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In situ antimicrobial behavior of materials with copper-based additives in a hospital environment

Humberto Palza [a,*], Mauricio Nuñez [b], Roberto Bastías [b], Katherine Delgado [c]

[a] Departamento de Ingeniería Química y Biotecnología, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile
[b] Laboratorio de Microbiología, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[c] Universidad Bernardo OHiggins, Santiago, Chile

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ABSTRACT

Copper and its alloys are effective antimicrobial surface materials in the laboratory and in clinical trials. Copper has been used in the healthcare setting to reduce environmental contamination, and thus prevent healthcare-associated infections, complementing traditional protocols. The addition of copper nanoparticles to polymer/plastic matrices can also produce antimicrobial materials, as confirmed under laboratory conditions. However, there is a lack of studies validating the antimicrobial effects of these nanocomposite materials in clinical trials. To satisfy this issue, plastic waiting room chairs with embedded metal copper nanoparticles, and metal hospital IV pools coated with an organic paint with nanostructured zeolite/copper particles were produced and tested in a hospital environment. These prototypes were sampled once weekly for 10 weeks and the viable microorganisms were analysed and compared with the copper–free materials. In the waiting rooms, chairs with copper reduced by around 73% the total viable microorganisms present, showing activity regardless of the microorganism tested.

Although there were only low levels of microorganisms in the IV pools installed in operating rooms because of rigorous hygiene protocols, samples with copper presented lower total viable microorganisms than unfilled materials. Some results did not have statistical significance because of the low load of microorganisms; however, during at least three weeks the IV pools with copper had reduced levels of microorganisms by a statistically significant 50%. These findings show for the first time the feasibility of utilizing the antimicrobial property of copper by adding nanosized fillers to other materials in a hospital environment.

1. Introduction

Environmental surfaces are a reservoir for pathogens and play a key role in the acquisition of healthcare infection [1,2]. In particular, hospital touch surfaces, such as door handles, touch plates, bed rails, call buttons, and toilet seats, can be highly contaminated with microbes [3]. This issue led some years ago to the use of copper-based alloys in healthcare settings as a mechanism to reduce environmental contamination and prevent healthcare-associated infections, as a complement to traditional protocols. This strategy is based on the evidence that copper and its alloys are outstanding antimicrobial surface materials that kill a wide range of microorganisms, including *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Listeria monocytogenes*, influenza A virus, and even *Clostridium difficile*, among others [4]. Starting with the ancient Egyptians around 2500 BC, there is considerable evidence confirming the relevance of copper in treating infection [5]. However, it is only during the last decades that the high biocidal activity of copper compounds has become scientifically clear. Moreover, metal surfaces with copper were more effective at reducing bacterial survival compared with other metals [6,7]. This activity depends on the material composition and the environment, as shown by comparing a set of alloys under different external conditions, where the efficacy in killing bacteria increases at higher copper content, temperature, and relative humidity [3]. The antimicrobial activity of copper compounds also depends on the surface structure; e.g., electroplated copper surfaces killed bacteria more rapidly than either polished copper or native rolled copper [8]. Indeed, when compared with silver, another well–known antimicrobial metal, copper has the advantage of being more active under typical indoor environments, such as dry conditions and low temperatures [9].

The antimicrobial activity of copper metal compounds is related mainly to the release of ions that attack both the membrane and the internal structures of bacteria [2,10–12]. Copper toxicity arises from several mechanisms based on the free ions forming reactive oxygen species (ROS) or organic complexes inside the bacteria. Excessive amounts of copper in the body in most humans can be

* Corresponding author. Departamento de Ingeniería Química y Biotecnología, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile.
E-mail address: hpalza@ing.uchile.cl (H. Palza).

