Physicochemical Properties and Antibacterial Activity of Nanostructured Copper Electrodeposited on Stainless Steel Surface

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Antimicrobial copper is beneficial in reducing hospital-acquired infections. Alteration of stainless steel by coating with copper makes the top surface antimicrobially active. In this study, nanostructured copper-coated stainless steel (Cu/SS) with excellent physicochemical properties and outstanding antibacterial activity was successfully prepared by electrodeposition technique. The physicochemical properties of the copper coating such as ultrafine nanostructured particles with grain diameter of 25 nm to 42 nm, rough surface with 24.23 nm, and low water contact angle (82^{o}) would contribute in enhancing the antibacterial activity of the coating. Adhesion strength between the coating and stainless steel substrate is very good, as indicated by 100% of retainment of copper on the stainless steel surface tested using Scotch® tape. The antibacterial activity showed no significant difference between nanostructured copper-coated stainless steel and C11000 copper in terms of reduction of *Staphylococcus aureus* (*S. aureus*). However, only 5 minutes was required to completely kill *Escherichia coli* (*E. coli*) using nanostructured Cu/, while longer time (i.e., 10 minutes) using solid copper. The dissolution rate of nanostructured Cu/SS in simulated hand sweat solution was 0.08048 mm/year. The nanostructured copper coating also has high surface roughness and low surface wettability (i.e., high hydrophobicity). These findings indicate that the modification of stainless steel with copper nanostructures can improve the antibacterial activity of stainless steel. The Cu/SS coating can be an ideal material to be used as touch surface in combating bacterial infection, especially in hospitals and public areas.

Key words: Antimicrobial copper; copper coating; electrodeposition; nanostructured coating

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Frequently-touched surfaces provide an intermediary for the transmission of infection, especially in hospital and healthcare settings [1-3]. Harmful pathogens can persist on frequently-touched surfaces for days, posing a threat to all who interact with these contaminated surfaces and promote transmission. Stainless steel is commonly used as touch surfaces due to its durability and appearance, however it is not intrinsically bactericidal. Pathogens can tolerate on stainless steel surface for several months, acting as an intermediate for transmission of pathogens, leading to increased infections [4,5].

Surface alteration for preventing infections has thus led to the development of antimicrobial coatings. Modification of surfaces can be done by coating with antimicrobial active metals like copper via physical or chemical processes [6-8]. Pathogenic microbes could be inactivated by coming into contact with copper surface. It has shown to kill a long list of microbes, including MRSA (Gram-positive bacteria that are resistant to antibiotics) and virulent strains of *E. coli* that cause food-borne illnesses [9-11]. Copper has received registration from Environmental Protection Agency (EPA) as an antimicrobial material, which is capable to inhibit biofilms and retain the antimicrobial activity even under typical indoor conditions [12]. Copper has been explored as an antimicrobial metal in laboratory testing, clinical trials and athletic facilities [13-15]. Pathogens that land on copper surfaces through a touch are rapidly destroyed, continuously [16,17]. Copper releases its ions to destroy DNA and materials inside the microbes, causing the membranes of the microbes to rupture, thus preventing the microbes from mutating and developing resistance [18,19]. While the antimicrobial properties of copper surfaces are now well-known, nanostructured copper offers superior benefits. Due to larger specific surface-area-to-volume ratios, the physical properties of nanostructured copper allow functionality that is not achievable with micro-structures [19,20].

Numerous studies have been carried out in the development of nanostructured copper coatings with good physicochemical properties and antibacterial activity to suit the application as antimicrobial touch surface. Ideally, copper coatings should be well adhered between the coating and substrate interface and work under typical indoor conditions. Although many publications revealed the methods to prepare nanostructured copper coatings with good antibacterial activity, however, there is limited discussion on nanostructured copper coatings by using electrodeposition technique. There are limited publications relating on electrodeposition of nanostructured copper coatings focusing on antimicrobial applications [21,22]. The main objective of this research is to fabricate nanostructured copper coating on stainless steel by electrodeposition technique with excellent physicochemical properties and outstanding antibacterial activity.

EXPERIMENTAL

Chemicals and Materials

A 304 stainless steel and a C11000 copper (20 mm × 20 mm × 1 mm) coupons were obtained from Sigma Aldrich. Acetone (C_3H_6O, molecular weight: 58.08), copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$, molecular weight: 249.68), sulfuric acid (H_2SO_4, molecular weight: 98.08), sodium hydroxide (NaOH, molecular weight: 39.99), and etylenediaminete-traacetic, EDTA ($C_{10}H_{16}N_2O_8$, molecular weight: 292.24) were supplied by R&M Chemicals. A silver-silver chloride (Ag/AgCl) electrode and a platinum rod (Pt) electrode were procured from Metrohm (Malaysia). All chemicals were of analytical grade and used as received.

