Appl Microbiol Biotechnol (2010) 87:1875–1879
DOI 10.1007/s00253-010-2640-1

Survival of bacteria on metallic copper surfaces in a hospital trial

André Mikolay · Susanne Huggett · Ladji Tikana · Gregor Grass · Jörg Braun · Dietrich H. Nies

Received: 12 March 2010 / Revised: 19 April 2010 / Accepted: 19 April 2010 / Published online: 7 May 2010
© Springer-Verlag 2010

Abstract Basic chemistry of copper is responsible for its Janus-faced feature: on one hand, copper is an essential trace element required to interact efficiently with molecular oxygen. On the other hand, interaction with reactive oxygen species in undesired Fenton-like reactions leads to the production of hydroxyl radicals, which rapidly damage cellular macromolecules. Moreover, copper cations strongly bind to thiol compounds disturbing redox-homeostasis and may also remove cations of other transition metals from their native binding sites in enzymes. Nature has learned during evolution to deal with the dangerous yet important copper cations. Bacterial cells use different efflux systems to detoxify the metal from the cytoplasm or periplasm. Despite this ability, bacteria are rapidly killed on dry metallic copper surfaces. The mode of killing likely involves copper cations being released from the metallic copper and reactive oxygen species. With all this knowledge about the interaction of copper and its cations with cellular macromolecules in mind, experiments were moved to the next level, and the antimicrobial properties of copper-containing alloys in an "everyday" hospital setting were investigated. The alloys tested decreased the number of colony-forming units on metallic copper-containing surfaces by one third compared to control aluminum or plastic surfaces. Moreover, after disinfection, repopulation of the surfaces was delayed on copper alloys. This study bridges a gap between basic research concerning cellular copper homeostasis and application of this knowledge. It demonstrates that the use of copper-containing alloys may limit the spread of multiple drug-resistant bacteria in hospitals.

Keywords Copper · Copper surfaces · Ciprofloxacin-resistant *Staphylococcus* (CRS) · Methicillin-resistant *Staphylococcus aureus* (MRSA)

A. Mikolay · D. H. Nies (✉)
Institut für Mikrobiologie,
Martin-Luther-Universität Halle-Wittenberg,
Kurt-Mothes-Str. 3,
06099 Halle, Germany
e-mail: d.nies@mikrobiologie.uni-halle.de
URL: http://bionomie.mikrobiologie.uni-halle.de

S. Huggett
MEDILYS Laborgesellschaft mbH,
Paul-Ehrlich-Str. 1,
22763 Hamburg, Germany

L. Tikana
German Copper Institute,
Am Bonneshof 5,
40474 Düsseldorf, Germany

G. Grass
School of Biological Sciences, University of Nebraska-Lincoln,
E141 Beadle Center,
Lincoln, NE 68588-0666, USA

J. Braun
Asklepios Klinik Wandsbek,
Alphonsstr. 14,
22043 Hamburg, Germany

Introduction: chemical reasons for biological use of copper ions

Copper is element number 29 of the periodic system with the theoretical electronic configuration of its 29 electrons $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^9$ (or Ar $4s^2\ 3d^9$). However, the negatively charged electrons in completely filled orbitals reside much closer to the positively charged atomic nucleus than their counterparts in incompletely filled orbitals. Thus, completely filled orbitals represent a state of low energy, and the actual electronic configuration of the copper atom is

Ar $4s^1$ $3d^{10}$, featuring a completely filled 3d orbital (Housecroft and Constable 2006).

