Antimicrobial Cotton Cloth Immobilized with Chemicals Derived from Plants

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Cotton cloths combined with chemicals of plant origin were prepared and their antimicrobial activities were investigated. Tannic acid and berberine, respectively, were fixed onto aminoethylated and carboxymethylated cotton cloths. Escherichia coli W3110 and Staphylococcus aureus IFO 13276 were used as the representatives of bacteria. Both aminoethylated- and carboxymethylated clothes, by themselves, showed a considerable antibacterial activity. Fixation of tannic acid and berberine onto the cloths enhanced the antibacterial activity. The cloths showed higher antibacterial activities against E. coli than against S. aureus. SEM observation after the test revealed that E. coli cells were totally collapsed on the surface of the cloth, while some S. aureus cells were observed to remain in the normal shape. The cloth fixed with tannic acid withstood 20 times of washing by a cloths-washer with laundry soap.

Key words: Antimicrobial Cotton Cloth/tannic acid/berberine/Escherichia coli/Staphylococcus aureus.

INTRODUCTION

Plants produce and accumulate various types of antimicrobial substances, which are specific to plants (prohibitins). They are also able to produce substances in response to the invasion by pathogenic bacteria (phytoalexins). They are mostly phenolic compounds and terpenoid compounds (linuma, 1998).

Various studies on prohibitins and phytoalexins have been done with the aim of utilizing them as a forestry resource (Yasue, 1995). Among them, hinokitiol has been spotlighted recently as a commercially profitable substance. Because hinokitiol has a strong antibacterial and antifungal activity against a broad spectrum of microorganisms, it has been used for various purposes such as an agent to prevent a plant disease, as a component of a preservative paint for wood, and as a preventive agent against dental caries (Okabe et al., 1990). Furthermore, hinokitiol is expected to be useful for prevention of methicillin-resistant Staphylococcus aureus (MRSA), which is becoming a major problem in hospitals since hinokitiol shows a growth inhibition effect at a concentration of only 100 μg/ml (Okabe et al., 1994).

As a plant antimicrobial substance with effectiveness almost equal to that of hinokitiol, we examined tannic acid, which is a phytoalexin of Rhus javanica and Quercus sp., and berberine, which is a prohibitin of Phellodendron amurense RUPR and Coptis japonica THUNB, long utilized as an oriental medicine (antidiarrheic), as an antimicrobial agent for cloth.

Up to now, there have been no reports of studies on
plant antimicrobial substances, including hinokitiol, bonded to fibers. Most antibacterial fibers used currently in medical institutions are synthetic fibers that have been mixed and kneaded with inorganic compounds such as silver as antimicrobial agents (Deitch et al., 1987; Nishino et al. 1997). Disposal of antimicrobial made from plant compounds will impose little environmental risk. Also, in medical institutions, as hospital-related infections and the care of aged patients with weakened immune function are becoming important issues, and the use of immunosuppression agents in organ transplant patients is increasing, there is a need for a novel approach to clinical control of pathogenic microbe activities. We expect that antimicrobial fibers made of plant components will be useful in this situation for fabricating patients’ cloths, bed sheets, ward curtains and other items.

Tannic acid is a tannin found in Chinese nutgall, Aleppo galls and other plant sources, and its chemical structure is still unclear. It is considered to be a mixture of ester compounds formed from glucose and galloyl gallic acid. Tannic acid is able to bind protein, metals and alkaloids, forming compounds with poor solubility. This characteristic makes tannic acid effective for convergence of skin, detoxication, and repair of facial burns, and it is now utilized as a drug (Ogata et al., 1986a). However, there is no detailed report about the antibacterial ability of tannic acid. Berberine is one of the alkaloids of the isoquinoline series. It has a quaternary ammonium structure (Ogata et al., 1986b), and is utilized as a drug in hydrochloride or sulfate form (Ogata et al., 1986b, 1986c and 1986d). It is reported that the minimum inhibitory concentration of berberine sulfate is about 25–100 ppm for pathogenic fungi and bacteria such as S. aureus, Vibrio cholerae, and Nisseria gonorrhoeae (Amin et al., 1969). Berberine is currently utilized as an oral drug and an ingredient of tooth powder.