Preparation of Nanostructurred Copper Coated on Stainless Steel

Prior to electrodeposition, the 304 stainless steel coupon was polished with silica carbide (SiC) paper, followed by ultrasonically cleaned in acetone, rinsed with ultra pure water, and dried at room temperature. The electrodeposition process of copper on stainless steel was performed in a typical three-electrochemical cell with polished stainless steel used as working electrode, platinum rod as counter electrode, and Ag/AgCl as reference electrode. The electrochemical studies were done by cyclic voltammetry (CV) and chronoamperometry (CA) using an Autolab Potentiostat (Aut302 FRA2), interfaced with NOVA software, as described in a previous study [22]. In this study, the electrodeposition of nanostructured copper on stainless steel was achieved in an electrolyte solution containing 0.01 mol/L CuSO4.5H2O-C10H16N2O8 (EDTA), adjusted to pH 8 by adding diluted H2SO4 and NaOH at – 1.1 Vvs Ag/AgCl for 15 min.

Characterization Methods

The surface morphology and elemental composition of the samples were investigated by Field Emission Scanning Electron Microscopy (FE-SEM, Carl Zeiss SMT Supra 40 VP) with a built-in Energy Dispersive X-Ray (EDX). The surface topography and roughness of the samples were evaluated using an Atomic Force Microscope (AFM, XE-100). Furthermore, the wettability of the samples was determined by a Contact Angle Goniometer (VCA 300TM). The adhesion test of nanostructured copper coating on stainless steel was tested by tape test using Scotch® tape.

Antibacterial Activity

Antibacterial activity of the samples was tested using intimate contact cell suspension test, modified according to the Japanese Industrial Standard JIS Z 2801:2000 Antimicrobial products – Test for antimicrobial activity and efficacy [23]. Two bacterial species, Gram-negative (E. coli ATCC 8739) and Gram-positive (S. aureus ATCC 6538P) were selected as test bacteria. The test bacteria were cultured in nutrient agar overnight at 37°C, then diluted in 10 mL of saline solution (0.9% NaCl) to an optical density OD625 of 0.1, which is equivalent to 1.5 × 108 cells/mL (compared with McFarland). Afterward, bacterial suspensions with approximately 105 cells/mL were prepared by serial 10-fold dilutions. To determine antibacterial activity of the samples, bacterial suspensions of 105 cells/mL of E. coli and S. aureus were placed, separately, onto sample surfaces for 30 min of exposure.

Dissolution Rate Measurement

For test solution, simulated hand sweat solution was prepared by dissolving 0.5% NaCl + 0.1% lactic acid + 0.1% urea, adjusted to pH 6.5 using ammonia solution. Dissolution rate of the samples in simulated hand sweat solution was evaluated by Tafel extrapolation method performed by an Autolab Potentiostat (Aut302 FRA2). Tafel extrapolation plot was drawn and the corrosion current density (icorr) was determined from the plot, and therefore the corrosion rate could be calculated. The Tafel plot was generated by initially scanning for corrosion potential (Ecorr) or open circuit potential (OCP), and then to -100 mV vs Ecorr (for a cathodic Tafel plot) and +100 mV vs Ecorr (for an anodic Tafel plot) with a scan rate of 0.1 mV/s. The resulting curve is a plot of the applied potential vs. the logarithm of the measured current. For the set-up experiment under this study, Tafel plots were scanned in a single scan, beginning with the cathodic plot and continuously to the anodic plot.

