Preparation of Antibacterial Sheepskin with Silver Nanoparticles: Potential for Use as a Mattress for Pressure Ulcer Prevention

by

Weite Yang1, Xuan Wang1, Ying Gong1, Hui Li1, Wuyong Chen1* And Carmen Gaidau2

[1] *National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, Sichuan, P. R. China*

[2] *Leather and Footwear Research Institute,*

93, Ion Minulescu St., sector 3, 031215, Bucharest, Romania

Abstract

In the study, sheepskin was treated with silver nanoparticles, which were prepared at the concentration of 2.4×10^{-2} g·L^{-1} with average particles size about 26 nm. And then, the adsorption of the silver nanoparticles by the sheepskin, as well as the morphology and the antibacterial effect of the treated sheepskin were investigated. The UV-vis absorption spectroscopy indicated nearly all of the silver nanoparticles were adsorbed by the sheepskin with this method. From scanning electron microscopy (SEM) observations, it was confirmed that the nanoparticles attached on the surface of the sheepskin. The antibacterial effect of the treated sheepskin was evaluated after repeated perspiration treatments. The results of antibacterial study showed that the treated sheepskin had an antibacterial inhibition of 99.9% against *Escherichia coli* and *Staphylococcus aureus*, even after 6 cycles of perspiration treatment, the sheepskin still exhibited a durable antibacterial effect with the inhibition rate above 79.4% and 67.1% respective to the leather and the wool. Thus, this study has provided an eco-friendly alternative for the preparation of antibacterial sheepskin without organic compounds, which would be potentially used as a mattress for pressure ulcer prevention.

Resumen

En este estudio, la piel de oveja fue tratada con nanopartículas de plata, que fueron preparadas en una concentración de 2.4×10^{-2} g·L^{-1} y con un tamaño de partículas promedio de 26 nm. Y luego, la adsorción de las nanopartículas de plata por la piel de oveja, así como la morfología y el efecto antibacteriano de la piel de oveja tratada fueron investigados. La espectroscopia de absorción UV-vis indica que casi todas las nanopartículas de plata fueron absorbidas por la piel de oveja en este método. Por observaciones mediante microscopía electrónica de barrido (SEM), se confirmó que las nanopartículas se fijaron en la superficie de la piel de oveja. El efecto antibacteriano de la piel de oveja tratada fue evaluado después de repetidos tratamientos de transpiración. Los resultados del estudio antibacterial mostraron que las pieles de oveja tratadas tenían una inhibición antibacteriana del 99,9% frente a Escherichia coli y Staphylococcus aureus, incluso después de 6 ciclos de tratamiento de transpiración, la piel de oveja exhibió aún un efecto antibacteriano duradero con tasa de inhibición por encima de 79,4% y 67,1% respecto del cuero y de la lana. Así, este estudio ha proporcionado una alternativa ecológica para la preparación de piel de oveja antibacteriana, sin compuestos orgánicos, lo que sería potencialmente usado como colchones para la prevención de úlceras por presión.

*Corresponding author e-mail: wuyong.chen@163.com

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INTRODUCTION

Pressure ulcers are very prevalent, especially among elderly people residing in nursing homes and patients perennially lying in bed.[1,2] They develop when blood circulation in capillaries is obstructed by prolonged pressure. Shear and friction are the main factors contributed for the development of pressure ulcers.[3] So, there are many interventions used to minimize pressure on bony prominences for the prevention of pressure ulcers,[4-6] in which, the usage of sheepskin has been a successful nursing aid since the early 1960's.[7] The high density pile of soft, springy resilient wool fibers provide a cushion to distribute the patient's weight and pressure points over a large area.[8] Each fiber acts as a "mini-spring" that deforms to the body contours. Meanwhile, the wool fibers are able to absorb 33% of their weight of moisture without damp feeling.[9] These properties of sheepskins result in improved comfort for the patients and reduction in the primary causes of pressure ulcers.

In daily use, nutrients from the patient's urine, feces and sweat, combined with the effect of temperature, may lead to the growth of microorganisms such as pathogenic or odor-generating bacteria and fungi on sheepskin. This could threaten the health of the patient. Thus, with a raising interest in hygiene, the antibacterial effects through using antibacterial agents to prevent or retard the growth of bacteria are becoming an increasingly desirable aim for sheepskin. Currently, the widely used antibacterial agents are organic compounds.[10] These organics have been introduced for decades to treat and prevent the development of mould, yeasts and bacteria. However, the organics are lack of antibacterial durability.[11] In addition, the excessive use of these chemicals has been companied by an increasing prevalence of micro-organisms that have acquired resistance to the chemicals.[12] Therefore, an ideal antibacterial agent for sheepskin should be safe and environmentally benign besides killing undesirable micro-organisms.

