Review:

Accelerated Antibacterial Inactivation on 2D Cu-Titania Surfaces: Latest Developments and Critical Issues

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Abstract:

This review focuses on Cu/TiO_2 sequentially and Cu-TiO_2 co-sputtered catalytic/photocatalytic surfaces leading to bacterial inactivation discussing their stability, synthesis, adhesion and antibacterial kinetics. The intervention of TiO_2, Cu and the synergic effect of Cu and TiO_2 on films prepared by a colloidal approach and other techniques is also reviewed leading to bacterial inactivation. Processes in aerobic media and anaerobic media leading to bacterial loss of viability on multidrug resistant (MDR) pathogens, Gram-negative and Gram-positive bacteria are described. Insight is provided for the interfacial charge transfer mechanism under solar irradiation occurring between TiO_2 and Cu. surface properties of 2D TiO_2/Cu and TiO_2-Cu films are correlated with the bacterial inactivation kinetics observed in the dark and under light. The intervention of these antibacterial sputtered surfaces in health-care facilities leading to MRSA-isolates is described in the dark and under the actinic light. The synergic intervention of the Cu and TiO_2 films leading to bacterial inactivation prepared by direct current magnetron sputtering (DCMS), pulsed direct current magnetron sputtering (DCMSP) and highly ionized pulse plasma magnetron sputtering (HIPIMS) is reported in a detailed way.

Keywords: DCMS, DCMSP, and sputtering, highly ionized pulse plasma magnetron sputtering (HIPIMS), bacterial inactivation kinetics, Cu and TiO_2 synergic effects, interfacial charge transfer

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1. Introduction

The focus of innovative antibacterial materials is to find composites in the colloid form or deposited on surfaces able to inactivate bacteria/pathogens almost instantly or within very short times and present long operational lifetime (high stability). Biofilms spreading bacteria in hospitals, schools and public places are the most common and dangerous form of infection by pathogens: bacteria, fungi and viruses. Pathogens are capable of living in any environment where minimal conditions of life are encountered since they have the ability to form biofilms, adhering to each other and to the surface spreading pathogenic bacteria continuously into closed environments most commonly in health facilities[1-9]. Healthcare- facilities associated infections (HCAI's) are becoming a worldwide problem since bacteria can survive on abiotic surfaces for long times. In this way they disseminate a wide range of infections. The Center for Disease Control and Prevention (CDC) estimates at approximately two millions the number of patients affected by HCAI's in the USA [10-11]. In Europe, HCAI's type infections affect ~3.2 million hospital patients/year leading to mortality or increasing the duration of the hospital stay and associated costs [12]. Nosocomial infection occurs by a simple touch the hands of healthcare workers by bacteria found in hospital clothing, equipment, air conditioners and hospital water distribution networks [13]. The use of gloves, gowns and masks as well as the patient isolation have limited the spread of infections, but by themselves are not able to avoid the transmission of HCAI's [14-15].

The levels of contamination in UK hospitals have been reported to be ~10^5 CFU/cm^2 in diabetic wound dressing room and in hospital residence rooms a density of 10^2 CFU/cm^2 only was found. This study will present several studies based on surfaces coated with TiO_2, Cu and Cu/TiO_2 that present the potential to decrease the environmental bacterial presence in hospital facilities since the reported level of bacterial contamination is not high. The added benefit of the same coatings is that they avoid to a certain degree biofilm formation [16-21]. When HCAI's are caused by multidrug-resistant (MDR) pathogens this problem becomes critical since antibiotics are not available or ineffective due to their prolonged application times make the pathogens resistant to its initially designed abatement effect.

At the present time more work is required on improved antibacterial coatings inducing fast bacterial reduction, high adhesion, robust layered structure and stability precluding HCAI's. The application of nanotechnology to produce innovative 2D-

surfaces of biomaterials useful in medicine posses at this time a significant potential for the prevention and treatment of infections. Nevertheless, concern exists about the field use of these new nano-particulate materials due to the incomplete knowledge of the toxicology. [22-23].

The present write-up reviews the recent work on TiO_2, Cu, and the recent films made up by Cu and TiO_2 leading fast bacterial inactivation kinetics and being able to preclude partly or totally biofilm formation. The spread of pathogenic infections will be decreased depending on the type of pathogen or its local concentration. Biofilm formation is at the origin of 80% of all microbial infections in the body making biofilms a primary health concern [1-9]. Biofilm pathogens adhere to a host surface, organize their community structure and remain there by producing extra-cellular polysaccharides (EPS) polymer matrix to cement the biofilm to the support in a permanent way. We report TiO_2-Cu inactivation of *E. coli* and *Methicillin-resistant Staphylococcus Aureus* (MRSA) a bacteria strain resistant to the effect of antibiotics and to a lesser degree on the abatement of other bacteria. What makes the problem even more complicated is that bacteria embedded in a biofilm can survive concentrations of antiseptic/antibiotics several times higher than the concentration able to kill planktonic cells of the same species [24].

2. Bacterial inactivation performance on TiO_2/Cu and surface properties

2.1. Short description of the main issues of concern affecting the TiO_2 photocatalysis application in microbial abatement

After the seminal report by Matsunaga et al. [25], a large number of papers appear devoted to the issue of microbial abatement in suspension or by supported TiO_2 surfaces. Lately, some reviews/monographs/reports have appeared on the TiO_2 photocatalysis involving a wide effort addressed to: a) the reporting in a detailed and systematic way the references on TiO_2 photocatalysis, b) the classification of the available data and c) the critical qualification of the validity and weaknesses of the data reported by the recent reviews.

Foster et al., have reported on the TiO_2 mediated disinfection of a large variety of pathogens [26]. The main barriers to the application of TiO_2 photocatalysis in suspension have been reported recently by Guillard et al., [27], Morawski et al., [28], Robertson et al., [29], Dalrymple et al., [30] and Gamage et al., [31]. Reviews on the

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antimicrobial mechanism mediated by TiO_2 composite films and suspensions haves been reported by several laboratories like Kubacka et al., [32] and Dalrymple's et al., [33]. TiO_2 upon band–gap light iirradiation photogenerates charges that react with adsorbed O_2, H_2Ov (at the solid-air interface) leading to reactive oxygen species (ROS) presenting a high oxidation potential to degrade organic compounds and bacteria [34-35]. These (ROS) radicals are $HO^{\bullet}$, $O_2^{\bullet-}$ and $HO_2^{\bullet}$ and have been documented and characterized for most of their properties like their concentration, lifetimes, spectra and redox potentials by numerous workers in a detailed way. Linsebigler et al., [36], Nakano et al., [37], Fujishima et al., [38], Douad et al., [39], Griesser et al., [40] and Hu et al., [41] studied the photocatalytic activity of TiO_2 thin films. More work using fast kinetics by femto- and picosecond spectroscopy in the visible region is still necessary to identify the nature and lifetime of these ROS species intervening in individual pathogen inactivation processes. Recent work involving fast kinetics of surfaces producing ROS under visible leading subsequently to *E. coli* inactivation has been reported recently by Kiwi et al., [42] and Rtimi et al., [43].

Photocatalytic degradation of pollutants and bacteria mediated by TiO_2 is a promising approach to face the increasing environmental contamination. However, because of its wide band gap (3.2 eV) TiO_2 can absorb mostly UV-light ($\lambda < 387$ nm) and only about 4-5% of the visible radiation of the solar spectrum. Sensitization of TiO_2 by doping anions or metal-cations/oxides like Cu /CuOx is been used to extend the TiO_2 response into the visible region. In this way the TiO_2 reaction kinetics under the visible light irradiation leading to bacterial inactivation on doped TiO_2, binary -oxides, TiO_2 composites or decorated 2D-surfaces has been recently reported by Dionysiou [44-45], Pillai [46-48], Kavitha [49], Dionysyou [50] and Bahneman [51].

Wettability plays an important role on the TiO_2 surface under band-gap irradiation. The surface wettability is evaluated by the water contact angle (CA). The CA (θ) defined as the angle between the solid surface and the tangent line of the aqueous solution at the liquid-solid interface. Hashimoto, Fujishima et al., [52-53] reported the importance of the hydrophilicity changes in TiO_2 under band-gap irradiation on the TiO_2 surface during pollutant/bacteria degradation. A low hydrophilic TiO_2 surface at times zero was reported to become highly hydrophilic or superhydrophilic ($\theta < 5^{o}$) under band-gap excitation. This surface will gradually revert to the original surface within realtively long times in the dark. This consideration is important in bacterial

inactivation processes since highly hydrophilic conversions lead to clean surfaces due to the destruction of bacteria adsorbed on the TiO_2 surface during the sample irradiation. The photo-generated holes have been shown responsible for the TiO_2 surface conversion to a high hydrophilic surface under light irradiation. These holes a) diffuse to the TiO_2 surface and the oxygen lattice sites reacting with bacteria or b) produce $HO^{\cdot}$-radicals reacting with the embedded $HO^{\cdot}$ of the TiO_2 surface. But a part of the photo-generated holes break the -Ti-O- lattice bonds by coordinating H_2O at the Ti-lattice sites. The coordinated water releases a proton (charge compensation) and a new HO° is formed increasing the number of surface HO° radicals [54].

