Antibacterial Activity of Cellulose Fabrics Modified with Metallic Salts

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ABSTRACT

This paper deals with the effect of a metallic salt treatment on the antibacterial activity of cellulosic fabrics against three kinds of bacteria: gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), gram-negative bacteria *Klebsiella pneumoniae* (*K. pneumoniae*), and methicillin resistant *Staphylococcus aureus* (MRSA). Two kinds of cellulose fabrics are treated with the metallic salts $CuSO_4$ and $ZnSO_4$. The fabrics are pretreated with succinic acid anhydride to make adsorption of metallic salts more effective. This pretreatment is very effective at increasing the amount of metal ions adsorbed, and is more prominent in Cu ion than in Zn ion. The degree of antibacterial activity of samples treated with Cu salt against one of the *S. aureus* increases with an increasing amount of adsorbed Cu ion. A similar tendency is observed for the samples treated with Zn salt, although its correlation is not as clear as for Cu ion. Antibacterial activity is also confirmed against *K. pneumoniae*. The degree of antibacterial activity of samples treated with Cu salt against MRSA is almost independent of the amount of Cu ion adsorbed, and the tendency is similar for samples treated with Zn salt. After ten laundering cycles, the effect of the treatment is maintained. We conclude that these treatments are very effective in providing antibacterial activities to cellulose fabrics.

Cellulosic fibers are most abundantly used as textile fibers, and their share of the textile market is almost 50%. Although cellulosic fibers have excellent properties as comfortable materials, they have some disadvantages such as the lack of form stability and wrinkle recovery, and several finishing treatments are used to correct these defects. Recently, some finishing treatments have been developed for cellulosic materials to provide antibacterial and deodorant properties [7, 13, 15, 23, 30]. These treatments are very useful for medical patients as well as for elderly people.

Antibacterial and deodorant finishes can be divided into two categories: one is part of the fiber forming process, and the other is incorporated in the finishing process [16, 17, 18]. The former is restricted to regenerated cellulosics, and the latter is widely adapted and classified into three different methods [16, 17, 18]: The first method is when antibacterial agents are directly adsorbed onto fibers [28]. Despite easy application, agents can be lost during laundering. The second method is when antibacterial agents are confined in the network structure of a reactive synthetic resin formed on the fiber surface [21]. The third method is when antibacterial agents are covalently bound to cellulosic fibers [8, 27, 29]. These last two methods have high durability against laundering, but unfortunately, they require heavy instruments and complex procedures and are therefore costly.

During the last two decades, workers have reported some bacteria with resistance to antibiotics such as meth-

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icillin originating from *Penicillium notatum* [1]. One of the most well known bacteria with resistance to antibiotics is methicillin resistant *Staphylococcus aureus* (MRSA), which causes infections in patients with low disease resistance. The number of patients infected with MRSA is reported to be increasing [4, 22, 26]. Infection of patients in the hospital is spread by MRSA through the clothes and/or hands of medical personel [1, 11, 12, 14, 19, 20, 24]. To avoid these infections, it is indispensable to provide clothing used in the hospital with antibacterial activity against MRSA [2, 5, 6, 25].

This paper deals with the modification of cellulosic fabrics by an easy, cost-effective method with laundering durability. For this purpose, we attempt to adsorb two kinds of metallic salts onto the surface of cellulosic fabrics after treatment with succinic anhydride, and we examine their antibacterial activities against gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), gram-negative bacteria *Klebsiella pneumoniae* (*K. pneumoniae*), and MRSA. We also examine their durability with laundering.

Experimental

The characteristics of the two original cellulosic fabric samples are shown in Table I. One is a plain weave fabric made of cotton fibers, and the other is a nonwoven fabric made of rayon fibers using the water-jet technique.

TABLE I. Characteristics of original cellulose fabrics.

Sample code	P	N
Fabric type	plain weave[a]	nonwoven fabric[b]
Cellulose type	cotton	rayon
Thickness, mm	0.231	0.814
Weight, g/m^2	125	139

[a] Made of 1/40 (15tex) cotton yarn. Numbers of warp and weft are 26/cm and 54/cm, respectively. [b] Prepared from 3 denier rayon fibers using water jet method (50kgf/cm^2).

