Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Technical note

Synthesis of hybrid carbon nanotube structures coated with *Sophora flavescens* nanoparticles and their application to antimicrobial air filtration

Gi Byoung Hwang\textsuperscript{a,b}, Kyoung Mi Sim\textsuperscript{a}, Gwi-Nam Bae\textsuperscript{a,*}, Jae Hee Jung\textsuperscript{a,**}

\textsuperscript{a} Center for Environment, Health and Welfare Research, Department of Energy and Environmental Engineering, Korea University of Science and Technology (UST), Korea Institute of Science and Technology (KIST), Hwasun-ro 14-gil 5 Seongbuk-gu, Seoul 136-791, Republic of Korea

\textsuperscript{b} Materials Chemistry Research Center, Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, United Kingdom

**Abstract**

Controlling airborne microorganisms has become increasingly important with increase in human indoor activities, epidemic disease outbreaks, and airborne pathogen transmission. Treatments using antimicrobial nanoparticles have shown promise because of the high surface-to-volume ratio of nanoparticles compared to their bulk counterparts, and their unique physical and chemical properties. In this study, hybrid nanostructures of multi-walled carbon nanotubes (MWCNTs) coated with antimicrobial, natural product (NP) nanoparticles were synthesized using a twin-head electrospray system (THES). The coated nanoparticles were then used in antimicrobial air filters to increase their antimicrobial efficiency. Electrosprayed droplets were converted to NP nanoparticles and MWCNTs through ethanol evaporation. Oppositely charged NP nanoparticles and MWCNTs were coagulated via Coulombic collisions to form hybrid nanoparticles that were deposited continuously onto an air filter medium. The size distribution and composition of the hybrid NP/MWCNT particles were characterized using a wide-range particle spectrometer (WPS) and transmission electron microscope (TEM). The concentration of hybrid NP/MWCNT nanoparticles was lower than that of NP nanoparticles but higher than that of MWCNTs and showed a bimodal size distribution with peak diameters of 21.1 and 49 nm. TEM analyses confirmed that the NP nanoparticles were attached to the MWCNT surface with a density of ~4–9 particles/MWCNT. When deposited onto the filter medium, NP/MWCNT particles formed dendrites on the filter's fiber surface. The filtration efficiency and pressure drop of the NP/MWCNT-coated filters were higher than those of pristine, NP nanoparticles-coated or MWCNTs-coated filters. The hybrid filter also exhibited stronger antimicrobial activity than those of NP or MWCNT-coated filters at identical deposited volumes (1.1 × 10^{-2} cm\textsuperscript{2}/cm\textsuperscript{2} fiber). Ninety-five percent of the tested bacterial aerosols were inactivated on the NP/MWCNTs filter while only <70% were inactivated on NP- or MWCNT-coated filters.

© 2015 Elsevier Ltd. All rights reserved.
1. Introduction

The recent outbreaks of epidemic diseases, including severe acute respiratory syndrome (SARS), swine-origin influenza A (H1N1), and the possibility of airborne pathogen transmission, have garnered both social and scientific attention (Lee et al., 2003; Smith et al., 2009; Tellier, 2009). Numerous methods to control transmission and subsequent outbreaks have been suggested, including the use of thermal energy (Jung, Lee, & Kim, 2009a; Jung, Lee, Lee, Kim, & Lee, 2009b), ultraviolet irradiation (Lin & Li, 2002; Peccia & Hernandez, 2004), air filtration (Pyankov, Agranovski, Huang, & Mullins, 2008; Yoon, Byeon, Park, & Hwang, 2008), and other treatments (Feng et al., 2000; Hwang, Jung, Jeong, & Lee, 2010).

