Using Copper to Fight Microorganisms

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Abstract: The manuscript reviews the biocidal mechanisms of copper and its current uses in the fight against transmission of health-associated (nosocomial) pathogens, foodborne diseases, dust mites loads and fungal and wound infections. The manuscript also discusses possible future applications such as filtration devices capable of deactivating contaminated blood products and breastmilk.

Keywords: Acaricidal, antiviral, biocide, copper, fungicide, nosocomial infections, wound healing.

BIOCIDAL PROPERTIES OF COPPER AND COPPER COMPOUNDS

The ancient Greeks in the time of Hippocrates (400 BC) were the first to discover the sanitizing power of copper. They prescribed copper for pulmonary diseases and for purifying drinking water. Since then, copper has been used as a biocide by many civilizations, such as the Celts, Phoenicians, Egyptians, Hindus, and Aztecs for treating sores and skin diseases and for purifying water [1]. By the 18th century copper had come into wide clinical use in the Western world for the treatment of mental disorders and afflictions of the lungs. Furthermore, in the 18th century it was discovered that no fungi grew on seed grains soaked in copper sulphate. Beginning in the early 1950s [e.g. [2-4]], the biocidal properties of copper and copper compounds were demonstrated in controlled laboratory studies. The wide range of microorganisms, including gram negative and gram positive bacteria, yeast, fungi and enveloped and non-enveloped viruses, shown to be killed by copper or copper compounds, are summarized in Table **1**. Notably, copper surfaces or copper compounds have also been shown to be efficacious against hard-to-kill spores [5-11].

Today, copper biocides have become indispensable and many thousands of tons are used annually all over the world for i) the prevention of roof moss formation [12]; ii) wood preservation [13]; iii) the control of green slime in farm ponds, rice fields, irrigation and drainage canals, rivers, lakes and swimming pools [14]; iv) the prevention of downy mildew on grapes [15]; and v) in antifouling paints [16-18].

Non-soluble copper compounds, such as degradable phosphate glass fibres impregnated with CuO [19, 20], glass coated with thin films of CuO [21], or metallic and copper alloys [10, 22-29] also exert potent biocidal properties, including against hard-to-kill spores [5-11]. Importantly, in March 2008 the U.S. Environmental Protection Agency (EPA) approved the registration of copper alloys as materials with antimicrobial properties, thus allowing the Copper Development Association (CDA) to make public health claims [30]. These public health claims acknowledge that copper, brass and bronze are capable of killing more than 99.9% of harmful, potentially deadly bacteria, such as Methicillin-resistant *S. aureus* (MRSA) within two hours, and continue to kill more than 99% of bacteria even after repeated contamination. MRSA is one of the most virulent strains of antibiotic-resistant bacteria and a common cause of hospital- and community-acquired infections. Copper is the only solid surface material to receive this type of EPA registration. This approval has now been given to 355 different copper alloys (including brass and bronze) following many years of independent laboratory testing based on rigorous EPA approved protocols.

Copper compounds, such as copper sulphate, copper nitrate and cupric chloride-bis-n-dodecylamine, are potent molluscicides [31-34]. Control of snails may be an important strategy in fighting some human diseases, such as bilharziasis. This disease is caused by a trematode parasite, *Schistosoma mansoni*, which uses snails and humans as hosts. Recently, copper metal nanoparticles have been found to control hematophagous parasites [35].

However, copper compounds may be toxic to fish and other organisms. This has led to a constant search for and production of chelated copper compounds. These compounds, on one hand, are biocidal, but on the other hand, do not react with other chemical constituents in water [e.g.[36-39]]. Therefore, chelated copper water-insoluble compounds, such as copper-8-quinolinolate and some of its derivatives are used to reduce environmental contamination of fungi in hospitals [14].

BIOCIDAL MECHANISMS OF COPPER

Exposure to copper may result in microorganisms' death even within minutes (e.g. [27, 40, 41]). Copper toxicity to microorganisms is achieved through several parallel mechanisms. These include plasma membrane permeabilization, membrane lipid peroxidation, alteration of proteins and inhibition of their biological assembly and activity, and denaturation of nucleic acids [42, 43]. It is likely that the first site

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Table 1. Demonstrated biocidal efficacy of copper.