METALLIC SALT TREATMENT

Each fabric sample (10 × 10 cm) was washed with acetone for 3 hours at room temperature and rinsed with distilled water. Fabric samples were pretreated with succinic anhydride in a solvent mixture of dimethyl sulfoxide (DMSO) and N,N-dimethylbenzylamide (DMBA) for 15 hours at 20°C. The composition of the solvent was 20:0.3 (DMSO : DMBA in volume), and the concentration of succinic anhydride varied from 0.1 to 0.3 *M*. The liquor ratio, that is, the ratio of the weight of the fabric (g) to the volume of the reaction medium (ml), was 1:20. After the reaction, samples were washed with 50 ml of acetone three times, rinsed with distilled water, and then dried.

Two kinds of metallic salts, $CuSO_4$ and $ZnSO_4$, were used to prepare the sample fabrics for the antibacterial activity tests. The pretreated fabrics were then treated with 0.5*M* metallic salt solution dissolved in 0.5*M* acetate buffer solution with a pH of 4.6 at room temperature and at a liquor ratio of 1:50 for 24 hours under intermittent stirring. The treated fabrics were then rinsed with distilled water five times and air dried. The amount of metal ion on the fabric was evaluated with atomic absorption spectroscopy.

ANTIBACTERIAL ACTIVITY TESTS

Three kinds of bacteria, *S. aureus* IFO 3060, *K. pneumoniae* IFO 13277, and MRSA ID 1677, certified for testing purposes, were used for the antibacterial activity tests.

A colony of preserved bacteria was taken with a bio-loop and inoculated on a nutrient agar plate, then cultivated at 37°C for 24–48 hours. One colony of bacteria on the agar plate was taken with a bio-loop and inoculated into 20 ml of broth and cultivated at 37°C for 18–24 hours with shaking. The number of living bacteria in the test tube was evaluated by measuring the optical density at 660 nm (the correlation between this and the number of bacteria had been confirmed). The bacterial number in the test tube was adjusted to $1–2 \times 10^6$/ml by dilution with nutrient broth. This broth (0.4 ml) was added to 20 ml of nutrient culture medium and cultivated at 37°C for 2 hours with shaking. The number of living bacteria was then adjusted to $1 \pm 0.3 \times 10^5$/ml by dilution with 1/20 nutrient broth, and this suspension was used as an inoculator and stored at 0°C until use.

The antibacterial activity test was done according to JIS L 1902 [9] as defined for evaluating textile products. Sample fabrics and an untreated sample (control), which were cut into pieces 18 × 18 cm, were put into a 30 ml vial with a screw cap. After sterilization in an autoclave at 121°C for 15 minutes, 0.2 ml of inoculator, the number of which was adjusted to $1 \pm 0.3 \times 10^5$/ml, was applied to both fabric samples and cultivated at 37°C for 18 hours. The number of living bacteria on the fabric was evaluated just after the inoculation and again after 18-hour cultivation by measuring the optical density of diluted suspensions.

Laundering durability tests were done according to the procedure of JIS L0217 130 [10]. Sample fabrics were laundered in a Sharp ES-28HI washing machine, which has a centrifugal squeezer and two baths (each volume of 36 L), using a commercial detergent (Lion Top) at a liquor ratio of 1:30. The time program for laundering was as follows: washing 5 minutes, squeezing 0.5 minutes, rinsing (by overflow) 2 minutes, squeezing 0.5 minutes, rinsing 2 minutes, and squeezing 0.5 minutes. This series of procedures, which is one laundering cycle, was repeated up to ten cycles, then subjected to antibacterial activity tests.

Results and Discussion

WEIGHT INCREASE AFTER SUCCINIC ANHYDRIDE AND METALLIC SALT TREATMENT

We used two kinds of cellulosic fabrics shown in Table I as original samples, and treated them with succinic anhydride and then with metallic salts under the conditions shown in Table II. The hydroxyl group of cellulose is succinoylated with succinic anhydride and produces a carboxyl group. Metal ion is introduced into succinoylated cellulose through that carboxyl group [18]. Weight increases after these treatments are also shown in Table II, and the sample codes appear in the left column. We evaluated the weight increases after each treatment. The weight after the succinic anhydride treatment increased with increasing concentration of reagent in the reaction medium. The relationship between fabric types and weight increase was not distinct.

