An Antimicrobial TiO_2 Coating for Reducing Hospital-Acquired Infection

Chi-Jen Chung,[1,2] Hsin-I Lin,[1] Hsi-Kai Tsou,[3] Zhi-Yuan Shi,[4] Ju-Liang He[1]

[1] Department of Materials Science and Engineering, Feng Chia University, 100 Wen-Hua Rd., Taichung 40724, Taiwan, Republic of China

[2] Department of Dental Laboratory Technology, Central Taiwan University of Science and Technology, 11 Buzih Lane, Beitun District, Taichung 40601, Taiwan, Republic of China

[3] Department of Neurosurgery, Taichung Veterans General Hospital, 160 Sec3 Chung-Kang Rd, Taichung 40705, Taiwan, Republic of China

[4] Department of Infectious Diseases, Taichung Veterans General Hospital, 160 Sec3 Chung-Kang Rd, Taichung 40705, Taiwan, Republic of China

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Abstract: Titanium dioxide (TiO_2) has been developed and applied extensively in the form of coatings, in particular for its unique properties such as non-toxicity, high photocatalytic activity, and strong self-cleaning ability. These coatings, which can be prepared via various processes, have not yet been proved to be antimicrobial. This research involves an arc ion plating method to produce TiO_2 film on medical grade AISI 304 stainless steel. Antimicrobial efficacy of the deposits is expected due to the photocatalysis action of the anatase phase presented in the deposit. The performance of the coating is evaluated by a JIS Z2801:2000 industrial standard. Experimental results show that TiO_2 film mainly consisting of anatase structure can be prepared with a high growth rate of 5 μm/h. Antimicrobial activity (R) of the deposited TiO_2 film against *Staphylococcus aureus* and *Escherichia coli* was 3.0 and 2.5, respectively, far beyond the value designated in JIS standard. This provides an effective antimicrobial surface coating method for medical implements thereby reducing the risk of hospital-acquired infections. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 85B: 220–224, 2008

Keywords: antimicrobial; coating(s); stainless steel; infection

INTRODUCTION

Increasing incidence and host risk of device-related infection resulting in morbidity and even mortality has been on record for some time.[1,2] Moreover, the spread of resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and the bursting *Clostridium difficile* are hospital-acquired infections and they are a worldwide problem.[3] In addition, the occurrence of SARS and avian influenza in recent years has drawn attention to the development and application of antimicrobial materials for preventive measures in contrast to the conventional concept of disinfection. Hence, it compelled us to develop an antimicrobial technique for medical implements in clinical use.[4]

Antimicrobial or antibacterial refers to the inhibition of bacterial growth and reproduction.[4] Antimicrobial function can be depicted essential materials themselves or through the use of coating materials. A first example of an essential antimicrobial alloy material is through the use of copper addition into stainless steel. ε-Cu precipitates and is distributed in the steel matrix. Copper ions can be dissolved into the surface passivated film, creating an antimicrobial effect on the stainless steel surface and inhibiting bacterial growth.[5] Historical trajectory of antimicrobial metal development follows this guidance, for instance, copper containing ferretic stainless steel, martensitic stainless steel, and austenitic stainless steels is used for antimicrobial purposes.[6–8]

As for coating materials, the idea of coatings incorporated with copper, silver, or other antimicrobial active metals was considered. Such a substrate/coating system may induce corrosion due to undesired bimetal effects and may be unsustainable during service. In this regard, photocatalytic titanium dioxide (TiO_2) material with anatase structure may have its benefits for antimicrobial purposes. The antimicrobial effects of TiO_2 are activated by its photocatalytic behaviour that was

Correspondence to: C.-J. Chung (e-mail: cjchung@seed.net.tw)

