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A facile method for electrospinning of Ag nanoparticles/poly (vinyl alcohol)/carboxymethyl-chitosan nanofibers

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ABSTRACT

A facile method to prepare silver nanoparticles (AgNPs) containing nanofibers via electrospinning has been demonstrated. AgNPs were *in situ* synthesized in poly (vinyl alcohol) (PVA)/carboxymethyl-chitosan (CM-chitosan) blend aqueous solution before electrospinning. UV–vis spectra, viscosity and conductivity of the electrospinning solution were measured to investigate their effects on the electrospinning procedure. The morphology of AgNPs/PVA/CM-chitosan nanofibers was observed by Field Emission Scanning Electron Microscopy. The formation and morphology of AgNPs were investigated by Transmission Electron Microscopy and X-ray Photoelectron Spectroscopy. The resulted nanofibers have smooth surface and uniform diameters ranging from 295 to 343 nm. The diameters of AgNPs mainly distributed in the range of 4–14 nm, and the electrostatic interaction between AgNPs and fibers was observed. Finally, *in vitro* Ag release from the nanofibers was measured and the antibacterial behavior of the nanofibers against *Escherichia coli* was studied by bacterial growth inhibition halos and bactericidal kinetic testing. The AgNPs/PVA/CM-chitosan nanofibers possessed certain antibacterial ability, which makes them capable for antibacterial biomaterials.

1. Introduction

Ag nanoparticles (AgNPs), which is well known as a broad-spectrum antibiotic with strong antibacterial properties, have attached intensive research interest in recent years [1]. Relevant research indicated that free radicals are derived from the surface of AgNPs, which could damage the membrane structure of bacteria [2]. Furthermore, AgNPs do not adversely affect viable cells and do not easily provoke microbial resistance [3]. Therefore, they have been widely used as additives of biomedical products, such as beads, gels, film and fibers, to enhance the antibacterial ability of the material [4–8]. Electrospinning is a simple approach to prepare polymer nanofibers with high porosity and large specific surface area [9]. The incorporation of AgNPs into electrospun nanofibers is well adopted to prepare antibacterial materials.

Poly (vinyl alcohol) (PVA) and carboxymethyl-chitosan (CM-chitosan) are proposed to be promising carriers of AgNPs for biomedical applications. PVA is widely used in biomedical field due to its excellent water-solubility, high biocompatibility and hydrophilicity, as well as sound mechanical properties [10]. As one of the most abundant natural polysaccharide, chitosan and its derivatives also have good biocompatibility and biodegradability [11]. CM-chitosan is a water-soluble chitosan derivative which has better biocompatibility than chitosan [12,13]. Its water-solubility could prevent the presence of organic solvents or acids residues, such as dimethylformamide or dichloromethane, in final products to avoid cytotoxicity. Moreover, CM-chitosan possesses antibacterial properties with board-spectra of activity and low toxicity toward mammalian cells [14].

The PVA and chitosan electrospinning nanofibers containing AgNPs, such as AgNPs/PVA [15,16], AgNPs/PVA/Chitosan [17,18], AgNPs/Chitosan/gelatin [19], AgNPs/Chitosan/poly (ethylene oxide) (PEO) [20] and AgNPs/N-carboxyethylchitosan/PEO [21] have been reported. The studies on the incorporation of AgNPs into electrospun nanofibers have been conducted by adding Ag nitrate ($AgNO_3$) into electrospun solution. Generally, there are two

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methods to reduce Ag^+ to AgNPs in electrospinning field. One is treating polymer solution containing Ag^+ by heating [8], microwave irradiation [22] or chemical reduction [23] before electrospinning. The other is reducing Ag^+ in electrospun nanofibers by heating [18], UV irradiation [15], microwave irradiation [24] or hydrogen reduction [25]. Considering the addition process and additive, it is desirable to develop a facile method for synthesizing AgNPs in electrospinning field.

