Supplementary Material (ESI) for Chemical Communications

< Supporting Information >

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

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Experimental Section

Materials. The materials used in the synthesis of the Ag-doped cotton fabrics were as follows. The fabric used in this study was bleached and sterilized 100% cotton fabric purchased from The Boots Company (Nottingham, England). Butylamine (99.5%) and silver nitrate (99%) were purchased from Aldrich (St. Louis, MO, USA) and used as received. Other chemicals, unless specified, were of reagent grade. High pure water (Millipore), of resistivity greater than 18.0 MΩ · cm, was used throughout.

For materials or bacteria used in the antibacterial test were as follows. *Escherichia coli* strain BL 21 (Novagen 70235-3) was obtained from Novagen (San Diego, USA). *Escherichia coli* strain

MG1655 (ATCC 47076) and *Staphylococcus aureus* strain (ATCC 10390) was obtained from ATCC (Manassas, USA). LB broth was purchased from Scharlau (Barcelona, Spain). Bacto agar was purchased from Becton, Dickinson, and Company (Sparks, USA).

Visible spectra were taken with SHIMADZU UV-1650PC to test growth inhibition in liquid LB media.

Preparation and Characterization of silver nanocoated fabrics

Silver deposition on cotton fabric was carried out employing the electroless plating method developed recently in our laboratory. Cotton fabrics in the size of 14 cm × 21.5 cm were placed inside the polypropylene reaction vessel, and a reaction mixture composed of ethanolic $AgNO_3$ and butylamine was added to it. The reason for using a polypropylene container as the reaction vessel was to avoid nonspecific silvering of the reaction vessel. The reaction vessel was incubated for 50 min at 45±1°C with vigorous shaking. As a reaction mixture, the concentration of $AgNO_3$ and butylamine in absolute ethanol was equally changed to 0.1, 0.2 and 0.5 mM in each experiment. The Ag-coated fabrics were finally rinsed with ethanol, and dried in the ambient.

X-ray diffraction (XRD) patterns were obtained on a Rigaku Model D/max03C powder diffractometer using Cu K (1.5419 Å) radiation. (**Supporting Figure** 1) The morphologies of the Ag-coated fabrics were examined by taking field emission scanning electron microscope (FE-SEM) images using JSM-6700F FE-SEM operated at 5.0 kV. The amount of silver deposited on cotton fabric was determined by ICP-AES (ICP-1000IV, SHIMADZU).

Bactericidal Test.

Growth inhibition test on LB agar plate of S. aureus

S. aureus (ATCC 10390) was inoculated on solid LB agar plate and then incubated at 30℃ for overnight. A single colony was inoculated into 5mL LB media and incubated overnight at 30□ with shaking at 200rpm until absorbance of cell suspension at 600nm (OD_{600}) reaches 0.8. The 50μL of first cultured cell suspension was inoculated into 5mL LB media. The diluted cell culture 100μL was plated onto LB agar plate. Then Fabric samples A, B, C, and D (15 strings x 15 strings) was plated onto the plate and was incubated at 30□ for 12h.

Trasnformation and Growth inhibition test on LB agar plate of E.coli (BL21)

The plasmid DNA (pGEX-2T) was transformed as follows. To 1 μL of plasmid DNA (pGEX-2T, 27 ng/mL) diluted in 4μL of ddH2O, 100 μL of freshly thawed CaCl2-competent BL21(Novagen 70235-3) in ice were added. After iced for 10 min, the cells were heat-shocked at 42℃ for 1 min, then iced for 1 min. The transformed cells were added into 500μL LB medium, and incubated at 37℃ with shaking at 200 rpm for 1h. 100μL of cell suspension was plated onto LB_{amp} agar plate. Then Fabric samples A, B, C, and D (15 strings x 15 strings) was plated onto the plate and was incubated at 37℃ for 12h.

Growth inhibition test on LB agar plate of E.coli (MG1655) by X-gal selection

E.coli MG1655 (ATCC 47076) was inoculated on solid LB agar plate and then incubated at 37℃ for overnight. A single colony was inoculated into 5mL LB media and incubated overnight at 37□ with shaking at 200rpm until absorbance of cell suspension at 600nm (OD_{600}) reaches 0.8. The

50μL of first cultured cell suspension was inoculated into 5mL LB media. 40μL of X-gal (2% w/v in DMSO) solution and 7μL of IPTG (20% w/v in sterilized water) were spreaded on to the antibiotics free LB plate. Then the plate was incubated at 37℃ until all of them has been absorbed. The 100μL of 10^5 fold diluted cultured bacterial suspension was inoculated to the plate and the fabric samples A, B, C, and D (15 strings x 15 strings) was plated onto the plate and was incubated at 37□ for 12h.