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controlled by either decreased absorption or increased excretion of its ions [13]. Therefore, copper found in current daily materials, such as medical products (i.e., dental and intrauterine devices) or even cosmetics, is not harmful [13]. Consuming copper-contaminated water or foods is associated with the development of acute gastrointestinal symptoms, but not with increased mortality from liver disease [13]. However, copper may cause cell damage and contribute to neurodegenerative disorders under certain medical conditions, predisposing the patient to increased copper concentrations [13]. Therefore, toxic effects associated with copper are rare in individuals who are not suffering specific diseases, such as Wilson's disease and idiopathic copper toxicosis, or not consuming high amounts of the metal, which has an estimated lethal dose of about 10 g [13,14].

Although the efficacy of copper in killing microbes has been the subject of extensive laboratory research, in vivo studies are limited [2]. The first in situ clinical trial tested three items for microbial contamination quantification: a toilet seat coated with a pure copper/resin composite (70% copper); a set of brass tap handles (60% copper), and a brass door push plate (70% copper) [4]. These results were compared against equivalent items with plastic, chrome-plated, and aluminum surfaces, respectively. The numbers of microorganisms found in the copper-containing items were between 90% and 100% lower than in the controls, showing that surfaces with copper may significantly reduce the numbers of microorganisms in a clinical environment. In another study, touch surfaces associated with push plates, doorknobs, and light switches were replaced by surfaces composed of metallic copper-containing alloys [15]. The total number of bacteria on copper surfaces was 63% lower than on control surfaces, with the largest difference found for doorknobs and the lowest for push plates. Another clinical trial found similar trends by using metal sheets of pure copper on touch surfaces associated with both desks and trolleys [16]. The study showed that the antimicrobial activity of copper touch surfaces reduced the environmental bioburden to a far greater extent than standard materials, and it would be beneficial in the healthcare environment. The effect of copper alloy-surfaced objects in reducing the risk of hospital–acquired infection (HAI) in intensive care units (ICU) was recently tested by measuring the rates of incident HAI and/or colonization with MRSA or vancomycin-resistant Enterococcus (VRE). The rate of HAI and/or MRSA or VRE colonization in ICU rooms with copper alloy surfaces was significantly lower than in standard ICU rooms [17].

During the last years, nanotechnology has utilized the antimicrobial effects of copper and highly biocidal nanoparticles have been synthesized [12,18]. Nanoparticles can dissolve faster in a given solution volume compared with larger particles; thereby releasing a larger amount of metal ions and presenting stronger antimicrobial effects than either microparticles or metal surfaces [12,19]. Indeed, cytotoxicity induced by nanosized copper particles is not only related to the released ions, but rather to the particles themselves, having higher toxicity than released metal ions, as these small particles can pass through the cell membrane more easily [20]. Copper nanoparticles can also adhere to the bacterial cell wall [21]. Copper ions from these nanoparticles destroy the bacterial cell wall and the cytoplasm is then degraded and disappears, resulting in cell death. Regardless of the kind of copper attacking the cells (either ions or nanoparticles), the ion is the final active agent, because even assuming that nanoparticles are the toxic agents, the intracellular dissolution attributed to pH effects is the final mechanism explaining their toxicity [22]. These nanoparticles can be either immobilized or coated on surfaces for application in several fields, including medical instruments and devices, water treatment, and food processing [18]. However, their combination with polymers to form composites better and more easily utilizes the antimicrobial activity of these nanoparticles. The advantage of these polymer nanocomposites is that the final material has the processability and properties of the polymer matrix together with the biocidal activity of the nanoparticle, thus extending the range of application of antimicrobial agents. Examples of antimicrobial polymer composites with copper nanoparticles can be found elsewhere, using a broad range of organic matrices from current thermoplastic materials to organic coatings [23–28]. Similar to metallic copper, the release of copper ions from these polymer composites can explain their biocidal behavior [12,29]. This mechanism explains why polymer/copper nanocomposites showed better antibacterial effects than equivalent microcomposites [19]. Despite the potential antimicrobial application of copper to materials other than metals and alloys, there are few studies validating the antimicrobial effects of these composite materials in clinical trials. To overcome this issue, two prototypes were produced in the present contribution: 1) waiting room chairs made of polypropylene thermoplastic with embedded copper metal nanoparticles [26]; and 2) metal hospital IV pools coated with an organic paint with nanostructured zeolite/copper particles [28]. Both materials showed antibacterial behavior under laboratory conditions, and in the current study they were tested in situ in a hospital environment.

