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The Influence of Surface Scratches on Copper's Antibacterial Activity

Samantha Douha[1]*, Emma Lacroix[3]*, Erin McClelland-Asu[3]*
Melissa Vermette[2]*, Kirsten A. Wilcox[1], Kevin Lam[1]†

[1]Simon Fraser University, *Department of Biological Sciences*
[2]Simon Fraser University, *Department of Chemistry*
[3]Simon Fraser University, *Department of Molecular Biology & Biochemistry*

Abstract

Copper metal is capable of killing more than 99.9% of bacteria upon contact, and has been researched as an alternative material to hospital surfaces with aim to prevent hospital-acquired infections. Because copper is a malleable metal, it is more likely to acquire surface scratches than other common materials in high-traffic health care facilities. To investigate the effects these scratches may have on copper's antibacterial activity, we inoculated scratched and unscratched copper C110 with either *Escherichia coli* ATCC 11303 or *Staphylococcus carnosus* 51365 and quantified the bacteria present at various time intervals. We found no significant difference in the antibacterial activity of copper between the two surface textures, with 99.9% of *E. coli* and the majority of *S. carnosus* bacteria killed within 10 minutes. This suggest that copper surfaces implemented in health care facilities would not require extensive maintenance of smooth surfaces to retain their bactericidal effects. Additionally, we found a significantly greater concentration of bacteria present on stainless steel compared to copper, supporting the claims that copper surfaces could help decrease the transmission of hospital-acquired infections.

Keywords — Hospital Acquired Infections, Surface Texture, Copper C110, Staphylococcus Carnosus 51365, Escherichia Coli ATCC 11303, Infection, Stainless Steel

1. Introduction

Health care facilities are the source of many infections [1, 2, 3], as bacteria can be deposited by a touch, sneeze, or cough from an infected patient [4]. Despite the various infection protocols implemented every year [1, 5, 6], hospital acquired infections remain prevalent today [3, 7, 8]. The Center for Disease Control estimates that 1 in every 25 patients in American acute care hospitals acquires an infection during their stay [9], with approximately 75 000 of these infections resulting in fatality in 2011 [9].

Frequently touched surfaces within health care facilities such as door knobs, hand-rails and light switches are often made from materials on which bacteria are able to survive and grow, such as aluminum or stainless steel [10, 11, 12]. Copper is classified by the Environmental Protection Agency as a solid antimicrobial material, and is capable

*Equal First-Authorship
†Corresponding Author: kevin_lam@sfu.ca

of killing viruses, yeasts and bacteria upon contact [10]. Because of its well-established antimicrobial properties [10, 13], copper metal and its alloys have been researched as an alternative material for these frequently touched surfaces [10, 14, 15, 16, 17, 18], with aim to decrease infection transmission. Although the mechanism by which copper kills bacteria is not fully understood, studies that replaced a variety of frequently touched surfaces with copper alloys saw a significant decrease in pathogens present on these surfaces [15, 19, 20].

Despite these promising results, copper is more malleable than aluminum or stainless steel, and therefore more susceptible to surface damage [13]. Because hospitals are high traffic facilities, copper surfaces are likely to obtain scratches and abrasions. If bacteria-metal contact is needed for efficient bacterial killing, we expect to see a decrease in the efficiency of copper's antimicrobial activity on scratched surfaces, as bacteria may become suspended in solution within these crevices. Our research aims to investigate the effects that surface scratches may have on copper's antibacterial property, which could indicate whether hospitals that replace frequently touched surfaces with copper should be vigilant in maintaining smooth surfaces.

2. Materials and Methods

2.1. Preparation of Bacterial Broths

We prepared a broth of each bacterial strain being tested (Table 1). Broth inoculation was done inside a biosafety class IIA/B3 Biological safety cabinet. First, we incubated each strain on Mueller Hinton agar (Forma Scientific IN2-MIC E-63 incubator; 37°C for 22-48 hours). Then, we isolated one bacterial colony on the agar plate and inoculated it in 10 ml Mueller Hinton broth (BBLTM Mueller Hinton Broth; 22 g broth powder per litre), and incubated each sample (Max Q 4000 Barnsteed Lab-Line incubator). All broths in this experiment were prepared with these methods, with incubation parameters depicted in Table 1.

