Physicochemical properties of copper important for its antibacterial activity and development of a unified model

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Contact killing is a novel term describing the killing of bacteria when they come in contact with metallic copper or copper-containing alloys. In recent years, the mechanism of contact killing has received much attention and many mechanistic details are available. The authors here review some of these mechanistic aspects with a focus on the critical physicochemical properties of copper which make it antibacterial. Known mechanisms of contact killing are set in context to ionic, corrosive, and physical properties of copper. The analysis reveals that the oxidation behavior of copper, paired with the solubility properties of copper oxides, are the key factors which make metallic copper antibacterial. The concept advanced here explains the unique position of copper as an antibacterial metal. Based on our model, novel design criteria for metallic antibacterial materials may be derived. © *2015 American Vacuum Society*. [http://dx.doi.org/10.1116/1.4935853]

I. INTRODUCTION

According to the latest Surveillance Report of the European Centre for Disease Prevention and Control and the WHO report on Patient Safety, on any given day, 80 000 patients in European hospitals have a hospital acquired infection and an estimated 100 deaths per day result from these infections.[1,2] In healthcare facilities, infectious bacteria can be transmitted via contact surfaces such as door handles, bed rails, armrests of chairs, bathroom fixtures, etc. It has been shown that many bacteria, including the dangerous methicillin resistant *Staphylococcus aureus*, can survive for months on plastic, stainless steel, and similar surfaces and constitute a source of infection.[3,4] Obviously, extensive cleaning and disinfection protocols are in place in all modern health care facilities. However, this does not provide full protection. Recontamination will occur between cleaning cycles, and disinfection can be flawed by human error or negligence. In addition, many common items like keyboards, computer mice, cell phones, and pens, are often not disinfected or not on a regular basis and can therefore be a significant source of bacterial contamination.[5] This calls for complementary strategies.

One such strategy is the use of materials which possess an intrinsic antibacterial activity. In this context, copper and copper alloys, due to their excellent, intrinsic antibacterial properties, have emerged as a *bona fide* solution to the problem. The antibacterial activity of copper is maintained in alloys as long as they contain 60% or more copper.[6] Classical copper-containing alloys are brass and bronze and have been used for centuries. Metallic silver surfaces exhibit only very poor antibacterial activity,[7] but silver or silver oxide in the form of nanoparticles efficiently kills bacteria, which is also true for copper or copper oxide nanoparticles. Here, nanoparticles will not be discussed in depth. The large surface to volume ratio of nanoparticles invokes very different physicochemical properties and near-surface phenomena. Additionally, nanoparticle–bacterial interactions may play an important role in the killing mechanism, which may thus not be comparable to that of solid, metallic surfaces (see Franci *et al*. for a recent review on nanoparticles[8]).

II. BRIEF HISTORY OF ANTIBACTERIAL COPPER

The use of copper as an antibacterial material can be dated back several millennia. Around 2400 BC, copper powder was used for wound disinfection in ancient Egypt.[9] Copper preparations continued to be used throughout the millennia until penicillin became widely available to the public in 1945. Anew interest in copper as an antibacterial agent was sparked by a 1983 study in a hospital, showing that viable *Escherichia coli* were present on stainless steel door knobs, while they were absent on brass door knobs.[10] Many studies on the antibacterial properties of copper have since been conducted, culminating in the registration of copper and copper alloys as antibacterial agents ("pesticides") with the U.S. Environmental Protection Agency in 2008.[6]

In the light of increasing hospital-acquired infections, this led to a number of studies employing copper for tables, armrests, IV poles, etc., in hospital wards in different

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countries and which show very promising results.[11–16] Such approaches are not only of interest in healthcare settings, but also in food-processing facilities.[17,18] Finally, copper finds increasing use in the design of antibacterial textiles.[19] While these efforts continue, new copper alloys have appeared on the market to meet modern design criteria in terms of color and material properties. Today, many fittings and objects made of copper or copper alloys are on the market, including door knobs, push plates, bathroom fixtures, toilet seats, copper pens, light switches, keyboards, etc. (Fig. 1). Also, antimicrobially functionalized materials have emerged, such as plastics doped with copper or silver, or fabrics impregnated with copper oxide. This has extended the scientific view from a purely microbiological approach to an interdisciplinary task, including materials science aspects.

