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Studies of the antimicrobial ability and silver ions migration from silver nitrate-incorporated electrospun nylon nanofibers

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ABSTRACT

Silver nanoparticles (AgNPs), reduced from $AgNO_3$ in a nylon–6 solution, were immobilized through electrospinning on polypropylene (PP) to form an AgNP–nylon–6/PP package film. The AgNP–nylon–6/PP films UV/VIS spectra exhibited absorption bands at 410 and 425 nm, which were typical surface plasmon polariton resonances of the two AgNP forms. The 2–5 nm AgNPs, which were face–centered cubic crystal with (111) plane–type structures contributing to antimicrobial ability, were uniformly distributed on the nanofibers. The inhibition percentage of AgNP–nylon–6/PP composite against *Escherichia coli* and *Staphylocccus aureus* could reach 100% when the accumulative release amount of Ag^+ was less than 0.62 μg/mL. The release ratio, *k* and *n* of the release kinetics evaluated by Fickian diffusion of Ag^+ in food simulants were in the order of 3% acetic acid > RO water > 10% alcohol > 95% alcohol.

1. Introduction

Nanomaterials produced using nanotechniques are new alternative materials for improving the polymeric properties of packaging materials to preserve food quality during transportation and storage and protect food from spoilage due to microorganisms (Bumbudsanpharoke & Ko, 2015). Nanofibers have been used to produce various types of food-contact packaging materials and containers with enhanced barrier properties, mechanical properties, and heat resistance. Among the numerous nanotechniques, electrospinning is a simple process for generating nanofibers. In electrospinning, the careful control of the operating conditions and polymer solution properties enables the production of highly porous structures with smooth nonwoven nanofibers (Ahmed, Lalia, & Hashaikeh, 2015; Lee et al., 2014).

Electrospinning is a facile, cost-effective, and flexible method through which electrically charged jets of a polymer solution are used to produce fibers at the submicron or nanoscale, which are stacked to form a network (Bhushani & Anandharamakrishnan, 2014). The extremely high specific surface area of electrospun nanofibers enables them to possess a high loading capacity for biological substances and active materials, which enhances the antimicrobial capacity of container materials (Lee et al., 2014). Therefore, electrospinning can potentially be used to prepare nanocomposites for active packaging materials and food containers (Vega-Lugo & Lim, 2009).

Nanocomposite materials containing silver nanoparticles (AgNPs) are often used in active packaging materials (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). AgNPs improve the antimicrobial characteristics of food-packaging materials and can kill more than 650 types of microorganisms including bacteria, fungi, and viruses (Jeong, Yeo, & Yi, 2005). AgNPs have a lower drug resistance than other antimicrobial agents (Rudramurthy, Swamy, Sinniah, & Ghasemzadeh, 2016), and have already been applied to numerous antibacterial food-packaging products available in the market (Bumbudsanpharoke & Ko, 2015). The antimicrobial activity of AgNPs is generally linked to their ability to alter cellular permeability and produce reactive oxygen species (ROS) (Ahamed, AlSalhi, & Siddiqui, 2010). Kim et al. (2008) reported that bacterial DNA and mitochondria are affected by ROS produced by the oxidation of AgNPs. Furthermore, the antimicrobial properties can be improved by increasing the surface-to-volume ratio of silver particle (Gupta & Chauhan, 2017; Kim et al., 2008). The antimicrobial activity of AgNPs smaller than 10 nm mainly arises from the nanoparticles themselves, whereas for larger AgNPs, it arises from the released silver ions. AgNPs penetrate the cells more effectively than the silver ions do (Durán et al., 2016).

Most studies related to nanosilver-containing electrospun fibers have focused on their antimicrobial ability and applications in sensors (Pupkevičiūtė, Adomavičiūtė, Pavilonis, Stanys, & Prosyčevas, 2015; Rujitanaroj, Pimpha, & Supaphol, 2008; Stanys & Prosyčevas, 2015; Zhao et al., 2012). However, increased human exposue to AgNPs increases the short- and long-term toxicity risks (Bumbudsanpharoke & Ko, 2015). AgNPs may harm human cells by modifying mitochondrial functioning, increasing membrane permeability, or generating ROS.

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However, adequate toxicological data are not yet available and safety assessments are still in progress (EFSA, 2016; Song, Li, Lin, Wu, & Chen, 2011). Korani, Ghazizadeh, Korani, Hami, and Mohammadi-Bardbori (2015) reported that AgNP penetration in the body mainly occurs through the respiratory system, gastrointestinal system, and skin. Thus, determining the role of the particle shape and size and different supply routes in the toxicity profile of AgNPs is vital and requires additional research. In recent years, several studies have reported the migration of AgNPs into foodstuffs. Most of these studies have focused on AgNPs in response to public and government concerns regarding their safety and health effects (Bumbudsanpharoke & Ko, 2015). Although the food industry has benefited considerably from antimicrobial nanomaterials, safety concerns should be taken into account before AgNPs are commerciallyapplied. The very high surface area of nanoparticles promotes their migration to contacting food and absorption through cell membranes. Hence, reducing the release and migration of AgNPs into foodstuffs has become an important research topic.

No studies have investigated the effect of the electrospinning parameters on the form of silver crystals and the release of silver from electrospun films into various food simulants. Therefore, this study investigated the immobilization of AgNPs from the electrospun nanofiber to reduce the migration of AgNPs into foodstuffs for developing safe and durable coating materials for antimicrobial containers. The effects of the electrospinning solution formula and operating parameters on the immobilization of AgNPs were investigated to evaluate their morphology, antimicrobial ability, and release into food simulants.

