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Antimicrobial activity of cuprous oxide-coated and cupric oxide-coated surfaces

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SUMMARY

Background: Disease can be spread through contact with contaminated surfaces (fomites). For example, fomites have been implicated in the spread of meticillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Antimicrobial surface treatments are a potential method of reducing disease transmission from fomites, and broad-spectrum activity is desirable.

Aim: To test cuprous oxide (Cu_2O) and cupric oxide (CuO) coatings for antimicrobial activity against 12 micro-organisms including bacteria and fungi.

Methods: We fabricated two surface coatings. The Cu_2O coating was fabricated in a simple two-step process using polyurethane to bind the active copper oxide particles; CuO was prepared by heat treatment of Cu_2O particles in air to produce cupric oxide (CuO) and to cause early-stage sintering to form a continuous coating. The antimicrobial activity was examined with 10 μL of microbial suspension droplets followed by counting cells as colony-forming units (cfu).

Findings: The coatings rapidly killed nine different micro-organisms, including Gram-negative and Gram-positive bacteria, mycobacteria and fungi. For example, the Cu_2O/PU coating killed 99.9997% of *P. aeruginosa* and 99.9993% of *S. aureus* after 1 h. Efficacy was not reduced after weekly cleanings. The antimicrobial activity of the Cu_2O coating was unchanged after abrasion treatment, and the coatings were not cytotoxic to human cells.

Conclusion: The combination of broad-spectrum antimicrobial activity, abrasion resistance, and low toxicity of the Cu_2O coating suggests potential use in healthcare settings.

Introduction

Micro-organisms infect millions of people throughout the world and cost billions of dollars to treat [1–3]. Serious infections are treated with antibiotics and thus antibiotic-resistant micro-organisms present particular problems. The

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United States (US) Center for Disease Control and Prevention (CDC) has listed the most serious microbial threats [4] and these include the yeast *Candida auris* (an Urgent Threat) and the bacteria meticillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (Serious Threats) [5,6].

Infection from micro-organisms occurs via several routes [7]. Microbes from the air, water, solids and food enter the body through inhalation, ingestion and skin contact. In this work we focus on disease transmission via contaminated solids (fomites) onto skin [8–11]. Studies have shown that micro-organisms are present on readily accessible surfaces in public areas [12–15] and hospitals [16,17] and that these micro-organisms can survive for periods as long as months [18]. Transmission of bacterial pathogens from surfaces in hospitals is well established [19]. Specifically, hospital-acquired infections have been reported due to transfer from surface-adherent *S. aureus*, [20] vancomycin-resistant and vancomycin-sensitive enterococci [21,22] and *P.* aeruginosa [19]. The US CDC estimated that in 2017 there were 323,700 MRSA infections in the US, causing 10,600 fatalities [4].

Methods to reduce transmission from fomites are currently based on treating surfaces with a disinfectant. However, that approach is limited by the fact that the surface can be immediately re-contaminated by contact with the pathogen. Frequent decontamination is labour intensive and, therefore, expensive, and the more time spent on decontamination, the less time the surface is available for use. One alternative to frequent disinfection is a surface coating that kills pathogens on contact. Such a coating would need to kill quickly, last for an extended period of time, and be able to kill pathogens after cleaning. Such coatings have been produced and evaluated against bacteria [23–26] and viruses, including SARS-CoV-2 [25,27–32]. Metals and their oxides, including copper, copper oxide, silver, silver oxide and zinc oxide [33–40], have been used as the active ingredients. A few surfaces have been studied that are not coatings but are modifications to the topology of the surface [41–44].

Here we describe the antimicrobial efficacy of coatings containing either cuprous oxide (Cu_2O) or cupric oxide (CuO) as the active microbicide, compared with uncoated stainless steel (a widely used surface in hospitals). These two copper oxides have broad antimicrobial properties, and are thus promising candidates for antimicrobial coatings [45–48]. Results are presented as relative survival where the survival of microbes on the coating is compared to the survival on stainless steel. During the COVID-19 pandemic, we described two coatings (Cu_2O/PU, consisting of a layer of cuprous oxide particles bound to a solid surface by commercial polyurethane, and sintered CuO, made by heat treatment of Cu_2O) that rapidly reduced the ability of SARS-CoV-2 on surfaces to infect human cells [27,29].

Materials and methods

Materials

Materials for preparation of antimicrobial copper surfaces

Cu_2O particles (Chem Copp HP III Type UltraFine -5) were obtained from American Chemet Corporation. Polyurethane (brand: Miniwax, Fast-Drying Polyurethane, clear satin) was purchased from Lowes Home Improvement Store. Stainless steel 301 (SS 301) shim (0.015 inches thick) was purchased from McMaster Carr. SS 301 was thoroughly washed with soap and water and rinsed with water. 200 PROOF ethanol and glass slides (25 × 75 × 1 mm) were obtained from VWR. For sample (coupon) preparation, deionized water was further purified by Milli-Q Reference system.

Fabrication of Cu_2O/PU coating [27]

A thin layer of polyurethane was applied to stainless steel 301 using a sponge; it was allowed to partially dry for a few min, then a suspension of Cu_2O in ethanol (10% w/w) was added dropwise, and it was left to partially dry for a few minutes at room temperature. Subsequently, the sample was heat treated at about 80°, blown with nitrogen gas, and washed thoroughly with deionized water. The coated stainless steel was cut into ≈ 12 × 12-mm pieces, and each piece was cleaned with argon plasma. The films were stored under water, unless otherwise stated. Scanning electron microscopy (SEM) showed that the coating was 10–16 μm in thickness. Optical images of the coatings and SEM images are available in Supplementary Figures S1 and S2, respectively, and the elemental composition is in Supplementary Table S1.

Fabrication of partially sintered CuO coating [29] a suspension of Cu_2O in ethanol (10% w/w) was deposited on SS 301 (≈ 12 × 12 mm), left to partially dry, and then heat-treated in a furnace at 700 °C for 2 h, followed by natural cooling to room temperature overnight. SEM showed that the thickness was about 32 ± 1 μm.

