E. coli in the Dark and under Light. The counting of the bacterial inactivation of E. coli was performed as described above in Section 2.4 [28, 36]. An actinic illumination lamp Osram Lumilux T8-L 18 W (4.0 mW/cm$^2$) was used in Figure 8 as a source of white light. This light is used for the indoors lightning in health facilities. The solar simulator (Heraeus, Hanau, Germany) emitting between 200 and 800 nm from a 100 W Xe-light resembling the solar spectrum and set at 50 mW/cm$^2$. The value of 50 mW/cm$^2$ is the average light dose reaching central European countries. Figure 8 shows the inactivation of E. coli by TiO$_2$, TiO$_2$-In$_2$O$_3$, and In$_2$O$_3$ samples.
low loading (<0.2%). Only a slight decrease in intensity of the 24.6° (101) anatase peak was observed in the In$_2$O$_3$/TiO$_2$ samples. No modification in the TiO$_2$ diffraction peaks due to In$_2$O$_3$ was observed, suggesting that no lattice modification due to In-doping takes place in the TiO$_2$ network.

3.2.4. Interfacial Charge Transfer Mechanism (IFCT) in TiO$_2$-In$_2$O$_3$ Composite Films. Coupling TiO$_2$-In$_2$O$_3$ induced a significant increase in the photocatalytic activity compared to TiO$_2$ alone and In$_2$O$_3$ as shown in Figure 8. This significant increase can be rationalized by the relative positions of the conduction band (cb) and valence band (vb) in In$_2$O$_3$ and TiO$_2$. The cb of In$_2$O$_3$ at −0.62 versus NHE [60, 61] transfers the cb electrons to TiO$_2$ (cb at 0.2 eV versus NHE for anatase). The UV component of sunlight generates TiO$_2$ holes able to transfer to In$_2$O$_3$ vb as shown in Figure 12(a). The subsequent spatial separation of photogenerated charge carriers and the e$^-$ and h$^+$ injection limit the e$^-$/h$^+$ recombination on TiO$_2$.

The highly dispersed In$_2$O$_3$ on the TiO$_2$ layers builds a Schottky barrier at the TiO$_2$/In$_2$O$_3$ interface. This precludes partly the recombination of electrons and holes in TiO$_2$. The
inducing cell wall damage with concomitant loss of bacterial viability in the dark. In 1985, Matsunaga et al., [63] reported that TiO₂ suspensions inactivate bacteria. After Matsunaga many studies have reported on the TiO₂ photocatalytic bacterial cell wall damage [64–66]. The bacterial inactivation by agents permeating into the cell structure has also been widely reported [5, 6, 67]. Few articles have reported cell wall damages by the photocatalysis by electron microscopy (EM) [5]. P. aeruginosa outer cell wall damages due to TiO₂ photocatalysis have been reported recently by P. Amezaga-Madrid et al., [68–74]. The TiO₂ interaction with the bacteria was reported to cause damages/disorganization in the cell wall morphology modifying its permeability and the capacity to regulate the outer layers osmotic pressures. The importance of the present study on antimicrobial surfaces relates to the fact that bacteria survive for long times in hospital facilities increasingly leading to hospital acquired infections (HAI). Precluding the infectious biofilm formation by the TiO₂-polyester surfaces in the dark is an effective way to reduce/suppress infections as will be reported in this study.

### 3.3. New Evidence for the Inactivation of TiO₂ Films with Bacteria in the Dark

This study addresses the design, preparation, and bacterial counting of the bacterial inactivation kinetics and characterization of TiO₂-polyester surfaces.

#### 3.3.1. Preparation of TiO₂-Polyester by the Hydrothermal Route and E. coli Loss of Viability in the Dark and under White Light

The TiO₂ on the polyester deposition by the hydrothermal route (HT) was carried out as follows: titanium tetraisopropoxide (TTIP) Sigma Aldrich p.a. was dissolved in isopropanol in a 1:3 volume ratio (1:3 molar ratio). This solution was poured into a beaker with 50 mL of 0.1 M HNO₃. The polyester samples 6 × 4 cm were immersed into the acid TiO₂ precursor suspension and heated under stirring in a reflux condenser for 2 hours at 80°C. The polyester fabric was removed from the suspension, rinsed with deionized water, and treated in ultrasound bath for 2 min to remove unbound TiO₂ particles. The last operation was repeated three times and the TiO₂-polyester fabric were subsequently dried for 2 hours at 70°C in air [75]. The inactivation of Escherichia coli (E. coli K12) on TiO₂ polyester samples was evaluated according to Section 2.4.

In Figure 12 no significant bacterial loss of viability was observed for bacteria on uncoated samples (Figure 13, trace (1)). A loading of 0.05% TiO₂ on the TiO₂-polyester in Figure 13, trace (2) did not contain enough TiO₂ to induce bacteria loss of viability. TiO₂ loadings between 0.1% and 5% led to similar bacterial loss of viability within 120 min. This indicates that the number of cells capable of forming colonies in contact with the cell wall surface attained a stable value for TiO₂-polyester surfaces loaded between 0.1% and 5.0%.

