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Protocols

Enhanced antimicrobial efficacy of thermal-reduced silver nanoparticles supported by titanium dioxide

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ABSTRACT

The antimicrobial efficacy of silver nanoparticles (AgNPs) is influenced by many factors, including the particle size, AgNP oxidation state and support materials. In this study, AgNPs are synthesized and supported by two types of TiO_2 powders (P25 and Merck TiO_2) using two heat-treatment temperatures (120 and 200 °C). The formation of well-dispersed AgNPs with diameters ranging from 3.2 to 5.7 nm was confirmed using transmission electron microscopy. X-ray photoelectron spectroscopy and X-ray diffraction indicated that the majority of the AgNPs were reduced from Ag^+ to Ag^0 at 200 °C. The AgNP antimicrobial activity was determined by the zone of inhibition against three fungi, *A. niger*, *P. spinulosum* and *S. chartarum*, and two bacteria, *E. coli* (Gram-negative) and *S. epidermidis* (Gram-positive). The antimicrobial activity of metallic AgNPs was more pronounced than that of silver nitrate and some antimicrobial drugs. The AgNPs exhibited optimal antimicrobial efficacy when the AgNP dispersion on the surface of TiO_2 was in the region between 0.2 and 0.7 μg-Ag/m^2. The minimum (critical) AgNP concentrations needed to inhibit the growth of bacteria (*E. coli*) and fungi (*A. niger*) were 13.48 and 25.4 μg/mL, respectively. The results indicate that AgNPs/TiO_2 nanocomposites are a promising disinfectant against both bacteria and fungi.

1. Introduction

Silver has historically been known as a potent antimicrobial agent and has a broad spectrum of antibacterial activity and low toxicity to mammalian cells [1]. Several studies have discussed the inhibitory effect of silver on bacteria [2–6]. Silver ions (Ag^+) are highly reactive with proteins, as they bind with the thiol groups (-SH) and cause structural changes in the bacterial cell wall and nuclear membrane, leading to DNA misrepresentation and cell death [4]. Moreover, Ag^+ can also inhibit bacterial replication by interacting with DNA and RNA, resulting in their denaturation [7]. Silver nanoparticles (AgNPs) have been extensively studied in recent years because they exhibit stronger antimicrobial activity than silver salts [5,8] The antimicrobial activity of AgNPs can be attributed to their large surface area, which enables better contact with microorganisms and the ability to release silver ions in bacterial cells, thus enhancing their bactericidal activity [9–11]. AgNPs can penetrate cell membranes and generate reactive oxygen species (ROS) that may induce oxidative stress on cell components and condense DNA molecules, inhibit DNA replication, and cause cell damage or death [12,13]. Titanium dioxide (TiO_2) is a common support for nanometal particles. TiO_2 exhibits many advantages, such as non-toxicity, stable physicochemical properties, and relatively low cost. Several studies have focused on promoting the antimicrobial activity of nanometals by doping metals on a TiO_2 support [14–17]. Combining TiO_2 and embedded silver compounds may promote and expand the antimicrobial functions of this nanocomposite. TiO_2 nanoparticles provide a large surface area for the dispersion of AgNPs and prevent their aggregation. The strong-metal-support interaction (SMSI) between AgNPs and the TiO_2 support can influence the oxidation state of AgNPs supported on TiO_2 nanoparticles [18]. The antimicrobial activity of AgNPs is strongly influenced by their oxidation states and particles sizes [10,13,19–22]. However, few studies have focused on the effect of the TiO_2 support on the antimicrobial activity of AgNPs.

This study examines the antimicrobial activity of AgNPs supported on TiO_2 (AgNPs/TiO_2) prepared by the impregnation method. Two different types of TiO_2 (P25 and Merck) are selected as the supports, and two heat treatment temperatures (120 and 200 °C) are used to test the effects of the support material and the heat treatment temperature on the antimicrobial activity of AgNPs/TiO_2. Two bacteria and three fungal strains were used as the target microorganisms, and the critical (minimum) concentrations of AgNPs required to inhibit the growth of target microorganisms

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were determined. The synthesized AgNPs/TiO_2 were characterized using X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), and transmission and scanning electron microscopy (TEM and SEM, respectively). The association between the characteristics and antimicrobial performance of the AgNPs/TiO_2 was systematically studied. The results of this research provide a better understanding of the effect of TiO_2 supports on the antimicrobial activity of AgNPs.

2. Materials and methods

2.1. Materials and microbial cultures

Two commercial TiO_2 powders were used as the support for AgNPs: (i) Degussa P25 (80% anatase, 20% rutile, Brunauer-Emmett-Teller (BET) specific surface area (S_{BET}): 53.3 m^2/g, particle diameters: 20–30 nm, Evonik (Degussa) Company, Essen, Germany) and (ii) Merck TiO_2 (pure anatase, BET specific surface area: 11.7 m^2/g, particles diameters: 200–500 nm, Merck Millipore Company, USA). Silver nitrate ($AgNO_3 \geq 99\%$ purity) and Tween 80 were purchased from Sigma-Aldrich Chemical Company. All of the solutions used in this study were prepared with distilled deionized water.

