Antimicrobial Properties of Nanostructured TiO₂ Plus Fe Additive Thin Films Synthesized by a Cost-Effective Sol–Gel Process

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We prepared thin films composed of pure TiO₂ or TiO₂ with an Fe additive (at concentrations of 0.2–0.8 wt%) via a simple and cost effective sol–gel process, and tested their antifungal properties (against *Candida albicans* (MTCC-1637), *Candida tropicalis* (MTCC-184), *Candida parapsilosis* (MTCC-2509), and *Candida glabrata* (MTCC-3019) and antibacterial properties (against *Staphylococcus faecalis* (NCIM-2604) *Staphylococcus epidermidis* (NCIM-2493), *Staphylococcus aureus* (NCIL-2122), and *Bacillus subtilis* (NCIM-2549)). The films were deposited on glass and Si substrates and subjected to annealing at 400 °C for 3 h in ambient air. The film structural and morphological properties were investigated by X-ray photoelectron spectroscopy profilometry and scanning electron microscopy, respectively. Antifungal and antibacterial tests were conducted using the drop test method. Among the species examined, *Candida albicans* (MTCC-1637), and *Staphylococcus aureus* (NCIL-2122) showed complete colony formation inhibition after exposure for 4 h for the TiO₂ loaded with 0.8 wt% Fe thin films. These results indicate that increasing the Fe concentration increased the antimicrobial activity, with complete inhibition of colony formation after 4 h exposure.

Keywords: Sol–Gel Process, TiO₂ Thin Films, Scanning Electron Microscopy, Antimicrobial Activity, X-Ray Photoelectron Spectroscopy, Fe Additive.

In developing countries, the threat of potentially harmful bacteria and fungi necessitates the development of materials that combat human pandemics. Various naturally occurring materials have excellent antimicrobial activities, for example cellulose fibers, chitosan, metals such as silver and copper, and metal oxides such as TiO₂ and ZnO. However, if such materials are to be used as antimicrobial agents in medical applications or in any context involving exposure to the environment, they must meet standards for biodegradability, biocompatibility, and nontoxicity. Cellulose has been explored as a substrate for composite materials because of the presence of several functional groups that may be employed in various activation processes.^{1–3} However, cellulose fibers provide an excellent environment

for microorganisms to grow. However, although cellulose itself has good antimicrobial activity, cellulose fibers have a large surface area and a high capacity for moisture retention, and hence provide an excellent environment for microorganisms to grow. As a result, such fibers must be incorporated into organic materials to achieve antimicrobial properties; however, these organic materials are toxic and not biocompatible. The fibers' large surface area and ability to retain moisture demand that they are incorporated into organic materials to introduce antimicrobial properties. These organic materials are toxic and are not biocompatible. Antimicrobial surfaces must be biocompatible, i.e., they should be environmentally friendly and must not be harmful to humans or the surrounding environment. They also must maintain their antimicrobial activity under a variety of environmental conditions and must not be removed during washing. Thus far, few antibacterial materials have been developed that meet the above criteria.

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Currently, several countries, including India, East Asian countries, Malaysia, Thailand, the Philippines, and China, use copper as an antimicrobial material. Silver and copper are used to purify water and are used as algacides, fungicides, nematocides, and molluscicides. They are also used as antibacterial and antifouling agents. A wide range of nanoparticles (Ag, Cu, Ni, Fe) with various structures can be immobilized onto fibers to introduce new properties into the final textile product. These textiles can be used for hygienic clothing, wound healing, and medical applications in hospitals and other places where bacteria present a hazard. However, to our knowledge, very few publications have described the production and application of copper-textile composites, with the exception of Gabbay et al.⁴ These authors reported that the copper-impregnated fibers of cotton and polyester containing 10 wt% Cu demonstrated significant antifungal and antimicrobial properties. They impregnated the cotton fibers with copper oxide via a multi-phase soaking procedure. Copper oxide was inserted into the polymer fibers during the master batch preparation stage using a previously prepared copper oxide powder.