This simple fact of the basic chemistry of copper has a variety of important chemical and biochemical consequences. (1) Copper cations are stable in the Cu(I) (Ar $4s^0$ $3d^{10}$) and the Cu(II) (Ar $4s^0$ $3d^9$) oxidation states in water, an exception among the transition metals of the first transition period. (2) The standard redox potential of the Cu(I)/Cu(II) couple at pH 7 and 25 °C is E_o'=−261 mV (Weast 1984). Since life depends on water, organismal redox biochemistry is mainly limited to a range between the redox potentials of the water constituents molecular hydrogen (E_o'=−414 mV) and molecular oxygen (E_o'=+815 mV). The Cu(I)/Cu(II) couple is among those metals with a standard redox potential falling within this range, in contrast, e.g., to Na, Mg, Ca, K, free Ni, or free Co. (3) Compared to Mn, copper-involving redox reactions are one-electron transfers. (4) Single Fe-dependent redox reactions are also one-electron reactions, but the Fe(II)/Fe(III) couple has a much higher standard redox potential (E_o'=+357 mV) than that of the copper couple. Therefore, electron transfer from Cu(I) to molecular oxygen is thermodynamically twice as favorable as that from Fe(II) (−104 versus −44.2 kJ/mol, respectively), making Cu(I) and exceptionally good redox reaction partner for O_2. These four items (1 to 4) determine the Janus-faced nature of copper cations: on one hand, copper is essential for a variety of biochemical reactions involving O_2 such as terminal oxidases or Cu/Zn superoxide dismutases. On the other hand, unbound copper cations participate in dangerous Fenton-like reaction (Haber and Weiss 1932), which lead to rapid oxidative damage of any cellular macromolecule by the production of the hydroxyl radical $OH^{\cdot}$.

(5) Similar to other transition metals, copper cations are able to form stable complex compounds. (6) The atomic radius of Cu^+ is close to that of Na^+ (960 and 950 pm, respectively) and that of Cu^{2+} close to that of Mg^{2+} (690 and 650 pm, respectively, (Weast 1984)). Therefore and due to higher similarity in the orbital sizes, Cu^{2+} cations form complexes with the non-metals N and O of the second period more easily than do other divalent cations of the first transition period. Conversely, Cu^+ is much larger than these cations and binds S as first shell complex ligand with ease. This explains a higher affinity of copper cations to first shell ligands of periods 2 and 3 compared to other metals of the first transition period. This property can be easily visualized when the solubility products of metals hydroxides are plotted against those of the metal sulfides (Nies 2007), and this is exactly the reason for the famous Irving–Williams series (Irving and Williams 1948): copper cations are able to remove most other transition metal cations from their complexes. Thus, items 5 and 6 determine yet another feature of copper: copper cations bind easily to thiol groups, e.g., of proteins, and consequently may remove other transition metals from their respective complexes.

Many bacteria use copper, at least in the periplasm if they possess a Gram-negative cell wall, and free-living bacteria are also able to handle the toxicity of copper cations up to a certain level (Dressler et al. 1991). Superfluous cytoplasmic and periplasmic copper cations are removed by efflux, bound to compounds such as glutathione or Cu^+, and are oxidized to the less harmful Cu^{2+} (Magnani and Solioz 2007). Nevertheless, bacteria were rapidly killed on dry metallic copper surfaces, and the killing rate increases with the copper content of the surface (Rogers et al. 1994; Noyce et al. 2006). In experiments with *Escherichia coli* (Santo et al. 2008), the cell's copper tolerance factors were able to decrease the killing rate but were not able to prevent inactivation of the cells. Copper chelating factors protected the cells, too. Killing of bacteria on metallic copper surfaces was therefore likely due to release of copper cations from the solid material. Factors that diminished reactive oxygen species also decreased the killing rate. Thus, the hydroxyl radical production by Fenton-like reactions contributed to inactivation. Anaerobic conditions decreased the killing rate but only by half. Likely, *E. coli* was inactivated by the highly toxic Cu(I) oxidation state under anaerobic conditions and by Cu(I)/Cu(II)/Fenton-like reaction under aerobic conditions, placing the bacterium between a rock and a hard place.

Bacterial contamination of touch surfaces especially in hospitals poses a serious threat. With all the knowledge about cellular copper homeostasis in mind, we demonstrated in this study that it was possible to inactivate bacteria in a "real life" setting, i.e., in a hospital environment. From these results, we anticipate that use of bactericidal surface materials made from metallic copper or its alloys constitute a way to support the use of antibiotics, disinfectants, and hand washing, thus minimizing the risk of emergence and spread of multiresistant germs in a place where bacterial contaminations of touch surfaces poses a serious threat.

