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Preparation and Antibacterial Properties of Nanocomposite Fibers Made of Polyamide 6 and Silver-doped Hydroxyapatite

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Abstarct: Nanocomposite fibers of polyamide 6 (PA6) and hydroxyapatite (HA) were prepared and doped with silver to investigate antibacterial activities due to good potential for textile modification. Nano-sized HA could be synthesized using agarose and ethanol as thickener and washing medium, respectively. The PA6/HA nanocomposite fibers could be doped with silver by dipping the fibers having HA in aqueous $AgNO_3$ solution containing 300 ppm of Ag ion for 1 min utilizing HA as a carrier to load silver through ion-exchange mechanism. It was found that silver was successfully doped to PA6/HA nanocomposite fibers from the EDS spectra. The nanocomposite fibers containing 3.3 wt% of HA after silver doping demonstrated such excellent antibacterial activities against *K. pneumonia* and *E. coli* that they are expected to serve as functional antibacterial materials in various application fields.

Keywords: Polyamide 6, Hydroxyapatite, Silver, Nanocomposites, Antibacterial properties

Introduction

Antibacterial properties are required to prevent three undesirable effects in textiles. The first includes the degradation phenomena like coloring, staining and deterioration of fibers [1]. Due to rapid improvement of hygienic living standard, the importance of controlling these phenomena aforementioned has been more emphasized. Therefore, many researches have focused on the antibacterial modification of textiles.

The interest in using bactericide, anti-viral and fungicide textiles to treat skin diseases has significantly increased during the last several years [2]. Since silver (Ag) has been known to be effective against bacteria and yet is known to be nontoxic to human cells, Ag has been most extensively studied and used to prepare antibacterial materials including textiles in the form of silver nano-particles or silver compounds [3-6]. Application of inorganic nano-particles as a carrier to load antibacterial silver nano-particles or ions and their nanocomposites would be a good passage open up a new opportunity for anti-microbial and multi-functional modification of textiles [7,8]. Because of its excellent biocompatibility and overall safety, and chemical structure similarity with the mineral found in hard tissues of the body, hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$), and their composites have attracted much attention as materials suitable not only for repairing and substituting for hard tissues, but for preparing materials as sorbents of metallic ions such as Ag, Cd^{2+} and Pb^{2+} [9-13].

As the use of silver-doped hydroxyapatite with antibacterial activity to polymers or fibers through nanocomposites is of good potential for textile modification, we prepared nano-composite fibers of polyamide 6 (PA6) and HA followed by doping with silver to investigate the morphology and antibacterial activities.

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Experimental

Materials

Polyamide 6 (D24, viscosity Index: 120 ml/g in 90 % formic acid) were provided by Rhodia Korea. Calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] and di-ammonium hydrogen phosphate [$(NH_4)_2HPO_4$] were obtained from Junsei and Yakuri, respectively. Reagent grade silver nitrate ($AgNO_3$) was purchased from Daejung. Thickners, agarose and poly(vinyl pyrrolidone) (PVP) were purchased from Wako and Daejung, respectively. Bacteria of *Staphylococcus aureus* (KCTC 1726), *Klebsiella pneumonia* (KCTC 1621) and *Escherichia coli* (ATCC 25922) were supplied by KCTC (Korea Collection for Type Culture).

Synthesis of Nano-sized HA

Nano-sized HA was synthesized as depicted in Figure 1. Calcium nitrate and di-ammonium hydrogen phosphate, ammonia solution, calcium hydroxide and agarose or poly(vinyl pyrrolidone) (PVP) were the reagents used for the synthesis according to the earlier report [14]. Diammonium hydrogen phosphate solution of 0. 5 M was prepared in 0.5 wt% agarose or PVP gel solution (w/v). The pH of the solution was adjusted to 10.5 by adding ammonia solution. 0.5 M calcium nitrate solution of pH 10.5 controlled by adding $Ca(OH)_2$ solution was added to the mixture dropwise. The mixture was continuously stirred at a pH of 10 and temperature 85 °C, followed by cooling and incubating at 40 °C for 24 h. After washing the resulting suspension in distilled water and filtering, it was washed again in water or ethanol. It was finally freeze dried.

