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Characterization and potent bactericidal effect of Cobalt doped Titanium dioxide nanofibers

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Abstract

We report the successful fabrication and characterization of novel cobalt doped titanium dioxide nanofibers and their antibacterial property. The Co-doped TiO_2 nanofibers were prepared by a facile electrospinning technique. The physicochemical characterization of the electrospun nanofibers was carried out by XRD, SEM-EDX and TEM. The antibacterial activity was tested against two common foodborne pathogenic bacteria such as *Staplylococcus aureus* (Gram-positive) and *Salmonella typhimurium* (Gram-negative). Bactericidal effects were determined by the minimum inhibitory concentration (MIC) method and TEM analysis respectively. Our results suggested that the Co-doped TiO_2 nanofibers severely inhibit the growth of the selected strains. The results of TEM investigation demonstrated that exposure of bacteria to the Co-doped TiO_2 nanofibers lead to disruption of cell membranes and respective membrane enzymes which caused bacteria to die eventually. The observed superior antimicrobial action of Co-doped TiO_2 as compared to virgin TiO_2 nanofibers is due to the synergistic impact of cobalt oxide and TiO_2.

Keywords: Electrospinning; *S. aureus*; Co-doped TiO_2; *S. typhimurium*

1. Introduction

Although remarkable progress has been made toward a better understanding of microbial transmission and pathogenicity of contaminated foods, however, the foodborne diseases still remain a universal problem [1]. Contamination of food by microbes can cause health hazards to human beings, particularly when food is contaminated by pathogenic microorganisms. The *S. aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. On the other hand, *Salmonella* is a major cause of bacterial foodborne diseases [2]. Recent reports have shown that different types of food and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs [3,4]. Therefore any method that reduces or eliminates food contamination will have a significant impact on the incidence of foodborne diseases [5]. A possible approach to reduce the microbial contamination on food surfaces and in food preparation environments is the use of nanomaterials. Antimicrobial nanocomposite systems are appealing, since materials in the nanoscale range have a high surface area to volume ratio as compared to their bulk counterparts. Nanomaterials are thus more efficient, since they are able to attach more copies of microbial assemblage and cells [6]. Titanium dioxide is one of the most studied materials in the field of antibacterial applications [7,8]. In the TiO_2 nanocomposites, biocidal efficiencies depend on their light absorbance. Therefore, most TiO_2 compounds exhibit no antimicrobial effect in the dark condition. Earlier workers have reported that the metal doped TiO_2 and coated titania compounds provide efficient antibacterial activities without light irradiation [9,10]. Nevertheless, wide investigations on the antibacterial effect of nanostructured titania has been

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carried out, however, the toxicity of Co-doped TiO_2 nanofibers has not been studied till now. The cobalt oxides (CoO and Co_3O_4) materials possess remarkable optical, electrical and magnetic properties and are therefore commonly used for photocatalysis and electromagnetic applications [11,12]. In the present study we have been exploring the electrospinning technique to successfully fabricate the Co-doped TiO_2 nanofibers. Electrospinning is a material processing technique that produces fibers with nanoscale range [13]. In the electrospinning process, a charged polymer jet is pulled out by applying electrostatic force and this jet at first extends in a straight line and then experience the whipping motion while traveling from the spinneret to collector and ultimately get deposited in the form of a non-woven fiber mat. Electrospinning is a simple and easy way to control the morphology of ultrafine fibers and the obtained fibers possess astonishing characteristics.

We communicate here the potential antibacterial effect of electrospun Co-doped TiO_2 nanofibers against *S. aureus* and *S. typhimurium*. Specifically, this study examines the effects of Co-doped TiO_2 nanofibers concentration and exposure time on the growth and viability of the bacteria. We used MIC method to test the antimicrobial activity. The MIC is the lowest concentration of an antimicrobial material that will inhibit the visible growth of a microorganism after incubation. To our knowledge, for the first time we report the fabrication of novel Co-doped TiO_2 nanofibers by facile electrospinning and offer supportive evidences to indicate that the nanofibers can inhibit *S. aureus* and *S. typhimurium* growth and even kill the bacterial cells by destroying the membranous configuration.