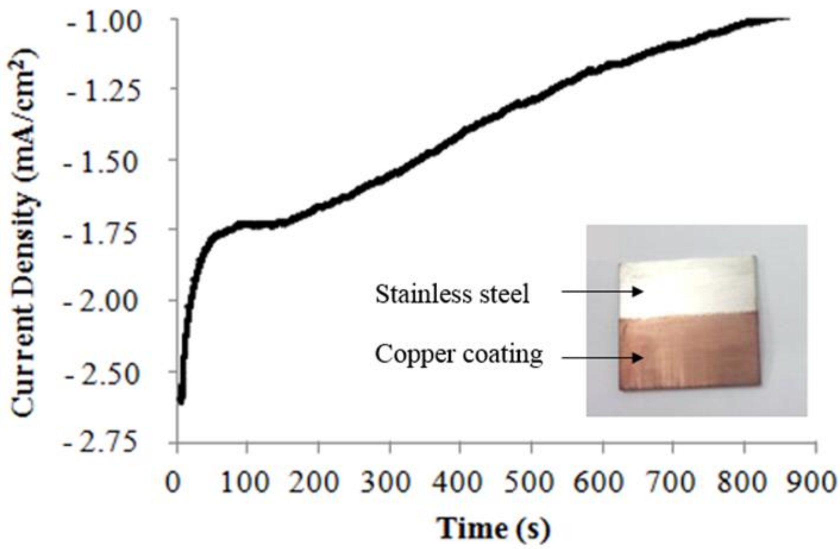


Figure 1. Chronoamperometry curve of nanostructured Cu/SS in electrolyte solution containing 0.01 mol/L CuSO4.5H2O-C10H16N2O8 (EDTA), adjusted to pH 8 by adding diluted H2SO4 and NaOH at –1.1 Vvs Ag/AgCl for 15 min. (Inset: Visual observation of copper coating on stainless steel substrate).

RESULTS AND DISCUSSION

Preparation of Nanostructured Copper Coated on Stainless Steel (Cu/SS)

The kinetics of nucleation and growth of nanostructured copper coating on stainless steel was analyzed from chronoamperometric curve (Figure 1), as reported previously [22]. The current density-time transient started with an abrupt decrease in cathodic current density for short times due to double-layer charging (non-Faradaic current). A continuous decrease in cathodic current density indicated the nucleation and growth of the copper deposited on stainless steel with the involvement of hydroxide. Simultaneously formation of hydroxide and reduction of copper in an alkaline electrolyte solution containing EDTA may affect the characterization of the copper coating. It can be visually seen from the inset in Figure 1, where the entire exposed stainless steel is covered with red-brown coating, indicating deposition of copper.

Characterization

Figure 2 shows surface morphologies of uncoated 304 stainless steel, stainless steel coated with copper (Cu/SS), and C11000 copper. The result showed that the morphology of uncoated stainless steel had a moderately smooth surface structure (Figure 2a). On the contrary, stainless steel coated with copper showed a homogenous coverage of ultrafine, smooth, and dense nanostructured grains with the diameter of 25-42 nm (Figure 2b). C11000 copper had a relatively smooth surface structure with negligible grooves in the polishing direction (Figure 2c). A comparative change in element composition of the samples was performed by EDX analysis, Figure 3. The analysis clearly showed there was no copper observed on uncoated stainless steel surface. Meanwhile, the weight percentage of copper on stainless steel coated with copper (Cu/SS) was 97.95 wt.%, which is nearly similar to copper composition of C11000 copper, which is 98.71 wt.%. Both copper surfaces (pure and coating) consisted of oxygen, as detected by EDX. For pure copper surface, the presence of oxygen cannot be avoided since copper is easily oxidized due to reaction with air in the environment. In the case of the copper coating, oxygen came from the disruption of hydroxide formed during electrodeposition process, since the electrolyte used was in an alkaline condition.

Surface topographies of the samples were captured by AFM (Figure 4) with comparable Ra-values, as presented in Table 1. For uncoated 304 stainless steel and C11000 copper, both surfaces had flat topographies with cracks due to the polishing effect. The average surface roughness was 1.644 nm for uncoated 304 stainless steel and 9.516 nm for C11000 copper. C11000 copper was rougher compared to uncoated stainless steel, since copper is softer in nature than stainless steel. In contrast, surface topography of nanostructured copper coated on stainless steel showed a crack-free, homogeneous, and compact structure with average surface roughness of 24.23 nm. It can be described that nanostructured Cu/SS increased in surface roughness due to the characteristics of the coating and morphology of the surface. Roughened surface has been receiving attention in antimicrobial surfaces due to its ability to induce advanced mechanical and physical characteristics on the surface through grain refinement, along with the effect of the characteristics on bacterial inhibition.