Preparation of PA6/HA Nanocomposite Fibers

Before melt-compounding of PA6 and HA, they were all dried under vacuum at 70 °C for 24 hr. The powdery HA (3-9 wt%) and PA6 chips were premixed using a programmable

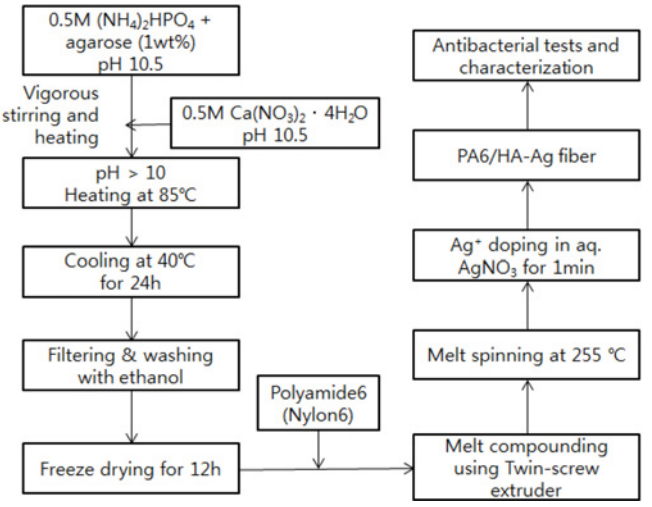

Figure 1. A schematic diagram for the preparation and test procedures of the study.

ball mill (Daihan Scientific Co., Korea) for 3 h in order to make HA powder adhered homogeneously on the surface of the polymer chips. The premixes were then melt-compounded using a co-rotating twin-screw extruder (BK-11, Bowtek Co., Korea). The screw length and diameter of the extruder were 440 and 11 mm, respectively. The temperature profile for the extruder was controlled in the range 190-240 °C. The extruded strands were cooled in a water bath, chopped into pellets, and dried under vacuum at 80 °C for 24 h. The pellets were melt-spun at 255 °C using a piston-type spinning machine equipped with a nozzle of 0.5 mm diamete.

Doping of Ag to PA6/HA Nanocomposite Fibers

Doping of silver to PA6/HA nanocomposite fibers was carried out as follows: The fibers were immersed in aqueous $AgNO_3$ solution containing 300 ppm of Ag ion for 1 min under stirring with a magnetic bar at room temperature. The resulting PA6/HA-Ag nanocomposite fibers were followed by washing in distilled water several times and collected for the further examination.

Tests of Antibacterial Activities

Antibacterial activities of PA6/HA-Ag nanocomposite fibers were investigated by shaking flask assay method (KS J4206:2008) with *S. aureus* (ATCC 6538) as the model Gram-positive bacteria, and *K. pneumonia* (ATCC 4352) and *E. coli* (ATCC 25922) as the model Gram-negative bacteria. A portion of the initially cultured bacteria were transferred into a nutrient broth (NB) medium to reach the concentration of bacteria to 10^6 colony forming units per milliliter (CFU/m*l*) and 1 m*l* of the bacterial solution were put into the test tube containing each samples. After incubation using shaking incubator (2 h, 37 °C, 200 rpm), 100 micro-liter of each bacterial suspension was spread on a nutrient agar plate and incubated for 12 h for counting the survival bacterial colonies. The antimicrobial activity was calculated using the following equation:

$$\text{Antibacterial activity (\%)} = (M_b - M_c)/M_c \times 100$$

Where, M_b and M_c are the average CFU of the control and the real samples, respectively.

Characterizations

The XRD pattern of the hydroxyapatite was obtained using a diffractometer (X-MAX/2000-PC, Rigaku) with Cu Ka radiation (λ=0.154 nm) at scan rate of 5 °/min. The DSC analysis of the material was carried out using a Perkin Elmer Diamond Series DSC at a heating or cooling rate of 20 °C/min. The morphology of the hydroxyapatite and its composites was observed using a Field Emission Scanning Electron Microscope (FE-SEM, JSM-6500F, JEOL) instrument. The samples were coated by gold before the observation. Identification of the hydroxyapatite and silver in the fibers was performed using an Energy Dispersive X-ray Spectrometer (EDS) which is attached to FE-SEM.