Measurement of antimicrobial activity

Growth of microbial strains

The microbial strains used are listed in Table I. With the exception of mycobacteria that were grown in 5 mL Middlebrook 7H9 Broth at 37 °C with aeration (60 rpm), all other bacterial strains were grown in 5 mL Tryptic Soy Broth (TSB) at 37 °C with aeration (60 rpm). *Bacillus anthracis* culture continued until the culture contained >95% spores on microscopic examination. Yeasts were grown in Potato Dextrose Broth (PDB) at 37 °C with aeration (60 rpm). *Aspergillus niger* was grown on PDA agar plates at 30 °C until the plate was covered with black-pigmented spores. Spores of both *B. anthracis* and *A. niger* were harvested by suspension in 5 mL sterile water, collected by centrifugation (5000 × g for 20 min) and resuspended in water. The purity and identity of cultures were verified by subculture.

Preparation of microbial cells for surface killing measurements

Cells were separated from the growth media and suspended in phosphate-buffered saline (PBS) as follows. After centrifugation (5000 × g for 20 min) the supernatant was discarded, and the cells resuspended in 5 mL of sterile PBS by vortexing for 60 s. Those suspensions were then subject to another round of centrifugation and resuspension, after which the cell densities were adjusted by dilution to equate to a McFarland No. 1 Standard. The number of colony-forming units (cfu)/mL of each suspension was then measured by spreading 0.1 mL (in duplicate) serial dilutions on agar; typical cell densities were 3.2×10^5–1.3×10^6 cfu/mL.

Measurement of surface-killing

A 10-μL test droplet of the cell suspension in PBS was placed on the test solid. Immediately afterwards, one surface of each type (i.e., control, Cu_2O/PU, Sintered CuO) was transferred to a sterile 50-mL centrifuge tube containing 5 mL of sterile PBS, vortexed for 30 s and sonicated for 5 min, and the cfu/mL of the suspension measured as described above. This was the zero time control that indicated our ability to recover bacteria. At later times (e.g., 1 h, 2 h, etc.), one sample of each material type was processed in the same way. Following 48 h incubation of the plates (14 days for *Mycobacterium* spp.), colonies were counted, the number of surviving cells expressed as cfu/mL of suspension calculated, and the % relative survival calculated as:

$$\frac{(\text{mean cfu coated})}{(\text{mean cfu uncoated})} \times 100\%$$

One coupon was measured for each time point, and results show the average of duplicate cfu measurements from the same bacterial suspension that was recovered from the coupon.

Cytotoxicity study

The cytotoxicity was assessed in human monocyte cells (white blood cells, Thp1, ATCC®TIB 202™) that form part of the immune system and in connective tissue cells (murine fibroblasts, L929 ATCC®CCL-1™) using a lactate dehydrogenase (LDH) assay as described in the Supplementary Material.

Results and discussion

Controls and validation

The following validation experiments are described in more detail in the Supplementary Material. (1) We tested whether viable bacteria remained on the stainless steel after our extraction procedure, in which case we would overcount killing. Results showed no significant difference between the number of viable cells plated onto the steel and the number extracted from stainless steel. (2) We tested whether there was carry over of antimicrobial liquid into the cfu assay. Such antimicrobial material could have provided an ongoing kill during the incubation for the cfu assay, thereby undermining our claim that killing was occurring on the coupon in 1 h. We did not find ongoing killing during the cfu incubation (Supplementary Material).

Both Cu_2O/PU and sintered CuO coatings have broad-spectrum antimicrobial activity

Measurements were made using 12 microbial species (Table I). The Cu_2O/PU coating was strongly antimicrobial: specifically, there were no surviving colonies (with the exception of *Mycobacterium* spp. and *Bacillus anthracis* spores) after 2 h of exposure to the surface. It is possible that the killing of the *A. niger* spores was a consequence of their germination. The sintered CuO coating was also effective, but less so than the Cu_2O/PU coating. The survival after 1 h of exposure of cells to the two *Mycobacterium* spp. was substantial. For example, survival on sintered CuO was 43% for *M. avium* and 29% for *M. abscessus*. *Mycobacterium* spp. is difficult to kill [49,50], probably due to the thick, lipid-rich outer membrane [51].

Table I
Relative survival of microbes on cuprous oxide/polyurethane (Cu_2O/PU) or sintered cupric oxide (CuO) coatings compared with stainless steel

Strain	Time[a] (h)	% Relative survival[c]	
		Cu_2O/PU	Sintered CuO
Staphylococcus aureus	0	0.16	51.0[b]
ATCC® 6538	1	0.3	0.3
	2	<0.1	<0.1
Staphylococcus aureus (MRSA)	0	0.1	3.0[b]
ATCC® 43330	1	<0.1	<0.1
	2	<0.1	<0.1
Escherichia coli	0	7.0	34.0
ATCC® 13706 DX	1	<1.4	<1.4
	2	<1.4	<1.4
Pseudomonas aeruginosa	0	<0.2	105.
ATCC® BAA-2111	1	<0.2	<0.2
	2	<0.2	<0.2
Stenotrophomonas maltophilia	0	173	158
ATCC® 17666	1	<2.0	<2.0
	2	<2.0	<2.0
Acinetobacter baumannii	0	14	172
ATCC® 2802	1	<1.4	22
	2	<1.4	<1.4
Bacillus anthracis Spores	0	79.1	79.4
Sterne Strain 34F2	1	78.2	100.1
(LLNL A0517)	2	57.4	82.8
Mycobacterium avium	0	108	98
ATCC® 25291	1	14	43
	2	14	29
Mycobacterium abscessus	0	23	44
ATCC® 23045	1	1.4	29
	2	<0.5	17
Candida albicans	0	23	110
ATCC® 10231	1	20	0.4
	2	<0.4	<0.4
Candida auris	0	14	105.
CDC & FDA AR	1	<0.3	<0.3
Bank # 0386	2	<0.3	<0.3
Aspergillus niger Spores	0	67	78
ATCC® 10535	1	19	33
	2	<1.4	<1.4

[a] Time since contact of the droplet with the sample; in practice, time = zero is actually a few minutes' processing time.
[b] The difference in results for the two *S. aureus* strains for CuO at zero time was large. To test whether the difference was significant, we performed a second round of experiments (see Supplementary Data) and concluded that there was not a significant difference.
[c] Compared with stainless steel. Data for stainless steel in Supplementary Table S10.

Anthrax spores are typically even more difficult to kill [50], and sintered CuO was ineffective. The Cu_2O/PU coating showed a mild effect against *Bacillus anthracis* after 2 h.

