Korean J. Chem. Eng., 39(8), 2165-2171 (2022)
DOI: 10.1007/s11814-021-1023-x

pISSN: 0256-1115
eISSN: 1975-7220

Coating of Au@Ag on electrospun cellulose nanofibers for wound healing and antibacterial activity

Kaleemullah Kalwar*, Juqun Xi, Chuanli Ren***, and Ming Shen*,†**

*College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, Jiangsu Province, P. R. China
**Institute of Translational Medicine, School of Medicine, Yangzhou University, Yangzhou 225001, China
***Department of Laboratory Medicine, Clinical Medical College of Yangzhou University,
No. 98 Western Nantong Road, Yangzhou 225001, China
(*Received 11 October 2021 • Revised 22 November 2021 • Accepted 23 November 2021*)

Abstract−Electrospun cellulose acetate nanofibers were synthesized and deacetylated by alkaline hydrolysis. Au@AgNPs were synthesized and decorated on the surface of cellulose nanofibers. The Au@AgNPs was decorated by dipping method on the surface of cellulose nanofibers to construct effective wound dressing material. Au@NPs and Au@AgNPs/cellulose nanofibers were characterized by scanning electron microscope and transmission electron microscope. Moreover, antibacterial activity was assessed against *E. coli* and *S. aureus* finding Au@AgNPs/cellulose excellent antimicrobial material. Moreover the wound healing experiment was carried out by using Au@AgNPs/cellulose as a wound dressing material, and determining great results.

Keywords : Electrospinning, Cellulose Acetate, Nanoparticles, Wound Healing, Antibacterial Activity

INTRODUCTION

The attributes of nanofibers can be organized to direct particular actions by utilizing materials and techniques of e-spinning. Novel nanomaterials can be gained by fit post-care from the e-spun technique. A diversity of nanoparticles (NPs) can also be integrated into the nanofibrous to contribute novel applications. The NPs can also be brought on the surface of e-spun nanofibers (NFs) by surface dethronement. The easiest technique is to plunge the electrospun nanofibers in a suspension having the properties of colloid NPs to acquire the NPs by chemical attaching [1,2], Moreover, many layers of charged NPs can be positioned on the NFs by a layer by layer method [3]. Such as gold nanoparticles with negative charge and lysozyme with positive charge which were subbed deposited on negatively charged cellulose nanofibers [4].

Cellulose acetate (CA) is an easy gettable significant gained from cellulose at a cheap rate and it is utilized in a wide range in sheets for food technology and medical applications because of its less toxicity, good hydrolytic constancy, and being environmentally well-disposed. Better wet attributes that make it extremely suitable in various coverings like tissue engineering [5-8]. Besides, exploitation in cellulose research proves that it is an anticipating biological material for tissue engineering, stem cell research, and regenerative medicine, such as antimicrobial electrospun cellulose acetate being examined for *in vivo* wound healing [9,10]. Bacterial cellulose is easily studied for bone regeneration and bio-based adhesive [11]. Thus, it does not suggest the quality to ensure the fabrics on the nano size or micro size that limits its pertinence in tissue engineering. Cellulose acetate is simple in e-spinning as equated to cellulose. Antimicrobic agents of CA, such as AgNPs and ZnNPs, have been described [12]. It has been described the synthesis of e-spun CANFs surface coated with AgNPs [13]. Only AgNPs are shown destructive for mammalian cells in high doses; the compounding of noble metals would be a good choice for biomedical applications such as Ag@Au and Au@Pt [14,15]. Aforementioned, skin functions as a safe roadblock for the inner organs of the human [16]. It could suffer its safety consequences when discredited that might extend to microorganism intrusion and dangerous health problem. Microorganism related injury can be identical to destructive [17] various bactericide nanomaterials, for instance, Ag [18,19]. ZnO, TiO, can be powerful versus microorganisms that can cause injury [20,21]. Above nanomaterials still can be cytotoxic or hemolytic [22]. Therefore, we investigated wound dressing materials that can defend versus injure, caused by bacteria and are safer towards the mammalian cell. Electrospun cellulose acetate nanofibers were synthesized, deacetylated by alkaline hydrolysis, and then decorated with Au@AgNPs by dipping technique. After that, cellulose nanofibers decorated with Au@AgNPs were utilized against bacteria and wound healing experiments were performed.

EXPERIMENTAL SECTION

1. Chemicals and Materials

Cellulose acetate (CA, 39.8 wt% of acetyl content, Mn=30,000) was bought from Sigma Aldrich and Co.; as acetone, N, N dimethylacetamide, NaOH, ethanol, silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), peptone, agar, yeast extract, sodium chloride ($NaCl_2$), CTAB (cetyl trimethyl ammonium chloride), $HAuCl_4{\cdot}3H_2O$, sodium citrate, and ascorbic acid were purchased from J&K Scientific Ltd. All other chemicals were of analytical grade and utilized without

†To whom correspondence should be addressed.
E-mail: shenming@yzu.edu.cn

further purification.

