The fabrication of the antibacterial paste based on TiO₂ nanotubes and Ag nanoparticles-loaded TiO₂ nanotubes powders

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ABSTRACT
We successfully synthesised TiO₂ nanotubes (TNTs) and silver nanoparticles (Ag NPs)-loaded TiO₂ nanotubes paste. These were coated on a glass substrate by spin coating method, and their antibacterial activities were surveyed. The morphology of materials was defined by transmission electron microscopy (TEM) image; the crystalline structure and the composition of the materials were determined by X-ray diffraction (XRD) pattern and X-ray photoelectron spectroscopy (XPS). Vibrational properties of the molecules existing in the sample were investigated by Fourier transform infrared (FTIR) spectroscopy, and the transmittances of films were determined by UV–Vis transmittance spectroscopy. This research shows that the structure and morphology of TNTs did not change after they underwent the processes of paste preparing and film coating on a glass substrate. Furthermore, the transmittance of TNTs film (about 75%) is higher than Ag NPs-loaded TiO₂ nanotubes (Ag/TNTs) film (about 65%) in the visible region. Moreover, the antibacterial property of Ag/TNTs film shows its effectiveness against Escherichia coli bacteria, and the antibacterial efficiency is 99.06% for 24 h-incubation period in the dark condition.

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1. Introduction

Bacteria concentrate the most on the surface of various objects such as fabric garment, sanitary equipment, and electronic devices. According to the London School of Hygiene & Tropical Medicine, the contaminated surface of mobile and hand electronic devices contains 82%–92% of bacteria, especially Escherichia coli [1]. E. coli is a common bacteria and can be found in intestinal microflora of a variety of animals including human, and can cause debilitating, urinary tract infections, meningitis and septicemia and sometimes fatal diseases in humans [2]. Recently, many scientists have used nanomaterials to fight against E. coli bacteria because the ideal physicochemical properties of nanomaterials are the small volume, large surface area and low toxicity [3–5]. There are many nanomaterials...
used to fight bacteria, but the combination of silver nanoparticles (Ag NPs) with TiO₂ has been studied a lot, recently [6–13]. Ag/TiO₂ not only possesses significant bactericidal activity under dark conditions but also enhances its self-cleaning [14–16]. The photoactivity of Ag/TiO₂ is greatly enhanced because Ag NPs are believed to both enhance photoactivity by facilitating electron–hole separation, and visible light absorption due to silver surface plasmons that are thought to induce electron transfer to TiO₂ [10,11,17,18]. Moreover, to improve the efficient surface area of this material system, TiO₂ was synthesised in nanotubes (TNTs) form due to the one-dimensional structure of TNTs. In addition, TNTs have an effectively large surface area, high mechanical strength, chemical stability, and are especially non-toxic [19,20]. Therefore, the photocatalytic and bactericidal ability of Ag/TNTs material system was significantly enhanced in the visible light [9,21–23].

Hydrothermal method is a simple method to synthesise TNTs powder with uniform structure, small diameter, and high crystallisation. However, the biggest disadvantages of the powder material are the limitation in the treatment of large surface and the retrieving of materials after use [24]. To overcome these difficulties, we transformed these materials from powder to a paste to facilitate the film- or spray-coating onto the surface of other materials and surveyed the structural stability, morphology as well as the transmittance and bactericidal ability against *E. coli*.

2. Experimental

2.1. Chemicals and materials

TNTs and 1% wt Ag/TNTs powder were successfully synthesised by using hydrothermal and photoreduction methods, respectively. The results had been published in [25,26]. Deionised (DI) water was purified using the Puris-Evo machine (South Korea); ethanol (Scharlau, Spain, 99.99% pure), acid acetic (Xilong Chemical, China, 99% pure), α-terpineol (Sigma-Aldrich, USA, 99.99% pure) and ethyl cellulose (HiMedia Labs, India) were purchased.