Survival was so low in some cases that our initial experiment did not resolve the full killing ability of the Cu_2O coating. To obtain improved resolution, we tested the survival for higher cell densities of *P. aeruginosa* (7.28×10^6 cfu/coupon) and *S. aureus* (3.76×10^6 cfu/coupon).

Survival after 1 h of exposure was 0.0003% for *P. aeruginosa* and 0.0007% for *S. aureus* (see Supplementary Table S7 for more

Table II
Repeated killing of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on rinsed and disinfected Cu_2O/PU coating

Week	Percent of bacteria surviving after 60 min exposure to Cu_2O/PU coating ($cfu_{coated,\ 1\ h}/cfu_{coated,\ 0h}$)	
	S. aureus	*P. aeruginosa*
0	0.07	0.5
1	0.07	<0.02
2	0.65	<0.02
3	<0.003	<0.03
4	<0.04	<0.17
5	<0.0067	<0.035
7	<0.009	<0.01
9	<0.008	<0.009
10	0.017	<0.002
20 (time = 1.5 h)	0.065	<0.0047

times). A full study of the killing as a function of cell density is described in the Supplementary Material and indicates that killing is at the detection limit at all but the very highest cell densities. This indicates both a high kill and the ability to kill different densities of cells. Killing at short times, 5 min and 10 min, is reported in Supplementary Table S8. Killing is rapid, particularly for *P. aeruginosa*.

Repeated disinfection/cleaning of the Cu_2O/PU coating with ethanol over 20 weeks did not reduce killing

We determined whether the copper surfaces retained antimicrobial activity after repeated, cleaning/disinfection (see Supplementary Table S3 for more details) over a period of 20 weeks. The results (Table II) demonstrate that killing was maintained at high levels throughout regular contamination and disinfection.

The PU/Cu_2O and sintered CuO coatings are not cytotoxic against human monocytes and murine fibroblasts

The cytotoxicity of the two coatings were assessed in human monocyte cells (white blood cells, Thp1) that form part of the immune system and in connective tissue cells (murine fibroblasts, L929) using a lactate dehydrogenase (LDH) assay as described in the Supplementary Material. Results in Figure 1 show that the lysis in the presence each of the coatings was negligible in comparison to full lysis.

Table III
Survival of *Pseudomonas aeruginosa* on fresh and abraded Cu_2O/PU coatings

	Time* (h)	Fresh coating % Survival	Abraded % Survival
Repeat 1	0	99.3	84.5
	1	<0.6	<0.6
Repeat 2	0	84.6	97.1
	1	<0.4	<0.4
Repeat 3	0	78.5	78.5
	1	<0.5	<0.5

Antimicrobial activity of Cu_2O/PU coating survive abrasion

We previously demonstrated the resistance of the Cu_2O/PU coating to scratching using a cross-hatch test [27]. Here we extended the durability testing using an abrasion test, modified from the standard US EPA test [52] (Supplementary Material). Cu_2O/PU coatings on glass that were stored in air were mechanically rubbed with a sponge and compared with non-rubbed samples. There was no difference in the microbiocidal activities of the rubbed and non-rubbed surfaces (Table III).

Are cells killed or are they simply trapped in the matrix?

Our antimicrobial coatings are porous (Supplementary Figure S2) and water permeable. The lack of viable bacteria that we detected in our cfu assay at zero time for *P. aeruginosa* and *S. aureus* (Table I) raises the question of whether bacteria were simply trapped in the porosity rather than killed, despite our vigorous efforts to shake them loose. We addressed this in two ways. First, we fabricated porous samples from glass, a material with no antimicrobial properties, and examined the recovery of *P. aeruginosa* from these samples. Recovery was determined using 2-μL suspension droplets placed on agar ($cfu_{suspension}$) or the glass coupon (cfu_{coupon}) for 1 min using the following equation:

$$\%\ \text{recovery} = \frac{\text{mean}(cfu_{coupon})}{\text{mean}(cfu_{suspension})} \times 100\%$$

The average recovery was 27% (Supplementary Table S4), demonstrating that that *P. aeruginosa* can be recovered from porous samples; 27% is much greater than the $<4.5\times10^{-4}$% that were recovered from the Cu_2O/PU coating, indicating that our results seem not to be explained by trapping of bacteria in Cu_2O/PU. However, we acknowledge that glass is a different material to the coating, and trapping could be different.

Second, if viable bacteria were simply trapped and not killed, it should be possible to culture the trapped bacteria. To detect residual viable bacteria within a (porous) Cu_2O/PU coupon after our extraction procedure, we placed the coated side of the coupon on TSB agar and incubated in contact for 48 h. We found at most only 1 or 2 cfu, compared with thousands of cfu that would need to have been trapped to account for our results.

The Cu_2O/PU coating is only approximately 15 μm thick: water and nutrients can easily be wicked or diffused over this distance. *P. aeruginosa* is motile, and could also migrate this distance [53]. Alternatively, viable, embedded cells could grow in diffused nutrients from the agar medium and progeny could reach the nutrient agar in time to grow a visible colony on the agar. This experiment shows that, given ideal growth conditions, trapped bacteria do not present an infection risk. Indeed, it suggests that viable bacteria are not present at all.

We also examined a Cu_2O/PU sample with no access to porosity to assess its capability to kill *P. aeruginosa*. This experiment is simple to perform because, with time, the PU matrix becomes