2. Materials and methods

2.1. Preparation of electrospun films

According to the study of Pant et al. (2011), nylon–6 powders (Sigma-Aldrich Chemical Co., St. Louis, MS, USA) were dissolved in HCOOH:CH_3COOH (4:1, 1:1, and 1:4 [w/w]) solution and stirred at 90 °C at 80 rpm for approximately 1 h and then cooled to an ambient temperature to become a 18% (w/w) nylon–6 electrospinning solution. $AgNO_3$ powder (Sigma-Aldrich Chemical Co.) was then added in the weight ratio 1%–4% to nylon–6 and dissolved in the electrospinning solution while it was magnetically stirred at 80 rpm for 3 h in a dark room. These solutions were fed into a positively charged spinneret attached to an electrospinning apparatus (NE-300, Falco Enterprise Co., Ltd., New Taipei City, Taiwan) operating at 22 kV. The electrospinning solution was placed in a 5-mL syringe with a No. 21 needle at a feed rate of 0.15 mL/h. A polypropylene screen, placed at 15 cm from the tip of the needle, was grounded and used as the counter electrode.

2.2. Preparation of the nonelectrospun film

One hundred microliter of the $AgNO_3$–nylon–6 solution was dropped on a 1.8 cm × 1.8-cm cover slip and spread at 1000 rpm for 20 s, which was increased to 3000 rpm for the last 60 s, to prepare the spin coating film in a photoresist spinner (King Polytechnic Engineering Co., Ltd., Taipei, Taiwan).

2.3. Physical property analysis

All treatments in the physical properties analysis were performed in triplicate at least.

2.3.1. Ultraviolet–visible spectrophotometry (UV/VIS)

UV/VIS absorption of the samples was measured at 300–600 nm by using a UV/VIS spectrometer (Hitachi U-2001, Tokyo, Japan) to determine the silver pattern.

2.3.2. X-ray diffraction (XRD)

Powders of the samples were pressed into a sample cell and placed on a glass slide. XRD patterns were then obtained using an X-ray diffractometer (Ultima IV, Rigaku, Houston, TX, USA) in the 2θ range of 35°–85° at a scan rate of 5°/min, a voltage of 40 kV, and a current of 20 mA.

2.3.3. Scanning electron microscope (SEM)

The electrospun films were lacerated into small pieces after freeze drying, and the dried specimens were mounted on aluminum studs and coated with a gold/palladium alloy under high vacuum conditions. The specimens were then examined using an ultra-high resolution field emission-SEM (NNS-230, Oregon, OR, USA) to observe the microstructure of ruptured surfaces, and the distribution of AgNPs was further analyzed by using an energy dispersive spectrometer.

2.3.4. Transmission electron microscopy (TEM)

AgNP–nylon–6 electrospun fibers were collected on a formvar-covered carbon-coated copper grid for 30 s. The morphology of AgNPs in the fibers was characterized through TEM (Tecnai G2 F20, FEI, OR, USA) by using an accelerating voltage of 120 kV.

2.3.5. Brunauer–Emmett–Teller (BET) surface area

Approximately 0.1 g of the fiber specimen was collected from the elecrospun films. The BET surface area of the specimen was measured using a surface area analyzer (ASAP 2010, Micromeritics Co., USA), and pore sizes were measured using the AutoPore IV 9500 (Micromeritics). The specimens were degassed overnight in vacuum at 150 °C; N_2 gas was used when detemining the BET surface area.

2.4. Food simulant tests

The exposure condition of nanosilver contained film should be carefully taken into account in the design of migration test to minimize potential negative impact on obtaining comparable results (Wu et al., 2017). According to European Regulation (2011), approximately 10 cm^2 pieces of the electrospun AgNP–nylon–6/PP film and spin-coated $AgNO_3$–nylon–6 film were immersed in 5 mL RO water, 3% acetic acid, 10% alcohol solution, and 95% alcohol solution to simulate contact with aqueous solution, acidic solution, alcoholic foods, and fatty foods. The specimens were immersed at 4 °C and 25 °C for 1.5, 3, 6, 24, 120, and 720 h and then removed and dried on a hot plate. The dried specimens were ashed for 5 h at 550 °C and restored using 1 N HNO_3 for atomic absorption (AA) spectroscopy analysis.

2.4.1. AA spectroscopy analysis

A quantitative analysis of AgNPs in the 0.22 μm membrane-filtered liquid specimen was performed through AA spectroscopy (Z-6100, Hitachi, Tokyo, Japan) under a lamp current of 9 mA, wavelength of 328.1 nm, and slit width of 1.3 nm. All treatments were performed in triplicate.

2.4.2. Silver release kinetics

Release ratio of silver ion from AgNPs-nylon6/PP composite film was calculated on the basis of the released amount of silver ion to the total amount of the silver in AgNPs-nylon6/PP composite film. The Ritger–Peppas equation (Ritger & Peppas, 1987) was utilized to elucidate the release kinetics of nanosilver from AgNP–nylon–6/PP in food simulants. The kinetic constant (k) was evaluated as:

$$M_t/M_\infty = kt^n$$

where M_t/M_∞ is the fractional release of the drug at time t, k is the kinetic constant, and n is the diffusion index of the nanosilver release systems.

According to Fick's second law, the one-way diffusion model was