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Antibacterial and Antifungal Dressings Obtained by Photochemical Deposition of Silver Nanoparticles

Federica Paladini,[1] Serena De Simone,[2] Alessandro Sannino,[1] Mauro Pollini[1]

[1]Department of Engineering for Innovation, University of Salento, 73100, Lecce, Italy

[2]Dhitech Scarl, Technological District Hi-Tech, 73100, Lecce, Italy

Correspondence to: Mauro Pollini (E-mail; mauro.pollini@unisalento.it).

ABSTRACT: The antimicrobial activity of silver against a wide spectrum of bacteria, fungi and viruses is known since antiquity. Silver has been used as topical antimicrobial agent in the treatment of wounds since many years and advanced silver-based dressings have been designed so far. The aim of this study was the development of low-cost antibacterial and antifungal dressings through the surface modification of conventional cotton gauzes. Different percentages of silver were deposited on textile substrates by adopting an innovative silver deposition technique based on the photochemical deposition of silver nanoparticles. The uniformity of the coating and the distribution of the silver clusters on the surface were evaluated by scanning electron microscopy (SEM). The amount of silver was quantified by thermogravimetric analysis (TGA) and the presence of metallic silver nanoparticles was also assesses through UV–Vis–NIR with integrating sphere and energy dispersive X-ray spectroscopy EDX. The effectiveness of the silver treated textile was verified on different microorganisms, namely *Escherichia coli, Staphylococcus aureus, Pseudomonas p43* and *Candida albicans* derived from a clinical isolate. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40326.

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INTRODUCTION

Chronic and acute dermal wounds are susceptible to infection due to sterile loss of the innate barrier function of the skin.[1] The presence of bacteria and fungi in wounds can contaminate and infect, seriously delaying the wound healing.[2–4] Particularly in burn wounds, the rise in the frequency of fungal infections represents a great concern because fungal infections are associated to excessive lengths of hospitalization and a high mortality rate.[5]

Antibiotic-resistant bacteria also represent an increasing concern in the management of wound infections, as wound colonization by these organisms is often associated to an aggressive management of the wound and to a limited choice of therapeutic antibiotics.[6] Wounds infected by antibiotic-resistant bacteria may cause further morbidity in the patient and result in additional treatment costs.[7] The dressing most commonly adopted in hospital is represented by plain cotton gauze, because of the gas permeability and wicking properties. Indeed, cotton is extensively used as primary and secondary wound dressing and is still preferred because of the low cost and the absorbent properties.[8,9] However, the interest in new advanced products that promotes protection of the wound environment from microorganisms is growing, and silver has long been recognized as a potential in the treatment of wounds.[10] Some advantages, such as the reduction of wound exudate and of bioburden levels in chronic wounds have been associated to nanosilver.[11] No *in vivo* evidence suggested the toxicity of the nanocrystalline silver to skin cells such as keratinocytes and fibroblasts.[12] Moreover, the strong bactericidal activity of silver against gram positive and gram negative bacteria,[13] including multiresistant bacterial strains has been widely demonstrated.[14,15] Even if not yet fully elucidated, different mechanisms of action of silver nanoparticle have been reported in literature.[16] Silver nanoparticles have demonstrated ability to penetrate the bacterial cell wall and to affect the vital functions of the microorganism.[17] The formation of free radicals, the generation of reactive oxygen species, the interaction with thiol groups, the inhibition of both respiration and DNA replication are some of the effects induced by silver nanoparticles on bacteria.[18,19] Silver ions also demonstrated a fungicide action against certain fungal strains such as *Candida albicans*.[20–22]

In this work, the use of silver-coated cotton gauzes is proposed in the management of wound infections associated to bacterial and fungal colonization. The antimicrobial dressings were produced by a silver deposition technology based on the photochemical deposition of silver particles on the cotton substrate.[23]

The distribution of the silver particles and the uniformity of the silver coating were evaluated by scanning electron microscopy (SEM). The presence of silver on the cotton fibers was also assessed by Energy Dispersive X-Ray spectroscopy EDX and by spectrophotometric analysis UV–Vis–NIR with integrating sphere. The amount of silver on the substrate was quantified by thermogravimetric analysis (TGA). The antibacterial capability of the silver treated gauzes was evaluated by agar diffusion tests and bacterial enumeration on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas p43*. The antifungal capability was also evaluated on *Candida albicans*. In order to simulate the specific application of the materials, the dressings were also tested from a microbiological point of view after conditioning in artificial exudate.