Table 1: *Naming system for bacterial broths used in this experiment.*

Bacterial Strain	Incubation Parameters	Name
E. coli strain ATCC 11303	190 rpm; 37 °C; 16 hours	Broth A
S. carnosus strain 51365	155 rpm; 37 °C; 19 hours	Broth B

2.2. Preparation of the Copper

We obtained 32 copper pieces (3 cm × cm C110 copper (99.9% pure copper); 0.064 inch thickness, Metal Supermarket).

Scratching the Surfaces of Copper Coupons

We scratched 12 parallel lines onto half of the copper pieces to introduce the variable of surface texture. To ensure all scratches were of comparable depth, we built a lever

(Figure 1) and placed a lead block (1.4515 kg, length: 10.1 cm, width: 4.9 cm, height: 2.4 cm) 2.54 cm from one edge of the board, and taped a piece of copper to be scratched 2.54 cm from the opposite edge. Using the corner of a razor blade (Personna 94-120-71 stainless steel razor blades), we applied pressure to the left upper corner of the copper piece until the wooden board was level to the ground. Then, we slid the blade towards the bottom edge of the copper piece, keeping pressure constant and the board level. We repeated this procedure 12 times from left to right for each copper piece, using a new blade for each square. Approximately 2% of the surface of the copper coupon was scratched.

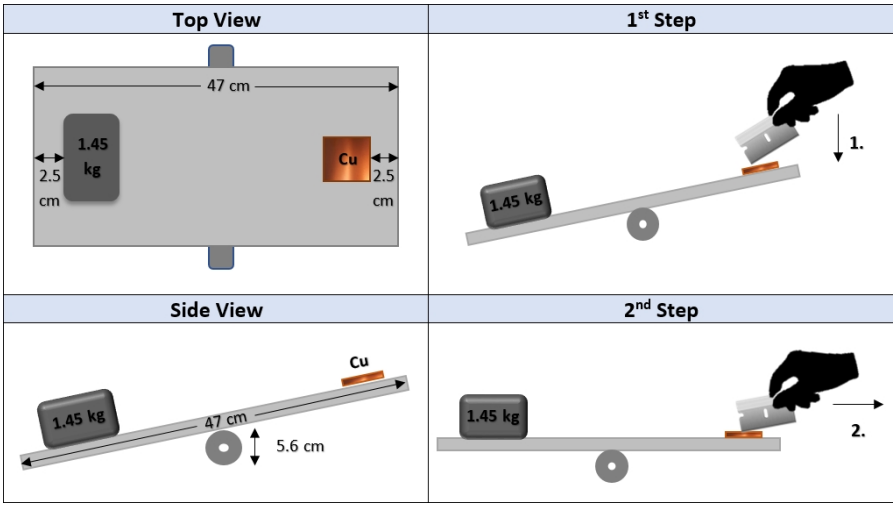

Figure 1: ***Overview of copper metal scratching mechanism implemented in this experiment.*** *Pictures not drawn to scale.*

Acetic Acid Wash and Sterilization

The scratches we introduced onto our copper pieces exposed copper that had not yet had contact with open air, resulting in variability in the oxidation levels of each copper piece. Previous studies have shown that oxide layers on copper surfaces affect copper's antibacterial activity [18, 21]. To account for this confounding variable, we performed an acid wash to ensure all copper being tested underwent the same level of oxidation.

We rinsed all copper pieces with distilled water, then 70% ethanol to remove dirt and debris. We immersed each copper square in glacial acetic acid for 15 seconds, as glacial acetic acid removes CuO layers without damaging the copper metal underneath [22]. Immersion time was based on results of a previous study [22]. After 15 seconds, we removed the copper pieces from the glacial acetic acid and let them air-dry. We placed 1 scratched and unscratched piece side by side in each glass petri dish to organize our copper such that the scratched and unscratched piece for each time interval were in the same environment. We autoclaved all the petri dishes with copper inside, then para-filmed each dish to prevent constant oxygen flow that could increase the rate of oxide layer formation [23].