Herein, we report the usage of silver nanoparticles as an antibacterial agent to prepare antibacterial sheepskin. Silver nanoparticles have attracted considerable interest from the chemical industry and medical field due to their unique properties, such as high electric conductivity,[12] high catalytic effect[13] and high antibacterial activity.[14-15] Various inorganic antibacterial materials containing silver nanoparticles have been developed and some are in commercial use. The antimicrobial activity of silver nanoparticles is comparable or better than the broad spectrum of most prominent antibiotics used worldwide.[16] Moreover, the biological safety of silver nanoparticles has been widely proved in many researches.[17-20] These advantageous properties of the silver nanoparticles might be potentially useful for their applications to the sheepskin as an alternative antimicrobial agent. Carmen Gaidau et al[21] have reported that silver nanoparticles were used for the treatment of leathers, and the products exhibited strong antifungal properties. In this study, sheepskin was treated with silver nanoparticles for the potential use of pressure ulcer prevention. The adsorption of the silver nanoparticles by the sheepskin, as well as the morphology and the antibacterial effect of the treated sheepskin were investigated in detail.

EXPERIMENTAL

Materials

Silver nitrate, sodium borohydride, sodium citrate and butylparaben were analytical grade and used as received. Benzalkonium bromide (5% solution, w/w) was purchased from Baiyun Pharmaceutical Company (China). Isothiazolinone (industrial grade) was bought from Huibang Chemical Company (China). Glutaraldehyde tanned sheepskin was bought from IKEA store in Chengdu (China).

Preparation of silver nanoparticles

A 1.5×10^{-2}g of $AgNO_3$ was dissolved in a 100mL of distilled water and placed in water bath at 30°C for 15min. A 100mL of benzalkonium bromide (2×10^{-2}g) solution was added dropwise to the $AgNO_3$ solution with intense stirring for 30min to form a combined solution. Subsequently, a 200mL of solution containing $NaBH_4$ (7.4×10^{-3}g) and benzalkonium bromide (4×10^{-2}g) was added dropwise for 1.5h to get silver nanoparticles. Then, the prepared silver nanoparticles were conserved at 30°C. Image of the silver nanoparticles was obtained using a transmission electron microscopy on copper grids (JEM-100CXII, Japanese electronics company, Japan). Size distribution of the silver nanoparticles was measured by DLS (Dynamic Light Scattering) technique using a nanoparticle size analyzer (Zetasizer NanoS90, Malvern company, England).

Antimicrobial activity of the silver nanoparticles

Various strains of bacteria including Gram-negative bacteria *Escherichia coli, Enterobacter hough, Acinetobacter*, Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* were used to evaluate the antibacterial activity of the prepared silver nanoparticles. Both butylparaben and isothiazolinone were used to compare the antimicrobial effect with the silver nanoparticles. Firstly, a 10mL of beef extract peptone medium and a 1mL of the bacteria suspension (1.0×10^5 cfu·mL^{-1}) were added in a culture dish. Then, the prepared antimicrobial agents were serially diluted in the culture dishes.[22] The minimum inhibitory concentration (MIC) was read after the culture dishes were incubated in a Mould Incubation Chamber at 37°C and 90% relative humidity for 24h.

Treatment of sheepskin with silver nanoparticles

Each sheepskin sample (3cm×3cm) was washed in 200ml water with 0.5g·L^{-1} SDS, and dried at 30°C. Then, the sheepskin was rewetted in a conical beaker with a 1200% float (based on the weight of the sheepskin) at 30°C overnight. Next, the sample was squeezed lightly to remove the excess water and soaked in the prepared silver nanoparticles solution (2.4×10^{-2} g·L^{-1}) and shaken in a shaker bath at 30°C for 3h. After this time period, the sheepskin was picked out, squeezed lightly to remove the excess water, and then dried at 30°C in an oven.