2. Methods

2.1. Materials

Metal copper nanoparticles of around 500 nm and lamellar morphologies were used as fillers of commercial grade isotactic polypropylene thermoplastic. For the plastic/metal composites, a melt mixing method was used at both laboratory and industrial scales. A commercial synthetic zeolite with copper nanocrystals was used as filler in a polyester surface coating paint. For the coating process, a voltage between 60 and 90 kV was used and later the products were placed in an oven at 200 °C for 15 min.

2.2. Laboratory scale tests

For antimicrobial tests at the laboratory scale, a polypropylene sample with 10 wt% of copper nanoparticles was prepared using a Brabender Plasticorder melt mixer. The sample of the antimicrobial coating was prepared by the standard method described above. In this case, samples with 0.2, 1.0, and 3.0 wt% of modified zeolite were tested.

For the antimicrobial tests, the samples were treated according to ISO 22196-2007 with few modifications. Briefly, *E. coli* (K-12) and *S. aureus* (isolated environmental strain) cultures were grown in LB medium at 37 °C. A pure culture of specified bacteria was grown overnight and then diluted in growth-supporting LB broth to an O.D. of 0.2 (~1×10^6 CFU/mL). The initial bacterial concentration was determined by incubating in a plate at 37 °C for 24 h. A 100-μL aliquot of the bacterial broth was poured into the sterilized sample surface and covered with a glass at 37 °C during a given time. After the prescribed time had elapsed, the samples were transferred to glass tubes with 5 mL saline solution (0.88 wt% NaCl) supplemented with 1 wt% of Tween 80, and vortexed to remove the bacteria. An aliquot of 100 μL of each sample was diluted 0.1, 0.01, and 0.001 times in saline solution, and then 100 μL of each dilution was inoculated in LB solid medium and incubated at 37 °C. After 24 h of incubation, bacterial colonies were counted to determine viable CFU/mL. The results were presented as % Inhibition: [(initial bacterial concentration)-(bacterial concentration after incubation)]/(initial bacterial concentration), and % reduction: [(bacterial concentration after incubation in control sample)-(bacterial concentration after incubation in sample with copper)]/(bacterial concentration after incubation in control sample). All the experiments were performed at least twice.

2.3. Prototypes for in situ tests

For the in situ validation of the antimicrobial effects of polymer/copper nanocomposites, two prototypes were produced at an industrial scale. The first prototype consisted of polypropylene waiting room chairs with 10 wt% of embedded metal copper nanoparticles. The second prototype was metal hospital IV pools coated with an electrostatic coating process using a polyester paint with 5 wt% of nanostructured zeolite/copper particles. Both prototypes were located in the La Florida Hospital in Santiago, Chile. The waiting room chairs were located in a public area, while the IV pools were located in the operating rooms. Together with these copper-based prototypes, both unfilled waiting room chairs and IV pools were evaluated as controls. Both the filled and unfilled prototypes were cleaned according to standard protocols without any modification. The samples were placed during January (summer time), and the microbial count was performed from March to May (autumn time), once a week for 10 weeks.