Bacteria	References
Acinetobacter baumannii	[29,98]
Acinetobacter calcoaceticus/baumannii	[6, 7, 126]
Acinetobacter johnsonii	[80]
Acinetobacter lwoffii	[127]
Bacillus cereus	[128-131]
Bacillus globigii	[5]
Bacillus subtilis	[126,131-137]
Bacillus macerans	[138]
Brachybacterium conglomeratum	[80]
Campylobacter jejuni	[28]
Citrobacter freundi	[126,139]
Clostridium difficile	[6,10,11]
Clostridium tyrobutyricum	[8]
Deinococcus radiodurans	[128]
Desulfovibrio desulfuricans	[140]
Edwardsiella tarda	[141]
Enterobacter aerogenes	[130,142]
Enterobacter cloacae	[130,137,143,144]
Enterococcus sp.	[6]
Enterococcus faecalis	[79,103,104,130,142,145,146]
Enterococcus faecium	[75,143,144,146,147]
Enterococcus gallinarum	[146]
Enterococcus hirae	[148]
Escherichia coli	[24,26,27,44,75,79,80,103,104,110,121,126,128,130-134,143,144,147,149-158]
Klebsiella pneumoniae	[29,79,126,158-160]
Kocuria marina	[80]
Kocuria palustris	[80]
Legionella pneumophila	[6,23,94,95,161,162]
Listeria monocytogenes	[23,104,142,163,164]
Mycobacterium tuberculosis	[29]
Micrococcus luteus	[80,143,144]
Morganella morganii	[139]
Pantoea stewartii	[80]
Photobacterium leiognathi	[79]
Proteus mirabilis	[159]
Proteus vulgaris	[130]
Pseudomonas aeruginosa	[29,60,79,98,129,130,133,134,137,143,144,165,166]
Pseudomonas fluorescens	[163]
Pseudomonas nitroreducens	[131]
Pseudomonas oleovorans	[80]
Pseudomonas putita	[167]
Pseudomonas striata	[138]
Salmonella spp.	[28,104,126,149]
Salmonella typhi	[136,139,156,159,167-169]
Salmonella typhimurium	[60,163,168,170]
Sarcina lutea	[129]
Serratia marcescens	[133]
Shewanella putrefaciens	[163]
Shigella dysenteriae	[159]
Shigella flexnerii	[126,136,139,169]
Sphingomonas panni	[80]
Staphylococcus aureus	[6,7,25,29,79,80,103,104,121,126,129-134,137,143,144,147,150,163,164,171-174]
Staphylococcus epidermidis	[20,80,130,157,160]

Table 1. Contd....

Staphylococcus haemolyticus	[80]
Staphylococcus hominis	[80]
Staphylococcus warnerii	[80]
Stenotrophomonas maltophilia	[98]
Streptococcus faecalis	[137]
Streptococcus pyogenes	[130]
Streptococcus sp.	[19,126]
Vibrio cholerae	[156,168,175]
Yersinia pseudotuberculosis	[142]
Xanthomonas compestris	[165]
Fungi/Yeast	
Alternaria brassicae	[165]
Aspergillus carbonarius	[176]
Aspergillus flavus	[9,134,167,169]
Aspergillus fumigatus	[9,177]
Aspergillus niger	[9,39,112,134,165,177-179]
Aspergillus oryzae	[39]
Candida albicans	[9,29,79,103,104,112,116,121,130,131,135,158,177,179-182]
Candida glabrata	[130,142,159,169]
Candida krusei	[130]
Candida parapsilosis	[130]
Candida tropicalis	[130,142]
Cronobacter sakazakii	[183]
Cryptococcus neoformans	[177]
Culvularia lunata	[160]
Epidermophyton floccosum	[177]
Fusarium culmonium	[9]
Fusarium oxysporium	[9,165]
Fusarium solani	[9,160,169]
Microsporum canis	[169,177]
Myrothecium verrucaria	[39]
Penicillium chrysogenum	[9]
Pleurotus ostreatus	[151]
Pycnoporus cinnabarinus	[151]
Rhizoctonia bataicola	[160,167]
Rhizoctonia solani	[178]
Rhizopus stolonifer	[167]
Saccharomyces cerevisiae	[41,131,182,184]
Torulopsis pintolopesii	[181]
Trichoderma viride	[39]
Trichophyton longifusus	[169]
Trichophyton mentagrophytes	[39,112,116,159]
Tricophyton rubrum	[116,177]
Tricophyton schoenleinii	[159]
Virus	
Avian Influenza	[122,171]
Adenovirus Type 1	[40,185]
Bacteriophages	[186-190]
Coxsackie Virus Types B2 & B4	[185]
Cytomegalovirus	[40]
Echovirus 4	[185]
Herpes Simplex Virus	[186,187]
Human Immunodeficiency Virus	[40,103,119,191]