Metallic salt treatments were given to the samples treated with succinic anhydride as well as the untreated samples. The weight increase after the metallic salt treatment depended on the degree of pretreatment with succinic anhydride. This effect was prominent for samples pretreated at the highest concentration (0.3 *M*) of succinic anhydride. The effect of pretreatment was not as clear at the concentration of 0.1 *M*.

ANTIBACTERIAL ACTIVITIES AGAINST *S. aureus* AND *K. pneumoniae*

We evaluated antibacterial activities of treated plain and nonwoven fabrics against *S. aureus*, and the results are shown in Figure 1 and Table III. The sample fabrics were inoculated with *S. aureus* by a bacterial suspension. The number of *S. aureus* on the fabric was controlled by adjusting the concentration of the bacterial suspension as well as its volume. In both Figure 1 and Table III, *a* and *b* denote the number of *S. aureus* in the untreated fabric

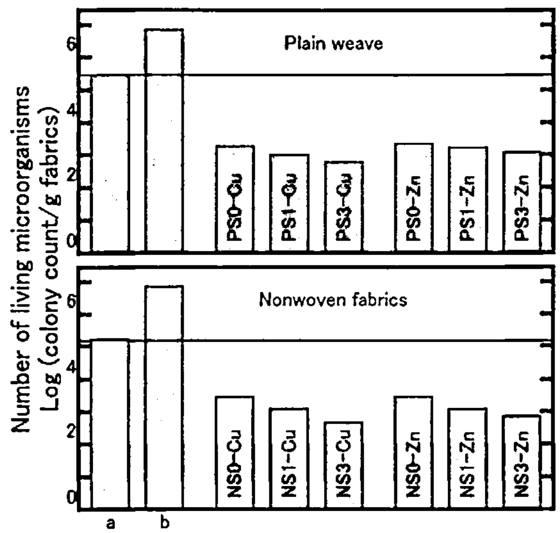

FIGURE 1. Number of living microorganisms after 18 hours incubation inoculated with *S. aureus* for the sample fabrics: (a) number of *S. aureus* in untreated fabric sample just after incubation, (b) number of *S. aureus* in untreated fabric sample after 18 hours incubation.

TABLE II. Preparation and characteristics of sample fabrics used for antimicrobial activity tests.

Sample code	Original sample	Treatment with succinic anhydride[a], concentration *M*	Weight increase after treatment, mg/g fabrics	Treatment[b]	Amount of metal ion absorbed[c], mg/g fabrics
P		–	–	–	–
PS0-Cu		0	–		1.286
PS1-Cu	plain	0.1	23.62	with $CuSO_4$	1.438
PS3-Cu	weave	0.3	62.81		3.700
PS0-Zn		0	–		0.843
PS1-Zn		0.1	23.62	with $ZnSO_4$	0.885
PS3-Zn		0.3	62.81		2.722
N		–	–	–	–
NS0-Cu		0	–		1.435
NS1-Cu	non-	0.1	26.75	with $CuSO_4$	1.685
NS3-Cu	woven	0.3	82.45		6.050
NS0-Zn	fabric	0	–		1.201
NS1-Zn		0.1	26.75	with $ZnSO_4$	1.234
NS3-Zn		0.3	82.45		2.924

[a] Liquor ratio [volume (ml) of reaction mixture to 1 g of fabrics] is 11. [b] Liquor ratio is 11, concentration of metalic salt is 0.5*M*.
[c] Evaluated by atomic absorption spectroscopy.

TABLE III. Results of antimicrobial activity tests for fabric samples inoculated with *S. aureus*.[a]

Sample code		Number of living microorganisms after 18 hours incubation, count/ml	Log (*b*/*c*)
P	*b*	7.2×10^{6} [b]	–
PS0-Cu	*c*	1.8×10^{3} [c]	3.60
PS1-Cu		1.2×10^{3} [c]	3.78
PS3-Cu		6.0×10^{2} [c]	4.08
PS0-Zn		2.3×10^{3} [c]	3.50
PS1-Zn		1.7×10^{3} [c]	3.63
PS3-Zn		1.5×10^{3} [c]	3.68
N	*b*	7.4×10^{6} [b]	–
NS0-Cu	*c*	2.8×10^{3} [c]	3.42
NS1-Cu		1.3×10^{3} [c]	3.76
NS3-Cu		4.0×10^{2} [c]	4.27
NS0-Zn		2.8×10^{3} [c]	3.42
NS1-Zn		1.1×10^{3} [c]	3.83
NS3-Zn		7.7×10^{2} [c]	3.98