Characterization of the treated sheepskin

The adsorption of silver nanoparticles was analyzed by UV-vis absorption spectroscopy (UV-2501PC, SHIMADZU Company, Japan). Images of the treated sheepskin were obtained using a scanning electron microscopy (S-480, Hitachi Company, Japan). The surfaces of the wool and the leather were coated with a layer of gold before SEM characterization. Energy dispersive spectroscopy analysis (EDS) was used to confirm the presence of silver particles.

Antimicrobial activity of the treated sheepskin

Antibacterial test

The antibacterial activity of the wool and the leather of the sheepskin were evaluated separately, using a shake flask method.[23] A typical procedure was as follows: 2g of the treated leather was cut into small pieces of approximately 1cm×1cm (each strand of the treated wool with total weight of 2g was cut into sections with the length around 1 cm), and 0.2mL bacterial spore suspension at the concentration of 1.0×10^5 cfu·mL^{-1} were added to a triangular flask containing 20mL sterilized physiological saline solution. Then, the flask was shaken for 8h at 200r/min in a water-bath oscillator. The colony forming units (cfu) of the remaining solution before and after oscillation was determined. The inhibition (*IR*) of the antibacterial sheepskin was calculated using the following formula:

$$IR = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

where C_0 and C_t are the colony forming units of the solution before and after oscillation, respectively.

Perspiration treatment:

The artificial sweat was prepared containing 5g/L hydroxylamine hydrochloride monohydrate, 5g/L sodium chloride and 2.5g/L disodium hydrogen phosphate dihydrate. The pH of the artificial sweat was adjusted to 8.0 with 0.1mol/L sodium hydroxide. The treated sheepskin sample (3cm×3cm) was completely immersed in the artificial sweat (100ml) and placed at 35°C for 24h. After this time period, the sheepskin was picked out and dried at 30°C for the antibacterial test.

RESULTS AND DISCUSSIONS

Degree of chemical dispersion and particle size analysis of silver nanoparticles

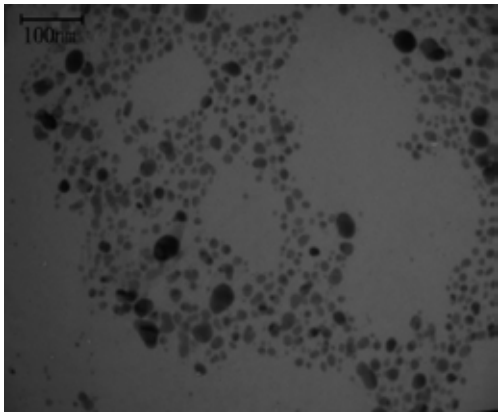

Figure 1: TEM image of the silver nanoparticles

The morphology and the degree of chemical dispersion of the silver nanoparticles were analyzed by TEM observation. The TEM image showed the silver nanoparticles were spherical in shape, and dispersed well in the solution, without agglomeration (Figure 1). As further analysis from the dynamic light scattering (DLS) method, the average particles size of the silver nanoparticles was about 26nm (Figure 2).

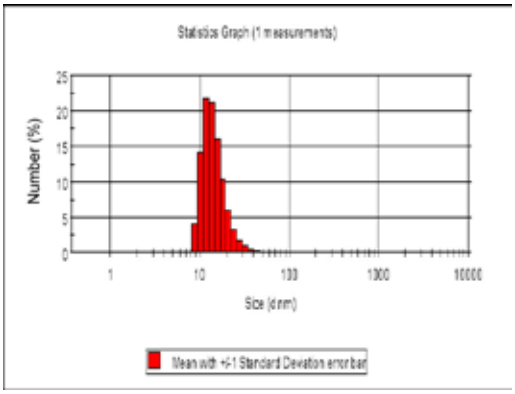

Figure 2: Size distribution of the silver nanoparticles
(Editor note: Contact author for better figure detail)

Antibacterial study of the silver nanoparticles

The dilution micromethod[22] was applied to study the antibacterial activity of silver nanoparticles on the agar plates. Both butylparaben and isothiazolin-ketone, which are the antibacterial agents commonly used in leather industry, were subjected to the antibacterial tests. MIC of the tested samples is summarized in Table I.