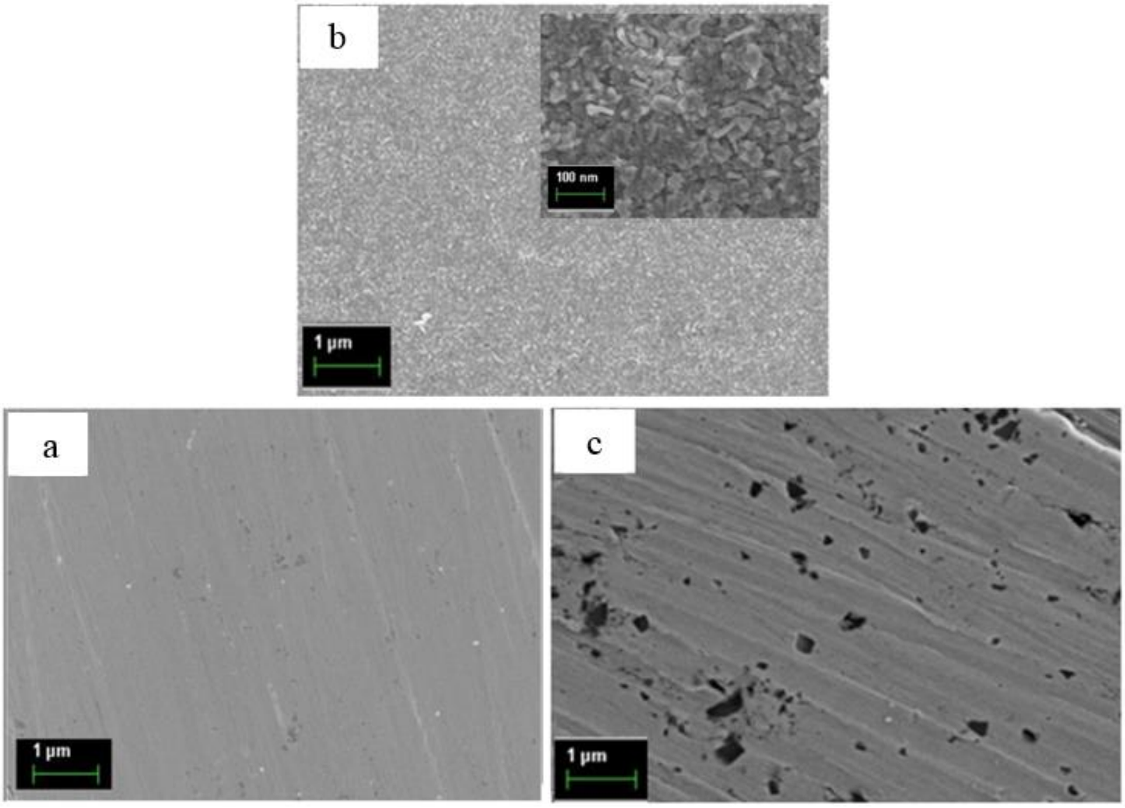
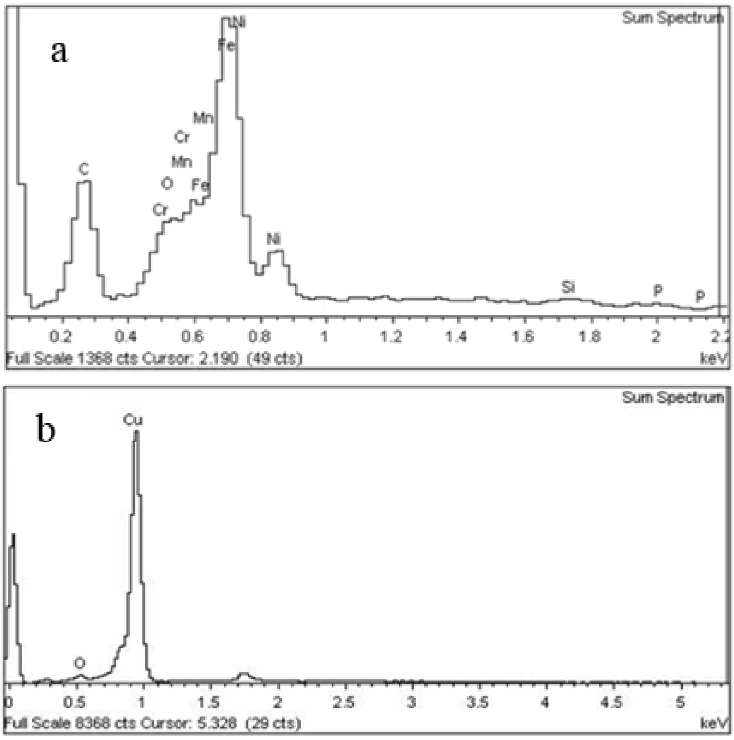


Figure 2. SEM images of (a) uncoated 304 stainless steel, (b) nanostructured Cu/SS, and (c) C11000 copper. Magnification: 5000x (inset: 50000x).