This manuscript described a facile method for electrospinning of nanofibers containing AgNPs. The AgNPs were easily formed in PVA/CM-chitosan blend aqueous solution before electrospinning. Penchev et al. synthesized AgNPs *in situ* in the PEO/N-carboxyethylchitosan electrospinning solution using HCOOH as a solvent. HCOOH could reduce Ag^+ to AgNPs in solutions [26]. In this work, CM-chitosan and PVA were served as reducing agent and stabilizer, respectively [27,28]. Further investigations such as the characterization of AgNPs, *in vitro* Ag ion release and antibacterial assessment were performed. The resulted AgNPs/PVA/CM-chitosan nanofibers showed sound antibacterial activity against bacterial. Therefore, it is expected to have potential for the application of biomaterials such as tissue engineering scaffold and wound dressing.

2. Experiment

2.1. Materials

PVA (M_W 88,000, degree of deacetylation 88%) was obtained from Acros Organics, Belgium. CM-chitosan (M_W 19,000, degree of substitution 0.64) was purchased from Zhejiang TengWang Chitosan Co., Ltd., China. Glutaraldehyde (50%) and $AgNO_3$ was purchased from Beijing Chemical Reagents Company. Other reagents were of analytical grade.

2.2. Preparation and characterization of electrospinning solutions containing $AgNO_3$

PVA and CM-chitosan powders were mixed in a weight ratio of 80/20. The mixed polymer powders were dissolved in aqueous $AgNO_3$ solution (5 or 10 mmol L^{-1}) with polymer concentration of 8 wt%. All the solutions were stirred for 12 h in room temperature to obtain a homogenous mixture.

The UV–vis spectra of PVA/CM-chitosan/$AgNO_3$ solutions were recorded by a Hitachi Model 3010 spectrophotometer. The scanning wave was in the range of 200–600 nm with an interval of 0.5 nm.

The conductivity of the electrospinning solutions was determined by an electric conductivity meter (DDS-307, Shanghai Precision & Scientific Instrument Co., Ltd., China). The measurement was performed at 25 °C.

The viscosity of the electrospinning solutions was measured by a rotational viscometer (Dial Reading Viscometer, Brookfield Engineering Laboratories, USA). The measurement was performed at 25 °C.

2.3. Preparation and characterization of AgNPs/PVA/CM-chitosan nanofibers

2.3.1. Electrospinning

The mixture solution was placed into a 5 mL syringe with a blunt ended metallic needle whose inner diameter is 0.5 mm. The rate of spinning was 1 mL h^{-1}. Electrospinning was performed at room temperature with a voltage of 15 kV (Model SL 60, 0–50 kV, Spellman High Voltage Electronics Corporation, USA). The electrospun nanofibers were collected on aluminum foil at a distance of 10 cm from the syringe needle.

2.3.2. Morphology of AgNPs/PVA/CM-chitosan nanofibers

The morphology of the fibers was observed by Field Emission Scanning Electron Microscopy (FESEM) using a HITACHI S-4800 (Japan). The samples were sputter coated with gold before observation. The average diameter of nanofibers was determined by measuring the diameters of 50 random selected fibers.

2.3.3. Characterization of AgNPs in nanofibers

The distribution and morphology of AgNPs in the fibers were observed using Tecnai F30 Transmission Electron Microscopy (TEM). The X-ray Photoelectron Spectroscopy (XPS) spectra were recorded on a Kratos Axis Ultra X-ray Photoelectron Spectroscopy using a monochromated Al Kα X-ray source. The average diameter of AgNPs was determined by measuring the diameters of 100 random selected AgNPs.

2.4. Crosslinking and in vitro Ag release

2.4.1. Crosslinking

The fibers were crosslinked in glutaraldehyde vapor at 80 °C for 12 h, and heated at 120 °C for another 12 h to remove residual glutaraldehyde.

2.4.2. In vitro Ag release

The AgNPs/PVA/CM-chitosan nanofibers (approximately 10 mg) were immersed in 50 mL phosphate buffer solution (PBS) after crosslinking. The samples were incubated at 37 °C and stirred at 90 rpm. After digested by HNO_3, the Ag concentration of the solution was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (PROFILE SPEC, Leeman, USA).

2.5. Antibacterial assessment

2.5.1. Bacterial incubations

The antibacterial activities of AgNPs/PVA/CM-chitosan nanofibers were examined by using *E. coli* (ATCC 25,922), which was cultivated at 37 °C in lysogeny broth (LB) medium (containing 10 g L^{-1} peptone, 5 g L^{-1} yeast extract, and 10 g L^{-1} NaCl) under oscillation at 150 rpm for 16 h. The bacterial suspension was diluted by LB medium into certain concentration. The solid medium was solidified by 15 g L^{-1} agar.