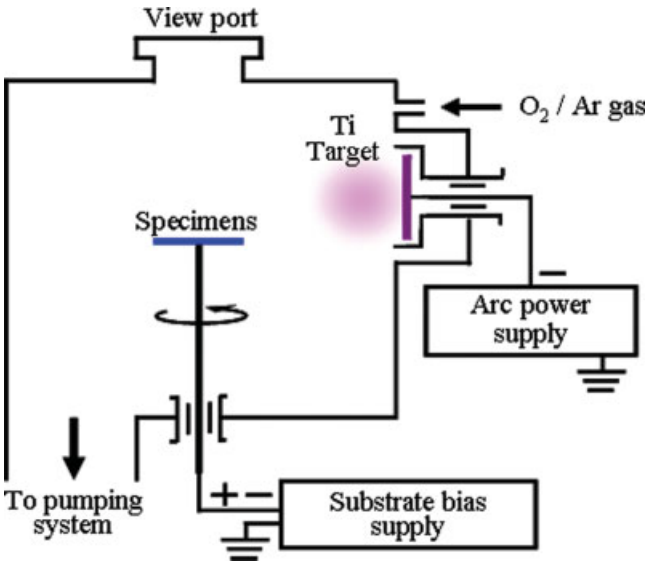

Figure 1. Schematic illustration of the AIP system. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

firstly discovered by Fujishima and Honda.[9] This has led to tremendous research on the mechanism and improvement of microstructure and photocatalytic performance.[10–12] The photocatalytic process of TiO_2 involves the generation of electron-hole pairs when exposed to light.[13] Aggressive oxygen radicals are generated by the electron attack, and the hole accelerates hydroxyl radical formation. These radicals eventually attack bacteria or viruses in terms of inhibiting DNA clonal processing.[14–16] Although photocatalytic coating techniques have been developed,[13,17–20] they have not been proved to be antimicrobial, until recently researchers successfully deposited TiO_2 films by wet processes[21,22] and sputter deposition.[23] These films however require real-time heating or post-annealing. The advantages of using arc ion plating (AIP) to prepare TiO_2 film include low-temperature deposition, high growth rate and strong film adhesion.[17,18] Our previous research in preparation of AIP-TiO_2 films indicated that the film deposited with maximum amount of anatase phase presented ultimate photocatalytic efficiency. This corresponds to a specific deposition condition with 100% oxygen pressure at 0.5 Pa for 60 min deposition. This study attempted to use an AIP method to deposit TiO_2 by using this optimized deposition condition on common medical grade AISI 304 stainless steel. Antimicrobial efficacy of the deposited specimens was evaluated according to JIS standard. The success may provide an effective antimicrobial surface coating method for medical implements to reduce the risk of hospital-acquired infections.

MATERIALS AND METHODS

Coating and Characterization

Figure 1 schematically illustrates the AIP-TiO_2 coating system. It consists of a deposition chamber, arc power supply, pumping system, and gas flow control system. During deposition, oxygen is admitted into the deposition chamber to react with titanium ions that are emitted from a titanium target via cathode arc spots. Crystal structure of the deposited TiO_2 film is controlled by the deposition parameters. Ultimate photocatalytic efficacy of the AIP-TiO_2 film resulting from maximized content of anatase structure has been demonstrated in an early study.[17] This would presumably provide superior antimicrobial efficacy. The whole deposition time takes 1 h.

AISI 304 stainless steel was used as a substrate and specimens were cut to a dimension of 50 mm × 50 mm × 1 mm to meet the demand for microstructure analysis and antimicrobial testing. The specimens were cleaned and dried before the deposition. An X-ray diffractometer was used to identify crystal structures of the deposited films, and a scanning electron microscope (SEM) was used to exam surfaces and cross sectional morphology of the deposits.

