A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications†

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Received (in Cambridge, UK) 28th February 2007, Accepted 1st May 2007
First published as an Advance Article on the web 18th May 2007
DOI: 10.1039/b703034g

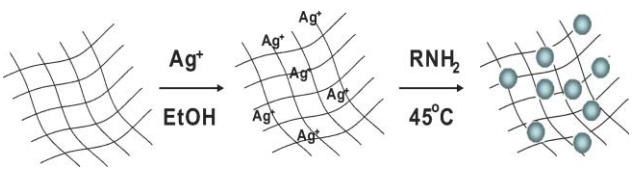
A novel and universal procedure has been developed for producing nanosized stable silver particles on cotton fabrics in a simple and cost-effective manner with complete control of the silver loading level on the fabrics; the antibacterial effect of Ag-nanocoated fabrics on various bacteria was evaluated by growth inhibition; for biomedical applications, skin irritation tests on guinea pigs were performed and no side effects were observed.

Many bacterial strains have developed resistance to various antibiotics, such as penicillin and ampicillin. Hence, it is necessary to develop novel antibacterial substances or chemicals. Silver has been known to be a bactericide since ancient times.[1] Recently, nanosized silver particles have been reported to exhibit antimicrobial properties.[2] It is believed that the high affinity of silver towards sulfur or phosphorus is the key element of this effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing proteins inside or outside the cell membrane, which in turn affects bacterial cell viability.[2a,3] It was also proposed that silver ions (particularly Ag^+) released from silver nanoparticles (Ag^0) can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulfur-containing proteins, leading to the inhibition of enzyme functions.[4,5]

Due to these novel properties, the incorporation of silver nanoparticles into various matrices has been intensively investigated in order to extend their utility in materials and biomedical applications.[6,7] Parikh *et al.* developed an antibacterial Ag/Na carboxymethyl cotton burn dressing by the partial cation exchange of sodium with silver.[8] Jeon *et al.* prepared Ag-doped thin silica films using Ag^+-incorporated tetraethyl orthosilicate (TEOS) *via* the sol–gel process.[9] Aymonier *et al.* showed that hybrids of Ag nanoparticles and hyperbranched polyethyleneimine can effectively adhere to a polar substrate and exhibit antimicrobial activity.[10] However, previous attempts required multiple steps and complex reagents to prepare the Ag-coated matrices. In the case of electroless Ag plating, additional protective reagents had to be added to minimize the corresponding bulk reactions of silvering.[11] Biomedical applications also require simple and eco-friendly procedures to minimize environmental biohazards. All such criteria require the novel and universal preparation of silver-coated matrices for biomedical applications. The scant literature on Ag-coated matrices also requires a systematic study of their biological evaluations.

We previously reported that stable and optically tunable Ag films can be simply and reproducibly fabricated by soaking glass substrates in ethanolic solutions of $AgNO_3$ and butylamine.[12] These Ag films showed an excellent homogenous morphology and surface-enhanced Raman scattering (SERS) activity. In this report, we demonstrate a universal procedure for depositing stable silver nanoparticles onto cotton fabrics for biomedical applications by using the same principle of Ag films. The practicality of this procedure is superb and readily applicable in clinical usages, since it can be produced by a one-pot reaction that requires only $AgNO_3$, butylamine and absolute ethanol (Scheme 1). In addition to its simplicity, the loading levels of silver nanoparticles on cotton fabrics are completely controlled by varying the concentration of the reactants.