2. Experimental

2.1. Materials and bacterial strains

Poly(vinyl acetate) (PVAc, Mw = 500,000) and Cobalt nitrate hexahydrate, ($Co(NO_3)_2 \cdot 6H_2O$) were purchased from Sigma-Aldrich, USA. *N, N*-dimethylformamide (DMF) and Titanium isopropoxide (TIP, 98.0 assay) were purchased from Showa and Junsei Co. Ltd., Japan respectively. The bacterial strains *Staphylococcus aureus* KCCM 11256 and *Salmonella typhimurium* KCCM 11862 were purchased from the Korean Culture Centre of Microorganisms (KCCM) and were cultured in Tryptone soy broth (TSB, Becton, Dickinson Diagnostic & Co., USA).

2.2. Fabrication of Co-doped TiO_2 nanofibers

The fabrication of Co-doped TiO_2 nanofibers was carried out by the simple electrospinning method. Briefly, PVAc (18 wt%) solution was prepared by dissolving PVAc in DMF under magnetic stirring (~6 h) at room temperature. 5 g of TIP was taken in a separate bottle and a few drops of acetic acid were added to it till the solution turns out to be transparent. The acetic acid was added to avoid precipitation of the solution. An alcoholic (ethyl alcohol) solution of $Co(NO_3)_3 \cdot 6H_2O$ (5 wt%) was added into the TIP solution. Subsequently, 6 g of PVAc solution was slowly mixed with the aforementioned solution and stirred vigorously. The obtained sol-gel was transferred into a 10 ml syringe. A voltage of 15 kV was applied and the distance between the tip of the needle and collector was fixed at 18 cm. The as-spun nanofibers were initially dried at 80 °C for 24 h under vacuum and then calcined in air at 500 °C with heating rate of 2 °C/min for 2 h.

2.3. Characterization

The XRD pattern of nanofibers was recorded on a Rigaku/Max-3 A X-ray diffractometer (XRD, Rigaku Co., Japan) with Cu Kα radiation ($\lambda = 1.540$ Å) over Bragg angles ranging from 20 to 80° and the operating voltage and current were maintained at 30 kV and 40 mA, respectively. To examine the microstructure, the powdered samples were uniformly sprayed on carbon tape and Pt coating was applied for 10 s. The images were acquired at various magnifications by scanning electron microscopy (SEM) (JSM6700, JEOL, Japan). The detailed microscopic features of doped nanofibers and exposed bacteria were analyzed by transmission electron microscopy (TEM-H-7650 Hitachi, Co., Japan).

2.4. Antibacterial test

The antibacterial activity of novel Co-doped TiO_2 nanofibers was studied following Li and co-workers [14] method against two common foodborne pathogenic microorganisms. Briefly, the bacterial strains were first grown on solid medium. From the agar plates fresh colonies were inoculated into TSB (100 ml). Growth was monitored by a UV–visible spectrophotometer (UV-2550, Shimadzu, Japan) till the optical density (OD) reached 0.1 at 600 nm (OD of 0.1 corresponded to a concentration of 10^8 CFU/ml of medium). Subsequently, 1 ml inoculum from the above culture was further added to the freshly prepared broth (100 ml) supplemented with 0, 10, 20, and 40 μg/ml of Co-doped TiO_2 and pristine TiO_2 (40 μg/ml) nanofibers. All the flasks were incubated at 37 °C in a rotary shaker at 150 rpm. The growth rate was monitored by OD measurement as mentioned above at an interval of 4 h for 20 h.

3. Results and discussion

Fig. 1 depicts the XRD pattern of the calcined electrospun nanofibers. All the reflections could be indexed based on the anatase TiO_2 phase (PDF- 89–4921) [15]. The peaks at 32.7 and 35.4 demonstrated the presence of some unidentified by-product. Fig. 2a, b shows the SEM images of calcined nanofibers at different magnifications. The nanofibers are having continuous, uniform and smooth surface with an average diameter of 350–450 nm. The presence of cobalt oxide in the doped nanofibers was further confirmed by EDX spectrum. The EDX spectrum (Fig. 2c) contains Ti, Co and O only; no other element impurity was detected. This indicates that the final product is free of impurity and composed of cobalt oxide and TiO_2 exclusively.

Fig. 3 illustrates the TEM image along with SAED pattern and FFT micrograph of the Co-doped TiO_2 nanofibers. The TEM image (Fig. 3a) further confirmed the fiber diameter ~400 nm. The dark black particles on the surface of Co-doped TiO_2 nanofiber may be due to the presence of cobalt oxide in the doped nanofibers. The FFT micrograph illustrated the parallel crystalline planes which confirms high crystallinity of the sample (Fig. 3b). The SAED pattern composed of some bright points which supports the polycrystalline nature of electrospun nanofibers (inset Fig. 3b).