The use of antimicrobial nanoparticles has also shown considerable promise (Ji et al., 2007; Kim et al., 2007; Pal, Tak, & Song, 2007) due to their high surface area-to-volume ratios compared with their bulk counterparts, their unique physical and chemical properties, and their applicability in a wide variety of fields (Vallejos et al., 2012). Silver (Ag) nanoparticles boast a broad antimicrobial spectrum (Panáček et al., 2009; Shrivastava et al., 2007) and have shown greater antimicrobial activity against bacteria and fungi than copper (Cu) (Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008) or titanium dioxide (TiO2) nanoparticles (Yun, Bae, Lee, Ji, & Kim, 2009). The antimicrobial efficacy of Ag nanoparticles depends on the size, shape, and concentration of particles and exposure time (Ji et al., 2007; Jung, Hwang, Lee, & Bae, 2011a; Lee & Lee, 2006; Lee, Yun, Jung, & Bae, 2010). As a result, Ag nanoparticles have been extensively studied with regard to indoor air quality and public human health. The airborne dispersion of Ag nanoparticles is an effective means of air purification (Ji et al., 2007; Jung, Hwang, Lee, & Bae, 2011a) and coating surgical masks with nanoparticles improves the bactericidal performance of the masks (Li, Leung, Yao, Song, & Newton, 2006). Ag nanoparticle-coated activated carbon filters exhibit both effective gas adsorption and antimicrobial activity (Yoon et al., 2008).

Recently, antimicrobial treatments using natural plant products have been studied as a means of improving indoor air quality (Pyankov et al., 2008). These natural products (NPs) are widely distributed and exhibit a wide range of biological activities such as anticancer, antibacterial, antifungal, and anti-inflammatory properties (Donaldson, Warner, Cates, & Gary Young, 2005; Kuroyanagi, Arakawa, Hirayama, & Hayashi, 1999; Young, Choi, & Baek, 2008). They are typically less toxic, inexpensive, and relatively environmentally friendly compared to inorganic materials such as Ag, Cu, and TiO2 (Carson, Hammer, & Riley, 2006). As such, NPs may be more appropriate for indoor environments. Jung et al. (2011b) suggested simple and continuous aerosol processes for synthesizing NP nanoparticles from Sophora flavescens extract and demonstrated satisfactory inhibition of bacterial aerosols when the particles were applied to air filters. Another study found that essential oil vapors of savory had a lethal effect on bacteria in ventilation systems and reported a bacterial reduction rate close to total disinfection (Pibiri, Goel, Vahekeni, & Roulet, 2006).

The synthesis of hybrid nanostructure/nanocomposites has also been suggested as a means of enhancing the antimicrobial activity of nanoparticles. Carbon nanotubes (CNTs) are known to be toxic to bacteria (Kang, Herzberg, Rodrigues, & Elimelech, 2008) and they have been used as a substrate to prepare antimicrobial Ag nanoparticle composites due to their excellent chemical and mechanical stability. Using a supercritical fluid technique, Niu, Han, Wu, Yu, and Xu (2010) synthesized one-dimensional (1D) carbon nanomaterials wrapped with Ag nanoparticles, which exhibited a high degree of antimicrobial activity against Escherichia coli. Rangari et al. (2010) showed that Ag/CNTs and Nylon-6/Ag/CNT hybrid particles were more toxic to microbes than pure Ag particles or CNTs alone. Jung et al. (2011a) demonstrated a simple aerosol technique for fabricating Ag/CNTs hybrids and antimicrobial filters. In addition, Aymonier et al. (2002) and Weickmann, Tiller, Thomann, and Mülhaupt (2005) synthesized various antimicrobial hybrid nanomaterials using polymers. They found that polymethyl methacrylate (PMMA) hybrid nanocomposites containing Ag nanoparticles and polyethyleneimine–Ag hybrid nanoparticles had inhibitory effects on several bacteria strains.

Numerous methods have been used to produce hybrid nanoparticles, including vapor deposition of a metal onto a surface-supported monolayer of colloids (Takei & Shimizu, 1997), partial modification of particles using a long-chain alkylsilane (Takahara et al., 2005), chemical modification of particles at a gas–liquid interface (Petit, Sellier, Duguet, Ravaine, & Mingotaud, 2000), deposition of thin polymer layers onto colloids using a Langmuir–Blodgett technique (Nakahama, Kawaguchi, & Fujimoto, 2000), and laser photochemical deposition (Hugonnout, Carles, Delville, Panizza, & Delville, 2003). However, most of these techniques required relatively dangerous chemical agents, multistep processes, and/or long-production times. Thus, the development of a simple and fast process without the use of chemical agents is desirable.