Dried cotton fabric, which consists of cellulosic walls, exhibits a very broad OH stretching band in the 3500 $\sim$ 3000 cm^{-1} region of the transmission infrared spectrum.[13] This indicates that the surfaces of cotton fabrics are terminated with OH groups. Thus, Ag can be readily deposited onto cotton fabric simply by soaking it in an ethanolic solution of $AgNO_3$ and butylamine; the Ag^+ ions can subsequently be adsorbed onto OH functional groups of the surface, and these ions in turn can act as seeds for the deposition of reduced Ag. As shown in the field emission-scanning electron microscope (FE-SEM) images (Fig. 1B), different levels of silver nanoparticles are deposited on the cotton fabric at various molar concentrations of reactants. When butylamine and silver nitrate were used at a low concentration (0.1 mM), silver nanoparticles with sizes of 41 $\pm$ 7 nm were formed sparsely over the cotton fabric (see Fig. 1B (b)). Following incubation of the cotton fabric in ethanol solution containing $AgNO_3$ (0.2 mM, 0.5 mM) and butylamine (0.2 mM, 0.5 mM), the size of the Ag nanoparticles increased with increasing concentration of the reactants, and the particles gradually aggregated. The deposition of silver nanoparticles on cotton fabric can also be confirmed by X-ray diffraction (XRD) data (see the ESI†). Four distinct XRD peaks are clearly observed at 2θ values of 38.1, 44.3, 64.4 and 77.3, corresponding to



Scheme 1 Practical and robust deposition of silver nanoparticles on cotton fabric.

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† Electronic supplementary information (ESI) available: Experimental procedures and spectroscopic data. See DOI: 10.1039/b703034g

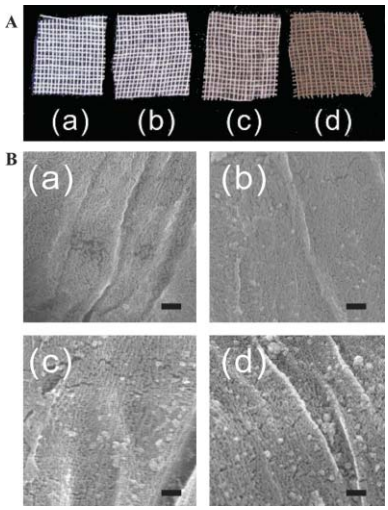

Fig. 1 Photographs of silver nanocoated cotton fabrics. A: (a) Uncoated cotton fabric, (b–d) cotton fabric coated with silver nanoparticles at various loading levels (low, medium, and high). B: FE-SEM image of each fabric sample (a–d) (scale bar = 200 nm).

the reflections of the (111), (200), (220) and (311) crystalline planes of cubic Ag, respectively. We can clearly state that any colloidal silver formed in bulk could not be identified; this indicates that no nucleation center existed in the solution phase. Butylamine is a very weak organic reductant, therefore nucleation centers are scarcely formed in the solution. However, once silver ions were bound to the anionic oxygen sites of cotton fabrics, silver nitrate could be reduced by butylamine anchoring on the surfaces of the cotton fabric. FE-SEM images suggest that Ag nanoparticles are formed on the cotton fabric at the initial stage (Fig. 1B (b)), and that these particles then provide nucleation sites for enlargement and aggregation of the nanoparticles (Fig. 1B (c and d)). This is also confirmed by the following optical properties of cotton fabrics. In fact, silver nanostructures show distinct surface plasmon absorption from the visible to the near infrared region. The four cotton fabrics, whose FE-SEM images are shown in Fig. 1B (a)–(d), are colored white, yellow, dark yellow, and brown (see Fig. 1A). In addition to qualitative estimations based on optical properties, we quantified the amount of silver nanoparticles loaded onto each cotton fabric by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The measured values were 0.61(b), 1.4(c) and 5.3(d) mg g^{-1} of silver nanocoated cotton fabrics corresponding to Fig. 1.