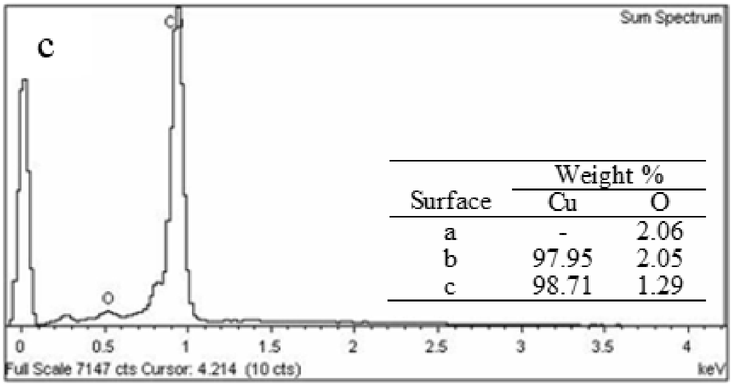


Figure 3. EDX spectra of (a) uncoated 304 stainless steel, (b) nanostructured Cu/SS, and (c) C11000 copper.

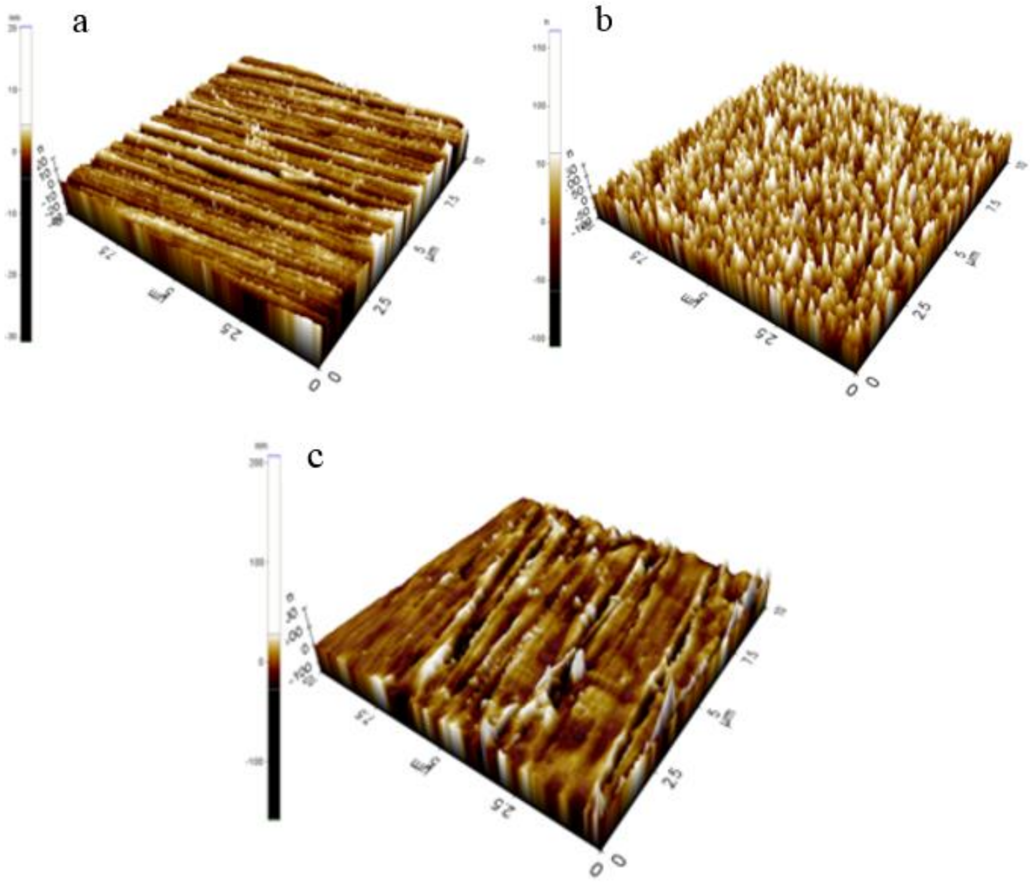


Figure 4. AFM images of (a) uncoated 304 stainless steel, (b) nanostructured Cu/SS, and (c) C11000 copper.