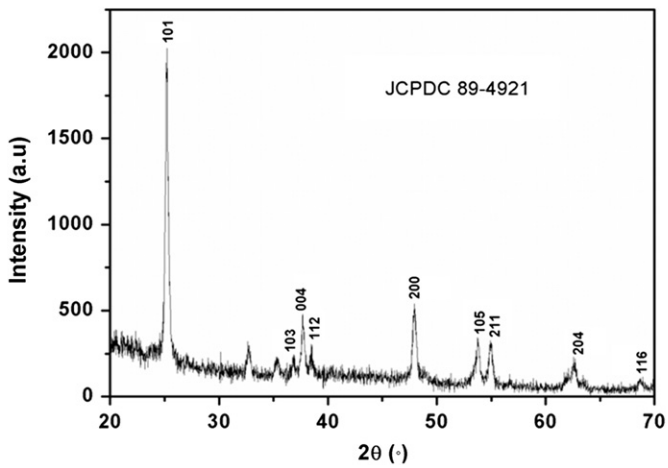

Fig. 1. XRD pattern of the electrospun nanofibers calcined at 500 °C.

To examine the antibacterial effect, double dilution method was used. Four different concentrations (0, 5, 10 and 20 μg/ml) of the Co-doped TiO_2 nanofibers have been used in the present study to determine the MIC. The observed MIC was found to be 5 μg/ml for both the tested pathogens (Fig. 4a and b) and a lower MIC is an indication of a better antimicrobial agent. Moreover, it has been noticed that with the increase in concentration of Co-doped TiO_2 solution, the inhibition rate has also been increased. The highest concentration of the Co-doped TiO_2 solution (20 μg/ml) has been found to completely inhibit the growth of pathogens. On the other hand the high concentration of pristine TiO_2 nanofibers (20 μg/ml) also demonstrated inhibition of bacterial culture as the contact time increased. The microbial strains grew up exponentially during the logarithmic phase (from 4 to 16 h) as observed in the case of control samples (Fig. 4a and b). On the other hand, Co-doped TiO_2 nanofibers have

