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Review

Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces

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ABSTRACT

The development of polymer nanocomposites with antimicrobial properties has been a key factor for controlling or inhibiting the growth of microorganisms and preventing foodborne diseases and nosocomial infections. Commercially available antibacterial products based on silver-polymer are the most widely used despite the fact that copper is considerably less expensive. The incorporation of copper nanoparticles as antibacterial agents in polymeric matrices to generate copper-polymer nanocomposites have presented excellent results in inhibiting the growth of a broad spectrum of microorganisms. The potential applications in food packaging, medical devices, textiles and pharmaceuticals and water treatment have generated an increasing number of investigations on preparing copper based nanocomposites and alternative polymeric matrices, as potential hosts of nano-modifiers. This review presents a comprehensive compilation of previous published work on the subject, mainly related to the antimicrobial activity of copper polymer nanocomposites. Within all the phenomenology associated to antibacterial effects we highlight the possible mechanisms of action. We discuss the differences in the susceptibility of Gram negative and positive bacteria to the antibacterial activity of nanocomposites, and influencing factors. As well, the main applications of copper polymer-metal nanocomposites are described, considering their physical and chemical characteristics. Finally, some commercially available copper-polymer nanocomposites are described.

Contents

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1. General background

The incorporation of metal nanoparticles in a polymer matrix generates new materials called nanocomposites. The combination of the different properties of these components (polymer and nanoparticles) can render a material with improved optical, electronic, mechanical and antimicrobial properties. Nanocomposites with antibacterial properties can be obtained either by incorporating nanoparticles with known antibacterial activity, or by enhancing the antibacterial properties that the polymeric matrix already has. In the latter case the substantial enhancement of biocidal capacity has been associated with a synergistic effect of the two components present in the composite. Therefore, the polymer not only provides a supporting matrix for nanoparticles, but can also enhance antibacterial performance and extend the possible applications of this material to meet several requirements in the biomedical field, water treatment, and food industry, among others.

There is an urgent need to develop materials that can control or prevent microbial colonization due to emerging infectious diseases that are affecting global economies and public health. Medical devices such as, endotracheal tubes, vascular and urinary catheters, and hip and knee prosthetics are responsible for over half the nosocomial infections in the United States [1]. These and other medical devices are made from polymeric materials [2]. The longer a nosocomial pathogen persists on the surface of a material, the longer it exists as a source of transmission and thus endangers susceptible patients or health workers [3]. The treatment of infections associated with medical devices can be difficult and expensive. In 2011, 1 in 25 hospital patients in the United States had at least one health care-associated infection (HAIs). Out of an estimated 722,000 cases, 75,000 patients died during hospitalization [4]. Central line-associated bloodstream infections were the most costly HAIs, followed by ventilator-associated pneumonia, surgical site infections, clostridium difficile infection, and catheter associated urinary tract infections. The total annual cost for these 5 major infections is $9.8 billion dollars [5].

In the food industry, pathogens and biofilms can proliferate on the surface of foods or packaging. Microbiological contamination costs the food industry millions of dollars annually in terms of lost or downgraded products [6]. Foodborne pathogens are a major contributor to human illness, hospitalization, and deaths per year. The costs of foodborne illness in the United States is estimated at $152 billion dollars per year for acute medical care and long-term health—related costs, and more than a quarter of these costs, an estimated $39 billion, are attributable to foodborne illnesses associated with fresh, canned and processed products [7]. According to the Centers for Disease Control and Prevention, in the United State alone, some 48 million illnesses and 3000 deaths are caused annually by bacterially contaminated foods. *Salmonella spp.*, *Listeria monocytogenes*, *Campylobacter spp.*, *Staphylococcus aureus* and *Toxoplasma gondii* are among the top pathogens causing foodborne illness and death [8].

Faced with this global public health problem, the incorporation of metal nanoparticles in polymeric materials is an excellent strategy to control bacterial growth. Metallic nanoparticles have been widely studied as antibacterial agents due to their recognized toxicity against bacteria, yeast and some virus. These biological properties depend of the metal, size, structure, and large surface of the nanometric particles. Metal oxide nanoparticles such as ZnO, TiO_2, CeO_2, MgO and CaO have been investigated as inorganic antibacterial agents, although the majority of research are currently centered on copper and silver. Examples of the first are studies on TiO_2 suspensions, which have proved to hold effective antibacterial properties towards *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa* and viruses and, in some cases, this behavior appears to be enhanced by UV light activation [9]. Photoactivation of TiO_2 can generate electron hole pairs that generate $O_2^{\bullet -}$ and $OH^{\bullet -}$ radicals. These radicals are very effective in degrading organic contaminations as well as in providing an antimicrobial function. However, the use of TiO_2 nanoparticles under UV light can produce genetic damage in human cells and tissues [10]. As with TiO_2, the antibacterial activity of Zinc oxide has been studied largely against pathogenic and nonpathogenic bacteria. ZnO nanoparticles are believed to be nontoxic, bio-safe, and biocompatible and have been also used as drug carriers, cosmetics, and also in medical devices. Silver nanoparticles are definitely the most popular inorganic nanoparticles as antimicrobial agents [11]. Their use as additives has been widely beneficial for the improvement of various plastic products, textiles and coating-based usages [12], therefore placing silver NPs as holders of a wide range of biomedical applications [13]. Several nanocomposites based on chitosan, poly(ethylene glycol), cellulose, PVP-alginate containing silver have been prepared for biomedical applications as antibacterial wound-dressing [14,15,16,17]. Silver nanocoatings could be effective in preventing hospital infections when deposited on intravenous catheters [18]. However, even if introduced 10 years ago in the US and five years ago in UK, the use of silver-coated urinary catheters has been sporadic in clinical practice, probably due to cost implications [19]. Copper nanoparticles, given their unique chemical, physical and biological properties are of great interest to potential applications in medicine [20–21]. At low concentrations copper is a cofactor for metalloproteins and enzymes, therefore, having the advantage of low toxicity when comparing to other metals. In addition, copper is inexpensive in relation to (3.6 USD/lb) other metals with antibacterial properties such as silver (30 USD/lb), therefore proving to be a cost-effective material [22]. Different polymers have been used as matrices to support copper nanoparticles and generate composite materials with antimicrobial properties. Among these polymeric matrices are: agar [23], bamboo-rayon [24], bovine serum albumin [25], carboxymethylcellulose (CMC) [26–27], cellulose [28–30], chitosan [31–37], cotton [38–40], cotton-cellulose [41], cotton silica [42], epoxy resin [43–44], glass (prepared by Sol-gel) [45], high-density polyethylene (HDPE) [46–47], hydrogel based on acrylamide and acrylic acids [48], linear low-density polyethylene (LLDPE) [49], low-density polyethylene (LDPE) [50], nylon [51], polyamine [52], polyaniline (PANI) [53–54], poly(D,L-lactide-*co*-glycolide) [55], poly(ethylene glycol diacrylate) hydrogel [56], polylactic acid [57], polymers based on acrylic acid, acrylonitrile and methyl methacrylate [58], polymethylmethacrylate (PMMA) [59], polypropylene [60–63], polystyrene (PS) [64], poly (styrene-co-sulfonic acid) [65], polythiophene [66], polyvinyl alcohol (PVA) [67], polyvinyl chloride (PVC) [68], polyvinylmethylketone (PVMK) [69–70], polyvinylidenefluoride (PVDF) [71], and silica [72–73].

Given the increase in the number related to the development and study of the antibacterial properties of the copper-polymer nanocomposites, their excellent antibacterial properties, and relatively low production cost, this review presents a comprehensive compilation of research, focused mainly on the antimicrobial activity of copper polymer nanocomposites. Within all the phenomenology associated to antibacterial effects we highlight the possible mechanisms of action. We discuss the differences in the susceptibility of Gram negative and positive bacteria to the antibacterial activity of nanocomposites, and influencing factors. Moreover the main applications of copper polymer-metal nanocomposites are described, considering their physical and chemical characteristics. Finally, some commercially available copper-polymer nanocomposites are described.

2. Copper-polymer nanocomposites

2.1. Copper nanoparticles

Copper has been used as an antimicrobial for hundreds of years. In ancient Egypt (2000 BCE), copper was used to sterilize water and wounds. During the Roman Empire, copper cooking utensils were used to prevent the spread of disease. The early Phoenicians nailed copper strips to ship hulls to inhibit fouling and thus increase speed and maneuverability. In the Second World War, Japanese soldiers put pieces of copper in their water bottles to prevent dysentery [74]. In the 1880s, mixtures of copper sulfate, lime, and water or copper sulfate and sodium carbonate were used in the US and France as fungicides [75]. However, copper ions and copper compounds can be toxic to microorganisms, humans and the environment [76–77]. In this respect, copper nanoparticles act as a reservoir for the controlled release of copper ions and thus inhibit their toxicity. Copper nanoparticles can easily be oxidized to form copper oxides. To prevent oxidation it is necessary to use an inert atmosphere of nitrogen or argon, and organic coatings [78]. Currently, there are several synthesis methods to obtain copper nanoparticles, including three main techniques: chemical, physical and biological [79]. The main chemical techniques to synthesize copper nanoparticles are chemical reduction [80], micro-emulsion (colloidal) [81], sonochemical reduction [82], electrochemical [83], microwave-assisted [84], and hydrothermal syntheses [85]. Biological synthesis is considered a type of chemical technique, although it generates nanoparticles by reducing metallic ions by biomolecules present in plants [86], plant extracts [87], fruits [88], marine algae [89], bacteria [90] and yeast [91]. Physical methods of copper nanoparticle synthesis are laser (pulse) ablation [92], metal vapor [93], pulsed wire discharge [94], and mechanical milling [95]. Physical methods enable the production of copper nanoparticles of different sizes and morphologies. However, the process requires costly equipment and the quality of the product is lower than the nanoparticles produced by chemical methods [79]. There are different working conditions to control the growth and geometry of nanoparticles during chemical synthesis, such as the choice of stabilizer, solvent and reducing agent. Although chemical synthesis generates nanoparticles with specific characteristics, the main disadvantage is the relative toxicity of these products. In this sense biological synthesis is more environment-friendly. Nevertheless, chemical synthesis is a much more popular technique.

Several studies have shown that nanoscale copper exhibits a broad spectrum of microbial activity, inhibiting the growth of bacteria, fungi, viruses and algae [28,69,96,97]. Early studies related to the antibacterial action of copper nanoparticles were conducted in systems with suspended nanoparticles (not incorporated into matrices). Antibacterial mechanisms have been associated with multiple toxicities, such as generating reactive oxygen species, lipid peroxidation, protein oxidation and DNA degradation in *E. coli* cells [98].

Large amounts of ROS (most likely superoxide anions) are produced directly on surface defect sites in nanocrystalline CuO. Applerot et al. propose that the combined actions of the strong adherence of the CuO particles to the bacterial cell membrane, together with ROS generation on the particle surface, cause an increase in cell permeability, leading to an uncontrolled transport of CuO particles through the cytoplasmic membrane and ultimately to cell death. This mode of operation predominantly increases in the case of small nanometric scale CuO particles given their higher surface-to-volume ratio, resulting in the formation of more ROS per unit weight, and a higher probability of cell penetration [99]. In addition Applerot et al. have demonstrated that Gram negative bacteria are more susceptible to the action of CuO NPs that Gram positive bacteria. This behavior is attributed to the presence of golden carotenoid pigments which provide integrity to the cell membrane and promote a more powerful oxidation resistance. A schematic illustration of the antibacterial mechanism of CuO NPs is shown in Fig. 1. When the bacterial defense mechanisms are overwhelmed by the CuO-induced ROS, the Programmed Cell death (PCD) genetic module is triggered, which in turn stimulates an outbreak of oxidative stress, and it is this burst of radicals that is lethal to the cells rather than the initial CuO-induced ROS [100,101]. Cu^{2+} ions originating from NPs may interact with phosphorus and sulfur-containing biomolecules such as DNA and protein, distorting their structures and disrupting biochemical processes [102,103].

