Effects of Surface Contamination and Cleaning with Hypochlorite Wipes on the Antibacterial Activity of Copper-Alloyed Antibacterial Stainless Steel

HIROSHI KAWAKAMI1,*, TAKATSUNA HAYASHI1, HIDEYUKI NISHIKUBO1, AKIFUMI MORIKAWA1, SATOSHI SUZUKI1,2, YOSHIHIRO SATO1, AND YASUSHI KIKUCHI1

1 Graduate School of Engineering, Osaka City University, 3-3-138, Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan
2 Stainless Steel & High Alloy R&D Dept., Nisshin Steel Co., Ltd., 4976 Nomura Minami-Machi, Shunan, Yamaguchi 746-8666, Japan

Received 26 June, 2013/Accepted 16 November, 2013

Effects of surface contamination and cleaning with hypochlorite wipes on the antibacterial activity of copper-alloyed stainless steel were studied. The antibacterial activity of copper alloyed stainless steel decreased with the increase in the amount of surface contaminant, and the bacterial counts from specimens contaminated with a contaminant, e.g. $1.6 \times 10^{-2} \mu g/mm^2$ of bovine serum albumin, were not significantly different from those from ordinary stainless steel specimens. The once contaminated surface could regain its antibacterial activity when it was sufficiently wiped clean with sterile wipes loaded with sodium hypochlorite solution.

Key words : Copper alloyed stainless steel / Sodium hypochlorite / Wipe cleaning / Environmental surfaces / Surface contamination.

INTRODUCTION

Pathogens remain viable on environmental surfaces, even in dry conditions, for considerable lengths of time (Dietze et al., 2001; Kusumanigrum et al., 2003; Kramer et al., 2006; Higashino and Kamiya, 2011). Contaminated environmental surfaces can serve as a reservoir of pathogens and play a role as the main pathway for the outbreak of pathogens. To prevent such outbreaks via environmental surfaces, materials that keep their surfaces disinfected should be developed.

Stainless steel is an iron-based alloy with surfaces that stay clean for long periods of time. That being said, stainless steel is not antibacterial: pathogens remain viable on stainless steel surfaces and present a contamination hazard for considerable periods of time (Kusumanigrum et al., 2003; Higashino and Kamiya, 2011). Alloing with copper invests stainless steel with antibacterial activity as has been well confirmed by several studies (Kielemoes and Verstraete, 2001; Hong and Koo, 2005; Noyce et al., 2006; Kawakami et al., 2010). Test surfaces of specimens used in those studies were in a fresh condition without contamination. Actually, even when antibacterial copper-alloyed stainless steel is used, surfaces are exposed to the surrounding environment and become contaminated by various substances in the surroundings. Surface contamination can reduce the antibacterial activity of copper alloyed stainless steel; cleaning henceforth must be carried out periodically on the contaminated surfaces.

To disinfect noncritical environmental surfaces, hypochlorites are the most widely used chlorine disinfectants as they have several advantages. For example, they possess a broad spectrum of antimicrobial activity (Denton et al., 2004), do not leave toxic residues and show a low incidence of serious toxicity (Rutala et al., 2008). In addition, they are unaffected by water hardness, inexpensive and fast...
acting, and capable of removing dried or fixed organisms and biofilms from surfaces. It is anticipated that such hypochlorites will function also as cleaning agents (Fukuzaki, 2006).

In this study, the effects of surface contamination and cleaning using sterile wipes loaded with sodium hypochlorite on the antibacterial activity of a copper-alloyed stainless steel were studied.

**EXPERIMENTS AND METHODS**

**Sample materials and preparation of specimens**

Samples subjected to experiments were copper-alloyed stainless steel (Nisshin Steel Co., Ltd., Tokyo, Japan) (Cu-SS) and type 304 stainless steel (type 304 SS). The copper content of the Cu-SS was about 3.8 mass%. Type 304 stainless steel was selected as a reference material for stainless steel as it is used for places often touched by hands, such as door handles, handrails, and frames of chairs and beds. The chemical compositions of the samples are summarized in Table 1.

