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BIOACTIVE COPPER NANOMATERIALS

The world-wide interest towards non-conventional antimicrobials is growing exponentially and metal nanomaterials are being frequently proposed as smart nano-reservoirs of bioactive ions. This review focuses on Cu-nanomaterials affording prevention against bio-contamination in several real-life applications.

The prevention of the spreading and growth of undesired or even pathogenic microorganisms is presently rising a great interest, as it involves several real-life applications, including food processing and packaging, health care and biomedical devices, surgery and implants, textiles, household furnishing and paints, disinfection of hospitals, airports and production facilities, and it might have implications even in more extreme scenarios, such as germ warfare, space exploration, and in the prevention of pandemic diseases like SARS, Influenza Viruses, etc.

Moreover, many undesired microorganism strains are being developing resistance towards antibiotics or disinfecting agents.
The development of non-conventional antibacterial and antifungal agents is therefore become a key research field and very recently, nanotechnology has been applied to the development of innovative nanomaterials containing metals with a marked bioactivity, such as silver, copper and zinc.
In most studies on metal-based nano-antimicrobials, the biological effectiveness of the material is not barely a result of the well known bioactivity of the metal itself, but it is demonstrated to be higher, and/or to have a prolonged effect, as compared to that of the homologous bulk metallic material.
This has been suggested to be due to several aspects, including the nano-metal size-dependent properties, as well as the high surface-to-volume ratio, and stabilizing issues tuning the ionic release and anti-bio-film properties.
Nowadays, the need for hygienic living conditions prompts new challenges for the development of affordable and efficacious antimicrobial materials that should be environmental friendly and absolutely non-toxic towards human beings, as well.
Finely dispersed bioactive nanomaterials are expected to exert an improved disinfecting effect due to their size, providing higher environmental mobility and allowing them to interact closely with bacterial membranes. On the other side, the same properties may give rise to severe risks for human health, thus greatly limiting the development of nanoparticle real-life applications.
The growing international concern in nanoparticle-toxicology towards humans is presently supporting the development of "smart" nano-antimicrobials. Such nontoxic nanomaterials can still reduce the risks of transmitting diseases by preventing microorganisms survival and proliferation in several application contexts, but are expected also to ensure environmental compatibility and absence of human toxicity, since they are impregnated or covalently linked or co-polymerized or somehow confined in a dispersing/supporting matrix. The latter component acts as an immobilizing carrier in a nanocomposite formulation or a nano-coating hybrid material and might also bring additional properties to the final product.
The usefulness of copper as an antibacterial agent has been known for a long time. In the metallic form, or as ionic/soluble species it has been shown to have excellent antimicrobial activity against a number of microorganisms including bacteria, fungi, algae and viruses, while it is relatively safe for humans. Copper appears to exert its killing effect by generating reactive hydroxyl radicals that can cause irreparable damage such as the oxidation of proteins, cleavage of DNA and RNA molecules, and membrane damage due to lipid peroxidation [1, 2].
Even if the bioactivity of copper, both as free or complex species, is also well-known and has been documented for many years, only few papers and patents [3-5] have been issued so far about the antibacterial properties of copper-containing nanosized materials.
The present critical review focuses on the synthesis and characterization of bioactive copper nanomaterials and their applications to the real life.

Biological properties of copper

After the completion of its genome sequence, *Saccharomyces cerevisiae* yeast has become a model organism for elucidating the mechanism and regulation of copper homeostasis [6]. Genetic screenings have identified genes that are responsible for Cu uptake under nutritional conditions, as well as for Cu distribution to appropriate subcellular compartments, and detoxification processes that are activated under toxic conditions, when Cu ions are present in excess [1]. In *S. cerevisiae*, copper is a cofactor of Cu/Zn-superoxide dismutase, cytochrome *c* oxidase (Cyt Ox), and of the FET3 and FET5 multicopper oxidases that are required for several essential biochemical processes.
Copper uptake in this model microorganism is mediated by two separate systems: the so-called *high-affinity* system is involved under conditions of Cu nutritional depletion, while the *low-affinity* system operates in the presence of an excess of Cu ions, under toxic conditions [6, 7].
The high affinity copper uptake system involves two plasma membrane reductase enzymes, named Fre1 and Fre2, capable to induce reduction of $Cu^{(II)}$ to $Cu^{(I)}$ and two high-affinity transporter proteins, Ctr1 and Ctr3, that operate on cuprous ions [1, 6, 8-10]. Ctr1 is a 406-amino acid long protein with three potential trans membrane domains, able to form a multi-meric complex representing its active form. The Ctr1 amino-terminal portion is particularly rich in methionine and serine moieties and contains several copper binding sites. There are three 19-amino acid repetitions and 11 shorter moieties, each of them containing the Met-X_2-Met sequence (where X is any amino acid). The role of this sequence is still under investigation; however, the presence of similar repetitions in bacterial copper-resistant proteins (CopA of *Pseudomonas syringae*, CopB of *Enterococcus hirae* and CutE of *Escherichia coli)* might suggest a role in binding and removing copper ions [7, 11-14]. Ctr3 is a 241-amino acid long protein with three potential trans