III. HOW BACTERIA ARE KILLED ON COPPER SURFACES

Contact killing by copper has been shown for at least 90 bacterial species, 30 types of fungi, and 20 different viruses, and it can safely be assumed that all bacteria and viruses are sensitive to contact killing.[6,20] Various possible mechanisms for contact killing by copper have been published.[6,21] Four toxicity mechanisms which participate in contact killing have been identified: (1) damage of the outer and/or inner bacterial membrane, (2) oxidative damage by reactive oxygen species (ROS), (3) inhibition of essential enzymes, and (4) degradation of deoxyribo nucleic acid (DNA). It appears that there is variation as to the key toxic principle, depending on the experimental system and the organism under investigation.

Release of ionic copper from the surface is clearly a key event in contact killing.[22] The second mechanistically important aspect is bacteria–metal contact.[23] These events are believed to induce severe damage of the outer and/or inner bacterial membrane as a key event in contact killing. Damage of the cell envelope has been documented for several organisms and by different techniques such as direct microscopic examination of cells, staining of cells with redox dyes, staining of respiring cells, or by showing membrane depolarization.[24–29] Figure 2 shows extensive structural damage inflicted on *Enterococcus hirae* after prolonged exposure to a copper surface in experiments conducted in our laboratories. A technique called "Live/Dead" staining has also been frequently used. It combines the green fluorescent SYTO9 DNA dye, which is membrane permeable and stains all cells, with red fluorescent propidium iodide, entering only damaged cells and staining cellular DNA with higher affinity than SYTO9. This changes the fluorescence of dying and dead cells from green to red.[28,30] Damage of the cell envelope of *E. coli* by metallic copper is also supported by proteomic profiling: proteins involved in cell envelope synthesis and capsule polysaccharide biogenesis are upregulated upon contact with copper.[31]

Following membrane damage, there is massive influx of copper ions into the bacterial cytoplasm.[25,29] Using a cytoplasmic copper sensor, Espírito Santo *et al.* measured the cellular copper content in *Staphylococcus* to be 2.6×10^{10} copper ions per cell 5 min after contact with a dry copper surface.[32] In line with this, it has been observed that copper chelators such as bicinchoninic acid, bathocuproine disulfonate, or ethylenediamine tetra acetic acid significantly inhibit or even prevent contact killing.[28,33] Also, *E. coli* cells unable to synthesize glutathione, which constitutes a major defense against toxic heavy metal ions, were more sensitive to contact killing than wild-type cells.[34] Of special interest is the recent observation that Cu^{+} is considerably more toxic to bacteria than Cu^{2+}, an aspect that directly bears on copper's antibacterial activity (see below).[35]

Clearly, the main toxicity mechanisms of copper and copper alloys is the release of copper ions into the aqueous

FIG. 1. Copper items available on the market. For a listing of suppliers, see http://www.antimicrobialcopper.com/uk/find-products-and-services/find-antimicrobial-copper-products.aspx.