Transparent, non-scattering PE-TiO_2 sputtered films inactivated *E. coli* on PE-TiO_2 (PE = polyethylene) sputtered for 8 min by direct current magnetron sputtering (DCMS) within one hour under low intensity solar simulated irradiation (52 mW/cm^2). The scheme of the sputtering unit is shown in Figure 1a. The PE was pretreated for 15 min by RF-plasma to graft a larger amount of polar negative groups able to bond the positive TiO_2 on the PE-surface [55-57]. The PE-TiO_2 samples pretreated for 15 min and sputtered for 8 min presented the highest amount of TiO_2 sites in exposed positions interacting leading to the favorable bacterial inactivation kinetics. A decrease from the initial PE-TiO_2 (CA) at zero time of ~121^{o}C to ($\theta < 5^{o}$) within 60 min inducing PE-TiO_2 super-hydrophilicity was observed to be concomitant with the time of bacterial inactivation. This seems to be a necessary condition to attain the optimal *E. coli* inactivation kinetics. Samples sputtered for times >8 min led to a thicker coating inducing charge bulk inward diffusion decreasing the charge transfer between the PE-TiO_2 and bacteria [58]. The rate of the hydrophobic to hydrophilic transformation was found to be 0.28 min^{-1}. The reverse reaction rate in the dark found was 8.7x10^{-3} min^{-1}. The reverse reaction time necessary to reach again a CA ~121^{o}C was completed within 24 hours. These rates were calculated by integrating “cos Θ” in the Young’s equation [53-54].

TiO_2 sputtering for 8 min led to a TiO_2 loading with the most suitable thickness for the charge diffusion generated in the TiO_2 reaching the bacteria. No bacterial re-growth was observed meaning that there was no bacteria adhered to the surface after the inactivation cycle. After each cycle, the samples were washed with distilled water and dried. Then, samples were kept in an oven at 60°C to avoid bacterial

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contamination. After washing the PE-TiO_2 samples were left standing for 24h before to regain the initial sample hydrophobicity.

According to Young's theory the "cos Θ" of a liquid droplet on a solid is a function of the interfacial energy between the solid and the liquid. The wettability is commonly evaluated in terms of the contact angle (CA), which is given by Young's equation [53]:

$$\gamma S = \gamma SL + \gamma L \cdot \cos \theta \quad (1)$$

In eq(1) γS and γL are the surface free energies per unit area of the solid and liquid respectively, and γSL is the interfacial free energy per unit area. In addition, γSL can be approximated using the Girifalco-Good eq. 2, with γS and γL, as

$$\gamma SL = \gamma S + \gamma L - \Phi(\gamma S / \gamma L)^{1/2} \quad (2)$$

Here, Φ is a constant parameter ranging from 0.6 to 1.1, depending on the solid. In addition, γL is the water surface free energy, which has a constant value of 74 mJ/m^2. Therefore, by combining (1) and (2), the CA can be simply expressed as:

$$\cos \theta = c\gamma S^{1/2} - 1 \text{ (c: constant)} \quad (3)$$

The highly hydrophilic state generated by UV light gradually returns to the initial hydrophobic state in the dark. The initial contact angle on RF-pretreated DCMS sputtered samples PE-TiO_2 decreased up to an angle $<5^o$ within 60 min irradiation. The hydrophobic or hydrophilic nature of a surface is important in the adhesion of the bacteria to a TiO_2 layered surface and the subsequent light or dark induced kinetic bacterial inactivation.

E. coli and *Staphylococcus aureus* present a preferential adhesion to hydrophilic surfaces [27, 59-62]. Efficient antimicrobial activity on Cu-surfaces has been recently reported against methicillin-resistant *staphylococcus aureus* (MRSA) and the bacterial reduction was evaluated by four methods. A reduction of $4\log_{10}$ in the initial MRSA concentration of 10^6 CFU for ten clinical MRSA isolates within one hour, suggesting relevance of the Cu-surfaces in preventing the transmission of these gram-positive bacteria [59]. The loss of viability results obtained by combining direct transfer on agar plates and stereomicroscopy relates to the information by two different methods to evaluate the bactericidal activity of the Cu-sputtered samples.

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Bacteria with hydrophobic surface properties like *S. epidermis* adhere preferentially to hydrophobic surfaces [61]. Hydrophobic bacteria have been reported to adhere to a variety of surfaces forming biofilms to a greater extent than hydrophilic bacteria [62]. Most of the bacteria acquire a negative electric charge in aqueous suspension or in the presence of air humidity, this aspect has revealed to be important in the bacterial adhesion to charged surfaces [63]. It has been known for a long time that surfaces with high surface energies like the ones encountered in hydrophilic surfaces are negative to a great extent and develop resistance to bio-adhesion [64-65]. These two last considerations have to be considered from case to case when inactivating a specific variety of pathogen on a well-defined antibacterial surface [1-8].

In the last 30 years, the TiO_2 mediated photocatalytic inactivation of pathogens has been the focus in the disinfection area. Inactivation by TiO_2 suspensions, supported photocatalysts and nano-rods under diverse light sources has been reported within minutes/hours [66]. In dark control experiments, TiO_2 has been reported to exert no microbial inactivation or a very low antibacterial activity. Recently, work has provided the evidence that under selected experimental conditions (pH, TiO_2 amount and particle size, bacterial concentration) that below the TiO_2 isoelectric point led to microbial inactivation in the range of about 10^4 CFU/ml for *E. coli* in the dark within 120 min. This was about 50% of the *E. coli* inactivation induced under the same experimental conditions when applying a UV-light centered at 366 nm. No photo-generated electrons/holes or ROS were photo-generated in the absence of band-gap irradiation [66]. This work showed that the electrostatic and or Coulomb interaction at pH's 4-4.5 suppresses the division of bacteria leading to *E. coli* inactivation/lack of cultivability.

A subsequent study presenting evidence for the lack of cultivability in dark due to the interaction between TiO_2 surfaces in the minute range has been recently reported [67]. By plate counting and by following in the time scale the bacterial cell interaction with TiO_2 aggregates by electron microscopy. The interaction of the TiO_2 in the dark with *E. coli* occurred within the time of events leading to cell wall damage/inactivation due to the electrostatic forces competing with the Van der Waals forces occurring at physiological pH-values and inducing damage/deformation in the cell-wall packing structure with an increase of the membrane permeability. TiO_2-polyester samples were

able of repetitive bacterial inactivation (loss of cultivability) in the dark. Bacterial inactivation in the dark occurred within 120 min compared to the *E. coli* mediated TiO_2 photocatalysis occurring within 60 min under an actinic light Osram Lumilux 18W/827 source radiating within 350 nm and 740 nm with an intensity of 4.1 mW/cm^2. This study reports an important issue in the field of the disinfection technology. The TiO_2-polyester led to disinfection processes carried out in a repetitive way. This is a more convenient approach compared to TiO_2 suspensions where the TiO_2 nanoparticles have to be removed from the solution after each cycle not allowing for a continuous disinfection treatment. Therefore, TiO_2-supported polyester surfaces show a practical potential to preclude pathogenic biofilm formation in the dark. Alteration of the *E. coli* cell permeability in contact with TiO_2 in the dark has also been reported recently and the morphological changes on the bacteria were further documented by Guillard et al. [27]. The damages in the bilayer cell envelope were ascribed to the lipo-saccharide (LPS) packing structure damage. In contrast, SiO_2 under the same conditions did not induce detrimental effects on the bacteria inhibiting its reproduction due to variance of its electrostatic charges/interaction at the reaction pH. TiO_2 nano-particles present a potential risk of these particles to penetrate in tissues and biological membranes. Ecotoxicology of TiO_2 has recently been addressed in several studies/reviews and have indicated the high stability of TiO_2 nanoparticles limiting their cytotoxic effect [22, 68-71].

The way light is applied to TiO_2 suspensions to inactivate bacteria has been reported recently in detail by Pulgarin et al. [72] to have significant effects in the inactivation kinetics. The way light is applied during bacterial disinfection may lead or not to complete bacterial removal: continuous irradiation lead to a rapid removal of bacteria compared to the same light dose applied intermittently. No bacterial re-growth was observed after illumination of a contaminated TiO_2 suspension. In contrast, without catalyst, illuminated bacteria in solution recovered its initial concentration after 3h in the dark. In a subsequent study, TiO_2 suspensions were shown to be effective in reducing *E. coli* when irradiated in the presence of a bacterial consortium. The inactivation rate was dependent on biological parameters such as: physiological state, pH, initial concentration of bacteria and the organic/inorganic impurities/residues present in the solution. After illumination periods with a different length, a "residual disinfecting effect" in the dark was observed after the light was turned of as a function

of the applied intensity (40–100 mW/cm^2). *Enterococcus* sp, a Gram-positive bacteria with cell-wall thicknesses between 30-80nm appear to be less sensitive than coliforms and other Gram-negative bacteria like *E. coli* with cell-wall thicknesses 10-20 nm to TiO_2 under light in suspension[73].