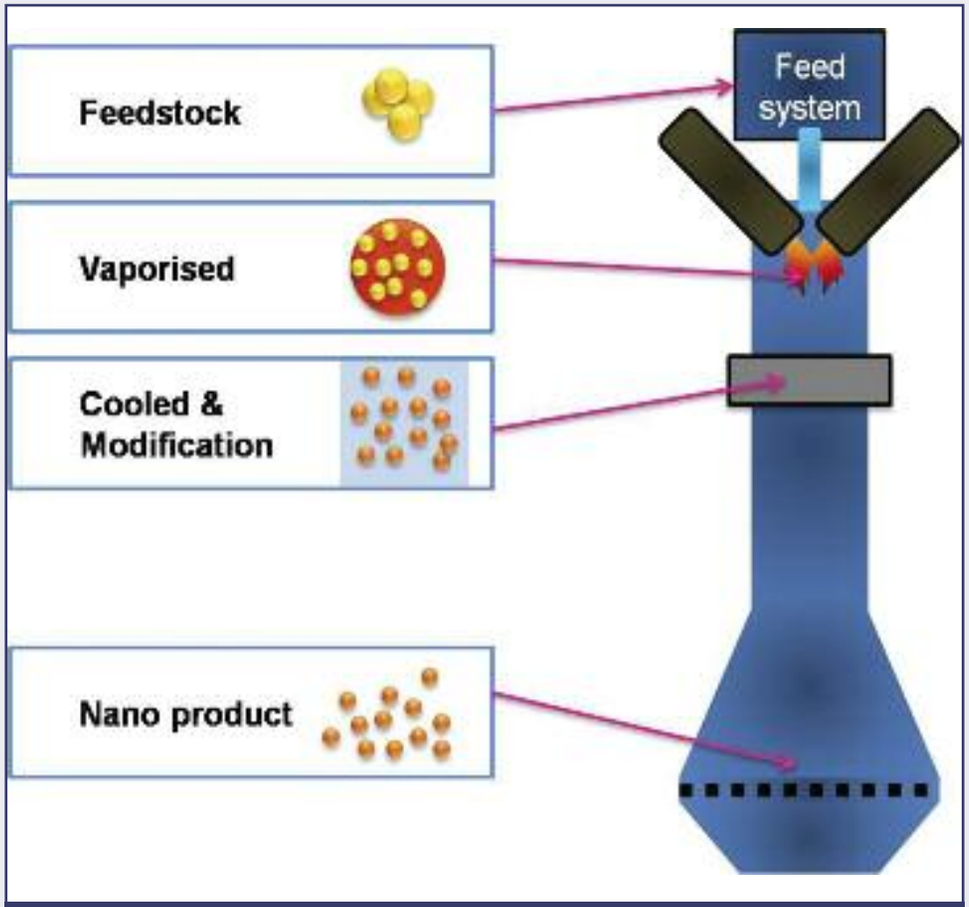

Fig. 1 - Tesima™ plasma process to generate nanoparticles, reprinted from International Journal of Antimicrobial Agents, **33**, G. Ren *et al.*, Characterisation of copper oxide nanoparticles for antimicrobial applications, 587-590. Reprinted 2009, with permission from Elsevier

membrane domains and strong sequence similarities, as compared to Ctr1 [7].

FRE1, FRE7, CTR1, and CTR3 species are downregulated by the transcriptional factor Mac1 [15-17], that is localized within the nucleus, apart from Cu concentration in the cell, and is characterized by cystein-rich carboxy-terminal moieties with trans-activation capability. Interestingly, Mac1 itself behaves as a Cu sensor, since its functioning may be repressed when $Cu^{(I)}$ binds to its activation domain [6, 15]. The excess of $Cu^{(II)}$ ions induces the expression of genes that encode proteins with a protective role, such as metallothioneins Cup1, Crs5 and superoxide dismutase Sod1. The expressions of CUP1, CRS5 and SOD1 genes is activated by the transcription factor Ace1 [6, 18].