2.2. Fabrication of TNTs and Ag/TNTs paste

Based on the synthesis process used by Seigo Ito et al. [27], we changed some parameters to meet with our materials. The detailed fabricating processes of TNTs and Ag/TNTs paste are shown in Scheme 1. First, 1 ml ethanol, 0.5 ml acetic acid and 0.5 ml DI water were added to the 3 gram TNTs powder (for TNTs colloid synthesis) or Ag/TNTs powder (for Ag/TNTs paste synthesis) and were mixed using ball milling with 0.5 mm diameter ZrO₂ balls for an hour. Second, ZrO₂ balls were filtered and the mixture was poured into a beaker. Next, the mixture was added with 100 ml ethanol, 20 grams of α-terpineol, and 30 grams of ethyl cellulose, respectively, and stirred to form a homogeneous mixture. To change the dispersion and viscosity of the paste, the above mixture is then dried at 100 °C for an hour to remove ethanol and DI water. On the contrary, the paste was added with ethanol to decrease their viscosity.
2.3. Process of film coating from the paste

A glass slide substrate (Merck, Germany) was cleaned by washing with soap and ultrasound in acetone and DI water, then dried and placed on a flat surface rotating axis perpendicular to the ground. The paste was diluted with ethanol to form the solution and then dropped onto the substrate. The TNTs and Ag/TNTs films were coated by spin coating method with a rate of 10,000 rpm for 90 s. Finally, the films were dried at 100 °C for 60 min in the normal atmosphere.

Scheme 1. An illustrate schematic of TNTs and Ag/TNTs paste process.

2.4. Characteristics of materials

X-ray diffraction (XRD) analysis was carried out using Burker D8 – Advance 5005, at a voltage of 45 kV with Cu Kα (λ = 15.406 nm) to examine the crystal structure of materials. The
morphology and shape of the material were recorded by transmission electron microscopy (TEM) on a JEM – 1400 instrument. The thicknesses of the thin films were measured by Dektak 6M Profiler. The vibrational characters of the molecules existing in the sample were determined by Fourier transform infrared (FTIR) spectroscopy from the Brucker Tensor 27 (US). Transmittance capabilities of the thin films were determined by the transmission spectra from 250 to 800 nm region of the spectrophotometer UV–Vis U2910 Hitachi (Japan). To analyse the composition of the material, X-ray photoelectron spectroscopy (XPS) (ESCA - 5600) with Al Kα monochromatic source (1486.6 eV) was operated at 13.9 kV.

2.5. Determination of the antibacterial materials

All samples were sterilised in an autoclave at 121 °C for the first 30 min. First, E. coli bacteria are grown in the Trypticase soy broth (TSB) medium. Then, the bacteria were incubated at 37 °C for 24 h under a relative humidity >90% in darkness to allow microbial growth to reach the concentration of 10⁶ CFU/ml. The concentration of the bacteria is determined by measuring of optical density (OD) at 600 nm wavelength throughout the spectroscopy machine. After that, the control sample (glass slide), TNTs and Ag/TNTs films were put into three dishes including E. coli bacteria in the same concentration. All samples were placed in the dark to test the antibacterial ability of each sample. The E. coli concentration of the samples was taken at different times from 0 to 24 h.

3. Results and discussions

3.1. The colour of paste

Figure 1 shows the colour of paste made from TNTs powder and Ag/TNTs powder. Because of the differences in the morphology and the chemical properties of the initial materials, TNTs paste is slightly yellow (Figure 1(a)) and Ag/TNTs paste is gray (Figure 1(b)).
3.2. TEM observation

Figure 2(a) shows that the morphology of nanotubes is uniform and the diameter of nanotubes is about 8–10 nm. The TEM image of Ag/TNTs sample (Figure 2(b)) shows the existence of small black dots representing better information about the presence of Ag NPs in the sample. In addition, Ag NPs have perfectly dispersed on the TNTs surface with their diameter about 3–5 nm. Moreover, Ag/TNTs paste (Figure 2(c)) shows the overlap of TNTs. The small black shapes, corresponding to organic compounds (α-terpineol, ethyl cellulose, ...), clouded the TNTs structure and can be identified through the dark areas of the TEM image.

Figure 2(d) is a TEM image of Ag/TNTs paste at 30,000× magnification (scale: 200 nm). It shows the black ranges covered on TNTs. These ranges indicate the existence of compounds such as α-terpineol and ethyl cellulose. The result is in agreement with the FTIR analysis which is explained in Section 3.3 (Figure 3) and the FE-SEM images (Figure S1) in the Supplemental information. The Ag NPs sizes remain the original size compared to the initial Ag/TNTs powder. This result indicates that the formation of Ag/TNTs paste did not affect the morphology of TNTs as well as of Ag NPs.