For the in situ microbial counts in the hospital, samples were collected as reported above, with modifications [30]. Briefly, every week, five control chairs/IV pools and five copper-modified chairs/IV pools were selected randomly. Samples were collected using sterile 100-cm^2 templates placed over the selected surface. Two samples were obtained from each chair using sterile swabs (Deltalab), with ten horizontal movements and ten vertical movements. Samples were transported to the laboratory at 4 °C in container tubes with phosphate-buffered saline (PBS) with 0.5% Tween 80, and then immediately analysed for total and specific viable microorganisms using classical microbiological methods. For total bacteria, each sample was diluted 0.1, 0.01, and 0.001 times, and then 100 μL of each dilution was inoculated in Agar Tripticase Soy (BD Difco™). The same volume was inoculated in Baird Parker Agar medium (BD Difco™) and Sabouraud Dextrose SDA agar medium (BD Difco™) to determine the viable *Staphylococcus* concentrations and molds concentrations. CFU counts were performed after 48 h of incubation at 37 °C. IV pool samples were collected in a similar way but using 20 cm^2 templates.

3. Results

3.1. Antimicrobial effects at the laboratory scale

Before the industrial production of both prototypes, and to evaluate the effect of the scaling-up, a polypropylene composite with 10 wt% of copper was produced at the laboratory scale, and its antimicrobial behavior against *E. coli* and *S. Aureus* was tested using a standard laboratory test. The same was done with a metal sample with a polyester coating paint filled with 0.2, 1.0, and 3.0 wt% of zeolite/copper. In the latter case, a set of filler concentrations was used, as previous results were not found for both modified zeolite particles and organic matrices. These results are displayed in Tables 1 and 2 for plastic and coating materials, respectively. Copper nanostructures embedded in the organic matrices provided strong antimicrobial effects, killing both kinds of bacteria. The results show that bacterial reduction was as high as 100% in the filled organic coating, probably due to the thin layer of polymer facilitating water diffusion. In both filled polymers, the antimicrobial efficiency barely differed according to the kind of bacteria studied, and our composites were able to kill both *E. coli* and *S. aureus*. A comparative study between both samples is not possible because not only are the thicknesses different, but also the kind of filler and its concentration differ.

Table 1
Inhibition and reduction of *E. coli* and *S. aureus* bacteria by polypropylene prototypes with 10 wt% of copper nanoparticles, evaluated at different times. A polypropylene prototype without copper additives was used as a control.

Bacteria	Time [h]	% Inhibition	% Reduction
Escherichia coli	6	100.0	89.1
	8	100.0	98.7
	24	100.0	99.3
Staphylococcus aureus	6	99.8	98.6
	8	99.9	98.2
	24	100.0	98.6

Table 2
Inhibition and reduction against *E. coli* and *S. aureus* bacteria of organic coating prototypes with different concentrations of copper nanoparticles evaluated at different times. An organic coating prototype without copper additives was used as a control.

Bacteria	Time [h]	% Inhibition For additive concentration of: 0.2/1.0/3.0 wt%	% Reduction For additive concentration of: 0.2/1.0/3.0 wt%
Escherichia coli	6	97.1/99.5/99.5	78.9/96.1/96.7
	8	99.9/100.0/100.0	99.3/99.9/100.0
	24	100.0/100.0/100.0	99.1/99.9/99.9
Staphylococcus aureus	6	98.5/99.3/98.3	95.3/99.7/94.5
	8	100.0/100.0/100.0	99.4/97.7/99.5
	24	100.0/100.0/100.0	99.8/99.6/100.0

Fig. 1 shows the ion release from our polypropylene/copper composites prepared at the laboratory scale after soaking in deionized water. The results clearly show that these composites release copper ions, which explains the antimicrobial effects shown in Table 1. Results from metal-coated samples are not presented, as the concentrations released were too low and the results were affected by other ions released from the metal sample itself.

The results in Tables 1 and 2 show that a product made of polypropylene containing copper particles, and a metal surface coated with a polyester resin containing zeolite/copper additives, could be useful in healthcare settings. Due to the variabilities in environmental conditions and bacterial concentrations and species, among other differences, it is expected that the high antimicrobial activity of these samples measured at the laboratory scale will not be replicated in a clinical trial. However, to our knowledge this kind of comparison had not been reported previously.