The protein Ace1 cooperatively binds $Cu^{(I)}$ to form a tetra-Cu cluster through specific cysteine residues within the amino-terminal DNA binding domain [1, 18, 19]. Copper binding leads to a conformational change in this domain resulting in the specific binding of Ace1 to the metal response elements (MREs) 5'-$TCY_{(4-6)}$GCTG-3' on the gene promoters involved in the copper detoxification and protection against oxidative damage [1, 20]. Three low molecular weight proteins have been identified, Cu-chaperone Atx1, Cox17, and Lys7, that are essential for the intracellular delivering of $Cu^{(I)}$ ions to cellular compartments. At least, one additional chaperone is expected to direct $Cu^{(I)}$ ions to the nucleus for regulation of Mac1 e Ace1 activities [6]. These proteins bind copper after it enters the cell and subsequently deliver it to the corresponding recipient proteins. Atx1 is a small protein containing one metal-binding site and is the specific copper chaperone asked for copper delivering to the Ccc2 P-type ATPase in a late Golgi vescicle for the incorporation into Fet3 [7, 21, 22]. Cox17 is the copper chaperone that delivers copper to the mitochondria for Cyt Ox [7, 23], while Lys7 is required to incorporate copper into Sod1 [7, 24].

Synthetic routes to bioactive copper nanomaterials

Several approaches to the preparation of copper nanomaterials are nowadays available [25]. In the present paragraph, the different studies dealing with bioactive copper nanomaterials have been classified as a function of the synthesis approach and of the nanocopper final form used in the biological tests. Four different classes have been defined and reported in the following: (i) unsupported copper nanoparticles (CuNPs); (ii) polymer dispersed copper nanomaterials prepared by multistep procedures; (iii) copper nanomaterials supported on inorganic substrates via multistep procedures; (iv) co-deposited copper-containing nanocomposites.

Syntheses of unsupported, matrix-free copper nanomaterials

This group of studies consists of approaches leading to copper nanoparticles (CuNPs) or colloids that have been used in the final application without being combined with a dispersing/supporting material. As outlined in the Introduction section, although extremely interesting as a proof-of-concept, this application principle has been now outperformed and is not expected to result into real-life applications as such, due to nano-toxicology issues related to the direct contact of humans with transition metal nanoparticles.

Among the simplest approaches, the direct reduction of a precursor into copper nanoparticles has to be mentioned. J.P. Ruparelia

et al. have reported on the synthesis of CuNPs involving the well known wet-chemical reaction between sodium borohydride and copper nitrate, used as precursor. The resulting nanoparticles were dried in a reducing environment and stored in air-tight containers until biological tests [26].

A different approach, making use of thermal plasma technology was proposed in 2009 by G. Ren and coworkers. A scheme of the process is reported in Fig. 1; the procedure allows the preparation of CuO, Cu, and Cu_2O nanoparticles with extremely high surface areas and unusual crystal morphologies [27]. On the other side, drawbacks are represented by the costs of the processing apparatus and, most of all, by the potential risks related to handling copper-based nanoparticulate matter that could be unintentionally inhaled.

A low cost approach to nanoparticles and nanoparticulate coatings has been proposed by H.M. Yates *et al.*, who used flame assisted chemical vapour deposition technique starting from aqueous solutions of cupric nitrate as precursor, leading to nanostructured metal oxide thin films [28].

Multi-step routes to polymer dispersed copper nanomaterials

In this section, the multistep synthetic approaches to copper nanoparticles or nanocolloids embedded into polymer dispersing matrices are reviewed. Following these multi-step processes it is possible to prepare CuNPs separately and then the product is embedded in a number of polymer matrices, as a function of the final application envisaged. This approach affords for a tighter control on the CuNPs synthesis parameters and on their morphological, chemical, and biological properties, consequently. Moreover, despite unsupported copper colloids are extremely active against a wide range of bacterial pathogens [29], embedded nanoparticles afford for a higher environmental compatibility and then for a wider range of applications, either as free-standing nano-composites or as deposited thin nanostructured films. Lipophilic nanoparticles embedded into water-insoluble polymers cannot be released as such in the environment or when let in contact with aqueous solutions. On the contrary, they can easily and quickly undergo oxidation and dissolution processes [30-32], producing bioactive metal ions that can be then released in solution. Noteworthy, the concentration levels of Cu ions release can be finely selected in order to kill or inhibit the growth of harmful species, without affecting the health of human beings [30].