Figure 14 shows the loss of bacterial viability under light irradiation within 60 min for TiO₂-polyester at loadings above TiO₂ 0.1%. The insert in Figure 14 shows the spectral emission of the actinic Osram Lumilux 18 W/827 light used with a dose of 4.0 mW/cm². The mechanism leading to bacterial inactivation under light has been reported by Foster et al., [4] and Tung and Daoud, [10]. More recently, our EPFL laboratory has reported in a detailed way the partial damage/degradation of the E. coli outer cell functional groups during TiO₂ photocatalysis by ATR-IR spectroscopy [50, 66].
By X-ray diffraction spectroscopy (XRD) the TiO$_2$ crystalline phases on the 5% TiO$_2$-polyester prepared by the HT-method show a significant anatase (A) peak at $2\theta = 21.5^\circ$ and a small rutile peak at $2\theta = 37.3^\circ$. These XRD results showing the formation of anatase and rutile peaks are due to the structure forming function of the polyester at low temperatures when colloidal TiO$_2$ suspensions were added. When heating TiO$_2$ suspensions by themselves anatase was formed $\sim$300$^\circ$C and rutile at around 600$^\circ$C in the absence of a structure forming surface like polyester [9, 12, 13]. These last temperatures are significantly higher compared to temperatures $\sim$80$^\circ$C required to prepare the TiO$_2$ with anatase and rutile phases as detected by XRD.

3.3.2. Transmission Electron Microscopy of the E. coli Cell Wall Interaction with TiO$_2$-Polyester. Sample Preparation and Process Kinetics. The samples of the TiO$_2$ polyester for the electron microscopy of the E. coli interaction with TiO$_2$-polyester fabrics were prepared in the following way: suspensions or E. coli were fixed in paraformaldehyde 2% + glut 0.2% in phosphate buffer for 30 min and centrifuged and pellet resuspended in low melting point agarose. This was then cut into small cubes, dehydrated, and stained for 20 min in 2% uranyl acetate then dehydrated in a graded alcohol series. The samples were then embedded in LR white resin in Beam capsules and polymerized overnight at 55°C. The resin blocks containing the E. coli on TiO$_2$-polyester were thin sectioned to a 70 nm thickness with an ultramicrotome (Leica UC7). The TiO$_2$-polyester fibers were embedded in epoxy and thin sectioned at a thickness of 80 to 100 nm.

The transmission electron microscopy (TEM) of the TiO$_2$-polyester (HT) samples interacting with E. coli is shown in Figure 15. The TEM in the upper left corner in Figure 15 shows the interaction of TiO$_2$-polyester (HT) sample with the E. coli K12 at time zero. The E. coli intact cell wall is seen as well as the aggregates and coaggregates of TiO$_2$ positioned at a distance from the cell wall in agreement with the DLVO theory of colloidal stability. TiO$_2$ nanoparticle aggregation sets in at a pH close to the isoelectric point (IEP) of 6-7 due to the attractive Van der Waals forces leading to TiO$_2$ aggregation within 30 min as shown in the TEM in the upper right corner [76]. The TiO$_2$ single particles present sizes between 40 and 60 nm and the hydrodynamic diameter of the aggregates was found to be 170–240 nm equivalent to 3-4 primary TiO$_2$ particles. After 30 min, the TiO$_2$ aggregates...
accumulate on the cell wall surface due to their almost neutral charge at physiological pH and this is shown in the right upper hand side Figure 15. Damage in the cell wall is localized in the contact area between the TiO$_2$ and bacteria due to (a) the weak attraction between the TiO$_2$ (IEP $\sim$6-7) and the negatively charged cell wall. Damage in the cell wall was observed at a pH close to the TiO$_2$ IEP (charge zero). The TiO$_2$ particles aggregate between themselves since they have an almost neutral charge. Concomitantly the Van der Waals attractive forces drive the interaction between the TiO$_2$-aggregates and the bacterial cell wall and (b) damage to the cell wall is also possible due to the abrasion by the TiO$_2$ rutile component of the E. coli envelope [77]. The effect of the cell wall in the dark after 30 minutes seems to be critical step in the loss of bacterial viability.

The lower central TEM in Figure 15 shows the damage to the E. coli outer wall cell within 120 min. After 120 min the wall outer layers present discontinuities in some regions and vanished in other regions. Cell wall damages leading to cell inactivation involve changes in cell morphology, cell-wall microstructure, and local pH [78]. The extensive damage to the outer cell layers after 120 min coincides with the time required for the total loss of cell viability in the dark shown in Figure 13. The bacterial cell cannot function anymore as a membrane regulating the in and out osmotic pressure and material flow. Sunada et al. reported E. coli inactivation by TiO$_2$ films damaging the cell wall membrane due to the leakage of internal cell components [79].

4. Conclusions

(i) This study accounts on self-disinfecting and self-cleaning TiO$_2$ films recently investigated in our laboratory. The design, preparation, evaluation, and characterization of uniform, adhesive, and innovative photocatalytic TiO$_2$ coatings are described.

(ii) It is possible by traditional DC, DCP, and HIPIMS sputtering to produce antibacterial films on 2D and 3D objects at low temperature on polymer films and textile fabrics not resisting higher temperatures.

(iii) The microstructure of TiO$_2$ films still has to be characterized to find the most suitable microstructure leading to disinfection in the minutes/seconds and not in hours.

(iv) An interdisciplinary approach is necessary when working in the field of self-disinfecting coatings as presented in this study.

(v) A considerable saving in Ti and deposition time (energy) was found with HIPIMS compared to conventional DC/DCP sputtering when coating surfaces.

(vi) TiO$_2$-polyester surfaces have been shown in this study to inactivate bacteria in the dark. This is an important point not addressed generally in bacterial inactivation studies addressing mainly TiO$_2$ photocatalysis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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