As can be seen in Table I, the silver nanoparticles had excellent antibacterial effect on all tested specimens against Gram-

TABLE I
MIC of the antibacterial agents

Sample	MIC (μg·mL^{-1})					
	Gram-negative bacteria			Gram-positive bacteria		
	Escherichia coli	Enterobacter hough	Acinetobacter	Staphylococcus aureus	Bacillus subtilis	Bacillus cereus
A[a]	50	100	200	50	50	25
B[b]	4	4	15	2	4	4
C[c]	1	4	6	4	6	2

[a] Butylparaben, [b] Isothiazolintone, [c] Silver nanoparticles

positive and Gram-negative bacteria. And also, the MIC of the silver nanoparticles was much less than butylparaben, and less than isothiazolinone overall. The antibacterial tests showed that the MIC of silver nanoparticles against *Escherichia coli* (1-ppm) was the lowest MIC value observed in this study. Against *Escherichia coli,* the MIC for silver nanoparticles was 50 times less than that of butylparaben and 4 times less than that of isothiazolinone. That is to say, to inhibit a same *Escherichia coli* strain, only a lower dosage of the silver nanoparticles is needed than that of butylparaben and isothiazolinone.

A likely mechanism for the effective antibacterial activity of silver nanoparticles is their weakening of the bacterial cell membrane, followed by invasion into the bacteria, and interaction with phosphorous-containing and sulfur-containing compounds such as DNA, leading to eventual death of the cell.[24] This activity suggests a suitable alternative to the use of organic antibacterial agents for treatment of sheepskins.

Antibacterial results of the treated sheepskin

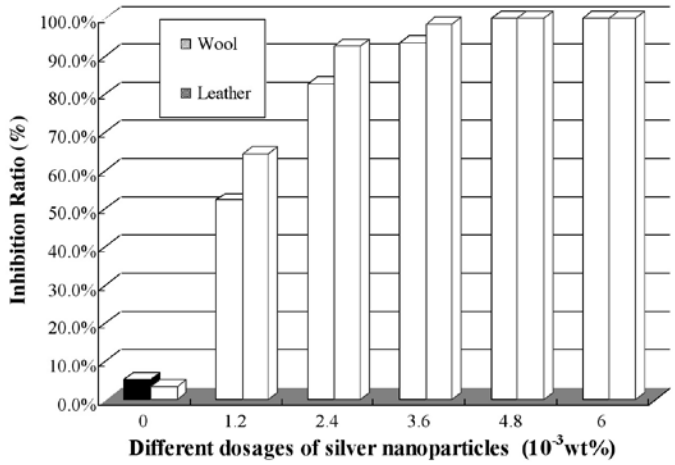

Figure 3: Inhibition ratio of the treated sheepskin with different dosages of silver nanoparicles against *Escherichia coli*

The antimicrobial properties of the sheepskin treated with different dosages of silver nanoparticles were quantitatively evaluated against *Escherichia coli*. The antibacterial effect of the wool and the leather of the sheepskin were tested separately. As expected, the wool and the leather without silver nanoparticles gave a negligible antibacterial activity of less than 6% (Figure 3). The antibacterial activity of the wool and the leather increased with increasing dosage of silver nanoparticles from 1.2×10^{-3}wt% to 4.8×10^{-3}wt% (base on the weight of the sheepskin). As the dosage was 4.8×10^{-3}wt%, the inhibition ratio reached 99.9% for both the leather and the wool, indicating the treated sheepskin had a better antibacterial activity against *Escherichia coli*, so, the sheepskin was treated with silver nanoparticles of this dosage for the further analysis.

Adsorption of the silver nanoparticles by the sheepskin

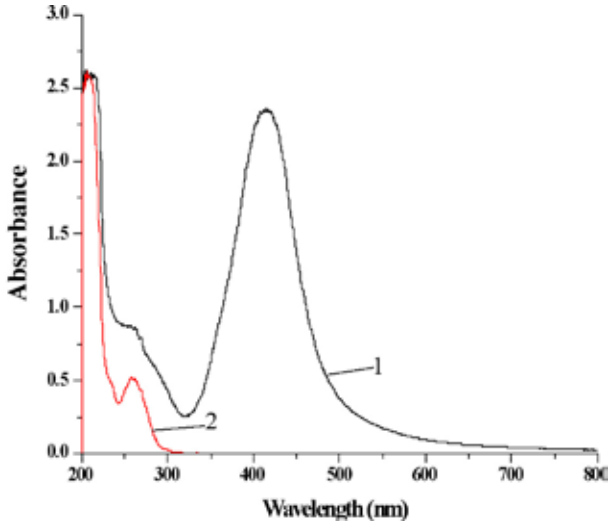

Figure 4: UV-vis absorption spectroscopy of the silver nanoparticles before and after antibacterial treatment, 1: before, 2: after.