2.3. Measuring the Rate of Bacterial Death on Scratched and Unscratched Copper

S. Carnosus

We removed the parafilm on each petri dish immediately before using them to keep oxygen exposure consistent for each copper piece. For each petri dish, we pipetted 10 µL of Broth B of *S. carnosus* onto the centre of the scratched and unscratched copper piece at the same time, using one plastic inoculating loop (VWR North American. Cat. No. 12000-810) per copper piece to spread the broth evenly over the copper surface. We used a cotton swab (Puritan Cotton Tipped Applicators 806-WC) and swabbed both copper pieces at either 1, 30, 60, or 90 minutes after bacteria was introduced then placed each swab into 1 mL Mueller Hinton broth. We performed 3 serial 10-fold dilutions of this broth, then plated 100 µL of each dilution on Mueller Hinton agar and incubated each agar plate (Forma Scientific IN2-MIC E-63 incubator; 37°C; 21 hours). (Figure 2) Data were collected by counting the number of visible colonies present on each plate. Colony counts greater than zero that followed a colony count of zero earlier in the dilution series were considered contamination and therefore excluded.

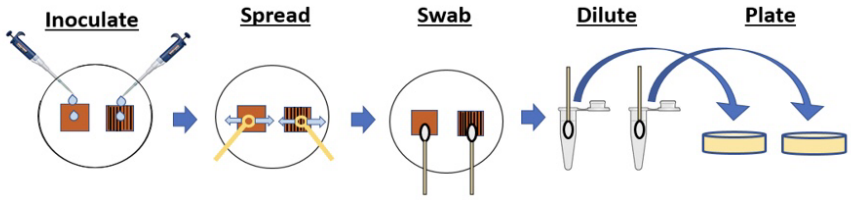

Figure 2: ***Overview of inoculation, spreading and plating of bacterial broth.*** *Pictures not drawn to scale.*

E. Coli

The same methods as used for *S. carnosus using Broth B were repeated* using Broth A of *E. coli* (Figure 2), following the time intervals and replicates depicted in Table 2. Preliminary results were more variable for *S. carnosus* than for *E. coli*, so the remainder of our time and resources were allocated to more replicates and an additional 10-minute time interval for *E. coli*. We performed a negative control (5 replicates) on stainless steel using these methods, swabbing only at 30 minutes to ensure the decrease in bacteria concentration was not a result of our copper preparation methods.

Table 2: *Number of replicates performed per time interval of E. Coli on C110 copper.*

Times swabbed (minutes after introduction of bacteria)	1	10	30	60	90
Number of replicates	5	5	5	2	2

2.4. Statistical Analysis

For each of our agar plates, we converted the number of visible colonies into colony forming units per millilitre (CFU/mL) to account for our dilutions. We then graphed the log of mean colony forming units per millilitre (with 95% confidence intervals) of both bacterial strains present on both scratched and unscratched copper pieces at all time intervals. We used unpaired t tests ($\alpha = 0.05$) with two-tailed p tests to look for differences in bacterial concentration between both bacterial strains on copper pieces of both surface textures. Statistical tests were performed on original, non-logarithmic data.