[a] Numbers of *S. aureus* in all fabric samples just after incubation were 2.6×10^5 and 2.2×10^5 for P-series and N-series fabrics, respectively. These values are regarded as *a* values for each series. [b] These values are regarded as *b* values. [c] These values are regarded as *c* values.

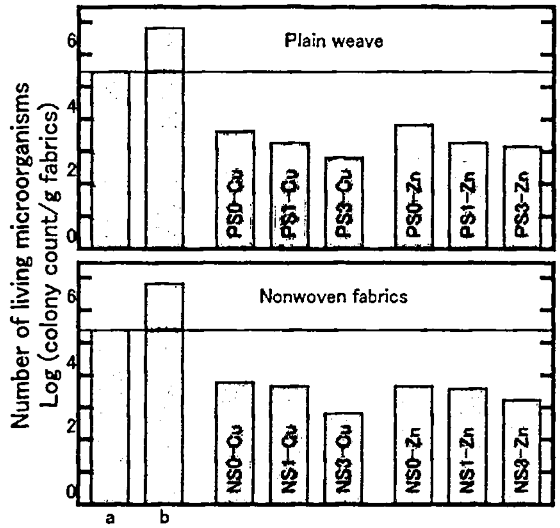

FIGURE 2. Number of living microorganisms after 18 hours incubation inoculated with *K. pneumoniae* for the sample fabrics: (a) number of *K. pneumoniae* in untreated fabric sample just after incubation, (b) number of *K. pneumoniae* in untreated fabric sample after 18 hours incubation.

sample just after incubation and the number after the 18-hour incubation, respectively. In the figure, the values are plotted as a logarithmic scale. The value of *a* is 2.6×10^5 and that of *b* is 7.2×10^6, indicating that the number of bacteria increased twenty-eight fold after the 18-hour incubation for the untreated fabric sample.

On the other hand, numbers of bacteria on all treated samples decreased after the 18-hour incubation, as shown in Table III and Figure 1. In the table, the values of *c* show the number of *S. aureus* in the treated fabric sample after the 18-hour incubation. The values of log(*b*/*c*), denoting the logarithmic ratio of *b* and *c*, correspond to an index for the degree of antibacterial activity. The higher this value, the higher the antibacterial activity of the sample.

It is clear from Figure 1 and Table III that the antibacterial activities of the fabrics treated with metallic salts increase considerably, and the increasing degree is sensitive to the concentrations of metal ions adsorbed, although concentration dependence is small. The improved antibacterial activity is almost independent of the kind of metal ions and the fabric type (plain weave or nonwoven).

We also evaluated the antibacterial activities of treated fabric samples against *K. pneumoniae,* and the results are shown in Figure 2 and Table IV. Sample fabrics were inoculated with *K. pneumoniae* by a process similar to that mentioned earlier for *S. aureus.* These tendencies were also similar to those for the samples inoculated with *S. aureus,* so it is evident that the antibacterial activities of cellulosic fabrics are effectively enhanced by the treatment with succinic anhydride followed by the metallic salt.

TABLE IV. Results of antimicrobial activity tests for fabric samples inoculated with *K. pneumoniae.*

Sample code		Number of living microorganisms after 18 hours incubation, count/ml	Log (*b*/*c*)
P	*b*	6.4×10^{6} [b]	–
PS0-Cu	*c*	4.0×10^{3} [c]	3.20
PS1-Cu		2.4×10^{3} [c]	3.43
PS3-Cu		8.0×10^{2} [c]	3.90
PS0-Zn		6.0×10^{3} [c]	3.03
PS1-Zn		2.4×10^{3} [c]	3.43
PS3-Zn		1.4×10^{3} [c]	3.66
N	*b*	5.2×10^{6} [b]	–
NS0-Cu	*c*	5.4×10^{3} [c]	2.98
NS1-Cu		4.4×10^{3} [c]	3.07
NS3-Cu		6.2×10^{2} [c]	3.92
NS0-Zn		4.2×10^{3} [c]	3.09
NS1-Zn		3.6×10^{3} [c]	3.16
NS3-Zn		1.6×10^{3} [c]	3.51