Results and Discussion

Preparation of HA Nanoparticle

It was reported that HA is formed from calcium nitrate and di-ammonium hydrogen phosphate through the reactions (1) and (2) as follows [15]:

$$Ca^{2+} + HPO_4^{2-} \rightarrow CaHPO_4 \quad (1)$$

$$10\ CaHPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 4H_3PO_4 \quad (2)$$

One of the main factors in the preparation of inorganic-organic composite is to suppress aggregation between inorganic particles and to disperse them uniformly in the organic matrix because the amount of inorganic particles and dispersity deeply affect the mechanical properties of the composites [16]. There is a so strong coagulation tendency of HA particles due to hydrogen bond and so it is difficult to separate HA particles once aggregated. In this study, for the purpose of preventing the aggregation of HA particles, we investigated the effect of thickener in aqueous medium during synthesis and washing medium of synthesized HA before freeze drying. Water soluble polymers, agarose and PVP were chosen as thickener and distilled water and ethanol as washing medium, respectively.

SEM micrographs in Figure 2 shows particle state of HA depending on the thickener during synthesis and washing medium after synthesis. It was found that agarose and ethanol were more effective for the better degree of individual particle separation than PVP or distilled water when they were freeze dried under same condition. Moreover, the apparent bulk density by using agarose and ethanol was much lower than the other cases, which also implies the

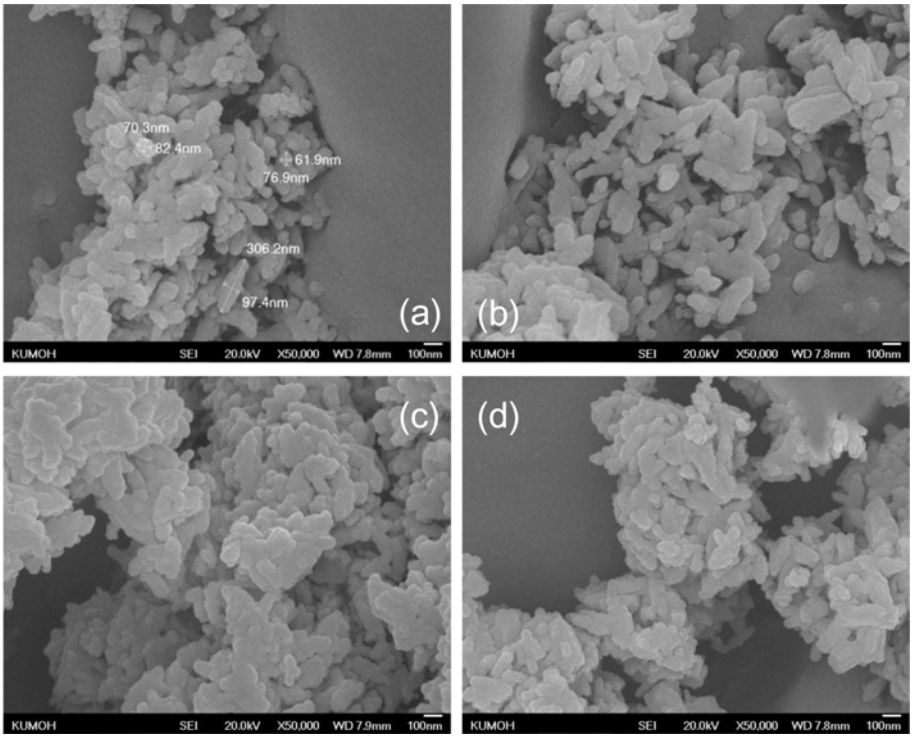

Figure 2. FE-SEM images of the hydroxyapatite nano-particles according to the thickener during synthesis and washing medium after synthesis; (a) agarose and distill water, (b) agarose and ethanol, (c) PVP and distill water, and (d) PVP and ethanol.