3.2. In situ antimicrobial effects in a hospital environment

Fig. 2 shows the total amount of viable microorganisms found in control waiting room chairs located in the hospital. The control chairs presented a load of microorganisms around 24.9 CFU/cm^2. Fig. 2 also displays the total amount of viable bacteria on waiting room chairs with copper particles. The results show an important

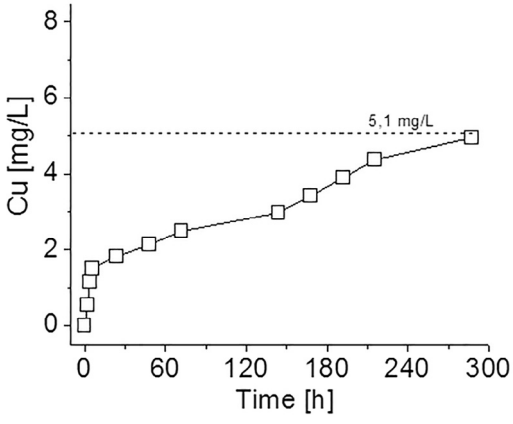

Fig. 1. Release of copper ions from a polypropylene sample with 10 wt% of metal copper nanoparticles soaked in water for different times.

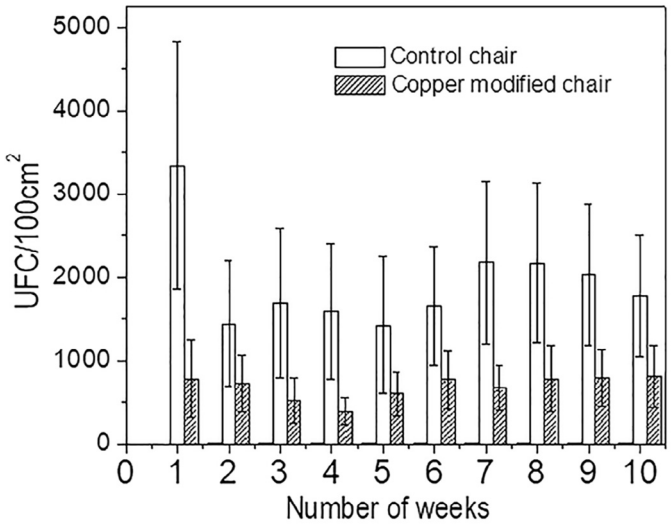

Fig. 2. Total amount of viable microorganisms present in waiting room chairs located in a non–clinical area of the hospital. Control chairs had an average over the 10 weeks of 24.9 CFU/cm^2, while chairs with copper presented an average of 6.8 CFU/ cm^2.

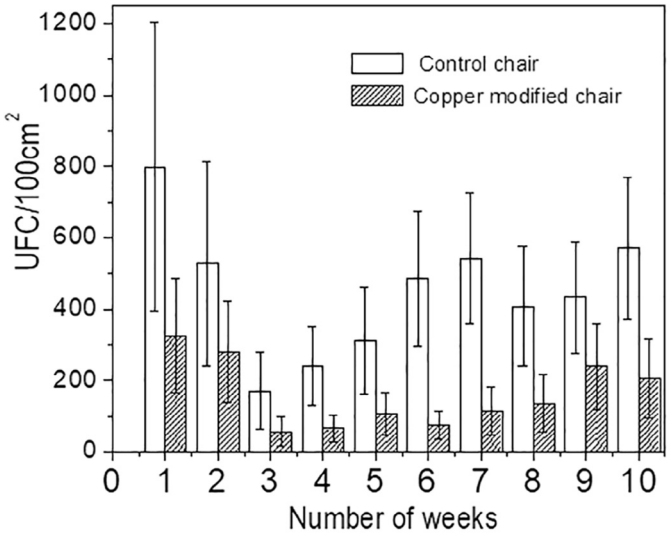

Fig. 3. *Staphylococcus* species present in the viable microorganisms from the waiting room chairs. Control chairs had an average over the 10 weeks of 4.5 CFU/cm^2, while chairs with copper had an average of 1.6 CFU/cm^2.