In this study, we prepared cotton cloths combined with tannic acid and berberine, respectively, and investigated their antimicrobial activities against Escherichia coli and Staphylococcus aureus.

MATERIALS AND METHODS

Materials
Tannic acid was purchased from Wako Pure Chemical Industries, Ltd. Berberine chloride was purchased from Sigma Chemical Co. Bleached cotton cloth used is one of the commercial goods sold in Japan. The agents for culture media were purchased from Difco Laboratories Ltd. All other chemicals were of chemical grade and were purchased from Wako Pure Chemical Industries, Ltd. Escherichia coli W3110 were provided by a researcher of our university and Staphylococcus aureus IFO 13276 were provided by the Institute for Fermentation, Osaka (Incorporated).

Preparation of antimicrobial cloth
Six kinds of cloth specimens including a control were prepared.

Control cotton cloth
Bleached cotton cloth was washed with hot deionized water and then air-dried.

Aminoethylated cotton cloth
Cotton cloth was cut into pieces of 4 cm x 4 cm in size, 0.4 g in weight. Fifty pieces (20 g) of the cloth were immersed in 0.4 L of an aqueous 1 M sodium hydroxide (NaOH) solution for 10 min at ambient temperature. Acrylamide (178 g) was added, and the mixture was kept at 40 °C for 2 h (carbamoylation). The carboxamoylated cloth, after being washed with deionized water, was immersed in 0.4 L of an aqueous 1.4 M sodium hypochlorite (NaOCl) solution for 1 h at 20 °C (N-chlorination), and then washed with deionized water. The N-chlorocarbamoylated cloth was then immersed in 0.4 L of an aqueous 1 M NaOH solution at 80 °C for 30 min (Hoffmann reaction). The aminoethylated cloth thus obtained was air-dried after being rinsed thoroughly with deionized water.

Cotton cloth combined with tannic acid
Ten grams of the aminoethylated cotton cloth were immersed in 0.4 L of an aqueous 0.5% tannic acid solution at ambient temperature for 16 h, washed twice over 12 h with a large amount of deionized water, and then air-dried.

Carboxymethylated cotton cloth
Fifty pieces of cotton cloth (20 g) were immersed in 0.4 L of isopropyl alcohol at ambient temperature for 10 min. NaOH was added to this mixture to a concentration of 1/30 M, and the mixture was kept at ambient temperature for 1 h. Then monochloroacetic acid was added to a concentration of 1/50 M, and the mixture was kept at 55 °C for 3.5 h. The mixture was then neutralized with acetic acid. The carboxymethylated cloth obtained was washed with diluted hydrochloric acid solution and methanol, and then air-dried.

Cotton cloth combined with berberine
Ten grams of the carboxymethylated cotton cloth were immersed in 0.4 L of an aqueous 0.1% berberine chloride solution at 80 °C for 1 h. Sodium bicarbonate
(0.088 g) were added to the mixture at immersion time of 5 min. The cloth was washed twice over 12 h with a large amount of deionized water, and then air-dried.

**A commercial antimicrobial cloth**

Cloth processed with octadecyl dimethyl (3-trimethoxysilylpropyl) ammonium chloride was used as a representative of commercially available antimicrobial cloths for comparison. The antimicrobial agent contained in this cloth is a quaternary ammonium compound.

**Chemical analyses of the antimicrobial cloths**

The amounts of aminoethyl groups and carboxymethyl groups in the cloth specimens were determined by acid-base titration and the results were shown in terms of the degree of substitution with respect to the hydroxyl groups of cellulose. The amount of tannic acid combined with the aminoethylated cotton cloth was calculated from the change in the absorbance (at 280 nm) of the tannic acid solution caused by the fixation process. The amount of berberine combined with the carboxymethylated cotton cloth was calculated from the change in the absorbance (at 340 nm) of the berberine solution caused by the fixation process.