2. Apparatus

Morphology was observed by field emission scanning electron microscopy (FE-SEM, S-4800II, and Hitachi, Japan), and high resolution transmission electron microscope images were checked out by TEM, Tecnai 12, and Philips, Netherlands. Absorbance was checked by a Uv-Visible spectrometer.

3. Synthesis of Gold@Silver Nanoparticles Au@AgNPs

First, gold nanoparticles (AuNPs) were synthesized according to the Frens technique and then Au@Ag core shell nanoparticles were synthesized by Lehui Lu et al. method [23]. While in 20 mL distilled water holding 0.2 mL of 25 mM CTAB and 1 mL of 100 mM ascorbic acid, 0.1 ml of 0.01 M $AgNO_3$ was combined. Later, 2 ml of freshly prepared AuNPs solution was added and 0.1 mL of 1 M NaOH was added dropwise in the solution during stirring. The temperature was kept at room temperature during the whole experiment.

4. Synthesis of Electrospun Cellulose Acetate Nanofibers and Incorporation of Au@AgNPs on Deacetylated Cellulose Nanofibers

Synthesis of cellulose acetate electrospun nanofibers and deacetylation of nanofibers is described in our previous study [24,25]. A simple dipping process was introduced for decoration of Au@AgNPs over nanofibers. In brief, dried deacetylated cellulose acetate electrospun nanofibers were placed in Au@AgNPs seed solution for an hour at room temperature and then dried at room temperature.

5. Antibacterial Activity

Antibacterial activity was assessed by the zone inhibition method. In brief, LB medium (1% peptone, 1% NaCl, 1% yeast extract, 2% agar, and pH 7 adjusted by NaOH) was used for growth of bacteria optimum temperature for the growth of *E. coli*. While 1 cm in diameter cellulose nanofibers were made and decorated with Au@AgNPs. Furthermore, 500 µL of bacterial broth culture was spread on the solid LB medium, and disc decorated with Au@AgNPs was placed in the center of Petri dish. After 24 hours, the inhibition zone was measured. Also, LB medium (1% peptone, 1% NaCl, 1% yeast extract, and pH 7 adjusted by NaOH) was used for growth of bacteria optimum temperature for the growth of *E. coli* and *S. aureus*. While 1 cm in diameter cellulose nanofibers was made and decorated with Au@AgNPs. Furthermore, the inhibitory effect of cellulose/Au@AgNPs was determined by culturing bacteria in broth media. The broth culture was incubated under aging process at 150 r min^{-1} for 24 h at 37 °C. The 96-well format microtiter plates were employed and cellulose/Au@AgNPs were put in the bottom of it, and 150 µl of broth culture was incubated at above mentioned conditions for 24 h. The optical density (OD) of each well was monitored by using a microplate reader before and after incubation time, and results were calculated according to our previously reported method [24].

6. Wound Healing Study

Studies were executed by the protocol authorized by the Institutional Animal Care and Use Committee of Yangzhou University. Rats were taken to assess the *in vivo* wound healing effects of the Au@AgNPs incorporated electrospun nanofibrous dressing. 0.8 cm skin was removed from back of a rat and the wound was created by spreading Staphylococcus aureus pathogenic bacteria on the wound. After one day Au@AgNPs/Cellulose nanofibers were applied for wound healing. 12 rats were divided into three groups as blank (pure gauze, commercially available), control (Cellulose nanofibers), and sample (Au@AgNPs/Cellulose nanofibers). Collected tissues of Rat wounds were determined with paraformaldehyde (4%) solution; afterward, the organ samples were dehydrated and inserted in paraffin, segmented, and defiled with hematoxylin and eosin (H&E). After *in vivo* investigation of wound medical care, all rats were killed and the key organs were also saved for toxicology investigation.

RESULTS AND DISCUSSION

1. Synthesis of Gold@Silver Nanoparticles (Au@AgNPs)

The Au@AgNPs were synthesized by adding a mixture on the seed's mixture of AuNPs utilizing 0.01 M concentration of $AgNO_3$ mixture. It enabled us to get Au@AgNPs as mentioned in previous literature [26,27]. When silver mixture was put into the gold mixture, silver ions with the +ve charge were sorbed onto the surface of AuNPs shaped Au@AgNPs by electrostatic characteristic. During Au@AgNPs synthesis process a change in color was observed. Moreover, orange yellowish color indicated complete synthesis of Au@AgNPs and light yellow indicated silver nanoparticles and dark red indicated AuNPs, respectively. The average size diameter of AgNPs and AuNPs was 18 nm and 18.5, respectively (see Fig. 1(A), (B)). Fig. 1(C) shows the core as Au and Ag as a shell; however, we did not find much change in AuNPs but the overall