[a] Numbers of *S. aureus* in all fabric samples just after incubation were 2.6×10^5 and 2.2×10^5 for P-series and N-series fabrics, respectively. These values are regarded as *a* values for each series. [b] These values are regarded as *b* values. [c] These values are regarded as *c* values.

ANTIBACTERIAL ACTIVITIES AGAINST MRSA

We evaluated the antibacterial activities of treated fabric samples against MRSA, and the results are shown in Figure 3 and Table V. In the figure and the table, the result for the commercial sample (PC) is also shown as a control. Sample fabrics were inoculated with MRSA by a bacterial suspension. In Figure 3 and Table V, the *a* and *b* denote the number of MRSA in the untreated fabric sample just after incubation and the number after the

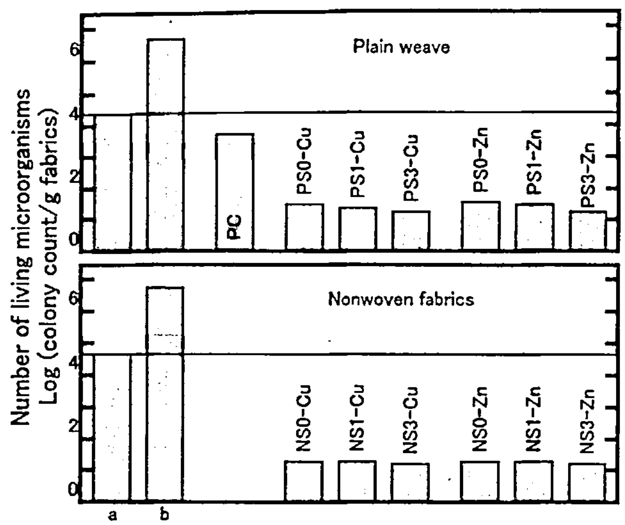

FIGURE 3. Number of living microorganisms after 18 hours incubation inoculated with MRSA for the sample fabrics: (a) number of MRSA in untreated fabric sample just after incubation, (b) number of MRSA in untreated fabric sample after 18 hours incubation.

TABLE V. Results of antimicrobial activity tests for fabric samples inoculated with MRSA.[a]

Sample code		Number of living microorganisms after 18 hours incubation, count/ml	Log (b/c)
P	b	4.45×10^6 [b]	–
PC	c	4.54×10^3 [c]	2.99
PS0-Cu	c	2.08×10^c	5.33
PS1-Cu		1.82×10^c	5.39
PS3-Cu		1.55×10^c	5.46
PS0-Zn		2.13×10^c	5.32
PS1-Zn		2.08×10^c	5.33
PS3-Zn		1.55×10^c	5.46
N	b	5.00×10^6 [b]	–
NS0-Cu	c	1.86×10^c	5.43
NS1-Cu		1.85×10^c	5.43
NS3-Cu		1.84×10^c	5.43
NS0-Zn		1.86×10^c	5.43
NS1-Zn		1.85×10^c	5.43
NS3-Zn		1.84×10^c	5.43

[a] Numbers of MRSA in all fabric samples just after incubation were 2.45×10^4 and 4.62×10^4 for P-series and N-series fabrics, respectively. These values are regarded as a values for each series. [b] These values are regarded as b values. [c] These values are regarded as c values.

18-hour incubation, respectively. In the figure, the values are represented as a logarithmic scale. The value of a is 2.45×10^4 and that of b is 4.45×10^6, indicating that the number of bacteria increased 180-fold after the 18-hour incubation for the untreated fabric sample.

On the other hand, the numbers of bacteria on all the treated samples decreased drastically after the 18-hour incubation, as shown in Table V and Figure 3. In the table, the values of c denote the numbers of MRSA in the treated fabric samples after the 18-hour incubation. The values of log(b/c) are the logarithmic ratio of b and c, which is an index of the degree of antibacterial activity.