2.5.2. Bacterial growth inhibition halos

For antibacterial assessment, 50 μL of bacterial solution with about 10^6 CFU mL^{-1} of *E. coli* was dispensed onto an agar plate, and then crosslinked AgNPs/PVA/CM-chitosan sample (1.5 cm × 1.5 cm) was covered on the surface of the plate. After incubation for 12 h at 37 °C, the bacterial growth inhibition halos were observed. PVA/CM-chitosan fibers were used as controls.

2.5.3. Antibacterial kinetic testing

Approximately 10 mg crosslinked AgNPs/PVA/CM-chitosan and PVA/CM-chitosan nanofibers were added into 50 mL bacteria nutrient solution with a bacterial concentration of ca. 10^4 CFU mL^{-1}. Bacteria nutrient solution with the same concentration was incubated at the same condition and used as control. The optical density (OD) of bacterial broth medium at 600 nm was measured by a UV–vis spectrophotometer. The inhibition ratios of the fibers containing AgNPs were calculated by Eq. (1):

$$\text{Inhibition ratio}\,(\%) = 100 - 100 \times \frac{A_t - A_0}{A_{con} - A_0} \quad (1)$$

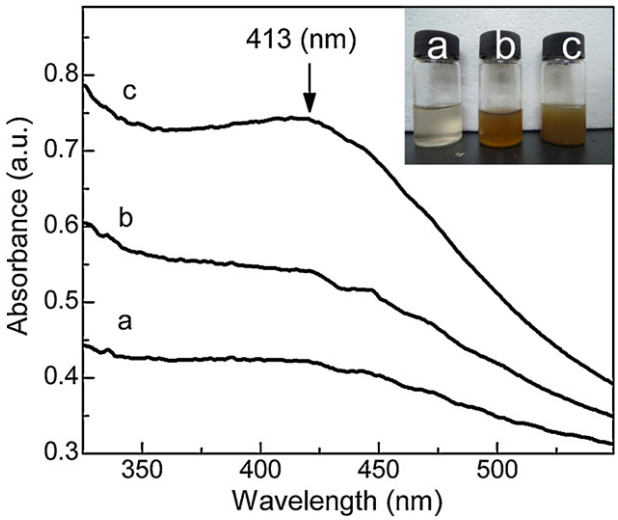

Fig. 1. UV–vis spectra of PVA/CM-chitosan aqueous solutions containing (a) 0 mmol L^{-1}, (b) 5 mmol L^{-1} and (c) 10 mmol L^{-1} of $AgNO_3$. The inset shows images of PVA/CM-chitosan solution with different concentrations of $AgNO_3$.

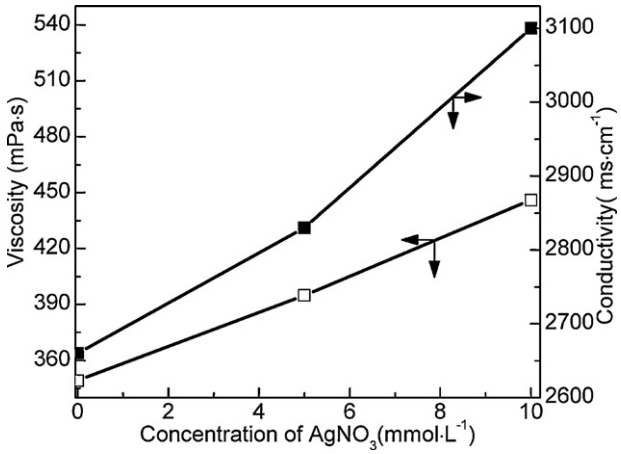

Fig. 2. Viscosity and conductivity of the PVA/CM-chitosan aqueous solutions containing different content of $AgNO_3$.

where A_0 was the OD of bacterial broth medium before incubation; A_t and A_{con} were the ODs of bacterial solutions after incubation of fibers and control sample for desired time respectively.