Electrospraying is a simple, versatile aerosol technique for producing airborne particles with a variety of chemical components and structures. Electrosprayed droplets can be shaped into solid particles by controlling solvent evaporation (Ganan-Calvo, Davila, & Barrero, 1997; Jung, Lee, & Bae, 2013; Yurteri, Hartman, & Marijnissen, 2010). Furthermore, co-electrospraying, which entails the use of two parallel nozzles, is capable of producing various hybrid nanoparticles, including coated, nano-laden, and nano-coated particles (Camelot, Marijnissen, & Scarlett, 1999). Using an oppositely charged, twin-head electrospray system (THES), Mou, Chen, Guan, Chen, and Jing (2013) built so-called Janus nanoparticles (those with two or more distinct properties) with controlled nanostructures. Langer and Yamate (1969) synthesized encapsulated (coated) aerosol particles. In addition, several researchers have fabricated spherical nanoparticles consisting of two chemical substances (Borra et al., 1999; Fu, Liu, & Chen, 2012).

In the present study, a THES was designed to produce antimicrobial hybrid nanoparticles consisting of S. flavescens NPs and multi-walled CNTs (MW-CNTs). The hybrid nanoparticles were synthesized in a continuous airflow and deposited onto an air filter medium. The hybrids were investigated using a wide-range particle spectrometer (WPS) and transmission electron microscope (TEM) for analyses of particle size and morphology. The surface morphology of hybrid nanoparticles...
deposited on air filter media was investigated by scanning electron microscopy (SEM). The antimicrobial and filtration efficiencies of the filter were evaluated and compared to filter media coated with NP nanoparticles or MWCNTs alone.

2. Materials and methods

2.1. Fabrication of NP/MWCNT nanoparticles using a THES

Fig. 1 shows the procedure used to prepare test suspensions of S. flavescens NPs and MWCNTs for electrospraying. The S. flavescens plant is a traditional herbal medicine that is widely distributed throughout northeast Asia. Its major chemical constituents are lavandulyl flavanones (kurarinone, 12.525%), prenylated flavonoids (sophoraflavanone G, 3.248%), and lavandulyl chalcone (kuraridin, 0.818%) (Jung et al., 2011a). These components are known for their antimicrobial, anticancer, antioxidant, and anti-inflammation properties (Kim et al., 2006; Kim, Son, Chang, Kang, & Kim, 2003; Kuroyanagi et al., 1999; Young et al., 2008). S. flavescens extract powder was provided by the Functional Food Center of the Korea Institute of Science and Technology, Gangneung Institute. The powder was dissolved in pure ethanol and sonicated for 10 min. The liquid was then filtered through a cellulose–acetate membrane filter (0.45-μm pore size; National Scientific Co., Rockwood, TN, USA) to eliminate insoluble residues, resulting in a 0.625% (w/v) solution of NP. A commercial MWCNT solution (1.0 wt% hollow CNT 50, in sterilized deionized water as the base solution, purity > 95%) was purchased from Nano Karbon Co. Ltd., Korea, and used to create MWCNT aerosols. The MWCNT suspension was vortexed with pure ethanol and diluted to a final concentration of 18.75% (v/v).