In the present study, we have developed a new method for preparing thin films of pure TiO₂, or TiO₂ with Fe as an additive, by a simple and cost-effective sol-gel process. Sol-gel processes have proven to be effective for the synthesis of thin films, nanoparticles, nanofibers (which can be coupled using an electrospinning unit), nanoporous materials,⁵ as well as for the deposition and insertion of nanoparticles on or into mesoporous ceramic and polymer supports, fabrics, and glass.^{6–8} The antibacterial activities of different metal oxides have been assigned to the photocatalytic production of reactive oxygen species, but this mechanism has so far been established only for TiO₂.⁹ In general, antibacterial activity has been demonstrated both in the dark and under UV illumination, but the differences in growth inhibition in these two cases depend both on the type of metal oxide as well as the type of bacteria (the same material yielded different responses for Gram-positive and Gram-negative bacteria).¹⁰ For some metal oxides, such as ZnO, exposure to ambient light and UV light resulted in a higher activity compared to dark conditions, but the optimal illumination conditions may be particle size-dependent.¹¹

In this work, we investigated the potential of sol-gel-based coatings of pure TiO₂ and TiO₂ with different concentrations of Fe additive. Films annealed at 400 °C for 3 h in ambient air were subjected to the antifungal and antibacterial drop test methods. These films are of interest for a number of potential applications, such as multifunctional coatings, as well as for coatings of nickel-plated tools, such as forceps and blades. For such applications, a simple fabrication method, such as the sol-gel method, is of significant interest. This process is simple, cost-effective, and easy to carry out.

The preparation and deposition processes of TiO₂ thin films by the sol-gel spin-coating process are shown schematically in the Appendix Figure A1. Titanium isopropoxide (Aldrich, 99.98% purity) was dissolved in 2-methoxy ethanol (99.98%) along with acetyl-acetone as a complexing and chelating agent. Cetyl trimethyl ammonium bromide (1.2 g) was dissolved in 20 mL 2-methoxy ethanol in a beaker, and the contents were added dropwise to the titanium isopropoxide sol with vigorous stirring. Iron was added to the main matrix of TiO₂ by addition iron chloride as the raw material, because it dissolves rapidly in 2-methoxy ethanol. Appropriate quantities of iron chloride were weighed (between 0.2 wt% and 0.8 wt% as a source of Fe in the TiO₂ matrix dissolved in 10 mL 2-methoxy ethanol, and added to the titanium isopropoxide solution. The contents were stirred at room temperature for 6 h. The molar ratio between titanium isopropoxide, 2-methoxy ethanol, and acetyl-acetone was 2.0:10.0:0.5. To the above contents were added, dropwise HNO₃ (1.2 mL, as a catalyst to increase the rate of reaction) and deionized water (1.5 mL to hydrolyze the sol). The solution was refluxed at 70 °C for 3 h to complete the reaction, followed by cooling to room temperature. The solution was filtered on a Whatman filter paper to remove any particulates formed during the reaction. The obtained stock solution was used for the deposition onto glass slides or quartz and Si substrates by the spin-coating process.

Si, quartz, or glass substrates were rinsed with deionized water and acetone, then were blown dry under N₂. Cleaned Si (100) substrates (3 × 3 cm²), quartz plates, and glass slides were immediately placed in the spin-coating chamber to prepare the thin films. A 50 microliters pipette was used to transfer 10–15 microliters of the stock solution to the surface of a Si (100) substrate, and the substrate was spun at 1000 rpm for 30 sec (cycle-1) to spread the solution over the entire surface of the substrate. This step was followed by spinning at 3000 rpm for 30 sec (cycle-2) to evaporate the solvent. The steps consisting of cycle-1 and cycle-2 were repeated 10 times to achieve a xerogel film with thickness of approximately 300 nm (as deposited). At the end of the process, the obtained xerogel films were subjected to annealing at 400 °C for 3 h in ambient air. A similar procedure was followed to deposit films onto quartz plates and glass slides. The annealed films were subjected to structural, compositional, and morphological characterization, followed by drop testing for antifungal and antibacterial properties. The drop test methods for both antifungal and antibacterial tests are illustrated in Appendix Figures A2(a) and (b), respectively.

Sabouraud agar was prepared by mixing 40 g dextrose, 10 g peptone, 20 g agar, and 1000 mL distilled water, and the pH was adjusted to pH 5.6. The agar medium was sterilized in aliquots of 15 mL at a pressure of 103.4 kPa for 15 min. This agar medium was transferred to sterilized Petri dishes in a laminar air flow unit and allowed

to solidify. One hundred microliters of a 24 h fungal suspension, grown in Sabouraud broth and standardized (0.5 McFarland standard), was added to each TiO₂ and Fe-coated plate, which were then kept in a UV chamber for 4 h. At regular intervals of 1 h, the exposed plates were washed with sterile saline or distilled water into the prepared Agar plates, and the cultures were spread using a glass spreader to ensure equal distribution of the organisms. The plates were incubated at 37 °C for 24 h, and colony forming units (CFU) which is a measure of viable bacteria were counted. Simultaneously, positive and negative controls were prepared. All the tests were repeated thrice and average value of colony forming units were taken as the final result.

