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Review

Copper alloys - The new 'old' weapon in the fight against infectious disease

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ABSTRACT

Exposure to dry copper alloy surfaces, such as brass, kills a wide spectrum of microorganisms including Gram-negative and Gram-positive bacteria and fungi, and permanently inactivates several types of viruses. A large body of published evidence reports that greater than 99.9% killing occurred within a 2-hour period when the microorganism was exposed to the copper alloy samples at room temperature and typical indoor humidity levels. Included in these studies were disease-causing bacteria such as *E. coli* O157:H7 as well as hospital "super-bugs" such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococci* (VRE). The results of these laboratory-based tests are reviewed here. The mechanism(s) of action of copper alloy surface killing is still under investigation and progress on this important area of research will be described. It is important to note that mutations that provide resistance to copper alloy surface exposure have not been reported. These results suggest that copper alloy surfaces could be a powerful tool against the transmission of infectious disease in public settings, most particularly hospitals. In a clinical trial, summarized here, the amount of live bacteria found on components made of copper alloys was compared to that found on components made from standard materials and shown to be 83% lower. Most significantly, when infection rates were tracked in these hospital rooms with the copper components and compared to rooms containing the standard components, it was found that the infection rates were reduced by a statistically significant 58%. Thus, the widespread deployment of copper alloy components to frequently touched surfaces, such as door knobs and hand rails, has the potential to significantly reduce the rate of transmission of infections in the clinical settings and public-use spaces such as schools and transit systems.

KEYWORDS: antimicrobial copper alloys, copper alloy surface contact killing, hospital-acquired infections

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INTRODUCTION

Infection control and prevention continues to be a serious challenge to public health officials in a variety of environments including hospitals and other healthcare associated facilities, schools, transportation facilities, food production facilities, restaurants, and cruise ships. In 2002, an estimated 1.7 million people acquired an infection during a stay in a U.S. hospital that resulted in approximately 100,000 deaths (or 271 fatalities per day) and cost between $35.7 and $45 billion in treatment expenses [1]. Bacteria are omnipresent in healthcare facilities and continue to cause healthcare-associated, or nosocomial, infections. The rise in drug resistant strains has only complicated the problem. The intrinsic ability of

microbial organisms, especially the spore forming ones, to survive on touch surfaces for extended periods of time facilitates their transfer from person to person. *Staphylococcus aureus* and other staphylococci strains are found on the skin of most people and are easily transferred between patients and visitors, to patients directly from healthcare workers, or to objects and then to patients. Thus, it is not surprising that the microbial burden of frequently touched surfaces in healthcare facilities appears to play a significant role in infection causality [2].

Current trends indicate that the emergence of multi-drug resistant bacteria is increasing at an alarming rate. Moreover, microbial contamination in many different environments leads to the rapid transmission of disease. Mason and colleagues carried out an extensive diversity study of the New York City subway system microbiome and report the presence of 1,688 bacterial, viral, archaea, and eukaryotic taxa, including disease causing organisms, suggesting that transit systems could serve as major sources of urban disease transmission [3]. Norovirus outbreaks are reported with alarming frequency in cruise ships, schools, restaurants and catering events, and healthcare facilities (http://www.cdc.gov/norovirus/trends-outbreaks.html). Pathogenic *E. coli* O157:H7 has become a household name due to numerous outbreaks of this potentially lethal infection that results from ingesting contaminated food from major national restaurant chains, distributors of ground beef and poultry products, and tainted supermarket-prepared foods throughout the United States, as reported by the U.S. Food and Drug Administration (FDA). All schools and institutions receiving federal funding are required to report confirmed cases of several communicable diseases. As a result, many of us are all too familiar with school closings due to outbreaks of N1H1 influenza, meningitis, measles, whooping cough, Multi-drug Resistant *Staphylococcus aureus* (MRSA) among school athletes, and other serious diseases. Keep in mind that communicable diseases affect not only the student but also unimmunized or immune-compromised members of their household.

While aggressive sanitation procedures, like hand washing and regular cleaning with disinfectants, go a long way to control infection they are not the whole answer. These types of infection control do not easily lend themselves to many public venues such as transportation systems. In this review, we describe a novel approach that compliments existing sanitation procedures but, unlike these methods, does not require behavioral changes and is continuously active without human intervention – which is the inclusion of copper alloy surfaces in the environment.

1. Metallurgy and ancient history of copper and copper alloys

Copper (Cu) is a member of the group of elements called metals. Metals, in general, are solid at ambient temperatures, denser in the native form than other elements, have a shiny surface when polished, conduct heat and electricity well, and are malleable. Chemically, metals react with oxygen to form oxides and positive ions forming salts like $CuCl_2$ and $CuSO_4$, as in the case of copper. Copper ions are most commonly found in two oxidation states, Cu^{+} or Cu^{++}, which are in equilibrium in solution in concentrations that favor Cu^{++}. This last point is particularly relevant to our discussion of the antimicrobial properties of copper.

An alloy is a homogeneous combination of two or more chemical elements, of which at least one is typically a metal. Ancient metallurgists realized that alloys often exhibit significantly greater strength or corrosion resistance than the pure metal component and devoted a great deal of attention to alloy development. For example, when zinc is mixed with copper, the result is brass, which has higher strength than copper. Other attributes of brass include a yellow gold-like appearance, acoustical properties desirable for musical instruments, and, typically, a lower cost than pure copper. Modern metallurgy continues to develop alloys for specific uses and over 800 copper alloys of a variety of compositions and properties are available today. Of these, 500 are registered with the U.S. Environmental Protection Agency (EPA) as having significant antimicrobial activity. Table 1 lists the composition of a few of the more important copper alloys.

Table 1. Copper alloy composition. The composition of several typical copper alloys and 304 stainless steel are listed in weight percent. Values are rounded to the closest integer. Intentional small additions of less than 1% are shown when they are required in the specification. Cu = copper, Zn = zinc, Sn = tin, Ni = nickel, Al = aluminum, Mn = manganese, Fe = iron, Cr = chromium, P = phosphorus, Si = silicon, and As = arsenic.

Alloy number (Universal number system)	Cu	Zn	Sn	Ni	Al	Mn	Fe	Cr	P	Si	As
High copper alloys											
C10200	99.95										
C11000	99.90										
Brasses											
C21000	95	5									
C22000	90	10									
C24000	80	20									
C26000	70	30									
C28000	60	40									
C68700	78	20			2						0.04
C68800	74	23			3						
Bronzes											
C51000	95		5						0.2		
C61500	90			2	8						
C63800	95				3					2	
C65500	97					1				2	
Copper-nickel alloys											
C70600	89			10			1				
C71000	80			20							
C71300	75			25							
C71500	69			30			1				
C72900	77		8	15							
Copper-nickel-zinc alloys											
C73500	72	10		18							
C75200	65	17		18							
C77000	55	27		18							
Stainless steel											
S30400				8			74	18			

Copper was one of the first metals to be used by humans ([4]; http://www.copper.org/education/history/60centuries/). Like gold, copper can be found in nature in a relatively pure elemental form that is sometimes exposed on the earth's surface. The identification of coins and jewelry dating to about 10,000 years B.C.E. in western Asia and what is now Iran indicates that Stone Age man knew how to work with copper. In fact, the more recent period of the Stone Age is often referred to as the Copper Age (3,500 to 2,300 B.C.E.) because it marked the transition to a culture based on technological advances in copper metallurgy. Early copper tools, such as axes, have been found alongside stone tools at sites in the Middles East and Europe. By about 5,000 to 4,000 B.C.E, the technology for mining and smelting copper from various copper-rich ores, like malachite (copper carbonate hydroxide), was well advanced. Ancient smelting kilns have been found in Iran, in the Negev Desert of current day Israel, and later in the Sinai peninsular of Egypt. Another important source of copper-rich ore was Cyprus from which is derived the word for copper and this was an important source of European copper.

Historical evidence indicates that the technique for copper casting, pouring molten copper into stone molds, was available in this region during this time period. As a result, a wider variety of shapes of solid copper implements were being manufactured than could be shaped by hammering copper sheet. With the introduction of large-scale production techniques by the Egyptians in about 1,200 B.C.E., copper production and trading became a major part of the region's economy. Knowledge of the technologies for copper smelting and casting moved throughout the Middle East, into Europe, to the Indus Valley of Pakistan, and eventually to China by about 1,600 B.C.E. Oetzi the Iceman, who was discovered in an Alpine glacier and dated to 3,300 B.C.E., carried a copper ax that was 99.7% pure. Clearly, copper metallurgy was utilized in Europe during this era.