Table 1. Surface roughness, Ra-values of samples

Surface	Roughness, Ra (nm)
304 stainless steel	1.644
Copper coating	24.23
C11000 copper	9.516

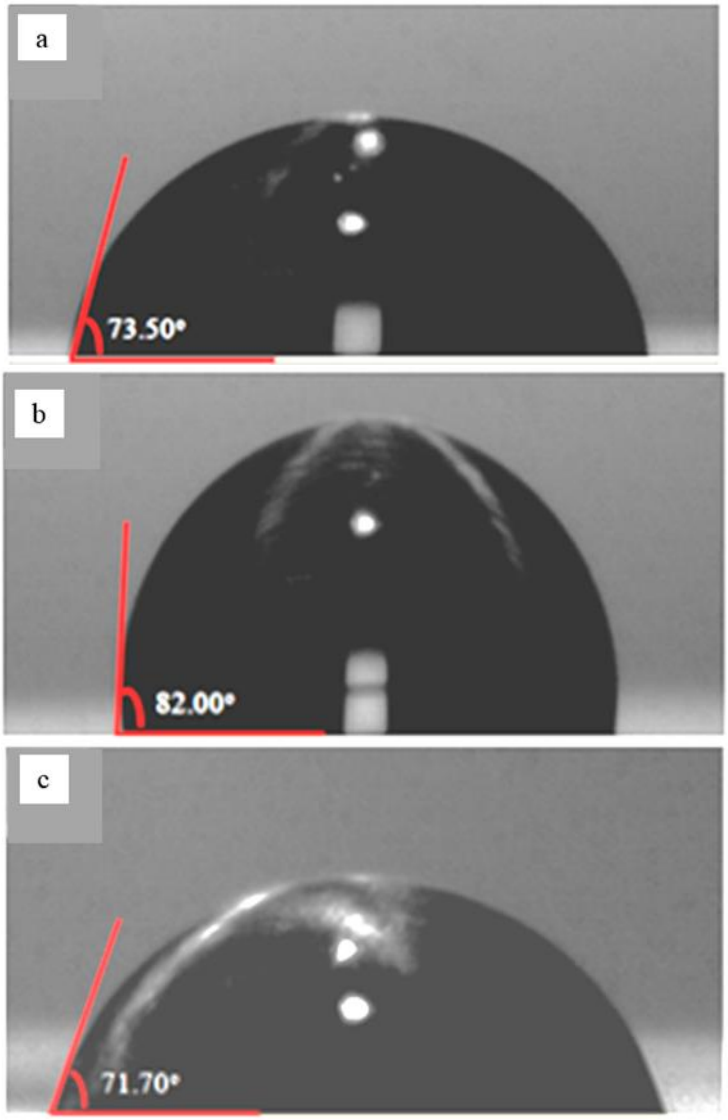


Figure 5. Contact angle images of (a) uncoated 304 stainless steel, (b) nanostructured Cu/SS, and (c) C11000 copper.

The surface wettability of the samples was evaluated based on water contact angle measurement using distilled water droplet of 4-5 µL (Figure 5). It was found that all samples had low surface wettability with water contact angles of 73.5o, 82.0o and 71.7o for uncoated 304 stainless steel, nanostructured Cu/SS and C11000 copper, respectively. Surfaces with contact angle measurement higher than 65o are usually recommended for antimicrobial applications due to the surfaces inhibiting cell attachment [24]. Therefore, nanostructured Cu/SS indicated that the surface is hydrophobic and suitable to be applied as touch surface.

Excellent adhesive strength between coating and substrate is one of the important factors for determining the mechanical behaviour and performance of a coating in order to be accepted for touch surface application. The adhesion property of nanostructured Cu/SS was investigated by tape test using Scotch® tape. The nanostructured copper has excellent adhesion strength on stainless steel, as indicated by 100% of retainment of copper on the stainless steel surface after Scotch® tape was removed. Moreover, the coating remained on the stainless steel even after rubbing aggressively with tissue papers. Metal-metal bond strength represents the adhesive force of the copper coating to the stainless steel substrate, generally correlates with the crystallographic coherency in the interface [25]. In addition, nano-sized grain structures can be 'filling in' the space and defect structures of stainless steel.