From Figure 3 and Table V, it is evident that the antibacterial activities of fabrics treated with metallic salts increased drastically. Such a drastic improvement is easily realized by comparing these results with the commercial sample PC, which was treated with octadecyl dimethyl(3-trimethoxysilylpropyl) ammonium chloride.

The improving effect of metallic-treated samples is almost independent of the concentrations of metal ions adsorbed. The improvement is also independent of the kind of metal ions examined. In addition, the fabric type (plain weave or nonwoven fabric) also has no effect on the degree of improvement. The antibacterial activity of cellulosic fabrics against MRSA is effectively enhanced by the treatment with succinic anhydride followed by the metallic salt.

LAUNDERING DURABILITY OF TREATED SAMPLES

It is very important to examine the durability of these finishing treatments after laundering, since fabrics are washed many times during wear life. Sample fabrics were laundered ten cycles in a home laundering machine, and subjected to antibacterial activity tests by a process similar to that mentioned earlier. The results for *S. aureus* are shown in Figure 4 and Table VI. The results for MRSA are shown in Figure 5 and Tables VII and VIII. These figures and tables reveal that the antibacterial activities of fabric samples are maintained even after ten laundering cycles, although the degree of antibacterial

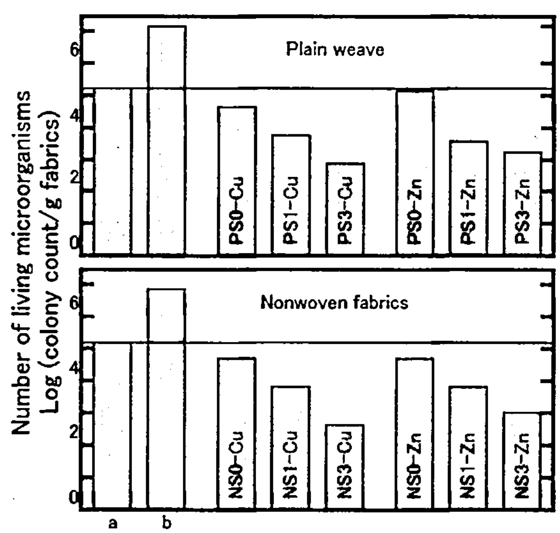

FIGURE 4. Number of living microorganisms after 18 hours incubation inoculated with *S. aureus* for the sample fabrics laundered ten cycles: (a) number of *S. aureus* in untreated fabric sample just after incubation, (b) number of *S. aureus* in untreated fabric sample after 18 hours incubation.

TABLE VI. Results of antimicrobial activity tests for fabric samples after ten laundering cycles.[a]

Sample code		Number of living microorganisms after 18 hours incubation, count/ml	Log (*b*/*c*)
P	*b*	1.4×10^{7} [b]	–
PS0-Cu	*c*	3.6×10^{4} [c]	2.59
PS1-Cu		6.4×10^{3} [c]	3.34
PS3-Cu		7.6×10^{2} [c]	4.27
PS0-Zn		1.3×10^{3} [c]	4.03
PS1-Zn		4.8×10^{3} [c]	3.46
PS3-Zn		1.7×10^{3} [c]	3.92
N	*b*	1.5×10^{7} [b]	–
NS0-Cu	*c*	9.0×10^{4} [c]	2.22
NS1-Cu		5.8×10^{3} [c]	3.41
NS3-Cu		4.6×10^{2} [c]	4.51
NS0-Zn		4.5×10^{4} [c]	2.52
NS1-Zn		5.8×10^{3} [c]	3.41
NS3-Zn		9.8×10^{2} [c]	4.18

[a] Fabric samples were inoculated with *S. aureus*. Numbers of *S. aureus* in all fabric samples just after incubation were 2.6×10^5 and 2.2×10^5 for P-series and N-series fabrics, respectively. These values are regarded as *a* values for each series. [b] These values are regarded as *b* values. [c] These values are regarded as *c* values.