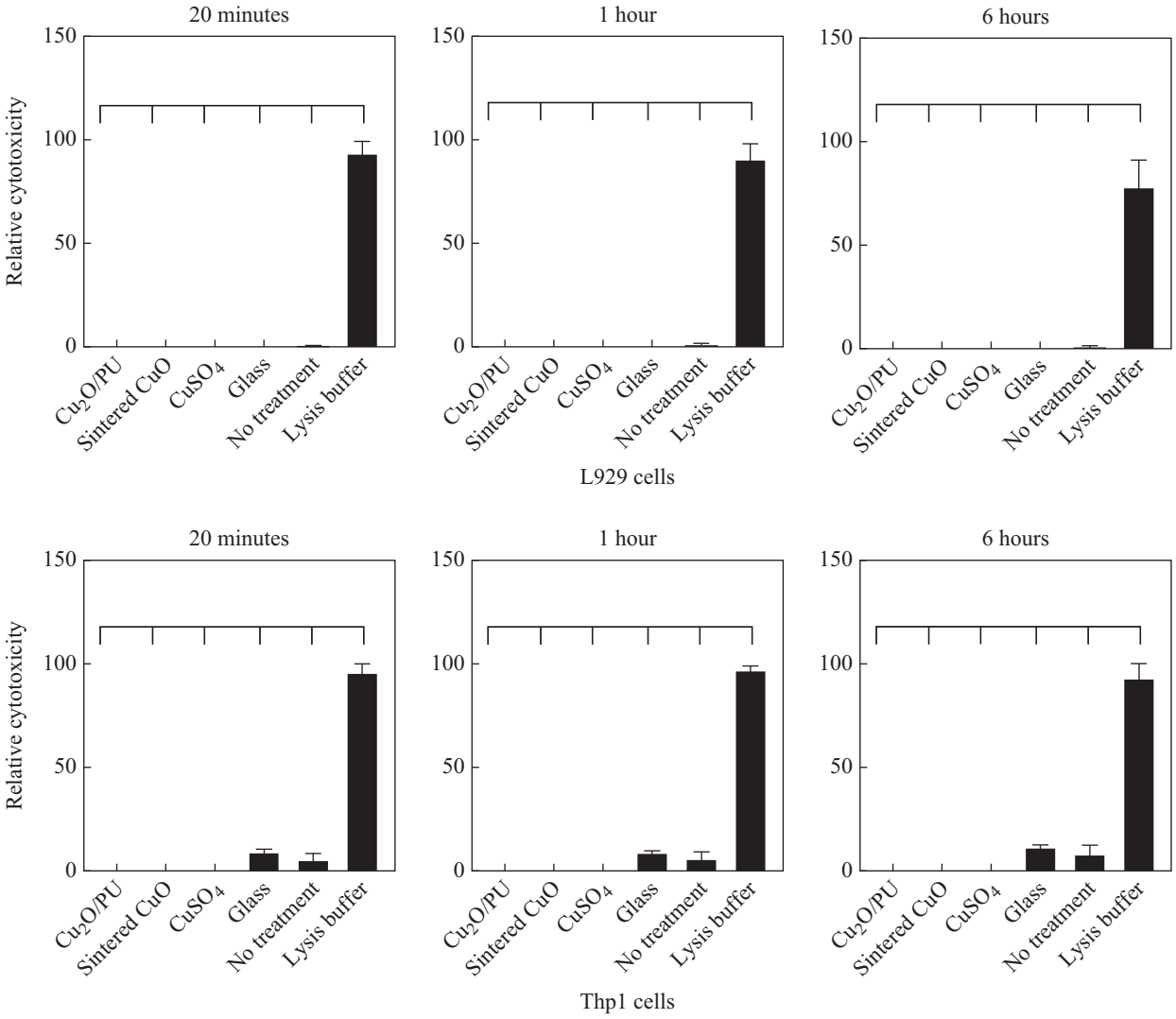

Figure 1. Cytotoxicity study of the coatings.

hydrophobic, preventing access to the porosity. The samples used for the abrasion testing were aged, and the measured zero-time survival was 87%. Without the other evidence described above, this might be considered evidence for trapping in the regular samples. However, we suggest that when bacteria penetrate into the porosity, the large contact area with the active ingredient, and short diffusion distances, provide a very rapid kill compared with an impermeable material.

Overall, we believe that bacteria are not trapped in the porosity, but that the porosity in fact contributes to bacterial killing in a shorter time period.

Widespread application of the Cu_2O/PU coating

Given the antimicrobial effectiveness of the coating, we investigated coating materials other than the stainless steel and glass reported previously [27]. We found that the coating could be easily applied on polypropylene plastic, ABS plastic, granite and wood. While we focused on a 1-h time-point throughout this work, commensurate with the US EPA standard [52], Cu_2O/PU kills bacteria much more rapidly, e.g., after 10 min the relative survivals of *P. aeruginosa* and MRSA on the coating are <0.2% and 14%, respectively (Supplementary Table S8).

In conclusion, Cu_2O and CuO coatings were both highly effective against a range of bacteria and fungi. The Cu_2O/PU coating was superior, leaving no survivors after 1 h for 8 of 12 organisms. The kill% was over 99.999% in 1 h in high-resolution experiments for *P. aeruginosa* and *S. aureus*. Survival was poor over a large range of cell densities, as high as 10^9 cfu/mL for *P. aeruginosa* and 10^8 cfu/mL for *S. aureus*. The efficacy of the Cu_2O/PU coating was unaffected by 10 repeat exposure/disinfection/rinse cycles over 20 weeks or by cycles of abrasion. The coating was less effective against mycobacteria and anthrax spores but these are typically difficult organisms to kill. The ability to pass durability testing and lack of cytotoxicity for human cells suggest that the Cu_2O/PU coating may be useful in hospital settings to reduce the transfer of microorganisms that are spread by contact.

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Conflict of interest statement

W.A.D. declares part ownership in a startup company that intends to produce surface coatings. Other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2022.07.022.

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Supplementary Material

Antimicrobial Activity of Cuprous Oxide and Cupric Oxide-Coated Surfaces

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1. Supplementary Materials and Methods

1.1. Abrasion Tests

Fresh Cu_2O/PU coating were abraded by a sponge (9.5 cm x 8 cm x 2.3 cm) mounted on an abrasion tester (Gardco D10V). The abrasion tester translates a sponge on the surface of the test sample (coupon) with a load of 0.45 kg, an amplitude of ~26 cm, and a period of ~5 s. The test was based on an United States (US) Environmental Protection Agency (EPA) protocol[1], which is employed to evaluate antimicrobial coatings for EPA registration for antimicrobial products to be distributed in the USA. Hospital surfaces are often cleaned with ethanolic solution, so our procedure diverged from the official EPA test by exposing the surface and sponge to 70% ethanol. Prior to conducting the tests, the sponge was wetted with 20 mL of 70% ethanol. An abrasion cycle was conducted as follows: the sponge was translated across the sample 8 times, then 70% ethanol was sprayed on the abrasion tester platform and the sample with a subsequent 30 minutes waiting period. 10 cycles (= 80 passes in total) were applied to the sample. A single sponge was used for the first 5 cycles and the sponge was replaced with a second sponge for the second 5 cycles.

