J Med Sci 2013;33(6):327-332
http://jms.ndmctsgh.edu.tw/3306327.pdf
DOI:10.6136/JMS.2013.33(6).327

Silver Nanoparticle-loaded Activated Carbon Cloth for Antimicrobial Applications

Tsung-Kun Lin[1], Rin-Hsiuang Ma[2], I-Ting Chen[3], Chiao-Hsi Chiang[3], and Jiin-Long Chen[3,4*]

[1]*Department of Pharmacy Practice, Tri-service General Hospital, National Defense Medical Center,Taipei;*
[2]*Chung Shan Institute Science and Technology, Taoyuan;*
[3]*School of Pharmacy, National Defense Medical Center, Taipei;*
[4]*Institute of Preventive Medicine, National Defense Medical Center, New Taipei, Taiwan, Republic of China*

Silver nanoparticle-loaded activated carbon cloths are prepared using a hydro-gel formula in this study. The final amounts of silver nanoparticle loaded range from 24.3 to 180.3 μg/25 cm^2 for the various formulas that inhibit bacterial growth by dynamic contact. For a static contact with Staphylococcus aureus, a 0.01% glycerin formula shows a good disinfectant effect, killing more than 99% of the bacteria after 24 h of contact. Moreover, after 1 h of water dipping, the formulas maintain their static-contact disinfectant effect. As broad-range disinfectants, the formulas are suitable for such diverse applications as water filters, masks, protective clothes and wound dressing.

Key words: Silver nanoparticle, activated carbon cloth, hydro-gel, disinfectant

INTRODUCTION

Silver has long been used as an anti-microbial reagent against a broad range of microorganisms, including Gram-positive, Gram-negative, yeast and fungi. In 1700, silver nitrate was used for the treatment of venereal diseases. In 1884, Crede used a 1% silver nitrate solution to treat ophthalmia neonatorum. Silver sulfadiazine has been used as an anti-bacterial agent for wound care for more than half a century.[1-3] Silver not only exhibits an antibacterial property on acute wounds, but also an anti-inflammatory effect on chronic wounds.[4]

In most accepted theories, silver ion plays a dominant role in the anti-microorganism activity of silver compounds, including silver sulfadiazine, silver salts and silver nanoparticle (silver NP). Silver ion interacts with thiol groups of proteins in the cell wall of a microorganism, thus causing cell wall damage. Subsequently, silver ion interacts with nucleic acid which inhibits the growth of or results in the death of microorganisms.[5-8]

Silver ion is highly toxic to bacteria. However, silver ion is easily removed by contact with a wound. Silver NP has also been used in anti-microbial applications. Silver ion is released from silver NP after the silver is oxidized and becomes silver oxide. The solubility of silver oxide is about 25 ppm.[9] The minimum inhibitory concentration (MIC) of silver NP when used as an anti-microorganism agent is less than 25 ppm.[10-12]

Activated carbon cloth (ACC) is highly porous and has a large surface area. It is used in filters or masks for removing water or air contaminants. ACC is also employed to make chemical-resistant clothing worn for protection against toxic gas. However, one drawback of ACC is the growth of microorganisms. In order to overcome this drawback, this study incorporated silver NP onto ACC and examined the effectiveness of using a water-soluble polymer as a silver NP carrier attached on ACC to inhibit growth of microorganisms.

MATERIALS AND METHODS

Materials

ACC was purchased from Taiwan Carbon Technology Co. Ltd. (1000-2300 m^2/g, Taichung, Taiwan). Methyl cellulose (MC, 25 cps), hydropropyl MC (HPMC, 4000 cps), sodium carboxymethyl cellulose (sodium CMC, 400-800 cps), sodium citrate, glycerin and ferrous sulfate were purchased from Sigma-Aldrich (St. Louis, MO,