Antimicrobial Test

JIS Z2801:2000[24] was employed as a standard to test the antimicrobial efficacy. Figure 2 summarizes the procedure

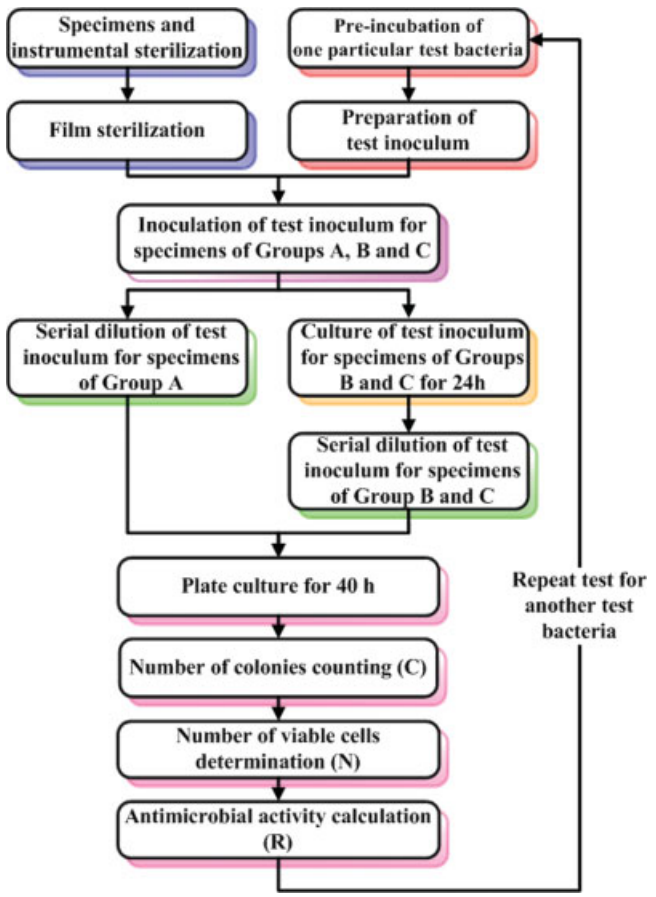

Figure 2. Antimicrobial test procedure according to JIS Z2801:2000. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

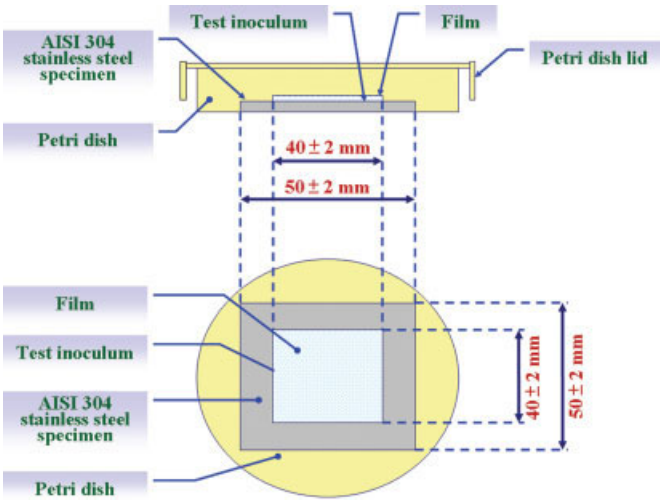

Figure 3. Test inoculum and film cover on the specimen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of doing this test. Group A and Group B consist of the uncoated stainless steel specimens, and Group C consists of TiO_2 coated stainless steel specimens. All specimens and test equipment were sterilized with pressurized steam prior to this test. Test procedure was conducted through an aseptic operation so as to be free from contamination.

The bacterial strains used in this test were gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* (American Type Culture Collection). Each of the tests was performed with an initial concentration of 4×10^5 bacteria/mL. Inoculation of the test onto specimen is shown in Figure 3, where the specimen is hosted in a sterilized Petri dish with surrounding temperature of 35°C ± 1°C according to JIS Z2801:2000[24]. The specimens of Group A immediately underwent serial dilution and plate culture after inoculation. Serial dilution of each corresponding specimen by the dilute buffer was performed to a concentration of 10^0, 10^1, 10^2, 10^3, and 10^4 fold, respectively. 1 mL of each diluted inoculum was taken for further plate culture for 40 h. Number of viable cells (N_A) was determined by counting the number of bacteria colonies (C_A) that grew in each Petri dish.

$$N_A = C_A \times D_A \times V_A \quad (1)$$

N_A, number of viable bacteria that correspond to Group A; C_A, number of bacteria colonies; D_A, fold of dilution; V_A, volume (mL) of the dilute buffer.