After developing a robust and practical procedure for producing Ag nanocoated cotton fabric, we evaluated the potential of this fabric as an antiseptic dressing or bandage, which are presently in high demand for biomedical applications. In particular, the development of a practical procedure for producing antiseptic dressings that can effectively inhibit both gram-positive and gram-negative bacteria is very important. To test the antimicrobial effect, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were selected as gram-positive and gram-negative test organisms, respectively. Firstly, we evaluated the antibacterial property of the silver nanocoated fabric by measuring the growth inhibition of bacteria on solid Luria–Bertani (LB) agar plates through the variation of the Kirby–Bauer test, a typical antibacterial screening system (see the ESI† for the detailed procedure). No *S. aureus* colonies grew under or near samples B, C and D, although a number of colonies were observed under sample A, which was a non-coated cotton fabric. The growth inhibition test on agar plates was also performed for *E. coli*. In this case, antibiotic resistance was introduced into the *E. coli* strain by transformation of the pGEX-2T plasmid into $CaCl_2$-competent BL21 (Novagen 70235-3) cells. Consistent with the result of the previous experiment, complete growth inhibition of *E. coli* was observed at the contact points or near samples B, C, and D. However, a number of colonies were observed near sample A, with some of them growing on the sample (data not shown).

For clear visualization of the growth differences between the non-coated cotton fabric (sample A) and the other silver nanocoated fabrics (B–D), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) selection was applied to the agar plate screening. As shown in Fig. 2, bacterial colonization was clearly observed only on cotton fabric A. Moreover, the bactericidal effect was observed not only on the silver nanocoated fabrics B, C and D, but also near the edge of these fabrics. However, we could not differentiate the trends of growth inhibition of *S. aureus* and *E. coli* among samples B, C and D.

To validate the dose-dependency of the bactericidal effect of silver nanoparticles on cotton fabrics, we performed growth retardation screening of *S. aureus* and *E. coli* in liquid LB media in the presence and absence of Ag-nanocoated fabrics. As shown in Fig. 3(a), the loading level of silver nanoparticles on cotton fabrics directly correlated with the growth retardation of *S. aureus* in liquid LB media. In the case of 50 and 70 mg of silver nanocoated fabric samples B, C and D, the growth of *S. aureus* was completely inhibited (see the ESI†). When the dose-dependent bactericidal effect of silver nanocoated fabric was evaluated using the ampicillin-resistant *E. coli* strain (BL21), complete growth inhibition was not observed with 30 and 50 mg of silver nanocoated fabric samples B, C and D. However, the 70 mg of silver nanocoated fabric in LB media clearly demonstrated a dose-dependent growth inhibition of *E. coli*. *S. aureus* without antibiotic resistance was more sensitive to silver nanocoated fabrics than antibiotic-resistant *E. coli*. Based on this experiment, we conclude that the silver nanocoated fabrics produced using our practical procedure demonstrate bactericidal activity in a dose-dependent manner through surface contact on agar plates and in liquid media, and the degree of bactericidal effect varies in different bacteria (gram-positive/gram-negative strains with/without antibiotic resistance).

For the practical application of Ag nanocoated fabrics, particularly as antiseptic dressings, we performed a skin irritation

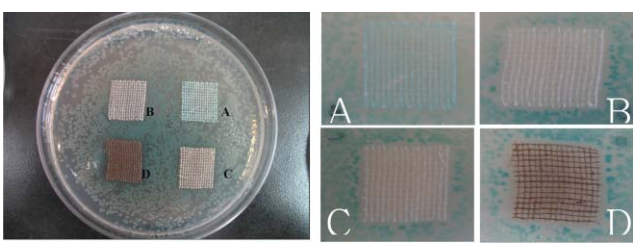

Fig. 2 Growth inhibition of *E. coli* by silver-coated cotton fabric on an agar plate with X-gal selection. A: cotton fabric. B, C and D: silver nanocoated fabric with low, medium and high loading levels, respectively.