Coupon specimens, 25 mm × 25 mm × 2 mm, were prepared from the supplied plates. The test surfaces of specimens were polished by 1,500 grit emery paper to obtain a uniform surface finish. The polished specimens were then subjected to ultrasonic cleaning in 70% ethanol aqueous solution followed by drying and disinfection under ultraviolet (UV) irradiation in a safety cabinet.

**Contamination and simulation of cleaning with wipes**

Two types of model contaminants were used in this study: one was bovine serum albumin (BSA), employed as a model protein contaminant, and the other was an artificial oily dirt as a model of a hydrophobic contaminant.

Bovine serum albumin was dissolved in distilled water at a final concentration of 0.2–200 μg/mL, depending on the amount of BSA to be applied to the surface of a specimen. 0.5 mL of the BSA solution was applied and spread on the test surface of the specimens. The specimens were then kept in thermostatic conditions at 40°C for 4 h in order to dry the contaminated test surfaces.

An artificial oily dirt was prepared with reference to JIS C 9606 (Japan Standard Association, 2007). The prepared artificial oily dirt contained oleic acid, triolein, blustery oleate, liquid paraffin, squalene, and cholesterol. The composition of the artificial oily dirt is shown in Table 2.

The artificial oily dirt was dissolved in 500 mL of ethyl alcohol, since the dirt was highly viscose and it was difficult to spread it on a test surface. The artificial oily dirt dissolved in ethyl alcohol was applied and spread on the test surface of the specimens. The specimens were then kept in thermostatic conditions at 40°C for 4 h in order to evaporate the ethyl alcohol.

Sodium hypochlorite solution was prepared by diluting commercially available sodium hypochlorite solution with distilled water. For the final sodium hypochlorite solution, the concentration of free available chlorine was adjusted to be 0.05 mass% (500 ppm), and the pH was controlled at 12.0 by sodium hydroxide solution.

A sterile wipe (ITW Texwipe, Kernersville, NC USA) was folded once to produce a rectangle of 80- by 145-mm. The sterile wipe was then loaded with 4.0 ml of the sodium hypochlorite solution or distilled water and fixed to a crockmeter (Atlas Electric Device Co., Chicago, IL USA) as shown in Fig. 1.

The specimen was held to the nib of the crockmeter with adhesive tape, and the test surface was pressed at a pressure of about 360 Pa to the sterile wipe. The specimen was then moved back and forth on the surface of the sterile wipe for a specific number of times to simulate cleaning with a wet wipe. The distance of the reciprocal movement was 75 mm. After being subjected to the cleaning, the specimen was removed from the crockmeter and the test surface was dried in a safety cabinet under UV irradiation in order to prepare for the following antibacterial test. Nomenclatures of specimens classified by preparation of their test surfaces.
surfaces are summarized in Table 3.

### Antibacterial tests

(The International Organization for Standardization, 2007)

The stains used in this study were *Escherichia coli* NBRC 3972 and *Staphylococcus aureus* subsp. *aureus* NBRC 12732. Bacteria were pre-cultured for 18 h at a temperature of 35°C. After pre-cultivation, the suspension was centrifuged for 10 min at about 3,000 g (5,000 rpm), and the precipitate was resuspended into fresh phosphate buffer (pH 7.0). This process was repeated four times, and concentration of the final suspension was adjusted to 1.0 ± 10^6 colony forming units per mL (cfu/mL).

The bacterial suspension of 0.050 ml was inoculated on the test surface of a coupon specimen placed on a sterilized Petri dish. The inoculum was then covered with a sterilized polyethylene film, 20- by 20-mm, in order to make the inoculum form a thin layer. In this study, sterilized Petri dishes served as control: a bacterial suspension of 0.050 ml was inoculated on a sterilized test surface and the polyethylene film, 20- by 20-mm, in order to make the inoculum form a thin layer. In this study, sterilized Petri dishes served as control: a bacterial suspension of 0.050 ml was inoculated on a sterilized Petri dish and the inoculum was covered with a sterilized polyethylene film.

The inoculated bacteria were incubated for 24 h under the same conditions as those for pre-cultivation. After the incubation of the test surface with the inoculum, the test surface and the polyethylene film were rinsed with Soybean Casein Digest broth with Lecithin and Polysorbate 80 medium of 3.0 mL and 2.0 mL respectively, and they were combined to recover bacteria exposed to the test surface. One mL of the rinse solution was diluted with phosphate buffer to obtain a diluted series of the bacterial suspension used for subsequent plate counting technique with standard agar medium. The number of viable bacteria was expressed as total colony forming units per specimen. Each experiment was repeated at least three times on different days.