**Determination of the minimal inhibitory concentrations (MIC)**

Peptone-Yeast extract (PY) liquid-culture medium (1L) was prepared from polypeptone 5 g, NaCl 5 g, powderd yeast extract 1 g and distilled water. Tannic acid and berberine chloride, respectively, were added to the PY liquid-culture media at fixed concentrations ranging from 0.005 to 0.05%, respectively. The mixtures were sterilized by autoclave at 121°C for 15 min. Bacterial cultures were prepared by inoculating *E. coli* and *S. aureus*, respectively, into PY liquid-media free from tannic acid and berberine in test tubes. The cultures were shaken for 12 h at 30°C and the absorbances at 660 nm were adjusted to about 0.5. Fifty μl of the bacterial liquid culture was inoculated into 5 ml of the sterilized PY cultures to which tannic acid and berberine had been added, and the mixtures were then shaken at 37°C. Absorbances at 660 nm of the liquid-culture media were measured every 2 h over 24 h, and bacterial growth inhibition by tannic acid and berberine were evaluated.

**Confirmation of viable cells in the culture medium of zero absorbance**

One ml of the medium was diluted successively 10-fold with a buffered physiological saline solution. One ml of the diluted solution at each phase was mixed with a dissolved standard agar medium (peptone 5 g, yeast extract 2.5 g, agar 15 g, purified water 1000 ml, pH 7.1) in a Petri dish, and then cultured at 37°C for 48 h, and the number of living bacteria was confirmed by counting the colonies in the medium.

**Antibacterial activity test of cloths**

A qualitative test (Halo test) for antibacterial cloths was done as following the method of JIS L 1902-1998. The bacteria were *E. coli* and *S. aureus*. All of the culture medium (slant medium for archives of strain, liquid medium for pre-culture, and agar medium for tests) were each bouillon-mixed medium. Bouillon solution prepared as follows; 5 g of meat extract, 10 g of peptone and 5 g of sodium chloride were dissolved in 1l of distilled water, the solution was adjusted to pH 7±0.2 by 1N NaOH afterwards.

One ml of bacteria liquid medium which was precultured at 37°C for 24 h was mixed with 15 ml of the sterilized agar medium by autoclave at 121°C for 15min. The mixed solution was hardened in a dish, and dried at ambient temperature for 2 h in a clean bench. The circular cloth of 28mm in diameter was sterilized by an autoclave at 121°C for 15min. Sample cloth was put onto the agar medium, and it was cultured at 37°C for 24 h in an incubator. The halo appeared around the test cloth was observed afterwards. The qualitative test was repeated 3 times.

A quantitative test (antibacterial ability test) was also done as following the prescribed method of JIS L 1902-1998. However, in cultivation after serial dilution, because the leached tannic acid reacted with bouillon, the color of culture media changed, and differentiation of the appeared colony was slightly difficult, the cultivation after serial dilution was done with the standard agar medium. Although the antibacterial ability of aminoethylated cloth was compared with a bouillon medium between a standard agar medium with both bacteria, there was the difference between neither.

Antibacterial activity test in this study was done as follows. Sen-i Seihin Eisei Kakou Kyougikai (Conference of Textiles Health Processing; SEK) provides several methods for evaluating the antimicrobial and deodorization abilities of various processed products (fibers, cloth etc.) (Sakagami, 1997a). According to the measuring method of microbial number of those methods, the antimicrobial abilities of cloths were evaluated.

The enumeration of bacteria of SEK has prescribed to measure using *S. aureus* and *Klebsiella pneumoniae* as bacteria. However, because we examined the use to bed, sheet and/or curtain for hospital and person of advanced age nursing in mind, *E. coli*
which was bacteria of coliform bacteria and excrements-infestation index bacteria same as K. pneumoniae was used.