In TiO_2 dispersions/suspensions made up of colloids or pre-formed powder nanoparticles, the photocatalysis led to many studies focusing on bacterial abatement [7, 25-35, 36-58, 66-67] and showing inactivation of spores [74-77], viruses [78], algae [79] and fungi [80]. The TiO_2 suspensions although successful to abate a variety of pathogens under light irradiation need times that seem to be too long to treat large water volumes. The second inconvenient is the separation of the suspension after the disinfection process from the bacterial residues. This is expensive in terms of time, labor and reagents. Therefore some laboratories begun to use supported TiO_2 materials in disinfection processes mediated by TiO_2. To avoid the separation step at the end of the bacterial abatement, Kuhn et al., [81] reported in 2003, that preformed TiO_2 nanoparticles grafted on a plexiglass surfaces inactivate *E. coli* under UV-light but only to a small extent. Nevertheless, these surfaces showed a very heterogeneous (non-uniform) TiO_2 coating. Generally, coating the with TiO_2 colloids thermal resistant materials like: glass, iron plates, ceramics, provide layers that are not reproducible, robust, and can be wiped out by a small rubbing using naked hands [82].

The synthesis of uniform 2D-coatings on supports applying direct current magnetron sputtering (DCMS), pulsed direct current magnetron sputtering (DCSMP) and highly ionized pulsed plasma magnetron sputtering (HIPIMS) have been reportedd to cover uniformly surfaces resistant to thermal stress. But sputtering methods have been used to coat textiles like cotton and polyester (PES) and low cost inert thin polymer films like polyethylene (PE) presenting low thermal stability ≤ 100-130^{o}C comprising 80% of the commercial market.

2.2. TiO_2/Cu synthesis leading to uniform, adhesive and antibacterial films.

Physical vapor deposition (PVD) is carried out in vacuum by condensation of a metal/non metal vapor onto the support surface that is generally in the reaction chamber at a relatively low temperature. This method involves high temperature vacuum evaporation of the material to be coated on the support. Anatase layers have been deposited this way showing a super-hydrophilic behavior under band-gap irradiation as reported by several workers in the field [83]. When using chemical vapor

deposition (CVD), the sustrate is exposed to volatile compounds decomposing on the substrate leading to the desired coatings [84-86]. A uniform, thin, adhesive, robust TiO_2 film has been deposited on glass [87] from Ti-chloride/ethyl-acetate at about 500 °C. CVD is able to deposit continuous films without the need of a post-annealing process to crystallize the TiO_2 nanoparticulate.

Diverse pulse magnetron sputtering (PMS) systems have been used to deposited TiO_2 films with thicknesses of 2 up to 300 nm and layers comprising preferentially anatase at temperatures ≤ 130°C. Using this approach, textiles presenting low thermal resistance, thin polymer films and polycarbonates (PC) have been coated by TiO_2, TiO_2-metal and TiO_2 metal-oxides [88-90]. The basic installation of a DCMS unit is shown in Figure 1.

One of the first reports on DCMS sputtered photocatalytic TiO_2 on Si-wafers and ceramic targets was reported by Miron et al., [91]. The utility of TiO_2 sputtered surfaces was already recognized many years ago. During the photocatalytic disinfection, the electron acceptor that is the O_2 in contact with the sputtered TiO_2 is readily available from the air and their concentration is replenished normally by the catalyst surface during disinfection. The efficiency of the antibacterial catalytic surface is influenced by the surface porosity. intensity of the light arriving on the sputtered surface, the sample roughness and uniformity controlling the bacterial adhesion, the type of bacteria either predominant Gram-negative or Gram-positive, the distance of the light source to the sputtered sample, the surface pH and the hydrophilic-hydrophobic balance of the sputtered sample.

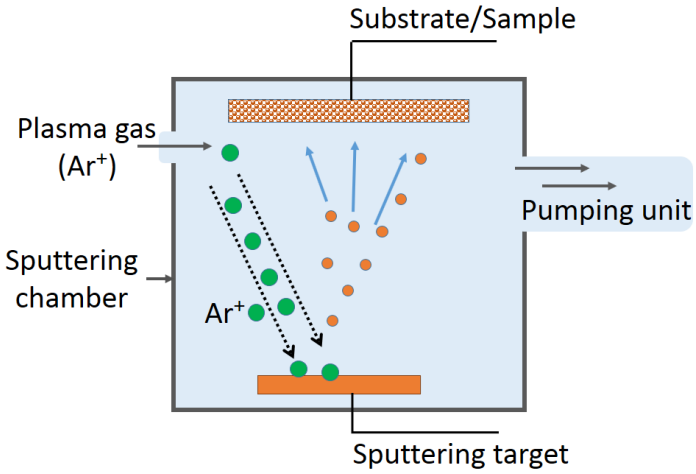


Figure 1a. Sputtering unit (one target)

2.3. Cu-loaded sputtered surfaces active in the dark and under light leading to microbial abatement

Antimicrobial nano-particulate films preparation is a topic of increasing attention since their objective is to reduce or eliminate the formation of infectious bacteria biofilms leading to hospital acquired infections (HAI) [92]. But more effective bacterial inactivation films on flat or complex shape 2D/3D surfaces are needed due to the increasing resistance of pathogenic bacteria to synthetic antibiotics administered for long times. Also nosocomial infections due to antibiotic resistant bacteria are becoming more frequent. [93].

Surfaces sputtered by DCMS/DCMSP containing metals, oxides, semiconductors either heat resistant or not, have been reported recently leading to effective, stable and uniform bactericide films [94-96]. The recently developed highly ionized pulsed plasma magnetron sputtering (HIPIMS) leads to films applying high electrical pulses 1-10A and up to 100V leading to layers presenting superior resistance and compactness against corrosion and oxidation. The HIPIMS sputtering on 3D-complex shape objects is one of the main problems encountered when depositing thin films on substrate by direct current pulsed magnetron sputtering (DCMS/DCMSP)[95] since the adhesion of the sputtered films are not strong. This is due to the relatively low bias voltage applied on the substrate within the sputtering time [96]. HIPIMS sputtering induces a strong interaction with 2D/3D substrate due to the higher fraction of the Cu^{+}/Cu^{2+}-ions up to 70% compared with DCMS/DCMSP [97]. The strong adherence of the sputtered atoms/ions on complex shape biased objects is due to the higher ion-arrival energy on the surface compared to the more traditional DC/DCP sputtering methods [97]. Cu-ions in ppb-amounts besides electrostatically interacting with the bacterial envelope lead partially to the unpacking/damage of the LPS of the outer *E. coli* bacterial cell wall, later penetrating the bacterial cell-porins with diameters of 1.1-1.3 nm [98]. Cu-ions will also diffuse through skin pores of ~100nm [1,7,26-22]. To preclude viral and diverse type of nosocomial infections caused by antibiotic resistant bacteria, Borkow and Gabbay [99-102] have reported studies on Cu-loaded textiles. These studies report in a detailed, systematic and comprehensive way the preparation and evaluation of the pathogens abatement on these innovative surfaces. Further colloidal Cu-coated textiles showing antibacterial properties reported by Gedanken's laboratory [103-104] focusing on ZnO and CuO colloids deposited on textiles by sonication. Cu-colloids were impregnated on polyester(PES) to inactivate bacteria under low intensity

sunlight. The PES was previously pre-treated by RF-plasma to increase the amount of negative carboxylic sites able to bond Cu-ions [105]. The non-uniform dispersion of the Cu/CuOx on the PES-surface moved us to work on the preparation by sputtering of Cu-antibacterial films to obtain adhesive, uniform and reproducible Cu/CuOx coatings. In this way we overcome the shortcomings of colloidal loaded PESs and this work will be described in the paragraph below.

Direct current magnetron sputtering (DC) sputtered samples were carried out with Cu-targets (99.9%) targets from Lesker AG, Hastings, East Sussex, UK sputtering the textiles/thin polymer films in a DCMS chamber at a pressure of 0.1-1 Pa. The distance between the Cu-target and the substrate was ~10 cm and the deposition current was 30-250 mA and the voltage was varied between 100-500V. The Cu-film thickness was determined with a profilometer (Alphastep500, Tencor). Cu-DCMS sputtering for 40s lead to the most favorable structure-reactivity relation on cotton inducing the shortest *E. coli* inactivation [106] Since the Cu-sputtering time did not have a direct relation with the bacterial disinfection time, the Cu-ions and not the amount of CuO sputtered on the substrate were responsible for the *E. coli* abatement. Taking 0.3 nm as the distance between Cu-atoms on the film surface and the thickness of an atomic layer ~0.2 nm, a Cu-coating 20 nm thick (about 100 layers) was deposited within 20s with a content of $1x10^{17}$ Cu atoms cm^2 at a rate of $5x10^{15}$ atoms/cm^2xs.

Direct current pulsed magnetron sputtering (DCP) of Cu with energy pulses between 5 and15 eV, led to a faster bacterial inactivation kinetics compared to DCMS [107]. More recently, higher energies per pulse compared to DCP involving HIPIMS sputtering was reported by, Petrov et al., [97]. HIPIMS sputtering on Cu-PES led to *E. coli* bacterial inactivation within 90 min when Cu was sputtered by HIPIMS pulses 60s long at 60A [108]. Effective bacterial inactivation was also observed in the dark. The energy and duration of the HIPIMS pulse was also limited by the heat resistant of the Cu-target. The amount of Cu-sputtered by HIPIMS inactivated *E. coli* with a Cu-loading three times lower compared to DCMS leading to *E. coli* bacterial inactivation within similar times. This shows the substantial Cu-metal savings when using Cu to prepare Cu-HIPIMS antibacterial films. A higher amount of Cu-ions was detected by X-ray photoelectron spectroscopy (XPS) on the textile surface for HIPIMS sputtered films compared to films sputtered by DCMS and DCMSP [59,106-108].