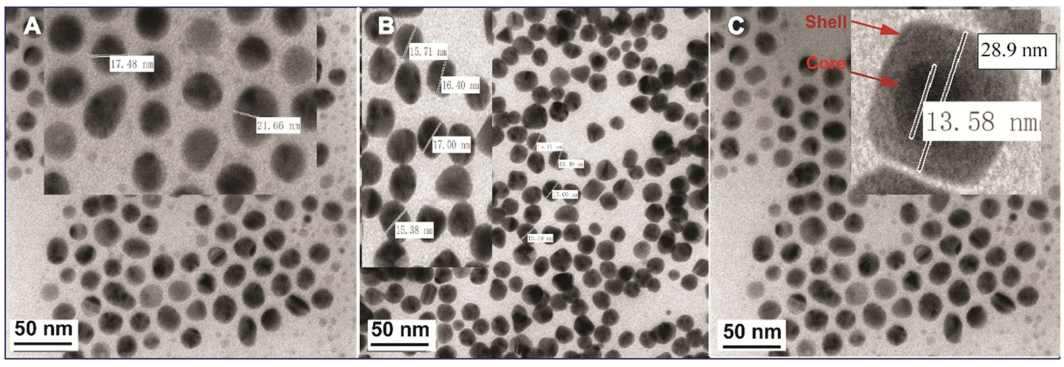

Fig. 1. TEM images of (A) Silver nanoparticles (AgNPs) (B) Gold nanoparticles (AuNPs) (C) Ag@AuNPs.

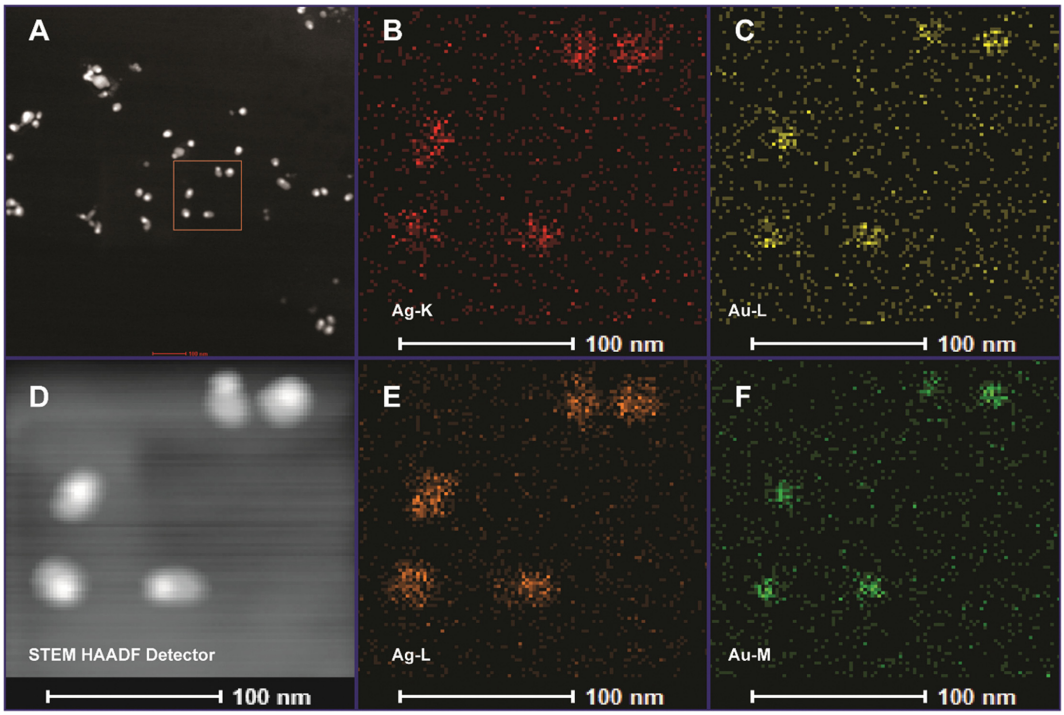

Fig. 2. HAADF-STEM image of Au@AgNPs; (A) Image scanning; (B) Ag-K; (C) Au-L; (D) STEM HAADF Detector; (E) Ag-L and (F) Au-M.

size increased to 30 nm on average. The black in the core of the nuclei represents the AuNPs and the lighter dark shells are representing AgNPs. The outcomes indicated that unlike thicknesses of the AgNPs shell can be given on the AuNP without aggregation by changing the concentration of $AgNO_3$. The outcomes further can be confirmed from previously reported work [28,29]. Furthermore, HAADF-STEM was utilized to examine core@shell Au@AgNPs, Core@shell is easily visible from Fig. 2. In Fig. 2(D), the white bright dot is representing the plane holding the heavy metal particles and the lighter white bright is representing that of the light metal particles. Due to the strong Z-sensitivity, the Au-atom Z=79 plane demonstrates a more prominent intensity than the Ag-atom Z=47 plane, which is in accord with what was detected from other crystalline particles, core@shell [30]. Moreover, element analysis shows in Fig. 2(B), (E) the red and yellow brown areas representing to the Ag shell; however, the yellow and greenish parts in Fig. 2(C), (F) match the Au core. Additionally, the UV-vis spectra are shown in Fig. 3. The maximum absorption wavelengths of Ag a, b, c, and d are at 410, 460, and 520 nm, respectively; that exhibits the features of AgNPs [31-34].