MATERIALS AND METHODS

Cotton substrates commonly adopted as dry dressings were treated with silver according to an innovative technology based on the *in situ* photo-reduction of a silver solution. The silver treatment consisted of dipping the materials in an alcoholic silver solution and exposing the wet substrates to UV lamp for 20 min in order to induce the photo-reduction of the silver precursor and the formation of silver clusters on the surface of the material. In this work, two concentrations of silver were tested in order to determine if any difference resulted in the treated materials in terms of antibacterial and antifungal capability. Hence, two silver solutions were obtained by dissolving respectively 0.1 wt/v % and 0.5 wt/v % of silver nitrate in a mixture of 10% v/v methanol and deionized water. Cotton gauzes were dip coated in these silver solutions and, then, the wet substrates were immediately exposed to UV irradiation ($\lambda = 365$ nm, $t = 20$ min). Then, the samples were washed with water to remove any trace of unreacted salt and dried in oven at 50°C for 1 h. The silver treated substrates were analyzed by scanning electron microscopy SEM (Zeiss) to study the morphology of silver coating and the distribution of the silver clusters. Moreover, energy dispersive X-Ray spectroscopy EDX (Bruker) was also performed during the SEM analysis in order to confirm the presence of silver on the surface. UV–Vis–Near IR spectrophotometric analysis was also performed with a Varian Cary 5000 spectrophotometer with integrating sphere and Teflon disc as reference, in order to verify the presence of metallic silver nanoparticles deposited on cotton. The percentage of silver deposited was quantified through thermogravimetric analysis TGA (Mettler) by heating the untreated and silver treated samples from room temperature to 900°C with a nitrogen flow rate of 50 mL/min. The amount of silver deposited was calculated as a difference between the residual combustion products of the silver treated and untreated cotton.

Table I. Composition of the Artificial Exudate Prepared for Dressings Conditioning

Bovine albumin	3.3%
NaCl	0.58%
$NaHCO_3$	0.33%
KCl	0.03%
$CaCl_2$	0.03%

The antibacterial and antifungal activity of cotton gauzes treated with 0.1 wt/v % and 0.5 wt/v % of silver were verified according to Standard 'SNV 195920-1992' through agar diffusion tests on *E. coli DH5(α)*, *S. aureus S1*, *Pseudomonas p43* and *Candida albicans* isolated from a patient. Textile samples were UV sterilized for 30 min and placed in contact with the microorganisms on agar plates. Samples treated with the different silver percentages as well as the untreated sample as control were incubated for 24 h at 37°C. After the incubation time, the inhibition zone around and beneath the samples was evaluated, and the activity of the samples was defined according to the antibacterial degrees provided by the Standard. Hence, if the size of the bacteria free area around a sample results larger than 1 mm, the antibacterial activity of the sample can be labeled as "good". If no bacteria free area is visible, it is labeled as "insufficient".[24]

Quantitative antibacterial tests were also performed through the serial dilution method on each bacteria strain. In triplicate, samples (2 cm^2) of treated and untreated gauze as control were incubated in 4 mL of Luria Broth agar inoculated with 100 μL of *E. coli* suspension (inoculating cell density 1.5×10^7), in 4 mL of nutrient agar inoculated with 100 μL of *S. aureus* suspension (inoculating cell density 3×10^7 CFU/ml) and in 4 mL of

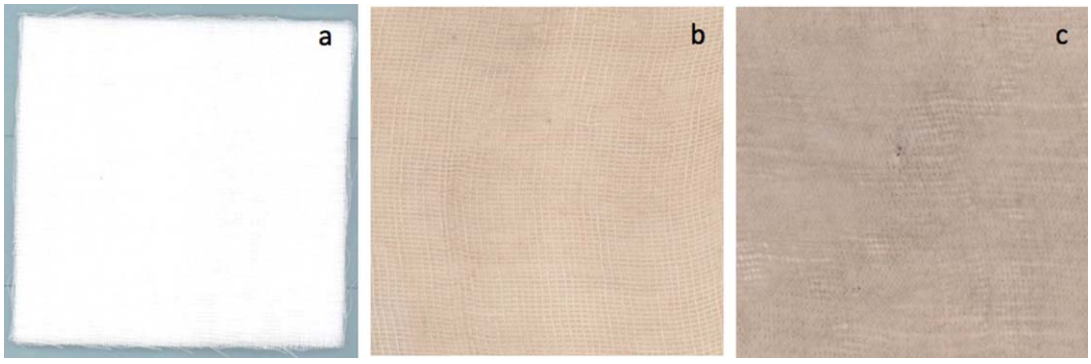

Figure 1. Visual comparison of untreated sample (a); cotton sample treated with 0.1 wt/v % Ag (b); cotton sample treated with 0.5 wt/v % Ag (c) showing the darkening of the substrate as function of the percentage of silver adopted for the treatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