Nutrient agar medium was prepared by dissolving peptone (5.0 g), beef extract (3.0 g), and sodium chloride (NaCl, 5.0 g) in 1000 mL distilled water, and the pH was adjusted to 7.0. Agar (15.0 g) was then added to the solution. The agar medium was sterilized in aliquots of 15 mL at a pressure of 103.4 kPa for 15 min. This nutrient agar medium was transferred to sterilized Petri dishes in

a laminar air flow unit and was allowed to solidify. One hundred microliters of a 24 h bacterial suspension, grown in nutrient broth and standardized (0.5 McFarland standard), was added to each TiO₂ and Fe-coated plate, and the plates were then kept in a UV chamber for 4 h. At regular intervals of 1 h, the exposed plates were washed with sterile saline or distilled water into the prepared agar plates, and the cultures were spread using a glass spreader to ensure equal distribution of the organisms. The plates were incubated at 37 °C for 24 h, and colony forming units (CFU) were counted. Simultaneously, positive and negative controls were prepared.

X-ray photoelectron spectroscopy (XPS) measurements of the TiO₂:Fe thin films, synthesized by a simple cost-effective process followed by annealing at 400 °C for 3 h in ambient air were performed using the PHI ESCA system equipped with a Mg X-ray source ($h\nu = 1253.6$ eV) and a hemispherical analyzer. XPS analysis of the TiO₂:Fe thin films surveyed the binding energy (BE) in range 0–1000 eV, over an 0.8 mm × 0.8 mm area, as shown in Figures 1(a) and (b). No contaminating species were

Fig. 1. XPS of a TiO₂:Fe thin films annealed at 400 °C for 3 h in ambient air. (a) General survey spectrum of TiO₂:Fe (0.2 wt%). (b) General survey spectrum of TiO₂:Fe (0.8 wt%). (c) Core level spectrum of Ti 2p. (d) Core level spectrum of Fe 2p.

observed within the sensitivity of the instrument apart from adsorbed atmospheric carbon. The binding energy (BE) spectra were calibrated with respect to the carbon 1s peak at BE = 284.8 eV. Peaks corresponding to Ti (2p, 2s), Fe (2p, 2s), O 1s, and C 1s were observed in the general survey spectrum for annealed thin TiO₂:Fe (0.8 wt%) films, apart from the Auger peaks of Ti, LMM, O, and KLL. The quantification of the survey spectra showed that superficial iron content increased from 2.3 at.% to 6.6 at.%. While the Oxygen concentration was above 70% for both extreme samples, indicating the full oxidation of the metal species. Figure 1(c) shows the Ti 2p peaks at 458.7 eV (2p_{3/2}) and 464.5 eV (2p_{1/2}) for the Ti in the TiO₂:Fe thin films which are consistent with the formation of stoichiometric TiO₂. Figure 1(d) shows the Fe 2p peaks located at 711.2 eV (2p_{3/2}) and 724.8 eV (2p_{1/2}) for the lowest and highest Fe containing samples, besides there are two shake-up satellite peaks located approximately at 8 eV above the Fe2p_{3/2} and Fe2p_{1/2} confirming that iron only exist in oxide state Fe³⁺.¹²

Morphological studies of the treated films of pure TiO₂ and TiO₂ with different concentrations of the Fe additive were conducted using a high resolution scanning electron microscopy (HR-SEM, Zeiss model). The deposited

and annealed films were uniform, homogeneous, crack-free, and highly dense. High magnification images of the pure TiO₂, TiO₂ loaded with 0.2 wt% Fe are shown in Figures 2(a) and (b), respectively. The uniform and continuous film textures relied on the use of 2-methoxy ethanol as a solvent (with a low evaporation rate), which produced crack-free films. Figures 2(a) and (b) images revealed that the annealed films were mostly nanostructured with grain sizes ranging from 15 to 20 nm. The addition of 0.8 wt% Fe to the TiO₂ matrix resulted in a porous morphology with large pits of diameter 200–250 nm, as shown in Figures 2(c–d).