Received: June 1, 2013; Revised: August 13, 2013; Accepted: October 8, 2013
*Corresponding author: Jiin-Long Chen, Institute of Preventive Medicine, National Defense Medical Center, Dapu, P.O. BOX 90048-700, Sanxia 237, New Taipei City, Taiwan, Republic of China. Tel: +886-2-81777038 ext 19910; Fax: +886-2-26736954; E-mail: cjl@mail.ndmctsgh.edu.tw; cjl531102@gmail.com

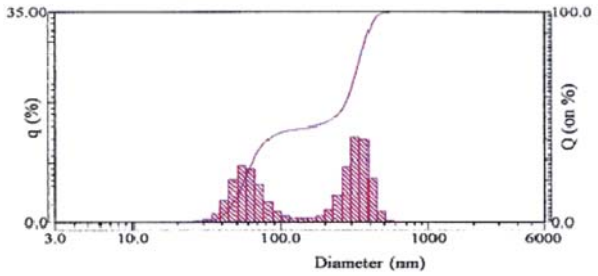

Fig. 1 Sizes of silver NP prepared

Table 1. Formulas of silver NP hydro-gel used on ACC

Formula of hydro-gel	H10	H20	M10	M20	C10	C20
Polymer	0.1% HPMC	0.1% HPMC	0.1% MC	0.1% MC	0.1% sodium CMC	0.1% Sodium CMC
Glycerin	0.01%	0.02%	0.01%	0.02%	0.01%	0.02%
Silver NP (ppm)	1000	1000	1000	1000	1000	1000

*1 mL hydro-gel was sprayed on 5×5 cm ACC

U.S.A). Other reagents were of analytical or reagent grade.

Preparation of silver NP

First, 98 mL of 40% sodium citrate aqueous solution was mixed with 70 mL of 30% ferrous sulfate solution under magnetic stirring. After 10 min of agitating, 70 mL of 10% silver nitrate was added drop by drop. The mixed solution was centrifuged at 6000 rpm for 1 h. The supernatant was poured out, and the remaining solid was mixed with 112 mL of 0.4M sodium nitrate and subjected to a 200 W ultrasonic shock for 50 min. The mixed solution was centrifuged at 6000 rpm for 1 h, and then the supernatant was poured out. The remaining solid was mixed with 60 mL of Milli-Q water and subjected to a 200 W ultrasonic shock for 1 h. The sizes of the silver NP prepared are shown in Figure 1, and the final concentration of silver NP was 1000 ppm as diluted by Milli-Q water.

Determination of silver content

One mL of silver sample and 20 mL of nitric acid were mixed and boiled for 10 min. The sample was cooled under ambient temperature and mixed with Milli-Q water to reach a total weight of 50 g. After filtrating, the filtrate was measured by atomic absorption spectroscopy (AA).

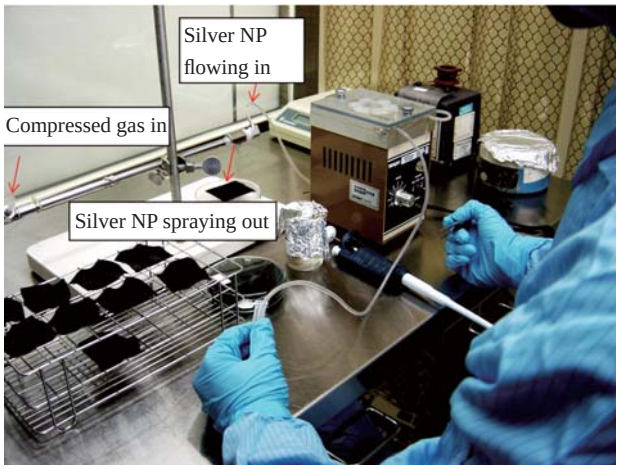

Fig. 2 Preparation of silver NP-ACC. Hydro-gel aqueous solution containing silver NP was sprayed on a 25 cm^2 ACC. The cloth was dried under ambient temperature for 48 h.

Determination of water content and surface area

The water content of the sample was evaluated by the weight loss method under heating at 110 ^oC in a vacuum for 3 h. The surface area of the silver NP-loaded ACC (silver NP-ACC) was measured by the Chung Shan Institute of Science and Technology using the Brunauer-Emmett-Teller (BET) gas adsorbed surface area method.