Respect to DNA degradation, *in vitro* interaction between plasmid pUC19 and copper nanoparticles revealed that DNA degradation was markedly inhibited by the presence of EDTA, a metal ion chelator, suggesting the active role of Cu^{2+} ions in degradation [98]. Other studies suggest that copper nanoparticles interact with –SH groups, leading to protein denaturation [104]. Bondarenko et al. showed that CuO nanoparticles induce the formation of anion superoxide, hydrogen peroxide and single-stranded DNA, already at very low sub-toxic levels (0,1 mg Cu/L) [105].

Other studies have demonstrated that the antibacterial properties of copper nanoparticles associated with the release of Cu^{2+}, are directly related to size. It has been observed that ion release from nanoparticles (diameters around 10 nm) embedded into polypropylene matrix increases quickly exhibiting a sharp maximum during the first day; meanwhile, in microcomposites (diameters around 45 μm), the release rate increases, slowly releasing ions. The antibacterial behavior of nanocomposites containing 5 v/v% of copper is able to reduce the concentration of *S. aureus* in 99.8% after 60 min, while microcomposites showed lower activity at the same time [61].

2.2. Polymers as a key for developing functional materials with antimicrobial capacity

As noted above, the use of polymers for developing nanocomposites with antimicrobial activity does not only provide a supporting function for nanoparticles, but can also enhance the antibacterial performance of nanocomposites. This behavior is related mainly to three phenomena: 1. synergy between the polymer and copper nanoparticles that increases the antibacterial capacity of the material (nanocomposite) over that of the separate components [53]; 2. The polymers capability for long term ion release, thus prolonging the antibacterial activity of nanocomposite [71]; 3. The effect of increasing the surface area, associated with the fine dispersion of copper nanoparticles in the polymer, on the level of antibacterial activity [46]; and 4. The increase in range of technological requirements for using copper nanoparticles. An example of the first case, Bogdanovic et al. reported a synergistic antibacterial effect between the nano-modifier (CuNps) and the host (PANI) in a copper-polyaniline nanocomposite (Cu-PANI) against *E. coli*, *S. aureus* and *C. albicans*. In effect, the nanocomposite exhibits greater antimicrobial activity than does either component separately. This observation can be explained as follows: [1] Physical interactions and electrostatic contacts play a role in determining the antimicrobial efficacy of the nanocomposite; [2] The PANI stabilizes the structure with steric hindrance, and decreases the potential aggregation of copper nanoparticles, ensuring a highly effective surface contact area to interact with cell surfaces; [3]

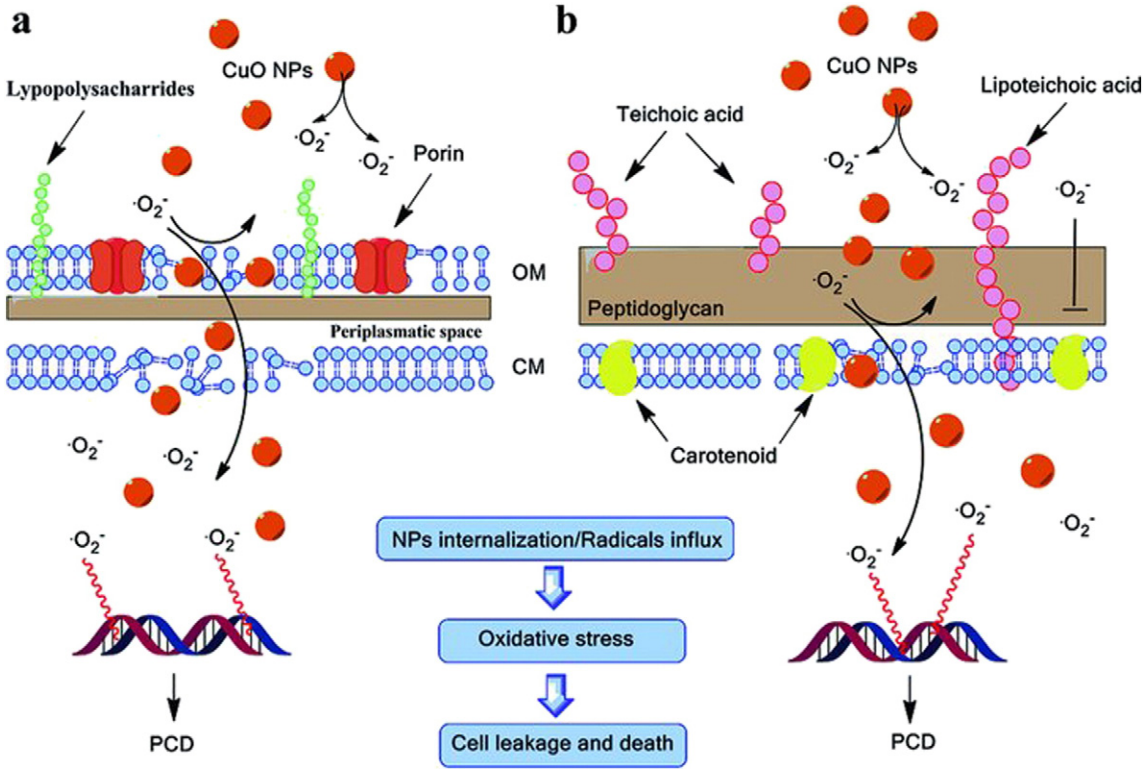

Fig. 1. Schematic illustration of the antibacterial mechanism of CuO NPs and the relative cellular structure of (a) *E. coli* (Gram negative) and (b) *S. aureus* (Gram positive). Carotenoid pigments of *S. aureus* provide integrity to its cell membrane and increases its protection against oxidative stress. The damage to the bacterial cell is mediated by the harmful superoxide anions formed by the cells attached/internalized CuO NPs.
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The network of PANI nanofibers increases the interface area between the nanocomposite and microbes, granting interaction between copper nanoparticles and functional groups of the cell walls; and [4] The slow oxidation of copper nanoparticles results in the release of Cu^{2+} ions from the surface of CuO, and in the partial reduction of these to Cu^{+} ions. The movement of Cu^{+} ions through the lipid bilayer is favored energetically, causing cell damage when taken in [53]. Cioffi et al. argued that nanostructured polymeric coatings are extremely attractive materials as they are capable of controlling the release of metal species and possess biostatic properties that can easily be tailored [71].

Currently, there numerous articles on the development of copper-polymer nanocomposites, which are summarized in Table 1. Among the most commonly used polymeric matrixes are natural polymers, such as cellulose, chitosan, and polyolefins like polyethylene and polypropylene. The most important properties and results related to the development of copper nanocomposites are described below.

2.2.1. Chitosan

Chitosan is a N-deacetylated derivative of chitin, which is a natural mucopolysacharide that forms the exoskeletons of crustaceans and insects. Chitosan has biocompatible, biodegradable, non-toxic, antioxidant, antibacterial, antifungal and adsorbent properties [111–113]. The presence of OH and NH_2 groups in its macromolecule can form various chemical bonds with metals and form stable chelate complexes that can withstand washing with organic solvents. Capability of chitosan as a chelating agent makes it a perfect material for metal nanoparticle synthesis [114]. In an acetic acid medium chitosan reacts with H^{+} ions to produce protonized chitosan with $-NH_3^{+}$ functional groups. The zeta potential of nanoparticles incorporated in polymers increases with chitosan concentration due to greater availability of protonized $-NH_3^{+}$ on nanoparticle surfaces, which increases electrostatic repulsion among the particles and therefore reduces the incidence of agglomeration, resulting in more stable nanoparticle dispersion [36].

Copper nanoparticles synthetized without chitosan have shown extensive aggregation and a high degree of polydispersity, while the finest and narrowest copper nanoparticle size distribution is obtained with chitosan. Antimicrobial results confirm marked growth inhibition, even after of 4 h of contact [34]. Mallick et al. synthetized copper nanoparticles using iodine as a stabilizing agent in a chitosan polymer. Electron microscopy and cytometry studies revealed that the nanocomposite was attached to the bacterial cell wall, causing irreversible damage to the membrane [31]. Copper nanoparticles/chitosan composite films prepared by the solution casting method showed effective antimicrobial activity against *S. aureus* and *S. enteric*, which is associated with the deformation and disintegration of bacterial cell walls (Fig. 2) [37]. Manikandan et al. synthetized copper nanoparticles using chitosan as a capping and reducing agent. Copper ions adsorbed in chitosan were reduced by using sodium hydroxide at specific pH to produce a CuO/chitosan nanocomposite, and applied on cotton fabric by the pad-dry cure technique. Copper-chitosan nanoparticles inhibit Gram negative and positive bacteria. Agar diffusion tests have shown greater inhibition of Gram negative than Gram positive bacteria, which the authors attributed to the differences in bacterial cell walls [32]. Zero valent copper has also been synthetized in chitosan matrices and its antibacterial activity has been evaluated against *S. epidermidis*, *E. coli*, and the spore form of *B. cereus* [35].

2.2.2. Cellulose and cotton

Cellulose is the most important skeletal component of wood. Cellulose-based materials are relatively cheap, renewable, abundantly available in a variety of forms, and have hydroxyl groups that are accessible for chemical modification [115–116]. Cellulose is widely used in biomedical devices, textiles and packaging. However, these cellulose-based materials do not have antibacterial activity *per se*. The preparation of hybrid composites based on copper nanoparticles-cellulose has generated a large number of materials with antibacterial activity. An

Table 1
Different copper-polymer nanocomposites and their antimicrobial effects.