Growth inhibition test in liquid LB media of S.aureus

A single colony of *S. aureus* was inoculated into 5mL LB media and incubated overnight at 30□ with shaking at 200rpm. After inoculation with 50μL of first cultured cell, 5mL of LB media was incubated at 30□ with shaking at 200rpm until it reach at OD_{600} = 0.8. Then 50μL of second cultured cell was inoculated into 5mL of LB media. Followed by the fabric was added (30mg, 50mg, and 70mg of fabric), the media was incubated at 30℃ with shaking at 200rpm. After 1 h incubation, OD600 was measured using a spectrophotometer at 30-min intervals.

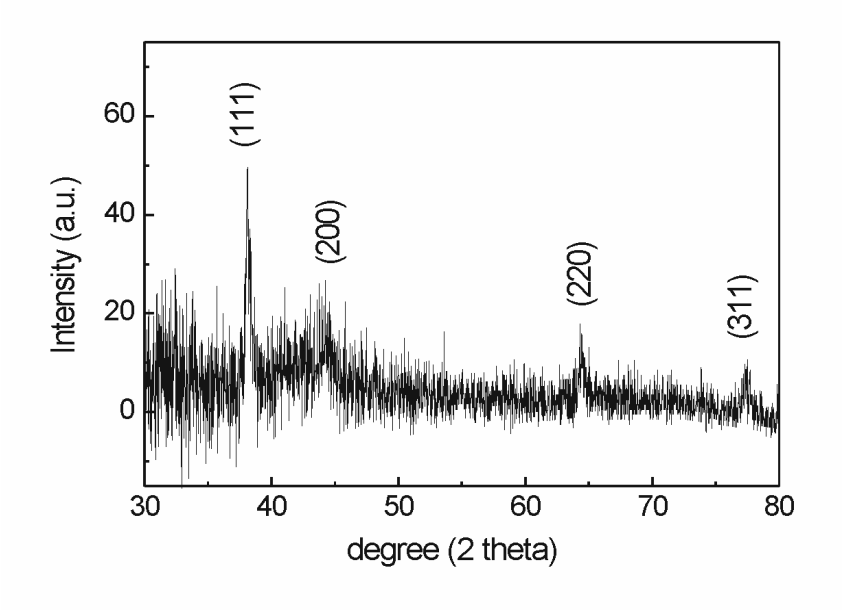
Growth inhibition test in liquid LB media of E.coli BL21

A single colony of transformed *E.coli*(BL21) was inoculated into 5mL LB_{amp} media and incubated overnight at 37□ with shaking at 200rpm. After inoculation with 50μL of first cultured cell, 5mL of LB_{amp} media was incubated at 37□ with shaking at 200rpm until it reach at OD_{600} = 0.8. Then 50μL of second cultured cell was inoculated into 5mL of LB_{amp} media. Followed by the fabric was added (30mg, 50mg, and 70mg of fabric), the media was incubated at 37℃ with shaking at 200rpm. After 1 h incubation, OD600 was measured using a spectrophotometer at 30-min intervals.

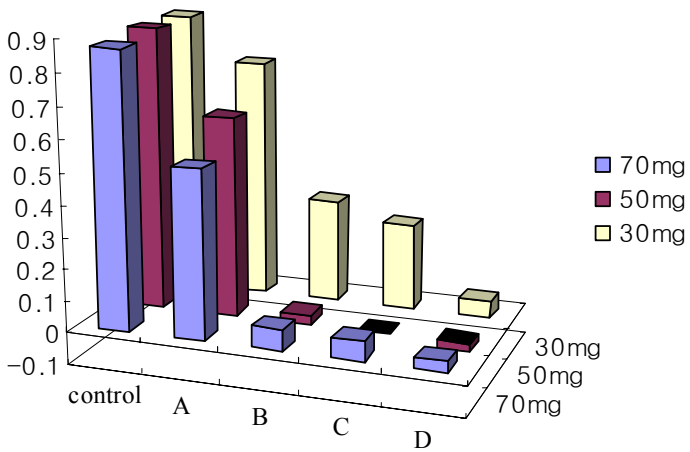
Skin irritation test with Guinea Pig

The back of guinea pigs was shaved as schematically shown in supporting Figure 4. One of the shaved areas was abraded, while the other shaved skin area was kept intact. After 24 h, silver nanocoated fabrics (2.5cm × 2.5cm) were wetted with absolute ethanol and placed on both the shaved areas. After 24 h of contact treatment, the silver nanocoated fabrics were removed, and skin irritation was examined by visual inspection at 4, 24, 48, and 72 h. (**Supporting Figure 4, Supporting table 1 and 2**)