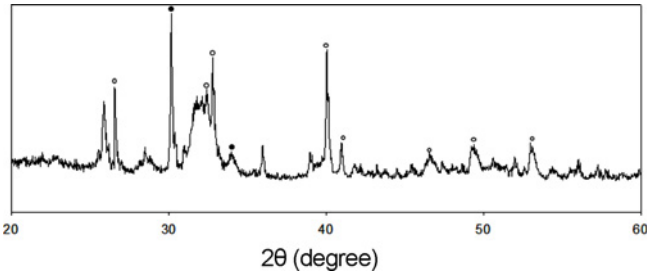

Figure 3. XRD patterns of the powder synthesized. The circle marks are the characteristic peaks of the hydroxyapatite reported in a reference [14].

same result as SEM images. This individually separated state is preferable to better dispersion in nanocomposites. Thus, all the HA used in the further experiments was prepared by using agarose and ethanol. The individual HA particles had a uniform elliptical shape, and their lengths and aspect ratios were approximately in the range of 150-200 nm and 1-2, respectively.

The XRD pattern of the as-prepared hydroxyapatite powder is presented in Figure 3. Compared with the pattern reported in a reference [14], the main characteristic peaks obtained in the synthesized powder is well matched to those of the HA from the reference. Therefore, it can be confirmed that the powder synthesized in the study is identified as HA.

Table 1. Actual hydroxyapatite (HA) content immobilized on the surface of PA6 chips after ball milling

Nanocomposite code	HA content (wt%)	
	Feed in ball mill	Actual content
PA6 100 %	-	-
PA6/HA 1.1 %	3	1.1 %
PA6/HA 1.6 %	6	1.6 %
PA6/HA 3.3 %	9	3.3 %

Preparation and Properties of PA6/HA Nanocomposites and Fibers Thereof

As PA6 was pellet form and HA fine powder form, it was needed to immobilize HA to the surface of PA6 chips in order to maintain uniform ratio during feeding to the extruder for melt compounding. Thus, the two materials were premixed in a ball mill for 24 h before feeding. As shown in Table 1, a part of HA was stick to PA6 chips after ball milling. The excessive HA was removed before feeding. The actual amount of HA in the premix was 1.1, 1.6 and 3.3 wt%, respectively.

Figure 4 shows FE-SEM images of the cross sections of neat PA6 and PA6/HA nanocomposites obtained through melt compounding. It can be clearly seen that HA was dispersed mostly individually in PA6 matrix. The fibers of PA6 and nanocomposites were prepared by melt spinning. As shown in Figure 5, the fibers became a little bit dull and