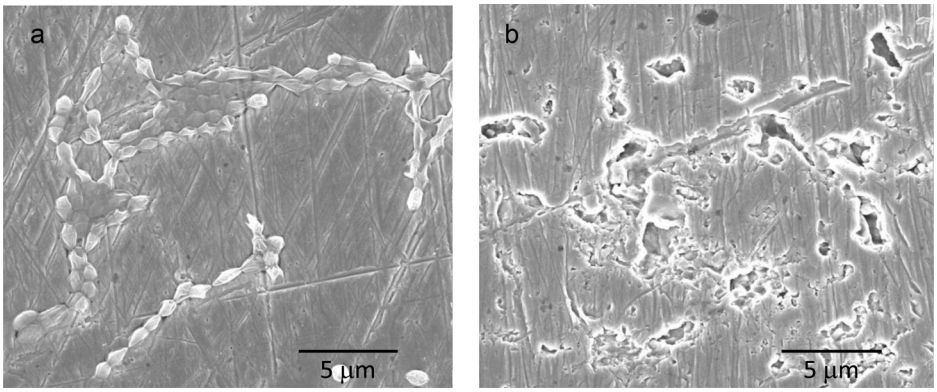

FIG. 2. Structural damage inflicted on *E. hirae* on a copper surface. (a) *E. hirae* immediately after contact with a metallic copper surface. Note how the cells tend to snug into groves on the copper surface. (b) Structural damage to *E. hirae* by the copper surface after 1 h of exposure. The pictures show scanning electron micrographs of unstained, air-dried cells.

phase; even in so-called "dry" applications of bacteria to copper surfaces, there will be an aqueous phase surrounding the bacteria. In line with this, surface roughness also influences contact killing: rough surfaces release more copper ions per time and are more antibacterial than polished surfaces.[36] But bacteria–metal contact also appears to play a role in contact killing. It was found that the killing of *E. hirae* on copper surfaces was drastically reduced when bacteria–metal contact was prevented by an inert grid, even though copper ion release remained unchanged.[23] This shows that contact killing by copper surfaces is not a simple phenomenon of copper ion toxicity to bacteria, but is influenced by bacteria–metal contact, corrosion phenomena, surface structure, nobility of alloying metals, and last, but not least, the bacterial species under investigation.

In the presence of oxygen, copper ions can cycle between the Cu^+ and Cu^{2+} oxidation state (1)

$$Cu^+ \rightarrow Cu^{2+} + e^- (E^\circ = -0.16\,V). \quad (1)$$

This reactivity can lead to the generation of ROS by a Fenton-type reaction (2)[37]

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^- + OH^\bullet. \quad (2)$$

Combined with the Haber–Weiss cycle [(3) and (4)], this can provide a rich source of ROS, particularly in lactic acid bacteria, which can produce large amounts of hydrogen peroxide[38,39]

$$H_2O_2 + OH^\bullet \rightarrow H_2O + O_2^- + H^+, \quad (3)$$

$$H_2O_2 + O_2^- \rightarrow O_2 + OH^- + OH^\bullet. \quad (4)$$

Reaction (4) by itself has a negligible rate constant, but is catalyzed by Cu(II) or Fe(III) complexes. ROS are well known to cause irreversible cell damage by a variety of mechanisms, such as by inhibition of respiration, lipid peroxidation, and oxidative damage of proteins.[31] ROS production was demonstrated for *E. coli* and *Salmonella* exposed to solid copper.[40,41] However, to what extent ROS production is important for contact killing remains an issue of debate. On one hand, ROS quenchers were found to inhibit contact killing to some extent, on the other hand, killing is apparently not impaired by anaerobic conditions which disfavor ROS production.[33,42]

With regard to copper toxicity mechanisms in bacteria, a distinction has to be made between cells in culture and cells exposed to metallic copper. In culture, cells are in a growing state while during exposure to copper surfaces, they are in a non-growing state. When studying copper toxicity in culture, only relatively low amounts of copper enter the cell. Under these conditions, the toxic effect in *E. coli* was shown to be the displacement of iron from [4Fe-4S] clusters of essential enzymes, rather than ROS production and oxidative damage.[43–46] Attack of [4Fe-4S] clusters was also demonstrated for Ag(I), Hg(II), Cd(II), and Zn(II) at concentrations which only minimally inhibited cell growth.[47] In line with this toxicity mechanism, the toxicity of metal ions was directly related to their thiophilicity (see below). Different principles obviously underlie the inactivation of viruses, where capsid and nucleic acid damage by copper have been demonstrated.[48–50] This will not be further considered here.