3. Results and discussion

3.1. Electrospinning of PVA/CM-chitosan solution containing $AgNO_3$

The formation of AgNPs in electrospinning solution can be detected by UV–vis spectra. The UV–vis spectra and images of PVA/CM-chitosan solution with different concentrations of $AgNO_3$ (0, 5 and 10 mmol L^{-1}) were shown in Fig. 1. The solution without Ag^+ was colorless. With the increase of Ag^+, the color of solution became brown. The UV–vis spectra exhibited an absorption band at around 410 nm, which is a typical surface plasmon absorption of AgNPs [29].

CM-chitosan, a typical water-soluble polyelectrolyte, draws persistent attention in metal nanoparticles synthesis due to its interaction with metal nanoparticles [27,30]. The CM-chitosan owns plenty of chelating groups such as amine, carboxylmethyl and hydroxyl groups, which could evenly disperse nanoparticles via chelation [30,31]. Furthermore, PVA was always used as stabilizer of the Ag nanoparticles due to its long pair electrons on the hydroxyl oxygen. It indicated that certain amount of AgNPs was formed during the preparation of PVA/CM-chitosan/$AgNO_3$ electrospinning solutions. Higher concentration of $AgNO_3$ (20 mmol L^{-1}) was attempted in this condition. However, the formation of dark precipitate could be observed in solution due to the coalescence of AgNPs.

Viscosity and conductivity are significant parameters during electrospinning process [9]. Fig. 2 shows the effect of $AgNO_3$ concentration on the viscosity and conductivity of the electrospinning solutions. It is obvious that the viscosity and conductivity increased with the increase of $AgNO_3$. The increase of Ag^+ concentration evidently induced the increase of conductivity. In addition, the CM-chitosan chain entanglement caused by the chelation between CM-chitosan and Ag^+/AgNPs may induce the increase of solution viscosity.

Fig. 3 shows SEM images of fibers electrospun from PVA/CM-chitosan solutions containing different content of $AgNO_3$ (0, 5 and 10 mmol L^{-1}). Similar morphologies and homogeneous appearance were observed. The average diameters of AgNPs/PVA/CM-chitosan nanofibers electrospun with 0, 5 and 10 mmol L^{-1} $AgNO_3$ were 393 ± 21, 343 ± 20 and 294 ± 23 nm respectively. It is well known that the morphology and diameter of fibers were affected by the viscosity and conductivity of mixture solution. High viscosity polymer solutions could prevent the fracturing of ejected jets during electrospinning process due to its longer stress relaxation time, which would increase the diameter of electrospun nanofibers [32]. However, high charge density forms on the surface of the ejected jet during electrospinning with the high conductivity solution. Then with the aid of high charge density, the high elongation forces are imposed to the jet, resulting in the decrease of the diameters of electrospun nanofibers. Under the synergy effects of viscosity and conductivity, the diameters of AgNPs/PVA/CM-chitosan nanofibers slightly decreased with the increasing concentration of $AgNO_3$.

Fig. 4 shows TEM micrographs of the AgNPs/PVA/CM-chitosan nanofibers with different content of Ag. The diameter distribution of AgNPs was also shown in Fig. 4. AgNPs can be observed on the surface of the fibers with round shape, which mainly distributed from 4 to 14 nm in both 5 and 10 mmol L^{-1} $AgNO_3$ fibers. Higher density of AgNPs could be observed in samples prepared with higher concentration of $AgNO_3$ (10 mmol L^{-1}). It is noticeable that some large particles (diameter around 23 nm) could also be observed on the surface of nanofibers electrospun from 10 mmol L^{-1} $AgNO_3$ solution, which is likely due to the increased incidence of coalescence of Ag atoms with high Ag concentration [33].