The culture of E. coli (or S. aureus) was shaken at 30°C for about 12 h in a PY liquid-culture medium in a test tube. When the absorbance of the culture medium (at 660nm) reached about 0.5, the culture fluid was diluted about 100-fold with the sterilized PY liquid-culture medium. This diluted solution was used as the pre-culture liquid. Each cloth was cut into pieces of 2 cm x 2 cm in size, 0.1 g in weight. Four pieces of cloth laid upon each other were used as a single specimen. Two control specimens were prepared for one antibacterial specimen. The three specimens were placed in 75-ml vials, and sterilized by autoclave at 121°C for 15 min. The pre-culture liquid (0.2 ml) was then inoculated onto each of the sterilized specimen. Twenty ml of a buffered physiological saline solution were added promptly to one of the control vials, and the viable cell count was measured. The other two vials were left to stand at 37°C for 18 h, 20 ml of the buffer solution were added to each vial, and the viable cell count was measured. The three vials to which the buffered physiological saline solution had been added were shaken for 3 min to allow the living bacteria to disperse in the fluid. The solutions containing the dispersed bacteria were diluted successively 10-fold with the buffered physiological saline solution. One ml of each diluted solution at each phase was mixed with the dissolved standard agar medium in a Petri dish. After culture at 37°C for 48 h, the numbers of living bacteria in the specimens were calculated by multiplying the number of the colonies by the degree of dilution. After the exposure of each bacterium to the cloths for 18 h, the numbers of living cells were determined. The antimicrobial ability was expressed as the increase-decrease differential value that was calculated by the following formula:

\[
\log B/A - \log C/A = \log B/C
\]

where A, Viable cell count in the sample solution immediately after inoculation onto the untreated cotton cloth. B, Viable cell count after inoculation onto the untreated cotton cloth and culture at 37°C for 18 h and C, Viable cell count after inoculation onto the antimicrobial treated cloth and culture at 37°C for 18 h.

The SEK stipulates that this antibacterial activity test is valid in the case where \(\log B/A\) is greater than 2.0, and that the cloth can be said to have an antimicrobial ability in the case where \(\log B/C\) is greater than 1.6.

In this study, the antimicrobial ability test was repeated 3 times.

The buffered physiological saline solution was prepared as follows: Five grams of sodium chloride, 72 ml of an aqueous 0.2 M disodium hydroygenphosphate solution, and 28 ml of an aqueous 0.2 M sodium dihydrogenphosphate solution were mixed, and the volume of the solution was adjusted to 1 l with distilled water.

**Measurement of pH**

The pH of each wet specimen after the antimicrobial activity test was measured with a pH test paper.

**Observation by scanning electron microscope**

After the antimicrobial activity test the specimens were observed with a scanning electron microscope (SEM). Each specimen of antimicrobial cloth, onto which a bacterium had been inoculated and cultured for the prescribed time, was placed on carbon paper tape stuck on the top of a specimen support. Gold was then vapor-deposited onto the cloth with an ion sputter apparatus for 5 min. The SEM used was JSM-5600 (JEOL Ltd.) operated at an accelerating voltage of 5 kV in high vacuum mode, and the secondary electron image (SEI) was observed.

**Durability to laundering**

Each cloth was washed ten or twenty times in an automatic washing machine, and then its antimicrobial activity was measured. One cycle of washing involved about 20 g of cloth with 20 ml of neutral-detergent (brand name: Monogen) in 16 l of water that had been passed through an activated carbon water-purifier. The cleaning cycle included washing 3 min, spinning for 3 min, and three rinses. Each rinse was done with 16 l of water and was accompanied by spinning for 3 min. finally the cloth was air-dried.