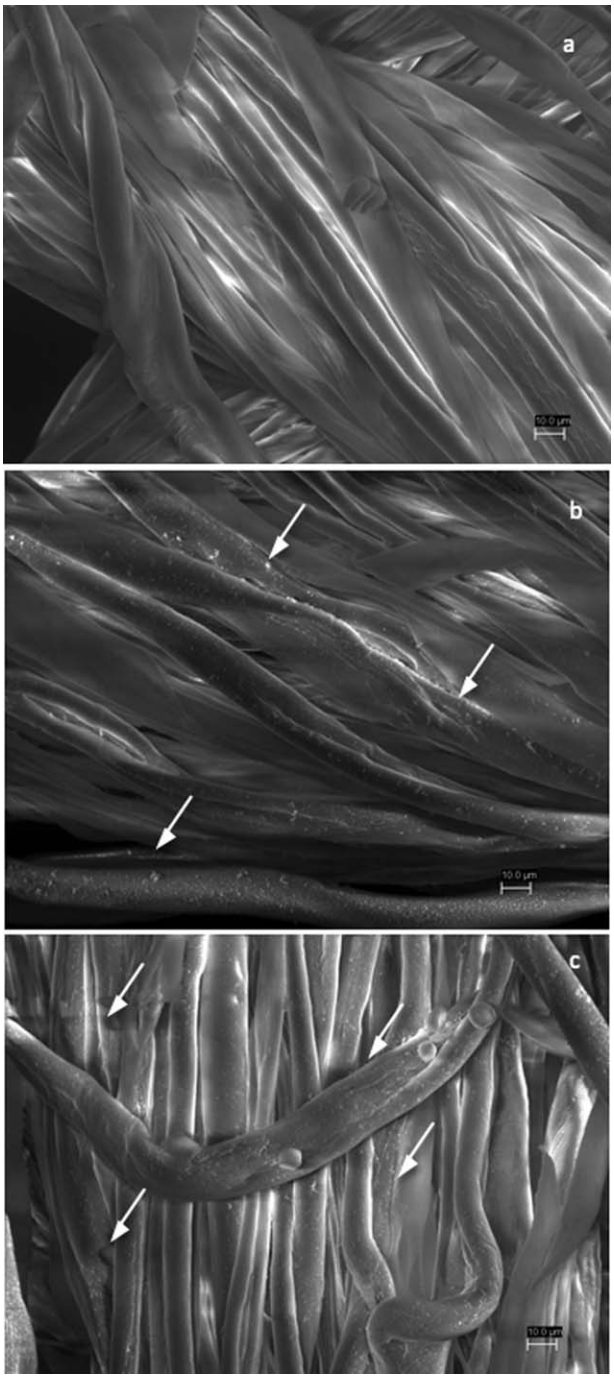

Figure 2. SEM analysis at 2000 magnifications on untreated cotton gauzes (a); cotton sample treated with 0.1 wt/v % Ag (b); cotton sample treated with 0.5 wt/v % Ag (c) showing the distribution of the silver particles on the cotton fibers.

nutrient agar inoculated with 100 µL of *Pseudomonas p43* suspension (inoculating cell density 6×10^7). After incubation for 24 h at 37°C, the samples were extracted from media and serial dilutions were performed in sterile phosphate buffered saline. From each dilution, 100 μL were extracted and plated on agar plates. The plates were incubated overnight at 37°C and the bacteria colonies grown on each plate were counted. The results were expressed as bacteria log reduction for each strain tested.

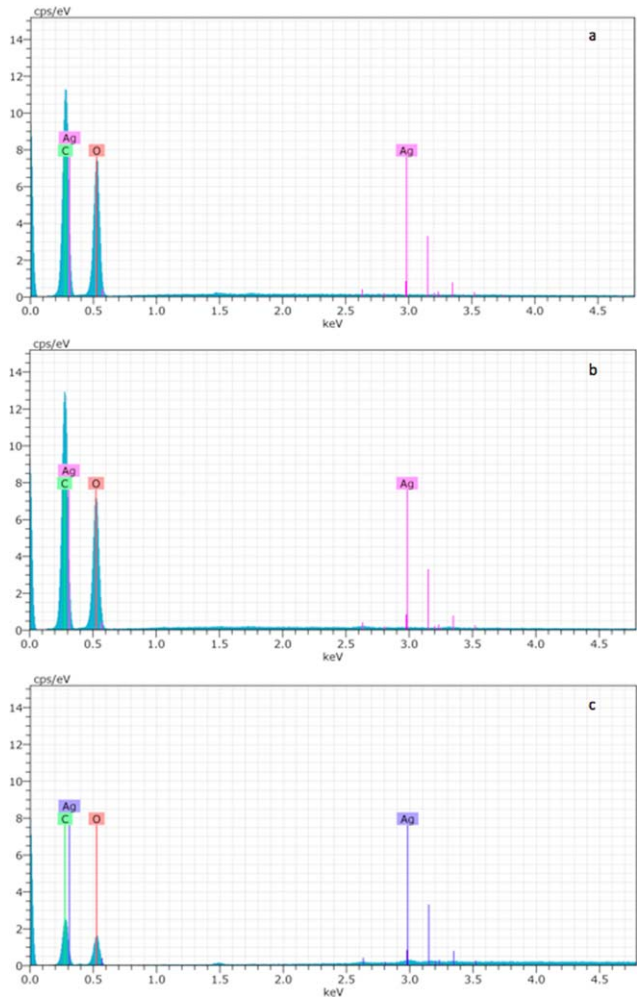

Figure 3. EDX analysis of untreated cotton gauze (a); cotton gauze treated with 0.1 wt/v % Ag (b); cotton gauze treated with 0.5 wt/v % Ag (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