reduction in bacterial content compared with the control. This reduction in bacterial load was observed during the whole sampling period and was statistically significant. The amount of CFU was reduced from an average of 24.9 CFU/cm^2 in control chairs to 6.8 CFU/cm^2 in chairs with copper particles, which is a 73% reduction of microorganisms. The observed reduction was similar to previous results from copper alloy surfaces. For instance, an overall 71% reduction in the bacterial load on copper alloy surfaces was found compared with that of control surfaces in a primary health care clinic [2,16]. In an oncology, respiratory, and geriatric ward, an average of 63% reduction in bacterial load on copper surfaces compared with the controls was also reported [15]. Therefore, the addition of copper particles to a polymer renders an antimicrobial behavior to the plastic material similar to that of copper metal alloys.

Of the different bacteria present in a hospital, *Staphylococci* are highlighted as an important cause of both nosocomial and community-acquired infections [31]. Indeed, in the last decade, *Staphylococcal* infection has reemerged as a cause for concern because of its numerical increase and the spread of MRSA isolates in the community. Molds, on the other hand, were previously considered to be of low virulence or even 'non–pathogenic', but during the last decades many medical institutions reported that they were becoming common pathogens in nosocomial infections [32]. Based on these reports, we analysed in Figs 3 and 4 the amount of *Staphylococci* and total molds present in the viable microorganisms from chairs. These results show that the amount of *Staphylococci* is around 4.5 CFU/cm^2, which represents 20% of the total microorganisms present on chairs. The total amount of molds is low, around 0.6 CFU/cm^2, which is close to the detection limit of the microbial analysis (0.1 CFU/cm^2). The copper additive in chairs reduced both pathogens, with values similar to those for total viable microorganisms. For *Staphylococci* the load decreased from 4.5 to 1.6 CFU/cm^2, representing a statistically significant 64% reduction during the whole period. Molds, on the other hand, were also reduced by 50% during the whole period in chairs containing copper from 0.6 to 0.3 CFU/cm^2, this was statistically significant only during the period between weeks 5 and 9 (Fig. 4). The error and the high standard deviations are associated with the low CFU values found for molds.

To further analyse the effect of copper additives on microorganism load, metal hospital IV pools were coated with an organic paint with nanostructured zeolite/copper particles and located in operating rooms. The idea was to analyse the effect of copper additives on the bacterial load in places with the highest hygiene protocols. The effect of the hygiene protocols is confirmed by the drastic reduction in the total amount of viable microorganisms found in the control IV pools compared with waiting room chair controls, decreasing from 24.9 to 1.3 CFU/cm^2, as displayed in Fig. 5. The organic copper coating showed no clear biocidal effect during six weeks because of the low bacterial load; the standard deviations were large enough to represent differences without statistical significance compared with the controls. Despite these experimental deviations there was a statistically significant reduction in bacterial load using the copper–modified surface from week 5 to 7: the bacterial load reduced by 53.0%, which is similar to the results from the waiting room chairs. These results confirm that a surface