Materials and methods

Experimental setting In a hospital in Hamburg, Germany (Asklepios Hospital Wandsbek), touch surfaces (patient bed rooms, rest rooms, and staff rooms) in an oncological/pneumological and a geriatric ward were exchanged for new surfaces composed of metallic copper-containing alloys. Pure copper was not used due to the rapid coloring and because the material could not as easily used to produce the desired items as the alloys. No alterations were made in control rooms. Aluminum surfaces on 48 push plates and 48 doorknobs were replaced by a CuZn21Si3 alloy, plastic on 48 light switches by a CuZn23Al3Co

Table 1 Colony-forming units on metallic copper surfaces compared to aluminum or plastic material

Surface	Metallic copper (%)	Control (%)	Degrees of freedom	Result *t* test
Total	66.0±13.3	100±18.8	30	>99.9%
	59.9±15.4	100±13.0	30	>99.9%
Door knobs	60.3±15.8	100±25.1	30	>99.9%
	60.3±15.8	100±16.3	30	>99.9%
Push plates	103±74	100±78	30	Random
	92.5±46.3	100±51.5	30	60%
Light switches	76.8±34	100±25.8	28	97.5%
	87.4±45.7	100±61.1	30	70%

For 16 weeks during summer (June to August 2008, first row in each cell) or winter (November 2008 to January 2009, second row in each cell), the number of aerobe, heterotrophic colony-forming units was determined once or twice per week on aluminum doorknobs and push plates, plastic light switches, and similar touch surfaces or metallic copper surface derivatives, respectively. The percentage of cfu/mm^2 on copper surfaces compared to those on standard surfaces is indicated

alloy. These materials were routinely cleaned each morning with surface disinfectant Incidin® PLUS (5 mL/L, active substance glucoprotamin, ECOLAB, Düsseldorf, Germany).

Probing During 16 weeks in the summer (summer probing event, June to August 2008) and 16 weeks in the winter (winter probing event, November 2008 to January 2009) of the year 2008/2009, the number of aerobic, heterotrophic colony-forming units (cfu) on these surfaces was determined once or twice per week (Table 1). Probing was done with a flexible Petrifilm™ for determination of aerobic cfu (3 M, Neuss, Germany) through direct surface contact by stamping. Before probing, 1 mL of sterile distilled water was added to the petrifilms, and they were incubated for 16 h at 4 °C to allow re-swelling of the petrifilm surface. Following probing, the number of cfu was counted after 36 h of incubation at 30 °C.

The colonies on the petrifilms were also replica plated onto a selective nutrient agar for determination of ciprofloxacin-resistant *Staphylococcus* (CRS, 8 mg/L ciprofloxacin, Baird-Parker agar, BD Biosciences, Franklin Lakes, NJ, USA), as a marker for the presence of resistant nosocomial microorganisms.

Results

During both test periods of 16 weeks (summer probing event, June to August 2008, and winter probing event, November 2008 to January 2009), the total number of cfu on metallic copper surfaces was 63% of that on control surfaces. The difference was highly significant as determined by *t* test and resulted from a highly significant difference in the cfu numbers on the doorknobs. In contrast, there was nearly no difference in the cfu numbers on the push plates, and the result concerning the light switches was in-between those on doorknobs and on push plates (Table 1). However, the total number of cfu counted on doorknobs (19,521 on copper, 26,999 on aluminum) was much higher than that on push plates (3,641 copper, 3,687 aluminum) or light switches (4,305 copper, 4,563 plastic). This may indicate that doorknobs were much more

Table 2 Colony-forming units of ciprofloxacin-resistant *Staphylococcus* (CRS) on metallic copper surfaces in a hospital compared to aluminum or plastic material

Surface	Metallic copper (%)	Control (%)	Degrees of freedom	Result *t* test
Total	77.7±15.5	100±18.1	8	99.5%
	59.9±15.4	100±13.0	30	>99.9%
CRS total	78.4±87.6	100±50.3	8	70%
	76.5±59.7	100±62.1	30	80%
%CRS	2.02±1.88	2.08±0.98	8	Random
	5.59±4.62	4.40±3.23	30	70%

The number of CRS was determined on Baird-Parker agar containing 8 mg/L ciprofloxacin. First row of each cell indicate the summer term, the second row, the winter term of the probing event. The percentage of cfu/mm^2 on copper surfaces compared to those on standard surfaces is indicated