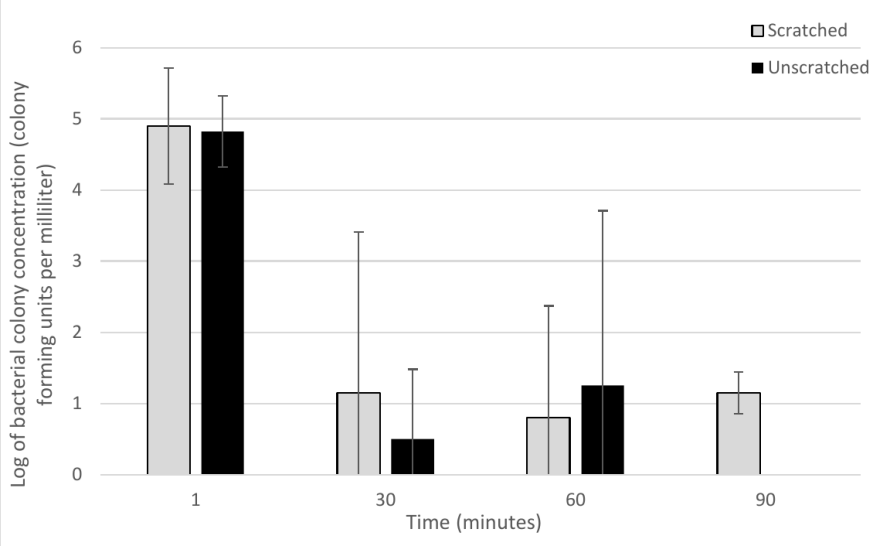

Figure 3: ***Mean S. carnosus strain 51365 colonies present on scratched and unscratched copper 1, 30, 60 and 90 minutes after introduction of bacteria.*** *Swab times are presented in minutes ± 15 seconds. Error bars represent 95% confidence intervals.*

3. Results

The antibacterial effectiveness of scratched and unscratched copper against the *S. carnosus* broth was determined by comparing the average logs of the bacterial colony forming units per millilitre on both types of copper. There was no statistically significant differences observed in bacterial concentrations between scratched and unscratched copper for any time interval tested (Figure 3; $t(2) = 0.42, p = 0.7181; t(2) = 0.95, p = 0.4408; t(2) = 0.87, p = 0.4755; t(2) = 2.8, p = 0.1074$). Non-logarithmic data for the 90-minute time interval showed no significant difference in bacterial concentrations on scratched and unscratched copper ($t(2) = 2.8, p = 0.1074$).

The effectiveness of scratched and unscratched copper against the *E. coli* broth was also determined by comparing the average logs of the bacterial colony forming units per millilitre on both types of copper. There was no statistically significant difference in the mean *E. coli* colony concentration on scratched versus unscratched copper at any time interval tested after introduction of bacteria onto the surfaces (Figure 4; $t(8) = 1.23, p = 0.2527; t(8) = 0.98, p = 0.3537; t(8) = 1.41, p = 0.1950$).

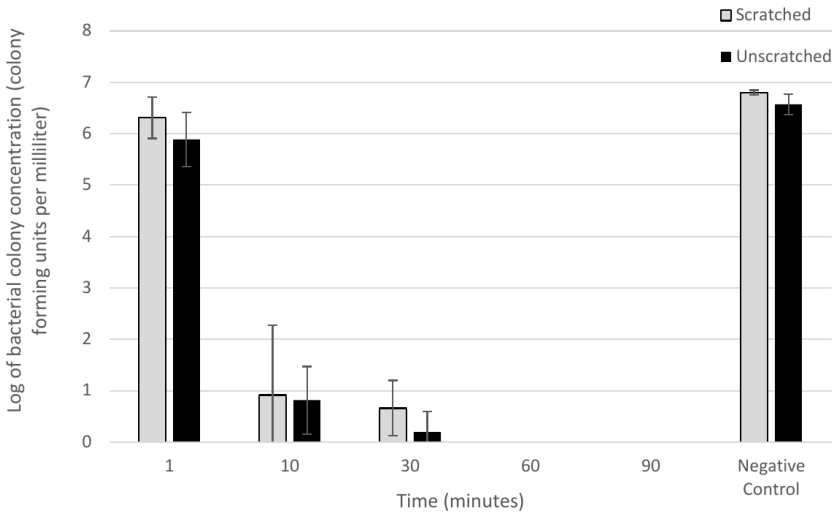

Figure 4: ***Mean E. coli strain ATCC 11303 colonies present on scratched and unscratched copper at times 1, 10, 30, 60 and 90 minutes after introduction of bacteria.*** *Negative control represents experiment done on stainless steel with swab taken 30 minutes after introduction of bacteria. Swab times are presented in minutes ± 15 seconds. Error bars represent 95% confidence intervals.*