A schematic diagram of the designed THES is shown in Fig. 1b. The system consisted of two separate spray chambers with separate capillaries, a particle hybrid chamber, positive and negative power supplies, and two syringe pumps. The NP and MWCNT suspensions were transferred using metal taper tips (New Objective, Woburn, MA, USA) and stainless steel tubing (IDEX Health & Science, Oak Harbor, WA, USA), respectively. The tip with an inner diameter of 100 μm was used for the NP suspension. For the MWCNT suspension, tubing with an inner diameter of 150 μm was used to increase the number of MWCNTs transferred and to reduce the likelihood of nozzle blockage by nanotubes of the undesired size. Two separate syringes (Hamilton, Reno, NV, USA) on two individual syringe pumps (KD Scientific, Holliston, MA, USA) were used to drive the two solutions. The pump flow rates were 0.08 mL/h (for the NP solution) and 0.8 mL/h (for the MWCNT suspension). Carrier air was filtered through a HEPA
filter and a diffusion dryer at a flow rate of 10 L/min prior to its introduction into the separate spray chambers of the THES. Upon application of positive and negative voltages to the separate capillaries via a DC high-power supply (Korea Switching, Seoul, Republic of Korea), the two suspensions were electrosprayed, and the generated droplets were transported immediately into the hybrid chamber (volume: 892.21 cm³) located directly below the orifice plate. The size of the liquid droplets was further reduced by solvent evaporation. Oppositely charged NPs (positive) and MWCNTs (negative) collided with each other via electric Coulombic attraction, coagulating into single NP/MWCNT nanostructures. The electrospray process was monitored using a light source (MLC-150; Motic Instruments, Richmond, BC, Canada), a microscopic charge-coupled device (CCD) digital camera (MARIN F-145C2; Allied Vision Technologies, Stadtroda, Germany), and a computer.

To fabricate the antimicrobial air filter, the synthesized NP/MWCNT hybrids were continuously deposited onto polyurethane resin fiber filters (Clean & Science Co., Ltd., Seoul, Republic of Korea) (medium grade; fiber diameter: 10–20 μm; thickness: 0.3 mm; packing density: 33%). Four sets of filters, including a pristine filter (control), a filter coated only with NP particles, one coated only with MWCNTs, and one coated with the hybrid NP/MWCNTs, were prepared and evaluated.

### 2.2. Measurements and sampling of hybrid particles

The size distribution of the nanoparticles was measured using a WPS (100XP, MSP Co., Shoreview, MN, USA) that is capable of measuring aerosol particles from 10 to 10,000 nm in diameter. The WPS system consisted of a differential mobility analyzer (DMA), condensation particle counter (CPC), and a laser light scattering spectrometer. The DMA-CPC system characterized the size distribution of the particles from 10- to 350-nm bases on the electrical mobility of the particles. The laser light scattering spectrometer also measured particle sizes (> 350 nm) based on the intensity of light scattered by the particles.

To assess the morphology of the particles, airborne NPs, MWCNTs, and NP/MWCNTs were deposited onto copper TEM grids (type A coated with a carbon film; Ted Pella Inc., Redding, CA, USA) using an electrostatic precipitating nanoparticle collector (model 4650; HCT, Icheon, Korea). The morphology and size of the nanoparticles were confirmed using an analytical TEM (CM30; Philips Electron Optics, Eindhoven, The Netherlands). An SEM (200 NANO SEM; FEI Co., Hillsboro, OR, USA) was used to examine the overall structure and morphology of the deposited nanoparticles on the filter surface.

### 2.3. Antimicrobial efficiency, filtration efficiency, pressure drop of hybrid nanoparticle-coated filters

The Gram-positive bacterium *Staphylococcus epidermidis* (KCTC 1917) was chosen for the antimicrobial air tests. *S. epidermidis* is common in indoor environments and generally nonpathogenic (Levinson, 2010). One bacterial colony was inoculated into nutrient broth media of 10 mL (Becton Dickinson, Franklin Lakes, NJ, USA) and cultured at 37 °C while shaking at 150 rpm. When the bacterial suspension reached an optical density of 0.7–0.8 at 600 nm, the bacteria were harvested by centrifugation (10,000 × g, 10 min) and washed three times with sterile water. To aerosolize the bacteria, a 30-mL aliquot was placed into a six-jet Collison nebulizer (BGI, Waltham, MA, USA). The bacterial density in the suspension was measured as ~ 10⁷ colony forming units (CFU)/mL using a traditional plate count method. As shown in Fig. 2a, the bacteria were nebulized at an airflow rate of 5 L/min. Dispersed bacterial aerosols were passed through a diffusion dryer to remove moisture and then introduced to a filter holder in which the fabricated antimicrobial filter was installed. The size distribution and concentration of the bacterial aerosol were measured using an aerodynamic particle sizer (APS 3321; TSI, Shoreview, MN, USA). The bacteria were allowed to reside on each filter for 5 min. The filter was then removed from the holder and soaked in a 50-mL conical tube containing 5 mL of phosphate-buffered saline (PBS, pH 7.4) with 0.01% Tween 80. Samples in the fluid were vortexed for 2 min and then sonicated in an ice bath for 10 min to ensure bacterial transmission from the filter to the PBS buffer. Finally, the suspension was serially diluted, and 100 μL of each resulting suspension were respectively plated onto nutrient agar (Becton Dickinson) and incubated at 37 °C for 24 h. Colonies that formed on the plate were counted after incubation.