Polymer matrix	NP Size (nm)	Microorganism	Innoculus (CFU/mL)	Incubation time/contact time (h)	Antibacterial Assay	Effect	Refs
Agar	40–250	*L. monocytogenes*, *E. coli*	10^5	12–16	Bacterial growth kinetics	High antibacterial activity against both Gram positive and Gram negative bacteria.	[23]
Bamboo-rayon	<100	*S. aureus E. coli*	10^5	24	ATCC 100 method	*S. aureus* and *E. coli* bacterial growth were reduced by 100%.	[24]
Bovine Serum albumin	5	*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *E. coli* ATCC, 35218, *S. aureus* ATCC 25923, *A. luteus* ATCC 21606, *S. aureus* 3A	10^7	18	Agar diffusion test, minimum inhibitory concentration (MIC), cytoplasm leakage analysis	Nanocomposites with 50 μg/mL of copper completely inhibited the growth of *E. coli*.	[25]
Carboxymethyl cellulose (CMC)	10–20	*E. coli* DH5α	10^8	10	Count of colony-forming unit	No detectable *E. coli* survivors.	[26]
Carboxymethyl cellulose hydrogel	40–75	*E. coli*, *S. aureus*	N. A.	24	Agar diffusion test	Antibacterial activity with inhibition zones of 19 and 14 mm against S. aureus and *E. coli*, respectively.	[27]
Cellulose	5	*A. baumannii*	10^5–10^8	0,16	ASTM method E2149–01, agar diffusion test	The nanocomposites showed a reduction of 8-log after 10 min. of contact.	[28]
Cellulose	25–60	*S. aureus*, *E. coli*	10^6	24	Count of colony-forming unit	After 1 h both bacteria were annihilated.	[22]
Cellulose	50	*S. cerevisiae*	10^6	24	Count of colony-forming unit	The nanocomposites showed a reduction of 4 log after 24 h of contact.	[29]
Cellulose vegetal, cellulose bacterial	36	*S. aureus*, *K. pneumoniae*	10^{-4}–10^{-5}	24	Count of colony-forming unit	Bacterial viability about 2 log bacterial growth over 24 h incubation.	[30]
Chitosan	8	*E. coli*, *B. cereus*	N.A	24	Determination of minimum inhibitory concentration (MIC)	The MIC values of Cu Nps for *E. coli* and *B. cereus* were 21,5 and 27,29 μg/mL, respectively.	[31]
Chitosan	20–30	*E. coli*, *Salmonella paratyphi*, *Bacillus*	N.A	24	agar diffusion test	More antibacterial activity was observed against Gram negative bacteria.	[32]
Chitosan	20	*S. aureus*, *E. coli*	$1.5–3 \times 10^5$	24	ATCC test method 147	RBC values of 99 and 96% for *S. aureus* and *E. coli*, respectively.	[33]
Chitosan	<40	*E. coli* ATCC 25922	10^4–10^5	4	N.A	Marked growth inhibition effect.	[34]
Chitosan	1–40	*S. epidermidis*, *E. coli*, *B. cereus* (spore form)	10^3–10^6	24	Count of colony-forming unit	No growth.	[35]
Chitosan		*E. coli*, *B. subtilis*	10^8	24	Minimum inhibitory concentration (MIC), minimal bactericidal concentration (MBC)	The MIC values for *E. coli* and *B. subtilis* were 0.433 and 0.33 mg/L. respectively.	[36]
Chitosan	10.6	*S. aureus*, *S. enterica*	N.A.	16	Count of colony-forming unit, Transmission electron microscopy	Cell wall deformation associated with the cytoplasmic volume decrease, and subsequent disintegration.	[37]
Cotton	15	*E. coli*, *S. aureus*	10^7	1–3	Count of colony-forming unit	After 3 h, the nanocomposites showed 100% reduction of bacterial growth for *S. aureus* and *E. coli*.	[38]
Cotton	50	*S. aureus*, *E. coli*	10^7	18–24	ATCC 100 and 147 methods	High antimicrobial efficiency with inhibition zones of 2,8 and 2,5 mm against *S. aureus* and *E. coli*, respectively.	[39]
Cotton	10	*E. coli*, *S. aureus*	N.A.	24	Count of colony-forming unit	No growth of bacterial colonies in the presence of copper nanocomposite.	[40]
Cotton-cellulose	29	*E. coli*	10^8	48	Agar diffusion test, colony-forming unit count, bacterial growth kinetics	Copper nanoparticle-loaded fibers show less biocidal action than copper alginate copper cellulose (ion).	[41]
Cotton-silica	40	*S. aureus*, *E. coli*	10^6	18	ATCC 100 method	The percentage of bacterial growth reduction was >70 and 90% for *E. coli* and *S. aureus*, respectively.	[42]
Epoxy resin	6,7	*S. aureus*, *C. Albicans*, *Chlorella sp.*	89.5×10^5	N. A.	Minimum inhibitory concentration (MIC), bacterial growth kinetics, agar diffusion test	Strong antibacterial activity against *C. albicans*, with inhibitory effect at the concentration range of 54–75 μg/mL.	[43]
Epoxy resin (BPSE)	10–20	*K. pneumoniae*, *S. aureus*	N.A.	24	Agar diffusion test	Nanocomposites with 3 wt% of copper nanoparticles exhibit inhibition zones of 26 and 23 mm for *K. pneumoniae*, *S. aureus*, respectively.	[44]
Glass (prepared by Sol-gel)	87	*E. coli* ATCC 25922	N. A.	24	Colony-forming unit count	Antibacterial activity for nanocomposites with 10 mol%.	[45]
High-density polyethylene (HDPE)	200–400	*E. coli* DHSa, *P. fluorescens* BS3, *S. aureus*	1.2×10^5	24	Count of colony-forming unit	Significant antibacterial effect for the nanocomposites containing 2,5–5 wt%	[46]

(continued on next page)

Table 1 (*continued*)

Polymer matrix	NP Size (nm)	Microorganism	Innoculus (CFU/mL)	Incubation time/contact time (h)	Antibacterial Assay	Effect	Refs
						Cu-nanofibers.	
High-density polyethylene (HDPE)	7.5–8	*E. coli* ATCC 8739, *S. aureus* ATCC 6538, *S. typhimurium* ATCC 14028, *P. fluorescens*, *B. cepacia*	10^6	N.A.	ATP test method	Bactericidal and bacteriostatic effects.	[47]
Hydrogel based on acrylamide and acrylic acid	20	*E. coli*, *S. aureus*	N. A.	24	Agar diffusion test	Inhibition zone of 19 and 25 mm for S. aureus and *E. coli*, respectively.	[48]
Hydroxypropylmethyl cellulose (HPMC)	53.28	*Streptococcus A.*, *S. epidermis*, *B. cereus*, *E. faecalis*, *Salmonella*, *P. aeruginosa*, *S. aureus*	N. A.	24	Agar diffusion test	High antibacterial activity against Gram positive bacteria with inhibition zone of 13 mm for *Streptococcus A.*	[106]
Linear low-density polyethylene (LLDPE)	20–50	*S. aureus* ATCC 25923	N.A.	16	JIS Z2801:2000 standard	Bactericidal rate of nanocomposite about 96%.	[49]
Low-density polyethylene (LDPE)	20	*E. faecalis*	1.5×10^8	24–96	Minimum inhibitory concentration (MIC), count of colony-forming unit	The number of bacterial colonies decreased to several hundred and dozen after 24 h.	[50]
Nylon	85	*S. aureus*	N. A.	24	ATCC 147 method	Inhibition zones.	[51]
Polyamine	3–15	*C. albicans*	10^8	48	Agar diffusion test	Inhibition zone of 12 mm against *C. albicans* at total concentration of copper of 600 g/100 L.	[52]
Polyaniline (PANI)	6	*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *C. albicans* ATCC 10259	$1.5–3 \times 10^5$	1–2	ASTM Standard E-2149-01	Bacterial growth was reduced by 99,9% for *E. coli* and *S. aureus*, and 97,9% for *C. albicans*, respectively.	[53]
Polyaniline (PANI)	50	*S. aureus*, *Bacillus*, *E. coli*, *Pseudomonas*	10^5	2–10	Agar diffusion test, viability assay, wet interfacial contact method	Disruption of bacterial cell membrane.	[54]
Poly(D,L-lactide-*co*-glycolide)	7	*E. coli*	10^8	4–16	Measurement of optical density, electron transmission microscopy	Composites showed complete inhibition of *E. coli*.	[55]
Polyethylene	2–4	*L. monocytogenes* ISP 6508	10^6	6–24	Colony-forming count, transmission electron microscopy, confocal microscopy	Bactericidal and bacteriolytic effects associated with penetration of nanoparticles and copper ion release.	[107]
Polyethylene	2–4	*E. coli*	10^6	16	Transmission electron microscopy, confocal microscopy	TEM image reveal damage to the bacterial membrane together with cytoplasmic material outside the membrane.	[108]
Poly(ethylene glycol diacrylate) hydrogel	3.2	*S. aureus*, *E. coli*	10^7	Overnight	Modified Kirby-Bauer test	Inhibition zone of 25 and 20 mm for *S. aureus* and *E. coli*, respectively.	[56]
Polylactic acid	36	*P. fluorescens*, *P. putida*	10^3	24	JIS Z 2801 method	6-log reduction of bacterial growth after 24 h of incubation.	[57]
Polymers based on acrylic	5–15	*Chlamydomonas* CD1 Red, *Synechocystis* PCC 6803, *Phaeodactylum tricornutum* CCMP 1327	N.A.	24	Bacterial growth kinetics	Nanocomposites exhibit antimicrobial activity similar to that of conventional copper based biocides (Fairmount Chemical Company).	[97]
Polymers based on acrylic acid, acrylonitrile and methyl methacrylate	25	*S. aureus*, *P. aeruginosa*	2×10^5	18	Minimum inhibitory concentration (MIC), Minimal bactericidal concentration (MBC)	The MIC value obtained is 200 y 400 μg/mL for P. aeruginosa and S. aureus, respectively.	[58]
Polymethylmethacrylate (PMMA)	14	*S. aureus* ATCC 25123	10^5	12–24	Minimum inhibitory concentration (MIC), count of colony-forming unit	The nanocomposite inhibits bacterial growth, but is not capable of killing the bacteria on its surface.	[59]
Polypropylene	30–60	*S. aureus* ATCC 6838, *P. aeruginosa* ATCC 13388	1.25×10^5	1–6	ASTM Standard E-2180-079	Antibacterial test, showed 95% of bacteria killed after 6 h of contact.	[109]
Polypropylene	5	*E. coli*	10^6	0.5–6	Colony-forming unit count	After 4 h, the nanocomposite killed >99.9% of bacteria.	[60]
Polypropylene	10	*S. aureus*, *P. aeruginosa*	10^5	0,5–1,5	Colony-forming unit count	Nanocomposites with 5 vol.% of copper, reduced the concentration of *S. aureus* by 99.8% after 60 min.	[61]
Polypropylene	20	*E. coli*	10^8	0.5–6	Colony-forming unit count	The nanocomposites eliminated >99.9% of *E. coli* after 4 h of contact.	[62]
Polypropylene	10	*E. coli*	10^8	0.5–6	Colony-forming unit count	After just 4 h of contact, the nanocomposites killed >95% of *E. coli*.	[63]
Polystyrene	7	*P. fluorescens* BS3, *B. circulens* BP2, *E. coli* DHSα, *S. aureus*	N.A.	N.A.	Agar diffusion test	Nanocomposite with 1,5% copper nanoparticles show the highest degree of inhibition against all four kinds of bacteria, but *Bacillus circulens* is more sensitive.	[64]
Poly(styrene-*co*-sulfonic acid)	10–120	*E. coli*, *S. aureus*	N. A.	24	Minimum inhibitory concentration (MIC)	After 24 h of incubation, the nanocomposites killed 99.99 and 98.45%	[65]

Table 1 (*continued*)

Polymer matrix	NP Size (nm)	Microorganism	Innoculus (CFU/mL)	Incubation time/contact time (h)	Antibacterial Assay	Effect	Refs
						of *E. coli* and *S. aureus*, respectively.	
Polythiophene (prepared by Sol-gel)	20–30	*E. coli*, *S. aureus*, *C. Albicans*	10^7	24	Agar diffusion test, minimum inhibitory concentration (MIC), minimal bactericidal concentration (MBC)	High antibacterial activity against C. albicans, the inhibition zone is 38 mm for nanocomposite with 80 wt%.	[66]
Polyvinyl alcohol (PVA)	9.2	*E. coli* DH5α	10^8	48	Count of colony-forming unit	Nanocomposite with 0.6 wt% copper nanoparticles showed a reduction of up to 5-log.	[67]
Poly(vinyl chloride) (PVC)	4	*E. coli* ATCC 25922	10^5	24–84	Agar diffusion test, adhesion assays	Antibacterial activity associated with the release copper ions is discarded. The adhesion ability of E. coli was inhibited after 4 days of incubation.	[68]
Polyvinylmethylketone (PVMK), Polyvinylchloride (PVC), Polyvinylidenefluoride (PVDF)	4.6–5.3	*S. cerevisiae*, *E. coli*, *S. aureus*, *L. monocytogenes*	N. A	4–24	Colony-forming unit count	Bacteriostatic activity associated with controlled copper release.	[69,71]
Poly-vinyl-methyl-ketone	1,7–6,3	*S. cerevisiae*, *E. coli*	N. A.	48	Colony-forming unit count	Significant decrease in the cellular growth with 21 and 0 CFU for E. coli and S. cerevisiae, respectively.	[70]
Silica	<10 nm	*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. cloacae* ATCC 29249, *C. albicans* ATCC 11282, *P. citrinum* ATCC 42504	10^6	24	Agar diffusion test	The antibacterial activity against Gram negative bacteria is superior to that against Gram positive bacteria.	[72]
Silica	20	*E. coli* ATCC 25922	10^6	24	Antibacterial drop-test	The antibacterial activity is better for Cu than CuO nanoparticles, in darkness and under light irradiation.	[73]
Silica	25	*E. coli*, *S. aureus*, *C. albicans*	10^6	24	Agar diffusion test, transmission electron microscopy, minimum inhibitory concentration (MIC)	High antibacterial efficiency with inhibition zones of 16.8, 16 and 10 mm against *S. aureus*, *E. coli* and *C. albicans*, respectively. TEM images show a dramatic effect on the morphology of the bacterial cell.	[110]