Antibacterial Activity

The antibacterial activity was confirmed using intimate contact cell suspension test, modified according to the standard method of JIZ S 2801 [23]. Figure 6 shows the reduction rate of viable bacteria within the nominated contact time under ambient room temperature and normal humidity conditions. Results showed no bacterial reduction against both E. coli and S. aureus exposed to uncoated 304 stainless steel surface, indicating that the surface exerted no lethal effect. However, both copper surfaces were active against E. coli and S. aureus. Although C11000 copper surface was able to kill E. coli and S. aureus within 10 min of exposure, nanostructured Cu/SS surface was able to kill both bacteria at relatively faster rates than C11000 copper surface. Nanostructured Cu/SS surface showed a noteworthy reduction of S. aureus after 5 min, with whole killing within 10 min of exposure; while for E. coli it required 5 min of exposure to kill completely. It showed that nanostructured Cu/SS surface is more sensitive to inhibitory effect compared to C11000 copper surface against tested bacteria.

Both bacteria were killed very fast on the copper surfaces, however, killing rate differed according to the surface modification and also type of bacteria. The antibacterial activity of the copper surfaces against S. aureus was less effective compared to E. coli. The extended time required to destroy Gram-positive S. aureus is attributed to their thick peptidoglycan layer, which makes them slightly tougher to contact killing on copper surfaces [26,27]. Furthermore, the modification of stainless steel by coating with nanostructured copper contributed to the fast contact killing rate compared to C11000 copper surface.

Dissolution Rate

As the concern on the release of copper ions might play a role for antimicrobial effect, it is vital to evaluate the long-term stability of nanostructured Cu/SS in order to make it reliable for touch surface application. The stability of nanostructured Cu/SS was evaluated via dissolution rate measurement in simulated hand sweat solution at ambient temperature by Tafel extrapolation method. Table 2 shows electrochemical parameters of the samples in the simulated hand sweat solution obtained from the Tafel plot. C11000 copper provided a high dissolution rate of 0.1292 mm/year, compared to nanostructured Cu/SS with 0.08048 mm/year. This indicates that the dissolution rate of nanostructured Cu/SS is very slow, even as compared to C11000 copper.

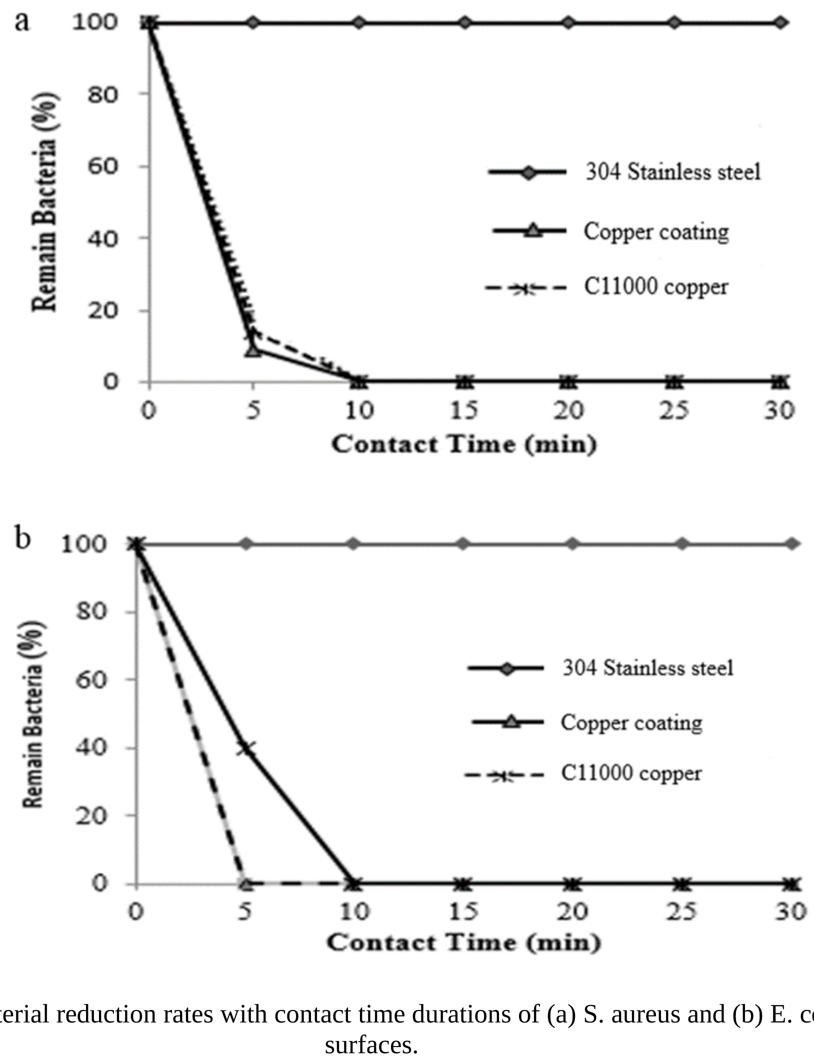


Figure 6. Viable bacterial reduction rates with contact time durations of (a) S. aureus and (b) E. coli on different surfaces.