Copper alloys are metallic materials in which copper is the primary component. The copper is typically combined with these other elements by melting and the alloys produced have novel properties that depend on the alloy composition. Bronze, commonly an alloy of copper and tin (but sometimes other elements), has greater strength and less malleability than pure copper, making this alloy a superior choice for implements like tools and weapons. The Bronze Age (3750 to 500 B.C.E.) initiated in the Near and Middle East and the knowledge of how to produce bronze slowly progressed to Europe (by 1,800 B.C.E.), India, China (by 1,600-1,200 B.C.E.), and later to Korea and Japan. The first bronze artifacts (in this case the type of bronze is an alloy of copper and arsenic and probably smelted from arsenic-containing copper ore) were found in the area of the Dead Sea and dated to about 3,000 B.C.E. Artifacts made from a very high quality bronze also date to about this same time and were found in the burial tombs of the ruling families of Samaria located in the city-state of Ur in the lower Euphrates valley. Throughout the Bronze Age, advances in metallurgy improved production techniques and expanded the uses of this versatile copper alloy.

The advent of the Iron Age (1,200-500 B.C.E.) relegated the use of copper and bronze to more decorative items like sculpture. The development of brass brought on a renewed interest in copper alloys, largely because of its gold-like appearance. Brass is an alloy of copper and zinc although the proportions vary significantly creating a range of different brasses with different characteristics. Copper-zinc alloys were known in Greece and the Near East in the third century B.C.E., in India in about the second century B.C.E., but perhaps as early as the fifth century B.C.E. in sites in China. These were the so-called "natural" alloys produced from copper and the available zinc-containing ores. Objects made of deliberately manufactured brass, made by melting pure copper and pure zinc, did not appear until Roman times, with the earliest dated to about 20 B.C.E. Roman coinage from the reign of Augustus was made of brass. Brass was also used for Roman military equipment.

2. Use of antimicrobial copper by ancient civilizations

Ancient civilizations exploited the antimicrobial properties of copper long before Louis Pasteur's discovery of bacteria and his concept of microbes as the causative agents of disease became common

knowledge in the 19th century. Much of the information described here comes from a thorough review of the medical uses of copper by Dollwet and Sorenson [5]. Copper was known to control infection as early as Egyptian times. Even the bible suggests that the water stored in copper/ bronze vessels were free of disease causing agents. In Exodus (30:17-20) Jehovah ordered Moses to place a copper basin on a copper stand and place it between the meeting tent and the altar where "Aaron and his sons are to wash their hands and feet with water from it. Whenever they enter the tent of meeting, they shall wash with water so that they will not die". The ancient Hindu tradition of Ayurvedic medicine dating back over 3,000 years recommends collecting and storing household water supplies in copper vessels for improved health. This tradition is consistent with recent studies confirming that overnight storage of contaminated water in copper vessels kills bacterial contaminants and makes the water safe for drinking [6].

For the most part, ancient medical treatments involved the use of copper salts and oxides but solid metallic copper, copper splinters, and shavings were used as well [5]. The first recorded medical use of copper is found in the Smith Papyrus, an Egyptian medical text written between 2,600 and 2,200 B.C.E. It describes how to treat an infected chest wound and promote healing with copper. The Papyrus speaks of a "green pigment" that is believed to be ground malachite (copper carbonate hydroxide), ground copper silicate (formed in a copper smelter), copper chloride (the "rust" scraped from metallic copper dipped into seawater), or "verdigris" (copper acetate formed on metallic copper exposed to concentrated vinegar vapors). The Ebers Papyrus, written around 1500 B.C.E., documents medicine practiced in ancient Egypt and in other cultures that flourished many centuries earlier. Copper compounds, in the form of metallic copper splinters and shavings and copper salts and oxides, were recommended for a variety of disorders including burns, itching of the skin, eye ailments, and to promote healing of infected wounds. Similarly, in the Hippocratic Collection (in part written by the Greek physician in 460 to 380 B.C.E.) leg wounds were treated with a poultice that included verdigris and red copper oxide among other oxides and natural products, all dissolved in wine.

The use of copper and its derivatives for the treatment of disease was well established during Roman times. The Roman physician Celsus, who began practicing medicine in the early part of the 1st century C.E., wrote in detail about the uses of copper filings, copper oxide, and copper salts in his six volume series *De Medicina*. Pliny the Elder (23-79 C.E.), a Roman author and naturalist, noted in his treatise *Naturalis Historia* that all kinds of ulcers are rapidly healed in individuals living or working in the vicinity of copper ores and mines. Ancient cultures outside of the Middle East and the Mediterranean region were also aware of the medical uses of copper. The Aztecs used a mouthwash containing a suspension of ground copper to treat sore throats, called "faucium calor". According to the 10th century Persian text *Liber Fundamentorum Pharmacologiae* and early Hindu texts, copper was widely used to treat lung diseases, venereal diseases, infected wounds, and skin ulcers.

Reports of medicinal copper continued into 19th and early 20th century Europe until the use of antibiotics became commonplace during World War II. The French physician Victor Burq detailed many uses for copper in medicine in his text *Metallotherapie* but perhaps his greatest contribution was his statistical analysis of cholera mortality and morbidity during two Paris epidemics (1865 and 1866). Burq found that only 16 deaths occurred among 30,000 workers in the copper industry while the death rate was 10-40 times higher among similar non-copper workers. The Swiss physician Köchlini popularized copper in the form of an ammonium salt for the treatment of disorders. He reported that "hammerschlag", the powder produced by hammering metallic copper, taken by mouth healed broken bones, and bone and muscle wounds. It was even reported by the German physician Werner Hangarter that Finnish copper miners did not suffer from arthritis, a common problem in Finland.

Medicinal uses of copper were not limited to copper oxides and salts. Copper and copper alloy surfaces were also reported to control infection and thereby promote healing. One example is seen in the choice of materials used for surgical instruments. Hippocrates is said to have recommended the use of bronze for medical

instrumentation, reportedly due to its long association with healing cults, and many examples of early bronze surgical instrumentation survive until today [7]. A large collection of bronze instruments made by the famous Roman surgeon Galen (130-200 C.E.) was found in Pompeii and Herculaneum. Ancient Egyptians used bronze surgical tools 6,000 years ago and the Phoenicians brought this technology to Europe. Another example of the antimicrobial properties of solid copper materials comes from the Far East. Ancient Chinese law prohibited the use of paper money in public drinking houses as a hygienic measure and mandated that payment be made with copper coins, based on the empirical knowledge that disease transmission rates were lower. An interesting observation consistent with the antimicrobial properties of copper came from World War I. It was found that, in a few cases in which fragments from a copper-containing projectile were not removed from a wound, the wound healed surprisingly free from infection [5].

3. Rediscovery of antimicrobial activity of copper alloy surfaces

While the antimicrobial activity of copper compounds like copper oxide and copper salts was well established in the scientific and medical community, knowledge of the antimicrobial activity of copper alloy surfaces had been lost until recently. In 1983, as part of a training program for hospital housekeeping and maintenance personnel, Kuhn [8] compared the bacterial contamination levels of brass and stainless steel doorknobs. Unexpectedly, only a rare few streptococcal and staphylococcal isolates were found on the brass doorknobs but the stainless steel ones were heavily contaminated with both Gram-negative and Gram-positive organisms. Kuhn [8] tested this observation further by showing little or no survival of *E. coli* and other bacteria when concentrated cultures are spread onto copper and brass metal strips whereas these organisms persisted at high numbers on the aluminum and stainless steel samples.

Nearly two decades later, the Kuhn report came to the attention of one of the authors, Harold Michels, who decided to explore these findings further in a more standardized laboratory environment. The results of the first study were reported in the Proceeding of the Copper 2003 - The 5th International Conference on Copper, Santiago, Chile and in Wilks *et al*. [9]. The testing protocol used is summarized as follows [9]. Microorganisms are exposed to the alloy surface using a 1-cm^2 metal sheet, referred to as a coupon. A small sample of a concentrated suspension of the microorganism of interest is spread over the surface of the coupon in a sterile environment at room temperature and ambient relative humidity. At specified time intervals, the microorganisms are washed from the coupon surface and survival determined by tittering. Stainless steel alloy S304 is typically used as the experimental control.