Bacteria which come into contact with a copper surface under contact killing conditions are starved for nutrients and are killed in the absence of growth. The most likely scenario of contact killing is thus the following: as a primary event, there is severe damage to the cell envelope and the cytoplasmic membrane; this is followed by massive influx of copper ions into the cell and damage of intracellular components, including DNA, by multiple mechanisms. The importance of each toxicity mechanism and the sequence of events seems to vary between species and between eukaryotes, prokaryotes, and viruses. For cells, it emerges that copper toxicity in contact killing is mechanistically different from the toxicity in growing cultures. The situation is also distinct from biofilms, which form on surfaces over extended periods of time and require growth and attachment of the cells to the substrate.

IV. THIOPHILICITY AND TOXICITY

In the "hard and soft acid and base" (HSAB) concept of Pearson, ions are classified as "hard" if they have a high

TABLE I. Selected properties of metals ordered by oxide solubility.

	Ionic properties		Corrosion behavior	
Element/ion	HSAB[a]	$pK_{S(MeS)}$[b]	E_0 (V)[c]	$pK_{S[MeO/Me(OH)]}$[d]
Ag(I)	S	50.2	−0.79	7.7
Cu(I)	S	47.6	−0.52	9.0
Cd(II)	S	26.1	−0.40	13.6
Co(II)	I	20.4	−0.28	14.2
Ni(II)	I	18.5	−0.26	14.7
Fe(II)	I	18.2	−0.41	15.1
Pb(II)	I	27.5	−0.13	15.2
Zn(II)	I	25.7	−0.76	16.4
Cu(II)	I	36.2	−0.34	23.5
Hg(II)	S	52.7	−0.80	25.4
Sn(II)	H	26.0	−0.14	26.2
Al(III)	H	Unstable	−1.66	32.9
Fe(III)	H	Unstable	−0.02	37.4
Au(III)	S	na[e]	1.49	na
Pt(II)	S	26.1	1.18	99.8[f]
Pd(II)	S	21.2	0.95	120.3[f]

Note: na, Value not available.
[a]Hard−soft character according to Pearson (Ref. 51): h, hard; i, intermediate, s, soft.
[b]pK_S-values for the solubility product K_S of the respective metal sulphide [$pK_{S(MeS)}$].
[c]Standard electrochemical potential.
[d]pK_S-values of the metal oxide or metal hydroxide equilibria {$pK_{S[MeO/Me(OH)]}$} (Ref. 52).
[e]The thiophilicity of gold appears to be high (Ref. 53).
[f]Values from Ref. 54.

charge density (high ratio of charge to radius) and are not easily polarized, and as "soft" if they have a low charge density (low ratio of charge to radius) and are therefore easily polarized.[51] Soft ionic species readily form compounds with sulphur, a property also termed "thiophilicity." An attempt was made here to correlate available formation constants for metal sulfides, $pK_{S(MeS)}$, with thiophilicity based on the HSAB concept (Table I). The correlation between hard/soft assignments and $pK_{S(MeS)}$ values does not hold up in all cases. Sn, for example, is classified as hard, but exhibits a relatively high $pK_{S(MeS)}$ of −26. However, it should be noted that widely differing values of $pK_{S(MeS)}$ can be found in the literature and the most recent available values were listed in Table I, assuming that they are the most accurate.

Ag^+, Cu^+, Cd^{2+}, and Hg^{2+}, which are all very toxic to growing bacteria, are assigned a soft character and exhibit the expected low $pK_{S(MeS)}$ values. Similarly, Cu^{2+}, which is classified as "intermediate" on the HSAB scale, has a $pK_{S(MeS)}$ of −36.2, and is less toxic to bacteria than Cu^+ with a $pK_{S(MeS)}$ of −47.6. Au, Pd, and Pt are classified as soft, but these metals are "noble" and do not form solute ions under ambient aqueous conditions, which is reflected by low solubility constants and high electrochemical standard potentials, E_0, of +0.95 to +1.49 V.

Overall, the combined consideration of all these parameters may allow some predictions of toxicity in culture or in contact killing. But other parameters like solubility, uptake by bacteria, and cellular defense mechanisms can play an overriding role, depending on the experimental conditions.