interesting application of cellulose nanostructures is as a template and stabilizer for metallic nanoparticles for packaging materials [117]. Copper nanoparticles incorporated in cellulose or cotton fibers have also been used for wound dressing. Antibacterial activity of vegetal and bacterial cellulose, based on nanocomposites may also be influenced by the structure of the polymer, for example, vegetal cellulose shows greater antibacterial effects than bacterial cellulose. The latter has a three-dimensional internal organization that acts as a protective cage for copper nanoparticles, which limits copper ion release compared to the more open structure of vegetal cellulose [30]. Cellulose or cotton fibers can be chemically modified with carboxylic groups on the surface to obtain chelation-controlled binding of cupric ions, followed by chemical reduction with sodium borohydride to generate nanostructured coating. This material shows excellent antibacterial properties against multidrug-resistant bacterial pathogens like *Acinetobacter baumannii* [28]. Copper nanoparticles in carboxymethylcellulose (CMC) demonstrated effective antibacterial properties against the non-pathogenic surrogate of foodborne *E. coli* [26]. Jia et al. elaborated copper nanoparticles embedded on cellulose film surfaces, and found strong and efficient antibacterial activity against *S. aureus* and *E. coli*, completely inhibiting the viability of bacteria within 1 h [22]. The antimicrobial activity of copper nanoparticles incorporated in biodegradable hydroxypropyl methylcellulose (HPMC) for applications as food packaging materials were evaluated against four Gram positive and four Gram negative bacteria. The bactericidal effect was greater for nanocomposite films against *S. aureus*, *B. Cereus*, *S. Epidermidis*, *Strep A.* containing 5 wt% of copper salt [106]. Copper oxide nanoparticles generated by borohydride reduction were incorporated in cellulose fibers. The antimicrobial activity of these nanocomposites was effective against *S. cerevisiae* fungi in melon and pineapple juice, while metallic copper micro/nanostructures generated by heat or heat/UV only showed strong antifungal activity in pineapple juice, while weak activity in melon juice, which was probably due to the pH level [29]. Copper oxide nanoparticles microencapsulated by an ionic gelation method were deposited and distributed on the surface of cotton fabric. The fabric was highly effective in reducing bacteria, 99.99% and 92.71% for *S. aureus* and *E. coli*, respectively, demonstrating its potential use in the manufacture of medical apparel [39]. Berendjchi et al. prepared cotton fabrics impregnated with silica sols doped to 0.5% with copper nanoparticles. The antibacterial assays showed a significant reduction in bacteria, over 70% for *E. coli* and 90% for *S. aureus*. Samples with 2% of copper nanoparticles had lower antibacterial activity. This result is associated with the agglomeration of nanoparticles due to a higher concentration [42]. On the other hand, antibacterial properties of copper oxide nanoparticles uniformly deposited on the surface of cotton fabrics using ultrasound irradiation (Fig. 3) showed a significant bactericidal effect against *E. coli* and *S. aureus* bacteria, reducing 99.9% of colony forming units of both bacteria after 1 h of incubation, demonstrating the potential application in wound dressing [38].

2.2.3. Polypropylene

Polypropylene (PP) is the most widely used thermoplastic polyolefin polymer, with applications in packaging, textiles and the automotive industry due its good processability and physical and thermal properties. These properties are governed by the type and amount of crystalline and amorphous regions formed from the polymer chains [118]. To date there have been some studies of antibacterial activity of copper polypropylene-nanocomposites. The main focus of these studies is the release of copper ions to completely eliminate bacterial growth in a few hours. When polypropylene copper nanocomposites are treated with argon plasma against *S. aureus* and *P. aeruginosa* [109], antibacterial activity is 400% higher than that of untreated films for 3 h of exposure. This enhancement has been attributed to the increased number of

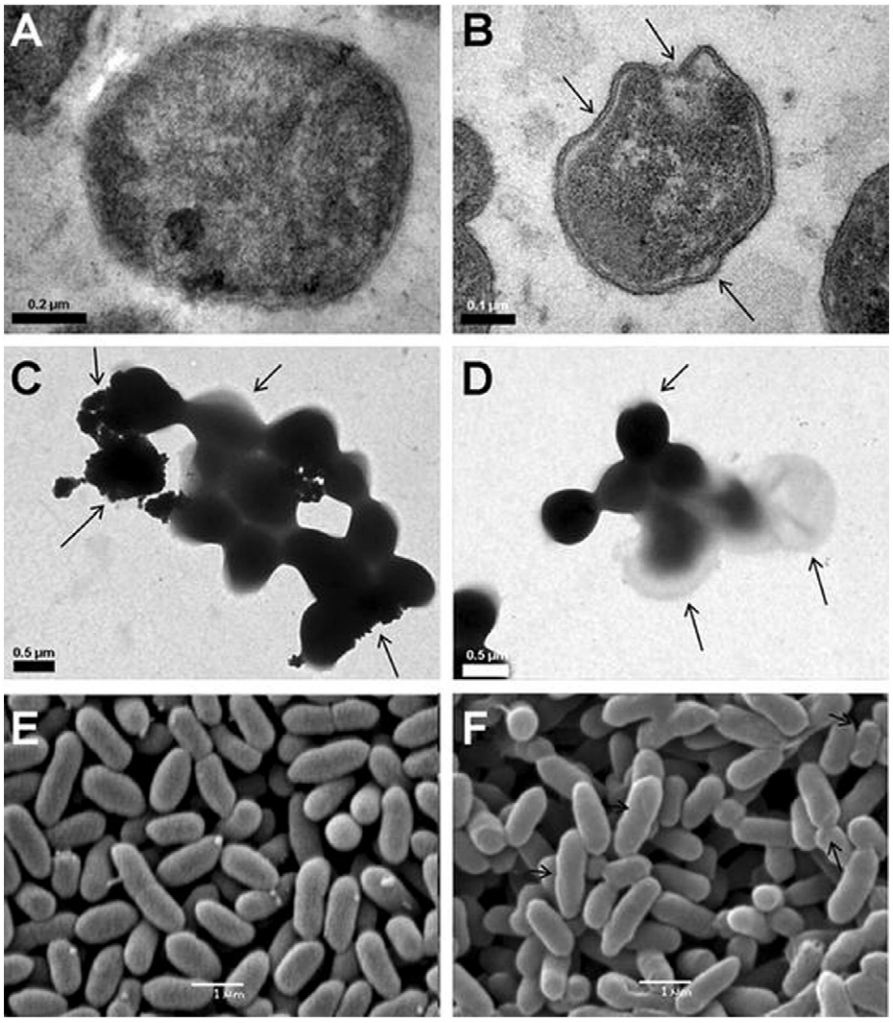

Fig. 2. (A) TEM micrograph of thin section of untreated *S. aureus*, (B) TEM micrograph of thin section of *S. aureus* treated with composite film, (C) and (D) TEM micrograph of deposition onto copper grid of S. aureus treated with composite film, (E) SEM micrograph of untreated *S. typhimurium* and (F) SEM micrograph of *S. typhimurium* treated with composite film. Reproduced with permission [37]. Copyright 2009, Springer.

nanoparticles with exposed surfaces and the increased surface roughness and hydrophilicity of the films. Therefore, the interaction between the surface of the nanocomposite and the pathogen promotes the antibacterial effect. Palza et al. prepared copper polypropylene-based composites by melt mixing. After 4 h this composite, with 1 v/v% of copper nanoparticles, killed >99.9% of *E. coli*. The time is reduced in half with 10 v/v%. Antibacterial activity in this nanocomposite is associated with copper ion release, which is facilitated by the incorporation of water molecules in the amorphous regions of the polypropylene [60,63]. Copper nanoparticles in polypropylene supported on organoclay has also been studied. Antibacterial activity was studied by the agar diffusion test, demonstrating inhibition clear zones surrounding the copper nanocomposite pastes. However, clear zones were not observed using film samples, suggesting that the copper ions are released slowly from the film.

2.2.4. Polyethylene

Jeziórska et al. prepared spherical silica containing immobilized copper nanoparticles in high-density polyethylene by melt blending and injection molding. They evaluated the antibacterial activity of nanocomposites against *E. coli*, *S. aureus*, *S. typhimurium*, *P. fluorescens* and *B. cepacia* and found good levels of activity against these bacteria due to significant ATP reduction (50–90%) for nanocomposites with higher copper content [47]. Bikiaris et al. also studied the antibacterial activity of HDPE-copper nanocomposites. HDPE nanocomposites containing copper nanofibers prepared by melt-mixing exhibited good antibacterial activity against *E. coli*, *P. fluorescens* and *S. aureus*. The antibacterial effect increased remarkably with the nanocomposites with 2.5 and 5 wt% of copper nanofibers after 24 h of incubation [46]. Linear low-density polyethylene (LLDPE), dickite, and copper nanoparticles were used to prepare a Cu/dickite/LLDPE nanocomposite by melt mixing and melt extrusion. The antibacterial rate for this nanocomposite is about 96% against *S. aureus*, which is attributed to copper release in bacterial suspension [49]. Vermiculite enriched with copper nanoparticles were incorporated into a low-density polyethylene matrix. The antibacterial test of these nanocomposites against *E. faecalis* showed that the nanocomposites inhibited bacterial growth [50]. Antibacterial activity of copper nanoparticles incorporated in polyethylene matrices have been evaluated against *L. monocytogenes*. Tamayo et al. showed that other antibacterial effects may occur; the nanoparticles can be released from polymer matrix and subsequently penetrate bacteria. TEM images of thin cross-sections of *L. monocytogenes* reveal the presence of copper nanoparticles in bacteria and the disruption cell walls associated with a bacteriolytic mechanism [107].

2.3. Copper-polymer nanocomposite preparation methods

Several techniques have been used to prepare copper-polymer nanocomposites with antimicrobial properties. For all techniques, *ex situ* and *in situ* method are the main routes of preparation.

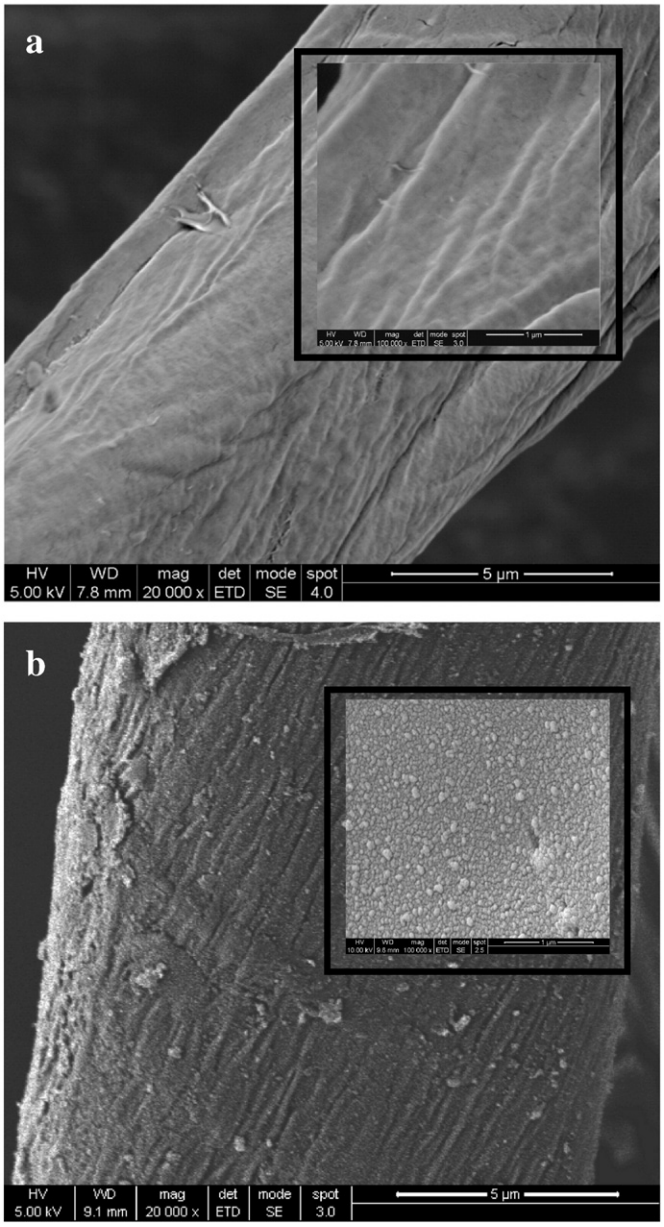

Fig. 3. (a) HR-SEM images of pristine cotton fabric (magnification ×20,000). Inset shows magnified image (×100,000) of the fiber (b) fabric coated with CuO nanoparticles (magnification ×20,000). Inset shows magnified image (×100,000) of the nanoparticles coated the fiber.
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2.3.1. Ex situ method

This method is based on synthesizing nanoparticles and polymerizing monomers separately and subsequently incorporating the nanoparticles in the polymer by one of two mixing techniques, either melt compounding by twin-screw extrusion or solution blending. Other nanocomposites have been prepared by deposition of nanoparticles on the polymer surface, by sprays or dip coating (immersion). Most studies on the preparation of cotton fabrics with antibacterial activity have used immersion. The fabrics are immersed in colloidal suspensions of copper nanoparticles under conditions of temperature for short periods of time followed by a final curing step [33,39,42]. After preparing the nanocomposites, the elaboration of fibers, scaffolds or films requires the use of mechanical, chemical, or physical techniques such as casting, pressing, salt leaching or electrospinning. For example, ultrafine HAP-garlanded PLGA fibers with incorporated CuO nanoparticles are prepared by solution blend, followed by electrospinning, to obtain ultrafine fibers with average diameters ranging from 1 to 1.2 μm, with uniform distribution of hydroxyapatite particles [55]. Casting is the simplest method to prepare nanocomposite films, since it only requires a flat surface to deposit the mixture. The films are then formed by evaporating the solvent at room or higher temperatures. Poly(vinyl chloride)/copper nanocomposites have been prepared by this method [68].