**Comparison of the measuring method of microbial number with the regularized test method for antimicrobial activity measurement**

The regularized test method for antimicrobial activity measurement was carried out for some antibacterial cloths to compare with the measuring method of microbial number. The regularized test method was provided as improvement of the measuring method of microbial number by SEK in 1994. The culture medium of the measuring method of microbial number was PY culture medium, whereas that of the regularized test method was nutrient broth (3 g of meat extract, 5 g of peptone and 5 g of sodium chloride were dissolved in 1 l of distilled water, the solution was adjusted to pH 6.8 ± 0.2 by 1 N NaOH afterwards). And the physiological saline solution utilized in the regularized test method was prepared as follows; 8.5 g of
TABLE 1. Inhibition of bacteria growth by tannic acid and berberine.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Culture time (h)</th>
<th>Conc of tannic acid (%)</th>
<th>Conc of berberine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.005 0.01 0.02 0.05</td>
<td>0.005 0.01 0.02</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td>0^a 0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.53 0.01 0 0</td>
<td>0.03 0 0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.57 0.38 0.24 0</td>
<td>0.48 0.08 0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.43 0.45 0.42 0</td>
<td>0.43 0.41 0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0</td>
<td>0 0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.35 0.09 0.08 0.07 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.47 0.25 0.24 0.14 0</td>
<td>0.08 0 0</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.42 0.35 0.33 0.17 0</td>
<td>0.39 0 0</td>
</tr>
</tbody>
</table>

^aA_50
^bNot done.

sodium chloride were dissolved in 1 l of distilled water, and 0.2 % (w/v) surface-active agents were added it only for washing.

RESULTS AND DISCUSSION

Minimal inhibitory concentrations (MIC)

Table 1 shows the minimal inhibitory concentrations (MIC) of tannic acid and berberine against E. coli and S. aureus. At a concentration of 0.02% both tannic acid and berberine were able to control the propagation of E. coli for 24 h. The MIC against S. aureus was 0.05% with tannic acid and 0.01% with berberine. The plate culture method at the culture medium of zero absorbance confirmed the presence of viable E. coli and S. aureus cells in the liquid culture medium containing tannic acid or berberine. In other wards, tannic acid and berberine acted as bacteriostatic agents against these bacteria even at the point of MIC.

S. aureus is a gram-positive bacterium, and E. coli is a gram-negative one. The difference is in the structure of the bacterial cell wall. The peptidoglycan layer of the cell wall of gram-positive bacterium is thick, while that of the gram-negative bacterium is thin, and gram-negative bacterium has an outer-membrane outside the peptidoglycan layer. Lipopolysaccharides are present as the outside layer of the duplex structure of this outer-membrane, and the surface is hydrophilic. This forms a permeability barrier for hydrophobic antimicrobial agents, and protects the cell from invasion (Neidhardt et al., 1990).

It is conceivable that tannic acid, which acts powerfully on the gram-negative E. coli, has an interaction with hydrophilic outer-membrane and protein of the cell wall causing malfunction of the cell wall. On the other hand, berberine, which is hydrophobic and acts powerfully on the gram-positive S. aureus having a hydrophobic cell wall, is considered to get through the cell wall and disrupt either one or both of the functions of the cell wall and cytoplasm.

Characteristics of the antimicrobial cloth

The degrees of substitution of aminoethyl groups and carboxymethyl groups with respect to the hydroxyl groups of the cellulose in the cloths are 0.044 of the degree of substitution (DS) and 0.055 of DS, respectively. It is defined that when all of the three hydroxyl groups of a glucose unit, the structural unit of cellulose, are substituted, the DS is 3. The amount of tannic acid combined with the aminoethyl group and that of berberine combined with carboxymethyl group are 0.028 as DS and 0.017 as DS in terms of the degree of substitution, respectively.

Tannic acid combined with 63.6% of the aminoethyl groups, and the berberine combined with 30.9% of the carboxymethyl groups.

Results of the antibacterial activity test

In the results of the qualitative test, the clear halo was not confirmed in all samples. Discoloration (about 1.5 cm in width) of culture medium by the leached tannic acid was observed around the aminoethylated cloth combined with tannic acid. Because, in the discolored range of color, there was a little colony count, it was estimated that the bacterial growth was inhibited by the leached tannic acid. That is to say, it was considered that the antibacterial ability of tannic acid-treated cloth appeared by both of antibacterial abilities of the fixed tannic acid on the treated cloth together with the leached-tannic acid.