The BSA was obtained from Sigma - Aldrich Co., USA. The Soybean Casein Digest broth with Lecithin and Polysorbate 80 medium was manufactured by Nihon Pharmaceutical Co., Ltd., Japan. Other chemicals, including the original sodium hypochlorite solution, were obtained from Wako Pure Chemical Industries, Ltd., Japan. All chemicals were used as obtained without further purification.

### Statistical Analysis

The Scheffé test was employed for the multiple comparison test and a p-value of less than 0.05 was considered statistically significant. Prior to application of the Scheffé test, analysis of variance was carried out to confirm the significance of the data.

### RESULTS AND DISCUSSION

Experimental results obtained from antibacterial tests against *E. coli*, in which BSA was used as a model contaminant, are summarized in Fig. 2 (a). In the control, i.e. the surface of sterilized Petri dishes, the bacterial count increased close to ten-fold in 24 h (Cont-24h). The bacterial count from the polished type 304 SS specimens was not significantly different from that of the Cont-24h, indicating that the type 304 stainless steel was not antibacterial. For type 304 SS specimens, none of the test surface conditioning treatments resulted in significant differences in bacterial counts in comparison with the Cont-24h.

The bacterial count recovered from polished Cu-SS specimens was about three orders of magnitude lower than those from all type 304 SS specimens, and the difference was significant. The bacterial count increased by increasing the amount of the BSA surface contaminant, and the bacterial counts from Cu-SS specimens contaminated with BSA of more than 1.6 × 10^7 μg/mm² (BSA-2 and BSA-1) were not significantly
Different from those from all type 304 SS specimens or the Cont-24h. Such trends in bacterial counts from the Cu-SS specimens were also found in experimental results obtained from antibacterial tests against *S. aureus* (Fig. 2 (b)): the bacterial counts from polished Cu-SS specimens were about three orders of magnitude lower than those from all type 304 SS specimens, and the bacterial counts from the Cu-SS specimens increased with the increase in the amount of BSA surface contaminant.

Experimental results obtained from the antibacterial test, in which the artificial oily dirt was used as a model contaminant, are shown in Fig. 3. The experimental results were qualitatively the same as those for the antibacterial tests for the BSA contaminated specimens. The bacterial counts from all type 304 SS specimens were independent to the amount of the artificial oily dirt and not significantly different from the values from the Cont-24. The bacterial counts from Cu-SS specimens contaminated with the artificial oily dirt of $3.0 \times 10^{-4} \mu g/mm^2$ were significantly lower than those from all type 304 SS specimens and the Cont-24, and the counts increased with the increase in the amount of the artificial oily dirt. The difference was not significant when the amount of the artificial oily dirt contaminant was more than $3.0 \times 10^{-2} \mu g/mm^2$ (O-2 and O-1).

These experimental results summarized in Figs. 2 and 3 indicate the Cu-SS specimens are antibacterial, and the antibacterial activity is reduced by surface contamination.

In order to study effects of cleaning with wipes on the antibacterial activity of contaminated Cu-SS specimens, the surfaces of the most heavily contaminated specimens, i.e. BSA-1 specimens and O-1 specimens, were wiped clean using sterile wipes loaded with sodium hypochlorite solution. Figure 4 shows experimental results obtained from antibacterial tests for specimens subjected to cleaning with wipes after contamination with BSA. In Fig. 4, the bacterial counts from specimens wiped clean six times with sterile wipes
SURFACE CONTAMINATION AND HClO CLEANING

We conclude that replacing general stainless steel with copper-alloyed antibacterial stainless steel and adopting hypochlorite-based cleaning methods will contribute to reduce levels of bacterial contamination of environmental surfaces.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Fukuzaki, Industrial Technology Center of Okayama Prefecture, Japan (recently, Professor of the Graduate School of Bioresources, Mie University, Japan), for his kind advice regarding the experiments. A part of this study was financially supported by the Financial Aid for Young Scientists, Osaka City University, Japan.
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