Table 1. Contd….

Infectious Bronchitis Virus	[192]
Influenza A	[22,40,122,193]
Junin Virus	[187]
Measles	[40]
Parainfluenza 3	[40]
Poliovirus	[189,194]
Pichinde	[40]
Punta Toro	[40]
Respiratory Syncytial Virus	[40]
Rhinovirus 2	[40]
Simian Rotavirus SA11	[185]
Vaccinia	[40]
West Nile Virus	[103]
Yellow Fever	[40]

that copper damages is the microorganisms' envelope. It was reported that copper containing steel adhered to *Escherichia coli* plasma membrane *via* the electrostatic forces exerted by Cu^{2+}, to a significantly greater extent than the austenitic stainless steel not containing copper [44]. This damaged the lipopolysaccharide patches on the outer plasma membrane causing it to collapse, while the inner part of the bacteria remained intact. Similarly, it was reported that Cu^{2+} elicits significant permeability changes in intact *Saccharomyces cerevisiae* cells [41, 45]. Extensive copper-induced disruption of membrane integrity inevitably leads to a loss of cell viability. However, even relatively small alterations in the physical properties of biological membranes can elicit marked changes in the activities of many essential membrane-dependent functions, including transport protein activity and ion permeability [46].

Copper may interact with several microbial proteins, such as with copper chaperones, without damaging them (e.g. [47, 48]). However, copper may damage many proteins, both on the microorganism envelope or within the cell. This may occur *via* displacement of essential metals from their native binding sites in the proteins, or *via* direct interactions with the proteins. In both cases, conformational changes in the protein structure or in the protein active site may occur, resulting in the inhibition or neutralization of the protein biological activities. For example, HIV-1 protease, an essential protein for the replication of the virus, is neutralized by stoichiometric concentrations of copper ions [49, 50]. Another example is the oxidation of the cysteine in the active site of vaccinia H1-related protein tyrosine phosphatase by copper ions, which results in complete inactivation of the protein activity [51]. Redox between Cu^{+} and Cu^{2+} results in the formation of hydroxyl radicals that may attack amino acids, especially histidine and proline, causing substantial protein alterations and even protein cleavage [52, 53].

Copper ions may interact with nucleic acids [54, 55]. For example, copper binding sites were found on average in every three nucleotides [56] in single-stranded DNA, such as that found in many DNA viruses, and in guanine residues in double stranded DNA [57, 58]. It was suggested that following copper binding to the nucleic acids, the repeated cyclic redox reactions between Cu^{+} and Cu^{2+} generated damaging OH radicals [59-61]. However, it was also suggested that although copper binds DNA *in vitro*, stronger competing ligands, such as glutathione and cysteine, may remove copper away from the DNA *in vivo* [62, 63]. Furthermore, recent studies using *E. coli* lacking copper export genes indicate that copper does not catalyze significant oxidative DNA damage *in vivo* [62]. Electron paramagnetic resonance spin trapping assays showed that the majority of H_2O_2-oxidizable copper was located in the periplasm, away from the DNA. However, it may still be the case that in some microorganisms, especially in viruses, copper oxidative damage to the genetic material may occur through Fenton mechanisms. Indirect toxic mechanisms have been suggested. For example, exposure to high concentrations of copper may increase the rate of H_2O_2 generation [64], which may accelerate iron-mediated oxidative DNA damage [62].