3.2. XPS analysis

The XPS spectra of Ag 3d and O 1 s for AgNPs/PVA/CM-chitosan nanofibers were shown in Fig. 5. The binding energies of Ag $3d_{5/2}$ and Ag $3d_{3/2}$ appeared at 367.8 eV and 374.0 eV, respectively (Fig. 5a1), which are between the binding energies of silver metal (368.2 eV for Ag $3d_{5/2}$ and 374.2 eV for Ag $3d_{3/2}$) and that of silver (I) oxide (367.5 eV for Ag $3d_{3/2}$ and 373.5 eV for Ag $3d_{3/2}$) [34]. The interaction between oxygen and silver particles may induce positive charges present on the surface of the silver colloids, which would cause a lower shift in Ag binding energies [29]. Therefore, the formation of AgNPs caused by the reduction of Ag^+ to Ag^0 during the electrospinning procedure was confirmed. Besides, the spectra of Ag 3d for AgNPs on crosslinked nanofibers indicated that the AgNPs were stable during the crosslinking process (Fig. 5a2).

The O 1 s photoemission spectrum of fibers containing AgNPs can be resolved into two peaks (Fig. 5b). One is at 532.2 eV (Fig. 5b1), which is assigned to the bond of C—O and C=O in PVA/CM-chitosan; the other is 532.6 eV (Fig. 5b2), which is attributed to the interaction between oxygen and silver particle [35]. Hence, the

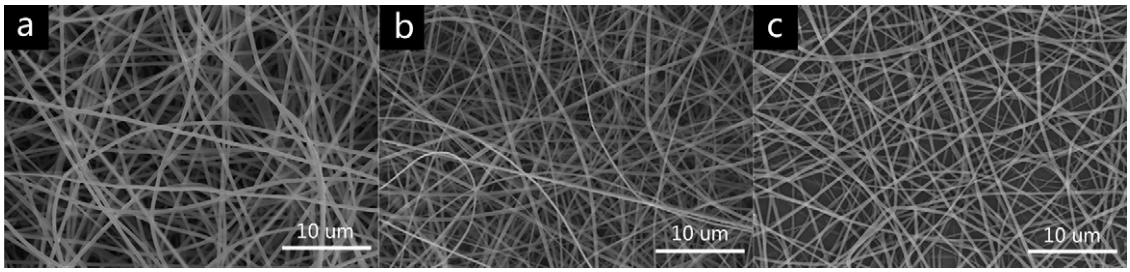

Fig. 3. FESEM micrograph of fibers electrospun from PVA/CM-chitosan aqueous solutions containing different content of $AgNO_3$: (a) 0 mmol L^{-1}, (b) 5 mmol L^{-1} and (c) 10 mmol L^{-1}.

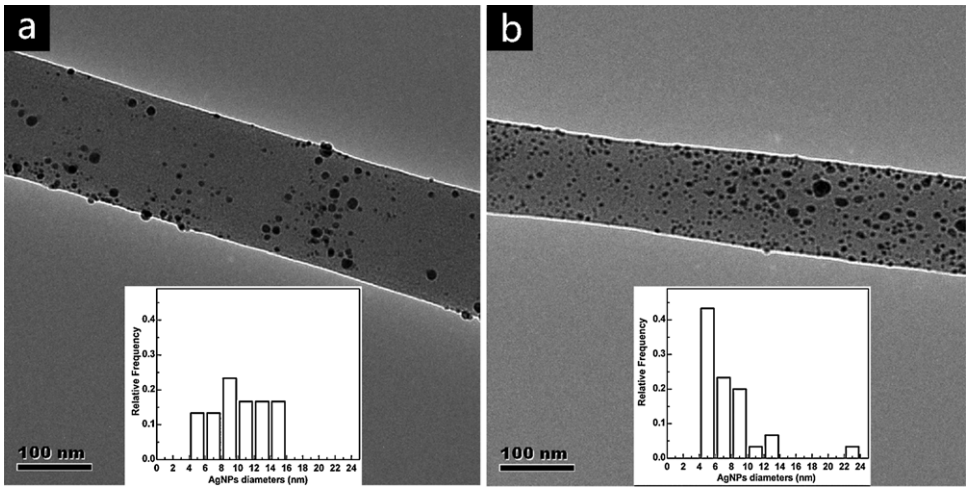

Fig. 4. TEM micrographs of the AgNPs/PVA/CM-chitosan nanofibers and size distribution of AgNPs prepared from PVA/CM-chitosan aqueous solutions containing different content of $AgNO_3$: (a) 5 mmol L^{-1} and (b) 10 mmol L^{-1}.

interaction between oxygen and silver particle was further demonstrated.