TABLE VII. Results of antimicrobial activity tests for fabric samples after ten laundering cycles.[a]

Sample code		Number of living microorganisms after 18 hours incubation, count/ml	Log (*b*/*c*)
P	*b*	4.45×10^{6} [b]	–
PC	*c*	4.75×10^{3}	2.97
PS0-Cu	*c*	2.22×10^{c}	5.30
PS1-Cu		1.82×10^{c}	5.39
PS3-Cu		1.55×10^{c}	5.46
PS0-Zn		2.28×10^{c}	5.29
PS1-Zn		2.08×10^{c}	5.33
PS3-Zn		1.55×10^{c}	5.46
N	*b*	5.00×10^{6} [b]	–
NS0-Cu	*c*	1.98×10^{c}	5.40
NS1-Cu		1.85×10^{c}	5.43
NS3-Cu		1.84×10^{c}	5.43
NS0-Zn		2.00×10^{c}	5.40
NS1-Zn		1.85×10^{c}	5.43
NS3-Zn		1.84×10^{c}	5.43

[a] Fabric samples were inoculated with MRSA. Numbers of MRSA in all fabric samples just after incubation were 2.4×10^4 and 4.62×10^4 for P-series and N-series fabrics, respectively. These values are regarded as *a* values for each series. [b] These values are regarded as *b* values. [c] These values are regarded as *c* values.

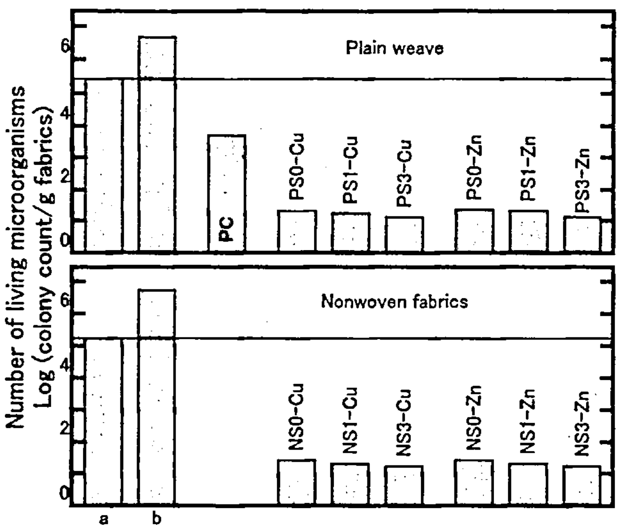

FIGURE 5. Number of living microorganisms after 18 hours incubation inoculated with MRSA for the sample fabrics laundered ten cycles: (a) number of MRSA in untreated fabric sample just after incubation, (b) number of MRSA in untreated fabric sample after 18 hours incubation.

TABLE VIII. Reduction of antibacterial activity after ten laundering cycles.[a]

Sample code	Antibacterial activity: log (*b*/*c*)[b] Before laundering [*BL*]	After 10 laundering cycles [*AL*]	Reduction of antibacterial activity, %[c]
PC	2.99	2.97	0.02
PS0-Cu	5.33	5.30	0.53
PS1-Cu	5.39	5.39	0.0
PS3-Cu	5.46	5.46	0.0
PS0-Zn	5.32	5.29	0.56
PS1-Zn	5.33	5.33	0.0
PS3-Zn	5.46	5.46	0.0
NS0-Cu	5.43	5.40	0.50
NS1-Cu	5.43	5.43	0.0
NS3-Cu	5.43	5.43	0.0
NS0-Zn	5.43	5.40	0.58
NS1-Zn	5.43	5.43	0.0
NS3-Zn	5.43	5.43	0.0

[a] Inoculated with MRSA. [b] Refer to Tables V and VII. [c] $([BL] - [AL])/[BL] \times 100$.

activity becomes less effective in some samples, such as NSO-Zn and PSO-Zn, with poor absorption of metal ions (see Figure 4 and Table VI).

We must refer to the preliminary result in this study. The antibacterial activity of fabrics was also enhanced in samples treated only with succinic anhydride (without the metallic salt treatment), although the result is not shown here. Unfortunately, the reason for this phenomenon is unclear at present. A detailed investigation of these samples is now in progress.

Conclusions

Antibacterial activities of cellulosic fabrics are easily achieved by treating them with succinic anhydride followed by metallic salts such as copper sulfate and zinc sulfate. The antibacterial activity of the fabrics is maintained after laundering at least ten cycles.

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