Preparation of silver NP-ACC

First, 0.02 g of HPMC, CMC or MC was dissolved in 20 mL of the aqueous solution containing 1000 ppm silver NP. Glycerin was used as the plasticizer (hydro-gel polymer:glycerin = 10:1 or 5:1 w/w). Table 1 lists all formulas. After autoclaving, 1 mL of the silver NP/hydro-gel solution was sprayed on a 5 cm×5 cm ACC, as shown in Figure 2. The cloth was dried under ambient temperature for 48 h.

Silver NP release test

A 5 cm x 5 cm silver NP-ACC was put in a magnetic agitator flask containing 20 mL of Milli-Q water. Then, 3 mL of supernatant was withdrawn and 3 mL of fresh medium was added. The withdrawn sample was measured by AA. All formulas were measured in triplicate.

Dynamic contact test

Culture broth was prepared using 5 g of peptone, 3 g of beef extract and Milli-Q water to a final volume 1000 mL. The pH value was adjusted to 6.8±0.1 by adding 1 N NaOH solution.

Table 2 Water and silver NP contents of silver NP-ACC after drying 48 h under ambient temperature inside laminar-flow hood

Formulas	Water content (%)ean±SD	Silver content (μg) ean±SD	Loading efficiency (%)	Surface area* (m^2/g)
Pure water	0.12±0.01	---	---	---
Original silver NP	---	693±2	100	---
H10	0.13±0.03	67.5±2.5	9.7	890
H20	0.21±0.06	180.3±13.0	26.0	620**
M10	0.11±0.02	24.3±0.5	3.5	961
M20	0.23±0.01	96.5±4.3	13.9	690**
C10	0.12±0.01	29.3±1.0	4.2	1028
C20	0.30±0.02	126.3±2.5	18.2	750**

*The original surface area of ACC was 1250 (m^2/g).

**The criterion of ACC surface area was > 850 (m^2/g).

*** Loading efficiency was calculated using the silver content of the formula/original silver NP content.

Culture agar was prepared using 5 g of peptone, 3 g of beef extract, 15 g of agar and Milli-Q water to a final volume 1000 mL. The pH value was adjusted to 7.1±0.1 by adding 1 N NaOH solution.

Staphylococcus aureus (ATCC 29213) was freshly cultured in culture broth with a final concentration of 2×10^5 cfu/mL. A 5 cm×5 cm ACC with/without silver NP was added to 100 mL of Staphylococcus aureus broth and shaken at 50 rpm. Then, 0.1 mL of the sample broth was withdrawn at suitable time intervals and dispersed on a culture agar plate. The plate was cultured at 37 °C for 24 h and the number of colony forming units (cfu) was counted.

Static contact test

Staphylococcus aureus (ATCC 29213) was freshly cultured in a culture broth with a final concentration of 2×10^7 cfu/mL. Then, 0.5 mL Staphylococcus aureus broth was absorbed by a 5 cm×5 cm ACC with/without silver NP and cultured at room temperature for 10 s (0 h) or 24 h. The remaining bacteria were washed out by 100 mL PBS, and a 0.1 mL PBS sample was dispersed on an agar culture plate. The plate was cultured at 37 °C for 24 h and the number of cfu was counted.

Static contact test for water dipped silver NP-ACC

A 5 cm×5 cm ACC with/without silver NP was dipped in 10 mL of bacteria-free water for 1 h. The cloths were taken out, dried, and then used in the static contact test described previously.