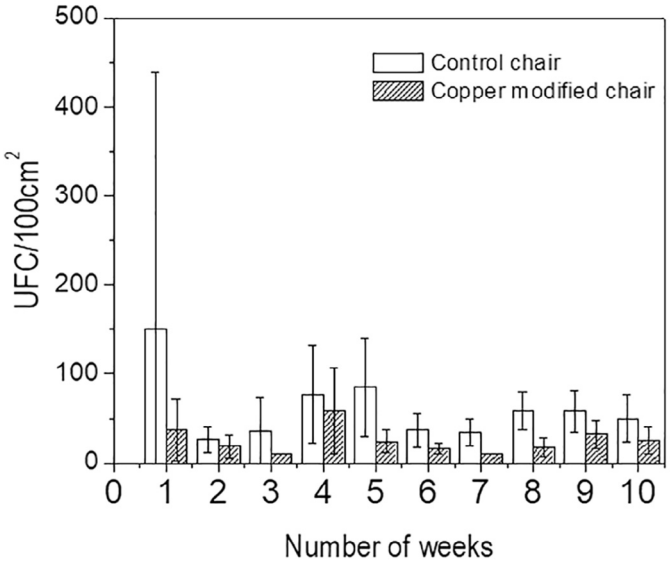

Fig. 4. Molds present in the viable microorganisms from the waiting room chairs. The low value found in this case is close to the resolution of the technique used for the microbial analysis. Therefore, some differences between the controls and copper-containing chairs do not have statistical significance. However, during statistically significant differences were found during weeks 5 to 9.

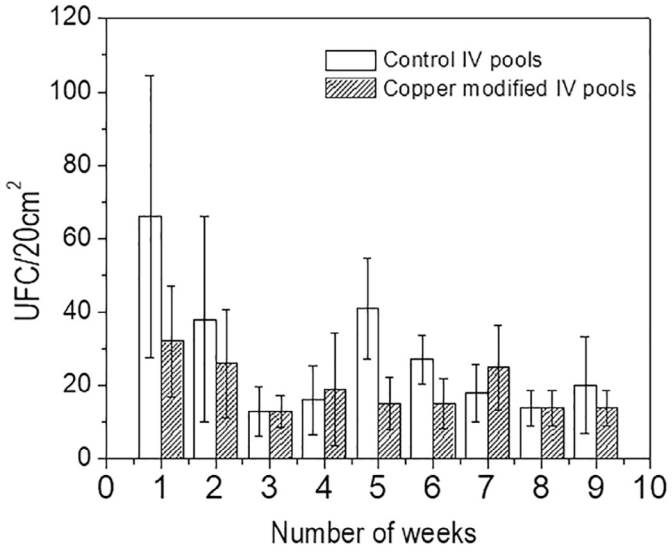

Fig. 5. Total amount of viable microorganisms present in metal hospital IV pools coated with an organic paint and located in operating rooms. Most of the samples did not display statistically significant differences because of the low amounts of microorganisms found. However, during weeks 5 to 7, a significant difference was found between the controls and the samples coated with copper additives.

modified with copper additive reduces the amount of total microorganisms present, even under extreme hygiene protocols. Due to the low bacterial load, details about the presence of *Staphylococci* and molds were not quantified, as the values were below the detection limit (0.5 CFU/cm^2).

4. Discussion

The release of copper ions from copper-based material seems to be the main mechanism for the antimicrobial behavior of this material [12]. Although Cu^+ contributes to the toxicity, producing highly reactive hydroxyl radicals by Fenton-like reactions [33], Cu^{2+} is considered the main biocidal agent [10]: it forms organic complexes with functional groups in the microorganisms, resulting in either defects in the conformational structure of nucleic acids and proteins or changes in the oxidative phosphorylation reactions and osmotic balance. Copper ions are therefore the real active compounds, either killing (cidal effect) or inhibiting the growth (static effect) of microorganisms [34]. A direct relationship between ion release rate and antimicrobial behavior was recently found in polymer/copper nanocomposites, showing that the inverse of the time needed to eliminate 50% of *E. coli* bacteria presented a linear relationship with the release rate of copper ions [29]. Therefore, samples releasing larger amounts of ions displayed better antimicrobial behavior, and in polymer/copper nanocomposites a tailored antimicrobial behavior can be achieved depending on the type and concentration of copper nanoparticle [19,27].

Fig. 1 shows that waiting room chairs with copper particles released metal ions, which explains the strong antimicrobial behavior observed (Table 1) despite the different conditions used for each test. Based on the relationship between metal ion release and killing effect, it is concluded that both the hospital and the in vitro environment promote the release of copper ions from the particles embedded in the polymer matrix. Moreover, comparing results from Fig. 2 and Table 1, the relevance of the antimicrobial test and its scale are highlighted. In a laboratory antimicrobial test, this sample presented almost 100% efficiency against *E. coli* and *S. aureus* tested in isolation, but in situ antimicrobial tests showed a 76% reduction of total microorganisms. The difference can be related to the environmental conditions (temperature and moisture), with the presence of bacterial biofilm limiting the action of antimicrobial agents, as reported elsewhere [35].