The bacterial inactivation rate was calculated using the following equation:

\[
\text{Inactivation rate} (\%) = \left(1 - \frac{\text{CFU}_{\text{Antimicrobial}}}{\text{CFU}_{\text{Control}}}\right) \times 100
\]

where CFU\text{Antimicrobial} is the number of bacterial colonies (CFU) counted from the antimicrobial filter and CFU\text{Control} is the number of bacterial colonies counted from the pristine (control) filter.

To investigate the filtration efficiency of the antimicrobial filters, the total number concentration of bacterial aerosols was measured at the inlet and outlet of the filter using an aerodynamic particle sizer.

Filtration efficiency was calculated using the following equation:

\[
\text{Filtration efficiency} (\%) = \left(1 - \frac{\text{Concentration}_{\text{Outlet}}}{\text{Concentration}_{\text{Inlet}}}\right) \times 100
\]

where Concentration\text{Outlet} and Concentration\text{Inlet} are the total particle concentrations (particle/cm³) of bacterial aerosols measured at the filter inlet and outlet, respectively.

As shown in Fig. 2b, pressure drop across the test filters was measured using a micromanometer (FCO12; Furness Controls Ltd., Bexhill, UK).
3. Results and discussion

3.1. Fabrication of NP/MWCNT hybrids using THES

THES was used to prepare NP/MWCNT hybrid nanoparticles. The procedure consisted of two steps: electrospraying two kinds of droplets followed by controlled ethanol evaporation and coagulation of particles resulting from Coulombic collisions between oppositely charged NPs and MWCNTs. In the first step, the droplets containing dissolved NPs experienced a transient phase change from a liquid to a solid (Camelot et al., 1999; Morozov, 2011; Tan, Wang, & Zhang, 2007). Fig. 3 shows the WPS size distributions and TEM images of the NPs, MWCNTs, and NP/MWCNTs. A significant transport loss of particles (> 90%) was observed when generating pure NP nanoparticles or pure MWCNTs via single-nozzle electrospraying without charge neutralization. These losses were due to the high electrical charge per particle. This phenomenon was similar to that shown in the experimental results of Fu et al. (2012). To mitigate this effect, as shown in Fig. 3a and b, NP nanoparticles or pure MWCNTs were generated using simultaneous electrospray of pure ethanol, the opposite charge polarity of which served to neutralize unipolarly charged NP nanoparticles or MWCNTs. The neutralized NP nanoparticles had a positively skewed size distribution of 10–100 nm with a peak diameter of 11.5 nm, a geometric standard deviation (GSD) of 1.63, and a geometric mean diameter (GMD) of 18.5 nm (Fig. 3a, Table 1) (Chen, Pui, & Kaufman, 1995; Feng, Bogan, & Agnes, 2001; Gomez & Tang, 1994; Hogan, Kettleson, Ramaswami, Chen, & Biswas, 2006; Ku & Kim, 2002; Rosell-Llompart & Fernandez de la Mora, 1994; Tang & Smith, 2001). The MWCNTs had a normal distribution of 10–700 nm with a peak diameter of 39.2 nm, GSD of 1.99, and GMD of 44.6 nm (Fig. 3b, Table 1). Hence, the concentration of < 40 nm particles observed by the particle sizer in response to the MWCNT solution was not caused by MWCNTs but rather by small debris and impurities such as catalyst particles. The concentration of NP/MWCNT nanoparticles was lower than that of NP nanoparticles but higher than that of MWCNTs (Table 1). The NP/MWCNT particles had a bimodal size distribution with peak diameters of 21.1 and 49 nm, GSD of 1.96, and GMD of 40.7 nm (Fig. 3c, Table 1). We hypothesize that most of NP/MWCNT nanoparticles at sizes < 30 nm were actually NP particles that did not attach to MWCNTs. Thus, the observed shift in peak size from 39.2 nm (MWCNTs) to 49 nm (NP/MWCNTs) resulted from the combination of NP and MWCNT nanoparticles. TEM was used to confirm the morphology of the nanoparticles. The NP particles were mostly spherical and non-agglomerated due to repulsion forces produced by their unipolar charge, with sizes from ~83 to 183 nm in diameter (Fig. 3a). The MWCNTs were ~0.4–20 μm in length and ~66–73 nm in diameter (Fig. 3b). Fig. 3c shows NP particles attached to the surface of MWCNTs. Successful combination of MWCNTs and NPs was confirmed by TEM. An analysis of 18 images containing NP/MWCNT nanoparticles showed ~4–9 NPs attached to each CNT. Fig. 3c suggests that a small portion of NP nanoparticles was attached to the MWCNTs and that significant transport losses reduced the number of NP nanoparticles in the mixing chamber. As described above, these losses were due to a high electrical charge on NP nanoparticles formed without neutralization, i.e., solely generated NP nanoparticles or MWCNTs in Fig. 3a and b.