In general, the redox cycling between Cu^{2+} and Cu^{1+}, which can catalyze the production of highly reactive hydroxyl radicals, with subsequent damage to lipids, proteins, DNA and other biomolecules [42, 65], makes copper highly reactive and a particularly effective antimicrobial. Other closely related metals, such as zinc and nickel, do not readily undergo redox-cycling reactions and are more stable in their various cationic states. Zinc, similarly to copper, is an essential trace mineral well metabolized by humans, displaying antifungal properties [66]. Zinc pyrithione, for example, is widely used as an antifouling agent in paints [67]. However, free zinc ion in solution is highly toxic to plants, invertebrates, and even to vertebrate fish [68] and in high dosage can promote oxidative toxicity in humans [69]. Nickel, which also has potent antimicrobial properties, is a known hematotoxic, immunotoxic, neurotoxic, genotoxic, nephrotoxic, hepatotoxic and carcinogenic agent [70] and is therefore not used.

Bacteria and fungi need to carefully control the intracellular copper loads. They have different means by which to remove excess copper (reviewed in [42]). These include intra- and extra-cellular sequestration by cell envelopes, exclusion by permeability barriers, active transport membrane efflux pumps, and extracellular chelation or precipitation by secreted metabolites. In addition, they can reduce the sensi-

tivity of cellular targets to copper ions, and upregulate relevant genes, such as of those coding for efflux pumps, when exposed to high concentrations of copper (e.g. [71, 72]). However, above a certain threshold and time of exposure, which differs between the microorganisms, they are overwhelmed by the copper overload and die. Importantly, in spite of copper being a part of the earth for millions of years, and being used by humans since the beginning of civilization, no microorganisms that are highly resistant to copper have been found, but only microorganisms with reduced copper sensitivity (~10 fold lower sensitivity to copper). This is in contrast to the resistance to antibiotics demonstrated by some microorganisms (e.g., 1000 fold less sensitivity to methicillin by methicillin resistant *S. aureus*). For example, *Enterococci* bacteria, isolated from the gut of pigs who were fed for many months with high concentrations of copper in their diet, were 7 fold less susceptible to copper than *Enterococci* bacteria isolated from pigs not fed with copper [73, 74]. Increased tolerance to copper is achieved by the induction of efflux pumps in the tolerant bacteria [74]. Outstandingly, the *Enterococci* and *E. coli* tolerant bacteria isolated from pig farms, following the use of copper sulfate as feed supplement, were rapidly killed when spread in a thin, moist layer on copper alloys with 85% or greater copper content or under dry conditions [75]. Tolerance, but not resistance, was found in nitrifying soil microorganisms exposed to Cu for nearly 80 years under field conditions [76]. Similarly, repeated yearly spraying of copper-containing compounds on vegetable and fruit crops, to limit the spread of plant pathogenic bacteria and fungi, has favored the spread of copper tolerant genes among saprophytic and plant pathogenic bacteria [77]. The increased tolerance to copper was found to be associated with the amount of soluble copper and not with the total amount of copper [78]. Thus, even in soils where the concentration of copper was extremely high, but in a non-soluble form, no increase in tolerance to copper was observed [78]. No resistant bacteria evolved *in vitro* when consecutively exposed to fabrics containing 1% copper oxide [79]. Interestingly, bacteria were isolated from copper-containing surfaces and some exhibited prolonged (1 to 3 days) survival on dry but not on moist copper surfaces [80]. None of these isolate strains was copper resistant in culture [80]. Survival on copper-containing surfaces appears to be the consequence of either endospore formation, survival on patches of dirt, or a special ability to endure a dry metallic copper surface.

The reason why no resistance but only tolerance to copper is found in microorganisms exposed to constant relatively high doses of copper, is probably because copper exerts its biocidal/antimicrobial activity not through one mechanism (as most antibiotics), but through several parallel non-specific mechanisms [42, 43]. In contrast to the highly resistant microbes that have evolved to antibiotics in less than 50 years of use, tolerant microbes to copper are extremely rare even though copper has been an ingredient of the earth for millions of years. Viruses are highly susceptible to copper induced damage since they do not have tolerance and repair mechanisms, such as DNA repair mechanisms present in bacteria and fungi.