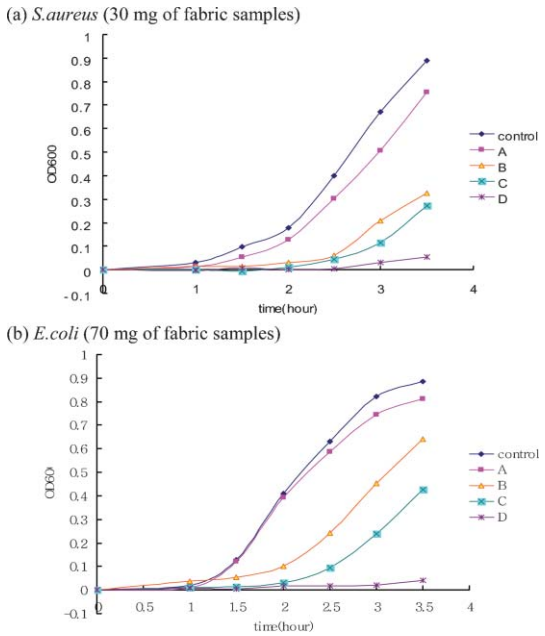

Fig. 3 Growth curve in liquid LB media: (a) *S. aureus* and (b) *E. coli*. Sample A: Non-coated cotton fabric. Samples B, C and D: 0.61, 1.4 and 5.3 mg silver per 1 g of silver nanocoated fabrics, respectively.

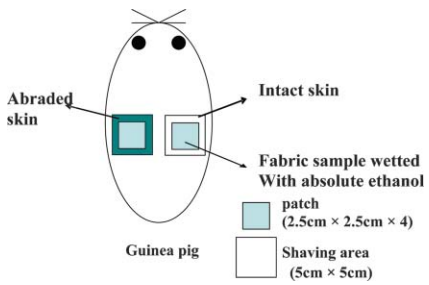

Fig. 4 Skin irritation test using guinea pigs.

experiment using guinea pigs in accordance with the Korean Functional Cosmetics Codex, which is the official guideline for skin irritation tests, regulated by the Korea Food and Drug Administration (KFDA).[14] The backs of guinea pigs were shaved, as schematically shown in Fig. 4. No erythema or edema were observed in any of the test areas on all of the guinea pigs (see the ESI†). Based on this experiment, we can conclude that silver-nanocoated cotton fabric does not cause direct skin irritation. Therefore, this fabric has high potential in medical applications. Additionally, due to its metallic texture, silver nanocoated cotton fabric can also be used in functionalized clothing, as well as in the design of new fashion clothes.

In summary, the fabrication procedure described in this report yielded a stable silver-nanocoated fabric in a very simple and cost-effective manner, with complete control of the silver loading level on the fabric. We validated the potential of this functionalized fabric as an antiseptic bandage by systematically evaluating the bactericidal effect on both gram-positive and gram-negative bacteria, and on an antibiotic-resistant strain. No side effects or skin irritation due to the silver nanocoated cotton fabric were observed in the skin irritation tests performed on guinea pigs in accordance with KFDA guidelines. Since this novel fabric exhibited excellent bactericidal effects on pathogenic bacteria found in wounded skin, for example *S. aureus*, it could be useful for the medical treatment of skin burns or cuts. Moreover, our proposed method is cost-effective, eco-friendly and suitable for the mass production of Ag coatings on various polar substrates[12] (*e.g.* glass, silica and carboxylated polystyrene), as well as cotton fabrics. Therefore, this novel preparation of silver-coated substrates can be directly used as a universal antiseptic apparatus for clinical applications.

This work was supported by the Korea Science and Engineering Foundation (KOSEF), Molecular and Cellular BioDiscovery Research Program from the Ministry of Science and Technology (MOST), and MarineBio21, Ministry of Maritime Affairs and Fisheries, Korea (MOMAF). H. Y. Lee is grateful for the Seoul Science Fellowship award. H. Y. Lee, H. K. Park and Y. M. Lee are grateful for the BK21 Fellowship award. We would like to thank Jung-Taek Kwon and Prof. Myung-Haing Cho for technical assistance in the skin irritation tests, and Prof. Yeong-Jae Seok for providing the MG1655 strain.

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