2.4. Behavior of Cu-sputtered surfaces in the dark and under hospital light leading to MRSA-isolates inactivation

Studies showing the reduction of the bacterial load on various Cu-surfaces are a growing focus of attention. The inactivation of Vancomycin resistant *enterococci* (VRE) and MRSA isolates is important due their strong infectious effects and the fact that they survive for months in health facilities [1-9, 18-21]. The inactivation of multidrug-resistant (MDR) pathogens like Gram-positive, Gram-negative and fungi on Cu-polyester (Cu-PES) has been reported [109-110]. The direct current magnetron sputtering (DC) of Cu was carried out on polyester (PES) for different times and led to uniform adhesive Cu nano-particulate films. Cu-PES samples sputtered for 160s as observed to induce a $6\log_{10}$ CFU loss of viability for MRSE and *A. baumannii* within 30 min. Loss of bacterial viability on the Cu-PES surface for other MDR bacteria proceeded within similar times in dark/light conditions suggesting that Cu/Cu-ions and not CuOx led to observed bacterial loss of viability. The use of gloves, gowns and masks as well as the patient isolation have limited the spread of infections, but by themselves are not able to avoid the transmission of HCAI's [111-112].

Copper is required by eukaryotic and prokaryotic cells at low concentrations as cofactors in metal-proteins and enzymes, but at high concentrations, Cu (II) has a toxic effect. Cu intervenes substituting essential ions and blocking of functional groups of proteins, inactivating enzymes, producing highly oxidative free radicals and altering the membrane integrity [25-35]. A number of studies have demonstrated the efficient killing of bacteria/fungi Cu-surfaces and Cu-alloys, both in vitro and in a clinical setting by Espirito Santo et al., [113-114], Grass et al., [115] and Casey et al., [116]. These studies show that Cu-surfaces rapidly and efficiently kill bacteria, in some cases accumulating Cu-ions on the cell wall membranes. This step seems to be followed by followed by Cu-uptake leading to cell wall loss of integrity and Cu-translocation. These studies also report that Cu/Cu-ions are effective against a wide variety of microbes but he mode of intervention of the Cu/Cu-ions and the inactivation mechanism still remains controversial. Experimental evidence for reactive oxidative stress (ROS) on the cytoplasm of Cu-stressed yeast is presented. New biomaterials for infection resistant surfaces considering additives interfering with biofilm formation, adhesive properties and microstructures driving the antibacterial action have been recently reviewed [117]. Testing the antibacterial action of many of metals on TiO_2 films, the metal-ion toxicity of Cu-ions was found much higher compared to: Ag-, Zn-, Co-, Al- and Hg-ions. The *in*

vitro growth inhibition revealed the higher activity of Cu-ions as antibacterial and bio-tolerant additive in ppb amounts [118]. Due to the fact that many bacteria grow easily on polyethylene widely used to wrap-up all kinds of objects: pharmaceuticals, perishables, surgical materials by Cu-polyethylene (Cu-PE) thin polymeric films our laboratory decided to sputter Cu on PE by DCMS and test bacterial inactivation performance [119]. Cu-coatings of 25 nm thick were sputtered at 60W and deposited 0.16% weight Cu/weight PE leading to bacterial inactivation under low intensity simulated sunlight (20% AM1). These films were also able to induce complete bacterial inactivation in the dark within 90 min and at a faster rate within 15 min under low intensity sunlight. Repetitive photo-induced bacterial inactivation was observed on the CuOx-PE. The Cu-released during the catalyst recycling in the ppb-range was determined by inductively coupled plasma mass-spectrometry (ICP-MS) and the showed the effect of the electrostatic interaction between bacteria and the CuOx-PE film. An increase in the applied light intensity accelerated the bacterial inactivation kinetics providing the evidence for the semiconductor behavior of the CuOx film. By X-ray photoelectron spectroscopy (XPS), the binding energy (BE) of the Cu-species was seen to shift after the bacterial inactivation cycle.

The use of intravenous catheters (IVCs) in patients who required vascular access is often associated with the development of potentially severe infections, including bloodstream infection (BSI), metastatic abscesses and infective endocarditis [120-121]. Catheters are commonly are colonized by microorganisms present on the skin at the time of intravascular insertion [122]. The infections caused by staphylococci, like *Staphylococcus aurous* are problematic in the case of methicillin-resistant *S. aurous* (MRSA) [123]. Polyurethane catheters currently used in Swiss hospitals do not generally infect the first day but are prone to infections over longer hospital stays [18-20, 92-93, 101, 110-112]. We generated Ag/Cu-coated catheters and investigated their efficacy in preventing methicillin-resistant *Staphylococcus aurous* (MRSA) infection *in vitro* and *in vivo* [124]. Ag and Cu were sputtered (67/ 33% ratio) externally on polyurethane catheters by DCMS. In vitro, Ag/Cu-coated and uncoated catheters were immersed for 18 h at 4^{o}C in PBS or rat plasma and exposed to 10^{4}-10^{8} CFU of MRSA ATCC 43300 for 90 min at 37°C. In vivo, Ag/Cu-coated and uncoated catheters were placed in the jugular vein of rats. Close by, MRSA (10^{7} CFU) was inoculated in the tail vein. Catheters were removed 48 h later and cultured. *In vitro*, Ag/Cu-coated catheters pre-incubated in PBS and exposed to 10^{4}-10^{7} CFU, prevented the adherence of

MRSA (0-12% colonization) compared to uncoated catheters (50-100% colonization; *P*<0.005). When pre-incubated in rat plasma, Ag/Cu-coated catheters retained their activity (0-20% colonization) while colonization of uncoated catheters increased (83-100%; *P*<0.005). Ag/Cu-coating protection decreased with 10^8 CFU in both PBS and plasma (50-100% colonization). In vivo, compared to uncoated catheters, Ag/Cu-coated catheters reduced the incidence of catheter infection (79% vs. 57%, respectively) and bacteraemia (68% vs 31%, respectively; *P*<0.05). Scanning electron microscopy (SEM) of explanted catheters suggests that the Ag/Cu catheters *in vivo* formed a dense fibrin sheath over their surface hindering the access of Ag/Cu to the infecting staphylococci. Their activity might be improved by limiting plasma protein adsorption on their surface. Ag/Cu-coating of catheters completely prevented catheter infection by MRSA *in vitro*.

Due to the interest of the subject and the recent advances on the Cu-inactivation of pathogens several studies have addressed this area of work combining recent progress in materials and microbiology. In some cases even reporting incipient clinical trials using Cu-surfaces in the dark or under light inactivating a variety of bacteria [125-133].

2.5. Photocatalytic/catalytic bactericidal effects on E. coli and MRSA by TiO_2/Cu thin films

Cu has been widely reported in the last decade to increase the bactericidal activity of TiO_2 [17,37-38,51,59,86,101-104,109-120,132]. This section presents some relevant developments (and references) of work in the bacterial inactivation mediated by TiO_2/Cu powders and TiO_2/Cu surfaces by catalysis and photocatalysis. Doping/decorating of TiO_2 by Cu/CuOx enhances the visible light absorption of TiO_2 and also it has been suggested also to enhance the separation of charges reaching the TiO_2 band-gap. The speciation of Cu on the TiO_2 surface and the mechanism of interfacial charge transfer (IFCT) between TiO_2 and Cu remains a controversial matter at the present time in spite of recent progress in this direction.

Inactivation of bacteria under UV- and visible light on TiO_2/Cu has been reported by the group of Applied Chemistry, University of Tokyo in the seminal references [136-139]. Hashimoto's laboratory has published widely on the preparation of TiO_2/Cu nanoparticles by sol-gel methods and evaluated their performance aunder UV- and visible light. Under weak UV-indoor lighting demonstrated th a notable activity increase

for bacterial inactivationof the TiO_2/Cu films with repect to TiO_2-films by themselves. The observed effect was ascribed to the ROS generated under band-gap irradiation attacking/damaging in the first step the LPS *E. coli* outer membrane followed by the effective uptake of the Cu-ions on the membrane cell wal leading to loss of membrane integrity. This in turn allows the translocation of Cu-ions into the bacteria cytoplasma leading to cell lysis. Subsequently, this group showed the increased effect of sunlight on Cu grafted WO_3-TiO_2 powders increasing the bacterial inactivation kinetics under light due the WO_3 interfacial charge transfer (IFCT) electron transfer to TiO_2 [138]. The bacterial inactivatiion of bacteriophages and viruses was reported on TiO_2/Cu recently [139] by the same group on foam samples used in aur cleaners. The results show that the pathogen count in the polluted air could be decreased using the approach suggested in this study improving the performance of air cleaners provided for with suitable TiO_2/Cu containing foams.