3.1. Copper alloys kill a broad spectrum of bacterial species

Escherichia coli O157:H7 was the first bacterium tested because it is responsible for numerous food recalls and outbreaks of severe gastrointestinal illness and kidney failure as a consequence of hemolytic uremic syndrome, often resulting in deaths [9]. Wilks *et al*. [9] demonstrated that *E. coli* O157:H7 was rapidly killed, over 7.5 logs decrease in survival, following about 45 minutes of exposure to a selection of 99-100% pure copper coupons and few if any survivors were observed by 100 minutes of exposure. Interestingly, only about 1 log of killing was seen during the initial 45 minutes of exposure. Hong *et al*. [10] observed similar results in a study carried out with a standard laboratory *E. coli* strain, shown in figure 1. The initial inoculum contained about 10^9 CFU (colony forming units) and essentially all were killed by 45 minutes. As seen by Wilks *et al*. [9], the killing curve was biphasic, that is, only about 1 log of killing was observed by the first time point (15 minutes), which coincided with the time required for the sample to dry on the coupon, and more rapid killing occurred between 15-45 minutes. No significant decrease in survival was observed in the stainless steel control during this same 45-minute time period. Hong *et al*. [10] compared a series of copper alloys ranging from nearly pure copper (C11000) to 60% copper (C28000). They found that the onset of very rapid killing correlated with the copper content of the alloy (Figure 1). *E. coli* cells exposed to the highest copper concentration alloys, C11000 and C24000, were killed immediately after the samples dried

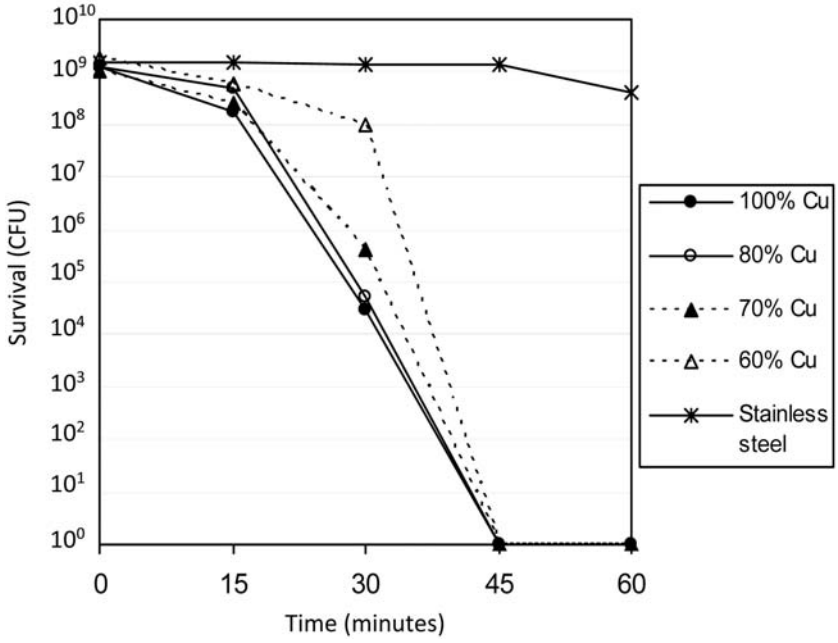

Figure 1. *E. coli* survival on copper–zinc alloy surfaces containing different copper concentrations. *E. coli* strain ATCC23724 was grown in Luria Broth to mid-log phase (OD600 0.3), harvested by centrifugation from 100 ml of culture, and resuspended in 0.85% NaCl to a final volume of 500 µl. 100 µl of concentrated cells were spread over the surface of metal coupons of 304 stainless steel (S30400), 99.90% copper (C11000), and copper-zinc alloys C24000, C26000, and C28000 containing 80%, 70%, 60% copper, respectively (alloy compositions listed in table 1). Following the indicated time of exposure, the cells were washed from the coupon surface with 100 µl of 0.85% NaCl and samples taken to titer survival. The results represent at least two independent trials. (Copyright © American Society for Microbiology, [Appl. Environ. Microbiol., 78(6), 2012, 1776, doi:10.1128/AEM.07068-11]).

but there was a decreased rate of killing observed in the lower copper concentration alloys. C28000 (60% copper) exhibited a nearly 30-minute delay in the onset of rapid killing. Several independent studies confirmed that copper alloys, both in sheets and cast, effectively killed *E. coli* and demonstrated a clear correlation between the copper content of the alloy and killing efficacy [11, 12, 13, 14].

Espírito Santo *et al*. [15] developed a so-called "dry inoculation method" that used a far smaller inoculum than the "moist" or "wet" application procedure, initially described by Wilks *et al*. [9]. This method was suggested to better simulate real-world surface use conditions by limiting the volume of the sample applied to a surface to 20 microliters (µl) or less thereby allowing the sample to dry very rapidly. The liquid quickly evaporates placing the microbe in direct contact with the copper alloy surface. When this 'dry inoculum method' was used, bacterial killing initiated immediately with no plateau. The kill time for alloy C11000 (99.9% copper) when challenged with *E. coli* O157:H7 was nine logs in only one minute [15]. The dry inoculum method was also used to test a copper-nickel-zinc alloy (C75200, 65% copper) and a brass (C28000, 60% copper). Both alloys resulted in over 9 logs of killing of *E. coli* within 15 min. These finding suggest that the biphasic killing curve observed by Wilks *et al*. [9] and Hong *et al*. [10] resulted from the time required for the larger sample applied to the coupon to dry and that this effectively delayed direct contact between the microorganism and the alloy surface.

Methicillin-resistant *Staphyloccocus aureus* (MRSA) infections cause about 126,000 hospitalizations each year in the U.S. [16]. Copper's efficacy against MRSA has been demonstrated in several independent studies [14, 17, 18, 13, 19]. Figure 2 illustrates not only that the copper surface is effective in killing the initial inoculum of MRSA

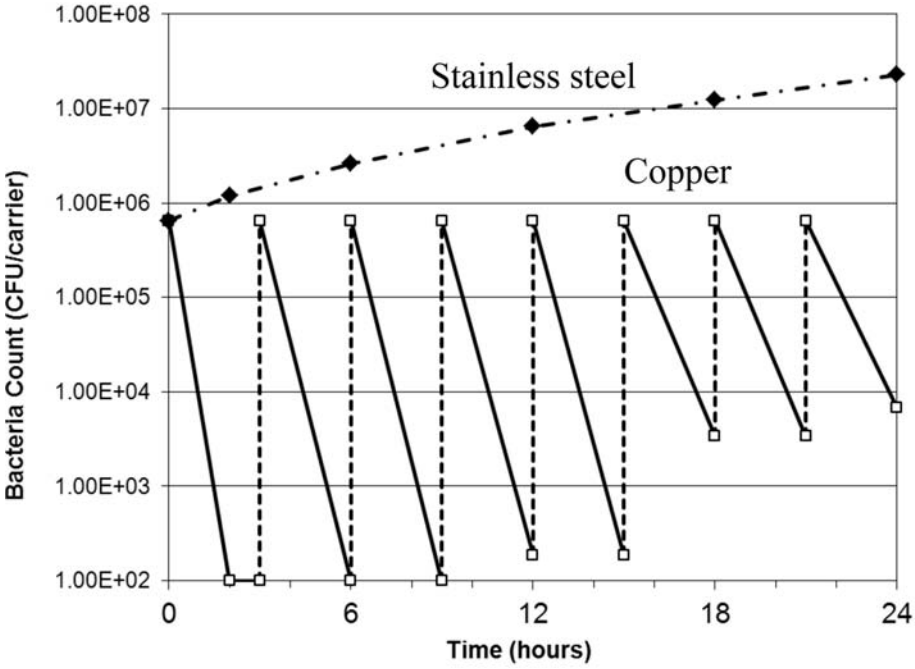

Figure 2. *Staphylococcus aureus* (MRSA) survival on copper (C11000) and stainless steel (S30400) following multiple inoculations. *Staphylococcus aureus* (MRSA) samples spread over the surface of metal coupons of 304 stainless steel (filled diamonds) and nearly 100% copper (open squares). Following the indicated time of exposure, the cells were washed from the coupon surface and samples taken to titer survival. (Reprinted with permission from Anderson, D. G. and Michels, H. T. 2008, Metal Ions in Biology and Medicine, P. Collery, I. Maymard, T. Thephanides, L. Khassanova and T. Collery (Eds.), John Libbey Eurotext, 185).

but that it maintains this efficacy after a total of 8 inoculations without any intermittent cleaning [11]. In this case, coupons consisting of nearly pure copper alloy (C11000) and stainless steel (S30400) were inoculated with a sample containing 10^6 CFU/in^2, allowed to incubate for about 1.5 hours, and then re-inoculated for up to a total of eight times in a 24-hour period without any intermittent cleaning. As shown in figure 2, greater than 99% of the MRSA was killed by the copper alloy surface even after an application of eight samples. In contrast, the stainless steel coupons continued to harbor viable, substantial, and increasing concentrations of MRSA after each inoculation. It should be noted that this result clearly demonstrates that the efficacy of copper alloys is maintained for over 24 hours and even after repeated applications of substantial concentrations of the pathogen.