3.3. *In vitro* Ag ion release of AgNPs/PVA/CMCTs nanofibers

In vitro Ag released amount of crosslinked AgNPs/PVA/CM-chitosan nanofibers as a function of time were shown in Fig. 6. The releases of Ag in both samples were fast at the 3rd hour and became relatively slow after that. Finally the concentrations of released Ag^+ reached equilibrium after 24 h in both samples. The accumulative Ag release amounts of nanofibers electrospun from 5 mmol L^{-1} to 10 mmol L^{-1} $AgNO_3$ group were similar.

Despite of the excellent antibacterial ability, the cytotoxicity of AgNPs has aroused the public attention. Therefore, the balance between antimicrobial activity and cytotoxicity should be considered in the design and application of AgNPs containing biomaterials [36]. It is reported that Ag^+ can inhibit the growth of bacteria when their concentration is above 0.1 ppb (part per billion) [37]. On the other hand, it is reported that the viability of HaCaT keratinocytes contacted with wound dressing containing AgNPs has exceeded 90% when release Ag ion concentration was

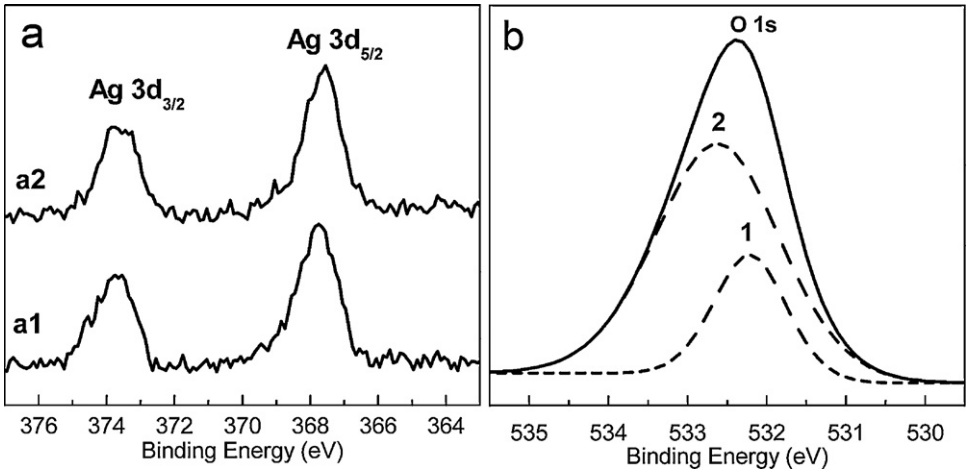

Fig. 5. XPS spectra of Ag 3d (a) and O 1 s (b) for AgNPs/PVA/CM-chitosan nanofibers with 10 mmol L^{-1} Ag. (a1) before crosslinked, (a2) after crosslinked.

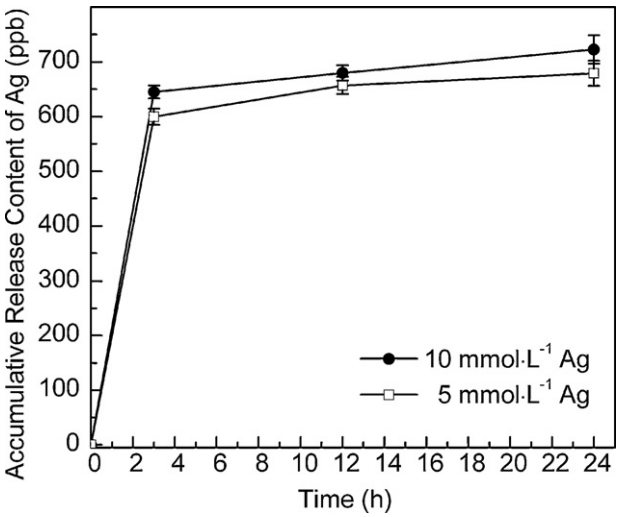

Fig. 6. *In vitro* Ag release curves of AgNPs/PVA/CM-chitosan nanofibers in PBS.