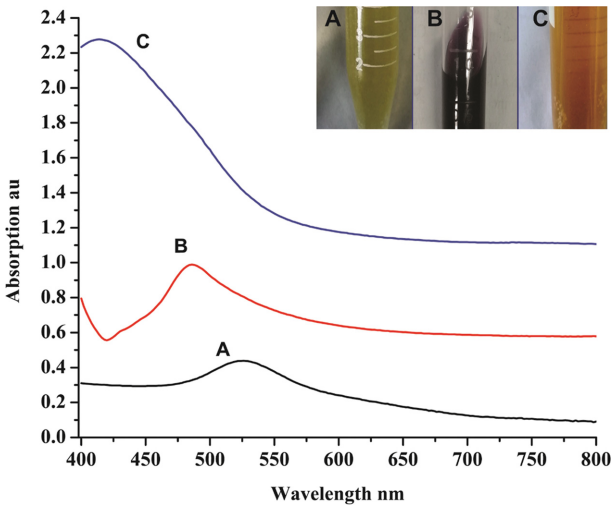

Fig. 3. UV-Spectra of (A) AgNPs (B) AuNPs (C) Au@AgNPs.

2. Decoration of Au@AgNPs on Deacetylated Cellulose Nanofibers

The Au@AgNPs were incorporated on electrospun cellulose nanofibers by adding seed solution on the surface of nanofibers. After adding Au@AgNPs seed solution, nanofibers were dried at room temperature and further characterized by SEM and TEM, as shown in Figs. 4 and 5. Cellulose electrospun nanofibers possess smooth morphology with a high surface area. Besides, the combining of the high specific surface area of electrospinning nanofibers with the biocidal activity of Au@AgNPs results in a superior and versatile antimicrobial material. The attachment of Au@AgNPs on the cellulose nanofibers is supposed to start with the contact of the cellulose with silver ions. Au@AgNPs solution was assimilated by the cellulose nanofibers because of the helping hydrophilic groups of cellulose and silver ions disseminated into the surface of fibers. Besides, Ag^+ made complexes with cellulose nanofibers because of the presence of OH and COOH groups onto the cellulose.

3. Antibacterial Activity

After immobilization of Au@AgNPs on the regenerated cellulose nanofibers, the antibacterial activity of cellulose and cellulose/Au@AgNPs was studied against *E. coli* and *S. aureus*; both bacteria are most usual infections, resulting in health problems [35,36]. The action of the bacteria was assessed in Fig. 6. We found the

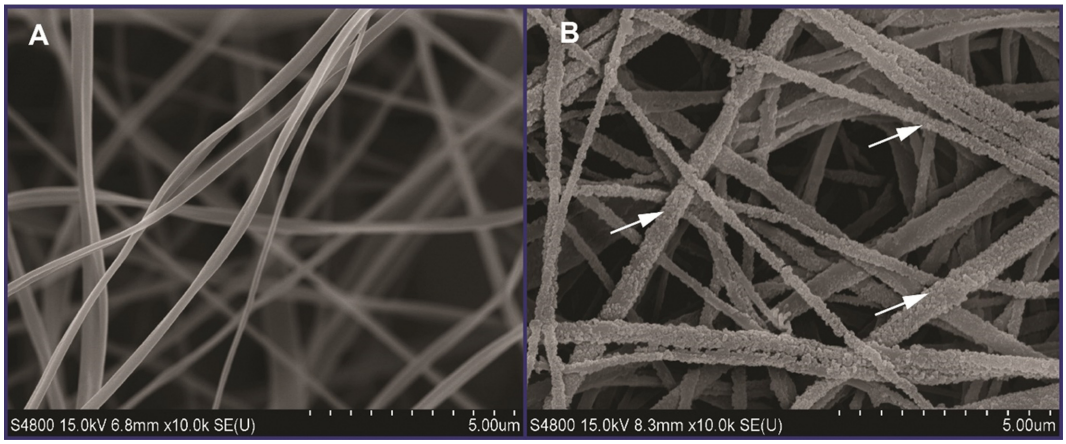

Fig. 4. SEM images of (A) Regenerated cellulose nanofibers (B) Ag@AuNPs incorporated regenerated cellulose nanofibers.