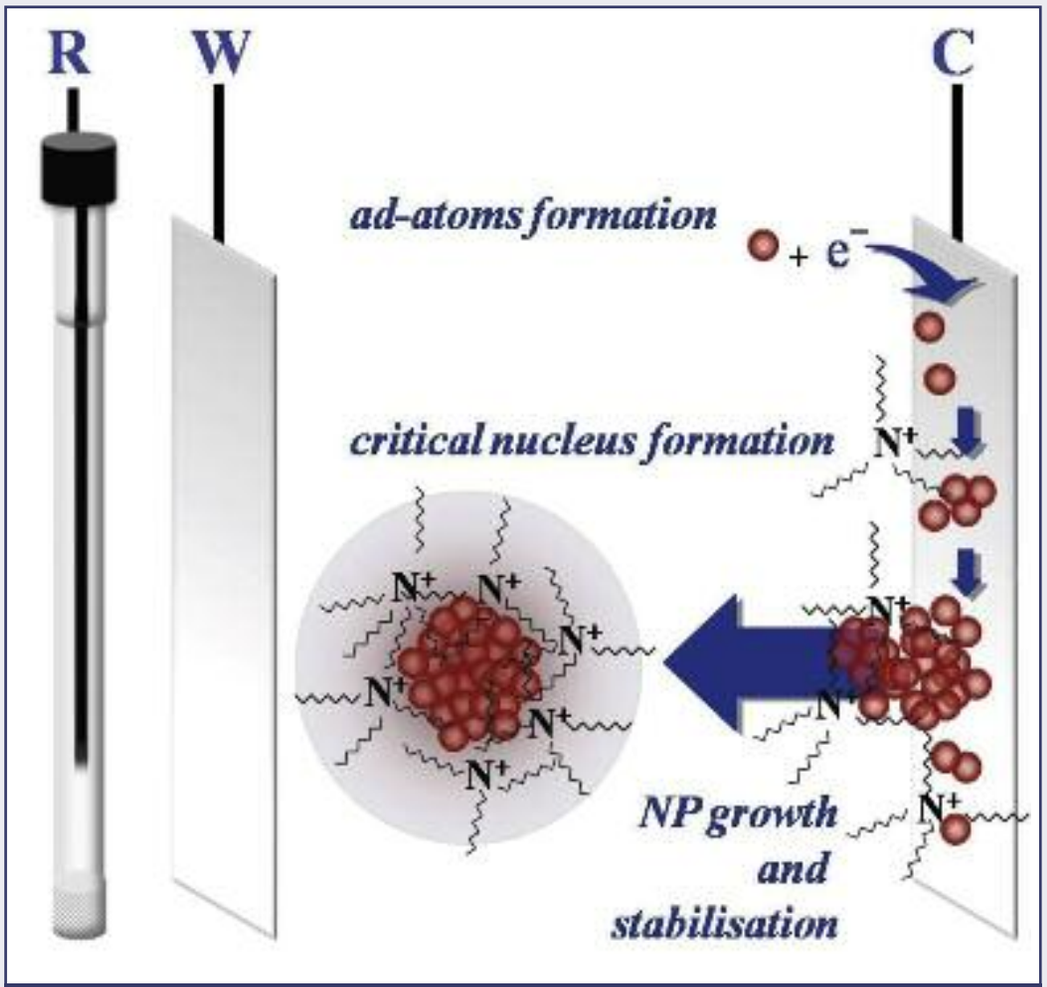

Fig. 2 - Schematic representation of the electrochemical cell used for the sacrificial anode electrosynthesis of core-shell copper nanoparticles (see text and references [13-16] for further details)

On the basis of these assumptions, in our laboratories we developed multi-step procedures for the preparation of core-shell CuNPs and CuNPs/polymer hybrid solutions that can be easily handled and used as precursor for spinnable nanocoatings and self-standing nanocomposites.