Many copper nanocomposites based on polyolefins like polyethylene and polypropylene have been prepared by melting compounds using twin-screw extrusion [46,47,60,109]. High temperatures (190–220 °C) and 30–110 rpm are needed to achieve the molten state of polymer. Nanoparticle dispersion is generally poor in nanocomposites prepared with polypropylene.

2.3.2. In situ method

The preparation of nanocomposites by this method consists of polymerizing monomers with nanoparticles. The nanoparticles must be dispersed in the monomeric solution or solvent, before polymerization. Another approach is to conduct ion reduction and polymerization simultaneously. *In situ* nanoparticle synthesis in a polymer matrix can improve nanoparticle stabilization and consequently control nanoparticle size. The high surface energy of the nanoparticles promotes thermodynamic stabilization through the interaction of the polymer chains with nanoparticle surfaces, thus controlling nanoparticle growth and agglomeration [119]. The good dispersion of nanoparticles significantly enhances the antibacterial capacity of nanocomposite, which is associated with increased ion release.

Although melt compounding is most often used to prepare copper nanocomposites with polyolefins, some studies have reported composite synthesis using *in situ* polymerization. Copper nanoparticles are incorporated into the reactor with an ethylene monomer, followed by the addition of a metallocene catalyst. The final product is a nanocomposite with homogeneous dispersion of the nanoparticles [107,108]. The preparation of copper/polyaniline nanocomposites is an example of *in situ* polymerization, wherein the nanoparticles and polymer are produced simultaneously. In this case copper salt dissolved in methanol and aniline are added to the reactor with stirring for 20 h at room temperature to obtain the nanocomposite. This method can be considered a simple and inexpensive route of preparation [53]. Copper-chitosan nanocomposites have also been prepared by a physical *in situ* technique. Bulk target copper is placed in an aqueous solution of chitosan that is irradiated by femtosecond laser ablation to obtain a copper-chitosan nanocomposite. The chitosan polymer acts as an *in situ* capping agent that generates ultrafine nanoparticle dispersion [34]. While most copper nanocomposites based on cotton fabrics are prepared *ex situ* by immersing the fabric, some studies have used *in situ* methods to modify the fabrics in a two-step process. In the first stage, the fabrics are immersed in a solution of copper ions, and in the second stage, the fabrics doped with copper ions are immersed in a reducing agent solution, generating copper nanoparticles in fabrics [41]. Two other methods have been developed that involve exposing cellulose fibers doped with copper ions to thermal treatment, and to heat/UV treatment to produce copper nanoparticles *in situ*. In both cases, no differences in the degree of dispersion of nanoparticles are observed [29].

2.4. Techniques of characterization of copper-polymer nanocomposites

2.4.1. Optical characterization

The most used technique for nanoparticle characterization, is UV–vis spectroscopy. Copper nanoparticles display an absorption band from 500 nm to 600 nm, associated to surface plasmon resonance phenomena [44,48]. Wavelength absorption maximum is related to size, shape and aggregation of nanoparticles (stability).

2.4.2. Morphological characterization

There are currently several techniques for characterizing copper-polymer nanocomposites. Transmission electron microscopy is the most used technique since it provides the means to identify nanoparticle size and material morphology. The samples must have a thickness of <100 nm and should be deposited on copper or nickel grid. If however, samples are in suspension, they are placed onto a carbon-coated grid. The morphology and microstructures are analyzed by field-emission scanning electron microscope. Some microscopes are equipped with energy-dispersive spectrometers (EDS), additionally contributing with the samples elemental analysis. In general, nanocomposites for EDS analysis should be sputtercoated with gold, palladium or carbon layers to prevent charging of the non-conducting sample.

2.4.3. Composition and structure characterization

X-ray diffraction (XRD) imaging discloses the solid structure of metal nanoparticles and polymer morphology. The polymer and the metal nanoparticles can be identified by their characteristic peaks in XRD patterns. This technique can indicate the degree of crystallinity of the polymer in the nanocomposite, showing sharper peaks due to a more ordered orientation of the polymer chains. According to this, some works have studied the changes of phase (crystalline and amorphous) depending on changes in the size of the nanoparticles [120].

X-ray photoelectron spectroscopy (XPS) provides valuable information on the surface composition, structure and charge transfer between metals and adsorbed ligands [121]. An increased nanoparticle binding energy is a confirmation of interaction between nanoparticles and the nanocomposite. In addition, this technique characterizes the change in nanoparticle content throughout the depth of the matrix [120]. Signals for Cu 2p2/3 are detected at 932.6 and 934.7 eV related to Cu^0 or Cu^+ (Cu_2O), and Cu^{2+} (CuO), respectively [122,123].

2.4.4. Thermal characterization

Thermal stability of the nanocomposites is evaluated by thermogravimetric analysis (TGA). TGA detects the amount of stabilizer chemisorbed on the surface of the nanoparticles [124]. The analysis of thermogram data helps visualize the influence of nanoparticles in the polymers thermal properties and degradation mechanism [47].

Thermal analysis obtained by differential scanning calorimetry (DSC) identifies nanocomposite melting temperature (Tm), heat flow (ΔHm) and crystallization pattern, thus conferring the possibility to study the influence of nanoparticles in the crystallinity of the polymer [47]. Some studies have shown, that metal nanoparticles act as nucleation centers in the orientation of polymer chains which would in turn increase the crystallinity of the polymer [125].

2.4.5. Mechanical characterization

Mechanical properties of the nanocomposites are evaluated by tensile or compressive measurements performed by mechanical testing (dynamometer). Values such as, Young's modulus, stress yield, elongation at break and break-point tensile or compressive strength can be determined by stress-strain curves. Given the fact that composite interfaces (boundary layer between nanoparticle surface and polymer matrix) exhibit local properties different than that of the bulk polymer matrix, It has been suggested that the presence of nanoparticles could significantly alter the mechanical properties of polymers [23,51].

2.4.6. Antibacterial assays

There are several methods for analyzing the antibacterial activity of nanocomposites. The Kirby-Bauer test, one of the most common of these techniques, analyses the ability of a sample to inhibit bacterial growth. After nanocomposite incubation in a seeded agar plate, the diameter of growth inhibition is measured in order to determine antibacterial properties [72]. For nanocomposites in suspension, the most popular evaluation methods are minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) determination. The MIC is established as the concentration of nanocomposite where no turbidity of the culture is observed, MBC on the other hand is defined as the lowest concentration of nanocomposite where no growth of bacteria is observed [31]. Bacterial growth in broth medium can be monitored by measuring optical density (OD) at 595 nm using UV–visible spectrophotometer.

Counting colony forming units is also used to evaluate the antibacterial activity of nanocomposites. In this method, an inoculum of bacteria is placed on the nanocomposite surface and incubated at 37 °C. Next, nanocomposite surface is washed with saline solution, an aliquot of which is transferred onto nutrient medium to be incubated at 37 °C, and finally perform colony counting.

Another method used for viability assays is flow cytometry with a propidium Iodide combination. Propidium iodide (PI) cells with membrane damage and binds to DNA, ~~and~~ emitting fluoresce at 620 nm when stimulated by a laser at 488 nm. In viable cells, PI remains in the medium and does not fluoresce [31].

Some standard methods, such as, ASTM E 2149 (ASTM Designation E 2149-01, 2001) [53], ATCC 147 (51), are also used to assess the antibacterial capability of nanocomposites. The antibacterial capability can also be evaluated by the morphological changes undergone by bacteria after they are exposed to the nanocomposite. Morphological changes could be observed by different microscopy techniques, such as TEM, SEM or AFM where samples are deposited on silicon wafer, mica or copper grids [37,53,107,108].

3. Mechanisms of action of copper polymer nanocomposites

3.1. Release of copper ions

In general, the antibacterial effectiveness of polymer-metal nanocomposites improves with a high surface to volume ratio, which increases the number of ions released from the nanoparticles into the polymer.

The copper corrosion mechanism in aqueous solutions and the resulting copper species vary with pH. In general two copper species are formed, Cu_2O and CuO, which can dissolve to copper ions [126].

Elemental metal particles require the presence of water and oxygen molecules to release a small number of ions. Therefore water and oxygen retained within the polymer is crucial for ion release. Some properties of materials like crystallinity and matrix polarity, which constitute the diffusion barrier to water molecules and ions during their propagation, can influence the rate of release [61]. Damm argues that ions released in silver-composites occur in the amorphous polymer ranges and therefore decrease with increased crystallinity [127]. On other hand, ion release increases with the polarity of polymer. The polar nature of the polymer facilitates the diffusion of water molecules. For example, atomic absorption spectroscopy (AAS) studies indicate that the concentration of Cu^{2+} released from copper polypropylene nanocomposites immersed in an aqueous medium for 6 h is around 5 ppm, while the concentration of chitosan-based copper nanocomposites tripled in only 1.25 h. The kinetic law in Damm and Munstedt [128] indicate that relaxation of polymer chains due to water penetration is a rate-limiting step in the transport process, indicating zero-order release kinetics if water acts as a plasticizer for hydrophilic polymers, while diffusion governs ion release in hydrophobic polymers. Experiments have shown that the rate-determining step for ion release is not the control exerted by the polymers or water penetrating molecules, but rather the dissolution of ions from the surface of the nanoparticle. This was demonstrated by Cioffi et al., who showed release curves from polymers with similar amounts of copper nanoparticles and copper salt. The authors observed very rapid ion release with copper salt [69]. XPS analysis show that copper ions released from nanocomposites cause the dissolution of CuO to soluble Cu(II), CuO is present on the surface of nanoparticles as a shell covering the metallic nanoparticle core. The release curves in this case exhibit first-order kinetics. The same behavior was observed by Longano et al., who found first-order kinetics resulting from the release of ions from polylactic acid-based copper nanocomposites (Fig. 4) [57].

Anusha Thampi et al. observed a different phenomenon where the antibacterial effect of copper oxide nanoparticles functionalized using PANI and immobilized on cotton fabrics is attributed to copper ion release. An analysis by atomic absorption spectroscopy demonstrated that a constant number of copper ions was released with the first 3 washes, but the detectable number was minimal after the third wash. Comparing the data analysis of the functionalized copper oxide to that of the non-functionalized, the Cu^{2+} ion content for all washes was lower for the functionalized copper oxide, indicating greater control over the release of copper ions from the PANI matrix. Therefore this material could function as an antimicrobial agent over a long period of time [54]. The cell walls of the viable bacteria are usually negatively charged due to the presence of functional group such as carboxylates present in lipoproteins [50]. Thus, according to the authors, bacteria are first attracted by the electrostatic forces exerted by copper ions and then immobilized on the copper oxide nanoparticle surface [54]. Copper ions could also dissociate and directly exert their antimicrobial effect on the bacteria. Some copper ions may enter the bacterial cells and bind with deoxyribonucleic acid molecules and become involved in cross-linking within and between nucleic acid strands, resulting in disorganized helical structures [54].