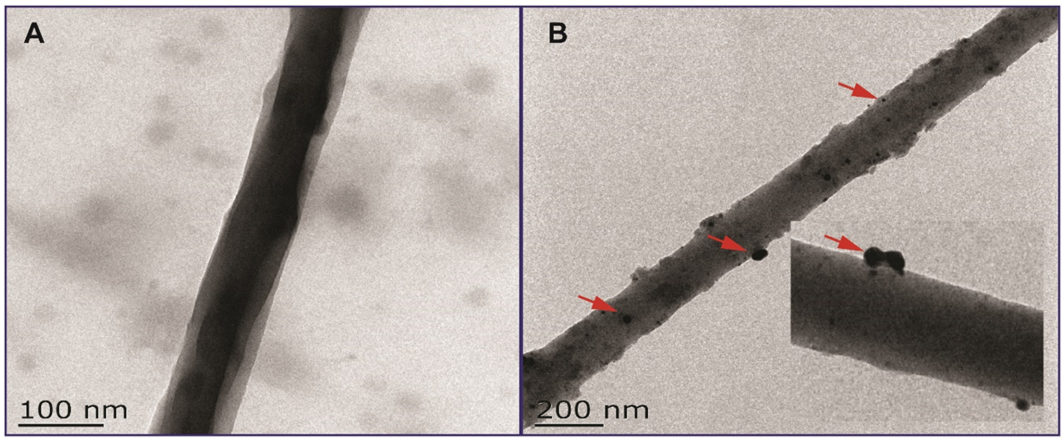

Fig. 5. TEM images of (A) Regenerated cellulose nanofibers (B) Ag@AuNPs incorporated on regenerated cellulose nanofibers.

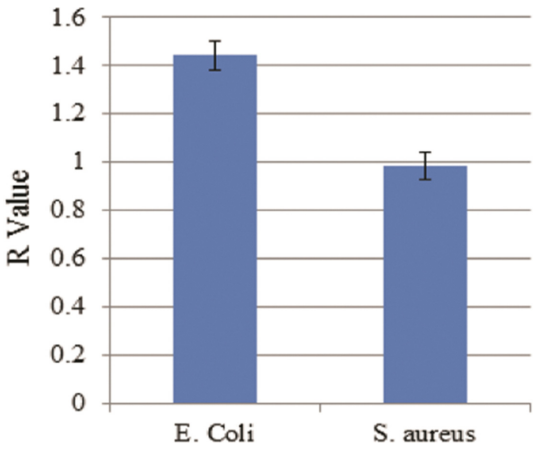

Fig. 6. Antibacterial activity.

perfect inhibition activity from cellulose/Au@AgNPs against both *E. coli* and *S. aureus* and the inhibition activity of the Au@AgNPs cellulose was more competent. Moreover, it can be suggested that the silver coating is creditworthy for bactericide activity, nearly because of the action of silver ions from the coat of the Au@AgNPs [37-39]. Silver ions are released from silver nanoparticles and diffuse in the cell wall of bacteria, which causes death. AgNPs might tie to the surface of the cell membrane and interrupt its role like permeability and respiration system [40]. Interaction of nanoparticles was observed under SEM, and it AgNPs may act with the external structure of bacterium as shown in Fig. 8, getting outer membrane change that extends to their demise. Moreover, regenerated cellulose nanofibers, the antibacterial activity of cellulose and cellulose/Au@AgNPs were studied against *E. coli* and *S. aureus* by zone inhibition method. The action of the bacteria was assessed in Fig. 7. We found the perfect inhibition zone after 24 hours from cellulose/Au@AgNPs and the inhibition activity of the Au@AgNPs cellulose was more competent.

4. Wound Dressing

The skin is a crucial roadblock to protecting the human body from the outside environment. Microorganisms once entered invade easily and start to form colonies, leading to severe wound infection and even life-threatening complications [41]. We employed pure gauze, cellulose nanofibers and cellulose/Au@AgNPs on the skin wounds of distinct classifies of rats Fig. 9. The skin injury was noticed severally at 1, 3, 5, 7, 10, and 12 days following the cure. It can be seen in Fig. 9 that injuries are cured well and quicker after

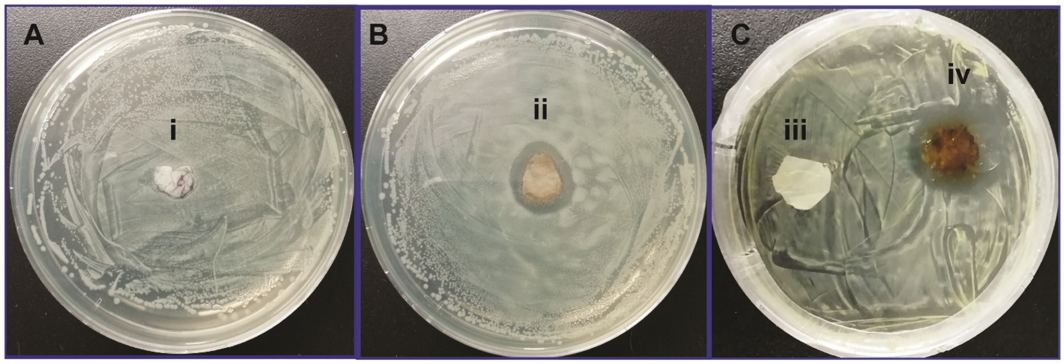

Fig. 7. Antibacterial activity of cellulose nanofibers and cellulose/Au@AgNPs against ((A) &(B)) *E. coli* and (C) *S. aureus*. Insets are *E. coli* and *S. aureus* i) control against *E. coli* ii) cellulose/Au@AgNPs against *E. coli* iii) control against *S. aureus* iv) cellulose/Au@AgNPs against *S. aureus*.