Copper nanoparticles were prepared using the sacrificial anode electrolytic process, performed by using a tree-electrode cell, equipped with an $Ag/AgNO_3$ reference electrode, a Cu anode and a Pt cathode. During the process, when the applied potential is sufficiently high, the anode dissolves under the form of metal ions that are subsequently reduced at the cathode surface in the presence of proper surfactants, such as tetra-n-alkyl-ammonium salts, which stabilize the nanoparticle under the form of a *core-shell* structure in which the

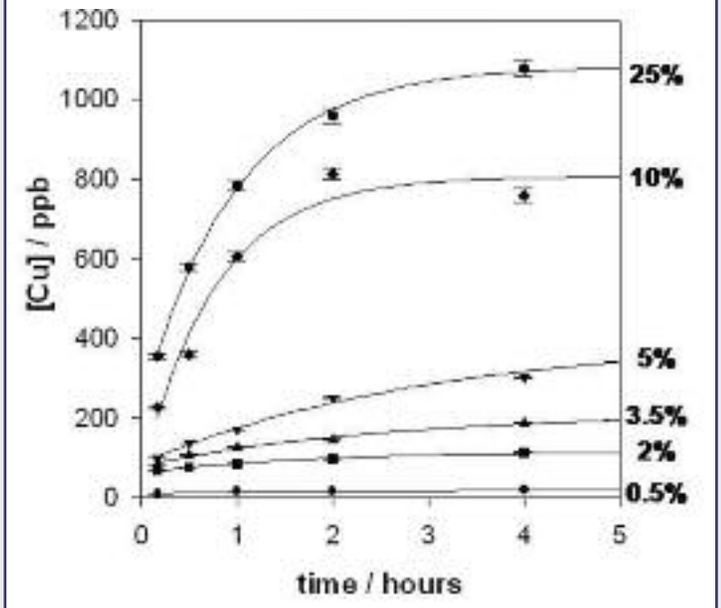

Fig. 3 - Copper release from CuNPs-polyvinyl-methylketone composite coatings with different CuNPs loadings (relative Cu weight percentage reported on the right). Lines are the result of a first-order kinetics release model. Reprinted with permission from N. Cioffi *et al., Chemistry of Materials*, 2005, **17**, 5255. Copyright 2005 American Chemical Society

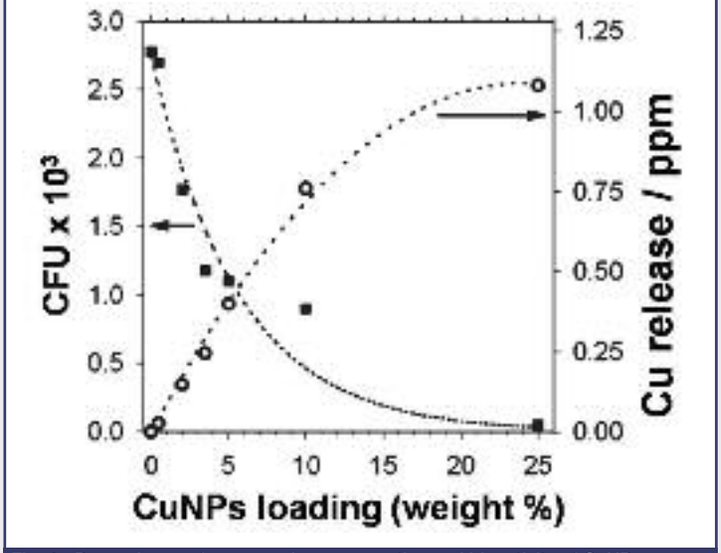

Fig. 4 - Dependence of copper release properties and bioactivity of CuNPs-polyvinyl-methylketone (PVMK) coatings upon CuNPs loading in the composite. The left Y axis reports the amount of the residual colony forming units (CFU)/ml developed after exposure to CuNPs/PVMK nanocomposites loaded with different bulk concentrations of CuNPs. The right Y axis reports the copper concentration released by the nanocomposites in a microorganism-free culture broth after the same exposure time. Reprinted with permission from N. Cioffi *et al., Applied Physics Letters*, 2004, **85**(12), 2417. Copyright 2004, American Institute of Physics

copper core is surrounded by quaternary ammonium ions (see Fig. 2 for a sketch of the process). *Core-shell* CuNPs were directly obtained as nano-colloidal dispersion in solution and then, they could be easily mixed to water insoluble polymers (such as poly-vinyl-methyl-ketone (PVMK), poly-vinyl-chloride, poly-vinylidene-fluoride, etc.) which were ultrasonically dissolved in the electrolysis solution. The resulting hybrid solutions - with different CuNPs loadings - were directly spin-coated on several substrates for further characterization or bioactivity studies.