The fungal species investigated were *Candida albicans* (MTCC-1637) (CA), *Candida glabrata* (MTCC-3019) (CG), *Candida tropicalis* (MTCC-184) (CT), and *Candida parapsilosis* (MTCC-2509) (CP), which have shown good resistance to pure TiO₂ as well as TiO₂:Fe thin films, as shown in Figures 3(a)–(d). Among the 4 fungal species, CG showed less resistance, even after exposure to the TiO₂:Fe (0.8 wt%) film for 4 h with a CFU of 100.4. The other three species showed CFUs of less than 60 after exposure for 4 h, particularly in the colony formation of CA, which was completely inhibited after 4 h exposure.

Fig. 2. HR-SEM images of nanostructured thin films of pure TiO₂ and TiO₂ with Fe additive, prepared by the cost-effective sol-gel process with annealing at 400 °C for 3 h in ambient air. (a) Pure TiO₂ (high magnification). (b) TiO₂ with Fe (0.2 wt%) as additive. (c) TiO₂ with Fe (0.8 wt%) as additive (low magnification). (d) TiO₂ with Fe (0.8 wt%) as additive (high magnification).

Fig. 3. Colony forming units as a function of time of exposure to the nanostructured thin films of pure TiO₂ and TiO₂ with different concentrations of Fe additives, prepared by the cost-effective sol-gel process with annealing at 400 °C for 3 h in ambient air, for each of the four fungal species (a) CA; (b) CG; (c) CT; (d) CP.

The bacterial species investigated were *Staphylococcus aureus* (NCIL-2122) (SA), *Staphylococcus faecalis* (NCIM-2604) (SF), *Bacillus subtilis* (NCIM-2549) (BS), and *Staphylococcus epidermidis* (NCIM-2493) (SE), which have shown consistent susceptibility to TiO₂:Fe thin films, as shown in Figures 4(a)–(d). As the concentration of Fe increased, the susceptibility also increased for all bacterial species. SA and BS showed dramatic falls in counts compared with the counts from the pure TiO₂ films (Figs. 4(a and c)). The results also showed a considerable decrease in microbial count as the concentration of Fe increased. The other species showed fewer changes in growth. Among the four species of microorganism used, SE was found to be the most resistant, but it showed a dramatic decrease in counts as the concentration of Fe increased from 0.6% to 0.8% (Fig. 4(d)). It is worth noting that the surfaces of the highest Fe-containing samples were very rough and showed extensive pitting. Such coatings are not effective antibacterial films. On the other hand, bacterial adhesion tests using SF showed a consistent reduction in the number of colonies formed when the concentration of Fe was

increased in the base matrix of the TiO₂ films. Photographs were taken for one species of bacteria, SA, grown on substrates with different iron concentrations in the base TiO₂ matrix. Figures 5(a) and (b) show photographs of the antibacterial drop tests for SA on TiO₂:Fe (0.2 wt% and 0.4 wt%) thin films deposited on glass (annealed at 400 °C for 3 h in ambient air). Figures 5(a)–(d) shows the antibacterial action of TiO₂:Fe 0.2 wt%, TiO₂:Fe 0.4 wt%, TiO₂:Fe 0.6 wt% and TiO₂:Fe 0.8 wt% thin films deposited on glass (annealed at 400 °C for 3 h in ambient air) respectively. Figure 5(a) clearly shows the antibacterial action on *Staphylococcus aureus* by (TiO₂:Fe 0.2 wt%) thin films which can be confirmed by decrease in CFU from 256 CFU to 92 CFU after 4 hrs contact, similarly Figure 5(b) (TiO₂:Fe 0.4 wt%) shows decreased bacterial growth from 223 CFU to 87 CFU at the end of 4 hrs exposure. Figure 5(c) (TiO₂:Fe 0.6 wt%) clearly shows that the CFU consistently decreased from 198 CFU to 61 CFU, and Figure 5(d) (TiO₂:Fe 0.8 wt%) shows that the number decreased from 144 CFU to 17 CFU. As the exposure time increased, the CFU decreased with increasing

Fig. 4. Colony forming units as a function of time of exposure to the nanostructured thin films of pure TiO₂ and TiO₂ with different concentrations of Fe additives, prepared by the cost-effective sol-gel process with annealing at 400 °C for 3 h in ambient air, for each of the four bacterial species (a) SA; (b) SF; (c) BS; (d) SE.

Fe concentration. For comparison purposes, a control is shown in all photographs.