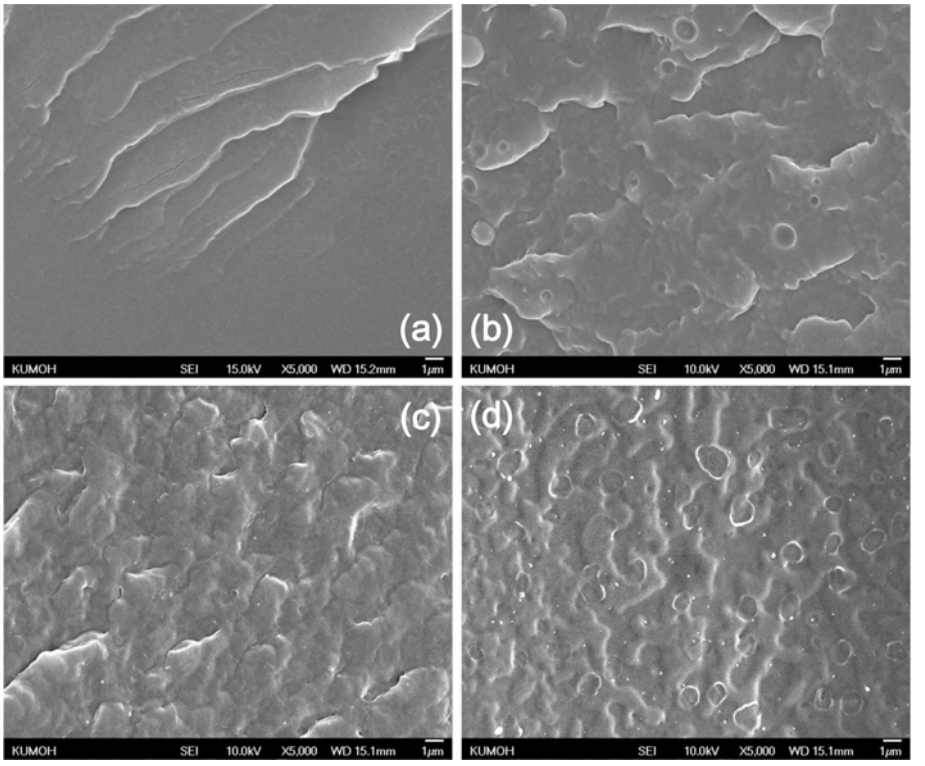

Figure 4. FE-SEM cross-sectional images of (a) PA6-100 %, (b) PA6/HA 1.1 %, (c) PA6/HA 1.6 %, and (d) PA6/HA 3.3 %.

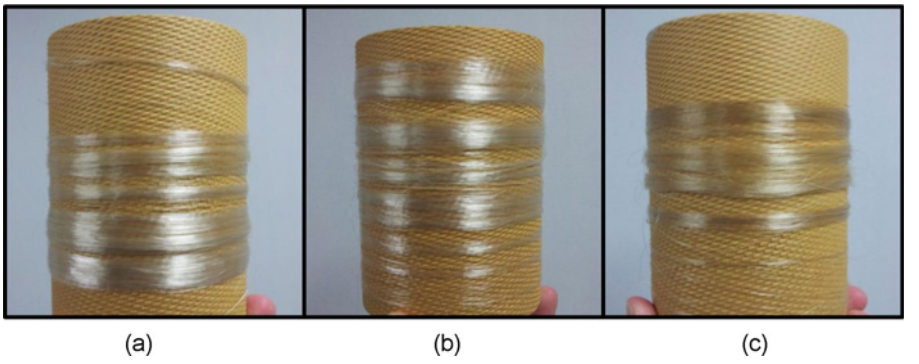

Figure 5. As-spun PA6 and the nanocomposite fibers; (a) PA6 100 %, (b) PA6/HA 1.1 %, and (c) PA6/HA 3.3 %.

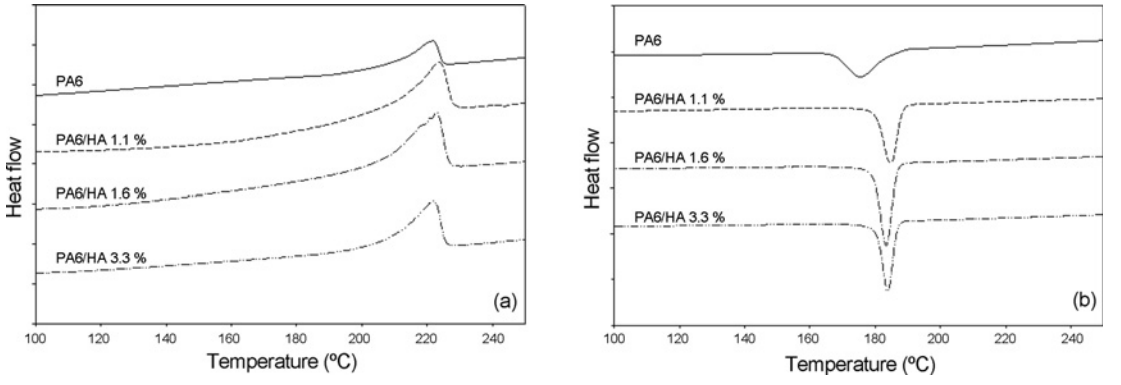

Figure 6. DSC thermograms showing melting (a) and crystallization (b) of PA6 and nanocomposite fibers.

grayish with increasing of HA content.