The adsorption of the silver nanoparticles by the sheepskin was determined by a UV-vis absorption spectroscopy, which is a very useful technique for the analysis of nanoparticles.[25] Figure 4 shows the UV-vis absorption spectroscopy of the silver nanoparticles before and after antibacterial treatment at the dosage of silver nanoparticles of 4.8×10^{-3}wt%. The absorption peak around 400nm is the characteristic absorption peak of silver nanoparticles.[26] It can be seen the absorption peak at 406nm was vanished completely after treatment, indicating that nearly all of the silver nanoparticles were adsorbed by the sheepskin and there are few nanoparticles in the solution. The same adsorption was observed when we used even silver nanoparticles dosage of 1.8×10^{-2}wt% to treat the sheepskin. This interesting phenomenon may be owing to the large surface area to volume ratio of the silver nanoparticles,[27] which would favor the adhesion of the silver nanoparticles on the sheepskin to reduce the surface energy.

Morphology of the treated sheepskin

Samples of sheepskin treated with silver nanoparticles at the dosage of 4.8×10^{-3}wt%, as well as their controls were investigated by SEM. Representative images of the micrographs of the wool investigated (Figure 5) are shown at different magnifications, both treated and control samples. The scale layer of the wool is clearly shown at low magnification (×10000). When comparing the treated samples with the control, it can be seen the surface of treated wool was obviously coarser than that of the control, indicating the attachment of the silver nanoparticles on the surface of the wool. Also, the EDS analysis showed the silver particles made up 3.01% of the sum of all elements on the surface.

The SEM images of the flesh side of the leather samples, both treated and control, were also presented at different magnifications (Figure 6). It can be easily observed that silver nanoparticles attached on the surface of the collagen fibrils in the comparision of the treated sample with the control. The EDS analysis showed the silver content of the all elements of the surface was of 6.43%. We also observed the cross section of the treated leather, but no silver particles were detected, showing the silver nanoparticles only attached on the surface of the leather rather than penetrated the fibrils.

Effect of perspiration on bacteriostasis of the treated sheepskin

Table II shows the effect of repeated perspiration treatment on the antibacterial activity of the sheepskin treated with silver nanoparticles at the dosage of 4.8×10^{-3}wt%. It is found that the treated sheepskin had an antibacterial inhibition of 99.9% against the two tested bacteria without perspiration treatment. As cycles of perspiration treatment increased, the antibacterial effect decreased gradually. However, even after 6 cycles of perspiration treatment, the sheepskin still exhibited a good antibacterial effect with the inhibition above 79.4% and 67.1% respective to the leather and the wool.

CONCLUSIONS

This study has shown a novel way to prepare antibacterial sheepskin with silver nanoparticles as an antibacterial agent. The UV-vis absorption spectroscopy indicated nearly all of the silver nanoparticles were adsorbed by the sheepskin in this way. The SEM images confirmed that the silver particles attached on the surface of the sheepskin. The results of antibacterial study showed that the treated sheepskin had an antibacterial inhibition of 99.9% against *Escherichia coli* and *Staphylococcus aureus*, even after 6 cycles of perspiration treatment, the sheepskin still exhibited a durable antibacterial effect with the inhibition above 79.4% and 67.1% respective to

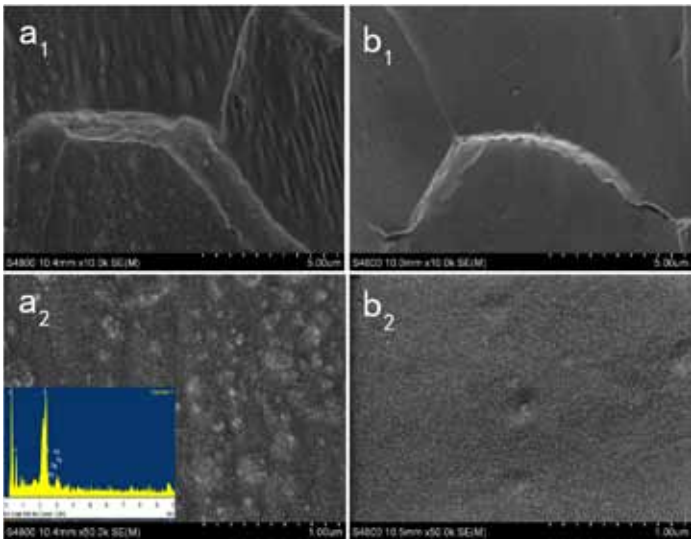

Figure 5: SEM images of the wool with silver nanoparticles (a_1, a_2) and control (b_1, b_2). EDS analysis shows peaks of silver (small panel).