V. CRITICAL PHYSICOCHEMICAL PARAMETERS IN CONTACT KILLING

A major issue to consider is the effect of copper surface oxidation. Under atmospheric conditions, copper oxidation takes place in several steps. The Pourbaix-diagram [Fig. 3(a)] describes the electrochemical corrosion behavior of copper in water from a thermodynamic point of view.[56] It shows how the speciation of copper depends on the reduction potential E_h (positive for oxidizing conditions, negative for reducing conditions) and the *p*H. Under conditions encountered in the biosphere [hatched areas in Fig. 3(a)], both, Cu_2O and CuO, can form. Oxidizing conditions (clean water in air, upper hatched area) favor formation of CuO, while reducing conditions (presence of organic matter, bacteria) favor Cu_2O formation. On the other hand, *p*H values below six and oxidizing conditions favor dissolution of copper oxides to aqueous Cu^{2+}. Thus, copper oxide formation and stability under real-life conditions can change markedly.

On a dry copper surface in ambient air and humidity, a layer of cuprous oxide (Cu_2O) is initially formed. This is followed by a second layer of cupric oxide (CuO).[57–60] The formation of the second CuO layer seems to be favored in a humid atmosphere and during long aging periods. Thus, atmospheric copper oxide layers consist of either Cu_2O or a Cu_2O/CuO double layer, depending on the oxidation time and the conditions. In the presence of chloride ions, for example, the formation of Cu^+, which is more toxic to bacteria than Cu^{2+}, is favored, and the formation of Cu_2O is shifted to more alkaline *p*H [Fig. 3(b)].[61] So, chloride ions from sweat could actually enhance the antibacterial activity of copper, but this remains to be demonstrated. Copper complexing agents such as ammonium, cyanide, or sulfur will alter reaction kinetics even more dramatically, making any prediction very difficult.

Various studies, most of them focusing on nanoparticles or coated textile fibers, demonstrate antibacterial activity for both types of copper oxides.[62–65] For metallic surfaces, the antibacterial activity of Cu_2O versus CuO was recently investigated.[66] It was found that Cu_2O exhibited strong antibacterial properties, whereas CuO showed much slower bacterial killing. Copper ion release from these surfaces correlated with the antibacterial potency. This suggests that the release of copper ions from copper oxides is a key parameter in contact killing.

The solvation of metal oxides is expressed by $pK_{S[MeO/Me(OH)]}$ in Table I, and the metals are ordered by decreasing values. Among the metal oxides for which solubility constants could be found in the literature, AgO and Cu_2O are the most soluble oxides, with pK_S values of −7.7 and −9, respectively. The solubility of CuO, on the other hand, is much lower and in the range of that of other metal oxides.