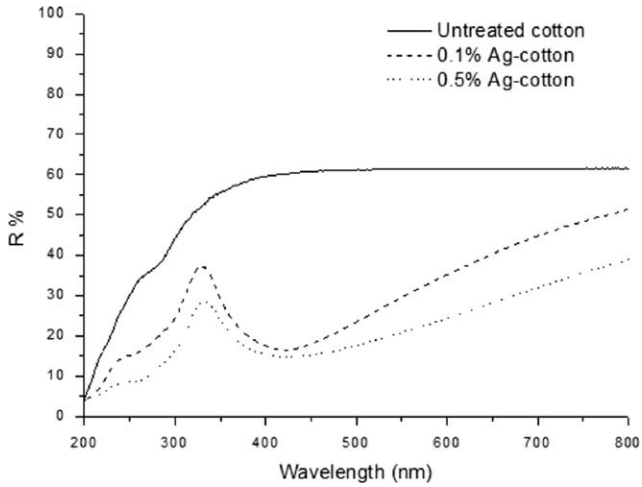

Figure 4. Reflection spectra of untreated cotton gauzes and cotton gauzes treated with different silver solutions, showing the presence of metallic silver on the substrate.

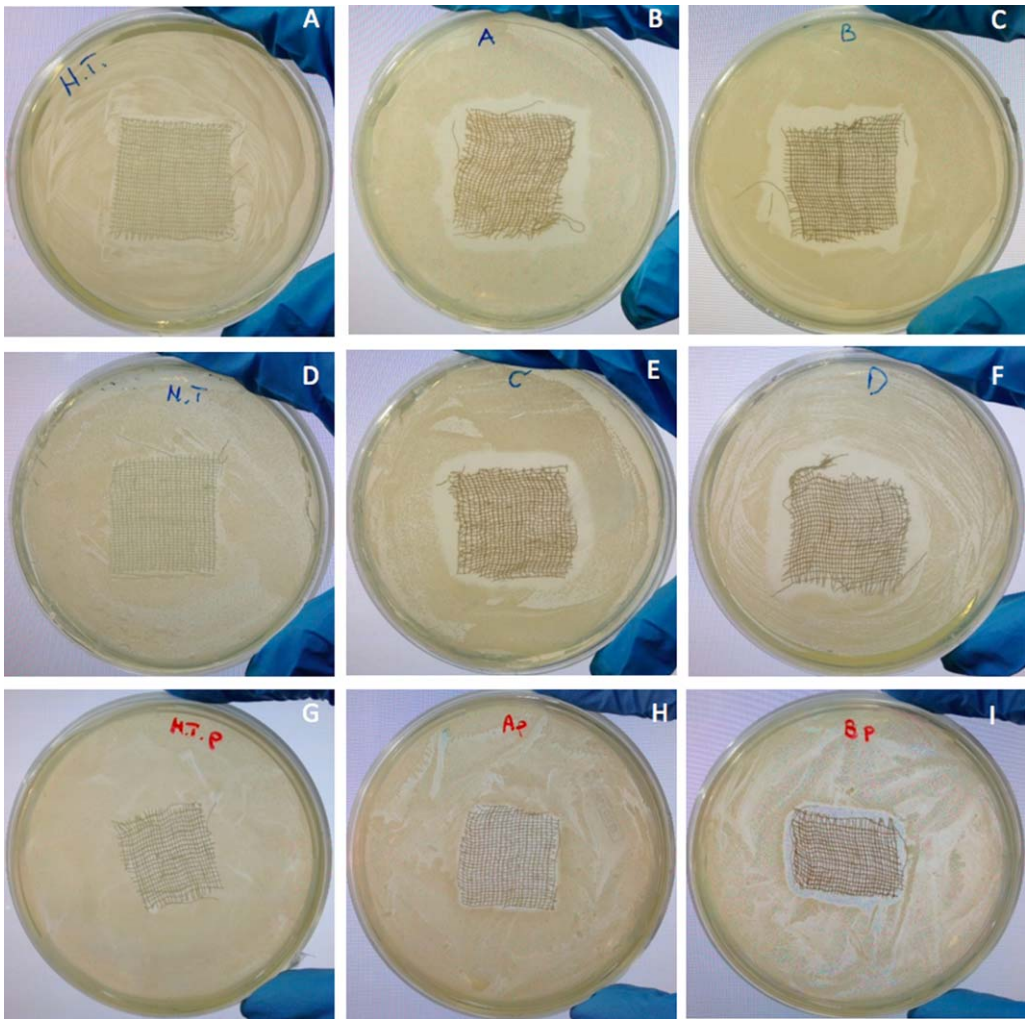

Figure 5. Agar diffusion tests on different bacterial strains. Tests on *E. coli* for untreated cotton (a), cotton gauze treated with 0.1 wt/v % Ag (b) and cotton gauze treated with 0.5 wt/v % (c); tests on *S. aureus* for untreated cotton (d), cotton gauze treated with 0.1 wt/v % Ag (e) and cotton gauze treated with 0.5 wt/v % (f); tests on *Pseudomonas p43* for untreated cotton (g), cotton gauze treated with 0.1 wt/v % Ag (h) and cotton gauze treated with 0.5 wt/v % Ag (i). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The antimicrobial activity of the silver treated dressings was also tested after incubation in artificial exudate at 37°C overnight in order to simulate the application of the material. The composition of the exudate adopted is reported in Table I.[25,26]

Two different procedures were adopted for testing the materials. The first procedure provided the incubation of the silver treated samples in the exudate only at 37°C overnight. Then, the samples were placed on agar plates inoculated with *S. aureus* and incubated for 24 h at 37°C. After incubation, the presence of bacterial growth around and beneath the silver treated sample was evaluated and compared with the control.