3.3. Fourier transform infrared spectra analysis

FTIR spectra of the TNTs and Ag/TNTs films are presented in Figure 3. This figure shows the absorbance band at the range from 450 to 900 cm\(^{-1}\), corresponding to the typical vibration of Ti–O–Ti bonding of TNTs. Besides, the peaks in the near region from 2974 to 2850 cm\(^{-1}\) present the bonding vibrations of C–H of CH\(_2\) group. In particular, the peaks at 2925 and 2872 cm\(^{-1}\) are assigned to the symmetric vibration mode of CH\(_2\) groups. The peaks at 1630 and 3453 cm\(^{-1}\) are characteristic for vibrations of O–H groups in H\(_2\)O and hydroxide, respectively [28,29]. Moreover, a peak at 1045 cm\(^{-1}\) recognises the vibration of C–O group in α-terpineol or ethanol solvent. The vibrations of the associated C–O–C or C–O–H of ethyl cellulose are associated with the peak at 1220 cm\(^{-1}\) wavenumber. The presence of acetic acid is ascertained with a peak at 1378 cm\(^{-1}\) wavenumber attributed to the original vibration of carboxyl [30]. The vibration peak of Ti–O–Ti group showed that TNTs remain their characteristic after coated on the glass.

![Figure 2](image-url) TEM images of TNTs powder (a), Ag/TNTs powder (b) and Ag/TNTs paste (c) (scale: 20 nm).
3.4. X-ray diffraction pattern analysis

X-ray diffraction evaluated the phase composition and crystal structure of our samples. In Figure 4(a), XRD patterns show that the crystal structure of TNTs powder has both anatase and rutile phases. The peaks at $2\theta = 25.27^\circ, 37.9^\circ, 48.2^\circ, 62.8^\circ$ and $76.2^\circ$ reveal the presence of anatase phase corresponding to the crystalline planes of (101), (004) (200), (204) and (301), respectively. Meanwhile, the peak at $2\theta = 27.5^\circ$ and $54.35^\circ$ are corresponding to the (110) and (211) crystalline planes of rutile phase, respectively. However, after the coating, the XRD pattern of TNTs film shows only a characteristic peak at $2\theta = 25.27^\circ$ corresponding to the (101) plane of anatase phase. In addition, Ag/TNTs sample (Figure 4(a)) has a similar result with TNTs sample. The XRD pattern of Ag/TNTs powder still appears as characteristic peaks of anatase and rutile phases. Besides, Figure 4(b) shows that these observations at $38.2^\circ$ and $44.4^\circ$ have represented (111) and (200) planes of the face-centred phase of silver metal [11,16]. These results demonstrated that the Ag NPs existed within the TNTs. XRD pattern of the TNTs film and Ag/TNTs films (Figure 4(a)) shows a peak at $2\theta = 25.27^\circ$, corresponding to the (101) plane of anatase phase. This phenomenon is explained by the absorption of acetic acid on the TNTs surface on forming paste (to functionalised TNTs) and TNTs dispersed in organic compounds. Moreover, the film is coated on the substrate having amorphous structure that affected the intensity of the diffraction peaks of TNTs.

3.5. XPS analysis of Ag/TNTs

Figure 5 shows the XPS results of the compositions of Ag/TNTs sample. The high-resolution XPS spectrum of the Ti2p shows that Ag/TNTs sample has two peaks at 459.4 and 465 eV which are characteristic of the configuration of Ti$^{4+}$ in the form of TiO$_2$. Besides, a peak at 456.4 eV was found with binding energy positions corresponding to Ti$^{3+}$.

In the XPS analysis results of the O1s in Ag/TNTs, there is a major peak at 531 eV with binding energy characterising for oxygen in TiO$_2$ and a smaller peak at 528 eV associated with characteristic for O$^-$. [31]
The bonding of Ag into TNTs was investigated by the high-resolution XPS of the Ag 3d. The results of the Ag3d XPS (Figure 5(d)) have the following spotlight: first, the sample had more common smaller peaks inside the two top large spectra. After using Gaussian fit to analyse the XPS spectra of Ag 3d, there is appearance of peaks at 367.5, 368.6, 371.8, and 375 eV with binding energy positions corresponding to Ag+ 3d5/2, Ag⁰ 3d5/2, Ag+ 3d3/2 and Ag⁰ 3d3/2, respectively [16,31]. This shows that there is an existence of two states of silver including Ag⁺ and Ag⁰. Consequently, one part of silver incorporated the TNTs and another part was silver atoms.