For accurate determination, only those C_A values that fell into 30–300 were taken and averaged from two parallel serial-dilutions and inoculated Petri dishes. The calculated N_A value if less then 10 was set at 10 according to the JIS test standard.

Those corresponding to specimens of Group B and C were incubated in an incubator where all specimens were exposed to regular indoor fluorescent lighting with an intensity of 372.5 lux for 24 h. Serial dilution of the inoculum was performed the same procedure repeated with different plate cultures to obtain N_B and N_C that corresponded to the number of viable bacteria for Group B and C, respectively. Antimicrobial activity (R) of the TiO_2 coated specimen was calculated as follows.

$$R = \left[\log\left(N_B/N_A\right) - \log\left(N_C/N_A\right)\right] = \left[\log\left(N_B/N_C\right)\right] \quad (2)$$

RESULTS

Microstructure of the Deposited TiO_2 Film

Cross sectional morphology of the deposited TiO_2 film in Figure 4 shows that fine crystal grains of TiO_2 grow in the initial growth stage and coarse columnar grains proceed further in the later growth stage. For a 1 h deposition as in this study, the deposited TiO_2 coating can be 5-μm thick as shown.

Figure 5(a,b) show the XRD patterns of the specimen without and with TiO_2 deposit. XRD pattern of the uncoated substrate shown in Figure 5(a) reflects austenitic crystal structure of AISI 304 stainless steel. The TiO_2-coated specimen shown in Figure 5(b) presents additional peaks attributed to the deposit that can not be ascribed to the substrate. By identification, the deposited film is mainly composed of anatase phase and a small amount of rutile phase. Although a strong A(101) peak appears at a diffraction angle of 25.3°, it resembles in the A(101) intensity of powdery anatase TiO_2 crystal structure found in JCPDS 78-2486.

Antimicrobial Efficacy of the Deposited TiO_2 Film

As revealed in Figures 6 and 7 for the case of *Staphylococcus aureus* and *Escherichia coli*, respectively, the Petri dishes corresponding to Group A and Group B (the uncoated specimens) present significant numbers of bacterial colonies as expected, while the TiO_2 coated specimens in Group C do not. This qualitatively describes the antimi-

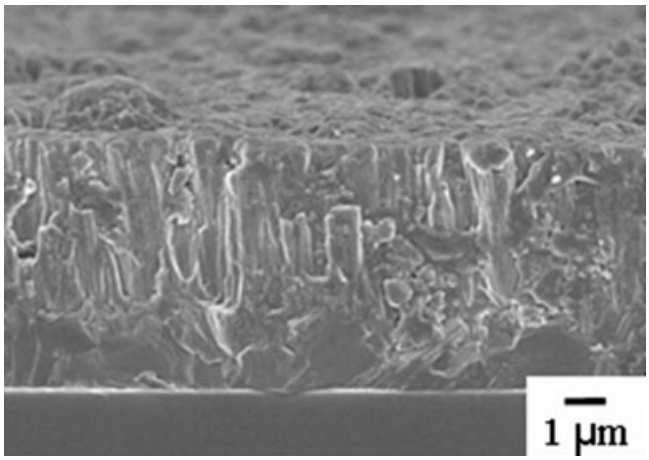

Figure 4. SEM cross-sectional morphology of TiO_2 film deposited for 1 h.