The effect of HA on the thermal properties of PA6 fiber was investigated using DSC. There was little effect of HA on the melting behavior of PA6 fibers as seen in Figure 6(a). On the other hand, crystallization temperature from melt was increased with existence of HA due to its role of nucleating agent in PA6 crystallization, as shown in Figure 6(b). Higher amount of HA than 1.1 wt%, however, was little further effect in increasing crystallization rate.

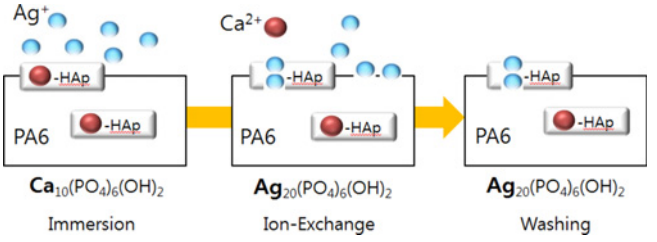

Figure 7. Schematic diagram showing silver doping to PA6/HA nanocomposite fibers through ion-exchange reaction between calcium ion and silver ion.

Evaluation of Antibacterial Activities of PA6/Ag-HA Nanocomposite Fibers

Fundamentally, HA has the very marked ability to adsorb easily various metal ions, organic molecules [17]. The mechanisms of the absorbance are suggested to be ion-exchange and complex formation. Ca^{2+} can be substituted by monovalent ions as well as divalent and trivalent cations [9, 18]. Among them, silver-doped hydroxyapatite demonstrating antimicrobial activity has been of great interest in view of its potential biomedical applications. Figure 7 shows a schematic diagram of silver doping to PA6/HA nanocomposite fibers by ion-exchange reaction between calcium ion of HA and silver ion in solution.

By using EDS, titanium and silver can be identified. Figure 8 represents FE-SEM images (left) and EDS (right) spectra thereof of PA6 (a) and PA6/HA 3.3 composites fibers before (b) and after (c) silver doping. From the EDS spectra, it can be concluded that silver was successfully doped to HA of nanocomposite fibers.

The mechanism of the antibacterial effect of silver-doped nanoparticles is not yet clearly elucidated [19]. A possibility is the gradual release of Ag^{+} ions from Ag-nanoparticles,