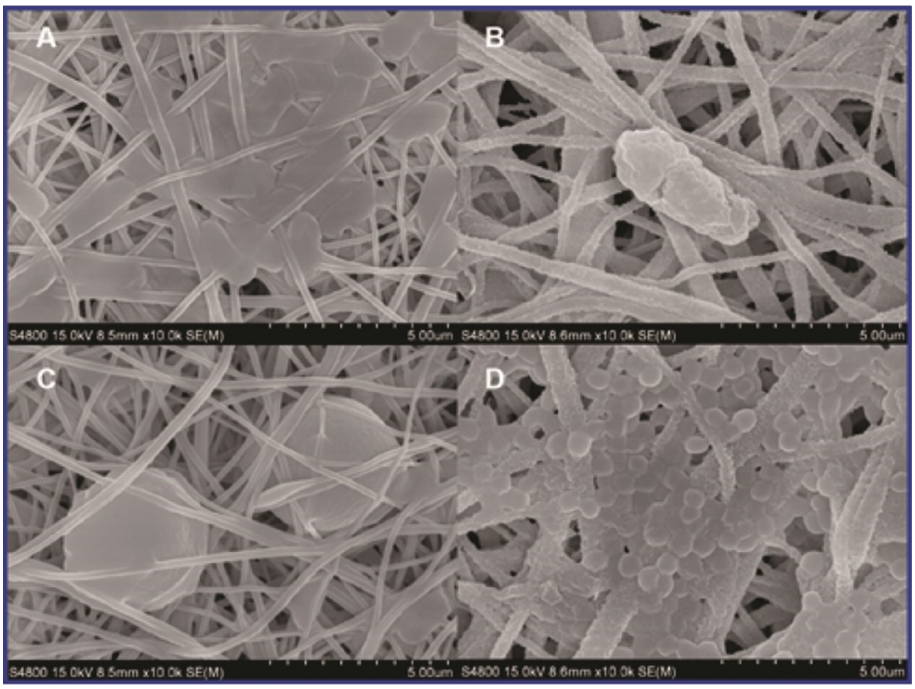

Fig. 8. SEM images of (A) *E. coli* on cellulose nanofibers; (B) *E. coli* on Au@AgNPs/cellulose nanofibers after interaction; (C) *S. aureus* on cellulose nanofibers and (D) *S. aureus* on Au@AgNPs/cellulose nanofibers after interaction.

being treated with cellulose/Au@AgNPs nanofibers than pure gauze and cellulose nanofibers. Because of bactericide activity of free Ag^{+}, it was noted slight inflammation and epithelium shriveling in the cellulose/Au@AgNPs on the seventh day after being treated. Afterward, with care for 12 days, substantial injury terminate and absolute re-epithelialization was evident in the cellulose/Au@AgNPs nanofibers, similar to pure gauze and cellulose nanofibers. A crude and weakened skin was shaped on the seventh day. Moreover, pure gauze and cellulose nanofibers depicted injury. Hence, injuries treated with electrospun cellulose/Au@AgNPs nanofibers demonstrated good fluid retentivity when equated to that treated with pure gauze and virgin cellulose nanofibers. The study determined that electrospun nanofibers were simpler to be treated than pure gauze. Injury curing is a dificult procedure demanding the cooperative exertions of many unlike tissues and cell lineages [42]. On the twelfth day, the rats were killed to execute a toxicologic investigation of tissue and organs shown in Fig. 10. There is no

Table 1. Size of animal wound

Days	Gauze (cm)	CNFs (cm)	Ag@AuNPs/CNFs (cm)
1	0.8	0.8	0.8
3	0.7	0.7	0.7
5	0.6	0.6	0.55
7	0.6	0.6	0.4
10	0.5	0.4	0.2
12	0.3	0.2	0 or healed

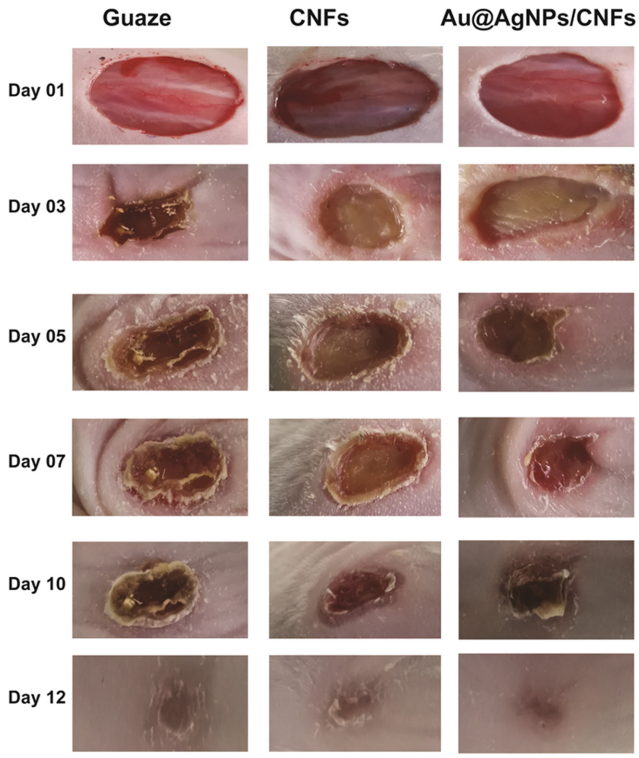

Fig. 9. Wound healing effect of gauze, Regenerated cellulose nanofibers and Ag@AuNPs incorporated regenerated cellulose nanofibers.