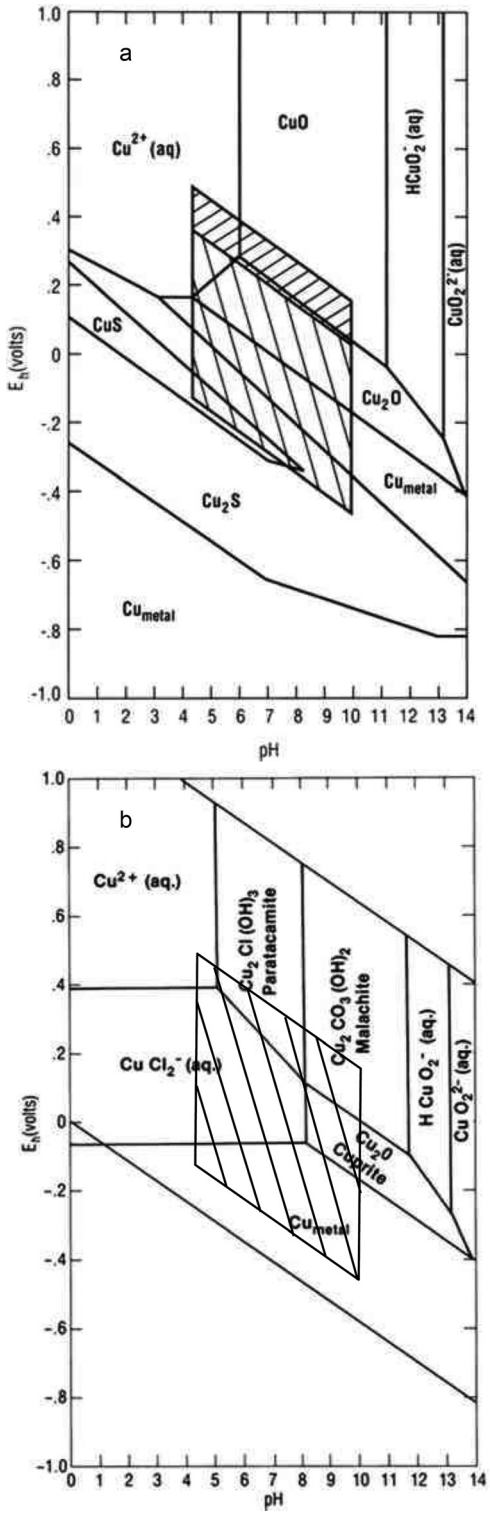

FIG. 3. Pourbaix diagrams for copper. The graphs show the speciation of copper as a function of reduction potential E_h and *p*H. (a) Copper in water without chloride and carbonate at 25 °C. The hatched area delineates conditions encountered in the biosphere (upper part: clean water in air, lower part: water with organic matter). (b) Copper in the presence of 0.3 M Cl^- and carbonate in equilibrium with air. The hatched area delineates conditions encountered in the biosphere and the upper and lower diagonal lines indicates the reduction potential of water saturated with oxygen or hydrogen, respectively, at 1 atm [Reproduced with permission from M. B. McNeil and B. J. Little, J. Am. Inst. Conserv. **31**, 355 (1992)] (Ref. 55).

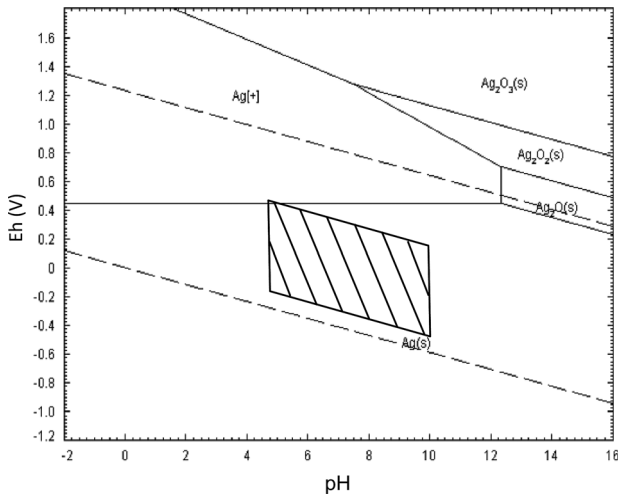

FIG. 4. Pourbaix diagram for silver. The graph shows the speciation of silver as a function of reduction potential E_h and *p*H. The upper and lower dashed lines indicate the reduction potential of water saturated with oxygen or hydrogen, respectively, at 1 atm, and the hatched area delineates conditions encountered in the biosphere.

The higher solubility of Cu_2O compared to CuO correlates with its higher antibacterial activity. By this approach, Cd is predicted to be a metal with good antibacterial activity: it is soft, does not have a noble character, has a high thiophilicity, is toxic to bacteria, and its oxide has a relatively high solubility, based on the $pK_{S[MeO/Me(OH)]}$ value of −13.6. To our knowledge, contact killing by cadmium has never been tested. Of course its toxicity precludes any practical applications, but it would be interesting from an academic point of view to know its contact killing activity.