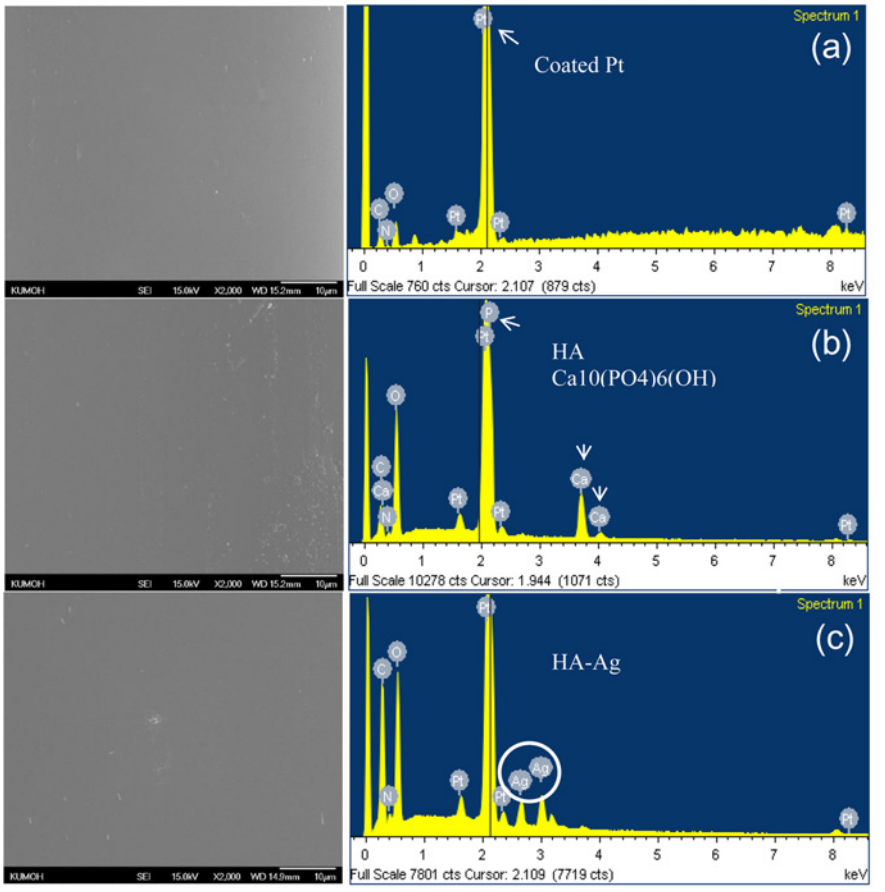

Figure 8. FE-SEM (left) images and EDS (right) spectra thereof of PA6 (a) and PA6/HA 3.3 composites fibers before (b) and after (c) silver doping.

Table 2. Changes of antimicrobial activity of PA/HA-Ag nanocomposite fibers according to HA content

Bacteria	HA content (wt%)	CFU	Antibacterial activity (%)
K. pneumoniae	0	1,210,000	-
	1.1	93,200	92.3
	1.6	10,700	99.1
	3.3	0	100.0
S. aureus	0	110,000	-
	1.1	9,120	91.7
	1.6	8,600	92.2
	3.3	8,000	92.7
E. coli	0	2,411,000	-
	1.1	149,400	93.8
	1.6	87,200	96.4
	3.3	0	100.0

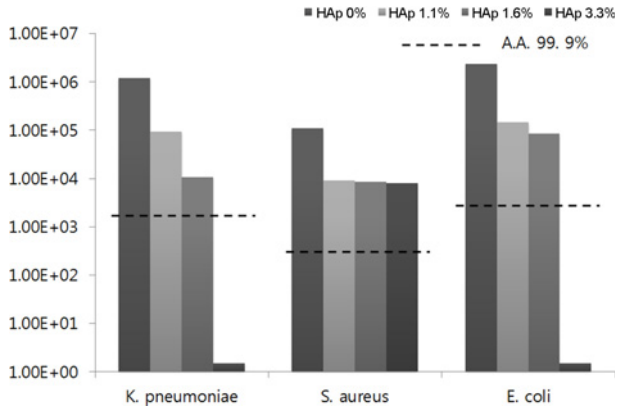

Figure 9. Antibacterial activities of silver-doped PA6/HA fibers against *K. pneumonia*, *S. aureus*, and *E. coli*.

followed by their disruption on ATP production and DNA replication. The antibacterial activity of silver-doped PA6/HA fibers against *K. pneumonia, S. aureus* and *E. coli* was examined by colony counting method. As summarized and shown in Table 2 and Figure 9, the numbers of *K. pneumonia* and *E. coli* were significantly reduced by the silver-doped nanocomposite fibers. Particularly, the silver-doped fiber having 3.3 wt% of HA demonstrated almost perfect killing of *K. pneumonia* and *E. coli* bacteria. On the other hand, the bacteriostatic effect against *S. aureus* was relatively less notable than that against the other bacteria.

Conclusion

In summary, we have successfully synthesized nano-sized HA using agarose and ethanol as thickener and washing medium, respectively. Nanocomposite fibers of PA6 and the nano-sized HA were prepared through melt compounding followed by melt spinning. It was found that the nanocomposite fibers could be successfully doped with silver by dipping in aqueous $AgNO_3$ solution containing 300 ppm of Ag ion for 1 min due to the carrier effect of HA in the fibers. From the EDS spectra, it could be concluded that silver was successfully doped to HA of nanocomposite fibers. The nanocomposite fibers containing 3.3 wt% of HA after silver doping demonstrated such excellent antibacterial activities against *K. pneumonia* and *E. coli* that they are expected to serve as functional antibacterial materials in various application field.

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