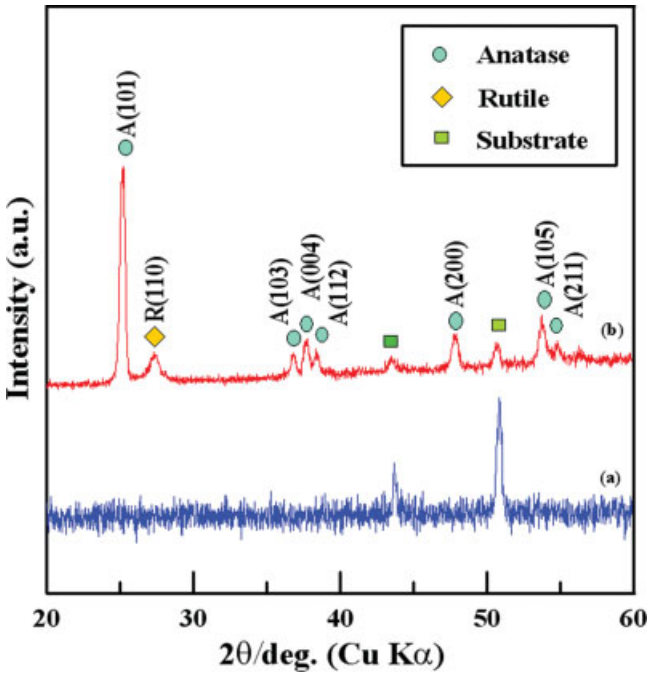

Figure 5. XRD patterns of AISI 304 stainless steel specimen (a) without and (b) with TiO_2 deposit. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

crobial ability of the TiO_2 coated specimens. Although only one Petri dish out of three was taken each for Group A, Group B and Group C to show in Figures 6 and 7, those Petri dishes (not shown) show a similar situation. This proves statistical accuracy of the antimicrobial test.

For both cases of *Staphylococcus aureus* and *Escherichia coli*, determination of the numbers of viable bacteria (N_A, N_B, and N_C) for Group A, B, and C are compared in Figure 8. The uncoated stainless steel specimens of Group A present N_A value of 2.85×10^5 and 1.06×10^5 bacteria, respectively for *Staphylococcus aureus* and *Escherichia coli*. The uncoated stainless steel specimens of Group B present an N_B value of 1.04×10^4 and 1.36×10^4 bacteria, respectively. For the TiO_2 coated specimen of Group C, no bacterial colony was observed in the case of *Staphylococcus aureus* and N_C value was designated as 10 bacteria according to the JIS test standard. For the TiO_2-coated specimen of Group C in the case of *Escherichia coli*, the

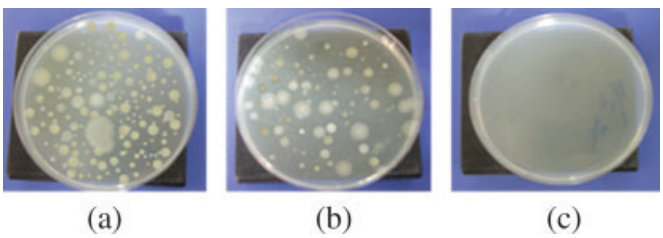

Figure 6. *Staphylococcus aureus* colonies formed on the Petri dishes after 40 h in corresponding to (a) Group A stainless steel specimen, (b) Group B stainless steel specimen, and (c) TiO_2 coated stainless steel specimen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

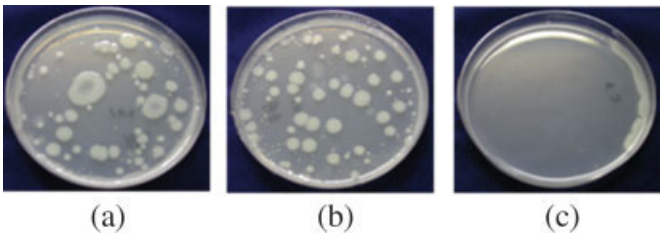

Figure 7. *Escherichia coli* colonies formed on the Petri dishes after 40 h in corresponding to (a) Group A stainless steel specimen, (b) Group B stainless steel specimen, and (c) TiO_2 coated stainless steel specimen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

N_C value was 4.30×10^1 bacteria. Using Eq. (2), to obtain an R value, antimicrobial activity, the TiO_2 coated specimen presented an R value of 3.0 and 2.5, respectively for *Staphylococcus aureus* and *Escherichia coli*. Such values are far beyond the index of 2 for JIS test standard.