According to the second procedure, the samples were incubated in 4 mL of artificial exudate inoculated with 100 μL of *S. aureus* suspension (stationary phase cells, inoculating cell density 3×10^7 CFU/mL). After incubation, the silver treated samples were removed from the exudate, placed on agar plates and incubated for 24 h at 37°C. After incubation, the effect of silver on bacterial growth was evaluated in comparison with the untreated sample.

RESULTS AND DISCUSSION

Infected wounds represent a significant problem for health care systems[11] and there is an urgent need to define efficient and cost-effective strategies for the management of infections in wound healing.[11] In recent years, a range of wound dressings with antibacterial properties containing silver compounds has been developed and marketed.[27–29]

In this work, an innovative technology to produce antibacterial and antifungal wound dressings was presented. The distinctive features of this technology are the versatility and the excellent adhesion of the coating to the substrate. Indeed, the technology has been already applied on many different natural and synthetic materials[30] and the durability of the coating has been demonstrated even in contact with flowing biological solutions.[31] The technology was based on the synthesis and deposition of silver nanoparticles on cotton fibers through the in situ photoreduction of silver nitrate. The textile substrates were impregnated in a silver solution containing different percentages of silver

nitrate dissolved in methanol and water. The presence of methanol is strictly required because methanol represents the reducing agent in the process; water can also be added in the silver solution to reduce the costs of the treatment. After impregnation, the wet substrates were exposed to UV irradiation in order to induce the photochemical deposition of silver nanoparticles on the cotton fibers. As clearly visible in Figure 1, the presence of silver particles induced a light darkening of the material and the effect is more evident for the sample treated with the highest percentage of silver. No discoloration areas were observed on any treated sample, thus demonstrating the efficacy of the impregnating technique on the material (Figure 1).

Before each characterization, the treated substrates were washed many times with water to avoid any presence of silver salt. The distribution of the silver particles, as well as the quality of the coating deposited, were analyzed through scanning electron microscopy (Figure 2) where the differences between the samples were also evaluated as function of the silver solution adopted for the treatment. As expected, the cotton substrate treated with the highest percentage of silver (0.5 wt/v %) demonstrated the best coverage of the fibers, the silver particles being quite homogeneously distributed on many cotton fibers [Figure 2(c), arrows]. On the other hand, the sample treated with 0.1 wt/v % of silver demonstrated a good coverage of the fibers, even if some neat fibers are still visible in Figure 2(b). The EDX analysis confirmed the difference in the amount of silver deposited. In Figure 3, the EDX spectra of the untreated gauze (a), of the gauze treated with 0.1 wt/v % Ag (b) and 0.5 wt/v % Ag (c) are reported. As expected, no peak associated to the presence of silver is visible in the untreated sample. Even if the silver peak in Figure 3(b) is not clearly visible, the presence of silver was quantified by the EDX analysis and resulted in 0.11 wt % with respect to the components of the substrate. A more evident silver peak is visible in the EDX spectrum reported in Figure 3(c), corresponding to a calculated percentage of 0.48 wt %.

The presence of metallic silver particles on cotton substrates was also confirmed by the results of the spectrophotometric analysis reported in Figure 4, where the reflection spectra of the untreated gauze and the gauzes treated with the different percentages of silver are reported. The signal associated to silver plasmon is clearly visible in the position expected,[32] and corresponds to 422 and 425 nm for 0.1 wt/v % Ag and 0.5 wt/v % Ag, respectively. Moreover, these wavelengths indicate the presence of silver nanoparticles with a diameter ranging between 35 and 80 nm, according to data reported in literature.[33]

TGA analysis also confirmed these data and the mean values obtained were $0.09 \pm 0.003\%$ and $0.6 \pm 0.005\%$, respectively, in agreement with the EDX results.

The results obtained from the microbiological characterization are reported in Figure 5(a–g) for *E. coli*, *S. aureus*, *Pseudomonas p43* and in Figure 6 for *Candida albicans*. In each figure, pictures of agar diffusion tests are reported for the sample treated with the different percentages of silver in comparison with the untreated sample. Particularly, no significant differences were demonstrated on *E. coli* and *S. aureus* by the sample treated with 0.1 wt/v % silver and 0.5 wt/v % silver [Figure 5(b,c) and 5(e,f)]. On the other hand, lower antibacterial capability was demonstrated by the sample treated with 0.1 wt/v % of silver on *Pseudomonas p43* [Figure 5(h)] and *Candida albicans* [Figure 6(b)], because different dimensions of the inhibition growth area can be observed around the samples. The average width of