COPPER HEALTH RELATED APPLICATIONS

Copper is an essential trace element involved in numerous physiological and metabolic human processes [81, 82], including wound repair [83]. Many over-the-counter treatments for wound healing contain copper [84, 85]. The National Academy of Sciences Committee established the U.S. Recommended Daily Allowance of 0.9 mg of copper for normal adults [86]. Copper is considered safe for humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices [87-90]. The risk of adverse reactions due to dermal contact with copper is extremely low [91, 92].

Due to their potent biocidal properties, copper and copper-based compounds are now routinely used in several health-related areas. These include 1) control of *Legionella* [93-97] and other bacteria [98] in hospital water distribution systems; 2) prevention of algae and other parasites growth in potable water reservoirs (e.g. [99, 100]); 3) reduction of caries in dentistry [101,102]; 4) reduction of foodborne diseases through the production and use of self-sterilizing metallic copper surfaces [23, 24, 26, 28] or materials containing copper [19, 21, 103-105], in which the food is kept, handled or transported. The addition of copper to drinking glasses has been shown to reduce biofilm formation of *Streptococcus sanguis*, thus reducing the risk of oral infections [19]; and 5) in birth control by using copper intrauterine contraceptive devices [90,106].

Novel uses of copper or copper-based compounds in health-related applications are being explored and/or implemented. One area is the reduction of transmission of health-associated (nosocomial) pathogens in hospitals, clinics and elderly homes, by i) manufacturing door knobs, bed rails, and intravenous stands, with metallic copper [10, 22, 25, 29], ii) manufacturing sheets, patient robes, patient pajamas, and nurse clothing, from copper-impregnated biocidal textiles [103,104,107], and iii) disinfecting contaminated clothes with copper-based biocides [6].

The significant contribution of copper surfaces to the reduction of bioburden in clinical settings has recently been demonstrated [108-111]. In a study performed in the UK [108] the efficacy of copper surfaces to reduce bioburden was examined in a busy acute medical ward. The median number of microorganisms harbored by the copper-containing items was between 90% and 100% lower than in their control equivalents (p values ranging from <0.05 to <0.0001). In a study conducted at a busy walk-in primary healthcare clinic in South Africa [110], the mean colony forming units (CFU) isolated from copper surfaces were 71% lower than those isolated from the matched control surfaces ($p<0.001$). And in a study conducted in an oncological/pneumological and a geriatric ward in Germany [109], the total number of CFU on metallic copper-containing surfaces was 63% of that on the control surfaces ($p<0.001$). Interestingly, after disinfection of the copper and control surfaces, microbial repopulation of the surfaces was significantly delayed on the copper alloys ($p<0.05$).

Copper oxide impregnated fabrics have been shown to be acaricidal [103,112]. Dust mites are a source of allergen that

results in perennial rhinitis and asthmatic attacks [113]. Thus, elimination of house dust mites in mattresses, quilts, carpets and pillows may improve the quality of life for those suffering from dust-mite related allergies.

Copper oxide impregnated socks prevent and treat fungal foot infections (athlete's foot) [114-116]. Wound dressings containing copper oxide reduce the dressing and wound contamination [79]. Application of wound dressings containing copper oxide to wounds inflicted in genetically engineered diabetic mice resulted in increased gene and *in situ* upregulation of pro-angiogenic factors (e.g., placental growth factor, HIF-1a and VEGF), increased blood vessel formation ($p<0.05$) and enhanced wound closure ($p<0.01$) as compared to control dressings (without copper) or commercial wound dressings containing silver [117]. Importantly, they enhance and allow wound repair, especially of diabetic ulcers in which conventional treatment modalities fail to close the wounds (unpublished data and [83]).