DISCUSSION

The substrate temperature is believed to be the main contributing factor to the film morphology. For the initial film growth stage, substrate is relatively cool and fine crystal grain growth is preferred. For the later growth stage, substrate temperature was elevated due to continuous titanium ion bombardment on the substrate surface as the titanium

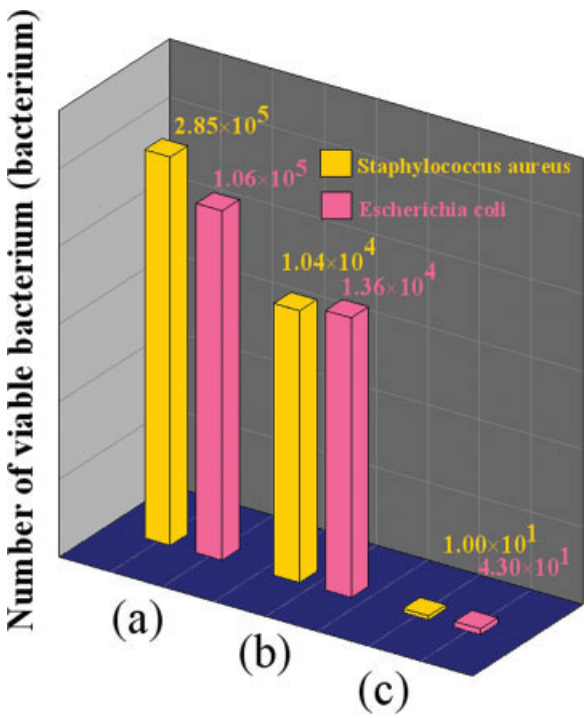

Figure 8. For both cases of *Staphylococcus aureus* and *Escherichia coli*, determination of the numbers of viable bacteria (a) N_A, (b) N_B and (c) N_C, for Group A, B, and C are compared. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ions are emitted from a titanium target. The ultimate substrate temperature occurs in the end of deposition time and reaches 150°C as measured. This shows the capability of utilizing low-temperature AIP deposition of TiO_2 on polymer substrates for antimicrobial application.

Apparently, the growth rate of 5 μm/h (Figure 4) is higher than other PVD processes can provide.[20,21] Such a high rate deposition technique could be beneficial in production when considering commercializing this technique. Previous studies indicate that a TiO_2 film thickness reaching 2.5 μm begins to possess photocatalytic efficiency and thus presumably antimicrobial efficiency.[17] Such a critical thickness for photocatalytic efficiency and thus presumably antimicrobial efficiency should be due to the increased amount of anatase phase (the effective phase) in the deposit and an increased effective surface area.

The success in growing anatase TiO_2 film (Figure 5) at such a relatively low substrate temperature may be due to the fact that anatase TiO_2 exists in the low temperature region of a thermodynamic equilibrium phase diagram. Whilst the intensified ions emitted from a target kinetically favors a rapid and complete reaction with oxygen to condense a solid TiO_2 film.

Obviously, the anatase phase-containing TiO_2 coatings gives rise to photocatalytic events and the subsequent inhibition of bacterial growth for both cases of *Staphylococcus aureus* [Figure 6(c)] and *Escherichia coli* [Figure 7(c)]. The R value shown in Figure 8 quantitatively categorize TiO_2 coated specimen to be an effective antimicrobial surface and arc ion plating is demonstrated to be an effective method to deposit TiO_2 films on stainless steel for antimicrobial purposes. It can be summarized that the deposited TiO_2 coating mainly consists of an anatase phase. The deposition can be carried out at relatively low substrate temperature with a high growth rate of 5 μm/h. The TiO_2 coating exhibits excellent antimicrobial efficacy against *Staphylococcus aureus* and *Escherichia coli* and could possibly serve as a new antimicrobial treatment for medical implements to reduce the risk of hospital-acquired infections.

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