Gould *et al.* [14] reports that three out of five clinically important MRSA strains tested on pure copper were killed within 60 minutes, and the remaining two strains were killed within 80 to 100 minutes. Furthermore, these authors also found similar efficacy against Community-Acquired Methicillin-sensitive *Staphylococcus aureus* (CA-MSSA). Anderson and Michels [11] tested alloys containing lower levels of copper, including brass and bronze, against MRSA and found greater than 99.9% kill within two hours when challenged with inoculation levels as high as 10^5 to 10^8 CFU/coupon. It should be noted that Noyce *et al.* [13] found that lowering the initial bacterial load to levels closer to those found in the clinical setting resulted in a shorter time for pure copper to kill MRSA. These results demonstrate the practical significance of using copper alloys in the typical clinical setting since the copper alloy surfaces will kill most of the bacteria that come in contact with the surface in a matter of minutes rather than hours.

Vancomycin-resistant *Enterococci* (VRE) are responsible for approximately one-third of entercoccal infections in intensive care units in the U.S. [20]. VRE is primarily transferred from environmental

surfaces to patients and healthcare workers by touch [21]. Thus, control of VRE contamination on hospital room surfaces should help control this serious cause of hospital-acquired infections (HAIs). Warnes *et al*. [20] demonstrated that strains of vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* were killed in less than an hour by copper alloys containing at least 90% copper but survived for several weeks on stainless steel. Gould *et al*. [14] reported similar results.

Clostridium difficile, an anaerobic spore-forming bacterium, is a highly resilient contaminant in healthcare facilities and is a particular problem for patients taking broad-spectrum antibiotics or are immune compromised. *Clostridium difficile* spores survive under extreme conditions and for up to five months on dry inanimate surfaces [22]. Additionally, *C. difficile* spores are not killed by all hospital-grade disinfectants (for example, quaternary ammonium based) and a *C. difficile* outbreak means significant additional costs for any institution. *Clostridium difficile* vegetative cells are highly sensitive to exposure to copper alloys but *C. difficile* spores are relatively resistant, although they are killed albeit at a reduced rate [23]. Weaver *et al*. [23] found that copper alloys ranging from 65%-100% copper were able to kill 10^5 *C. difficile* spores in between 24 and 48 hours while a similar loss in viability was not seen on stainless steel even after 168 hours of exposure. While seemingly ineffective, this finding is promising in view of the fact that *C. difficile* spores can germinate even after months of exposure to ambient oxygen on inanimate surfaces [22]. By inducing *C. difficile* spore germination, Wheeldon *et al*. [24] were able to get a greater than 99% reduction (over 10^6 CFUs/cm^2) within three hours of exposure to a pure copper surface, suggesting that including germinant in cleaning procedures could enhance the antimicrobial efficacy of copper alloys against *C. difficile* spores.

Studies of copper alloy sensitivity of spore-forming *Bacillus* species found similar results, highly sensitive vegetative cells and resistant spores [25, 26]. San *et al.* [26] observed very rapid killing of a sporulation-defective strain of *Bacillus subtilis* on copper alloy surfaces containing 60-100% copper. Exposure of the isogenic sporulation-competent parental strain exhibited only 1 log of killing, consistent with the presence of about 90% spores in the inoculum.

3.2. Copper alloy surfaces inactivate viruses

Influenza, SARS, norovirus, smallpox, measles, chickenpox, and several other childhood diseases are responsible for epidemics and often lead to hospitalization and even death [27, 28]. Persons with weakened immune systems are especially vulnerable to acquiring a secondary infection when hospitalized and secondary infections are the cause of significant mortality and morbidity in the elderly and other groups at high risk. Noyce *et al*. [27] reported that copper samples inactivated 75% of Influenza A (H1N1) in one hour and almost 100% after six hours. Norovirus is transferred by hand-to-hand contact, touching environmental surfaces, and ingesting contaminated food. Moreover, infected individuals also shed norovirus for up to three weeks after symptoms cease. Presently, neither a vaccine nor an effective treatment is available and the only method available to control outbreaks is to clean with bleach. Human norovirus currently cannot be cultured in the laboratory but murine norovirus, MNV-1, has been identified as a close surrogate. Warnes *et al*. [28] showed that murine norovirus was no longer infective after as little as 30 minutes of exposure to copper (99.9% copper) and 60 minutes of exposure to copper-nickel (90% copper) surfaces but retained infectivity when exposed to stainless steel for even longer times. When using the dry inoculum method, inactivation rates were found to be 5 minutes for both copper (99.9% copper) and copper-nickel (90% copper) surfaces. In a subsequent study, Warnes *et al*. [29] observed that capsid integrity was compromised upon coming in contact with copper alloys.

3.3. Yeast and other fungal species are sensitive to copper alloy surface killing

There is significant variability in the sensitivity of fungal species to copper alloy surface exposure [30, 31]. *Candida albicans* and *Saccharomyces cerevisiae* vegetative cells were both extremely sensitive to copper alloy surface exposure when tested using either the wet or dry exposure technique [31]. Weaver *et al*. [30] tested a number of spore-forming species including *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Penicillium chrysogenum*,

and *Fusarium culmonium*, *F. oxysporium*, and *F. solani* on copper and aluminum coupons. The spores of all of these species were extremely resistant to copper surface exposure. No survivors were detected for *Penicillium chrysogenum* and the *Fusarium* species after 24 hours of exposure to copper but no decrease in viability was observed in the spores exposed to the aluminum surface. In *Aspergillus* species, complete killing of the spores of *A. flavus* and *A. fumigatus* required 4-10 days of exposure and, amazingly, *A. niger* spores were still viable with no evidence of killing even after 10 days of exposure. Vegetative growth of *A. niger* was tested by placing a coupon on solid growth medium inoculated with spores. Hyphae were unable to grow over a copper coupon, demonstrating significant inhibition, but were able to grow up to and over the surface of the aluminum coupon. Thus, growth and survival of vegetative cells of these fungal species appears to be sensitive to copper alloy surface exposure but spores are extremely resistant.

3.4. Survey of microorganisms sensitive to copper alloy surface killing

In addition to the previously discussed organisms, copper alloy surfaces have demonstrated antimicrobial efficacy against a wide range of other microorganisms including prokaryotes, viruses, and fungi, many of which are listed in table 2, along with literature references for specific details. Table 2 should not be considered exhaustive [52]. More species have been reported in the literature and more will likely be added as researchers explore the potential of copper alloy surfaces in controlling infection in healthcare facilities, schools, and other public spaces and the antimicrobial properties become more widely known. Specific statements regarding the level of sensitivity are not included in table 2 because the studies referenced used different exposure protocols thereby making direct comparisons difficult if not impossible. Researchers used wet and dry application techniques, copper alloys that differed in copper content from 60 to 100%, metal samples with potentially different surface structures due to variations in processing procedures, different culture methods that could affect the physiology and/or cell envelope structure of the microorganism, and different temperature and humidity conditions, all of which are known to affect the kinetics of killing. Nonetheless, we can make some general statements based on the information in table 2. Primarily, all bacterial species reported in table 2 exhibit significant sensitivity to copper alloy surface exposure. The only exceptions are the endospores of those spore-forming species such as *Clostridium difficile* and *Bacillus anthracis*. It should be noted that table 2 includes both Gram-negative and Gram-positive species. Particularly notable is *Deinococcus radiodurans*, an "extremophilic" Gram-positive bacterial species with an elaborate cell envelope, reminiscent of Gram-negative strains. *D. radiodurans* is highly resistant to ionizing radiation, desiccation, and oxidizing and electrophilic agents but exhibits a similar degree of sensitivity to exposure to copper alloy surfaces as other bacterial species [15, 53].

Second, a review of the literature indicates that the rate of killing is faster on alloys with higher copper content [9, 10, 23, 26]. Third, spore formation provides significant protection against copper alloy surface killing [23, 25]. Microorganisms produce spores as a means of withstanding environmental extremes like desiccation and starvation. Several of the bacterial and fungal species listed in table 2 produce spores that are quite distinct in structure yet all demonstrate significant resilience to killing by exposure to copper alloy surfaces. Studies of viral sensitivity to copper alloy surfaces are still in the early stages. Only a few viruses have been tested and they fall into very different groups based on their capsid-type. To determine whether the broad range of inactivation kinetics exhibited by the different viruses is a function of the capsid or genome structure, the studies would need to be carried out with a larger group of related viruses using a single protocol.