The peculiarity of these materials relies on the stabilized structure of the NPs, which allows a gradual and controlled copper ions release, when the nano-coating is exposed to aqueous solutions. The release extent of bioactive ions was demonstrated to be easily tunable by a proper selection of preparation parameters, such as, for instance, the metal loading in the film. In Fig. 3 typical curves of $Cu^{(II)}$ release in physiological solutions are reported as a function of time (abscissa axis) and of the Cu weight percentage in the nano-coating. The release kinetics were modeled as a pseudo-first order process and provided useful information on the release mechanism. In separate experiments with twin samples, a clear correlation was shown between the extent of ionic release in solution and its inhibition effects towards the growth of yeasts colony forming units (CFU). In Fig. 4, the results of such a characterization are reported for a set of CuNPs/PVMK samples with different copper loading. It can be noted that the higher the NP loading in the coating, the lower is the number of CFU, i.e. the stronger the antifungal effect [30-33].

The incorporation of smaller inorganic particles into larger polymers is desirable in order to combine the key properties of both the materials. The group of C. Neckers has recently reported on the synthesis of functionalized CuNPs/polymer composites. The method used to functionalize the CuNPs was a modification of the Brust's procedure, introducing a polymerizable acrylic functionality into the NPs shell [34].

Among the wide variety of polymer matrices, biopolymers are optimal candidates as CuNP-dispersing matrix, since they are readily available, inexpensive, environmental friendly and fully compatible with scaling up industrial needs. Moreover, the biopolymer oxygen-rich functionalities and their affinity towards metals make them ideal candidates for the stabilization of nanoparticles [29]. For example, agarose has been utilized to prepare bioactive composite films containing metal/semiconductor nanoparticles by introducing a metal/-semiconductor precursor solution followed by a reducing agent, such as hydrazine hydrate, during the agarose gelation process [29]. Recently, G. Mary *et al.* have prepared CuNP-loaded fibers [35]. They have proposed a novel cellulose-based fiber, obtained by periodate-induced oxidation of cotton cellulose fibers to give di-aldehyde cellulose, followed by covalent attachment of $-NH_2$ group of chitosan through a coupling reaction. This novel chitosan-bound cellulose fiber has been used for the immobilization of $Cu^{(II)}$ ions and subsequently of copper nanoparticles produced by borohydride-induced reduction of $Cu^{(II)}$ [35]. The same authors have also developed copper nanoparticles-loaded composite fibers, which were

prepared by immersing cotton fibers in aqueous solution of sodium alginate, followed by cross-linking of alginate chains in presence of $Cu^{(II)}$ ions; finally, the fibers were reduced with sodium borohydride [36]. Very recently a new method for preparing antibacterial cotton bandages has been developed by treating coating fabric surface with CuO nanoparticles obtained via ultrasound irradiation [37].

Finally, G. Cárdenas *et al.* have described the preparation and characterization of colloidal Cu nanoparticles/chitosan composite films by a solution-casting technique assisted by microwave heating [38].

Multi-step syntheses of copper nanomaterials supported on inorganic substrates

Compared to organic hybrid antibacterial materials, fully inorganic antibacterial materials may have the following advantages: long lasting action period, chemical stability, thermal resistance, simplified manufacturing and storing issues [39]. The preparation of inorganic antibacterial materials has attracted interest in recent years, although it should be noted that - in case of weak interactions - this approach might suffer for NP leaching/releasing in aqueous media, that might give rise to nano-toxicology drawbacks.

H. Weickmann *et al.* produced poly-methyl-methacrylate (PMMA)/bentonite/Cu nanocomposites by an electroless copper plating processes involving aqueous Pd/bentonite dispersions that were firstly blended with a PMMA dispersion and then treated with an electroless plating bath. As a result, 14 nm copper nanospheres were selectively immobilized at the bentonite nanoplatelet surface [40].

Inclusion of copper into vermiculite (chemical formula: $(Mg,Ca)_{0.6\text{-}0.9}(Mg,Fe^{3+},Al)_{6.0}[(Al,Si)_8O_{20}](OH)_4{\cdot}nH_2O$) has been investigated by B. Li *et al.* [41]. Vermiculite has high magnesium cation exchange capacity and Cu ions may easily replace it because of similar charge density and ionic radius. The Cu-containing nanomaterial was prepared by impregnating copper ions into the nanometer openings between vermiculite layers.