In the results of the quantitative test, the changes in the viable cell count of E. coli on the antimicrobial cloths are shown in (A) of Fig. 1. The pH changes of the antimicrobial cloths over the period of culture with E. coli are shown in (B) of Fig. 1. Every antimicrobial cloth examined diminished the viable cell count of E. coli in less than 1 h. The aminoethylated cloth combined with tannic acid, the carboxymethylated cloth and the carboxymethylated cloth combined with
berberine annihilated the bacteria in 1 h. When washings of the test specimens were cultured by the plate-culture method no colony of viable E. coli was observed. However, in the case of the aminoethylated cloth, although the count of viable bacteria reached zero once in 1 h, the count began to increase 3 h later. That is, the aminoethylated cloth lost its antimicrobial activity as the culturing time increased. It may be due to the accumulation of the dead bacterial cells bound to the aminoethyl groups. The pH changes of the antimicrobial cloths suggest that the propagation of E. coli is favored in weakly alkaline media but is hindered in acidic media.

In the case of S. aureus (Fig. 1 C), the propagation was suppressed by every antimicrobial cloth in a similar manner to the case of E. coli. However, the antibacterial activities of the cloths against S. aureus were slightly less than those against E. coli. Berberine showed a lower minimal inhibitory concentration (MIC) for S. aureus than for E. coli (Table 1). However, this was not the case when berberine was bound to cloth, and S. aureus, unlike E. coli, was still able to propagate at a low pH (Fig. 1 C and D).

It must be noted that tannic acid bound to an aminoethyl group showed a antimicrobial activity as high as that of free tannic acid, but berberine bound to a carboxymethyl group showed a less antimicrobial activity than expected from that of free berberine. It is considered that tannic acid is fixed on the cloth by ionic bonding between the carboxyl group of gallic acid which was isolated from the tannic acid and the aminoethyl group of the cloth, or by hydrogen bonding between the phenolic hydroxyl groups of tannic acid or the hydroxyl groups of glucose group of tannic acid and aminoethyl groups (see Fig. 2 A). Because of the abundance of the phenolic hydroxyl groups, tannic acid bound to an aminoethyl group is considered to have some free phenolic hydroxyl groups to which the antibacterial activity is ascribed. In contrast, it is considered that the bonding between berberine and the cloth is an ionic bond between the quaternary ammonium ion of the berberine and the carboxyl group of the cloth (see Fig. 2 B). If the antibiotic site of the berberine is a quaternary ammonium ion, berberine will not show the antibacterial activity when it is fixed and blocked. Thus it is considered that the berberine is liberated from the cloth when it comes across a bacterium, and then acts as the antibacterial agent.

![Graphs showing viable count and pH changes](https://example.com/graphs.png)
FIG. 2. Schematic representation of tannic acid or berberine fixed onto the chemically modified cloth. A, Aminoethylated cloth combined with tannic acid; B, carboxymethylated cloth combined with berberine.

TABLE 2. Antibacterial abilities of the clothes.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Cloth</th>
<th>Log B/A</th>
<th>Log B/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Control cloth</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth combined with tannic acid</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carboxymethylated cloth</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carboxymethylated cloth combined with berberine</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marketing antimicrobial cloth</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>Control cloth</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth combined with tannic acid</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carboxymethylated cloth</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carboxymethylated cloth combined with berberine</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marketing antimicrobial cloth</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

* The test is valid when log B/A is greater than 2.0.
* When log B/C is greater than 1.6, the cloth can be said to have antibacterial ability.

Table 2 summarizes the antibacterial activities of the clothes. It became clear that the cloths prepared in this study other than the commercially available one had good antimicrobial activities against both bacteria. The antibacterial activities of these cloth samples were higher for E. coli than for S. aureus. The aminoethylated cloth combined with tannic acid showed a splendid antimicrobial activity against S. aureus.

**SEM observation**

Scanning electron micrographs were taken of the surface fibers of the cloth samples. The appearances of bacteria on the aminoethylated cloth combined with tannic acid and carboxymethylated cloth combined with berberine are shown in Fig. 3.