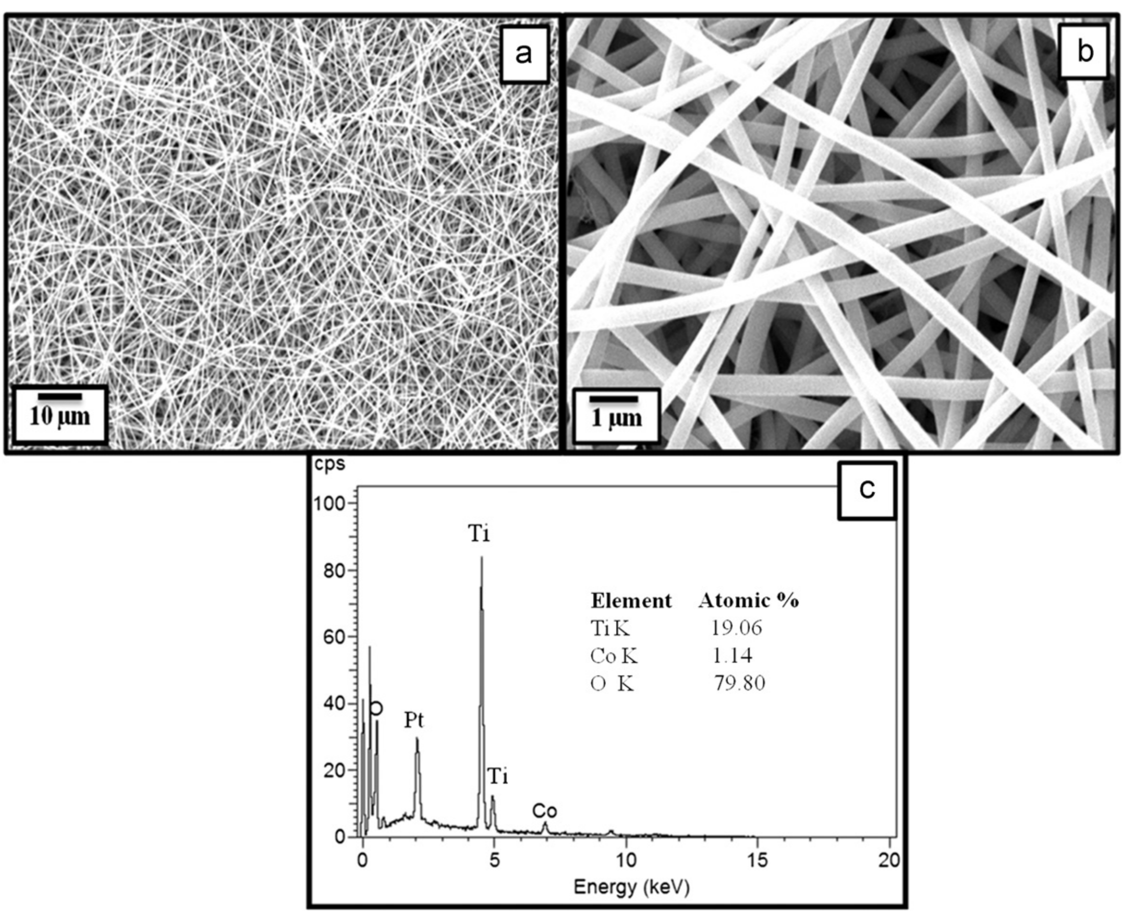

Fig. 2. SEM images at different magnifications (a and b) and EDX spectrum (c) of the electrospun Co-doped TiO_2 nanofibers.

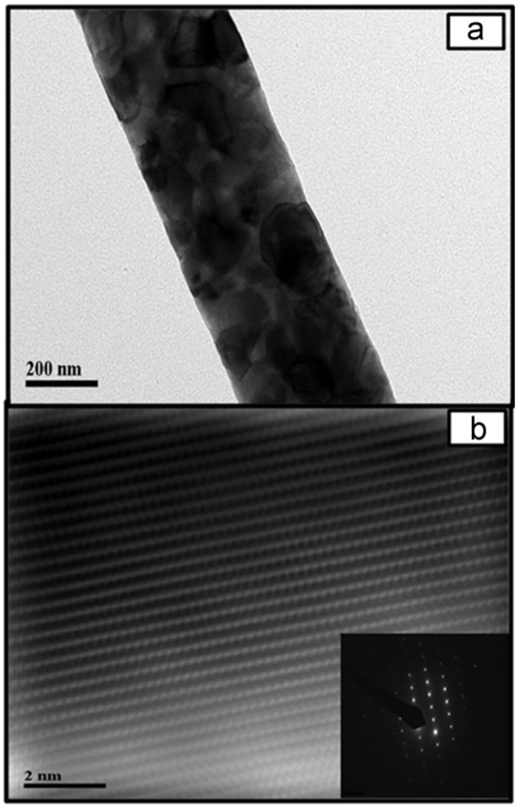

Fig. 3. TEM image (a) FFT micrograph (b) of the electrospun Co-doped TiO_2 nanofibers, the *inset* represents the SAED pattern.

demonstrated effective toxicity against both the Gram-positive and Gram-negative strains. The obtained results indicate that the inhibition is both a time and concentration-dependent manner. Moreover the doping of Co in TiO_2 promotes the bactericidal effect. Our findings are in good agreement with the earlier findings [7–10]. To gain insight into mechanisms we performed TEM. TEM analysis is suitable tools for investigation of the morphology and microstructure of the cells. Fig. 4 (c and d) shows the strains treated with Co-doped TiO_2 nanofibers. The cell structure of exposed bacteria was damaged drastically. Significant changes in the morphology occurred (Fig. 4c and d). Different scientists have specified different opinions or/and mechanisms such as leakage of internal contents, rupture of bacterial membranes [16], disturbance of osmotic balance, oxidative stress and so on for bacterial toxicity and death. However exact mechanism is still unresolved. In the present study we suppose that the dispersed Co-doped TiO_2 solution ensures continuous release of ions into the growth medium. The Co ions interacted with the negatively charged bacterial cells and adhered to the bacterial cell walls. The bacteria and the Co-doped TiO_2 nanofibers developed electrostatic forces and we consider this electrostatic attraction may be the reason for their adhesion and acute toxicity. The penetration of the released ions through ruptured bacterial cell walls and their complexation with enzymes in the cell membrane resulted in the inhibition of the enzymatic