4. What do we know about the mechanism of killing on copper alloy surfaces?

Table 2 clearly demonstrates that exposure to surfaces composed of copper or copper-containing alloys but not stainless steel, the typical experimental control alloy, results in efficient and rapid killing of bacteria and fungi and inactivation of viruses. The extensive and growing literature on antimicrobial activity of copper alloy surfaces reported here

Table 2. Microorganisms shown to exhibit sensitivity to copper alloy surface contact killing.

Microorganism	**Reference**
Bacterial species	
Acinetobacter species (MDR, other strains)	[17], [32], [33], [34]
Bacillus anthrax, *B. cereus*, *B. subtilis* (vegetative cells, not spores)	[15], [25], [26]
Brachybacterium conglomernatum	[15]
Brucella melitensis	[25]
Burkholderia species	[25], [35], [36]
Campylobacter jejuni	[37]
Clostridium difficile (vegetative cells, not spores)	[23], [24]
Deinococcus radiodurans	[15]
Enterobacter species	[11], [32], [38]
Enterococci species (vancomycin – resistant, other strains)	[14], [20], [39], [40], [41]
Escherichia coli (various strains)	[9], [10], [12], [14]
Francisella tularensis	[25]
Klebsiella pneumonia	[17], [32], [42]
Legionella pneumophila	[30], [43], [44]
Listeria monocytogenes	[45], [46]
Mycobacterium tuberculosis	[17]
Pantoea stewartii	[34]
Pseudomonas species	[11], [14], [32], [33], [34]
Salmonella enterica	[37], [42], [47]
Staphylococcus aureus (MRSA, other strains); *S. pneumoniae*, other species	[13], [14], [18], [33], [48]
Yersinia pestis	[25]
Viruses	
Coronavirus 229E (human)	[49]
Influenza A	[13]
Norovirus (murine, human)	[28], [29], [50]
T2 bacteriophage	[51]
Vaccinia, Monkeypox	[25]
Fungi	
Aspergillus species	[30]
Candida albicans	[17], [30], [31]
Fusarium species	[30]
Penicillium chrysogenum	[30]
Saccharomyces cerevisiae	[31]

has been confirmed by standardized testing in an approved "Good Laboratory Practices" facility, and the US Environmental Protection Agency registered over 500 copper alloys as "pesticides" having antimicrobial activity against 6 different bacteria. Clearly, the publication by Wilks *et al*. [9] opened whole new vistas for the uses of copper and copper alloys as passive antimicrobial sanitizing agents. In view of the potential importance of copper alloy surfaces in the battle against the spread of infectious disease, it is essential to understand the mechanism of contact-mediated killing by copper, particularly to gain insights into the possibility that microorganisms could become resistant. The ability of bacteria to acquire antibiotic resistance by simple genetic changes, often to multiple antibiotics in a single event, has limited their use and forced the pharmaceutical industry to a constant battle to develop new antibiotic agents with different mechanisms of action. As we discuss below, resistance to copper alloy surface killing appears to be a greater challenge to microorganisms.

4.1. Copper ion formation on copper alloy surfaces is the first step

Several lines of evidence point to the formation of copper ions, Cu^{+} and Cu^{++}, as the first step in copper alloy surface killing [12, 15, 34, 41, 54, 55]. Molteni *et al.* [41] used the moist exposure method to test killing of *E. hirae* cells. The cells were suspended in different buffers that release copper ions from the alloy surface at different rates. They found that the rate of killing varied directly with the ability of the buffer to dissolve copper from the metal surface. Consistent with this, investigations of copper surface killing on different copper alloys demonstrated a clear correlation between the efficacy of killing and the copper content of the alloy [9, 10, 23, 26]. Espíritu Santo *et al.* [15] followed the uptake of copper ions after moist and dry exposure of *E. coli* to a copper alloy surface. Intracellular copper accumulated at a significant rate under moist exposure but even greater (27-fold higher) levels of copper accumulated following dry exposure. This higher rate correlated with far more rapid killing. In addition, copper levels remained very high for up to 90 minutes suggesting that the cells were likely overwhelmed by these excessive levels [15]. Zeiger *et al*. [55] compared different copper alloy surface structures and found that surfaces that exhibited a higher rate of copper ion release also exhibited higher rates of copper killing. Based on these findings, researchers explored the possibility that copper alloy surface killing resulted from the accumulation of toxic levels of intracellular copper ions.

4.2. Does perturbation of copper homeostasis mechanisms impact resistance to copper alloy surfaces?

Copper's ability to transition between two oxidation states, cuprous Cu^{+} (reduced) and cupric Cu^{++} (oxidized), allows it to function as a catalytic co-factor in biological systems. It is required for several essential biological processes that exhibit remarkable structural and functional conservation from bacteria to human [56, 57]. However, while copper is an essential micronutrient at appropriate concentrations, at higher concentrations it is toxic. In excess, copper affects nucleic acid, protein, and lipid biochemistry as a result of disturbances in copper homeostasis and the oxidation of macromolecules, particularly proteins and components of the plasma membrane, reportedly leads to a rapid decline in membrane integrity [58, 59, 60, 61, 62].

Current understanding of the mechanisms of copper acquisition, distribution to intracellular compartments and the periplasmic space, and homeostasis comes from studies of bacterial, yeast, and mammalian model systems [reviewed in 56, 57, 59, 63, 64, 65, 66, 67, 68]. Cellular functions that contribute to copper homeostasis are very complex and much remains unknown. Included are a network of interconnected process such as: (1) the copper import and efflux systems that control the transport of copper ions across cellular membranes and between the cytoplasm and periplasm; (2) the superfamily of cuproenzymes, like cytochrome c oxidase, that are found in the plasma membrane and periplasm; (3) copper chaperones that are responsible for trafficking copper ions to the cuproenzymes and facilitating their transfer to these copper-dependent proteins; (4) copper chelators like metallothionein that sequesters free copper ions in a physiologically benign form possibly for storage or copper sensing;

and (5) the transcription regulators controlling the expression of these functions and related copper sensors. Given the broad spectrum of copper requirements and the potential for toxicity, it is no surprise that species have evolved tightly regulated mechanisms for copper homeostasis. Therefore, researchers first considered the possibility that overwhelming the mechanisms controlling copper homeostasis could be the possible basis for copper alloy surface toxicity.

In support of the hypothesis, several groups demonstrated that levels of intracellular copper increased soon after exposure to a copper alloy surface and that this played a role in toxicity [12, 15, 40, 41, 48]. Consistent with this, studies found that strains carrying null mutations in the genes encoding components of the plasma membrane P-type ATPase copper efflux transporter CopA, the tripartite outer-inner membrane spanning copper efflux system encoded by CusCFBA, and the periplasmic multicopper oxidase CueO exhibited increased sensitivity to growth in media containing copper ions [69, 70, 71]. Additionally, copper-resistant mutants were identified in bacteria isolated from a variety of environments such as mining effluents, manure from animal farms, and the surface of copper coins [12]. Reports that identified the responsible resistance genes found them to encode components of the copper efflux systems that were being overexpressed from plasmids [66, 72, 73].

4.3. The membrane is the primary target of copper alloy surface exposure

The role of intracellular copper levels in copper alloy surface killing was brought into question following an investigation of the contribution of copper resistance systems to survival of *E. coli* on copper surfaces. Espírito Santo *et al*. [12] found that, despite their more rapid accumulation of intracellular copper, mutant strains lacking the copper detoxification systems CopA, Cus, and CueO were only marginally more sensitive to copper surface exposure. Conversely, a copper-resistant strain harboring the multi-copy plasmid-borne *Pco* operon survived only 3-times longer than the parental strain on a copper alloy surface but, nonetheless, succumbed to dry copper surface killing in a matter of minutes. Estimates of the amount of copper ion released during these brief exposures were orders of magnitude less than the copper ion concentrations to which these strains are resistant when exposed in solution [12]. Thus, the copper ion resistance of these mutant strains had little impact on their survival on copper surfaces and, more importantly, did not prevent killing. Espírito Santo *et al*. [15] concluded that the toxic effects of high levels of intracellular copper appear to play a minor role in copper alloy-mediated contact killing. Instead, they suggested that the effects of copper ions at the membrane and/or in the periplasm mediate copper alloy contact killing and that an as-yet unidentified component of the bacterial membrane is the primary target [reviewed in 60].