1.2. Statistical Analysis

Hypothesis testing was done with Student t-tests, where *p*-values less than about 0.05 were indicative of significant differences from the null hypothesis. In some instances, colony counts of the copper coupons were below the limit of detection. In statistical tests these were calculated at the detection limit (<1 CFU/plate), which overestimates the mean and underestimates the standard deviation.

1.3. Cytotoxicity Experiments

Lactate Dehydrogenase (LDH) is an enzyme naturally present in the cytosol of many cells. When cells die, the plasma membrane degrades, allowing LDH to be found outside of the cell membrane. For cell culture, this means that LDH is released into the media. The colorimetric assay used utilizes a two-step enzymatic reaction to produce a red formazan product that can then be read spectrophotometrically at 490nm[2]. Importantly, the level of the produced formazan byproduct is directly proportional to the level of LDH in the sample media, which can be inferred as a measure of cytotoxicity. In this assay, the use of a 10x Lysis buffer provides a positive control by disrupting the plasma membrane, allowing the release of cytosolic LDH into the media. By lysing a high percentage of cells, it provides a maximum baseline of LDH in the media which can be compared to treatment groups to determine the relative level of cytotoxicity induced by each treatment. These studies were performed in two cell lines- Thp1 cells and L929 cells. Thp1 cells (ATCC®TIB 202™) are human monocyte (white blood) cells that form part of the immune system, and generally do not adhere to solids. To perform a valid cytotoxicity experiment, we needed to obtain contact between the cell and the test solid. To force contact, a test solid (coupon) and a cell suspension were placed in a centrifuge tube and the tube was centrifuged in order to force contact with the test solid. The copper compounds did result in a visual color change in the media. In order to correct for this, we measured the absorbance of leachate from the cuprous oxide and cupric oxide as well as the copper sulfate in media in the absence of cells, and we subtracted the appropriate baseline absorbance from the absorbance in the presence of cells.

2. Supplementary Results and Discussion

2.1. Characterization of Coatings

Figure S2 shows cross section scanning electron microscope (SEM) image of each coating. Both coatings were uniform in thickness, even though they were made by hand. The thickness of the Cu_2O/PU coating was 10-16 µm and the sintered CuO coating was ~32 µm. The expected stochiometric ratio for the coatings was observed as shown by X-ray photoelectron spectroscopy (XPS) data in Table S1. XPS samples only the outer few nanometers of the coating.

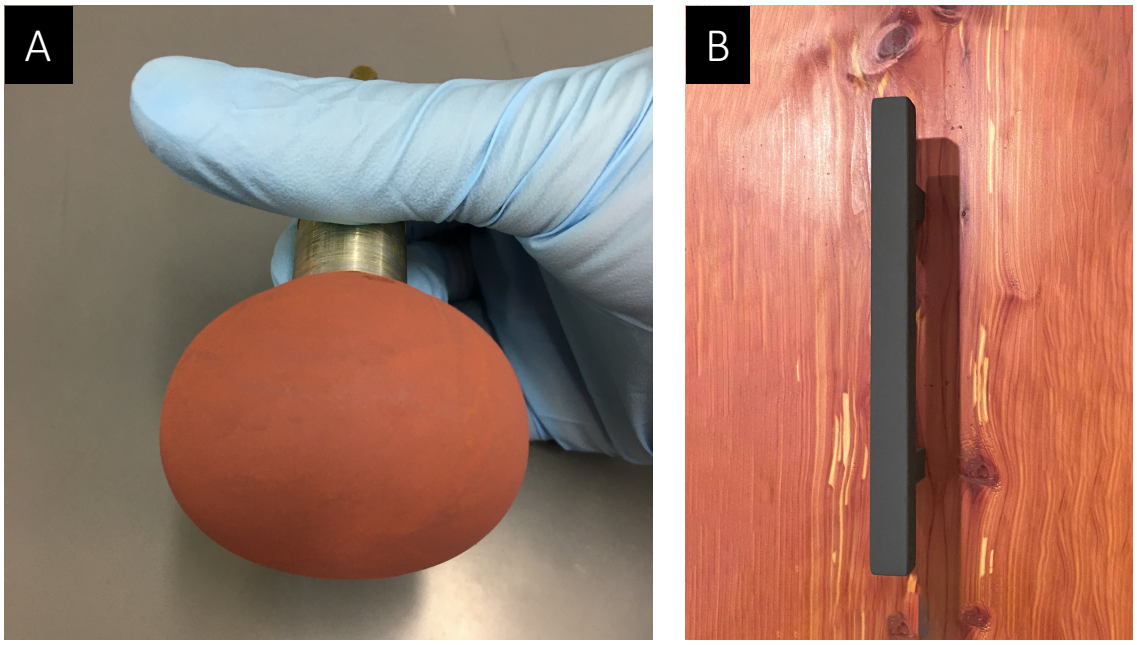

Figure S1. A) Optical images of the coatings. A) Cu_2O/PU that was made on a doorknob B) Sintered CuO that was made on a door handle.