Some antimicrobial agents are extremely irritating and toxic, and current investigations are targeted toward formulating new types of safe and cost-effective biocidal materials. Nanostructured thin films based on inorganic oxides, such as TiO₂ or ZnO synthesized by the sol-gel process, have been demonstrated to be biocompatible and to provide excellent supports for a wide variety of compounds.¹³ The size-dependent interactions between Ag/TiO₂ nanoparticles and gram-negative bacteria and virus have been demonstrated.^{14, 15} The electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is fundamental to bactericidal activity.¹⁶ Coleman et al.¹⁷ reported that polyvinyl chloride (PVC) interacts with titania and can be used as a bactericide. Titania can be surface-modified to improve its anti-bacterial properties and provide a high surface area. Dispersed antibacterial particles on a support increase the contact interface between the bactericidal material and bacteria. Because TiO₂ has photocatalytic properties, some

researchers have employed this property together with the antibacterial properties of metals to prepare metal supported titania materials as photocatalytic bactericides. Our results indicate that iron is in an oxide nanostructure form, as indicated by the XPS results in Figure 1, and with increasing iron concentrations, an increased antimicrobial activity in the thin films is observed due to the strong and antimicrobial interactions. However, since all fungal species tested were susceptible over time to both pure TiO₂ films and TiO₂:Fe films, as shown in Figures 3 and 5(a), it is an indication of the oxide production of ROS, which can include superoxide anions O₂⁻, hydroxyl groups OH⁻, and hydrogen peroxide H₂O₂.^{18–20} and can result in oxidative damage of bacteria and microorganisms and could be a helpful mechanism increasing in part the antifungal and antibacterial activity. Previous studies^{20–24} have demonstrated that with or without illumination, the creation of reactive oxygen species (ROS) results in oxidative damage to the cell membrane or internal cell structure, which appears to be a likely mechanism of the antibacterial activity of metal oxides that could be either transitional

Fig. 5. Photographs of SA bacteria adhered to samples with increasing Fe concentrations after various exposure times. (a) 0.2 wt% Fe, (b) 0.4 wt% Fe, (c) 0.6 wt% Fe, (d) 0.8 wt% Fe.

metal oxides or other non-transitional metal oxides.^{21, 23} These ROS could be hydroxyl groups (OH⁻), superoxide anions (O₂⁻) or hydrogen peroxide (H₂O₂). Mainly, hydrogen peroxide release has been identified as a mechanism of antibacterial activity for some metal oxides, such as ZnO,²² while for others, such as MgO and TiO₂, antibacterial activity was assigned to O₂⁻ species.²¹ Destruction or decomposition of cell membranes upon exposure to metal oxide nanoparticles has also been demonstrated,²⁰ consistent with the hypothesis of oxidative damage of the membrane by ROS. In some cases, penetration of metal oxide nanoparticles into the cell has also been observed. With the hypothesis of ROS mediated oxidative damage of cell membranes,^{18–20} all nanocomposite can be valid antimicrobial coating candidates.

In this research study, thin films of pure TiO₂ and TiO₂ containing Fe additive (at different concentrations) were synthesized using a simple and cost-effective sol–gel process. The films were deposited onto glass and Si substrates and were subjected to annealing at 400 °C for 3 h in ambient air. The annealed films were characterized for their structural and morphological properties using XPS and HR-SEM, respectively. The annealed films were tested for antibacterial and antifungal properties using the drop test method. Interestingly, the results indicated that increasing the Fe concentration increased the antimicrobial activity, and that CFU formation stopped after 4 h exposure for the TiO₂ loaded with 0.8 wt% Fe thin films. Complete inhibition of CA and SA were observed. The

results are encouraging for practical applications. Experiments are underway to introducing coatings and optimize the films on TiAlV alloy surfaces. Because infection is one of the most common clinical problems associated with high rates of death in a variety of surgical contexts, thin film coatings on surgical instruments or implants provides a useful method for reducing such infections during surgery.

APPENDIX

Fig. A1. Flow chart for the preparation of nanostructured thin films of pure TiO₂ and TiO₂ with Fe additives via the cost-effective sol–gel process.

Fig. A2. Drop testing procedure for the nanostructured thin films of pure TiO₂ and TiO₂ with Fe additives, prepared by the cost-effective sol-gel process with annealing at 400 °C for 3 h in ambient air. (a) Fungal species: CA, CG, CT, and CP (top). (b) Bacterial species SA, SF, BS, SE (bottom).

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