The microbial strains for the antimicrobial experiments were shipped from the Bioresource Collection and Research Center (BCRC) of Taiwan in freeze-dried form. These strains contain three fungi, *Aspergillus niger* (BCRC 30190), *Penicillium spinulosum* (BCRC 31302), and *Stachybotrys chartarum* (BCRC 31862), and two bacteria, Gram-negative *Escherichia coli* (BCRC 11634) and Gram-positive *Staphylococcus epidermidis* (BCRC 11030). Those model strains are prevalent indoor species, which can be found in building materials, house dust and the air conditioners, and have been shown to be strongly associated with human health. The bioaerosol prevalence in indoor basements is an important factor for biocidal use in exposure assessments performed in indoor environments. According to the instruction guide, these strains require activation and a purity check before experimentation. Malt extract agar (MEA) for the cultivation of fungi was obtained from Becton and Dickinson Company (BD Co., USA). Tryptone soya agar (TSA) and lysogeny broth (LB) medium used for the cultivation of bacteria were also purchased from BD Company, USA.

Before use in the antifungal experiments, the three fungal cultures on MEA plates were incubated at 25 °C for more than 5 days to produce fungal spores. To harvest the fungal spores, 10–15 mL of 0.05% Tween 80 solution was added to the culture plates, and the spores were scraped with a glass spreader. The fungal spore suspensions were transferred to test tubes with a screw cap and adjusted to an approximate concentration of 2×10^6–2×10^5 CFU/mL before use in the experiments.

To prepare bacterial suspensions for the antimicrobial experiments, a single colony of each bacterium strain was inoculated into 20 mL of LB medium. The cultures were incubated at 37 °C with a shaking rate of 200 rpm for 12–15 h, and the final bacterial concentration was approximately 5×10^7 CFU/mL.

2.2. Preparation and characterization of silver nanoparticles by TiO_2

AgNPs/TiO_2 with different silver contents were prepared by the impregnation method [23]. Briefly, the silver nitrate solution was mixed with TiO_2 powders, and the mixing ratio of water solution to Merck and P25 TiO_2 powders was 0.8 and 1.1 mL of H_2O/g-TiO_2, respectively. After 60 min of stirring, the resultant mixture was heated at 120 or 200 °C for 24 h. Then, the agglomerate of AgNPs/TiO_2 was obtained, which was ground into a fine powder with an agate mortar.

The field-emission electron microscopy (FE-SEM), high-resolution transmission electron microscopy (HR-TEM), and field-emission transmission electron microscopy (FE-TEM) images of the prepared samples were recorded using a LEO 1530 microscope, a JEOL 2011 microscope, and a JEOL JEM-2100 microscope, respectively. XPS was performed using an Omicron ESCA spectrometer with a monochromatic Al Kα X-ray source. All spectra were calibrated by the C 1s spectrum at 284.5 eV. The XRD measurements were performed using Bruker D8 tools advance, operated with Cu Kα radiation (λ = 1.5406 Å) generated at 40 keV and 40 mA. The specific surface area (S_{BET}) was measured with a Micromeritics ASAP 2020 HD88 instrument, using the adsorption of N_2 at the temperature of liquid nitrogen. Prior to measuring, all of the samples were degassed at 100 °C for 8 h and finally outgassed to 10^{-5} Torr.

2.3. Antimicrobial efficacy of AgNPs/TiO_2

The antimicrobial efficacy of AgNPs/TiO_2 was determined by the zone of inhibition (ZOI) against five selected strains. The AgNPs/TiO_2 powder was suspended in distilled deionized water with sonication before use in the antimicrobial experiments. At the beginning of the ZOI assay, 15 mL of MEA (for fungi) or TSA (for bacteria) was poured into disposable sterilized Petri dishes (9 mm in diameter) and allowed to solidify. Then, 1 mL of the fungal or bacterial suspension was spread uniformly on the MEA and TSA plates, respectively. The reservoir well for the AgNPs/TiO_2 suspensions was hollowed on each agar plate, and the AgNPs/TiO_2 suspensions (100 μL) with various Ag concentrations were added to the wells. The $AgNO_3$ solution and sterile distilled water were used as the positive and negative controls of the antimicrobial experiments, respectively. The MEA (fungi) and TSA (bacteria) plates were incubated at 25 °C for 48 h and 37 °C for 24 h, respectively, and then, the ZOI was measured. All of the assays were performed in quintuplicate.

The critical (minimum) concentration (Cc) of AgNPs required to inhibit the growth of microbes was determined according to the following equation [23]:

$$(r_2 - r_1)^2 = 4Dt\ln\left(\frac{C}{C_C}\right) \quad (1)$$

where r_1 is the diameter of the reservoir well for the AgNPs/TiO_2 suspension; r_2 is the ZOI diameter; D is the diffusion coefficient of the AgNPs (in MEA or TSA); t is the incubation time; C is the Ag concentration; and Cc is the critical concentration of AgNPs. The plot of lnC versus $(r_2 - r_1)^2$ gives a slope of (1/4Dt) and a y-intercept of $\ln C_C$, as demonstrated in Fig. 4.

3. Results and discussion

3.1. Characterization of the silver nanoparticles (AgNPs) supported by TiO_2

3.1.1. Transmission electron microscopy (TEM)

There are many uniformly dispersed AgNPs on the surface of agglomerated TiO_2 powders, as shown in the TEM images of the as-prepared AgNPs/TiO_2 (Fig. 1). These AgNPs are mainly spherical, with a mean diameter of 3.02–5.74 nm, and the size of the AgNPs increases with increases in the weight percentage of silver (Ag wt%). This phenomenon may result from the agglomeration of AgNPs due to the relatively high silver loading. The AgNPs supported by P25 TiO_2 treated at a higher temperature of 200 °C (AgNPs/P25-200 °C) have a narrower size distribution and smaller sizes than those treated at 120 °C (AgNPs/P25–120 °C), as shown in Fig. 1(a)–(f). Due to the SMSI between AgNPs and TiO_2, the heat treatment at