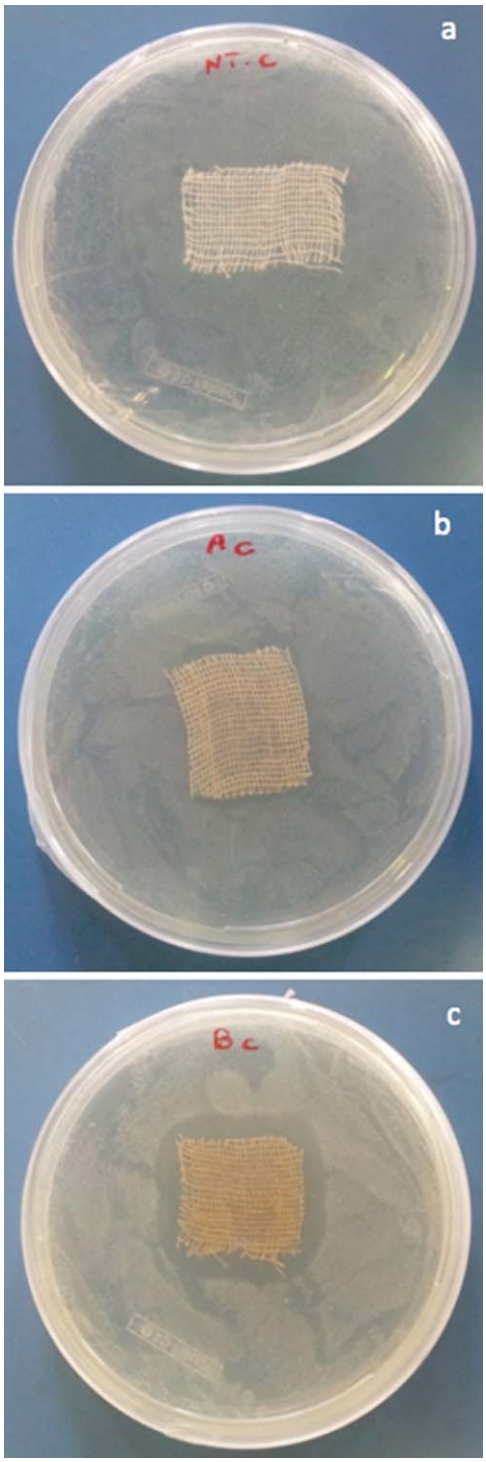

Figure 6. Agar diffusion tests on *Candida albicans*: untreated cotton (a), cotton gauze treated with 0.1 wt % Ag (b) and cotton gauze treated with 0.5 wt % Ag. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Inhibition Growth Area Resulted by Agar Diffusion Tests on the Different Microorganisms Tested

	E. coli	*S. aureus*	*Pseudomonas*	*C. albicans*
Untreated sample	–	–	–	–
0.1 wt/v % Ag	5 mm	4 mm	1 mm	1 mm
0.5 wt/v % Ag	5 mm	4 mm	3 mm	5 mm

Table III. Antibacterial Activity Expressed as Log Reduction Resulted by Quantitative Tests on the Different Strains Tested

	E. coli	*S. aureus*	*Pseudomonas*
Untreated sample	–	–	–
0.1 wt/v % Ag	1.73	1.07	0.47
0.5 wt/v % Ag	2.03	1.13	0.83

the inhibition growth area calculated around each sample is reported in Table II for each microorganism tested. In line with these results, the log reductions obtained by quantitative antibacterial tests (Table III) indicated a lower antibacterial efficacy on *Pseudomonas* for both the silver concentrations tested, when compared with the antibacterial capability demonstrated by the samples on *E. coli* and *S. aureus*. Moreover, no significant differences resulted on *E. coli* and *S. aureus* between the samples treated with 0.1 and 0.5 wt/v % silver solutions, whilst the efficacy on *Pseudomonas* resulted lower in the sample treated with 0.1 wt/v of silver.

In order to reproduce the conditions of use and to test the materials in an environment favorable to bacteria, the antibacterial efficacy of the silver treated gauzes was also tested after conditioning in artificial exudate. It has been prepared by selecting an appropriate composition for the specific application, because of the presence of both salts and proteins. The fluid prepared contained plasma constituents such as inorganic electrolytes and albumin, thus well reproducing the wound environment.[25,26]

The antibacterial tests were conducted according to two different methods. The first one consisted in conditioning the gauzes in exudate and placing them in contact with bacteria directly on the agar plate, the other in incubating the gauzes in artificial exudate inoculated with bacteria. The results are reported in Figure 7, for the first [Figure 7(a–c)] and the second method [Figure 7(a′–c′)], respectively. As visible in the images of the silver treated samples, an evident reduction on the bacterial colonization of the samples can be observed. Indeed, the untreated samples did not demonstrate any capability against bacteria, as visible in Figure 7(a–c′) where a complete colonization of the agar plate can be observed. All the experiments demonstrated that the presence of exudate on the gauze promoted the bacterial growth on the untreated textile substrate whilst a reduced presence of bacteria can be observed on all the silver treated samples.