RESULTS

Characteristics

The final water content of each formula is shown in Table 2. ACC is porous and has a large surface area. In the control group, ACC was sprayed with 1 mL of pure water. The final water content of the ACC when dried was 0.12%. The water contents of the H10, M10 and C10 groups with a glycerin content of 10% polymer were 0.13%, 0.11% and 0.12%, respectively, which were close to the result of the control group. Following the increase of glycerin to 20%, the water contents of the H20, M20 and C20 groups also rose to 0.21%, 0.23% and 0.30%, respectively. The silver NP loading of each formula shared the same tendency as that of water content (H20 > H10, M20 > M10, and C20 > C10). The silver NP loading was also related to the viscosity of the polymer used. The viscosity of the three polymers was in the order of HPMC (4000 cps, 2% in water at 20 °C) > Sodium CMC (400-800 cps, 2% in water at 25 °C) > MC (25 cps, 2% in water at 20 °C). The silver NP loading also showed the same order (H20 > C20 > M20, H10 > C10 > M10).

After being dipped in pure water, the silver NP released from each formula was collected and analyzed by AA. The results shown in Figure 3 present the total silver, including both silver NP and silver ion, in the medium. As seen in the plots, the 0.02% glycerin formula (20% of polymer) released a lower amount of silver than the 0.01% glycerin formula (10% of polymer) (amount of silver released: H10 > H20, M10 > M20, C10 > C20). The amount of silver loaded in the 0.02% glycerin formula exceeded that of the 0.01% glycerin formula. These phenomena may have resulted from the higher adhesive property of the 0.02% glycerin formula. A large surface area allows the ACC to adsorb anything from the environment, such as polluted air. However, the surface area was seriously reduced in the 0.02% glycerin formula, as shown in Table 2.

Dynamic contact with bacteria

The original Staphylococcus aureus solution of 1 mL 2.1×10^7 cfu/mL was diluted to 100 mL with culture medium to a final concentration of 2.1×10^7 cfu/100 mL for use in the dynamic contact test. In the control group without silver NP loading, bacteria grew from 2.1×10^7 cfu/100 mL (1 h) to 4.5×10^9 cfu/100 mL (4 h), an increase of two orders in 3 h. Between the six formulas, there were large differences on silver loading (such as

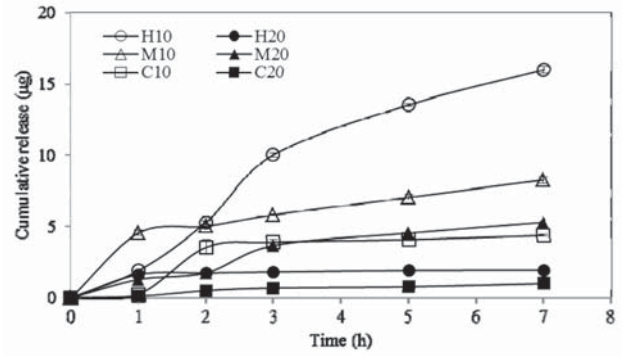

Fig. 3 Cumulative amount of silver NP released on NP-ACC (n = 3). The cumulative release of silver presented the total silver, including both silver NP and silver ion, in the medium. In each formula, 0.01% glycerin or 0.02% glycerin was used (Formula code H, M, C means HPMC, MC, sodium CMC. 10 or 20 indicates 0.01% or 0.02% glycerin/0.1% polymer = 10% or 20%). From the plots, the amount of silver released using the 0.01% glycerin formula was obviously higher than that of the 0.02% glycerin formula (amount of silver released: H10 > H20, M10 > M20, C10 > C20). Even the silver loaded in the 0.02% glycerin formula exceeded that of the 0.01% glycerin formula.

H20/M10 = 7) and silver releasing (such as H10/C20 = 14 at 4 h). However, the dynamic contact results of the six formulas were similar; that is, the amount of bacteria remained almost the same for 1 to 4 hours, as shown in Table 3. The dynamic contact test result suggested that the growth of Staphylococcus aureus was inhibited, but the bacteria were not killed. Staphylococcus aureus is easily killed by silver ion. As shown in Figure 3, the total silver released was predominantly silver NP and not silver ion.