Physical vapor deposition (PVD) and Chemical vapor deposition (CVD) have been widely used as mentioned in references [83-86] to coat glass, silicon, polymers and steel with TiO_2 and other metal and metal/oxides. Work along this lines coating TiO_2/Cu by CVD on silica at 500 °C has been reported [140]. Since a growing interest has developed recently in the uniform, adhesive, robust antibacterial coatings of textile, thin polymers like polyrthylene (PE) polyurethane (PU) and textiles resisting much lower temperatures up to 160 °C, sputtering methods (SPM) working below this temperature and applied for very short times have developed recentla as a field of research gaining constantly in interest in view of the ulterior applications of the 2D/3D sputtered materials in health facilities, garments and many artifracts used in hospitals facilities [18-21]. Pioneering work in this area has been carried out for the two decades by P. Kelly in Manchester (MMU), looking in a detailed and comprehensive way to the effect of a variety of physical parameters set during the sputtering of surfaces and their effect on the antibacterial performance. In conjunction with J. Verran's work, they have reported work contributing to the mechanistic understanding of the microstructure of well defined sputtered surfaces on the abatement of pathogens [141-143].

Recently S. Pillai [144] has carried out work on the ability of TiO_2/Cu nanoparticulate co-doped with fluorine (F) under visible light to bate *Staphylococcus aureus* (MRSA). F introduced visible light absorption in TiO_2, induce Ti^{3+} leading to O-vacancies and preclude e^-/h^+ recombination in TiO_2. A sol-gel synthesis using aqueous

titanyl acetate and titanyl trifluoroacetate with added Cu-nitrate was prepared according to US-patent 9,210,934[145] The sol was then deposited onglass platesby dip-coating, and after drying heated to 550 °C. In the intial anatase network the O-sites were expected to be substituted partially by F and the Ti by Cu [38, 51]. The addition of F and Cu improved significantly the inactivation of MRSA under visibele light irradiation as available in hospital faciities. When TiO_2 was doped only with F, the photocatalytic activity was observed to be below the one registered for TiO_2/F/Cu illustrating the synergistic effect of the combined doping of F and Cu. Cu-doping improved thebacterial inactivation on TiO_2, but TiO_2-F was ineffective in MRSA inactivation under dark conditions.

2.6. Recent work on TiO_2/Cu sputtered surfaces by DCMS, DCMSP and HIPIMS, bactericidal effects and thin film properties

The thin uniform TiO_2/Cu films on cotton about 78 nm thick were sputtered in the unit showed below. The most suitable films leading to bacterial inactivation consisted in Ti-sputtered for 10 min sequentially sputtered by Cu for 40 s at 300 mA, under a partial pressure of O_2 0.08 Pa and a partial pressure of Ar of 0.4 Pa. This Cu-doped sample inactivated *E. coli* within about 30 min in the dark.

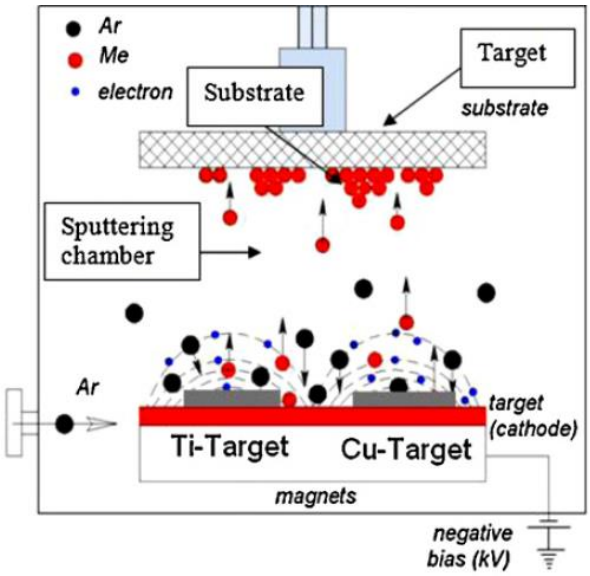


Figure 1a. Sputtering unit (two target)

Bacterial inactivation was completed within 5-6 min under low intensity actinic light >400nm providing evidence for a) the synergic interaction between Cu and TiO_2 and b) the interfacial charge transfer (IFCT) occurring between TiO_2 and Cu under band-gap irradiation [146]. The bacterial inactivation involved redox catalysis, This is the

variation of the Cu-oxidation state during the bacterial process due to the contact of the bacteria with the topmost Cu/CuO layers in the TiO_2/Cu sample. The evidence for this occurance was obtained by X-ray photoelectron spectroscopy (XPS) for variations >0.2 ev in the spectrograms for Cu, O, and C observed for samples at time zero and samples after the 5-6 minute disinfection period induced under actinic light irradiation.

The DRS spectra of this sputtered TiO_2/Cu sample show that Cu does not substitute Ti^{4+} in the TiO_2 crystal lattice. Ti and Cu-ions were released in ppb amounts during the period of bacterial disinfection providing for the evidence that the bacterial inactivation involves predominantly an oligodynamic effect [147-148]. The small amounts of Cu-relased during disinfection electrostatically bind to the S- and N- and COO^- negative groups of the bacteria cell wall before translocating onto the bacterial cytoplasma. This mechanism was originally suggested by the Tokyo University photocatalysis laboratory [136-139]. Figure 2a shows the Cu-released during the disinfection cycles by a TiO_2/Cu (10min/40s) sputtered sample irradiated by actinic 4mW/cm^2 light. After each cycle, the samples were washed throughly before starting the next repetitive cycle. Afrer the 8th recycling, Figure 2a shows Cu released at ppb levels < 8 ppb.These Cu-levels are not considered cytotoxic to mammalian cells [129, 149].

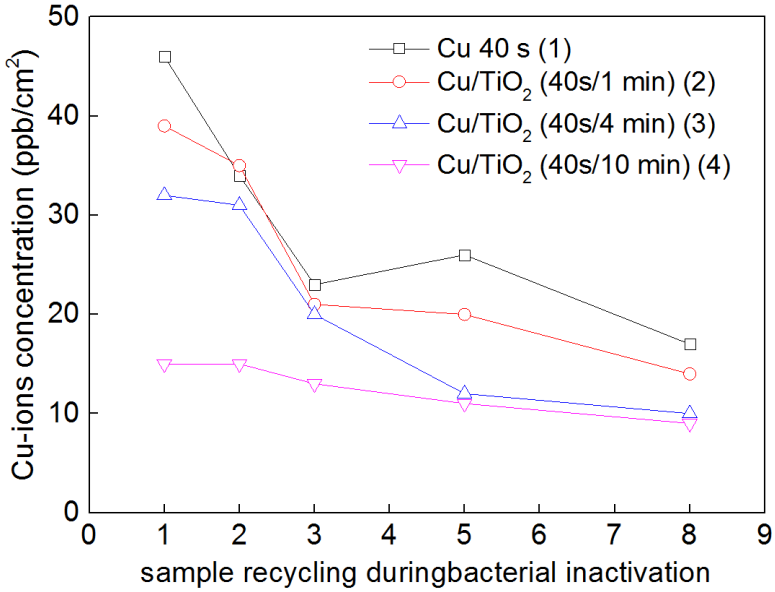


Figure 2a. Cu ions release from diverse Cu-cotton and TiO_2/Cu-cotton sputtered films as a function of catalyst cycling up to the 8th cycle.

Figure 2b shows a concomitant lower release of 8 ppb Ti-ions during bacterial disinfection dropping to about 4 ppb after the 8th cycling. This lower Ti-ions release is consistent with the very high stability reported for TiO_2 up to pH ~13.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 September 2016 doi: 10.20944/preprints201609.0050.v1

Peer-reviewed version available at Coatings 2017, 7, 20; doi:10.3390/coatings7020020

TiO_2/Cu cotton samples increased the *E. coli* inactivation kinetics with the increase of the applied light intensity [149]. Therefore, the predominant effect leading to bacterial inactivation seems to be due to the photo-generated charges either to: a) the TiO_2, b) the intermetallic TiO_2/Cu & TiO_2-Cu phases and finally c) the CuO.

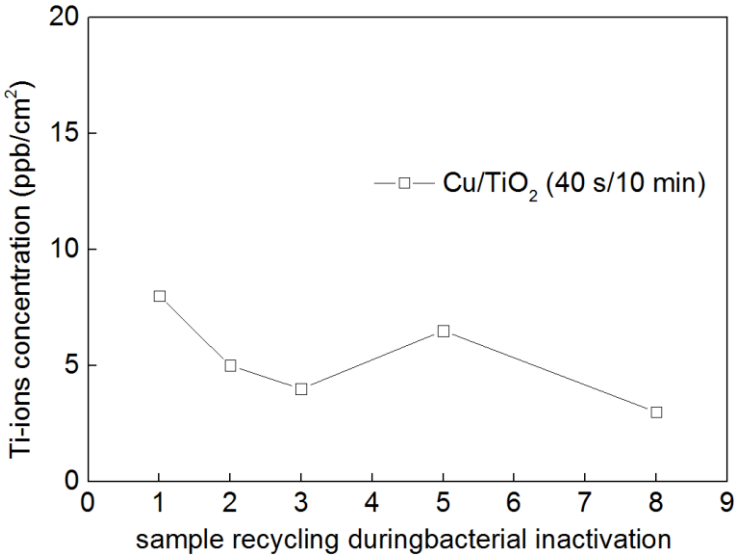


Figure 2b. Ti-ions release from diverse TiO_2/Cu (10 min/40 s) cotton sputtered sample as a function of catalyst cycling up to the 8th cycle.