Figure 4. XRD patterns of TNTs and Ag/TNTs (powder and film samples): (a) original image, (b) magnified image of powder samples.
3.6. The transmittance of TNTs and Ag/TNTs films

The TNTs and Ag/TNTs are coated on a glass substrate from TNTs and Ag/TNTs paste, respectively, by the spin coating method. After that, the thickness of the films is measured by the Dektak 6M machine. The results show that the thickness of the films is non-uniform and their average thickness is about 3–5 μm. The photo of films in Figure 6(a) shows that although the thickness of the films is large, the transmission of these films are still relatively high; and they are suitable for coating on device’s surface to apply in the antibacterial field. Besides, Figure 6(a) shows that the transmittance of TNTs films is higher than that of Ag/TNTs film. Furthermore, the transmission spectra of films (Figure 6(b)) show that the TNTs film has high transmittance, approximately 75% in the visible region, while Ag/TNTs film has lower transmittance than TNTs film with about 65%.

3.7. Antibacterial activity

After analysing the physicochemical properties of films made from TNTs and Ag/TNTs paste, we surveyed the bactericidal ability of the Ag/TNTs film for E. coli bacteria. The results of the bacterial concentration are shown in Figure 7 and Table S1 (Supplement information). The result indicates that the bacteria concentration is significantly high for the reference samples (glass slide) while the TNTs film killed some
Figure 6. Photo (a) and transmittance spectra (b) of TNTs, Ag/TNTs films on the glass slide.

Figure 7. The concentration of *E. coli* bacteria and the antibacterial efficiency according to the incubation period.
E. coli bacteria. This shows that the concentration of bacteria on TNTs film sample decreased about 3.4 times compared with the reference sample after a 24 h-incubation period. Especially, the antibacterial activity against E. coli of Ag/TNTs film is greatest, showing that the bacterial concentration decreased 106.1 times with the same condition.

Figure 7(b) shows the antibacterial efficiency of TNTs and Ag/TNTs films with respect to the incubation period. It can be identified that Ag/TNTs film killed almost all bacteria after 12 h of incubation, and its antibacterial efficiency reached 98.6%, whereas the antibacterial ability of TNTs film reaches the maximum after 12-h incubation and this effect decreases in the next period. Especially, TNTs film killed only 70.7% of E. coli bacteria after 24-h incubation. This result proves that the presence of Ag in TNTs improved the antibacterial efficiency.

The antibacterial mechanisms are mainly based on the generation of reactive oxygen species (ROS), such as hydroxyl radicals (•OH), superoxide anion (•O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) [32,33]. Besides, according to XPS (Figure 5), the sample contain Ti$^{3+}$ (on the surface of TNTs), Ag$^+$, and Ag$^0$. Therefore, the presence of Ti$^{3+}$ ion that forms ionised oxygen vacancies can lead to the formation of ROS.

$$\text{O}_2 + e^- \rightarrow \cdot \text{O}_2^-,$$

$$\cdot \text{O}_2^- + \text{H}_2\text{O} \rightarrow \cdot \text{HO}_2 + \text{OH}^-,$$

$$\cdot \text{HO}_2 + \cdot \text{HO}_2 \rightarrow \text{H}_2\text{O}_2,$$

$$\text{H}_2\text{O}_2 + \cdot \text{O}_2^- \rightarrow \text{O}_2 + \cdot \text{OH} + \text{OH}^-.$$

Oxygen in the air can react with electron on surface of the sample to form superoxide and hydroxyl radicals.

This mechanism is approximate with studies that were recently published, such as in the research of Nigel S. Leyland et al., the formation of Cu$^{2+}$ ions is associated with an accelerated formation of ROS and this accelerates the consumption of antioxidants, reducing the capacity of the cell wall to absorb damage from these species and to self-repair [34]; V. Lakshmi Prasanna et al. [35] demonstrated that in nano-ZnO involving superoxide species facilitated, by surface defects, to form ROS as in above equations. Moreover, Ag$^+$ ions can directly involve in contact-killing bacterial cells or indirectly by interacting with protein thiol groups, and then damaging the DNA to kill bacterial cells [13,36–38].

4. Conclusions

In this paper, we have successfully fabricated TNTs and Ag/TNTs paste with high viscos-ity and easy to use for antibacterial applications. TNTs morphology is preserved after coating. The characteristics of the molecular vibration and the crystal structure of TNTs have not changed after coating. In addition, the TNTs film has high transmittance, approximately 75% in the visible region. Furthermore, the Ag/TNTs film can kill 99.06% of E. coli bacteria after a 24 h-incubation period under devoid-of-light condition. In the future, TNTs and Ag/TNTs paste can be fully used in bactericidal applications as well as in photocatalyst applications.
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Disclosure statement

No potential conflict of interest was reported by the authors.

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