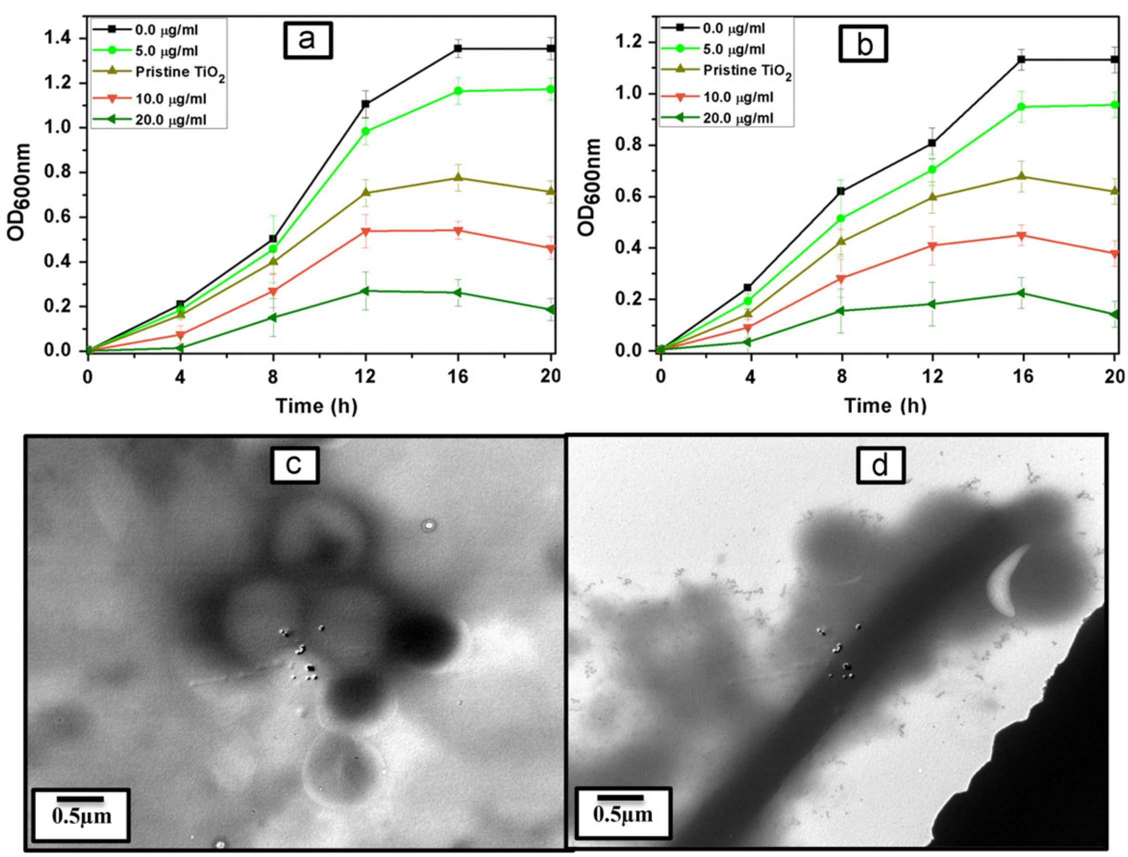

Fig. 4. Growth curves of (a) *S. aureus* (b) *S. typhimurium* exposed to different concentrations of Co-doped TiO_2 nanofibers. Data are average from triplicate experiments. Representative transmission electron microscope images of *S. aureus* (c) and *S. typhimurium* (d) treated with calcined nanofibers.

activity and ultimately death of the bacteria. The current work suggests that the enhanced antibacterial effect of the Co-doped TiO_2 nanofibers might be explained by a synergistic effect of Co and TiO_2 components

4. Conclusion

Novel Co-doped TiO_2 nanofibers were prepared via electrospinning process. The morphology and crystallinity of the synthesized nanofibers was described by SEM, TEM, and XRD pattern respectively. Whereas the elemental composition was determined by EDX spectroscopy. Throughout the above results, there is no doubt on the bactericidal effect of Co-doped TiO_2 nanofibers. In the present investigation the antibacterial activity was attributed to the release of ions which caused the damage of cell membrane and interior molecules of the bacteria. Our results clearly demonstrated the enhanced antibacterial activity even in the absence of light. The superior antibacterial effect of the Co-doped TiO_2 is attributed to the synergistic outcome of Co and TiO_2. In summary, our work leads to a latest trend in the design of multifunctional materials for enhanced bactericidal effect, which might help to combat the pathological bacteria and diseases. The prepared Co-doped TiO_2 nanofibers are depicted as promising candidates for the development of new bactericides to be used in food packaging.

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