Static contact with bacteria

Surface area of formulas affects the function against toxic gas. That of the three formulas with 0.02% glycerin were less than 850 m^2/g as shown in table 2. Only the H10, M10 and C10 formulas were evaluated using the following static contact test. The original Staphylococcus aureus solution of 0.5 mL 2.1×10^7 cfu/mL was adsorbed by silver NP-ACC. After contact of 0 h (10 sec) and 24 h, the bacteria-adsorbed cloth was washed using 100 mL of PBS. The amount of bacteria remaining in the washing solution was measured, as shown in Table 4. Following 24 h of contact, more than 99% of the Staphylococcus aureus was killed by all three formulas. In this test, some

Table 3 Dynamic contact effect of silver NP-ACC on Staphylococcus aureus

Formulation	Total Staphylococcus aureus (cfu)			
	1 hr	2 hr	3 hr	4 hr
Bacteria only	3.7×10^7	9.1×10^7	5.3×10^8	4.5×10^9
H10	9.8×10^6	9.8×10^6	6.7×10^6	5.2×10^6
H20	9.5×10^6	7.6×10^6	7.6×10^6	7.4×10^6
M10	9.8×10^6	9.5×10^6	6.5×10^6	5.4×10^6
M20	1.0×10^7	1.0×10^7	8.9×10^6	8.9×10^6
C10	9.8×10^6	9.9×10^6	8.8×10^6	8.9×10^6
C20	1.5×10^7	1.6×10^7	1.3×10^7	1.4×10^7

Staphylococcus aureus contacts with silver NP-ACC in a shaking bottle. The original Staphylococcus aureus concentration was 2.1×10^7 cfu/mL. After one mL of the medium containing the bacteria was diluted with 100 mL medium, silver NP-ACC was added and the bottle was shaken at room temperature. A control group (coded as bacteria only), bacteria without ACC, was also cultured in the same condition for monitoring the growth of bacteria.

Table 4 Static contact effect of silver NP-ACC on Staphylococcus aureus

Formula	Total Staphylococcus aureus (cfu)	
	0 hr	24 hr
H10	4.8×10^6	2.0×10^4
M10	9.3×10^6	0
C10	3.4×10^6	0

0.5 mL 2.1×10^7 cfu/mL Staphylococcus aureus was penetrated into silver NP-ACC for 0 and 24 h. The remaining bacteria were washed out and the amount was determined.

silver NP may be washed from the cloth during measurement. However, the influence of the silver NP thus washed away could be ignored, as suggested from the results of the release test (Figure 3) and the dynamic contact test (Table 3).

In order to evaluate whether the silver NP-loaded formulas could still kill bacteria after washing, the formulas were gently agitated in 10 mL of bacteria-free water for 1 h, then taken out and dried. These dried formulas were used in the static contact test. The results in Table 5 show that more than 99% of the Staphylococcus aureus was killed within 24 h of contact by all three formulas.

DISCUSSION

Silver has long been used as an antimicrobial agent. Moreover, the appearance of antibiotic-resistant bacteria has renewed the focus on the effect of silver against

Table 5. Static contact effect of washed silver NP-ACC with Staphylococcus aureus

Formula	Total Staphylococcus aureus (cfu)	
	0 hr	24 hr
H10	1.5×10^{7}	0
M10	4.1×10^{6}	0
C10	1.1×10^{7}	6.0×10^{4}

Silver NP-ACC was dipped in 10 mL bacteria-free water for 1 h. After drying, 0.5 mL 2.1×10^{7}cfu/mL Staphylococcus aureus was penetrated into the washed silver NP-ACC for 0 and 24 h. The remaining bacteria were washed out and the amount was determined.

multi-drug-resistant bacteria.[13] Silver is not only helpful on acute wounds, but it also reacts with matrix metalloproteinase which assists in the healing of chronic wounds.[4] The spraying method and the dipping method are most commonly used for the silver coating process on cloth in industry. The high pressure of the spraying caused the low loading efficiency in this study. Owing to the higher viscosity with a higher resistance against the high pressure, the silver NP loading seemed to be related to the viscosity of the hydro-gel used in the formulas. However, the higher polymer content in the formulas (H20, M20 and C20) showed a stronger sealing of the surface pores in silver NP-ACC, leading to reduction of the surface area, as shown in Table 2.