3.2. Application of NP/MWCNTs to antimicrobial filters

Fabricated NP, MWCNT, and NP/MWCNT nanoparticles were continuously deposited onto filters at a rate of 10 L/min. For each filter to have an identical volume of nanoparticles, the deposition time was adjusted depending on the number and volume of particles and their filtration efficiency. Since the NP, MWCNT, and NP/MWCNT nanoparticles had different sizes and densities, determining the amount of particles deposited by volume rather than by number or mass concentration was...
The particle deposition efficiencies of the control filter against NP, MWCNT, and NP/MWCNT nanoparticles were 92, 95, and 94%, respectively. Thus, the total particle volume per cross-sectional filter area was $1.1 \times 10^{-2}$ cm$^3$/cm$^2$. Fig. 4 shows the surfaces of pristine filters and antimicrobial filters onto which NP, MWCNT, and NP/MWCNT nanoparticles were neutralized.
MWCNT nanoparticles were deposited. The surface of the pristine filter (Fig. 4a, b) was smooth and clear, while the surfaces coated with NP (Fig. 4c, d) and MWCNT (Fig. 4e, f) nanoparticles were relatively uneven. Compared to the MWCNT coatings, the NP coatings were uniformly deposited onto the filter surface. In contrast, the NP/MWCNT-coated filters (Fig. 4g, h) had a more uneven surface.

Table 1

Concentration, GSD, GMD, and peak diameters of nanoparticles (n=3).

| Type of nanoparticles | Concentration (× 10^4 particle/cm³) | GSDᵃ | GMDᵇ (nm) | Peak diameter (nm) |
|-----------------------|-------------------------------------|------|------------|--------------------|
| Natural product (NP)  | 217 ± 278                           | 1.63 ± 0.11 | 18.5 ± 0.26 | 11.5 ± 2.13        |
| MWCNT                | 1.06 ± 0.04                         | 1.99 ± 0.01 | 44.6 ± 0.99 | 39.2 ± 2.26        |
| NP/MWCNT             | 1.74 ± 0.07                         | 1.96 ± 0.03 | 40.7 ± 1.08 | 21.1 ± 1.10        |

ᵃ GSD, geometric standard deviation.
ᵇ GMD, geometric mean diameter.
ᶜ The first peak diameter.
ᵈ The second peak diameter.

Fig. 4. Scanning electron micrographs of (A) the pristine (control) air filter and (B) NP nanoparticle-coated, (C) MWCNT-coated, and (D) NP/MWCNT-coated filters.