Table 2. Electrochemical parameters of copper surfaces in simulated hand sweat solution.

Surface	E_{corr} (mV)	i_{corr} (μA/cm^2)	Dissolution rate (mm/yr)	Rp (kΩ)
Copper coating	-103.800	6.9405	0.08048	11.2300
C11000 copper	-101.0000	11.1460	0.1292	9.3012

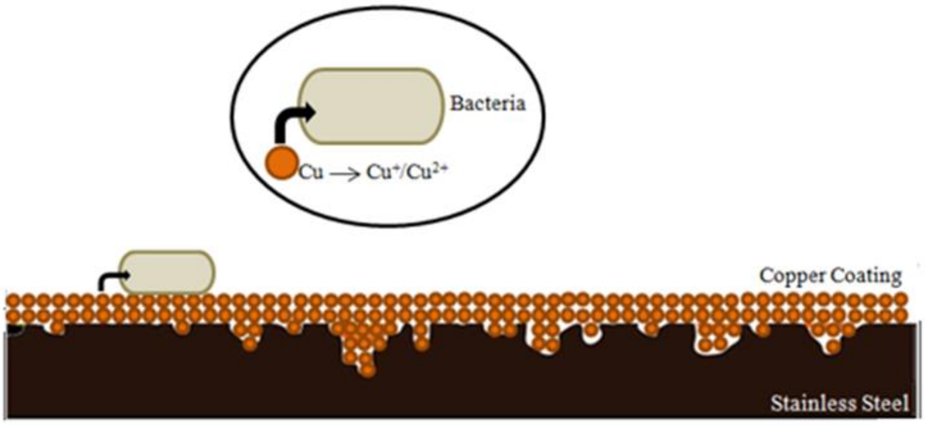


Figure 7. Schematic diagram of biocidal copper surface.

Theoretically, a more efficient killing rate in a tested solution may thus be due to the accelerated dissolution of copper [18]. It can be imagined that, from Figure 7, when bacteria and copper come into contact, copper ions weaken the outer membrane of the bacterial cells through the process of oxidation. The released copper ions could effectively kill the bacteria by collapsing their outer cell membranes. Copper is toxic to the inside of a cell, and eventually copper ions cause cell rupture, making the cell lose its vital structures and dies [28]. It should be noted that pure copper has a supposedly high killing rate compared to the copper coating (based on dissolution rate). However, based on antibacterial testing against E. coli and S. aureus, the copper coating represented better contact killing. From the outcome, it can be said that the bactericidal effect was not only because of the release of copper ions (i.e.: Cu+/Cu2+), but the contact killing also played an important role [29]. It is suggested that copper accumulation within the cell, cell death and DNA damage assays all indicate that copper has lethal effects towards bacteria.

The copper coating with nanostructures resulted in a better and more efficient contact killing than solid copper (C11000) by allowing copper structure to interact closely with bacterial membranes. Although the definite antibacterial mechanism of nanostructures remains a controversy, most probably high surface area-to-volume ratios provide more efficient means for antibacterial activity, which allow copper structure to interact closely with bacterial membranes [30,31]. Besides that, the interaction of bacteria and touch surfaces also depends on various factors like morphology, roughness, wettability, and also the characteristics of bacteria and the surrounding environment, such as medium and temperature [32,33]. This indicates that killing rate does not only depend on the release of copper ions, but also from the contribution of surface properties. The material surface characteristics including ultrafine nanostructures, appropriate copper element composition on the surface, high surface roughness, and low water contact angle will assist in accelerating the contact killing properties of touch surfaces.

CONCLUSION

Nanostructured copper coating was successfully deposited onto stainless steel surface using electrodeposition method (i.e., chronoampero-metry). The well-adhered nanostructured copper coating onto the stainless steel surface has high surface roughness and low surface wettability. These properties contributed to the enhancement of antimicrobial activity of the copper coating in killing bacteria.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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