There was a significant decrease of bacterial concentration on the copper surfaces over time between 1 minute and 10 minute intervals (Figure 4; $t(8) = 2.88, p = 0.0206$), however there were no statistically significant changes in bacterial concentration on the copper surfaces between 10 and 30 minutes (Figure 4; $t(8) = 0.99, p = 0.3500$). The bacterial concentration on copper surfaces decreased to 0 by 60 minutes. The stainless steel negative control had no significant difference between bacterial concentration present on scratched and unscratched surfaces after 30 minutes of contact (Figure 4; $t(5) = 1.08, p = 0.3293$), however there were significant differences in bacterial concentrations between stainless steel and copper at 30 minutes, for both scratched and unscratched surfaces (Figure 4; $t(5) = 33.56, p < 0.0001; t(8) = 3.71, p = 0.0060$). There were no significant differences in bacterial concentrations on stainless steel at 30 minutes and copper at 1 minute, for scratched or unscratched copper (Figure 4; $t(5) = 1.97, p = 0.1064; t(8) = 2.12, p = 0.0673$).

4. Discussion

Many mechanisms have been proposed to explain copper's antibacterial properties; some theorize that direct contact is required between bacteria and copper for effective bactericidal action, [17, 24] whereas others suggest that copper ions in solution may play a more important role [24, 25]. Our results support the latter, as there was a significant decrease in *E. coli* concentration after only 10 minutes of exposure to copper C110, yet liquid broth was still present on the copper surfaces at this time. Because liquid was still present on the copper surfaces when the majority of bacteria had been killed, it is possible the liquid facilitated the movement of Cu^{2+}, allowing bacteria to be killed without direct contact between the *E. coli* and the solid copper surface.

In previous studies working with *E. coli* strain O157 on copper alloys, it was found that a similar procedure resulted in little to no bacteria present on copper's surface after 75 minutes of exposure [17], a longer kill-time than recorded in this experiment. This time difference could be due to the volume of broth used and copper alloy composition. Seventy five minute kill-times were obtained using 20 µL of broth per 1 × 1cm 95% copper square, [17] whereas our data were collected using 10 µL of broth per 3 × 3cm 99% copper square, a much lower volume of liquid per unit surface area of copper. Further research is needed to investigate the surface area of copper needed per unit of volume of bacteria in liquid medium. Moreover, it is possible that *E. coli* strain ATCC 11303 is more susceptible to copper's antibacterial action than *E. coli* strain O157. *E. coli* O157 is a pathogenic strain [26], whereas strain ATCC 11303 used in this study is not. Various pathogenic microorganisms have been shown to secrete siderophores [27], which are small chelators used to bind iron molecules necessary to for cellular functions [28, 29]. Uropathogenic *E. coli* has been found to produce a siderophore able to bind copper, and protect cells against copper toxicity [30]. Additional research is needed to determine if this protective mechanism is a contributor to the longer exposure time needed to kill *E. coli* O157, and the extent to which this mechanism can protect pathogenic *E. coli* against coppers antibacterial activity.

Due to limited resources, we were only able to perform additional replicates for *E. coli*, which could be the reason for the larger error bars observed in the *S. carnosus* data. During future research into this topic, increasing the number of replicates may also increase the accuracy of the 95% confidence interval bars for the *S. carnosus* data. This may reveal new trends in the *S. carnosus* data not observed in this study.

For both *E. coli* and *S. carnosus*, there were no statistically significant differences in the concentration of bacteria on scratched versus unscratched copper at any time interval tested (Figure 3, Figure 4). This suggests that hospital surfaces replaced with copper metal may not require extensive maintenance of their smooth surfaces, provided that the oxidative effects of acquired scratches do not have a significantly negative effect. Further research investigating the relationship between surface scratches and the oxidation of copper surfaces, and the effects they may have on copper's antibacterial activity could help assess the benefits of implementing copper surfaces into hospitals in a real-world, uncontrolled environment.

5. Conclusion

Overall, our results suggest that copper surfaces implemented in health care facilities are an effective antibacterial measure whether visible surface scratches are present or not, as we observed no difference in copper's antibacterial activity when scratched at this magnitude. Additionally, as we saw significantly less bacteria present on copper when compared to stainless steel at the same time interval (Figure 3). Our results support the claims that the replacement of frequently touched hospital surfaces with copper metal could be beneficial in decreasing the transmission of hospital acquired infections.

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