After 15 min of the antimicrobial activity test E. coli cells were damaged in an altered morphological state. Even 18 h after bacterial inoculation, no bacterial cells retaining their forms could be found on the aminoethylated cloth combined with tannic acid, the carboxymethylated cloth and the carboxymethylated cloth combined with berberine.

In all the antimicrobial ability tests, almost all of S. aureus cells were eradicated in less than 1 h of bacterial inoculation. However, some bacteria with normal configurations remained viable 1 h later on the aminoethylated cloth combined with tannic acid and also on the carboxymethylated cloth combined with berberine.

**Durability to laundering**

The antibacterial activity of each cloth before and after cleaning is shown in Fig. 4. Even after 20 cleaning cycles, the aminoethylated cloth and the aminoethylated cloth combined with tannic acid...
**FIG. 3.** Appearance of *Escherichia coli* (A, B and C) and *Staphylococcus aureus* (D, E and F) on the cloth. Samples were control cloth (after 15 min culture) (A and D), aminoethylated cloth combined with tannic acid (after 15 min culture) (B), carboxymethylated cloth combined with berberine (after 15 min culture) (C), aminoethylated cloth combined with tannic acid (after 1 h culture) (E), carboxymethylated cloth combined with berberine (after 1 h culture) (F).

**FIG. 4.** Durability to laundering. *A. coli*; *B. aureus*. Laundering cycles are 0 (□), 10 (■) and 20 cycles (▲). Samples are aminoethylated cloth (A-cloth), aminoethylated cloth combined with tannic acid (A-cloth-T), carboxymethylated cloth (C-cloth), carboxymethylated cloth combined with berberine (C-cloth-B).

**FIG. 5.** Comparison of the results of the measuring method of microbial number with the results of the regularized method test method. Symbols: ○, Viable count of *E. coli* on control cloth; ▲, Viable count of *E. coli* on aminoethylated cloth; □, Viable count of *E. coli* on aminoethylated cloth combined with tannic acid; ●, Viable count of *S. aureus* on control cloth; △, Viable count of *S. aureus* on aminoethylated cloth; ■, Viable count of *S. aureus* on aminoethylated cloth combined with tannic acid.
TABLE 3. Antibacterial abilities of the clothes according to the regularized test method.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Cloth</th>
<th>Log B/A †</th>
<th>Log B/C ‡</th>
<th>Log A/C §</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Control cloth</td>
<td>2.6 (2.2)</td>
<td>3.9 (4.8)</td>
<td>1.3 (2.6)</td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth</td>
<td></td>
<td>5.4 (7.5)</td>
<td>2.8 (5.3)</td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth combined with tannic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>Control cloth</td>
<td>2.5 (2.1)</td>
<td>2.4 (2.2)</td>
<td>−0.1 (0.1)</td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth</td>
<td></td>
<td>3.2 (4.8)</td>
<td>0.7 (2.7)</td>
</tr>
<tr>
<td></td>
<td>Aminoethylated cloth combined with tannic acid</td>
<td></td>
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* The test is valid when log B/A is greater than 1.5.
† Bacteriostatic activation value.
‡ Bactericidal activation value.
§ The data in parentheses are according to the measuring method of microbial number.

Antimicrobial abilities of the clothes retained good antimicrobial activities against both bacteria. However, although the carboxymethylated cloth and the carboxymethylated cloth combined with berberine had high activities before cleaning, the activities were lost after ten cleaning cycles. The reason for this difference in durability remains to be examined.

Comparison of the measuring method of microbial number with the results of the regularized test method for antimicrobial activity measurement

The relationship between results of antibacterial activity measurement according to the regularized test method and the measuring method of microbial number was shown in Fig. 5. The antibacterial activation values of the clothes according to the regularized test method were shown in Table 3. It was indicated that there is good correlation between the two antimicrobial tests using different culture medium. Therefore it is said that the results of antibacterial activity test in this study according to the measuring method of microbial number are general and reproducible.

Recently, antimicrobial abilities of clothes are evaluated mainly according to the regularized test method in addition to the measuring method of microbial number that is conventional method.

REFERENCES