evident cytotoxicity noticed for the key organs, considering heart, liver, spleen, lung, and kidney. This is due to the bactericidal attributes of the Ag^{+} that suppress the seditious reaction across the in0 jured region. At 12 days after first handling of cellulose/Au@AgNPs nanofibers group, skin injuries were entirely recovered with a new epithelium and size of wound from day 1 to day 12 shown in Table 1.

CONCLUSIONS

The skin encounters the environment directly and it is the most prominent organ of the body. Therefore, the skin is dangerous to respective harm, especially wound healing. Thus, in this work, cellulose acetate electrospun nanofibrous material was synthesized for antibacterial activity and wound healing. The Au@AgNPs were decorated by dipping method on the surface of cellulose nanofibers to construct effective wound dressing material. Additionally, Au@AgNPs was characterized by UV-Vis spectra, SEM and TEM. The cellulose/Au@AgNPs e-spun nanofibrous material was detected to obtain suitable bactericide action against Gram negative and Gram positive bacterium. The combination of noble metals would be a better choice for biomedical applications such as Au@AgNPs. Furthermore, in the finding, cellulose/Au@AgNPs has great antibacterial activity and wound healing efficiency.

ACKNOWLEDGEMENT

This work was funded by the National Natural Science Foundation of China (grant number 21673201), top-notch Academic Programs Project of Jiangsu Higher Education Institutions of China (TAPP) and the Priority Academic Program Development of Jiangsu Higher Education Institutions of China.

CONFLICT OF INTEREST STATEMENT

It is stated that authors declare no competing financial or conflict of interest.

REFERENCES

1. D. Li, J. T. McCann and Y. Xia. *Small*, **1**, 83 (2005).
2. J. Wang, H. B. Yao, D. He, C. L. Zhang and S. H. Yu, *ACS Appl. Mater. Interf.*, **4**, 1963 (2012).
3. K. Saetia, J. M. Schnorr, M. M. Mannarino, S. Y. Kim, G. C. Rutledge and T. M. Swager, *Adv. Function. Mat.*, **24**, 492 (2014).
4. B. Zhou, Y. Li, H. Deng, Y. Hu and B. Li, *Colloid. Surf. Biointerf.*, **116**, 432 (2014).
5. A. W. Jatoi, I. S. Kim and Q. Q. Ni, *Carbohyd. Polym.*, **207**, 640 (2019).
6. H. Lee, M. Nishino, D. Sohn, K. S. Lee and I. S. Kim, *Cellulose*, **25**,

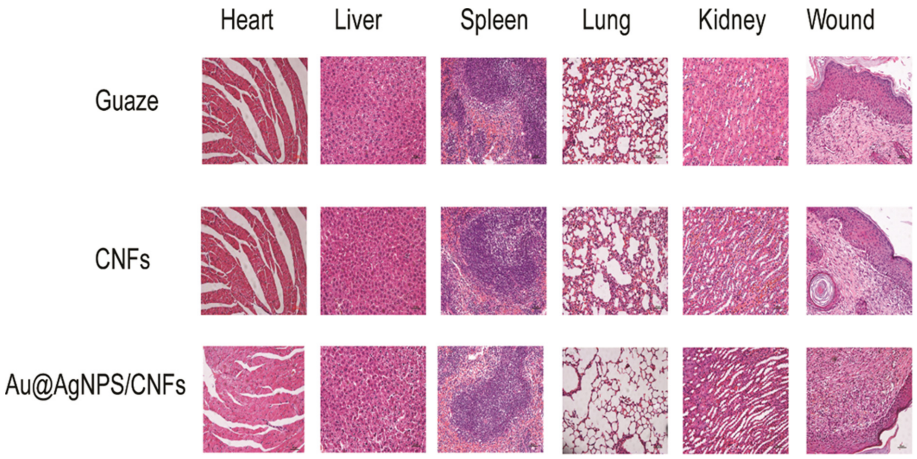

Fig. 10. The histologic analyses.