In comparison to copper, metallic silver surfaces are dramatically less effective in contact killing, and to call them "antibacterial" is not really justified. To observe efficient contact killing by metallic silver, long exposure times at elevated temperature in aqueous environments have to be used, e.g., 24 h at 37 °C, as specified by the Japanese test procedure JIS Z 2801:2000. This procedure was used by Kawakami *et al.*[67] and lead to the misleading conclusion that silver is essentially as active in contact killing as copper. But if short exposures (e.g., 1–2 h) at room temperature are used, silver is essentially ineffective in killing bacteria or viruses.[68–70] Silver is more noble than copper and does not readily oxidize under ambient conditions, as can be seen from its Pourbaix diagram (Fig. 4). Only under conditions which promote the formation of silver oxides (e.g., high *p*H and oxidizing conditions), the antibacterial activity of silver surfaces is induced, presumably because the ensuing AgO has a high solubility, even greater than that of Cu_2O (cf. Table I), and gives off toxic Ag^+ ions.[68] This also explains the excellent antibacterial activity of silver oxide nanoparticles. Taken together, for a solid metal surface to be antibacterial, it must (1) readily oxidize under ambient conditions, and (2) the oxide must be relatively soluble to release toxic metal ions.

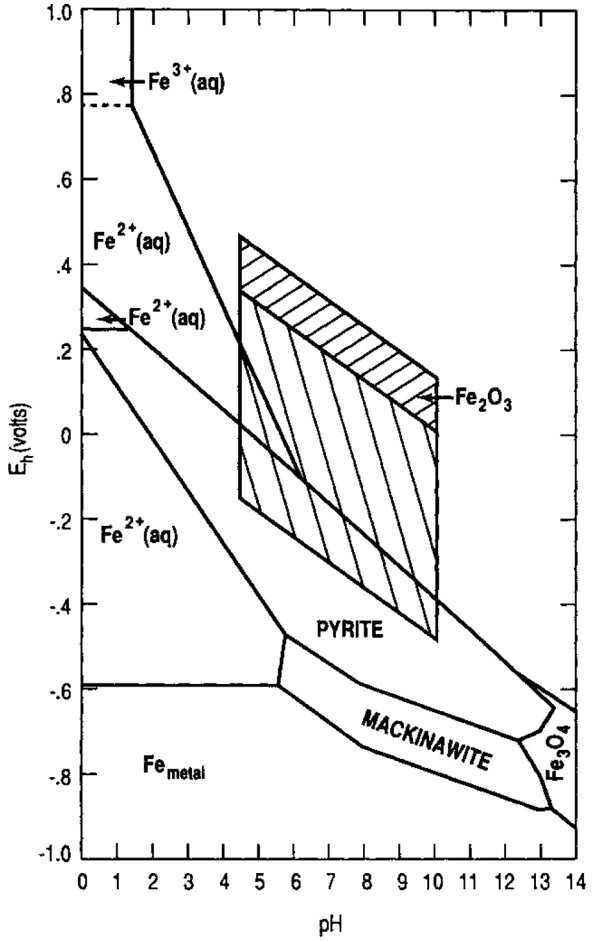

FIG. 5. Pourbaix diagram for iron. The graphs shows the speciation of iron as a function of reduction potential E_h and *p*H. The hatched areas delineate conditions encountered in the biosphere [upper part: clean water in air, lower part: water with organic matter; reproduced with permission from M. B. McNeil and B. J. Little, J. Am. Inst. Conserv. **31**, 355 (1992)] (Ref. 55).

VI. ELECTROCHEMICAL PHENOMENA IN CONTACT KILLING

It was shown that a copper alloy containing 10% silver exhibited enhanced contact killing compared to pure copper.[71] This correlated with increased copper ion release. Jing *et al*. also demonstrated increased bactericidal activity of copper coated with a thin silver layer.[72] These studies were conducted in humid environments, where corrosion can take place. This suggests that electrochemically driven mechanisms can also enhance the release of copper and/or silver ions, a strategy that could be further exploited to design more effective antibacterial materials.