Silver is also a well-known antimicrobial reagent against a broad range of microorganisms, including Gram-positive, Gram-negative, yeast and fungi.[1] Brady et al. (2003)[14] reported that silver and quaternary ammonium disinfectants had a significant effect against Staphylococcus aureus, but only silver was effective against Pseudomonas. Jo *et al*. (2009)[6] reported that 100% of a rice blast fungus, Magnaporthe grisea, was killed using 5 ppm silver NP for 1 h.

The results of this study indicated that silver NP-loaded products showed a bacterial inhibitory effect in the dynamic contact test and a disinfectant effect in the static contact test. Silver ion plays a dominant role in antimicroorganisms from silver compounds; however, both solubility and diffusibility of silver NP are low. Petrus et al. (2011)[11] reported that the minimum inhibitory concentration (MIC) of silver NP was 2-10 ppm for Staphylococcus aureus strains SA2b, SA22a, SA37a, SA5a and 50 ppm for Staphylococcus aureus strain SA25c when using a combination of silver NP in the bacterial culture media. Ruparelia et al. (2008)[15] reported that the minimum inhibitory concentration (MIC) of silver NP was 120 ppm for Staphylococcus aureus strains NCIM 2079, 5021 and 5022 obtained as using the disk diffusion test. These results matched the current observations as to the disinfectant effect of static contact.

CONCLUSION

This study prepared successfully silver NP-ACC with a good static-contact disinfectant effect and a large surface area in ACC using the hydro-gel formula. The NP-ACC could be applied to water filters, masks, protective clothes and wound dressings as a broad-range disinfectant.

DISCLOSURE

All authors declare no competing financial interests.

ACKNOWLEDGEMENTS

This study was supported in part by grants TSGH-C98-97 and 96VCAA4V-D.

REFERENCES

1. Lansdown A. Silver. I: Its antibacterial properties and mechanism of action. J Wound Care 2002;11:125-130.
2. Rai MK, Yadav A, Gade AK. Silver nanoparticles as a new generation of antimicrobials. Biotech Adv 2009;27:76-83, doi: 10.1016/j.biotechadv.2008.09.002.
3. Prabhu S and Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters 2012;2:32-41.
4. Dowssett C. The use of silver-based dressings in wound care. Nursing Standard 2004;19:56-60.
5. Lansdown A. A review of the use of silver in wound care: facts and fallacies. British J Nursing 2004;13:s6-s19.
6. Jo Y-K, Kim B-H, and Jung G. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis 2009;93:1037-1043.
7. Assar NH, Hamuoda HM. Colloidal silver as a new antimicrobial agent. Int J Microbiol Res 2010;1:33-36.
8. Xiu Z-M, Zhang Q-B, Puppala HL, Colvin VL, Alvarex PJJ. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett 2012;12:4271-4275, doi: 10.1021/nl301934w.
9. http://en.wikipedia.org/wiki/silver_oxide, 2013.

10. http://microbewiki.kenyon.edu/index.php/silver_as_an_antimicrobial_agent, 2013.
11. Petrus EM, Tinakumari S, Chai LC, Ubong A, Tunung R, Elexson N, Chai LF, and Son R. A study on the minimum inhibitory concentration and minimum bactericidal concentration of nano colloidal silver on food-borne pathogen. Int Food Res J 2011;18:55-66.
12. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, and Park YH. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Appl Environ Microbio 2008;74:2171-2178, doi: 10.1128/AEM.02001-07.
13. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 2012;112:841-852, doi: 10.1111/j.1365-2672.2012.05253.x.
14. Brady MJ, Lisay CM, Yurkovetskiy AV, and Sawan SP. Persistent silver disinfection for the environmental control of pathogenic bacteria. Am J Inf Control 2003;31:208-214.
15. Ruparelia JP, Chatterjee AK, Duttagupta SP, and Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater 2008;4:707-716, doi: 10.1016/j.actbio.2007.11.006.