2829 (2018).
7. F. Truffa Giachet, M. Periolatto, D. O. Sanchez Ramirez, R. A. Carletto, A. Varesano and C. Vineis, *Textil.*, **48**, 384 (2019).
8. A. D. Fuchs and J. C. Tiller, *Angew. Chem. Int. Ed.*, **45**, 6759 (2006).
9. P. Kurt, L. Wood, D. E. Ohman and K. J. Wynne, *Langmuir*, **23**, 4719 (2007).
10. X. Liu, T. Lin, Y. Gao, Z. Xu, C. Huang and G. Yao, *Appl. Biomater.*, **100**, 1556 (2012).
11. L. Yue, F. Liu, S. Mekala, A. Patel, R. A. Gross and I. Manas-Zloczower, *ACS Sustain. Chem. Eng.*, **7**, 5986 (2019).
12. R. Konwarh, N. Karak and M. Misra, *Biotechnol. Adv.*, **31**, 421 (2013).
13. W. K. Son, J. H. Youk and W. H. Park, *Carbohyd. Polym.*, **65**, 430 (2006).
14. R. Wang, Y. Yao, M. Shen and X. Wang, *Colloid. Surf. Physicochem. Eng. Aspect.*, **492**, 263 (2016).
15. G. Zhang, H. Zheng, M. Shen, L. Wang and X. Wang, *J. Phys. Chem. Solid.*, **81**, 79 (2015).
16. S. G. Priya, H. Jungvid and A. Kumar, *Tiss. Eng. Rev.*, **14**, 105 (2008).
17. S. B. Levy and B. Marshall, *Nat. Medicine.*, **10**, S122 (2004).
18. W. Bing, Z. Chen, H. Sun, P. Shi, N. Gao and J. Ren, *Nano Res.*, **8**, 1648 (2015).
19. Z. M. Xiu, Q. B. Zhang, H. L. Puppala, V. L. Colvin and P. J. Alvarez, *Nano Lett.*, **12**, 4271 (2012).
20. W. Li, K. Dong, J. Ren and X. Qu, *Ang. Chem. Int. Ed.*, **55**, 8049 (2016).
21. Z. Wang, K. Dong, Z. Liu, Y. Zhang, Z. Chen and H. Sun, *Biomaterials*, **13**, 145 (2017).
22. S. Kittler, C. Greulich, J. Diendorf, M. Koller and M. Epple, *Chem. Mater.*, **22**, 4548 (2010).
23. L. Lu, H. Wang, Y. Zhou, S. Xi, H. Zhang and J. Hu, *Chem. Comm.*, **2**, 144 (2002).
24. K. Kalwar, M. A. Bhutto, L. Dali and D. Shan, *Mater. Res. Exp.*, **4**, 105405 (2017).
25. K. Kalwar, L. Hu, L. D. Li and D. Shan, *Polym. Adv. Technol.*, **29**, 394 (2018).
26. M. Banerjee, S. Sharma, A. Chattopadhyay and S. S. Ghosh, *Nanoscale*, **3**, 5120 (2011).
27. T. Laksanasopin, T. W. Guo, S. Nayak, A. A. Sridhara, S. Xie and O. O. Olowookere, *Sci. Translational Med.*, **7**, 273 (2015).
28. T. T. Tsai, T. H. Huang, C. J. Chang, N. Y. J. Ho, Y. T. Tseng and C. F. Chen, *Scientif. Rep.*, **7**, 3155 (2017).
29. V. Prabhawathi, P. M. Sivakumar, T. Boobalan, C. M. Manohar and M. Doble, *Mater. Sci. Eng. C.*, **94**, 656 (2019).
30. S. Pennycook and L. Boatner, *Nature*, **336**, 565 (1988).
31. S. Chen and D. L. Carroll, *J. Physical Chem.*, **108**, 5500 (2004).
32. R. Jin, Y. Cao, C. A. Mirkin, K. Kelly, G. C. Schatz and J. Zheng, *Science*, **294**, 1901 (2001).
33. T. J. Norman, C. D. Grant, D. Magana, J. Z. Zhang, J. Liu and D. Cao, *J. Physical Chem.*, **106**, 7005 (2002).
34. X. Tian, W. Wang and G. Cao, *Mater Lett.*, **61**, 130 (2007).
35. Y. An and R. Friedman, *J. Hosp. Infect.*, **33**, 93 (1996).
36. K. Kalwar, W. X. Sun, L. D. Li, X. J. Zhang and D. Shan, *React. Function. Polym.*, **107**, 87 (2016).
37. S. H. Jeong, Y. H. Hwang and S. C. Yi, *J. Mater. Sci.*, **40**, 5413 (2005).
38. D. Lee, R. E. Cohen and M. F. Rubner, *Langmuir*, **21**, 9651 (2005).
39. J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri and J. T. Ramírez, *Nanotechnol.*, **16**, 2346 (2005).
40. A. Panáček, L. Kvitek, R. Prucek, M. Kolář, R. Večeřová and N. Pizúrová, *J. Physical Chem.*, **110**, 16248 (2006).
41. A. Meddahi-Pellé, A. Legrand, A. Marcellan, L. Louedec, D. Letourneur and L. Leibler, *Ang. Chem. Int. Ed.*, **53**, 6369 (2014).
42. P. Martin, *Science*, **276**, 75 (1997).