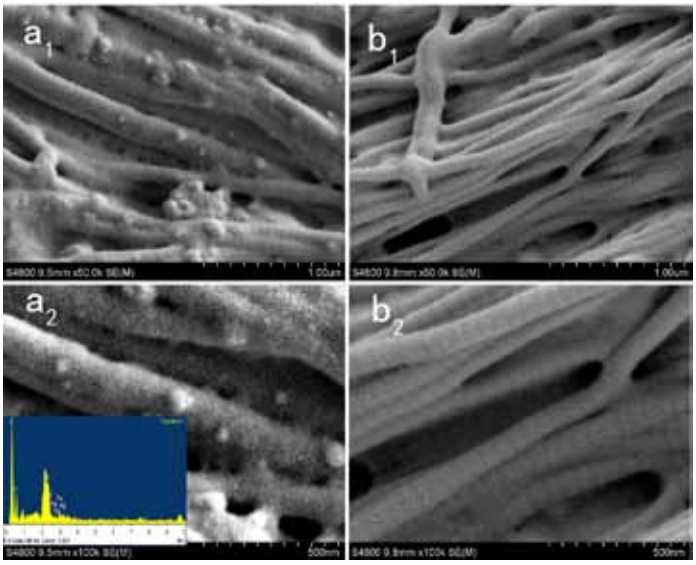

Figure 6: SEM images of the leather with silver nanoparticles (a_1, a_2) and control (b_1, b_2). EDS analysis shows peaks of silver (small panel).

TABLE II
Antibacterial inhibition (%) of the sheepskin treated with sliver nanoparticles after different cycles of perspiration treatment

Sample	Perspiration Treatment	Bacterial Strains	
Wool	0 cycles	99.9	99.9
	3 cycles	88.1	74.7
	6 cycles	83.2	67.1
Leather	0 cycles	99.9	99.9
	3 cycles	90.6	81.3
	6 cycles	87.5	79.4

the leather and the wool. Thus, this study has presented an eco-friendly alternative for the antibacterial treatment of sheepskin without organic compounds, which would be potentially used as a mattress for pressure ulcer prevention.