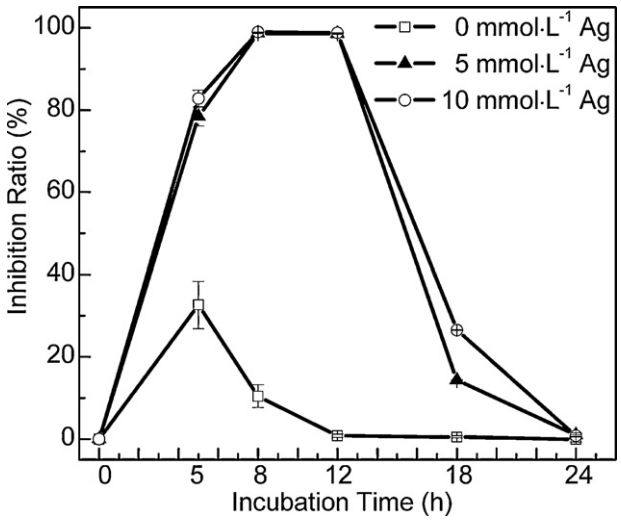

Fig. 8. Bactericidal kinetic study against *E. coli* for the AgNPs/PVA/CM-chitosan nanofibers with different content of Ag.

below 2.3 ppm (part per million) [38]. Another study showed that AgNPs with a concentration of 1.7 ppm in media did not decrease the viability of human epidermal keratinocytes (HEKs) [39]. A chitosan/nano-hydroxyapatite/nano-silver scaffold prepared with 50 mM $AgNO_3$ was found to be non-toxic to rat osteoprogenitor cells and human osteosarcoma cell line [40]. It indicated that a suitable concentration of AgNPs can have efficient antibacterial activity and acceptable cytotoxicity.

In this study, approximately 10 mg nanofibers were immersed in 50 mL PBS. The same condition was applied in bactericidal kinetic testing (approximately 10 mg nanofibers immersed in 50 mL bacteria nutrient solution). The maximum Ag^+ concentration of the solution after Ag releasing in this condition was around 600–700 ppb, which is much higher than 0.1 ppb and lower than 1.7 ppm. It suggests that these fibers should have sound antibacterial ability and infinitesimal cytotoxicity.

3.4. Antibacterial assessment

The antibacterial abilities of AgNPs/PVA/CM-chitosan nanofibers against gram-negative *E. coli* were explored by bacterial growth inhibition halos and bactericidal kinetic testing. Fig. 7 shows the inhibition halo images of AgNPs/PVA/CM-chitosan nanofibers against *E. coli*, using PVA/CM-chitosan as control. The inhibition halo surrounding AgNPs that contained fibers can be clearly observed (Fig. 7b and c). However, the PVA/CM-chitosan nanofibers without AgNPs (Fig. 7a) did not show inhibition halo. With the increase of AgNPs amount, the area of inhibition halo expanded. The results manifested that the antibacterial ability depends on the presence of AgNPs in fibers. When the amount of AgNPs increased, the antibacterial effect of fibers containing AgNPs was amplified.

Fig. 8 shows the bactericidal kinetic testing of AgNPs/PVA/CM-chitosan nanofibers with different content of Ag. With approximately 10 mg nanofibers in 50 mL bacteria nutrient solution with an initial *E. coli.* concentration of ca. 10^4 CFU mL^{-1}, the inhibition ratios with Ag content of 0, 5 and 10 mmol L^{-1} were 32.6%, 78.4% and 82.8% at the 5th hour (Fig. 8), respectively. As a contrast, the PVA/CM-chitosan control merely showed certain antibacterial ability, which can be ascribed to the antibacterial ability of CM-chitosan [41]. When the incubation time reached 8 h, the fibers without AgNPs lost inhibition ability. However, nanofibers with Ag content of 5 and 10 mmol L^{-1} showed similar inhibition ratios (98.7% and 98.9%, respectively), which was agreed with the results of *in vitro* Ag release study. The inhibition ratios of 5 and 10 mmol L^{-1} groups at the 12th hour were 98.6% and 98.8% which were equal to the inhibition ratios at the 8th hour. After 18 h of incubation, the inhibition ratios with Ag content of 5 and 10 mmol L^{-1} decreased to 14.3% and 26.5% respectively, which indicated the termination of inhibition. As the bacteria would consume Ag ion during the incubation, the higher inhibition ratio of 10 mmol L^{-1} group in 18 h was likely attributable to the higher capacity of Ag in fibers. The results showed that the AgNPs/PVA/CM-chitosan nanofibers have sound antibacterial effects against *E. coli* up to 12 h in our experimental

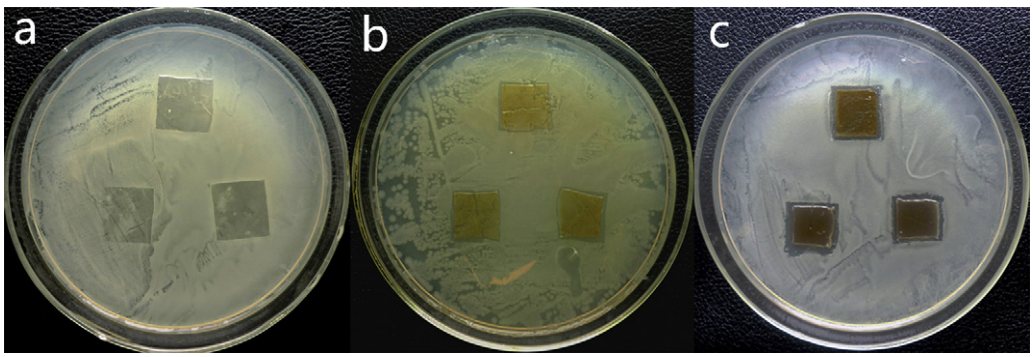

Fig. 7. The bacterial growth inhibition halos against *E. coli* for (a) PVA/CM-chitosan nanofibers, (b) AgNPs/PVA/CM-chitosan nanofibers with 5 mmol L^{-1} Ag and (c) AgNPs/PVA/CM-chitosan nanofibers with 10 mmol L^{-1} Ag.

condition, which support their future application as antibacterial materials.

PVA/CM-chitosan fibers showed certain antibacterial ability in antibacterial kinetic study; however, its inhibition halo was indiscernible. Similar results were obtained in our previous work on AgNPs/gelatin/CM-chitosan hydrogel [42]. It is reported that the CM-chitosan with negatively charged on the surface could inhibit the transcription from DNA when it contact with bacterial, which imparted certain antibacterial activity to PVA/CM-chitosan fibers [14]. However, the fibers are fixed on the surface of LB medium in inhibition halos test, which interfered the contact of CM-chitosan with *E. coli*. In the case of fibers containing AgNPs, silver was released into the solution, which was verified by *in vitro* Ag ion release study, to form inhibition halos.

In our previous work, a series of antibacterial AgNPs/gelatin/CM-chitosan hydrogel were fabricated. The results of bactericidal kinetic testing in approximate conditions indicated that nanofibers had higher antibacterial activity than that of hydrogel with equivalent weight, Ag concentration and AgNPs size [43]. AgNPs on the surface of nanofibers could have more effective contact antibacterial property than hydrogel due to the smaller dimension and higher specific area, which showed the advantages of applying AgNPs containing nanofibers as antibacterial materials.

4. Conclusions

AgNPs/PVA/CM-chitosan nanofibers were successfully fabricated by electrospinning of PVA/CM-chitosan/$AgNO_3$ aqueous solution with an $AgNO_3$ concentration of 5 and 10 mmol L^{-1}. The AgNPs were *in situ* formed in PVA/CM-chitosan blend aqueous solution before electrospinning. The resulted AgNPs/PVA/CM-chitosan nanofibers have smooth surface and uniform diameters, and the average diameter of fibers slightly decreased with the decrease of Ag content. The AgNPs can be observed on the surface of the fibers with round shape, which mainly distributed from 4 to 14 nm in the nanofibers. With approximately 10 mg nanofibers in 50 mL bacteria nutrient solution with an initial concentration of ca. 10^4 CFU mL^{-1}, the AgNPs/PVA/CM-chitosan nanofibers had an inhibition ratio of 98% against *E. coli* up to a contact time of 12 h. The comprehensive results of this study suggest that AgNPs/PVA/CM-chitosan nanofibers have the potential to be utilized as antibacterial biomaterials.

Acknowledgements

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