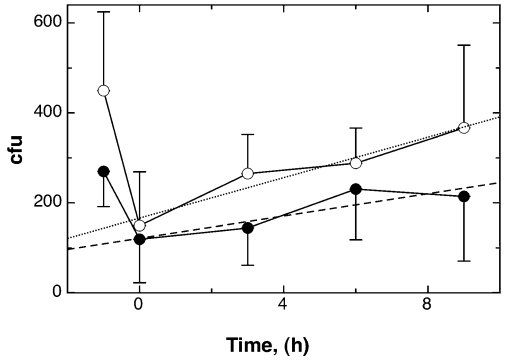

Fig. 1 Repopulation of touch surfaces after cleaning. The total number of aerobe, heterotrophic bacteria determined as colony-forming units (cfu) on metallic copper surfaces (*closed circles*) and on other surfaces (aluminum, plastic, *open circles*) was determined before cleaning (t=0), directly after cleaning and 3, 6, and 9 h later. Result of the summer probing event. Mean values and deviation bars of the total cfu of 270 samples per material and time point are shown. The mean values were significant with a t test result of >99.9% (before cleaning), 70% (after cleaning), >99.9% (3 h), 90% (6 h), and 99% (9 h). The increase in mean cfu after cleaning was 12.4 cfu/h on metallic copper and 22.5 cfu/h on other surfaces

frequently touched and contaminated with bacteria than the other two surfaces. The differences in cfu of CRS from metallic copper and other surfaces were not statistically significant but merely a trend (Table 2).

For bacterial inactivation time courses, all control standard and metallic copper touch surfaces were disinfected once a day. The total number of cfu was also determined directly before and after cleaning, and 3, 6, and 9 h later (Fig. 1). This was done at each of the 16 probing events in the summer and winter term, respectively. Prior to cleaning, the total number of cfu was significantly different on metallic copper surfaces compared to the control (Fig. 1). Cleaning resulted in a decrease of cfu on either surface, and the numbers were no longer significantly different. In the hours after cleaning, the surfaces were repopulated at a different rate, 12.4 cfu/h for the metallic copper surfaces (summer probing event, winter probing event 14.2 cfu/h, data not shown) and 22.5 cfu/h (winter 33.0 cfu/h) on the other surfaces. This clearly suggests that metallic copper efficiently decreased the repopulation of touch surfaces by aerobe, heterotrophic bacteria.

Discussion

The presented data clearly indicate that use of copper alloys for touch surfaces decreases the number of living bacterial cells adhering to these surfaces. Further, these surfaces probably decreased the repopulation rate of these germs. The antibacterial effect on CRS was not significant and was therefore not followed up by more detailed characterization of the bacteria involved, e.g., methicillin-resistant *Staphylococcus aureus*. The percentage of CRS cfu was not different between copper and standard surfaces; however, the total number of cfu was different, providing indirect evidence that the number of CRS cfu may also have been decreased on copper compared to other surfaces.

Silver cations are more toxic to bacteria than copper cations (Nies 1999). Since silver cations bind even more strongly to thiols than copper cations (Nies 2007), it may also be speculated that silver surfaces may be also suitable to limit the spread of bacteria in a hospital or elsewhere. However, silver is not stable in the Ag(II) oxidation form and should not catalyze a Fenton-type reaction. In our hands, silver surfaces did not kill bacteria (Nies and Nies, unpublished observations). In line with this observation, copper alloys indeed were much more efficient as antimicrobial materials than silver-containing materials at temperature and humidity levels typical for indoor environments such as hospitals (Michels et al. 2009).

In our hospital experiment, the cfu number of heterotrophic aerobe bacteria was merely decreased by one third on copper surfaces compared to the control in the hospital environment. In contrast, *E. coli* was killed within minutes under laboratory test conditions on dry copper surfaces (Santo et al. 2008). *Pseudomonas aeruginosa* strain PAO1 was also rapidly killed on different copper alloys (Elguindi et al. 2009). Killing was also influenced by temperature. Reminiscent to the situation on *E. coli*, genes conferring resistance to copper cations also influenced survival on copper alloys (Elguindi et al. 2009). This indicates again that copper cations released from the metallic copper surface were responsible for the "killing" process.

In another study performed in parallel to our experiments, a set of tap handles and a ward entrance door push plate each containing copper were sampled for the presence of bacteria and compared to non-copper-containing items on the same ward. In this experiment, the decrease in the median number of microorganisms surviving on copper surfaces was above 90% and thus even higher than in our experiment (Casey et al. 2010).