A significant approach is that of synthesizing CuNPs in the presence of a supporting oxide, such as alumina, titania, and mesoporous silica. Based on the chemical stability of the interactions between CuNPs and the supporting oxide, the copper release properties can be delayed for a long time so that Cu-supported materials become appealing for antibacterial applications. Y.H. Kim *et al.* reported on the synthesis and characterization of a well dispersed Cu-SiO_2 nanocomposite that was obtained by depositing copper nanophases onto preformed SiO_2 NPs [42]. In this process $CuCl_2$ was added to a SiO_2 NPs slurry with or without making use of catalysts, at room temperature, and under vigorous stirring. The schematic diagram for the tentative mechanism of the preparation is shown in Fig. 5.

Very recently, X. Wu *et al.* prepared mesoporous copper-doped silica xerogels (m-SXCu) by a sol-gel process in which tetra-ethoxy-orthosilicate and $CuSO_4{\cdot}5H_2O$ were used as precursors of Si and

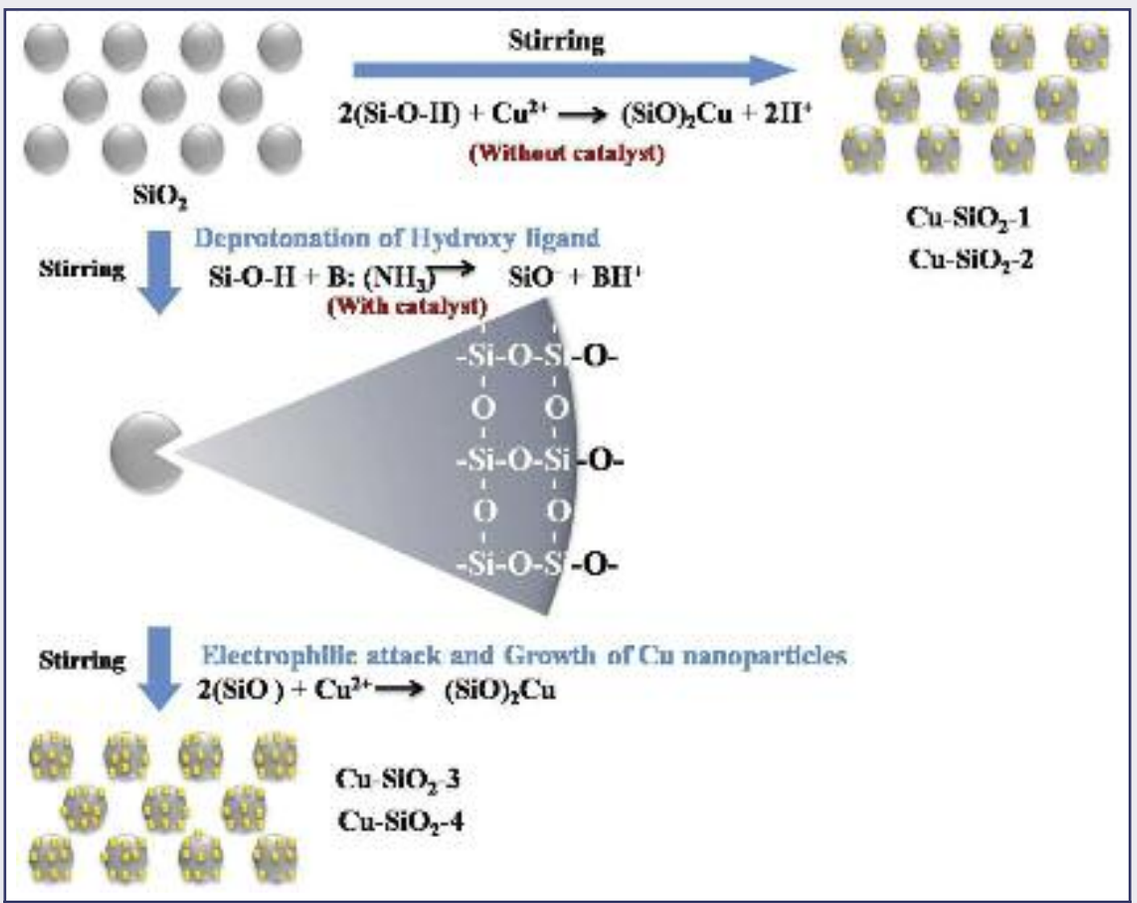

Fig. 5 - Tentative mechanism of Cu deposition on the surface of SiO_2 nanoparticles with and without making use of catalysts. Adapted with permission from Y.H. Kim *et al., J. Phys. Chem. B*, 2006, **110**, 24923, Copyright 2006 American Chemical Society