Therefore, it is not the highly oxidative nano-particulate Cu present initially in a defined amount that determines the mechanism of the Cu-intervention during bacterial degradation, but the semiconductor character of CuO that generates photo-induced charges as a funcion of the aplied light dose. Since the photocatalytic mechanism of TiO_2 has been widely reviewed [25-35, 38-42, 43-51], we can suggest for CuO mechanistic steps leading to charge separation:

$$CuO + h\nu\ (\text{sunlight}) \rightarrow CuO(cbe\text{-}),\ CuO(vbh+) \qquad (1)$$

Under photon energies exceeding the CuO band-gap, the cbe- electron could either react directly with the O_2 forming O_2^- eq(3) or reduce the Cu^{2+} to Cu^+ as noted in eqs.(3-5):

$$CuO(cbe\text{-}) + O_2 \rightarrow CuO + O_2^- \qquad (3)$$

$$CuO(cbe\text{-}) \rightarrow CuO(Cu^+) \qquad (4)$$

$$CuO(Cu^+) + O_2 \rightarrow CuO(Cu^{2+}) + O_2^- \qquad (5)$$

The cbe- in eq(2) is produced by a CuO (p-type) with band-gap energy of 1.7 eV, a

flat-band potential of -0.3 V SCE (pH 7), and a valence band at +1.4 V SCE [151].

$$CuO(Cu^{+}) \rightarrow CuO\ vacancy + Cu^{+} \quad (6)$$

2.7. TiO_2-Cu films by highly ionized pulse plasma magnetron sputtering (HIPIMS): Comparison of the bacterial inactivation performance with DCMS-films

In a recent study, the EPFL laboratory reported the sputtering by HIPIMS on polyester (PES) showing that thinner TiO_2-Cu HIPIMS sputtered films (~40 nm) induced bacterial inactivation of *E. coli* within 9-12 min in circumstances that the inactivation time required about 600 nm by TiO_2/Cu sequentially sputtered by DCMS/DCMSP [152]. For the HIPMS co-sputtering, the target used was 60%Ti / 40%Cu by weight and the pulse used 5A and 350 V with a power of 1750 W for the 100 microseconds per pulse. DCMS sputtering of TiO_2 0.3 A followed by Cu-sputtering by DCMSP with pulses 0.3 A/622 V during 10 microseconds at an average power of 187W/pulse. Figure 3a presents the loss of viability kinetics *vs* thickness for DCMS/DCMSP and HIPIMS TiO_2-Cu sputtered films. Figure 3a shows the much thinner TiO_2-Cu layer was necessary for complete bacterial inactivation sputtered by HIPIMS compared to the one by DCMS/DCMSP.

Sputtering by DCMS involves Cu-ions grafted on the PES at energies much lower compared to HIPIMS, it was expected that the DCMS TiO_2/Cu sample would release Cu-ions at the a more favorable rate compared to the HIPIMS samples leading to a more accelerated bacterial inactivation. The result obtained reported in Figure 3a was the opposite to the result expected while planning this experiment. A possible reason for this is the higher amounts of Cu-ions generated by HIPIMS grafted on the PES substrate compared to the Cu-ions generated by DCMS. More work to clear quantitatively the amount and nature (Cu-oxidation sates) by XPS and allied techniques on the PES surface is needed at the present time.

Figure 3b, left hand side presents a scheme for the DCMS sputtering proceeding with an ionization of the Cu-ions of 1% [153]. The DCMSP sputtering is schematically presented in Figure 3b (middle section) and proceeds with ionization of Cu-ions well above the values attained by DC [154]. Next, Figure 3b (right hand side) shows in a schematic way the nature of the films obtained by HIPIMS sputtering. In this alter case leading, a Cu-ionization of ca. 70% and an electronic density of ~$10^{18\text{-}19}$ e-/m^3 has been reported [94]. DCP sputtering generates Cu^{+}/Cu^{2+} with an ionization of 5% with

an electronic density of ~$10^{15\text{-}16}$ e-/m^3 [154]. The HIPIMS higher energy increases the amount of ionized Cu-ions in the reaction

$$Cu^0 \rightarrow Cu^+/Cu^{2+} \quad (7)$$

The Cu-ionization by DCMS has been reported to be on the average 1-5%. For DCMSP the Cu-ions ionization can go up to 10% and up to 70% by HIPIMS [94,153-154]. This increased arrival energy of the Cu-ions on the substrate allows the alignment of the Cu-ions on the irregular/rough surfaces [155-156]. The polyester roughness could not be quantified by atomic force microscopy (AFM) since it is beyond the limit of the AFM unit of ~10 microns.

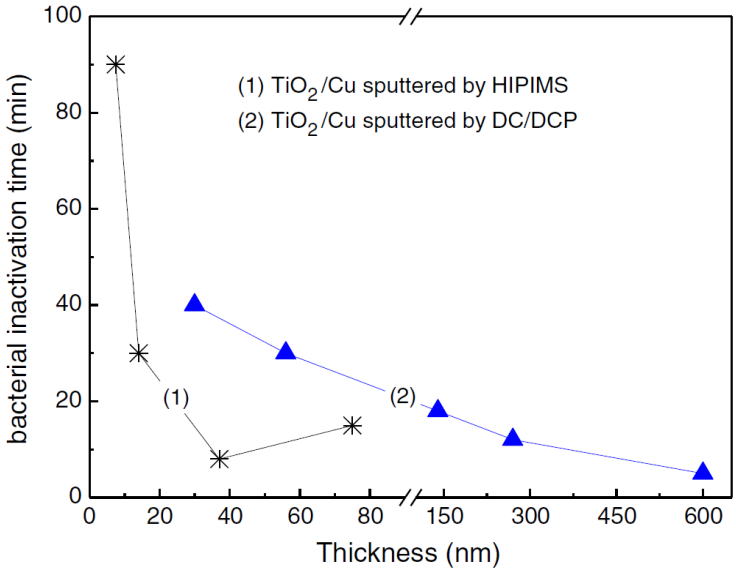


Figure 3a. Bacterial inactivation time vs nominal thicknesses for TiO_2-Cu-HIPIMS sputtered films and TiO_2/Cu by DC/DCP sputtered layers under Osram Lumilux 18 W/827 actinic lamp (4 mW/cm^2) irradiation.

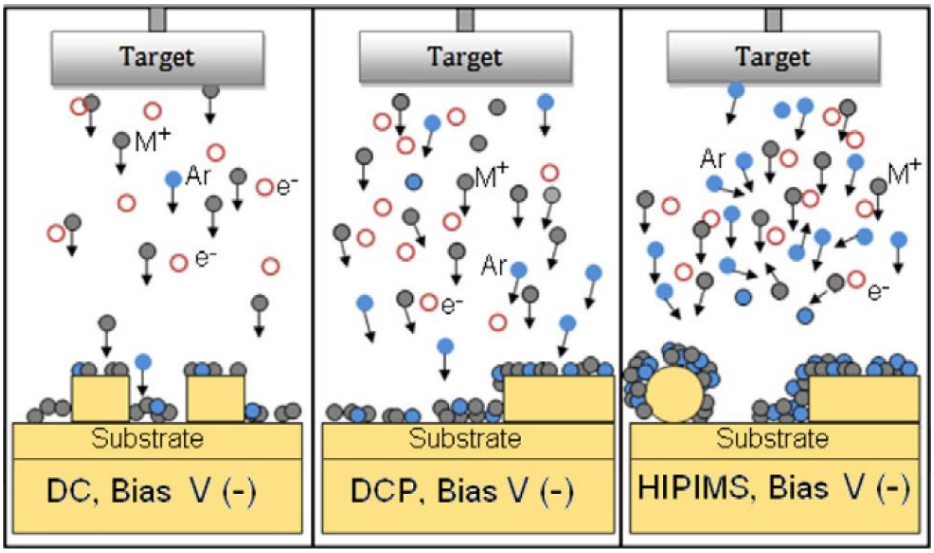


Figure 3a. Schematics of the a) DC, b) DCP and c) HIPIMS sputtering of metal-ions (M+) on polyester 3-D surfaces.

HIPIMS sputtered TiO_2 for 10 min on PES inactivated bacteria within 50 min, and Cu sputtered for 40-60 s on PES inactivated *E. coli* under low intensity actinic light within 60 min. More important was the observation of the release of Cu-ions inactivating *E. coli* from the HIPIMS sputtered TiO_2-Cu PES as a function of catalyst recycling showed by ICP-MS. After the 8^{th} recycling, the release of Cu- was ~8 ppb/cm^2. This value is lower than the Cu-release from the Cu-sputtered PES by HIPIMS reaching up to ~18 ppb Cu/cm^2 after the 8^{th} cycle. This shows the protective/adhesive effect of TiO_2. These Cu ppb-levels are not considered to be cytotoxic to *mammalian* cells and have been suggested to proceed through an oligo-dynamic effect [129,147-148]. It is known that the Cu-nanoparticle size determines the reactivity of photocatalytic surfaces [150].