Quaranta *et al*. [31] explored the mechanism of copper alloy-mediated contact killing in the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Using the dry exposure technique, they demonstrated complete killing of *S. cerevisiae* and *C. albicans* in 30 seconds and 5 minutes, respectively. Mutant strains carrying alterations in various copper homeostasis genes were tested. These included an *S. cerevisiae* strain with hyperactivity of the copper uptake transporter encoded by *CTR1*, an *S. cerevisiae* strain carrying a deletion of *ACE1*, the activator of *CUP1* encoding metallothionein, and a *C. albicans* strain carrying a deletion of *CRP1*, encoding a copper efflux P-type ATPase. They found that increased levels of intracellular copper increased the exposure time required for killing in both yeasts by several fold. Nonetheless, all of the mutants tested remained significantly sensitive to copper alloy-mediated killing and no survivors could be detected after an exposure of only 20 minutes for *S. cerevisiae* and 60 minutes for *C. albicans*. Quaranta *et al*. [31] observed that copper surface exposure caused extensive loss in cell membrane integrity in both species and stress to subcellular compartments. They concluded that, similar to studies in bacterial strains, the primary target of copper alloy surface exposure is the cell membrane.

4.4. Peroxidation of membrane lipids as the primary target of copper alloy surface killing

What membrane component is the target of copper alloy surface killing? One clue can be garnered from the diversity of microorganisms

sensitive to copper-mediated contact killing (Table 2) – the component(s) is common to the membrane of all of these organisms. Another important clue comes from the finding that rare survivors of copper surface contact killing when re-tested were shown to be as sensitive to copper alloy surface exposure as the original strain [9]. Thus, these sporadic survivors had not acquired a heritable change and are likely to have survived simply for stochastic reasons, sometimes referred to as "persisters". The apparent lack of mutants resistant to copper alloy contact is very intriguing, especially to a geneticist. It implies that (1) mutants resistant to copper surface exposure are extremely rare or (2) mutations that allow survival following copper surface exposure are lethal events. A mutant strain of the desired phenotype can be rare for several reasons. Perhaps multiple genetic changes are required to give the desired phenotype or, alternately, the mutation is so special that it is limited to a particular site in a particular gene. Only "conditional" mutations, such as temperature sensitive mutations, can be isolated in genes encoding essential functions.

Based on this thinking, Hong *et al*. [10] suggested that the unsaturated fatty acids, which are a component of some of the phospholipids in the lipid bilayer of the plasma membrane, met these criteria. First, unsaturated fatty acids are essential and irreplaceable components of biological membranes [74, 75, 76, 77]. Alterations in fatty acid composition affect membrane fluidity and thereby indirectly regulate the activity of membrane proteins, which in the case of prokaryotes includes the cytochromes and the enzymes for phospholipid biosynthesis. Estimates have reported that *E. coli* requires that at least 15 to 20% of membrane fatty acids be unsaturated [74]. Therefore, genetic alterations that eliminate or severely restrict the level of unsaturated fatty acids to less than this critical minimum would be expected to be lethal. Second, transition metals such as iron and copper are capable of catalyzing the formation of reactive oxygen species (ROS), particularly hydroxyl radicals (•OH), via the Fenton reaction shown below [78, 79, 80, 81].

$$Cu^{+} + H_2O_2 \rightarrow Cu^{++} + \bullet OH + OH^{-}$$

The unpaired electron of the hydroxyl radical is highly reactive and capable of causing oxidative damage to cellular macromolecules including lipids, proteins, and nucleic acids. Membrane phospholipids are composed of a polar head group (glycerol 3-phosphate and ethanolamine, serine, or choline) covalently bonded to two long-chain fatty acids (typically 14 to 20 carbons in length) that may contain one or more unsaturated double bonds as well as other modifications [61]. The biochemistry of enzymatic and non-enzymatic lipid peroxidation is reviewed in [58, 82, 83]. ROS, such as the hydroxyl radical formed by the Fenton reaction, drive the non-enzymatic peroxidation of unsaturated double bonds and result in the formation of unstable lipid hydroperoxides that fragment to shorter species, form cross-links, bend, or even circularize creating distortions of the phospholipid bilayer. Ultimately, the biophysical characteristics of the membrane are disrupted leading to a concomitant loss of membrane integrity impeding its role as a selectively permeable barrier that defines the limits of the cell, cell lysis, and ultimately cell death.

Hong *et al*. [10] explored their hypothesis that peroxidation of the unsaturated fatty acids of membrane phospholipids is the initiating event in copper alloy surface-mediated killing by following lipid peroxidation in *E. coli* upon exposure to copper alloy surfaces. *E. coli* cells were exposed to a series of copper-zinc alloys containing between 99.9% copper to 60% copper and the kinetics of killing and lipid peroxidation monitored. They found that the more rapid the decrease in survivors the faster the rate of lipid peroxidation. Moreover, the timing of cell death and lipid peroxidation correlated with the loss in membrane integrity, as measured by the Live/Dead BacLight assay. To test the significance of this correlation, Hong *et al*. [10] utilized an *E. coli* mutant strain having an increased ratio of unsaturated to saturated fatty acids and found sensitivity to copper alloy contact killing, and the rate of lipid peroxidation increased in this mutant strain compared to that observed in the parental strain. As proposed by Hong *et al*. [10], these results clearly implicate the peroxidation of unsaturated fatty acids in the *E. coli* membrane as the cause of the rapid, efficient, and catastrophic cell death observed in cells exposed to dry metallic copper alloy surfaces. San *et al*. [26] extended this work to the Gram-positive *Bacillus subtilis*. Although

B. subtilis spores were resistant to copper alloy surface exposure, they took advantage of a sporulation-defective mutant strain to demonstrate that, similar to *E. coli*, lipid peroxidation correlated with cell death.

4.5. Other potential targets of copper toxicity

The redox properties of copper ions can lead to additional damage, other than the impact on lipids and one must consider these as possible intracellular and/or inner and outer membrane targets responsible for copper toxicity. Proteins and enzymes that functionally depend on free cysteines or disulfide bonds present possible oxidation/reduction targets [61, 84, 85]. Keevil and coworkers reported that chromosomal fragmentation was associated with copper alloy surface exposure and suggested that DNA degradation was key to copper alloy surface killing [20, 39, 86]. This suggestion was not supported by the work of others [12, 70]. Studies on *Deinococcus radiodurans* also indicate that DNA damage is not the primary target of copper alloy surfaces. *D. radiodurans* is highly resistant to ionizing radiation because of its amazing capability to repair DNA damage [53]. Espíritu Santo *et al*. [15] argue that this capability should translate into reduced sensitivity to copper alloy surface killing if DNA damage played a critical role. Instead, they found that the kinetics of copper killing in *D. radiodurans* were essentially the same as for *E. coli*. The work of Hong *et al*. [10] and San *et al*. [26] provides the best evidence that the DNA degradation observed in cells exposed to high copper content surfaces is not the primary cause of killing. Using the copper-zinc alloy C28000, the alloy with the lowest concentration of copper that was still capable of effective killing, both studies were able to identify an exposure time point at which killing was 100% complete (no survivors detected) but, nonetheless, there was no evidence of DNA degradation. It is now widely accepted that genomic DNA degradation is a secondary event in the process of copper-mediated contact killing in bacteria. Additional studies focusing on fungi and viruses are needed.

5. Efficacy of copper alloy surface killing in the clinical setting and public spaces

Although laboratory studies have clearly demonstrated that bacteria and other microorganisms die on contact with copper alloy surfaces, it is critical to determine whether these antimicrobial characteristics translate to the healthcare setting. The most thorough and well-controlled clinical trial of antimicrobial copper's ability to control nocosomial infections is reported in Salgado *et al*. [87]. It was carried out in three major U.S. hospitals by a U.S. team of infectious disease specialists, microbiologists, and statisticians and will be described in detail here. Salgado *et al*. [87] designed the clinical trial to answer the following key questions. Do the surfaces of components made from standard (non-copper) materials, such as stainless steel, plastics, and wood, harbor bacteria in the clinical environment? Similarly, do the surfaces of components made from copper alloys harbor bacteria in the clinical environment? Will a reduction in bacterial contamination levels be observed on copper alloy surfaces of components installed in the clinical environment in comparison to components made from standard (non-copper) materials? And, most importantly, will any observed reduction in microbial burden on the copper alloy surfaces of installed components translate into a reduction in the acquisition of infections by patients in these so-called "copper rooms" compared to patients in standard (non-copper) rooms?