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REFERENCES

1. Horn, S. D., Bender, S. A., Ferguson, M. L., Smout, R. J., Bergstrom, N., Taler, G., Cook, A. S., Sharkey, S. S., and Voss, A. C.; The National Pressure Ulcer Long-Term Care Study: pressure ulcer development in long-term care residents. *J. Am. Geriatr. Soc.* **52**, 359, 2004.
2. Woodbury, M. G., and Houghton, P. E; Prevalence of pressure ulcers in Canadian healthcare settings. *Ostomy Wound Manage* **50**, 22, 2004.
3. Bryant, R.; Acute and chronic wounds nursing management, Mosby -Year Book, St. Louis, 1992.
4. Link, A.; Anti-decubitusmaterialen onder druk, CVZ College voor Zorgverzekeringen, Amstelveen, 2002.
5. Cullum, N., McInnes, E., Bell-Syer, S. E., and Legood, R.; Support surfaces for pressure ulcer prevention. *Cochrane Database Syst. Rev.* **3**, 1735, 2004.
6. Deitermann, B., Kemper, C., Hoffmann, F., and Glaeske, G.; GEK-Heil- und Hilfsmittel-Report 2006, Gmund: Gmunder Ersatzkasse, 2006.
7. Ewing, M. R., Garrow, C., and McHugh, N.; A sheepskin as a nursing aid. *The Lancet* **30**, 1447, 1961.
8. Clark, M.; Continuous interface pressure measurement using an electropneumatic sensor: the SCP monitor. *Care-Science and Practice* **5**, 5, 1987.
9. Denne, W. A.; An objective assessment of the sheepskins used for decubitus sore prophylaxis. *Rheumatology and Rehabilitation* **18**, 23, 1979.
10. Yea, W. J, Leungb, M. F., Xina, J., Kwonga, T. L., Leeb, D. K. L., and Lib, P.; Novel core-shell particles with poly(n-butyl acrylate) cores and chitosan shells as an antibacterial coating for textiles. *Polymer* **46**, 10538, 2005.
11. Bohringer, A., Rupp, J., and Yonenaga. A.; Antimicrobial textiles. *International textile Bulletin* **46**, 12, 2000.
12. Lee, K. J., Jun, B. H., Choi, J., Lee, Y. I., Joung, J., and Oh, Y.S.; Environmentally friendly synthesis of organic-soluble silver nanoparticles for printed electronics. *Nanotechnology* **18**, 335601, 2007.
13. Krutyakov, Y.A., Kudrinskii, A. A., Olenin, A.Y., and Lisichkin, G.V.; Synthesis of highly stable silver colloids stabilized with water soluble sulfonated polyaniline. *Russ. Chem. Rev.* **77**, 233, 2008.
14. Le, A.T., Huy, P. T., Tam, P. D., Huy, T. Q., Cam, P. D., Kudrinskiy, A. A., and Krutyakov.Y. A.; Green synthesis of finely-dispersed highly bactericidal silver nanoparticles via modified Tollens technique. *Current Applied Physics* **10**, 910, 2010.
15. Chudasama, B., Vala, A. K., Andhariya, N., Mehta, R. V., and Upadhyay, R. V.; Highly bacterial resistant silver nanoparticles: synthesis and antibacterial activities. *J. Nanopart Res.***12**, 1677, 2010.
16. Roy, R., Hoover, M. R., Bhalla, A. S., Slaweekl, T., Dey, S., Cao, and W., Li, J.; Ultradilute Ag-aquasols with extraordinary bactericidal properties: role of the system Ag-O-H_2O. *Mater. Res. Innov.***11**, 3, 2008.
17. Silver, S., Phung, L. T., and Silver, G.; Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. *J Ind. Microbiol Biotechnol* **33**, 627, 2006.

18. Ji, J. H., Jung, J. H., Kim, S. S., Yoon, J. U., Park, J. D., Choi, B. S., Chung, Y. H., Kwon, I. H., Jeong, J., Han, B. S., Shin, J. H., Sung, J. H., Song, K. S., and Yu, I. J.; Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol* **19**, 857, 2007.
19. Kim, Y. S., Kim, J. S., Cho, H. S. Rha, D. S., Kim, J. M., Park, J. D., Choi, B. S., Lim, R., Chang, H. K., Chung, Y. H., Kwon, I. H., Jeong, J., Han, B. S., and Yu, I. J.; Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol* **20**, 575, 2008.
20. Vizuete, M. L., and Venero, J. L.; An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. *Biomaterial* **25**, 4383, 2004.
21. Gaidau C., Petica A., Dragomir T., Iovu H., and Andronescu C.; Ag and Ag/TiO_2 nano-dispersed systems for treatment of leathers with strong antifungal properies. *JALCA* **106**, 102, 2011.
22. Libor, K., Ales, P., Jana, S., Milan, K., Renata, V., Robert, P., Mirka, H., and Radek, Z.; Effect of Surfactants and Polymers on Stability and Antibacterial Activity of Silver Nanoparticles (NPs). *J. Phys. Chem. C* **112**, 5825, 2008 .
23. Gu, H. B., Chen, W. Y., Zhao, C. Q., and Hao, X.; New antimicrobial complex of copper (II) with benzothiazole derivative: synthesis and application in leather industry. *JALCA* **103**, 114, 2008.
24. Le, A. T., Tam, L. T., Tam, P. D., Huy, P. T., Huy, T. Q., Hieu, N. V., Kudrinskiy, A. A., and Krutyakov, Y. A.; Synthesis of oleic acid-stabilized silver nanoparticles and analysis of their antibacterial activity. *Materials Science and Engineering C* **30**, 910, 2010.
25. Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R., and Minaian, S.; Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. *Nanomedicine: Nanotechnology, Biology, and Medicine* **3**, 168, 2007.
26. Liu, J., Lee, J. B., Kim, D. H., and Kim, Y.; Preparation of high concentration of silver colloidal nanoparticles in layered laponite sol. *Colloids Surf. A* **302**, 276, 2007.
27. Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T., and Yacaman, M. J.; The bactericidal effect of silver nanoparticles. *Nanotechnology* **16**, 2346, 2005.