HIPIMS sputtered for 30s show Cu-nanoparticles between 8-15 nm. The TiO_2 samples sputtered for 150s present sizes between 8-12 nm, and the TiO_2-Cu samples sputtered for 150s presented particles 5-10 nm. TiO_2 has been suggested to bind, disperse and stabilize the Cu-clusters on the PES surfaces. The distribution of TiO_2 and Cu-nanoparticles on the PES in Figure 4a was found to be uniform not presenting any cracks. Figure 4b (left hand side) presents the contrasted high angular annular dark field (HAADF) TiO_2/Cu microscopy showing the Cu-nanoparticles to be immiscible with Ti. In the right hand side, Figure 4b shows the mapping/distribution of the Cu, Ti and O on PES.

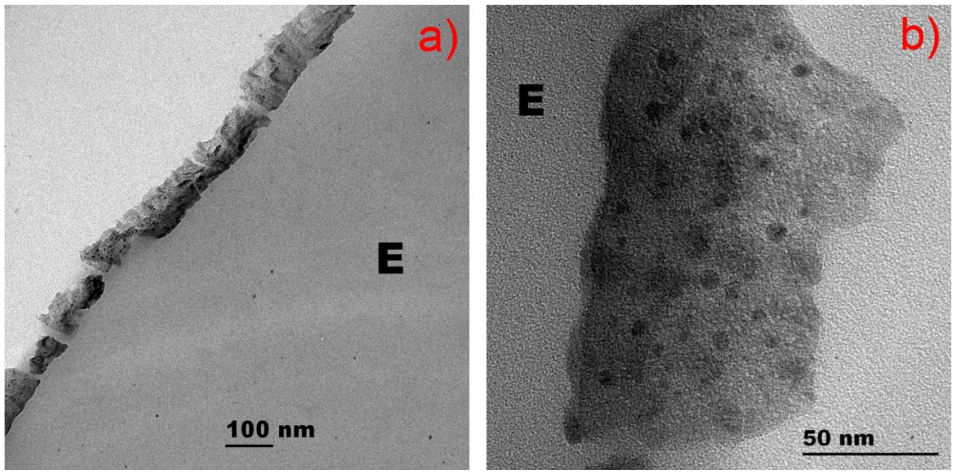


Figure 4a. Transmission electron microscopy (TEM) of: a) Cu sputtered for 150 s by HIPIMS on polyester, b) Cu-TiO_2 sputtered for 150 s by HIPIMS on polyester.

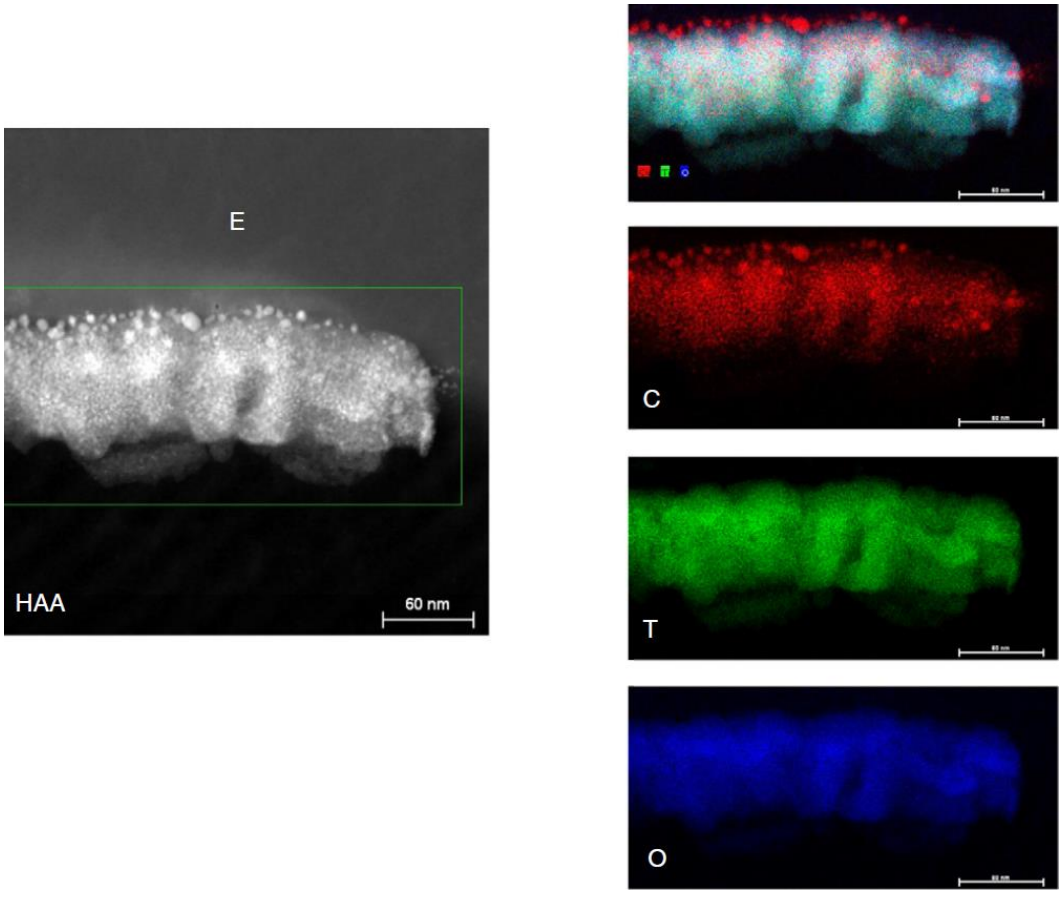


Figure 4b. High-Angle Annular Dark-Field imaging (HAADF) images of Cu-TiO_2 HIPIMS sputtered for 150 s showing the complete sample and the mapping of Cu, Ti and O by Z-contrast imaging.

Due to its size, the CuO/Cu nanoparticles with particle size > 8 nm are not able to penetrate into the bacteria core through the cell wall porins with diameters of 1-1.3 nm [98]. But Cu-ions are able to diffuse through the cell wall porins leading to cytoplasm/DNA damage and finally to the loss of bacterial viability.

2.8. Interfacial charge transfer (IFCT) suggested on TiO_2-Cu films leading to Gram-negative and Gram-positive bacterial inactivation

Figure 5 shows the interfacial charge transfer between TiO_2 and Cu in the under simulated solar irradiation. In the TiO_2 semiconductor the irradiation with solar simulated light in the cavity of the solar simulator has been suggested to transfer e^- and h^+ from TiO_2 to CuO. The potential energy levels of the TiO_2cb and TiO_2vb lie above the CuOcb and CuOvb levels as shown in Figure 5. The partial recombination of e^--h^+ in TiO_2 would be hindered by the charge injection into CuO. The interfacial charge transfer (IFCT) to TiO_2 from the CuOvb at +1.4 eV to the TiO_2vb at +2.5 eV *vs* SCE, pH 0, proceeds with a considerable driving force due to the large difference between these two vb levels. This leads to the fast *E. coli* inactivation times of 10 min shown in Figure 5. These TiO_2vb holes react with the surface $^-$OH of the TiO_2 releasing OH-radicals to inactivate bacteria. But CuO can be reduced to Cu_2O and the Cu_2O can reduce O_2 via a multi-electron process and re-oxidize to CuO as shown in Figure 5.

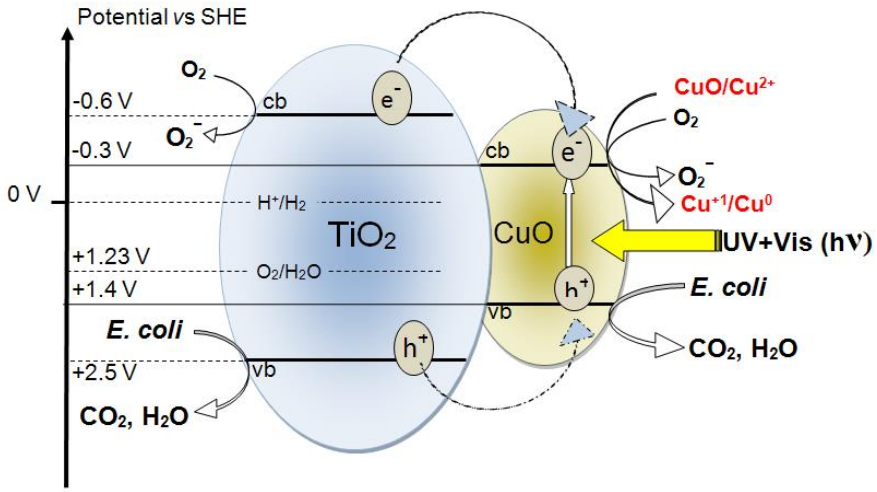


Figure 5. Scheme of bacteria inactivation under light on Cu-TiO2 films on polyester.