The Medical Intensive Care Unit (ICU) was selected as the most appropriate setting to conduct a clinical trial because their patients often have compromised immunity, and are thus more susceptible to acquiring infections. The consensus was that if copper alloys were found to be antimicrobial in the clinical setting, the introduction of copper would be most beneficial in this ICU setting. The following three hospitals were selected to participate in the clinical trial: Medical University of South Carolina, in Charleston, SC; Memorial Sloan Kettering Cancer Center, in New York City, NY; and Ralph H. Johnson Veterans Administration Medical Center, Charleston, SC. The trial consisted of the following three phases, which were conducted sequentially. Phase 1 measured the baseline microbial burden on existing components made from conventional standard (non-copper) materials located throughout the medical ICU patient rooms. In Phase 2, a suite of components with copper alloy surfaces was

installed in random patient rooms. Following several weeks of adaptation to the ICU environment and then regularly throughout the clinical trial, the microbial burden found on the suite of components with copper alloy surfaces in the "copper rooms" was compared to that found on the same suite of components made from standard (non-copper) materials in the "non-copper rooms". Phase 3 measured infection rates of patients in rooms with standard (non-copper) components, and compared these with the infection rates measured in rooms with the copper components.

On the basis of the microbial burdens found on a variety of components in Phase 1, the following six components were found to be the most contaminated: the bed rails, the nurses' call button, the arms of the visitor's chair, the over-the-bed patient tray table, the intravenous (IV) pole, and the computer data input device, which varied by hospital (mouse, laptop, or the bezel on a touch screen patient monitor). Not so coincidently, these are the components closest to the patient. It was decided that the same six components would be fabricated in copper alloys and installed in random ICU rooms for Phase 2 and Phase 3.

5.1. Phase 1 of the clinical trial: Determine baseline microbial burden on standard components

During this phase, sampling techniques were developed, with the objective of optimizing reproducibility, as well as maximizing the amount of bacteria picked up and released from the sampled area of the surfaces of the components. The final selected technique consisted of a sterile template, which was placed over each surface, and the exposed area was wiped 5 times horizontally and 5 times vertically [88]. It should be noted that the measurement techniques were developed in the medical ICU setting, where the sampling in subsequent phases would occur. The ICU consists of single-patient rooms containing a variety of components constructed from a variety of conventional or standard (non-copper) materials such as plastics, wood, coated steel, aluminum, and stainless steel. These standard (non-copper) components subsequently served as experimental controls in ICU rooms in Phases 2 and 3.

5.2. Phase 2 of the clinical trial: Comparison of microbial burden on components made from standard materials or copper alloys

In order to achieve randomness, patients admitted to the ICU were placed in the first available room, without regard to which rooms contained copper surfaces. Bed control personnel were not informed which rooms contained copper, but treatment teams, who had no role in room assignment, were informed. Personnel, who were also masked or blinded as to which rooms contained copper components, recorded data on patient demographics and clinical characteristics. Each of the hospitals continued to follow existing cleaning procedures as well as protocols prescribed for terminal cleaning. No new cleaning procedures were initiated, nor were additional cleaning cycles implemented, during the trial. Trial personnel had no role in the frequency, time of day, methods or products used for cleaning. Hand hygiene was also monitored at each hospital. In addition, no outbreaks of hospital-acquired infections (HAIs) occurred during the trial. Samples were taken weekly at random times from the six objects in each ICU room. The trial consisted of a total of eight copper rooms and eight control rooms, or a total of sixteen rooms for all three hospitals. Samples were taken from the surface of the components made from copper alloys in copper rooms, as well as standard (non-copper) components made from conventional materials in the standard (non-copper) control rooms. To monitor cleaning, one standard (non-copper) object, the rail at the foot of the bed, was sampled in both the copper and standard (non-copper) rooms, unbeknownst to the participating clinicians, environmental services, or the healthcare teams, to control for bias. In regard to the individual standard (non-copper) components, the bed rail is the most contaminated item in the standard room, followed by the call button, chair arms, IV pole, tray table, and data input device, as shown in figure 3 (taken from [89]). The consensus of experts in the field is that a microbial burden below 250 CFU/cm^2 is generally accepted as benign [89, 90, 91, 92, 93, 94]. It should be noted that the 250 CFU/cm^2 is an approximation, and varies by organism.

While the average microbial burden in the copper rooms, at 465 CFU/cm^2, is almost double the

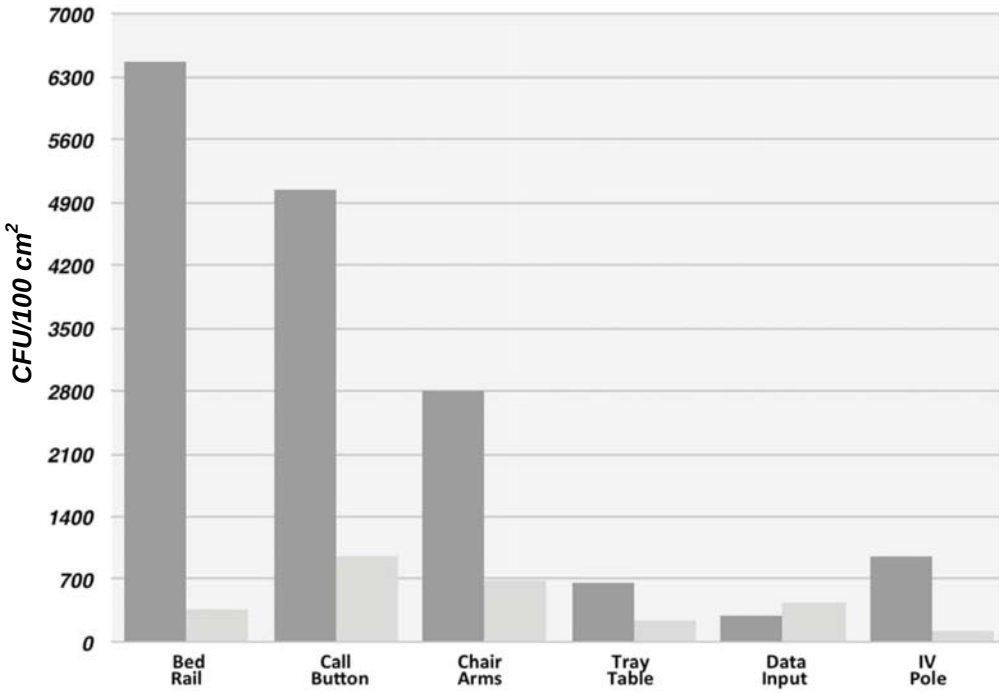

Figure 3. Microbial burden found on six objects from hospital intensive care unit (ICU) rooms. Sample were taken from the standard non-copper ICU rooms (dark gray bars) and from the copper component ICU rooms (light gray bars). (Copyright © American Society for Microbiology, [J. Clin. Microbiol., 50(7), 2012, 2217, doi: 10.1128/JCM.01032-12]).

above-mentioned 250 CFU/cm^2 level generally accepted as benign, the average amount measured in the standard rooms, at 2,674 CFU/cm^2, is an order of magnitude higher than 250 CFU/cm^2. On average, the amount of bacteria measured on the copper components is 83% lower than that found on the standard (non-copper) components. In contrast to the standard (non-copper) components, relatively small differences in microbial burden can be seen between the components in the copper rooms, as shown in figure 4, where the copper call button has the highest level of contamination, followed by the copper chair arms, copper data input device, copper bed rail, copper tray table, and copper IV pole. It should be noted that the level of bacterial contamination seen on the standard (non-copper) bed rails is the highest seen in the trial, as shown in figure 4. It is significant because the bed rail is the focal point of activity in the room. It is a major area of interaction between the patient, healthcare workers, and visitors.

All the copper components have lower contamination levels compared to the standard (non-copper) components, except for the data input device, as can be seen in figure 4. The data input device shows an unexplained anomaly. The microbial burden on the copper data input device is slightly higher than that seen on its standard (non-copper) counterpart. The contamination levels on both data input devices are quite low, plus the difference in contamination levels is the smallest when compared to the other five objects. It should be noted that the data input device is exclusively for the use of the healthcare professionals and not touched by patients. Healthcare professionals as a group are more cognizant of the consequences of infections and this may account for the abnormally low contamination levels seen on both the standard (non-copper) and copper data input devices rather than any difference in the frequency of cleaning.