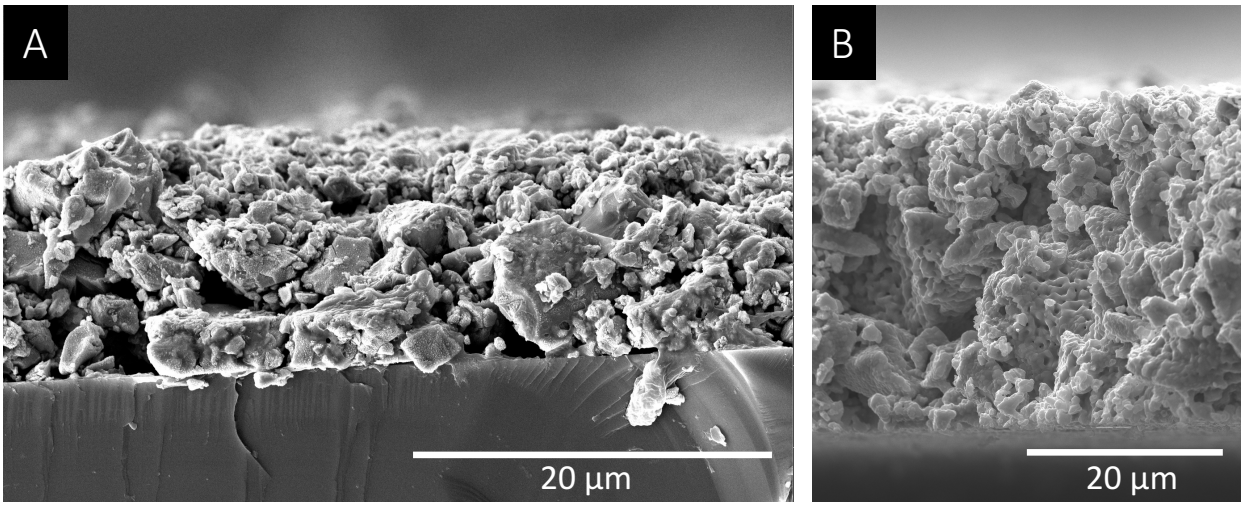

Figure S2. Cross section SEM images of the coatings. A) Cu_2O/PU and B) Sintered CuO.

Element	Cu_2O/PU	Sintered CuO
Copper	1.8	0.99
Oxygen	1	1

Table S1. XPS data of coatings. Both stoichiometric ratio are similar to theoretical ratios.

2.2. Tests of Microbial Survival on Surfaces

2.2.1. There Is a High Rate of Recovery of Microbial Cells from Stainless Steel Samples

We examined the hypothesis that we failed to recover all viable, colony-forming microbial cells from the stainless-steel samples. This hypothesis was tested by comparing the number of CFUs that were recovered from stainless steel to the number when a bacterial suspension was plated directly on agar. The number of CFUs from stainless steel were measured in the normal way, i.e., a bacterial suspension droplet was first placed on stainless steel and then the stainless-steel coupon was immediately transferred to 5 mL PBS, vortexed 30 secs and sonicated for 5 min and

then 0.1 mL samples of the suspension spread on the agar medium surface. For all organisms (Table S2), the CFUs are similar: we performed a Student's t-test to check whether there was a significant difference between the recovered and the original CFU count and the p-value was > 0.05. For the two cases where the *p*-value was low, *C. albicans* and *M. abscessus,* the number of bacteria extracted from the stainless steel was *greater* than the input (rather than lesser), so the hypothesis of "failure to extract" should still be rejected.

Microbe strain	Colony-Forming Units/Plate (%)		Paired t-test
	Suspension	Stainless	p-value
S. aureus	582 ± 62 (100)	531 ± 47 (91)	0.6286
MRSA	33 ± 13 (100)	68 ± 4 (206)	0.2103
E. coli	45 ± 14 (100)	43 ± 8 (96)	0.9208
P. aeruginosa	1278 ± 25 (100)	1494 ± 71 (117)	0.2157
A. baumannii	157 ± 23 (100)	110 ± 11 (70)	0.3193
S. maltophilia	33 ± 7 (100)	31 ± 11 (94)	0.8743
C. albicans	55 ± 13 (100)	73 ± 8 (133)	0.1190
C. auris	165 ± 29 (100)	163 ± 0.7 (99)	0.8834
M. abscessus	79 ± 0.7 (100)	110 ± 11 (139)	0.1414
M. avium	1917 ± 86 (100)	1656 ± 223 (86)	0.4444

Table S2. Recovery of different microorganisms from stainless steel coupons.

Week	Cleaning/disinfection Treatment
1	The coupon was rinsed 3 times in DI water, then immersed in 70% ethanol for 30 mins. After which the coupon was left in air to dry.
2	Same as Week 1
3	Same as Week 2
4	The coupon was immersed in 3% Lysol for 30 mins, then rinsed 3 times in DI water.
5	Same as Week 4
7	Same as Week 4
8	The coupon was rinsed with H_2O; vortexed in H_2O; vortexed in ethanol, rinsed with H_2O, vortexed in H_2O.
9	The coupon was immersed in 3% lysol for 30 mins, then it was immersed in 70% ethanol for 1 min (3 times). After which it was rinsed 3 times in DI water (each for 1 min).
10	Same as Week 9
20	Same as Week 9

Table S3. Details of cleaning/disinfection cycles for each week. Note that in each case, the disinfectant was removed by either rinsing with water or for ethanol by air drying before subsequent testing of the antimicrobial

2.2.2. There Is a High Rate of Recovery of Microbial Cells from an Inactive Porous Medium

We examined the possibility that we achieved poor recovery from the porous materials. We examined this hypothesis by measuring recovery from an inert porous material, porous glass (size = ~12x12mm), to help to distinguish the role of porosity from an active surface. 2µL of *P. aeruginosa* was placed on the porous glass sample, and after 1-2 minutes, the sample was vortexed (for 1 min), sonicated (for 1 min), and again vortexed. Then the suspension was plated as mentioned in Materials & Methods section. The experiment was repeated 3 times, and the data is shown in Table. S4. We found that 27% of the bacteria is recovered from an inert sample.

		CFU/plate
Porous glass	Repeat 1	197
	Repeat 2	388
	Repeat 3	198
Suspension	---	1494

Table S4. Recovery data of porous glass and the suspension used.

2.2.3. Microbial-killing is Not Affected by Carryover of Antimicrobials from the Coupon to the CFU Assay

Our reported killing times indicate the time period during which droplets of bacterial suspension remained on the antimicrobial test solid. It was possible, however, that dissolved ions or particles from the copper oxide surface that are shaken loose by sonication and vortexing could be carried over into our CFU assay and there inhibit colony formation. Colony formation occurs over 48 h, which is much longer than the 1-2 h of contact with the test solid, and this large

difference in time-scales means that subtle carry-over effects may influence our results. In a test of an antimicrobial *solid* almost all of the antimicrobial is removed by physical removal of the antimicrobial solid before the CFU assay is performed. This is very different to a test of an antimicrobial *liquid* where the antimicrobial liquid is much more difficult to extract. However, even for a solid, there remains that possibility of some antimicrobial carryover.

To determine the possibility of carryover, we utilized Dey-Engley (DE) buffer, which is specifically formulated to contain compounds that are able to block or inhibit killing by a wide variety of antimicrobial agents, including metals [3]. This type of buffer is sometimes referred to as a neutralizer. We compared CFU counts when elution from the solid was done with PBS and DE buffers. Two bacteria, *P. aeruginosa* and MRSA were examined; all statistics were calculated in Microsoft Excel.

In contrast to PBS, DE buffer contains nutrients and other species that may aid the survival of the bacterial, so we first compared bacterial survival in the buffer (i.e., no coupon used). Our results, shown in Table S5A, show that that there is greater survival in DE buffer for both organisms, and that the effect is larger for MRSA (linear regression using two factors, buffer (p = 0.0035) and bacterium (p = 0.0066)). To control for this increased survivability, in further statistical results in section 2.2.3, we normalized results by the average survivability for the particular bacterium and buffer combination.

To test for the effect of "neutralizing" antimicrobials from the solid, the bacteria were initially suspended in PBS and then a 10 µL droplet was placed on the solid for a specified time. For elution, the coupon was transferred to either 5 mL of PBS or 5 mL of DE Broth, vortexed for 10 sec and then sonicated for 1 minute before spreading 0.1 mL samples on Tryptic Soy Agar plates. Those manipulations took about 3 min. Samples were incubated at 37° C overnight and then colonies were counted. The results, shown in Table S5B, demonstrate that both bacteria are killed by Cu_2O, even when the DE buffer was used.

We performed a regression analysis (Table S6) with the following variables: bacterium (*P. aeruginosa* vs MRSA), surface type (stainless steel vs Cu_2O/PU), buffer type (PBS vs DE), and time (0 vs 60 min). The *p*-value of 0.95 for buffer is consistent with no effect of the buffer and therefore no effect of carryover.

The resolution of the effect of buffer on the 60 minute samples was, however, diminished by the very poor survival in both buffers. In the above analysis, results at the detection limit were analyzed as if they were at the detection limit, which inflates the *p*-value for buffer. When we excluded the 60 min samples from the analysis and repeated the regression, the *p*-value for the buffer dropped to 0.87 but remained high. This is consistent with the idea that extraction of species during the sonication and vortexing of the copper coupons did not cause a carry-over effect.

Strain	Buffer	Log(CFU)
P. aeruginosa	PBS	6.36
		6.33
		6.34
	DE	6.53
		6.49
		6.49
MRSA	PBS	5.28
		5.19
		5.30
	DE	6.46
		6.35
		6.43

Table S5A. Effect of buffer on CFU counts with no coupon present.

Bacterium	Surface	Time* (min)	Repeat	Log(CFU)	
				PBS	DE broth
Pseudomonas aeruginosa	Cu_2O/PU	0	1	6.47	6.51
			2	6.30	6.56
			3	6.29	6.51
		60	1	<	<
			2	<	<
			3	<	<
Pseudomonas aeruginosa	Stainless steel	0	1	6.43	6.54
			2	6.27	6.53
			3	6.35	6.48
		60	1	5.09	6.08
			2	5.016	5.95
			3	5.07	6.15
MRSA	Cu_2O/PU	0	1	5.21	6.30
			2	4.94	6.39
			3	5.53	6.43
		60	1	<	3.32
			2	<	<
			3	<	<
MRSA	Stainless steel	0	1	5.50	6.36
			2	5.18	6.42
			3	5.44	6.37
		60	1	5.13	6.31
			2	5.33	6.31
			3	5.35	6.37

Table S5B. Effect of buffer on Cu_2O coupon experiments. Bacteria were inoculated on stainless steel or Cu_2O/PU coupons, and extracted into PBS or DE broth. < indicates below the detection limit, which was 1.70 in this experiment. Regression analysis of this data (Table S6) found that effect of buffer is not significant.

Variable	Coefficients	P-value	note
Bacterium	-0.378	0.232	not significant
Surface	-1.98	1.087E-07	significant
Buffer	-0.0212	0.946	not significant
Time	-0.0397	1.50E-09	significant

Table S6. Regression analysis results for data in Table S5.

2.3. Survival of *P. aeruginosa* and *S. aureus* at Higher Cell Densities

To obtain a clear resolution, we repeated the experiment in 3.3. with two bacteria (*P. aeruginosa and S. aureus)* that had much higher cell densities. The results are shown in Table S7 and they clearly show that the Cu_2O/PU coating is very effective. Only 0.0003% of *P. aeruginosa* and 0.0007% of *S. aureus* survived After one hour of contact with the coating.

Strain	Time* (hr)	% Survival	
		Cu_2O/PU	Sintered CuO
Pseudomonas aeruginosa (Density = 7,280,000 CFU/coupon)	0	57.1 ± 0.02	71.4 ± 0.01
	1	< 0.0003	34.3 ± 0.01
	2	< 0.0008	73.6 ± 0.03
	3	< 0.0007	55.0 ± 0.04
Staphylococcus aureus (Density = 3,760,000 CFU/coupon)	0	94.1 ± 0.02	133.0 ± 0.04
	1	< 0.0007	37.3 ± 0.02
	2	< 0.0007	6.5 ± 0.02
	3	< 0.001	30.7 ± 0.03

Table S7. Survival of *P. aeruginosa* or *S. aureus* on cuprous oxide/polyurethane (Cu_2O/PU) or sintered cupric oxide (CuO) coatings with high initial loading of bacteria. The initial density of *P. aeruginosa* was 7,280,000 CFU/coupon and for *S. aureus* 3,760,000 CFU/coupon. Even at this high load, no colonies grew for Cu_2O/PU.

2.4. Bacterial Killing as a Function of Cell Density

It is advantageous for a coating to kill bacteria in suspensions with a range of bacterial densities. To measure whether cell killing was reduced at higher cell densities, the survival of a 10-fold dilution series of *P. aeruginosa* and MRSA cells was prepared. 60 min after a 10 µL droplet was placed on the Cu_2O/PU coating, the survival of cells was measured by colony-formation. Results (Figure S3) show that survival was reduced by 3 logs for all the dilutions where we had resolution. For very dilute suspensions, there were insufficient survivors to resolve 3 logs. The coating killed all of the *S. aureus* in 60 minutes except for the highest cell density (10^7 CFU/10 µL). Likewise, all of the *P. aeruginosa* was killed in 60 min except at the highest concentration (10^7 CFU/10 µL) and after 180 minutes there were no survivors.

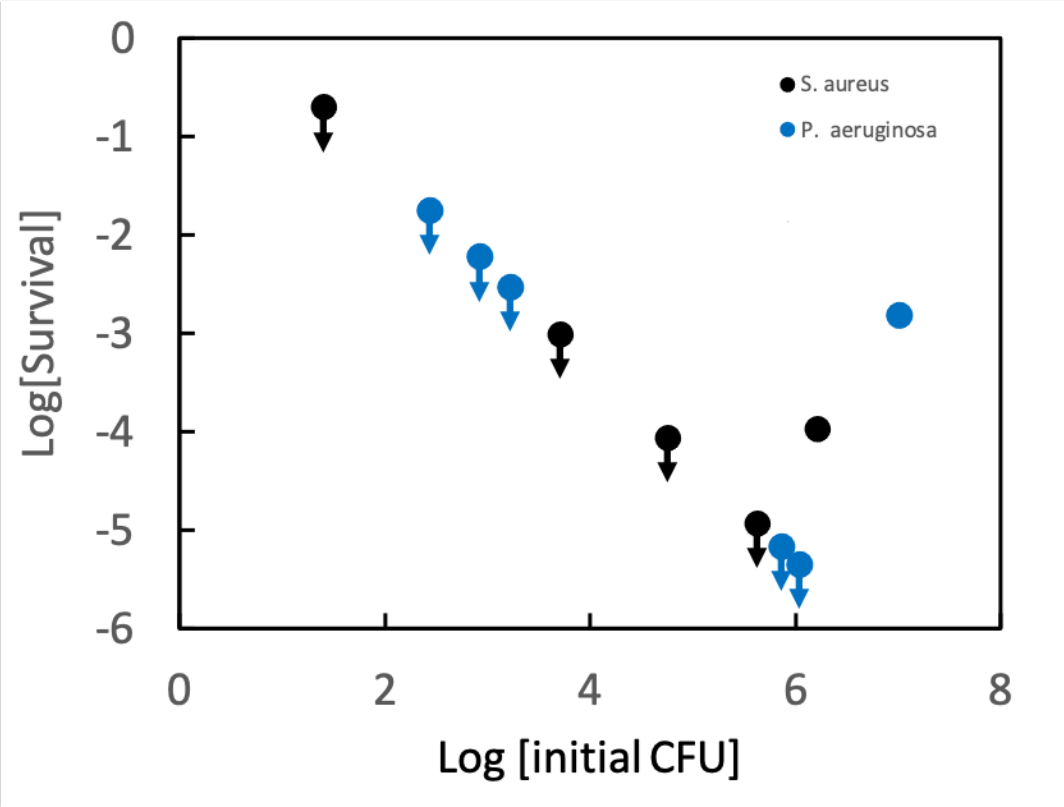

Figure S3. Effect of initial bacterial concentration on survival of *S. aureus* or *P. Aeruginosa* on the Cu_2O/PU coating after 60 minutes. The horizontal axis shows the measured CFU from a series dilution of bacteria, starting at 1.9 x 10^6 and diluted by 1/10 for each data point going from right to left. The vertical axis shows the survival, measured by CFU count of the bacteria on the coating. Fewer than 5 bacteria were found for all but the most concentrated bacterial suspension, so the data shows an upper bound as indicated by an arrow. Many upper bounds fall on a line because the dilution changes by a factor of 10 while the resolution of colonies remains constant. Results are the same if the bacteria are on the coating for 180 min., except that for *P. Aeruginosa* in the most concentrated suspension, no cells survived.

2.5. Cu_2O/PU Coating Kills Bacteria Rapidly

The EPA standard period for 3 logs (99.9%) killing of bacteria is1 hr [1]. We tested our Cu_2O/PU coating at shorter timepoints, and found that it kills bacteria very rapidly (See the Table below).

As a reminder, relative survival compares the survival on the coated and uncoated samples:

$$\%\ \text{relative survival} = \frac{\text{mean}(\text{CFU}_{\text{coated}})}{\text{mean}(\text{CFU}_{\text{uncoated}})} \times 100\%$$

Bacterium	Time* (min)	Repeat	Relative survival (%)	Average Relative survival (%)
Pseudomonas aeruginosa	0	1	7.92	7.6
		2	7.37	
		3	7.42	
	5	1	0.07	0.04
		2	0.01	
		3	0.06	
	10	1	<0.01	0.20
		2	0.52	
		3	0.07	
MRSA	0	1	31.87	29
		2	29.94	
		3	26.16	
	5	1	20.11	17
		2	14.60	
		3	15.08	
	10	1	17.27	14
		2	8.57	
		3	16.23	

Table S8. Relative Survival of bacteria on Cu_2O/PU coating. Within 10 minutes, relative survival of *P. aeruginosa* and *MRSA* are <0.2% and 14%, respectively.

2.6. Comparison of killing of *S. aureus* and Methicillin-resistant *S. aureus* by Sintered CuO Coating at 0 Time Point

In our survey of multiple organisms, there was a large, unexpected, difference in results for the two *S. aureus* strains on CuO at zero time. We followed initial experiments with further experiments to examine the null hypothesis that these the results for the two strains at this specific condition were the same. The results for the repeat test are shown in Table S9, and the result for the Student's t-test was $p = 0.77$, so we were unable to reject the hypothesis that the two stains have the same killing rates.

Strain	Time (min)	Repeat	Relative survival (%)
Non-resistant	0	1	118.3
		2	62.7
		3	142.5
		average	108
Resistant	0	1	118.6
		2	167.2
		3	71.0
		average	119

Table S9. Zero-time survival of *S. aureus* by Sintered CuO for two strains.

2.7. Survival of bacteria on uncoated stainless-steel control

Bacteria can die on their own on uncoated surfaces at a slow rate. Table S10 reports the data for survival of bacteria on the uncoated stainless steel control.

Bacterium	Time* (min)	Average (Colony-Forming Units/Plate)
Pseudomonas aeruginosa	suspension	547
	0	669
	5	631
	10	658
	suspension	878
	0	594
	60	686
MRSA	suspension	287
	0	244
	5	269
	10	271
	suspension	1075
	0	1196
	60	870

Table S10. Survival of bacteria on uncoated stainless-steel control

References:

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