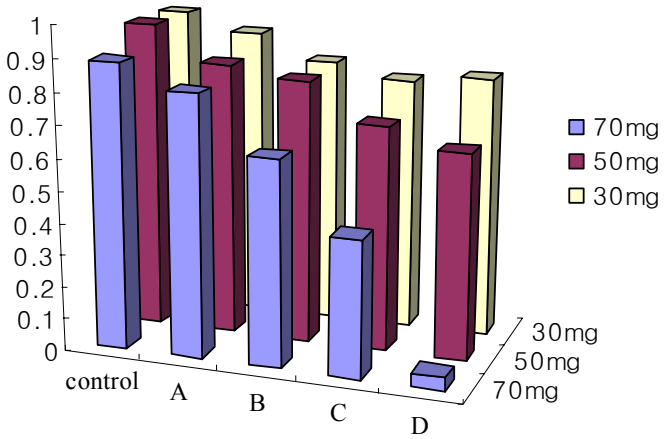
Supporting Figure 1. X-ray Diffraction patterns of Ag-coated cotton fabric



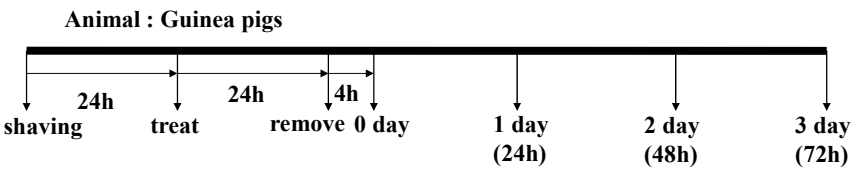
Supporting Figure 2. Growth inhibition of *S. aureus* after 3.5h shaking incubation

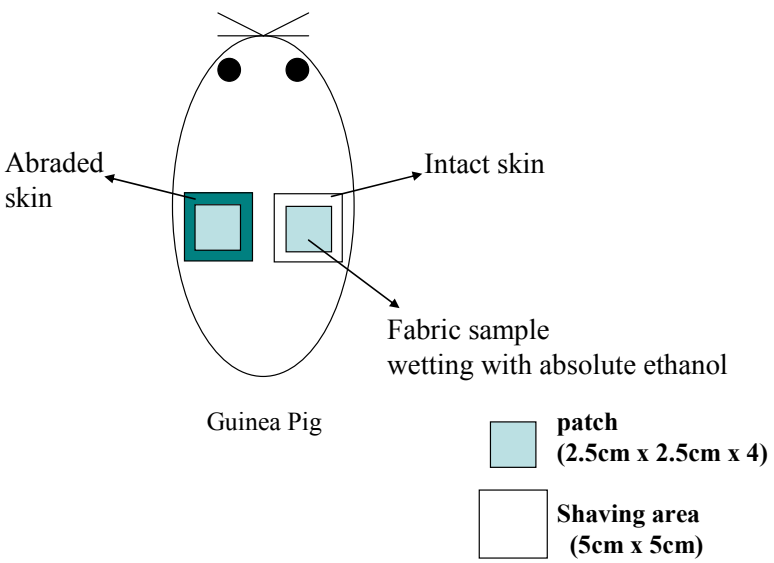


Supporting Figure 3. Growth inhibition of *E.coli* with ampicilin resistency after 3.5h shaking incubation



Supporting Figure 4. Skin irritation test





Supporting Table 1. Skin irritation test performed on guinea pigs in accordance with the Korean Functional Cosmetics Codex regulated by Korea Food and Drug Administration

		0 h		24 h		48 h		72 h		Total
		A	B	A	B	A	B	A	B	
Con	S-N	0	0	0	0	0	0	0	0	0
	S-S	0	0	0	0	0	0	0	0	0
Low	S-N	0	0	0	0	0	0	0	0	0
	S-S	0	0	0	0	0	0	0	0	0
Mid	S-N	0	0	0	0	0	0	0	0	0
	S-S	0	0	0	0	0	0	0	0	0
High	S-N	0	0	0	0	0	0	0	0	0
	S-S	0	0	0	0	0	0	0	0	0

A: erythema, B: edema, S-N: intact skin after shaving, S-S: abraded skin after shaving, Con: control (fabric A) Low: fabric B, Mid: fabric C, High: fabric D

Supporting Table 2. Scoring system of skin irritation test[13]

Score	skin reaction
Erythema or scrab	
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)
Edema	
0	No edema
1	Very slight edema (barely perceptible)
2	Slight edema (edges of area well defined by raising)
3	Moderate edema (raised approximately 1mm)
4	Severe edema (raised more than 1mm and extending beyond area of exposure)