5.3. Phase 3 of the clinical trial: Infection rates

The objective of Phase 3 was to determine if the introduction of copper components impacted infection rates. In other words, will the reduction of microbial burden, measured on the copper components in the copper rooms, result in fewer

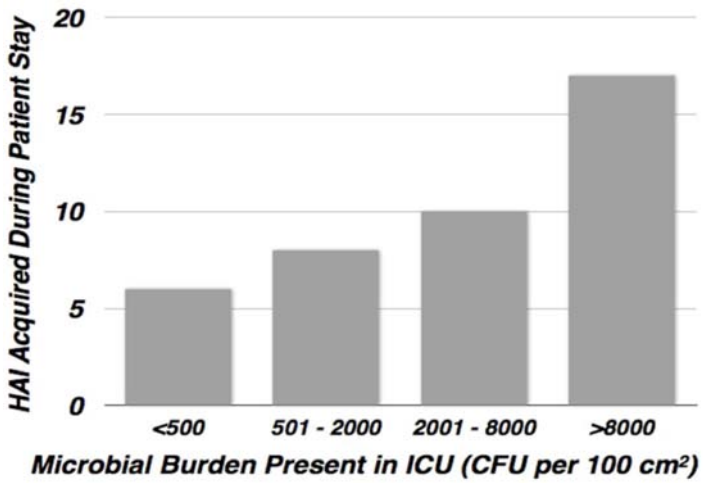

Figure 4. Distribution of healthcare-associated infection as a function of microbial burden. The number of hospital-acquired infections (HAI) acquired during the patient stay is plotted versus the microbial burden of the intensive care unit (ICU) rooms during the patient stay. Standardized procedures were used to swab and titer the level of bacterial contamination on the surface of various components from standard (non-copper) and copper medical ICU patient rooms. (Copyright © Cambridge University Press [Salgado, C. D., Sepkowitz, K. A., John, J. F., Cantey, J. R., Attaway, H. H., Freeman, K. D., Sharpe, P. A., Michels, H. T. and Schmidt, M. G. 2013, Infect. Control Hosp. Epidemiol. Off. J. Soc. Hosp. Epidemiol. Am., 34(5), 479] 'Reprinted with permission').

HAIs, when compared to the number of infections observed during the same period in the standard (non-copper) control rooms without copper surfaces? Clinicians at each hospital determined incidents of HAIs, according to National Healthcare Safety Network definitions, after examining relevant clinical information. However, they were masked or blinded as to patient identity, and the type of room, copper or standard (non-copper), to which the patient was assigned. Demographics and clinical characteristics between patients admitted to copper and standard non-copper rooms were comparable. The infection data recorded in the copper rooms and standard non-copper rooms at the end of Phase 3 were: 3.4% in the copper rooms (10 infections in 294 patients) and 8.1% in the standard (non-copper) rooms (26 infections in 320 patients). This equates to a highly statistically significant (p value of 0.013) reduction of more than 58% in hospital-acquired infection (HAIs) due to the introduction of only six copper components into the copper rooms [87]. It should be noted that the six copper components comprised less than 10% of the surface area of the room. These results are a strong indication of the ability of antimicrobial copper surfaces to continuously kill bacteria in the clinical setting between routine cleanings.

Note the relationship between microbial burden and risk of HAIs shown in figure 4, which illustrates that the risk of acquiring an infection increases as microbial burden increases. In other words, high microbial burden favored acquiring an infection. This observation applies to all the rooms, both copper and non-copper, and is statistically significant (p value of 0.038). However, the cumulative microbial burden was lower in the rooms containing the copper components, which, based on the results in Salgado *et al*. [87], translated into fewer infections. Only 17% of the total 4,450,545 CFUs of bacteria identified from all rooms were recovered from the copper surfaces, while the remaining 83% were found on the standard non-copper surfaces. Thus the microbial burden was almost five times higher on the standard (non-copper) versus the copper surfaces. This implies that cleaner surfaces, meaning lower bacterial burden, favors lower infection rates.

5.4. Limitations and caveats of the clinical trial

This was the first study of its kind that demonstrated that the deployment of an active antimicrobial environmental surface could improve patient outcomes, meaning reduce HAIs. Therefore, as in all scientific studies, additional trials are

needed to confirm and verify the findings reported by Salgado *et al*. [87]. However, the findings reported here are sufficiently striking that they should provide the impetus to hospital decision makers to specify antimicrobial copper for their hospitals. The Salgado *et al*. [87] trial was designed as an intent-to-treat randomized control trial. Blinding healthcare workers is impractical because of the unique appearance of copper alloys. However, the copper objects were in place for nine months prior to the collection of clinical data related to infection status. As the healthcare workers became accustomed to seeing the copper surfaces every day, it is unlikely that the presence of copper had a lasting influence on their behavior. Furthermore, the ICU staff was not told that Phase 3 of the study, to measure infection rates, had commenced. The collection of samples from surfaces continued during this period. Thus, the ICU staff and others had no indication that the last phase of the trial, related to HAIs, had started. For additional information, it is suggested that the original paper be consulted [87].

5.5. Conclusions to be drawn from the Salgado *et al.* clinical trial

Taken collectively, these studies clearly make a strong argument for incorporating antimicrobial copper alloys into infection control practices in hospitals. Publication of the Salgado *et al*. [87] study demonstrating that copper surfaces reduced the acquisition of HAIs represents the first instance in which a continuously active antimicrobial material was shown to significantly reduce infections that are contracted by hospitalized patients. In addition, it is the first illustration of the correlation between microbial burden and infection rates, which increase as microbial burden increases, resulting in more infections. Incorporation of copper into essential items within the built environment of hospitals offers the potential for a unique, passive solution to control and limit HAIs, reduce medical costs and save lives.

5.6. Efficacy of antimicrobial copper in public spaces

Grand Central Terminal in New York City is a major transportation hub, through which 750,000 commuters and travelers pass each day. A ten-week pilot study was conducted in 2012, just prior to Grand Central Terminal's 100th anniversary in 2013. The objective was to explore whether architectural components made from copper alloys retained their antimicrobial characteristics after decades of being touched by humans, even while recognizing that the surfaces in the mass transit facility are not subjected to the same cleaning frequency as hospitals. Bacterial levels were measured on three types of surfaces, brass railings versus non-brass railings (wood, marble, and stainless steel), brass shelves versus marble ticket counters, and brass door pulls and push bars versus glass and wood surfaces. The bacterial reduction was comparable or better than those found in the clinical trial. Specifically, a 97% reduction in bacteria was found in the brass rails, a 92% reduction in brass shelf, and an 84% reduction on the door hardware, when compared to the surfaces made from conventional non-copper materials. The pilot study demonstrated that copper alloys retained their ability to kill bacteria after over an extended time measured in decades, even when they are not subject to the cleaning frequency seen in hospitals.

6. Future for antimicrobial copper

Our goal in writing this review is to inform scientific, medical, architectural, and engineering professionals about antimicrobial copper. Clearly, widespread deployment of copper alloy components to frequently touched surfaces, such as doorknobs and handrails, has the potential to significantly reduce the rate of transmission of infections in the clinical setting and public-use spaces. There is a great deal more to do. More research is needed to further explore the array of microbial species sensitive to copper alloy surface exposure. Along with this, it is essential that the mechanism of killing in these species be determined. Mechanism is of particular importance because the ease of becoming resistant to copper alloy surface exposure is a serious consideration when deciding to incorporate copper components into the built environment of public spaces and medical facilities. Additional clinical trials are required to strengthen and extend the results of Salgado *et al*. [87] and confirm the efficacy of including copper components in reducing the spread of hospital-acquired infections. Similarly, well-designed scientific investigations should also be undertaken in public

venues like schools, cruise ships, and transportation systems to determine whether the spread of infectious disease can be better controlled by adding copper components in these environments.

Antimicrobial copper also has the potential to stave off the impending crisis caused by the rise in antibiotic resistance. The U.S. Center for Disease Control reports that widespread abuse and overuse of antibiotics, particularly in raising animals for human consumption, is the major contributor to the emergence, persistence, and spread of resistant bacteria (http://www.cdc.gov/drugresistance/). The use of subclinical doses of antibiotics to improve growth rates of cattle, chickens, and others, has made food-producing animals a major reservoir of antibiotic-resistant bacteria that are then transmitted to humans through the food supply [95]. The introduction of antimicrobial copper into the built environment of food production and animal husbandry could reduce the need for antibiotics. In past decades, antibiotics were too frequently prescribed for humans and animals without first confirming the presence of a sensitive bacterial infection. Additionally, too often patients are noncompliant and stop taking the antibiotic as soon as their symptoms disappear. Over-the-counter availability of antibiotics in many countries has further complicated the problem.

The major roadblock for expanding the use of antimicrobial copper is the difficulty that has been encountered in getting this information to the public, although government regulatory agencies have also slowed progress. We are confident that, once these difficulties are surmounted, antimicrobial copper will be commonplace in the built environment and will serve as a shining signal to the public that their health is being protected.

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CONFLICT OF INTEREST STATEMENT

None to indicate.

ABBREVIATIONS

HAIs, Hospital-acquired infections; Cu, Copper; B.C.E./C.E., Before the common era/Common era.

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