3.2. Release of copper nanoparticles

The most substantial results regarding the possible antibacterial mechanism associated with copper-polymer nanocomposites are reported by Mallick et al. The authors studied the antibacterial effect of iodine-stabilized copper nanoparticle chitosan composite (CS-Cu NP composite) against *E. coli* by cytometry measurement. Flow cytometric assessment of bacterial cell viability in response to a bactericidal effect reveals four states of cell populations. The first state is living cells, the second is committed cells, and the third and the fourth are dead and lysed cells. The results suggest that nanocomposites cause irreparably damage to the membrane of bacterial cells, with the effect starting as soon as 2 h after the treatment. *E. coli* expressing recombinant green fluorescent protein (GPF) was used to test bactericidal efficacy. Field emission scanning microscopy (FESEM) images of untreated and treated *E. coli* are displayed in Fig. 5a and b. The first image shows bacteria with normal and intact surface morphology, while the second image is of a cell covered with particles with diameters between 5 and 15 nm. Fig. 5c and d show a TEM image of bacteria incubated for 3 h in the presence of a CS-Cu NP composite. The darker areas in the micrograph indicate the presence of copper nanoparticles attached to the cell wall. The image also shows damaged bacteria, which the authors attributed to the development of porosity in their cell walls. Further, the author suggested that the negative surface charge of *E. coli* cells, possibly related to the presence of functional groups with negative charges, interacts with the positively charged nanocomposite, mainly through electrostatic interactions [31]. A gel retardation assay indicated the possibility of binding between DNA and the nanocomposite, suggesting that DNA of the bacterial cell is accessed by composites after cell wall perforation, promoted by copper nanoparticles in the nanocomposite. The antibacterial effect associated with copper ions from nanocomposites was also investigated by atomic absorption spectroscopy (AAS). The results show that the freshly prepared nanocomposite at MIC (130.84 μg/mL composite containing 21.55 μg/mL of copper nanoparticles) slowly released Cu^{2+} ions into the aqueous medium. Thus, after ~1.5 h the concentration of Cu^{2+} in solution was 17.8 ppm, increasing up to 32.2 ppm in ~4 h of exposure. However, the authors concluded that released Cu^{2+} ions did not play an important role in killing the bacteria, which is similar to other experiments where similar amounts of copper ions from $CuSO_4$ did not retard bacterial growth. Mallick et al. suggested that the CuNps from the iodinated CS-Cu NP composite adhere to the cell wall, piercing and causing leakage of intracellular proteins and other species, which eventually causes the death of the bacteria [31].

On the other hand, the antibacterial effect of copper nanoparticles incorporated in polyethylene polymers have shown two morphologic effects on *Listeria monocytogenes*: 1. The cell wall separates from the cytoplasmic membrane, but is not destroyed, with the formation of low molecular weight regions in the center of the bacteria; 2. Cell wall and membrane damage, with release of cytoplasmic material (bacteriolytic effect). In bacteria with intact cell walls, the presence of nanoparticles inside bacteria is revealed (Fig. 6) [46]. There are two mechanisms of nanoparticle penetration of bacterial cells: endocytosis and direct diffusion [129].

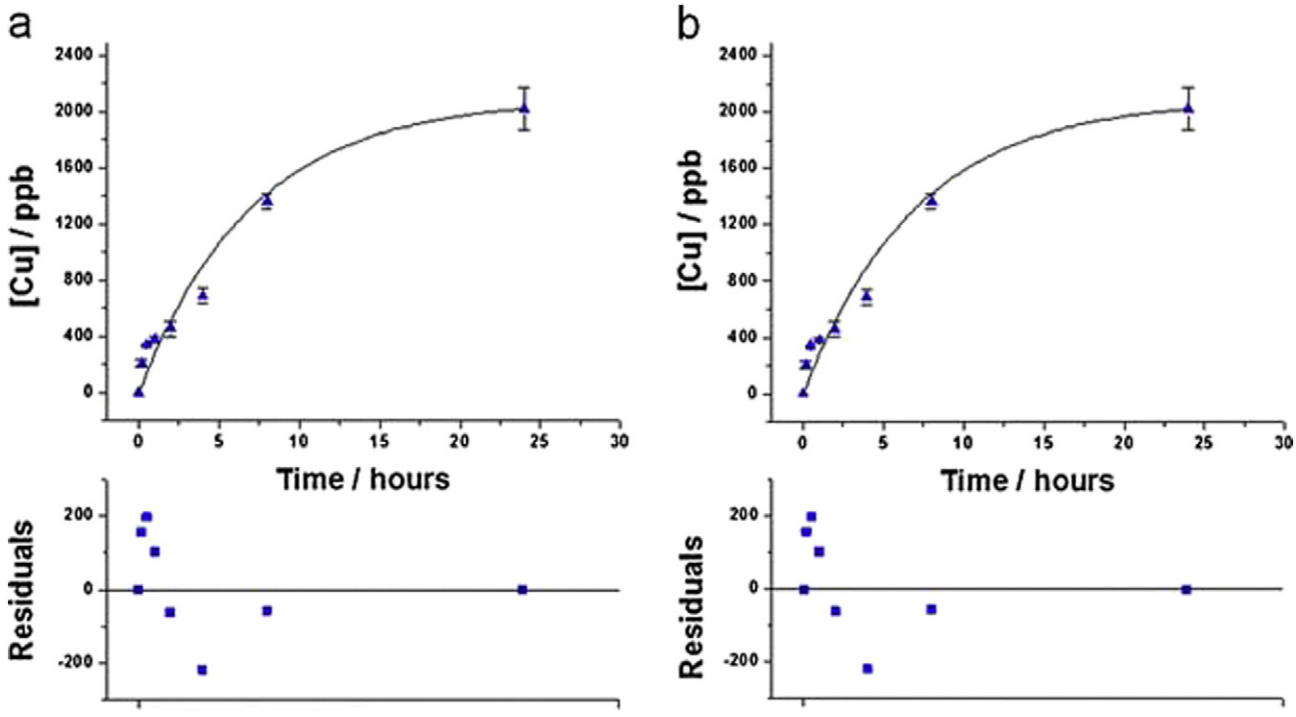

Fig. 4. Copper release from CuNPs-C-PLA nanocomposite is plotted as function of time according to a first-order equation (a) and a two-fold exponential equation (b). Reproduced with permission [57]. Copyright 2012, Springer.

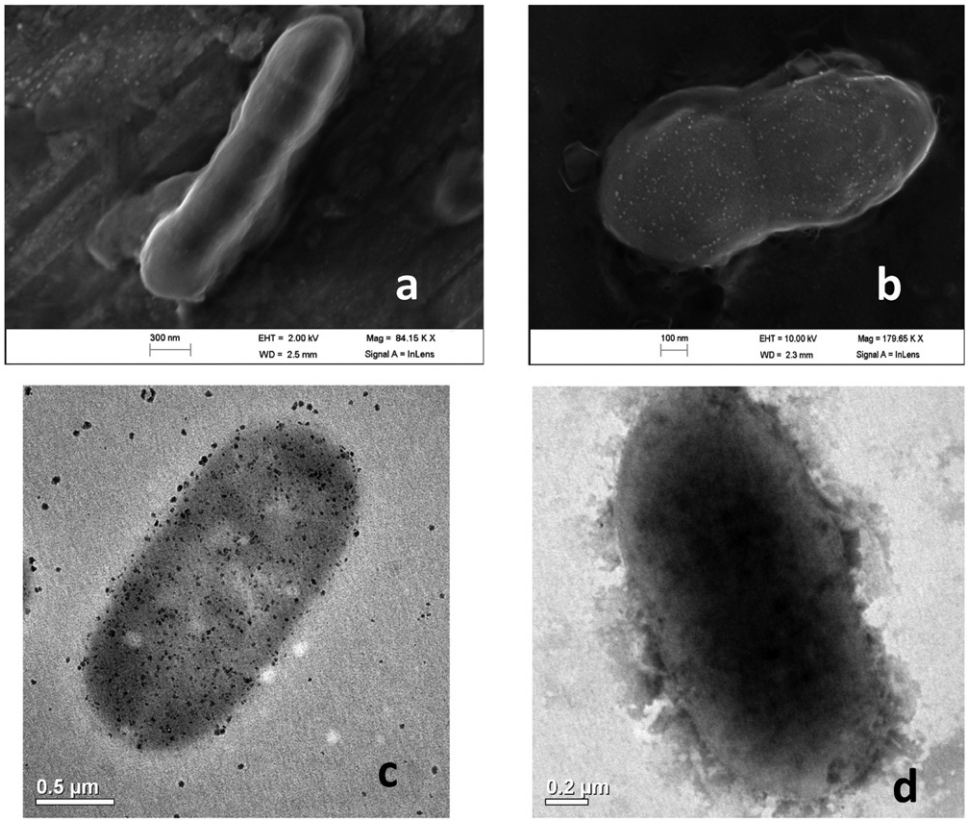

Fig. 5. (a) Field-emission scanning electron micrograph of green fluorescent protein (GFP) expressing recombinant *E. coli* cells and (b) *E. coli* cells treated with 130.84 μg/mL (MIC) iodinated CS-Cu NP composite in liquid LB medium for 3 h (c) TEM image of a GPF expressing recombinant *E. coli* cell treated with 130.84 μg/mL (MIC) iodinated CS-Cu NP composite in liquid LB medium for 3 h (d) TEM of a cell treated with MIC of ionidated CS-Cu NP composite showing damage.
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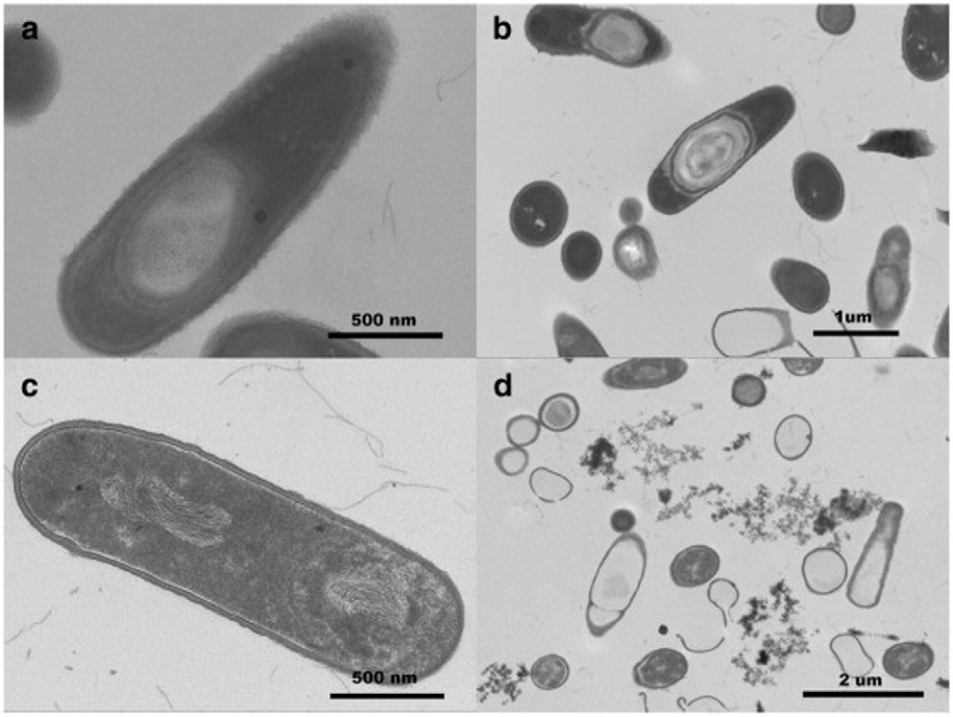

Fig. 6. *Listeria monocytogenes* (ISP 6508) incubated for 16 h on the nanocomposite surface: (a, b) PE-AgNp nanocomposite; (c, d) PE-CuNp nanocomposite.
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Nanoparticles between 10 and 100 nm usually cross the membrane by endocytosis [130], which occurs in three stages: first particles stick to the membrane, second, the membrane wrap around the nanoparticles forming a particle-lipid complex, and finally the particle-lipid complex detaches from the membrane. In the case of very small nanoparticles with diameters of only several nanometers or even smaller, endocytosis is not an effective way of penetration due to lower adhesion energy, which comes mainly from ligand-receptor interaction [131]. Increasing the energy of bending and stretching resulting from the deformation of the membrane cannot be compensated and in this case, the nanoparticles may aggregate to be endocytosed [132].