In our study, the surfaces were cleaned with a solution containing glucoprotamin as an active substance since Cu^{2+} works synergistically with quaternary ammonium compounds to eradicate biofilms of *E. coli*, *S. aureus*, *Salmonella enterica*, and *Pseudomonas fluorescens* (Harrison et al. 2008). In contrast, the British study used sodium dichloroisocyanurate with 1,000 ppm available chlorine and detergent for the same purpose (Casey et al. 2010). Instead of enhancing killing, the glucoprotamine may have generated a thin layer between the metallic copper surface and the bacteria, increasing copper endurance of the bacteria. Survival of bacteria on copper-containing coins, which are

routinely contaminated with grease from human skin during handling, has been shown (Santo et al. 2010). It might therefore be only a matter of appropriate cleaning procedures that have to be optimized to develop copper alloys as useful additional tool to decrease the spread of multiple drug-resistant bacteria in hospitals, in addition to standard hygiene procedures already established and in practice.

Acknowledgments We thank the German Copper Institute for financial support. We thank Ayla Nies for performing the silver experiment.

References

Casey AL, Adams D, Karpanen TJ, Lambert PA, Cookson BD, Nightingale P, Miruszenko L, Shillam R, Christian P, Elliott TSJ (2010) Role of copper in reducing hospital environment contamination. J Hosp Inf 74:72–77

Dressler C, Kües U, Nies DH, Friedrich B (1991) Determinants encoding multiple metal resistance in newly isolated copper-resistant bacteria. Appl Environ Microbiol 57:3079–3085

Elguindi J, Wagner J, Rensing C (2009) Genes involved in copper resistance influence survival of *Pseudomonas aeruginosa* on copper surfaces. J Appl Microbiol 106:1448–1455. doi:10.1111/j.1365-2672.2009.04148.x

Haber F, Weiss J (1932) Über die Katalyse des Hydroperoxydes. Naturwissenschaften 20:948–950. doi:10.1007/BF0150471

Harrison JJ, Turner RJ, Joo DA, Stan MA, Chan CS, Allan ND, Vrionis HA, Olson ME, Ceri H (2008) Copper and quaternary ammonium cations exert synergistic bactericidal and antibiofilm activity against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 52:2870–2881

Housecroft CE, Constable EC (2006) Chemistry (3rd edn). Pearson Education Limited, Essex, England

Irving H, Williams RJP (1948) Order of stability of metal complexes. Nature 162:746–747

Magnani D, Solioz M (2007) How bacteria handle copper. In: Nies DH, Silver S (eds) Molecular microbiology of heavy metals. Springer-Verlag, Berlin, pp 259–285

Michels HT, Noyce JO, Keevil CW (2009) Effects of temperature and humidity on the efficacy of methicillin-resistant *Staphylococcus aureus* challenged antimicrobial materials containing silver and copper. Lett Appl Microbiol 49:191–195. doi:10.1111/j.1472-765X.2009.02637.x

Nies DH (1999) Microbial heavy metal resistance. Appl Microbiol Biotechnol 51:730–750

Nies DH (2007) Bacterial transition metal homeostasis. In: Nies DH, Silver S (eds) Molecular microbiology of heavy metals. Springer-Verlag, Berlin, pp 118–142

Noyce JO, Michels H, Keevil CW (2006) Use of copper cast alloys to control *Escherichia coli* O157 cross-contamination during food processing. Appl Environ Microbiol 72:4239–4244

Rogers J, Dowsett AB, Dennis PJ, Lee JV, Keevil CW (1994) Influence of temperature and plumbing material selection on biofilm formation and growth of *Legionella pneumophila* in a model potable water system containing complex microbial flora. Appl Environ Microbiol 60:1585–1592

Santo CE, Taudte N, Nies DH, Grass G (2008) Contribution of copper ion resistance to survival of *Escherichia coli* on metallic copper surfaces. Appl Environ Microbiol 74:977–986

Santo CE, Morais PV, Grass G (2010) Isolation and characterization of bacteria resistant to metallic copper surfaces. Appl Environ Microbiol 76:1341–1348. doi:10.1128/aem.01952-09

Weast RC (1984) CRC handbook of chemistry and physics, 64th edn. CRC Press, Inc., Boca Raton