The microbiological characterization of the silver treated cotton gauzes demonstrated the good efficacy of the silver deposition

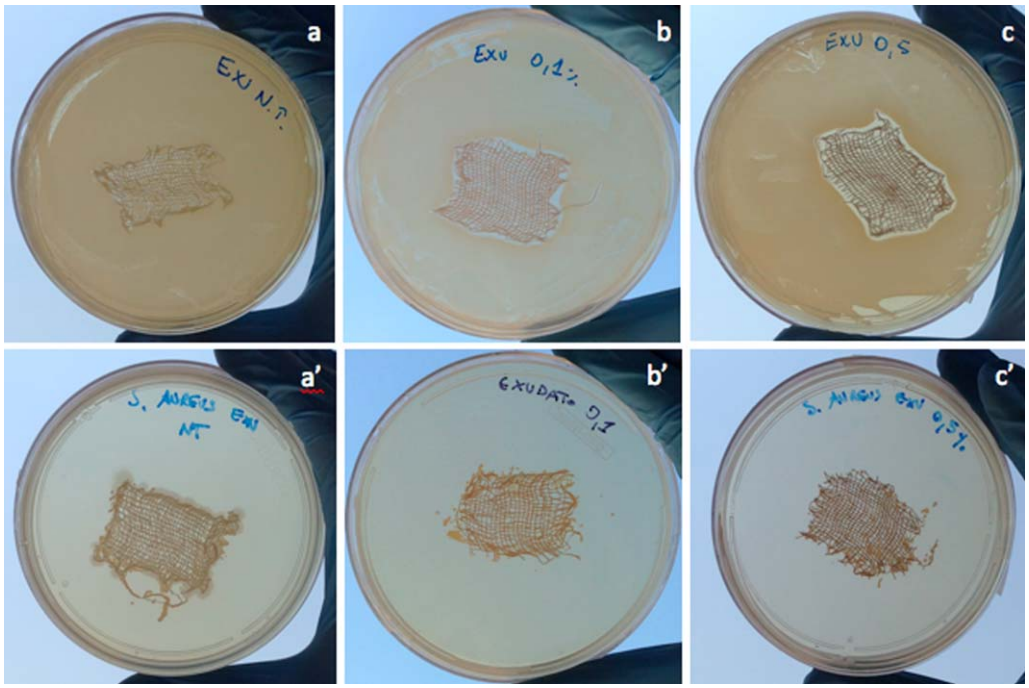

Figure 7. Agar diffusion tests on *S. aureus* after incubation in artificial exudate of untreated cotton (a–a′), cotton gauzes treated with 0.1 wt/v % Ag (b–b′) and cotton gauzed treated with 0.5 wt/v % Ag (c–c′). A set of cotton gauzes was incubated in exudate, then placed in contact with bacteria on the agar plate and incubated (a–c); another set of cotton gauzes was incubated in artificial exudate inoculated with bacteria, then placed on agar plate and incubated (a′–c′). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

technology adopted in reducing the microbial colonization of biomedical textile. Moreover, low percentages of silver and low-cost traditional gauzes have been adopted for the treatment, thus indicating that the introduction of these devices in hospital practice can be proposed with no significant impact on the health care costs. Indeed, even if the percentage 0.5 wt/v % can be preferred because it resulted more effective than 0.1 wt/v % on certain microorganisms tested, the costs of the treatment can be considered very low in terms of chemicals adopted and of equipment required for the process. However, the process parameters, such as the percentage of silver deposited, can be selected as function of the antibacterial or antifungal properties required to the biomedical product for its specific application. A silver solution containing 2 wt/v % of silver precursor was also deposited on cotton substrates and tested in a previous work, where the strong adhesion of the silver coating and the antibacterial capability on Gram positive and Gram negative bacteria were assessed.[34] The treated cotton was also *in vivo* tested to evaluate any effect of the silver coating in terms of skin irritation and hypoallergenicity.[34] Interestingly, even if the percentage of silver (2 wt/v %) was higher than the percentages adopted in the present work (0.1 and 0.5 wt/v %), no skin irritation and hypoallergenicity resulted on the selected subjects.

These data indicate that the silver coatings developed could be considered effective, low cost and no dangerous on human health in terms of skin reactions. Moreover, in the clinical management of wounds, the presented material can be proposed as an interesting tool for the prevention of bacterial and fungal infections associated to delayed wound healing. The silver treated gauzes can be also considered as a part of more complex devices, as they can be easily impregnated with emollients, hydrogels or other substances during their application on the patient in order to reduce the pain associated to the replacement of the dressing.

CONCLUSIONS

In this work an effective technology for the treatment of textile substrates for application as wound dressing has been developed and presented. The ease of the silver deposition and the excellent adhesion of the silver coating to the substrate are distinctive features of this technology. The silver deposition treatment on cotton gauzes resulted in a homogeneous distribution of silver particles on the cotton fibers, accordingly to the percentage of silver precursor adopted for the preparation of the silver solution. The antimicrobial capability of the silver treated materials was assessed on different microorganisms, such as Gram positive and Gram negative bacteria and also on a fungal strain. Even if the percentage of silver adopted for the treatment was very low, a good antimicrobial capability was demonstrated, thus indicating the potential of this technology in terms of cost-effectiveness.