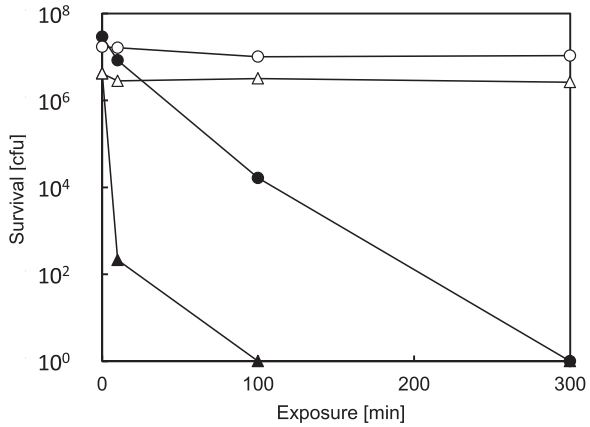

FIG. 6. Killing of bacteria on iron in the presence of copper. Cells (1–3 × 10^7) of *E. hirae* wild-type or a Δ*copAB* mutant deficient in copper export were spread on iron coupons in the absence or presence of 4 mM $CuSO_4$ and survival was determined at different times. Shown are survival for *E. hirae* wild-type without Cu (○), *E. hirae* wild-type plus 4 mM Cu (●), a Δ*copAB* mutant without copper (Δ), and a Δ*copAB* mutant with 4 mM Cu (▲).

Another electrochemically driven phenomenon in contact killing was demonstrated in a study by Mathews *et al*.[42] Metallic iron surfaces are not antibacterial, which is again apparent from the Pourbaix diagram: under ambient conditions, iron does not form any oxides which are soluble (Fig. 5). However, if a subtoxic concentration of $CuSO_4$ is added to the system, rapid killing of bacteria occurs (Fig. 6). The effect was shown to be due to the reduction of Cu^{2+} to the more toxic Cu^+ by the iron surface.[42] A mutant deficient in Cu^+ export was considerably more sensitive to killing than wild-type cells, underlining the importance of copper ions in the killing process. Cu^+ ions could also play a key role in "normal" contact killing by copper, where the dissolution of copper oxides could lead to the generation of Cu^+. This would be an interesting aspect to address experimentally.

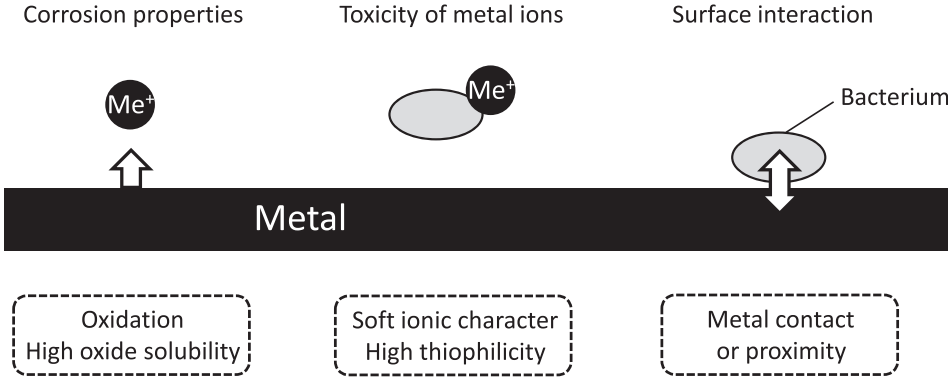

FIG. 7. Schematic of the key factors required for the antibacterial activity of a metal.

VII. SUMMARY AND CONCLUSION

Copper is the only element for which rapid contact killing of bacteria by a solid metallic surface has been demonstrated. This remarkable phenomenon appears to rest on three key properties of copper: (1) copper oxidizes under ambient conditions, e.g., in air of moderate humidity, (2) the copper oxides thus formed are soluble in the aqueous phase, and (3) the copper ions released by the oxides are toxic to bacteria due to their soft ionic character and their thiophilicity. Surface contact or proximity may play an additional role (Fig. 7). Even in so-called dry exposure of bacteria to copper, there will be an aqueous phase surrounding the bacteria, allowing the above processes to take place. Taking these concepts into consideration, it should be possible to develop alloys in which additional electrochemical effects lead to even higher antibacterial activity than that displayed by pure copper. Based on our analysis, cadmium is predicted to display good contact killing and should be tested for proof-of-principle.

ACKNOWLEDGMENTS

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