The electronic transfer between the TiO_2/Cu and *E. coli* depends on the length of the charge diffusion in the TiO_2/Cu. The diffusion is a function of the TiO_2 and Cu particle size and shape [94,155]. The interfacial distances between TiO_2 and Cu/CuO on the polyester surface range from below 5 nm and up. This allows the interfacial charge transfer (IFCT) as shown in Figure 5 to proceed with high quanta efficiency depending on the applied light intensity and nano-particulate size and surface properties [136-139]. Quantum size effects occur in particles with sizes ~10 nm having

about 10^4 atoms as shown Figure 4a [156]. The surface composition and properties of the TiO_2-CuO play a role in the charge transfer kinetics involving: a) surface defects, b) surface imperfections and c) dangling bonds at on the edge of this composite. By XPS and atomic force microscopy (AFM) information on the surface composition and roughness has been partially reported [157]. In TiO_2-Cu the charge recombination is short due to their small particle size. The small particle size decreases the space for charge separation. Also, the semiconductor space charge layer in both TiO_2 and CuO decreases further the potential depth available for the charge injection at the TiO_2-Cu heterojunction. This decreases the difference in energies between the two components for the charge injection and does not favor a rapid charge injection [38, 51, 151]**.** The conduction band of CuO at -0.30 V vs SCE (pH 7) in Figure 5 is at a more negative potential than the potential required for the one electron oxygen reduction

$$O_2 + H^+ + e^- \rightarrow HO_2^o \quad -0.22\ V \qquad (8)$$

[136-139,152]. Furthermore, the Cu^{2+} can also react with O_2^-

$$Cu^{2+} + O_2^- \rightarrow Cu^+ + O_2 \qquad (9)$$

A recent study addressed the inactivation of *E. coli* and MRSA on co-sputtered TiO_2-Cu to compare the PES sputtered samples in the dark and under light on Gram-negative and Gram-positive bacteria [158]. The TiO_2-Cu-PES was obtained by direct current magnetron co-sputtering for 3 min and induced Gram-negative *E. coli* inactivation within 120 min in the dark within 30 min under low intensity actinic light. The inactivation of MRSA ATCC 43300 on co-sputtered TiO_2-Cu attained $5\log_{10}$ (99.99%) starting with a concentration of 10^6 CFU/ml was observed to proceed within 120 min in the dark and under actinic light irradiation. Cu was released in ppb amounts during the bacterial inactivation as detected by inductively coupled plasma-mass spectrometry (ICP-MS) suggesting an oligo-dynamic effect. Figure 6a shows the bacterial inactivation under actinic light irradiation and in dark as a function of TiO_2-Cu co-sputtering time. In the dark, the *E coli* bacterial inactivation proceeds within 120 min.

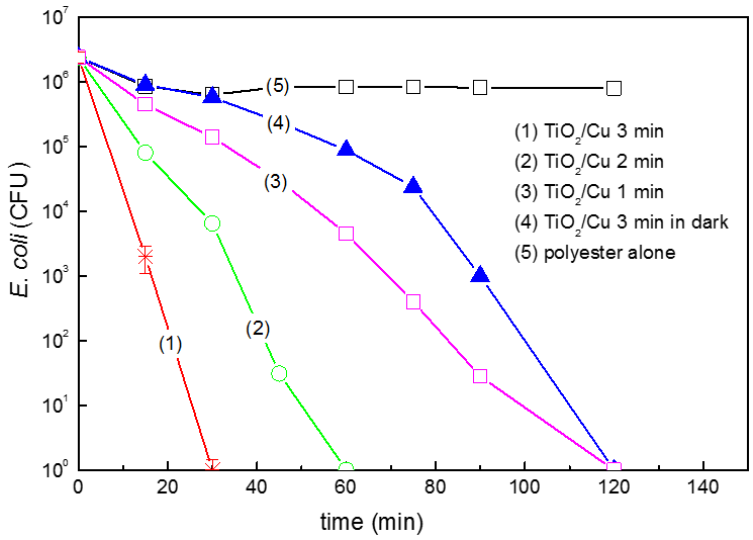
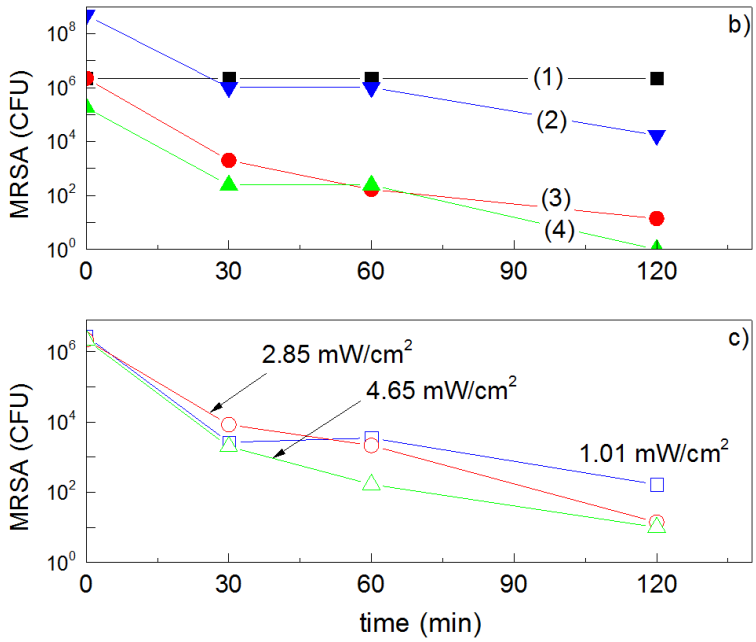


Figure 6a. *E. coli* inactivation on TiO_2-Cu co-sputtered for different times on PES as indicated in the traces: (1) 3 min, (2) 2 min, (3) 1 min, (4) co-sputtered TiO_2-Cu for 3 min in the dark and (5) PES-alone. The bacterial reduction under light irradiation used a lamp Philips Master-18W/865 (4.65 mW/cm^2).

Figure 6a, trace 1 shows bacterial inactivation within 30 min for TiO_2-Cu co-sputtered for 3 min. A sufficient amount of TiO_2-Cu was sputtered on the PES to interact rapidly with bacteria. Co-sputtering for 1 and 2 min did not induce the necessary amount of TiO_2 and Cu sites leading to an accelerated bactericidal activity. Next we address the issue of the MRSA bacterial inactivation kinetics and relate the inactivation time to the applied light intensity. A longer inactivation time was observed for MRSA compared to *E. coli* under light irradiation. This is shown in Figures 6c and 6a. The difference in wall thickness, number of layers, peptidoglycan content, lipid and protein content and lipopolysaccharide (LPS) content, seems to account for the different bacterial inactivation kinetics observed for *E. coli* and MRSA [1-4, 59, 113, 117]. A different electrostatic interaction of the Cu NPs-positive-ions interacting with the negative lipopolysaccharide (LPS) of *E. coli* and the same positive Cu-NPs ions interacting with the positive outer layer of MRSA. Figure 6b show the MRSA inactivation time in the dark under different conditions and for different MRSA loadings. Next, Figure 6c shows MRSA inactivation under three different light intensities. The MRSA inactivation in the dark and under actinic light irradiation occurred within similar times suggesting an inactivation mechanism completely different to the one leading to the inactivation of *E. coli* (Figure 6a). Some studies have appeared recently in the

open-literature on the inactivation of Gram-negative and Gram-positive bacteria on Cu-composites [159-160].

During the last two years some studies have been directed to sputtering of binary-oxide-Cu-promoted films on a variety of supports. Reports on their bacterial inactivation kinetics, adhesion and mechanical strength seem encouraging at this stage based on synergistic effects taking place in these composite activating bacterial reduction. The long-term operational lifetime and inactivation mechanism have been reported along the amount of Cu-released by these innovative during bacterial inactivation films in the dark and under light. These studies have shown a drastic enhancement of the bacterial inactivation kinetics of *E. coli* when Cu was added on these semiconductor binary-oxides in percentages from 0.01% (decoration) up to 1% (doping) [161-163]. A variety of TiO_2-Cu preparations and the evaluation of their activity for diverse applications is an area of current interest [165-168].



The relevance of the TiO_2-Cu catalysts and photocatalysts addressing the issue of bacterial inactivation is reflected in the increasing number of studies focusing on the antibacterial effects of TiO_2-Cu and TiO_2/Cu in the form of 2D-coatings, suspensions, spheres, alloys, and doped binary oxides [169-175].

3. Conclusions

The Cu and TiO_2 semiconductor interact with the bacterial wall and the cytoplasm in the dark and under light by mechanisms that remain controversial. This review focused on uniform, stable reproducible Titania-Cu films obtained by sequential or by co-sputtering. Accelerated bacterial inactivation kinetics was observed by the co-sputtered films compared to Cu or TiO_2 sequentially sputtered films in the dark or solar simulated light irradiation. The evaluation of the bacterial inactivation kinetics, the disinfection stability and last but not least the properties of the sputtered films leading to the bacterial inactivation were addressed in detail. A considerable saving in metal and deposition time (energy) was found by HIPIMS sputtering compared to conventional DC/DCP-sputtering. Increasing demand for Cu decreases rapidly the known Cu-world reserves. It seems that the biocide properties of Cu drastically increase the bactericidal properties of TiO_2. Progress in the knowledge of the molecular mechanism implicated in the bacteria-TiO_2/Cu is needed to design and synthetize more effective antibacterial biomaterial-composites with a well-defined and more performing microstructure providing an alternative to existing antibiotics. This is an important point in view of the current limitations in the use of antibiotics over long-time periods and the increasing resistance of many toxic pathogens to antibiotic/antiseptic formulations.

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Graphical abstract: