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# Hydrothermal synthesis of bacterial cellulose/AgNPs composite: A "green" route for antibacterial application

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ABSTRACT

Antibacterial materials based on cellulose have been widely used in many fields. In this work, an environmentally benign and facile approach for production of silver nanoparticles (AgNPs) was proposed for the first time by hydrothermal synthesis using bacterial cellulose (BC) as both the reducing and stabilizing agent, without any chemical reagents introduced. Some key reaction parameters were optimized to achieve a high antibacterial activity of the BC/AgNPs composite. Under the optimal conditions, a small size and a narrow distribution of AgNPs, $17.1 \pm 5.9$ nm, was formed on the BC matrix, with a silver content of 1.78% (w/w) and a MIC value of $1.30 \times 10^{-4}$ μg/CFU. Moreover, a sustained release of silver and a prolonged antibacterial performance of the composite against *Staphylococcus aureus* were found over a long period time of 72 h, which were important for practical applications.



## 1. Introduction

Natural cellulose, the most abundant biopolymer on earth, has been used for thousands of years and their use continues today as one of the most exploited renewable raw materials. In recent years, a new cellulose material, bacterial cellulose (BC), has attracted more and more attentions in both scientific and industrial applications. Bacterial cellulose is a kind of natural cellulose synthesized by some species of bacteria, especially *Gluconacetobacter xylinus* (formerly *Acetobacter xylinum*). Unlike celluloses of plant or wood origin, bacterial cellulose features a distinctive three dimension structure of an ultrafine nanofiber network (Czaja, Young, Kawecki, & Brown, 2007). Besides, it owns some distinguished physical and mechanical properties, e.g. free of lignin and hemicelluloses, high purity and degree of polymerization, excellent textile strength, high porosity as well as good biocompatibility, making it a suitable candidate for producing high quality paper (Cheng, Catchmark, & Demirci, 2011) and various biomedical materials such as temporary wound dressing (Czaja, Krystynowicz, Bielecki, & Brown, 2006), bone graft (Zaborowska et al., 2010) and artificial blood vessel (Fink et al., 2010).

However, for many applications of cellulose based materials especially those in biomedical and food package fields (Costerton, Stewart, & Greenberg, 1999; Hall-Stoodley, Costerton, & Stoodley, 2004), microorganism pollution is a major concern from basic household maintenance to public health safety, since cellulose do not have inherent antibacterial property. A lot of researches have been made in the last decades in order to develop antibacterial celluloses. Silver has been utilized for the antibacterial applications for thousands of years due to its broad efficacy against bacteria and other microorganisms (Morones et al., 2005; Sharma, Yngard, & Lin, 2009) and its relatively low toxicity to humans (Klasen, 2000a, 2000b). In the present century, silver nanoparticles (AgNPs) have come up as one of the most effective antibacterial agents because of their large surface area to volume ratio comparable to the bulk form (Rai, Yadav, & Gade, 2009). They are one of the most commercialized nanomaterials with broad applications in textile (Falletta et al., 2008), wound dressing (Vlachou et al., 2007), bone implant (Schneider, Loher, Brunner, Schmidling, & Stark, 2008), water and air purification (Oyanedel-Craver & Smith, 2008; Yoon, Byeon, Park, & Hwang, 2008) and self-sterilizing polymer films (Loher, Schneider, Maienfisch, Bokorny, & Stark, 2008; Marini et al., 2007). Various methods for the preparation of such nanoparticles have been developed over the past years. However, most of them reported to date rely heavily on the use of organic solvents and toxic reducing agents like sodium borohydride (Van Hyning, Klemperer, & Zukoski, 2001), hydrazine (Sakai, Kanda, Shibata, Ohkubo, & Abe, 2006), N,N-dimethylformamide (Pastoriza-Santos & Liz-Marzán, 2002), and/or a capping/surfactant agent (Kvítek et al., 2008). All these chemicals are highly reactive and bring potential environmental and biological safety risks. In view of the awareness towards green chemistry and sustainable strategy, the development of an eco-friendly approach for the synthesis of silver nanoparticles is necessary. Some reports have utilized natural

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polymers like polysaccharide (Cai, Kimura, Wada, & Kuga, 2009; Raveendran, Fu, & Wallen, 2006; Vimala et al., 2010) as the reducing and stabilizing agents to prepare silver nanoparticles. It was revealed that the use of hydroxyl-containing polymers made it possible to produce silver nanoparticles and to control their size and size distribution (Vimala, Samba Sivudu, Murali Mohan, Sreedhar, & Mohana Raju, 2009).

In our previous work, bacterial cellulose was used as the template to in situ synthesize silver nanoparticles by chemical reduction using sodium borohydride (Yang, Xie, Hong, Cao, & Yang, 2012). It was found that the extensive hydroxyl groups on the BC microfibrils acted as the anchoring sites for the silver ions, and the nanosized pores of the BC material served as the nanoreactors for the nucleation and growth of the AgNPs, restricting the growth of particles within the pores and inducing a small size and a narrow size distribution. By considering the inherent reduction ability to metal ions by hydroxyl groups on BC material, in this work, a green and environmentally benign approach for production of silver nanoparticles was proposed by hydrothermal synthesis in a gently heated system, using bacterial cellulose as both the reducing and stabilizing agent, without any chemical reagents introduced. To the best knowledge of the authors, no such reports have been published until now. This process is hopeful to be expanded in the synthesis of other metal-contained cellulose composites, especially those exhibit antibacterial efficacies for biotechnological applications. Besides, a green AgNPs-contained cellulose product, i.e. BC/AgNPs composite, is promising to be offered and used in various antibacterial applications, especially those involving direct interactions with human cells such as medical devices, health care, self-sterilizing textiles, water purification and food packages.

## 2. Material and methods

### 2.1. Bacterial strain and culture medium

*G. xylinus* (formerly *Acetobacter aceti* subsp. *xylinus* or *A. xylinus*) (strain 1.1812) and *Staphylococcus aureus* (strain 1.128) were purchased from Institute of Microbiology Chinese Academy of Science and maintained on solid agar medium at 4 °C. Both the seed medium and fermentation medium used for BC production contained 2.5% (w/v) maltose, 0.3% (w/v) tryptone and 0.5% (w/v) yeast extract. Prior to sterilization at 121 °C, the pH value of the medium was adjusted to 5.0.

### 2.2. Preparation and purification of BC membrane

The preparation of BC membrane was conducted according to the previous work (Yang et al., 2012). Briefly, a single *G. xylinus* colony was transferred into 100 ml of seed culture medium and cultivated agitatedly for 24 h at 30 °C. Then, 6 ml of the cell suspension was introduced into a 250-ml Erlenmeyer flask containing 100 ml of fermentation culture medium, and incubated statically at 30 °C for 5–7 days. After that, the BC membrane was harvested and purified by boiling them in 1.0% NaOH solution for 2 h, and subsequently in distilled water for another 2 h. The two steps were repeated for three cycles. Finally, the membrane was thoroughly washed with distilled water until the pH of the washing liquid was neutral and finally immersed in the distilled water prior to use.

### 2.3. Hydrothermal synthesis of BC/AgNPs composites

The BC/AgNPs composite was prepared as follows: one piece of the purified BC membrane (0.1 g, dry weight) was rinsed in 25 ml of 1 mM aqueous $AgNO_3$ solution under agitation at 100 rpm and 30 °C for 0.5 h protected from light, then picked up and washed by $3 \times 10$ ml of distilled water to remove the excess $AgNO_3$ solution. Subsequently, the membrane was placed in a Teflon-sealed glass bottle and heated at 80 °C for 4 h. After cooling to room temperature, the as-prepared composite was thoroughly washed with distilled water and freeze-dried (LYQQLEST-55, Telstar, Spain) for 24 h before use. The concentration of $AgNO_3$ solution and immersion time varied from 1 to 5 mM and 0.5 to 24 h, respectively.

### 2.4. Characterization

The FE-SEM analysis was carried out to observe the morphology of the BC/AgNPs composite. The freeze-dried samples were coated with a thin layer of gold before observation, and the images were taken using a HITACHI, S-4800 field emission scanning electron microscope (Japan). The particle sizes of the AgNPs were measured using ImageJ software. In order to measure the particle size accurately, those uncovered particles by the cellulose microfibrils were chose for measurement, and at least 100 particles of the sample from different FE-SEM images were analyzed. The amount of silver in the composite was quantified by atomic absorption spectrometer (AAS, ZEEnit700, Analytik Jena, Germany) with the sample dissolved in 95% nitric acid. Elemental maps of the composite were obtained using scanning electron microscopy-energy dispersive X-ray spectrometer (SEM-EDX; SEM, JEOL, JSM-5600LV; EDX, IE 300X, Oxford). Triplicate experiments for each sample were carried out.

### 2.5. Assay of antibacterial activity

The antibacterial activity of the BC/AgNPs composite was determined against the common pathogenic bacteria *S. aureus.* as the model microorganism, which was pre-cultured at 37 °C to reach a concentration of $1.6–1.9 \times 10^7$ colony forming unit/ml (CFU/ml). All the BC/AgNPs composite samples were cut into circular discs (15 mm in diameter) for use. Two methods, i.e. the zone of inhibition and minimum inhibitory concentration (MIC) test were adopted to evaluate the antibacterial activity. The zone of inhibition method was performed according to our previous work (Yang et al., 2012). The MIC test was carried out to evaluate the antibacterial activity quantitatively. In the test, nine groups of BC/AgNPs disc samples (sample amount varied from 1 to 9 pieces) were added into 20 ml of *S. aureus.* nutrient broth, seeded with 0.1 ml of test strains ($1.6–1.9 \times 10^6$ CFU of total bacteria number) and incubated at 37 °C for 24 h. After that, the bacterial survival numbers were determined by plating serial dilution of the microbial suspension on plate count agar. The MIC was recorded as the lowest concentration of AgNPs in the composite sample that showed no growth on agar plates. MIC assay was performed in triplicate with appropriate controls (uninoculated medium and medium without BC/AgNPs composite).

### 2.6. The release of silver from BC/AgNPs composite

The release behavior of silver from the BC/AgNPs composite was studied as follows: 0.05 g of the test sample was immersed statically in 50 ml of the physiological saline solution at 37 °C over a period time of 72 h. The liquids withdrawn from the solution as a function of immersion time were analyzed quantitatively by atomic absorption spectrophotometer (AAS). The obtained data were calculated carefully to obtain the cumulative amounts of released silver, and expressed as the releasing profile.

### 2.7. Antibacterial kinetics

The prolonged antibacterial property of the BC/AgNPs composite was investigated by determination of the growth curve of *S. aureus* in the culture medium containing the sample. The *S. aureus*

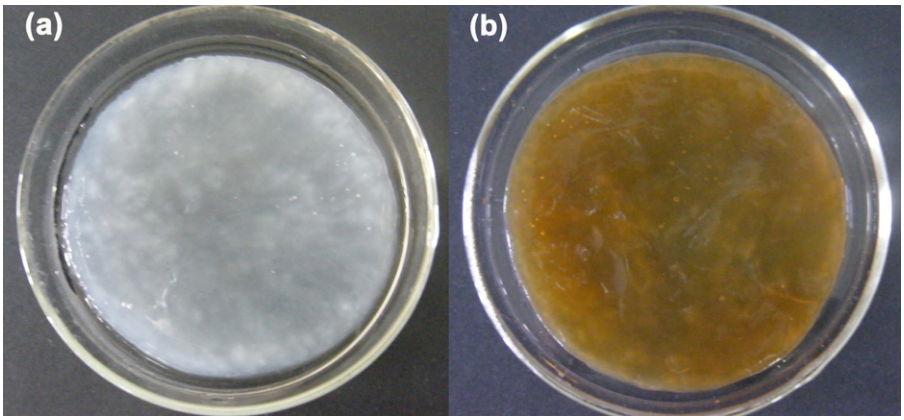


**Fig. 1.** Photographs of a pure BC membrane (a) and a BC/AgNPs composite prepared by hydrothermal reduction (b).

cell suspension was prepared as described in Section 2.5. In the test, 0.02 g of the BC/AgNPs composite was immersed in 25 ml of the culture medium, seeded with 0.1 ml of bacterial suspension, and then incubated at 37 °C and 100 rpm. After 24 h and 48 h incubation the same amount of fresh bacteria was reinoculated, respectively, and the total incubation time lasted for 72 h. At defined time intervals, the cell suspensions were withdrawn and analyzed spectrophotometrically by measuring the absorbance at 600 nm. The growth of *S. aureus* in the culture medium without BC/AgNPs composite was also investigated as control.

## 3. Results and discussion

### 3.1. Preparation of AgNPs on BC matrix by hydrothermal synthesis

In our previous work, bacterial cellulose has been successfully utilized as the template to in situ synthesize the AgNPs by chemical reduction, i.e. $NaBH_4$ as the reductant (Yang et al., 2012). It was found that the structure of the three-dimensional network and large amount of the nanosized pores in the BC matrix acted as the nanoreactors for the nucleation and growth of the AgNPs, resulting in a small size and a narrow size distribution. The chemical regent $NaBH_4$ is highly reactive for the process. However, its inherent toxicity caused potential biological and environmental risks. Actually, the surfaces of the BC microfibrils possess plenty of hydroxyl groups, which could supply the reducing power for metal synthesis (Vimala et al., 2009). Based on these backgrounds, in this work, a green and eco-friendly approach, i.e. hydrothermal synthesis, was attempted to prepare the BC/AgNPs composite, with no extra chemicals involved. For the first step, different reaction temperatures were tried in order to realize the formation of silver nanoparticles after rinsing the BC membrane into the silver precursor, $AgNO_3$ solution. It was found that no apparent color change of the membrane happened when the $Ag^+$-contained BC membrane was incubated at room temperature for more that two days, and a relatively slow reaction was observed at 50 °C. When the temperature increased to 80 °C, the reaction could be easily monitored from the color change of the membrane. Visual observation showed that as the reaction proceeded, the color shifted from white to brown yellow, signifying the formation of the silver nanoparticles in the membrane. Fig. 1a and b illustrates the photographs of a pure BC membrane and a AgNPs-contained BC composite prepared by hydrothermal reduction. The formation of AgNPs was further confirmed by SEM-EDX analysis (Fig. 2).

### 3.2. Optimization of reaction conditions

In order to achieve higher antibacterial activity under lower silver content of the BC/AgNPs composite, some key reaction parameters, e.g. the concentration of $AgNO_3$ solution and immersion time, were investigated, both of which were picked up based on the research results in the pre-experiments.

Three BC/AgNPs composites were prepared by different concentrations of $AgNO_3$ solution, i.e. 1.0, 2.5 and 5.0 mM. The zone of inhibition test was first carried out to evaluate qualitatively the antibacterial activity of the BC/AgNPs composites. As seen in Fig. 3a–c, every test BC/AgNPs composite exhibited an obvious inhibition zone, and higher width of the zone was obtained by the sample prepared in higher $AgNO_3$ concentration. Additionally, the silver contents of the three BC/AgNPs composites were determined by elemental analysis, and the results were listed in Table 1. The mass contents of silver in the composites ranged from 1.78 to 2.88%, showing a positive trend with the concentrations of $AgNO_3$ solution used. By associating the silver content with the inhibition zone of the composite, it could be found that the width of the inhibition zone was in correlation with the silver content of the sample. Furthermore, the minimum inhibitory concentration test was conducted to evaluate the antibacterial activity quantitatively. The results of MIC assay were summarized in Table 1 (sample 1–3). From the data it could be seen that lower $AgNO_3$ concentration, i.e. lower silver content of the composite, gave smaller MIC value, signifying stronger antibacterial efficacy. A MIC value of $1.30 \times 10^{-4}$ μg/CFU of silver was achieved when the $AgNO_3$ concentration was 0.1 mM. The reasons behind this phenomenon might attribute to the formation process of silver nanoparticles. It was reported that the formation of AgNPs on cellulose matrix involved

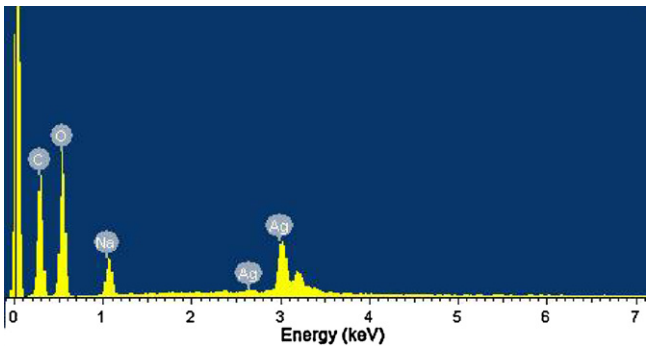


**Fig. 2.** EDX spectrum of the BC/AgNPs composite.

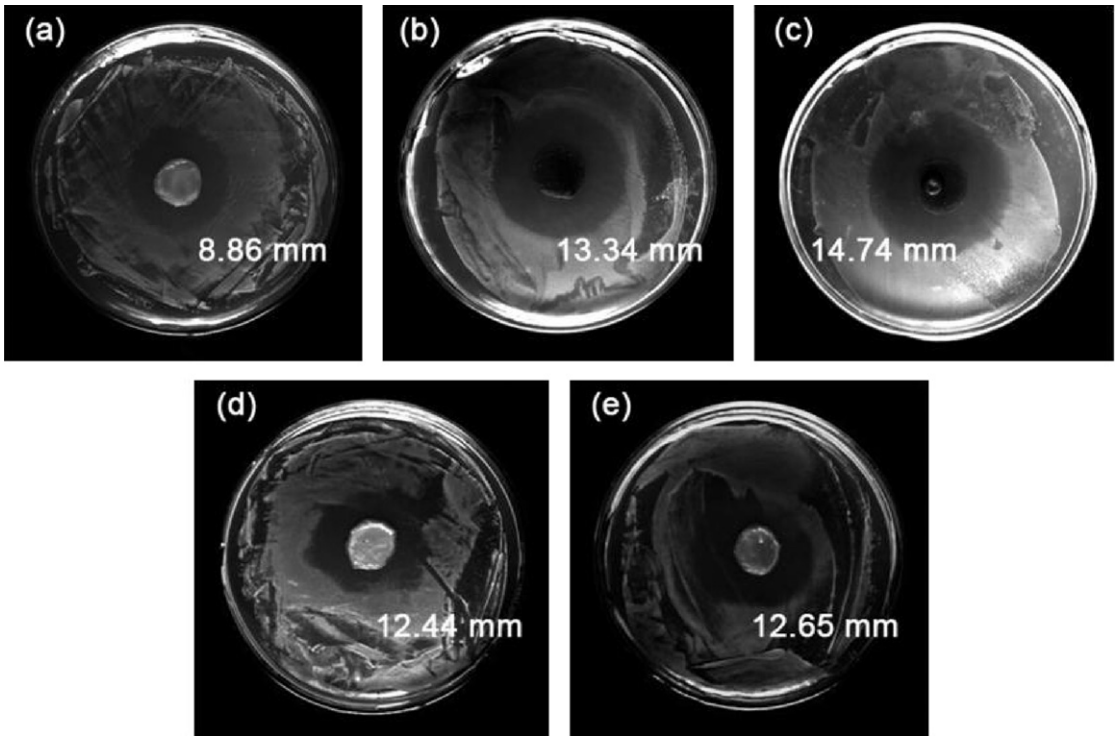


**Fig. 3.** The inhibition zones of the BC/AgNPs composites under $AgNO_3$ concentration of 1.0 mM (a), 2.5 mM (b) and 5.0 mM (c) by 0.5 h of immersion time, as well as immersion time of 12 h (d) and 24 h (e) under $AgNO_3$ concentration of 1.0 mM.

the nucleation, growth and aggregation procedures (Barud et al., 2008; Cai et al., 2009; Maneerung, Tokura, & Rujiravanit, 2008). Taking BC material as an example, when BC was immersed in the aqueous $AgNO_3$ solution, silver ions diffused into the matrix through the porous structure and anchored on BC microfibrils probably via electrostatic interactions by the electron-rich oxygen atoms of hydroxyl and ether groups of BC; then the absorbed silver ions inside the BC matrix were reduced to the metallic silver nanoparticles ($Ag^0$) under some reaction conditions (Barud et al., 2008; Cai et al., 2009; Maneerung et al., 2008). In this case, when BC matrix was immersed in higher $AgNO_3$ concentration, more silver ions would attach to it, and be reduced into silver clusters during the heating treatment, which might not be stabilized but form aggregations, thus resulting in lower antibacterial activity.

Different BC/AgNPs composites rinsed in 1.0 mM $AgNO_3$ solution but various immersion times, i.e. 0.5, 12, and 24 h were

**Table 1**
The silver contents and MIC results of the BC/AgNPs composites under different reaction conditions.

| Sample No. | $AgNO_3$ concn. (mM) | Immersion time (h) | Silver content (%, w/w) | Concn. of Ag ($\times 10^{-4}$ μg/CFU) | *S. aureus*[a] (24 h) | MIC ($\times 10^{-4}$ μg/CFU) |
|---|---|---|---|---|---|---|
| | | | | 0.65 | + | 1.30 |
| | | | | 0.97 | + | |
| 1 | 1.0 | 0.5 | 1.78 ± 0.13 | 1.30 | – | |
| | | | | 1.62 | – | |
| | | | | 1.95 | – | |
| | | | | 0.79 | + | 1.98 |
| | | | | 1.19 | + | |
| 2 | 2.5 | 0.5 | 2.18 ± 0.22 | 1.59 | + | |
| | | | | 1.98 | – | |
| | | | | 2.38 | – | |
| | | | | 1.05 | + | 2.10 |
| | | | | 1.57 | + | |
| 3 | 5.0 | 0.5 | 2.88 ± 0.24 | 2.10 | – | |
| | | | | 2.62 | – | |
| | | | | 3.15 | – | |
| | | | | 0.70 | + | 1.76 |
| | | | | 1.05 | + | |
| 4 | 1.0 | 12 | 2.21 ± 0.19 | 1.41 | + | |
| | | | | 1.76 | – | |
| | | | | 2.11 | – | |
| | | | | 0.73 | + | 1.83 |
| | | | | 1.10 | + | |
| 5 | 1.0 | 24 | 2.31 ± 0.18 | 1.46 | + | |
| | | | | 1.83 | – | |
| | | | | 2.20 | – | |

[a] "+" meant growth and "–" meant no growth.

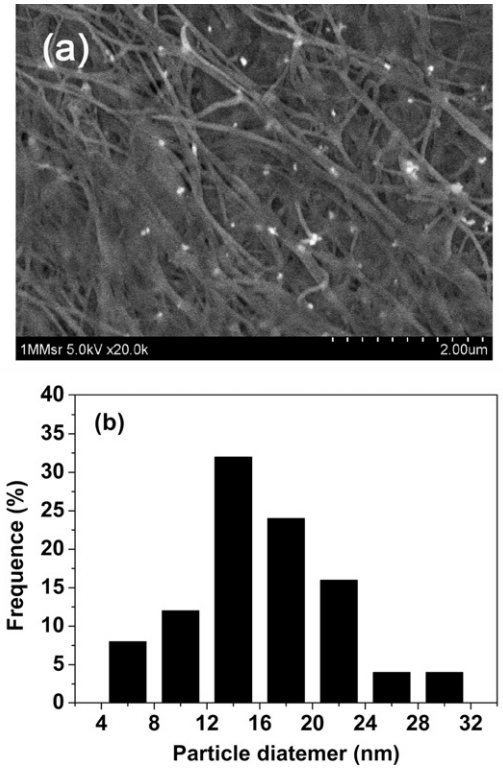


**Fig. 4.** The FE-SEM image (a) and particle size distribution histogram (b) of AgNPs formed in BC matrix.

prepared. The qualitative evaluation of the antibacterial activity of the composites by zone of inhibition test was shown in Fig. 3a, d and e, and their silver contents and MIC values were exhibited in Table 1 (sample 1, 4 and 5). It could be concluded that the silver content of the composite increased obviously by prolonging the immersion time from 0.5 to 12 h, while no significant increase happened from 12 to 24 h. A good correlation between the silver contents and the widths of inhibition zones of the test samples could be established, which was mentioned above. With respect to the MIC values, the sample immersed by 0.5 h presented the lowest MIC value of $1.30 \times 10^{-4}$ μg/CFU, followed by the ones of 12 h and 24 h which were almost the same with each other. Based on the formation process of the AgNPs in the BC matrix, it was thought that the immersion time influenced probably the diffusion of silver ions from the external solution into the matrix. Increasing the immersion time, e.g. from 5 h to 12 h, more silver ions would diffuse into the inner part of the matrix, thus bringing a higher silver content. However, it seemed that 12 h was enough for the silver ions to penetrate into the matrix, since no obvious changes happened in the silver content when the immersion time prolonged to 24 h.

### 3.3. Morphology and long-term antibacterial performance studies

After optimization of the main reaction parameters, the sample with the lowest MIC value of $1.30 \times 10^{-4}$ μg/CFU was chosen for further investigations. Fig. 4 demonstrates the FE-SEM image of the BC/AgNPs composite. From the picture it could be observed that, the silver particles were homogeneously dispersed on the surface of the BC microfibrils. The size and size distribution analyzed based on the FE-SEM images illustrated that the AgNPs exhibited a small size and a narrow size distribution with an average size of $17.1 \pm 5.9$ nm. This result confirmed that the BC material was indeed a pattern of suitable template matrix for the synthesis of metal nanoparticles,

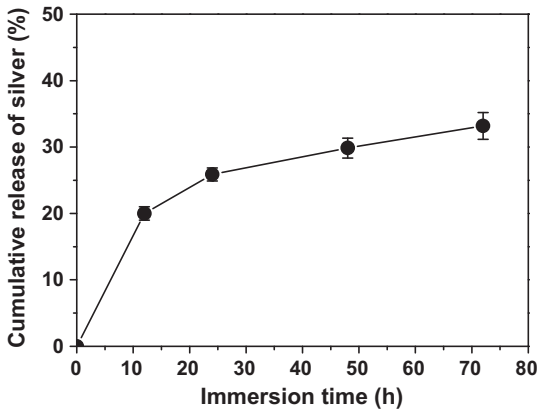


**Fig. 5.** The silver releasing profile of the BC/AgNPs composite.

which were also referred by some other reports (Barud et al., 2008; Cai et al., 2009; Maneerung et al., 2008).

Long-term release performance of silver is considered as an important factor of the silver-contained antibacterial materials from the viewpoint of practical applications. Fig. 5 exhibits the silver releasing behavior of the BC/AgNPs composite. As seen in the picture, a relatively rapid release of silver happened in the first 12 h with a mass ratio of 20% released. Then the releasing rate slowed down, and a total mass ratio of 33% released after 72 h. It was thought that the silver particles located in the surface region of the BC matrix was ready to release when rinsed in physiological saline solution, and explained the rapid release of silver in the initial time. The silver particles impregnated in the inner part of the composite were hard to diffuse out due to the porous structure of BC, thus the silver went into a sustained release way. This implied that a prolonged antibacterial effect was promising to be achieved by this BC/AgNPs composite.

This expectation was evidenced by determination of the growth curve of bacteria in contact with the composite in the culture medium system over a long period time of 72 h. In order to highlight the long-term antibacterial performance of silver, two times of reinoculation of fresh bacteria were supplied to maintain a steady number of live bacteria in the test culture medium. Moreover, a relatively small amount of composite sample with a silver content of $2.22 \times 10^{-4}$ μg/CFU (1.7 folds of the MIC value) was utilized in this test. The growth curve of *S. aureus* without BC/AgNPs composite in the culture medium was also determined as control. As shown in Fig. 6, a sharp contrast existed between the two growth curves. For instance, a fast growth of bacteria for the

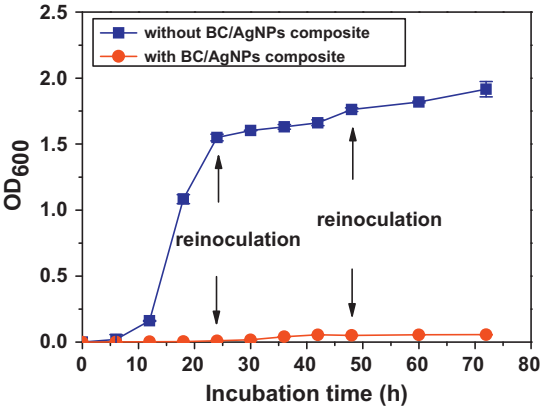


**Fig. 6.** Comparisons of growth curves of *S. aureus* between with and without BC/AgNPs composite in the culture medium system.

control sample was observed reflected by the $OD_{600}$ values in the first 24 h, while no growth happened by that with the BC/AgNPs composite. Besides, although twofold amounts of new bacteria cells were reinoculated in the next 48 h, almost zero growth was found by the sample exposed to the composite with the $OD_{600}$ values close to zero, while further growth happened by the control one. Therefore, it was revealed that the BC/AgNPs composite could realize a complete inhibition of *S. aureus* growth over a prolonged period time of 72 h, and a good bactericidal efficacy for a longer period would be expected from the result herein. One advantage of this property would be the lack of excess availability of silver, which relieves fears of potential toxicity to human, and the reduction of the labor costs due to the frequent replacements of the antibacterial materials.

## 4. Conclusions

In this work, an environmentally benign material and process for antibacterial application was proposed, i.e. BC/AgNPs composite prepared by hydrothermal synthesis in a gentle heating system. The BC matrix covered with extensive hydroxyl groups on the microfibrils served as both the reducing and stabilizing agent for the formation of AgNPs, and a small size of AgNPs with a good antibacterial performance was obtained. This process is hopeful to be expanded in the synthesis of other metal-contained cellulose composites, and the product would be used for various antibacterial applications including medical devices, health care, self-sterilizing textiles, water purification and food packages.

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## References

Barud, H. S., Barrios, C., Regiani, T., Marques, R. F. C., Verelst, M., Dexpert-Ghys, J., et al. (2008). Self-supported silver nanoparticles containing bacterial cellulose membranes. *Materials Science and Engineering C*, *28*, 515–518.

Cai, J., Kimura, S., Wada, M., & Kuga, S. (2009). Nanoporous cellulose as metal nanoparticles support. *Biomacromolecules*, *10*, 87–94.

Cheng, K., Catchmark, J. M., & Demirci, A. (2011). Effects of CMC addition on bacterial cellulose production in a biofilm reactor and its paper sheets analysis. *Biomacromolecules*, *12*, 730–736.

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: A common cause of persistent infections. *Science*, *284*, 1318–1322.

Czaja, W., Krystynowicz, A., Bielecki, S., & Brown, R. M. (2006). Microbial cellulose—the natural power to heal wounds. *Biomaterials*, *27*, 145–151.

Czaja, W. K., Young, D. J., Kawecki, M., & Brown, R. M. (2007). The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules*, *8*, 1–12.

Falletta, E., Bonini, M., Fratini, E., Nostro, A. L., Pesavento, G., Becheri, A., et al. (2008). Clusters of poly(acrylates) and silver nanoparticles: Structure and applications for antimicrobial fabrics. *The Journal of Physical Chemistry C*, *112*, 11758–11766.

Fink, H., Faxäv, L., Molnár, G. F., Drotz, K., Risberg, B., Lindahl, T. L., et al. (2010). Real-time measurements of coagulation on bacterial cellulose and conventional vascular graft materials. *Acta Biomaterialia*, *6*, 1125–1130.

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: From the natural environment to infections diseases. *Nature Reviews-Microbiology*, *2*, 95–108.

Klasen, H. J. (2000a). Historical review of the use of silver in the treatment of burns: I. Early uses. *Burns*, *26*, 117–130.

Klasen, H. J. (2000b). Historical review of the use of silver in the treatment of burns: I. Early uses. *Burns*, *26*, 131–138.

Kvítek, L., Panáček, A., Soukupová, J., Kolář, M., Večeřová, R., Prucek, R., et al. (2008). Effect of surfactants and polymers on stability and antibacterial activity of silverNanoparticles (NPs). *The Journal of Physical Chemistry C*, *112*, 5825–5834.

Loher, S., Schneider, O. D., Maienfisch, T., Bokorny, S., & Stark, W. J. (2008). Micro-organism-triggered release of silver nanoparticles from biodegradable oxide carriers allows preparation of self-sterilizing polymer surfaces. *Small*, *4*, 824–832.

Maneerung, T., Tokura, S., & Rujiravanit, R. (2008). Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate Polymers*, *72*, 43–51.

Marini, M., De Niederhausern, S., Iseppi, R., Bondi, M., Sabia, C., Toselli, M., et al. (2007). Antibacterial activity of plastics coated with silver-doped organic–inorganic hybrid coatings prepared by sol–gel processes. *Biomacromolecules*, *8*, 1246–1254.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., et al. (2005). The actericidal effect of silver nanoparticles. *Nanotechnology*, *16*, 2346–2353.

Oyanedel-Craver, V. A., & Smith, J. A. (2008). Sustainable colloidal-silver-impregnated ceramic filter for point-of-use water treatment. *Environmental Science & Technology*, *42*, 927–933.

Pastoriza-Santos, I., & Liz-Marzán, L. M. (2002). Synthesis of silver nanoprisms in DMF. *Nano Letters*, *2*, 903–905.

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, *27*, 76–83.

Raveendran, P., Fu, J., & Wallen, S. L. (2006). A simple and "green" method for the synthesis of Au, Ag, and Au–Ag alloy nanoparticles. *Green Chemistry*, *8*, 34–38.

Sakai, H., Kanda, T., Shibata, H., Ohkubo, T., & Abe, M. (2006). Preparation of highly dispersed core/shell-type titania nanocapsules containing a single Ag nanoparticle. *Journal of the American Chemical Society*, *128*, 4944–4945.

Schneider, O. D., Loher, S., Brunner, T. J., Schmidling, P., & Stark, W. J. (2008). Flexible, silver containing nanocomposites for the repair of bone defects-antimicrobial effect against *E. coli* infection and comparison to tetracycline containing scaffolds. *Journal of Materials Chemistry*, *18*, 2679–2684.

Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science*, *145*, 83–96.

Van Hyning, D. L., Klemperer, W. G., & Zukoski, C. F. (2001). Silver nanoparticle formation: Predictions and verification of the aggregative growth model. *Langmuir*, *17*, 3128–3135.

Vimala, K., Murali Mohan, Y., Samba Sivudu, K., Varaprasad, K., Ravindra, S., Narayana Reddy, N., et al. (2010). Fabrication of porous chitosan films impregnated with silver nanoparticles: A facile approach for superior antibacterial application. *Colloids and Surfaces B: Biointerfaces*, *76*, 248–258.

Vimala, K., Samba Sivudu, K., Murali Mohan, Y., Sreedhar, B., & Mohana Raju, K. (2009). Controlled silver nanoparticles synthesis in semi-hydrogel networks of poly(acrylamide) and carbohydrates: A rational methodology for antibacterial application. *Carbohydrate Polymers*, *75*, 463–471.

Vlachou, E., Chipp, E., Shale, E., Wilson, Y. T., Papini, R., & Moiemen, N. S. (2007). The safety of nanocrystalline silver dressings on burns: A study of systemic silver absorption. *Burns*, *33*, 979–985.

Yang, G., Xie, J. J., Hong, F., Cao, Z. J., & Yang, X. X. (2012). Antimicrobial activity of silver nanoparticle impregnated bacterial cellulose membrane: Effect of fermentation carbon sources of bacterial cellulose. *Carbohydrate Polymers*, *87*, 839–845.

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 & Technology*, *42*, 1251–1255.

Zaborowska, M., Bodin, A., Bäkdahl, H., Popp, J., Goldstein, A., & Gatenholm, P. (2010). Microporous bacterial cellulose as a potential scaffold for bone regeneration. *Acta Biomaterialia*, *6*, 2540–2547.