A possible application of copper due to its potent virucidal properties is its use in filtration devices that can deactivate viruses in contaminated solutions, such as contaminated blood products and breastmilk [118]. Recently, the deactivation of HIV-1 and other viruses in suspension by copper-based filters has been reported [40, 119]. Furthermore, the deactivation of HIV-1 in breastmilk obtained from HIV-1 infected women has been demonstrated [120].

The capacity to impregnate copper into different textile products, as well as into latex and other polymeric materials [103,104,121] allows for the production of personal protective equipment (PPE) with antimicrobial and antiviral properties that can be used by first responders and laboratory personnel, who may be exposed to pathogens. PPE such as gloves, masks and disposable robes, may increase the safety not only of those using these products but also of the immediate environment and assure safer disposal of the used items. Similarly, the use of biocidal uniforms by police or health-workers that can be exposed to infectious solutions, such as contaminated blood, may reduce the risk of pathogens transmission. Recently, the production of antiviral copper containing respiratory masks has been reported [122].

In contrast to the above copper health related applications, copper is not appropriate for use for systemic infections, mainly because once copper is ingested, it readily interacts with transport proteins as well as small molecular weight ligands [123,124], making it unavailable as an antimicrobial. Furthermore, in cases where no efficacious copper metabolism occurs, the unligated free copper in the body may be involved in disease pathogenesis, such as in Alzheimer's disease [125]. Another limitation of copper may be its price, which has recently escalated. However, this is of special relevance mainly when whole surfaces or products are made with copper or copper alloys. It is significantly less prohibitive when copper compounds, such as copper oxide, are impregnated in low percentages in soft or hard surfaces used in hospital environments and medical devices. In any case, when compared to the alternatives or the consequences of not using copper-containing products, e.g., increased nosocomial infections and food poisoning and the related costs of treatments, the issue of the copper cost is not significant.

In conclusion, the safety of copper to humans and its potent biocidal properties allow the use of copper in many applications (Fig. (**1**)), including several that address medical

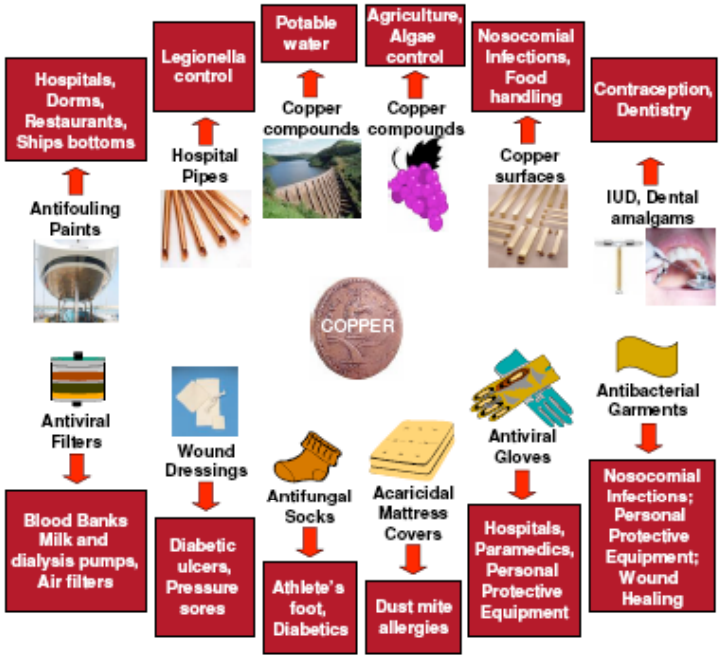

Fig. (1). Current and future potential applications of copper and copper compounds in different areas, which are based on copper's biocidal properties.

concerns of the greatest importance. While some of these applications are already being amply used, novel possible applications of copper may have a major effect on our lives.

ABBREVIATIONS

CDA = Copper Development Association

EPA = U.S. Environmental Protection Agency

MRSA = Methicillin-resistant *Staphylococcus aureus*

HIV-1 = Human Immunodeficiency Virus Type 1

PPE = personal protective equipment

CONFLICT OF INTEREST

G.B. is the Chief Medical Scientist of Cupron Scientific. Cupron is a company that uses copper oxide in its medical and consumer applications.

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