Fig. 5. Images and pressure drops of the test filters (n=3).
formed dendrites on the filter surface, which suggests that the unique shape of the NP/MWCNT particles contributes to the formation of dendrites on the filter surface and that the deposition structure increased the contact surface area between the antimicrobial substance and bacteria. There was no additional separation unit for NP and NP/MWCNT nanoparticles. Therefore, the coating on NP/MWCNT-coated filters also contained NP nanoparticles. However, the NP/NWCNT nanoparticles were expected to show a greater antimicrobial activity than that of either the pure NP nanoparticles or pure MWCNTs.

Prior to conducting this study, we collected filter samples with similar pressure drops at a face velocity of 8 cm/s to prevent false results from filter variations alone. As shown Fig. 5, the pressure drops of the antimicrobial filters were higher than those of the pristine filters (6.6 mmH₂O). The MWCNT- (11.1 mmH₂O) and NP/MWCNT-coated filters (11.6 mmH₂O) showed a significant increase in pressure drop compared with the NP-coated filters (7.1 mmH₂O). The surfaces of the NP- and MWCNT-coated filters were yellow and black, respectively. The NP/MWCNT-coated filter was gray due to the combination of NP nanoparticles (yellow) and MWCNTs (black).

The filtration efficiencies of pristine, NP, MWCNT-, and NP/MWCNT-coated filters were tested using *S. epidermidis* aerosols. Fig. 6a shows the normalized aerodynamic particle size distribution of tested bacterial aerosols generated by the nebulizer. The size distribution of the aerosols was a monomodal curve with a peak diameter of 0.898 μm, a GMD of 0.914 μm, and a GSD of 1.17. The concentration of *S. epidermidis* in the aerosols was $1.33 \times 10^3$ particles/cm$^3$. As shown in Fig. 6b, although the differences were minor, the filtration efficiencies of the NP (~98.8%), MWCNT (99.7%), and NP/MWCNT-deposited filters (99.8%) were statistically higher than that of the pristine filter (~98.3%) (SPSS t-test: $p < 0.05$). The NP/MWCNT-coated filters had the highest filtration efficiency.

To ensure that each filter had the same density of bacteria, the deposition time was controlled in accordance with the aerosol concentration and filtration efficiency. The total density of bacteria deposited on each filter was $\approx 2.35 \times 10^7$ particles/cm$^2_{\text{filter}}$. Fig. 7 shows that the NP/MWCNT filters had a higher antimicrobial activity than the NP and MWCNT filters. Under identical experimental conditions, the NP/MWCNT filters inactivated 95% of the test bacteria while the other filters inactivated < 70% of the bacteria (SPSS t-test: $p < 0.05$). These results are consistent with the results of previous studies showing that Ag/CNT nanoparticles or NYLON-6/Ag/CNT hybrid polymer nanocomposite fibers had significant antimicrobial activity against various bacterial species including *E. coli*, *Staphylococcus aureus*, and *S. epidermidis* (Jung et al., 2011a; Rangari et al., 2010). Such inhibition appears to be due to the high contact surface area between bacteria and antimicrobial substances. Factors that have been shown to influence the antimicrobial efficacy of such coated filters.
include the size, concentration, and shape of the coating materials (Ji et al., 2007; Kim et al., 2007; Pal et al., 2007). The NP nanoparticles used in this study were composed primarily of flavonoids. Flavonoids exhibit an antibacterial activity by reducing the fluidity of the inner and outer layers of bacterial membranes, thereby inhibiting DNA and RNA synthesis and deterring energy metabolism (Cushnie & Lamb, 2005). In addition, direct contact between CNTs and bacteria cells causes damage to the outer bacterial membrane and subsequent cell death. Short CNTs are more effective in damaging bacterial cells due to increased interactions with the cell membrane. The open ends of nanotubes can induce further bacterial cell damage (Kang et al., 2008). The primary reason for the enhanced antimicrobial activity of the NP/MWCNT filters was the increased contact surface area resulting from the high surface area of the NP particles and the presence of the MWCNTs. The net effect was the high antimicrobial efficacy of filters coated with hybrid NP/MWCNT nanoparticles.