Theoretical studies have shown that the cellular uptake of ligand-coated NPs is strongly size-dependent, Zhang et al. identified three regimes separated by two characteristic particle radii R_{min} and R_{max} When $R < R_{min} \sim 20$ nm, endocytosis almost never occurs because adhesion energy is too low to compensate for bending energy. If $R > R_{min} \sim 60$ nm, endocytosis rarely occurs, and almost all nanoparticles are only partially wrapped, because of the depletion of the free receptors (lipids). However, $R_{min} < R < R_{max}$ is an optimal nanoparticle radius at which cellular uptake of nanoparticles is maximized. The optimal radius falls in the range of 25–30 nm for reasonable values of the membrane bending rigidity and the ligand-receptor binding energy, where the ligand is a capping agent [133].

Dynamic simulations of nanoparticles with ligands (capping agent) on their surfaces also show that nanoparticles can spontaneously penetrate membranes. This is composed of amphiphilic lipids consisting of a head group containing three connected hydrophilic beads and two tails with four hydrophobic beads each, while a ligand is composed of four connected beads, three of which are hydrophobic and the other is hydrophilic (Fig. 7a) [134]. The simulations adopt reversible non-covalent bonds to generate aggregation (P_{on}) and detachment (P_{off}) between nanoparticles and ligands. When the bond length between the bead of the ligand head is longer than its initial length, the bond is broken, with a probability being P_{off}, and when the length is shorter than its initial length, the bond forms with a probability P_{on}. The ratio $P_a = P_{on} / P_{off}$ can replace the reaction equilibrium constant $K_a = K_{on} / K_{off}$ (Fig. 7b), where K_a determines the final balance of the nanoparticle-ligand complex. When the medium is hydrophobic, the nanoparticle ligand-complex can be formed (Fig. 7c), but when P_a is large, the formation of the nanoparticle ligand-complex is metastable. Because the complex surface is hydrophobic, it can spontaneously enter the membrane, while amphiphilic ligands remain in the membrane and arrange themselves along the lipid distribution. Hydrophilic nanoparticles do not remain in the hydrophobic environment in the bilayer and tend to leave with ligands detaching from the nanoparticle (Fig. 7d). Li et al. argued that the hydrophilic and hydrophobic properties of nanoparticles are an important factor for their interactions with membranes, due to the lipid head group-nanoparticle interaction [135]. When the interaction is strong, the nanoparticle can enter the membrane [136], while hydrophobic nanoparticles can enter membrane, driven by their preference for the hydrophobic tail of the lipid.

3.3. Biofilm inhibition

Among the factors influencing surface bacterial adhesion, and therefore antimicrobial response, we find: chemical composition, surface charge, hydrophobicity, and surface roughness. Depending on the hydrophobicity of both bacteria and the materials surface, bacteria differently adhere to substrates with modified superficial properties [137].

Chapman et al. have studied the antifouling performance of copper macro- and nano- particles doped into sol-gel. Slime detection assays showed that the macro copper samples have the most slime attachment, while copper nanoparticles have showed the least slime levels. Similarly, copper nanoparticles have also been the best performing antifouling dopants for polydimethylsiloxane (PDMS), where the least amount of slime and mass for this polymer group were observed. The authors argue that when copper nanoparticles are introduced into both PDMS and the sol–gel matrices, a notable shift in wettability is observed. This behavior is attributed to the drying procedure of this sample, since it causes particle movement to surface, which achieves its activation before its immersion in water. In other words, generating a material where the active agents are present in the contact surface and not in the depth of the polymer matrix improves the antifouling properties of nanocomposite [138].

Cell surface hydrophobicity (CSH) has been observed to play a major role in the attachment of bacterial cells to surface. Some studies have evaluated the effect of copper nanoparticles on CSH of *P. aeruginosa* using the bacterial adhesion to hydrocarbons (BATH) assay. The results showed a significant reduction of CSH to ~99%. Similarly, copper nanoparticles have showed a significant inhibition of extracellular polymeric substances (EPS) which also play role in biofilm formation and maturation [139].

Finally the studies published to date have shown that biocidal effects observed in copper-polymer nanocomposites are based on three phenomena: I. Release of copper ions, II. Release of copper nanoparticles from nanocomposites and III. Biofilm inhibition. A scheme of three phenomena is shown in Fig. 8 and are listed below.

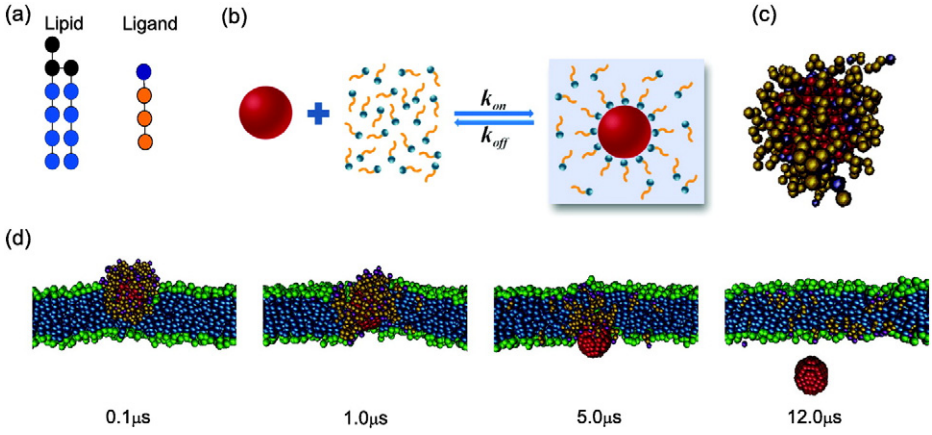

Fig. 7. (a) Architecture of lipid and ligand molecules. Green head beads of lipid; blue, tail beads of lipids; hydrophilic of ligand; orange hydrophobic beads of ligand. Schematic representation of the equilibrium between nanoparticles and ligands, where K_{on} and K_{off} denote on rate constant for forming a nanoparticle-ligand complex (NLC), respectively. (c) Snapshot of-self-assemble NLC in the oil. (d) Snapshot of four stage of the NLC interacting with the lipid bilayer, where the nanoparticle diameter is 3 nm and the surface ligand density is 3.0 nm^{-2}.
Reproduced with permission [134]. Copyright 2012, ACS.

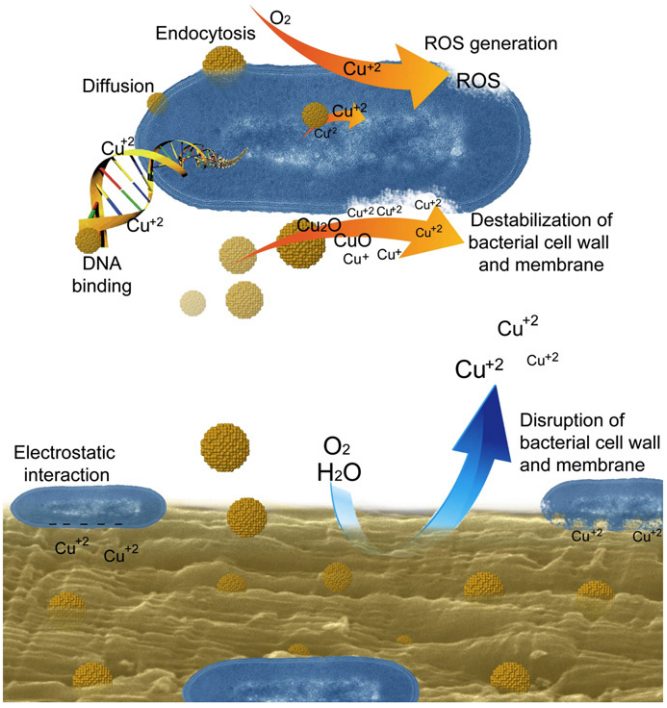

Fig. 8. Antibacterial effects of copper-polymer nanocomposites.

I. *Release of copper ions*

1. The copper ions released reach the outer membrane or cell wall of bacteria and interact with:
   - Sulfhydryl groups causing denaturation of proteins in the bacterial membrane.
   - Amines and carboxyl groups in *N*-acetylglucosamine and *N*-acetylmuramic acid in the peptidoglycan layer.
2. In all cases these interactions destabilize or break and subsequently disintegrate the bacterial cell wall and membrane, which is known as the bacteriolytic effect.
3. Copper ions released in the bacteria bind to DNA and become involved in cross-linking within and between nucleic acid strands, resulting in disorganized helical structures, with the result that the cell cannot replicate.
4. Copper ions generate reactive oxygen species, lipid peroxidation and protein oxidation.

II. *Release of copper nanoparticles*

1. The copper nanoparticles adhere to the surface of the bacteria through electrostatic forces and molecular interactions.
2. The nanoparticles penetrate the bacteria through the outer membrane or cell wall by one of two means: Endocytosis and direct diffusion.
3. Nanoparticles between 10 and 100 nm cross the membrane by endocytosis in three steps; nanoparticles stick to the membrane, the membrane wraps around the nanoparticles and finally the particle-lipid complex detaches from the membrane.
4. When the nanoparticle is <10 nm, its hydrophobic or hydrophilic nature plays a more important role. If the interaction is strong, the nanoparticle enters the membrane driven by its preference for the lipid head-group or tail.
5. Parallel to these mechanisms, nanoparticles release ions and simultaneously trigger the effects associated with ion release.

III. *Biofilm inhibition*

1. Copper nanoparticles move to the surface of the nanocomposite, rendering a much more active outer layer.
2. Surface nanoparticles reduce significantly the CSH, altering the attachment of bacteria.
3. In addition, surface nanoparticles reduce the EPS, which also plays role in biofilm formation and maturation.

4. Controversial antibacterial activity of copper polymer nanocomposites associated with Gram classification

The bacterial response to copper nanocomposites can be influenced both by the structure and chemical composition of its cell wall, therefore playing a central role towards tolerance or susceptibility of certain antibacterial agents. According to the structure, components, and functions, the bacterial cell wall can be divided into two main categories according to tests: Gram positive and Gram negative. Gram negative bacteria have a rigid cell wall that limits the entry of large and/or hydrophobic molecules to reach the cytoplasm. The mechanical strength of Gram negative bacteria is provided by a thin peptidoglycan layer (7–8 nm) consisting of glycan chains of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues that are cross-linked by short peptide chains [140]. This layer is overlaid by an outer membrane, a bilaminar structure composed of tightly packed phospholipids and lipopolysaccharides containing membrane proteins that increase the negative charge of the cell membrane. The variety of proteins found in Gram negative outer membranes is limited, but several of the proteins are in high concentrations, many of the proteins traverse the entire lipid bilayer and are thus transmembrane proteins. A group of these proteins is known as porins because they form pores that allow the diffusion of hydrophilic molecules <700 Da in mass through the membrane, but it is a barrier against larger or hydrophobic antibiotics and proteins. On the other hand, Gram positive bacteria contains a thick layer of peptidoglycan (20–50 nm) consisting of glycan chains of GlcNAc and MurNAc cross linked by a peptide bridge. This layer is attached to teichoic and lipoteichoic acids and complex polysaccharides [141]. The peptidoglycan is sufficiently porous to allow diffusion of metabolites to the plasma membrane.