The hospital environment may be a significant reservoir for potential pathogens that can then be extended by vectors such as air turbulence, aerosolized moisture, unwashed hands, or direct contact with an inanimate object, equipment, or material [1]. Moreover, this dynamic environment can be further affected by the season, weather conditions, indoor ventilation system design and operation, intrusion of moisture, the outdoor microbial load, the number of occupants and visitors, and human activities in general [36]. Even in clinical areas, the hands of healthcare workers represent a mode of transmission where exemplary hand hygiene cannot break the chain of infection when the environment is heavily contaminated. Considering the presence of a high visitor density without any hand hygiene protocols, the bacterial load will probably increase [36]. The bacterial load found in these control samples was in the range of previous results coming from frequent-touch surfaces from both clinical and non–clinical areas in a hospital, having values between 0 and 35 CFU/cm^2 [37]. Another study reported values ranging from 2.5 to 40 CFU/cm^2 depending on the location and surface analysed [38]. These studies concluded that higher microbial counts are found in the non–clinical areas. Therefore, the bacterial load found in the control chairs in our study agrees with previous reports. These results associated with the total amount of microorganisms presented in public waiting room chairs stressed the relevance of the use of copper additives, as they were able to reduce significantly the large amount of microorganisms present in these surfaces. This contribution shows that under large bacterial load and real environmental conditions, materials with copper additives are effective in killing microorganisms, with reductions similar to those of copper-based metals and alloys.

Although Tables 1 and 2 did not display any strain specificity, this effect should be considered in the discussion of the behavior of antimicrobial materials. For instance, the biocidal behavior of copper ions depends on the bacteria tested as a double membrane and periplasmic space decrease ion transport in Gram-negative bacteria thereby reducing the biocidal effect of copper compared with Gram–positive bacteria [39,40]. In a study including 569 different bacterial isolates, the *Salmonella* isolates were generally less susceptible to copper sulfate, followed by *E. coli* and the Gram-positive species [41]. This strain specificity is confirmed with copper metal nanoparticles, where the Gram- positive *Bacillus subtilis* MTCC 441 strain was found to be more sensitive than *E. coli* and *S. aureus* [42].

The toxicity of copper metal nanoparticles is well known to be highly correlated with the particle size/specific surface area by in vivo studies in animals [43]. The mechanism is not related to the particle directly, but to the accumulation of excessive alkalescent substance and copper ions, culminating in metabolic alkalosis and copper ion overload [43]. Copper nanoparticles can further trigger both intrinsic and extrinsic apoptotic pathways in oxidative stress-mediated kidney dysfunction [44]. However, these studies focused on particles in solution, whereas our materials used nanoparticles embedded into a polymer matrix without any cell cytotoxicity effect even at concentrations as high as 20 wt% [45]. There is little particle migration of nanoparticles in these dense polymer matrices [45].

5. Conclusions

Our results show for the first time in a clinical trial that copper additives are a viable route for applying the antimicrobial effects of copper to organic materials, such as plastics and organic coatings. Plastic waiting room chairs with embedded copper metal nanoparticles, and metal hospital IV pools coated with an organic

paint with nanostructured zeolite/copper particles were produced and tested in a hospital environment. In the waiting rooms, copper additives reduced the total viable microorganisms by around 73%, showing an activity independent of the microorganism tested. These antimicrobial results are similar to those found in copper alloys. The IV pools containing copper that were installed in surgical rooms presented lower total viable microorganisms than unfilled materials, although some results did not show statistical significance because of the low load of microorganisms. However, for at least 3 weeks, the IV pools with copper were able to statistically significant reduce (by 50%) the amount of microorganisms.

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Ethical approval: Not required.

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