4. Conclusions

NP/MWCNT hybrid nanostructures were produced using a THES technique. The particle size distribution and TEM and SEM analyses showed that NP particles were successfully combined with MWCNTs. The hybrid nanoparticles were continuously deposited onto filter surfaces using an aerosol process. The NP/MWCNT-coated filters exhibited higher antimicrobial activity than those coated only with NP particles or MWCNTs. The fabrication technique described is simple and the resulting hybrid particles have demonstrated a potential for applications in indoor air and/or water purification systems. However, the experimental results also showed that electrical charge optimization on the particles was necessary to increase the synthesis rate of the hybrid nanoparticles. Thus, future studies will include electrical charge optimizations for efficient hybrid nanoparticle synthesis.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2013K000386), the Railway Technology Research Project funded by the Ministry of Land, Infrastructure and Transport (14RTRP-B081249-01), Republic of Korea, and was partially supported by the KIST Institutional Program (2E25290)

References

Aymonier, C., Schlotterbeck, U., Antonietti, L., Zacharias, P., Thomann, R., Tiller, J.C., & Mecking, S. (2002). Hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibiting antimicrobial properties. Chemical Communications, 2002, 3018–2019.
Borra, J., Camelot, D., Chou, K.-L., Kooyman, P., Marijnissen, J., & Scarlett, B. (1999). Bipolar coagulation for powder production: micro-mixing inside droplets. Journal of Aerosol Science, 30, 945–958.
Camelot, D., Marijnissen, J., & Scarlett, B. (1999). Bipolar coagulation process for the production of powders. Industrial and Engineering Chemistry Research, 38, 631–638.
Carson, C., Hammer, K., & Riley, T. (2006). Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. Clinical Microbiology Reviews, 19, 50–62.
Chen, D.-R., Pui, D.Y., & Kaufman, S.L. (1995). Electrospraying of conducting liquids for monodisperse aerosol generation in the 4 nm to 1.8 μm diameter range. Journal of Aerosol Science, 26, 963–977.
Cushnie, T.P.T., & Lamb, A.J. (2005). Review: antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 26, 343–356.
Donaldson, J.R., Warner, S.L., Cates, R.G., & Gary Young, D. (2005). Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods. Pharmaceutical Biology, 43, 687–695.
Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N., & Kim, J.O. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of Biomedical Materials Research, 52, 662–668.
Feng, X., Bogan, M.J., & Agnes, G.R. (2001). Coulomb fission event resolved progeny droplet production from isolated evaporating methanol droplets. Analytical Chemistry, 73, 4499–4507.
Vallejos, S., Umek, P., Stoycheva, T., Annanouch, F., Llobet, E., Correig, X., De Marco, P., Bittencourt, C., & Blackman, C. (2012). Single-step deposition of Au- and Pt-nanoparticle-functionalized tungsten oxide nanoneedles synthesized via aerosol-assisted CVD, and used for fabrication of selective gas microsensor arrays. *Advanced Functional Materials*, 23, 1313–1322.

Weickmann, H., Tiller, J.C., Thomann, R., & Mülhaupt, R. (2005). Metallized organoclays as new intermediates for aqueous nanohybrid dispersions, nanohybrid catalysts and antimicrobial polymer hybrid nanocomposites. *Macromolecular Materials and Engineering*, 290, 875–883.

Yoon, K.Y., Byeon, J.H., Park, C.W., & Hwang, J. (2008). Antimicrobial effect of silver particles on bacterial contamination of activated carbon fibers. *Environmental Science and Technology*, 42, 1251–1255.

Young, H.E., Choi, H.J., & Baek, S.H. (2008). Antimicrobial effect of falavanones from Sphora flavescens Ait. *Yakhak Hoeji*, 52, 274–278.

Yun, S.H., Bae, G.-N., Lee, B.U., Ji, J.-H., & Kim, S.J. (2009). Evaluation of antifungal activities of nanoparticles against Cladosporium cladosporioides spore bioaerosols. *Journal of Korean Society for Atmospheric Environment*, 4, 255–263.

Yurteri, C., Hartman, R., & Marijnissen, J. (2010). Producing pharmaceutical particles via electrospaying with an emphasis on nano and nano structured particles—a review. *Kona Powder and Particle Journal*, 28, 91–115.