There is no consensus among studies in the last decade related to the antimicrobial activity of copper-polymer nanocomposites regarding the susceptibility of some kinds of Gram positive and Gram negative bacteria. For example, copper nanoparticles incorporated in a chitosan matrix exhibit inhibitory activity towards *E. coli*, *S. paratyphi* (Gram negative) and *Bacillus* (Gram positive). However, the observed antibacterial activity was higher for Gram negative than for Gram positive bacteria. The authors attributed the different behaviors to the thickness of the peptidoglycan layer. While Gram negative bacteria have a single peptidoglycan layer, Gram positive bacteria have several peptidoglycan layers [32]. The same behavior is observed for copper nanoparticles deposited on silica nanospheres, the antibacterial assays against *E. coli* (Gram negative) and *S. aureus* (Gram positive), showed that the inhibition zone for *E. coli* is superior to that for *S. aureus* [72]. A similar response was observed by Mallick et al., who found that the minimum inhibitory concentration (MIC) values for B. cereus were higher than for *E. coli*, requiring a lower dose of nanocomposite to inhibit the growth of the Gram negative bacteria [31]. TEM images showed the adhesion of the nanocomposite on the surface of the bacteria, which is associated with the negative surface charge of *E. coli* cells that adhere to the positively charged iodinated copper nanocomposite through electrostatic interaction.

The antibacterial activity of copper nanoparticles incorporated in hydroxypropyl methylcellulose showed an opposite response; the antibacterial evaluation against *Streptococcus A.*, *S. epidermidis*, *S. aureus*, *B. cereus*, *E. coli*, *E. faecalis*, *Salmonella* and *P. aeruginosa*, demonstrated that copper nanocomposites showed a high antibacterial activity against Gram positive bacteria but not against Gram negative bacteria [106]. The same behavior is observed in antibacterial activity of silica doped with copper nanoparticles, showing a slightly higher percentage of reduction against *S. aureus* than *E. coli* [42]. The copper hydrogel

nanocomposite against *E. coli* and *S. aureus* shows greater zone inhibition for Gram positive than Gram negative bacteria [48]. An interesting phenomenon is observed in the antibacterial effect of copper nanoparticles incorporated in polyethylene against *E. coli* and *L. monocytogenes*. Although the antibacterial effectiveness is similar with both bacteria, nanoparticle penetration occurs only with the Gram positive bacteria. TEM images show nanoparticles in *L. monocytogenes* [107], but not in *E. coli* [108]. It is likely that the antibacterial effect of copper nanoparticles is governed by the nature of the stabilizing agent. In this case, the negative charge of citrate ion used as a stabilizer may be repelled by the outer membrane of *E. coli*, specifically by the negatively charged carboxylate groups and thereby preventing penetration by copper nanoparticles.

Theoretical studies argue that the hydrophilic/hydrophobic properties of nanoparticles are an important factor for the interaction with cell membranes (lipid bilayer). Hydrophobic nanoparticles are embedded in the hydrophobic core of the membrane and the resulting lateral distribution of lipids around the inclusion is usually not homogeneous. Hydrophilic nanoparticles present a different mechanism that promotes their adsorption on the surface of the bilayer rather than inclusion in core [135]. In experimental studies the hydrophilic or hydrophobic characteristic of nanoparticles is caused by the nature of the stabilizing agent, which is oriented around the nanoparticles.

In summary, the susceptibility of bacteria to the biocidal action of the copper nanoparticles depends mainly on the following factors: 1. Composition of the bacterial cell wall and membrane, 2. Particle size, 3. Hydrophobic/hydrophilic characteristic of the nanoparticle, 4. Electrostatic affinity between the nanoparticle surface and the bacterial cell wall and membrane. The latter two are governed by the nature of the stabilizing agent.

5. Applications of copper polymer nanocomposites

The large number of polymers used to prepare copper-based nanocomposites generates materials with various characteristics that can be used in a wide field of applications. The antibacterial property of copper-based nanocomposites suggests application in manufacturing medical devices, the textile industry, food packaging and water decontamination. Table 2 presents some of the main applications. For example, the antimicrobial activity of CuO nanoparticles impregnated on fabrics (woven and non-woven) has shown excellent response with Gram positive and Gram negative bacteria, demonstrating potential use for nosocomial purposes [54]. Several nanocomposites based on cotton fabrics and copper nanoparticles have been developed for use in medical apparel, wound dressing, bedding and active bandages [38–40]. One example is copper nanoparticles with alginate-cotton cellulose composite fibers which have shown fair mechanical strength and antibacterial properties, and are now used as dressing material [41]. CuNps-based water repellent coatings were tested against Gram negative and Gram positive bacteria. The coatings, produced by aerosol assisted chemical vapor deposition, showed significant antibacterial activity against both types of bacteria upon a treatment time range of 15–60 min. In addition, all the film samples results in a significant reduction in bacterial cell adhesion related to their superhydrophobicity [142]. In a recent study, the antibacterial activity of starch hydrogel with copper nanoparticles was tested against Gram positive and Gram negative bacteria. The gels were highly active in ~~a~~ dermal toxicity tests and showed that the material could be scored as slightly irritant, proving its biocompatibility [143]. Ag/Cu-coated catheters were investigated for their efficacy in preventing methicillin-resistant *Staphylococcus aureus* (MRSA) infection *in vitro* and *in vivo*. Although bacterial adherence was reduced and the overall material presented low toxicity, the films were hindered by deposition of plasma proteins and fibrin sheath formation over the surface of the catheter. In this regard, the development of catheters which combine Ag/Cu coatings with compounds that limit and/or prevent plasma protein adsorption, becomes an interesting challenge [144]. For potential applications in desalination plants, membranes with biocidal copper nanoparticles are expected to be effective alternatives to thin-films. Ben-Sasson et al., demonstrated strong antibacterial activity of CuNps on these membranes, which led to a 90% reduction of *Escherichia coli* when compared to pristine reverse osmosis membranes. This kind of study demonstrates that *in situ* grafting of CuNps on reverse osmosis membranes is a potential alternative to reduce biofouling [145]. BioGlass and epoxy resins have been studied for application in medical implants [43,45]. For example, BioGlass shows properties of bone regeneration, while epoxy resin has exhibited non-cytotoxic properties in rat heart cells. Olefins like polyethylene and polypropylene with copper nanoparticles have two major potential applications, medical devices and food packaging owing to excellent levels of antibacterial activity and good mechanical and barrier properties [46, 109]. A copper nanocomposite based on polyaniline has been developed with excellent antibacterial activity against *E. coli*, *S. aureus* and *C. albicans*. It is used as a biological indicator of water contamination, demonstrating potential application as a water disinfectant [53].

Table 2
Main applications of copper-polymer nanocomposites.

Polymer matrix	Microorganism	Application	Refs
Cellulose fabrics	*S. aureus Bacillus E. coli P. aeruginosa*	Nosocomial purpose	[54]
Polyethylene	*E. coli*	Medical devices	[108]
Cellulose	*S. cerevisiae*	Food packaging	[29]
Hydroxypropyl methylcellulose	*Streptococcus A., S. epidermis, B. cereus, E. faecalis, Salmonella, P. aeruginosa, Staphylococcus aureus*	Food packaging	[106]
Cotton	*E. coli ATCC 25922 S. aureus ATCC 25923*	Medical usage	[42]
Cotton	*S. aureus ATCC 6538, E. coli ATCC 11230*	Medical apparel	[39]
Polylactic acid	*Pseudomonas spp.*	Food packaging	[57]
Agar	*L. monocytogenes, E. coli*	Food packaging	[80]
BioGlass	*E. coli ATCC 25922*	Medical implant	[45]
Polyaniline	*E. coli, S. aureus, C. albicans*	Water disinfection	[53]
Cotton	*E. coli*	Dressing materials	[41]
Bamboo rayon	*S. aureus, E. coli*	Medical clothes	[24]
High density polyethylene	*E. coli* DHSα	Food packaging	[46]
Epoxy resin	*Staphylococcus aureus, C. Albicans, Chlorella sp.*	Implantable antimicrobial biomaterial	[43]
Cotton	*E. coli, S. aureus*	Wound dressing, bed lining and active bandages	[38]
Polymers based on acrylic	*Chlamydomonas CD1 Red, Synechocystis PCC 6803, Phaeodactylum tricornutum CCMP 1327*	Marine antifouling coatings	[97]
Polypropylene	*S. aureus, P. aeruginosa*	Medical and health care applications	[109]
Chitosan	*E. coli, S. paratyphi, S. aureus, B. cereus*	Pharmaceutical and biomedical applications	[32]
Cotton	*E. coli, S. aureus*	Medical cloths, protective garments, bed spread	[40]

There are some products on the market based on polymeric copper materials. The company CUPRON® offers a variety of products for medical, industrial and military applications. In the medical field, products such as woven and non-woven fabrics help reduce healthcare-associated and nosocomial infections and improve healing/quality of life for specific patient groups. Other products, such as linens for hospital services, building materials, heating and cooling equipment (HVAC) and filtration systems, airline textiles and food services, industrial uniforms, garments and footwear, packaging, military clothing and food wear are also available. The Harvest SPF textile company Ltd. specializes in manufacturing healthy functional textile, has among its products Enerup® antibacterial socks made by Nano copper and silver powder evenly spreading in the nylon or polyester fiber, the antibacterial rate to *E. coli*, *S. aureus* and *C. albicans* can reach 99%. Although there are some companies dedicated to the manufacture of copper-based products, some websites, as alibaba.com, show that there is a wider variety of available silver-based products than those based on copper.

The addition of copper nanoparticles into polymers is a way to profit from their substantial antimicrobial properties and produce novel materials for applications in medicine, food packaging, and water purification, among others. In this context, copper nanoparticles emerge as an inexpensive alternative for the production of a broad range of polymer nanocomposites with high antimicrobial activity in time. Although there is a great amount of research related to the development of copper nanoparticles embedded materials, further research is needed to support the development of novel bioactive polymeric materials. Such materials are needed particularly for the production of hospital equipment or prostheses that will prevent, among others, hospital acquired infections. In this context, further research must consider scaling up processes, optimization of the biocidal activity against different kinds of microorganism, the prevention biofilm formation, and the reduction of toxic effects. Finally, *in vivo* studies of these coatings are crucial for gaining full understanding of their properties and real range of application.

6. Environmental risk of the copper-polymer nanocomposites

Although copper-based nanocomposites have diverse applications, copper ions and nanoparticles can be released into the environment. For this reason it is important to consider the risks and implications for Environmental Health Safety (EHS). Some studies have reported long-term chronic effects, bioaccumulation and toxicity in non-target organisms exposed to copper nanoparticles (example: fish, plants, nematodes, algae, mammalian cell lines, etc.) [146–156]. However, this particular kind of research is very difficult to analyze, since the experimental conditions vary from article to article. A very interesting review [157] collected the nanoecotoxicity data on CuO NPs and AgNPs for different organisms and showed that median LC50 values of CuO NPs were 2 to 3 mg/L for crustaceans and algae and 10–100 mg/L for fish and most of the organisms studied. Copper ions resulted more toxic than CuO NPs to all organisms (LC50: 0.024 mg/L for crustaceans and algae; 0.7 to 53 mg/L for the organisms studied) [157]. In comparison, AgNPs and Ag ions were more toxic median LC50 values for most organisms studied was below 10 mg/L [157]. Even though copper nanoparticle toxicity has been reported, more notably in regard to CuO NPs little is known of copper-based nanocomposite toxicity [158–160]. In consequence, emphasis should be placed on studying the ecotoxicity of released nanomaterials from nanocomposite with ~~a~~ special focus on uptake routes, bioaccumulation, toxicity, test protocols, and a wider range of organisms depending on the application.

7. Conclusion

To date, there have been a significant number of published articles related to the antibacterial activity of copper-polymer nanocomposites generating wider knowledge on three main topics; [1] Possible mechanisms of antibacterial action, [2] The most commonly used polymeric matrices and how they influence the antibacterial activity of the resulting material, and [3] Potential applications in accordance with the additional features of these nanocomposites. Although there are several applications for these nanocomposites, it is clear from articles cited in this review that paradoxically there are relatively few commercially available products compared to the number of silver-based products available, and this despite the fact that copper is 8 times as economic as silver. In this regard, a strategic approach might be to invest more effort in generating new commercial products based on copper-polymer nanocomposites. However, it is necessary to first deepen the cytotoxic evaluation of these nanocomposites. Despite this, the excellent antibacterial properties that have presented the nanomaterials based on copper against a broad antibacterial spectrum, combined with their low cost with respect to other metals like silver, make it an outstanding biocide.

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