REFERENCES

1. Percival, S. L., Hill, K. E., Williams, D. W., Hooper, S. J., Thomas, D. W., Costerton, J. W. *Wound Rep. Reg.* **2012**, *20*, 647.
2. Adams, A. P., Santschi, E. M., Mellencamp, M. A. *Vet. Surg.* **1999**, *28*, 219.
3. Chambers, C. W., Proctor, C. M., Kabler, P. W. *J. Am. Water. Works Assoc.* **1962**, *54*, 208.
4. Brook, I. *J. Otolaryngol.* **1996**, *25*, 249.
5. Wright, J. B., Lam, K., Hansen, D., Burrell, R. E. *Am. J. Infect. Control.* **1999**, *27*, 344.
6. Phillips, E., Young, T. *Br. J. Nurs.* **1995**, *4*, 1345.
7. Wright, J. B., Lam, K., Burrell, R. E. *Am. J. Infect. Control.* **1998**, *26*, 572.
8. Murphy, P. S., Evans, G. R. D. *Plast Surg Int.* **2012**, *10*, 1904.
9. Edwards, J. V., Yager, D. R., Cohen, I. K., Diegelmann, R. R., Montante, S., Bertoniere, N., Bopp, A. F. *Wound Repair. Regen.* **2001**, *9*, 50.
10. Silver, S., Phung, le T., Silver, G. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 627.
11. Gottrup, F., Apelqvist, J., Price, P. *J. Wound Care.* **2010**, *19*, 237.
12. Poon, V.K., Burd, A. *Burns.* **2004**, *30*, 140.
13. Amato, E., Diaz-Fernandez, Y. A., Taglietti, A., Pallavicini, P., Pasotti, L. *Langmuir.* **2011**, *27*, 9165.
14. Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N. *J. Phys. Chem. B.* **2006**, *110*, 16248.
15. Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B. and Ramirez, J. T. *Nanotechnology.* **2005**, *16*, 2346.
16. Rai, M. K., Deshmukh, S. D., Ingle, A. P., Gade, A. K. *J. Appl. Microbiol.* **2012**, *112*, 841.
17. Sondi, I., Salopek-Sondi, B. *J. Colloid Interface Sci.* **2004**, *275*, 177.
18. Li, W. R., Xie, X. B., Shi, Q. S., Zeng, H. Y., Ou-Yang, Y. S., Chen, Y. B. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1115.
19. Rai, M., Yadav, A., Gade, A. *Biotechnol. Adv.* **2009**, *27*, 76.
20. Fox, C. L., Jr.: *Arch. Surg.* **1968**; *96*, 184.
21. Betbeze, C. M., Wu, C. C., Krohne, S. G., Stiles, J. *Am. J. Vet. Res.* **2006**; *67*, 1788.
22. Monteiro, D. R., Silva, S., Negri, M., Gorup, L. F., De Camargo, E. R., Oliveira, R., Barbosa, D. B., Henriques, M. *Lett. Appl. Microbiol.* **2012**, *54*, 383.
23. Pollini, M., Sannino, A., Maffezzoli, A., Licciulli A. Antibacterial surface treatments based on silver clusters deposition. EP NO 20050850988, **2008**.
24. Paladini, F., Pollini, M., Deponti, D., Di Giancamillo, A., Peretti, G., Sannino, A. *J. Mater. Sci. Mater. Med.* **2013**, *24*, 1105.
25. Cutting, K. F. *Br. J. Community Nurs.* **2003**, *8*, 4.
26. Widmaier, E. P., Raff, H., Strang, K. T. In Vander's Human Physiology: The Mechanisms of Body Function, Vander, Ed.; McGraw-Hill: Boston, **2001**, Chapter 14, pp 373.
27. Ip, M., Lui, S. L., Poon, V. K. M., Lung, I., Burd, A. *J. Med. Microbiol.* **2006**, *55*, 59.

28. Melaiye, A., Youngs, W. *J. Expert Opin. Ther. Pat.* **2005**, *15*, 125.

29. Monafo, W. W., West, M. A. *Drugs.* **1990**, *40*, 364.

30. Pollini, M., Paladini, F., Licciulli, A., Maffezzoli, A., Sannino, A., Nicolais, L. *J. Coat. Technol. Res.* **2013**, *10*, 239.

31. Paladini, F., Pollini, M., Talà, A., Alifano, A., Sannino, A. *J. Mater. Sci. Mater. Med.* **2012**, *11*, 2005.

32. Vittorino, E., Giancane, G., Manno, D., Serra, A., Valli, L., Sortino, S. *J. Colloid Interface Sci.* **2012**, *15*, 191.

33. Solomon, S. D., Bahadory, M., Jeyarajasingam, A. V., Rutkowsky, S. A., Boritz, C. *J. Chem. Educ.* **2007**, *84*, 322.

34. Paladini, F., Sannino, A., Pollini, M. *J. Biomed. Mater. Res. B Appl. Biomater.* **2013**, DOI: 10.1002/jbm.b.33085.