Tab. 1 - Overview of the different biological tests performed using bioactive copper nanomaterials against the growth of bacteria

Target bacteria	Biological test	Nanomaterial	Ref.
Escherichia coli	Contact with culture broth	CuNPs/fluoropolymer films	45
	Contact with culture broth	CuNPs/polymer composites	32
	Contact with culture broth	CuNPs//polymer composites	31
	Contact with culture broth	Cu agarose nanocomposites	30
	Agar diffusion assay/Contact with culture broth	CuNP-loaded fibers	35
	Plate-counting	CuNPs embedded into polyethylene	47
	Agar diffusion assay	Cu/vermiculite nanocomposite	29
	Agar diffusion assay	Cu-SiO_2 nanocomposites	42
	Plate counting	Cu-doped mesoporous silica xerogels	43
	Minimum Agar diffusion assay/Minimum inhibitory concentration/Minimum bactericidal concentration	Cu nanoparticles	26
	Minimum bactericidal concentration	Cu oxide nanoparticles	27
	Contact with culture broth	Cu oxide films	28
	Contact with culture broth	CuO-cotton nanocomposite	37
	Agar diffusion assay/Plate-counting	CuNP-loaded fibers	36
	Plate-counting	n-CuO doped glass	44
Staphylococcus aureus	Contact with culture broth	CuNPs/fluoropolymer films	45
	Contact with culture broth	CuNPs/polymer composites	32
	Contact with culture broth	CuO-cotton nanocomposite	37
	Agar diffusion assay	Cu-SiO_2 nanocomposites	42
	Plate counting	Cu-doped mesoporous silica xerogels	43
	Minimum Agar diffusion assay/Minimum inhibitory concentration/Minimum bactericidal concentration	Cu nanoparticles	26
	Minimum bactericidal concentration	Cu oxide nanoparticles	27
	Plate counting	CuNPs/chitosan composite films	38
Pseudomonas aeruginosa	Minimum bactericidal concentration	Cu oxide nanoparticles	27
Synechocystis sp. PCC 6803	Contact with culture broth	CuNPs/polymer composites	34
Synechococcus sp. PCC 7002	Contact with culture broth	CuNPs/polymer composites	34
Enterobacter cloacae	Agar diffusion assay	Cu-SiO_2 nanocomposites	42
Bacillus subtilis	Minimum Agar diffusion assay/Minimum inhibitory concentration/Minimum bactericidal concentration	Cu nanoparticles	26
Staphylococcus epidermidis	Contact with culture broth	Cu oxide films	28
	Minimum bactericidal concentration	Cu oxide nanoparticles	27
Lysteria monocytogenes	Contact with culture broth	CuNPs/polymer composites	32
Salmonella enterica	Plate counting	CuNPs/chitosan composite films	38
Micrococcus luteus	Plate counting	n-CuO doped glass	44

Cu, respectively [43]. Cu ions were demonstrated to be released from m-SXCu into the simulated body fluid at release rates depending on the nanocomposite composition. Finally, in a very recent work by Esteban-Tejeda *et al.*, a low melting point soda-lime glass powder containing copper nanoparticles has been obtained following a bottom-up route starting from n-Cu sepiolite as source of the copper nanoparticles [44]. The glass was milled down and homogeneously mixed in isopropyl alcohol with the corresponding fraction of n-Cu sepiolite to obtain a copper doped glass that was further treated thus obtaining glass bars or pellets, that were finally milled down to <30 μm in an agate planetary mortar [44].

Co-deposition approaches for copper-containing nanocomposites

The last class of routes to nanocopper-based antimicrobials is based on physical approaches leading to the simultaneous deposition of both CuNPs and of a proper dispersing medium (either organic or inorganic). The main difference between this approach and those described in previous paragraphs is that it does not provide NP stabilization with a chemisorbed layer of surfactants or capping agents. The co-deposited CuNPs are just dispersed into a matrix, and this often is not enough to reach a fine tuning of the nanomaterial ionic release properties.

In 2005, we used the ion beam

Tab. 2 - Overview of the different biological tests performed using bioactive copper nanomaterials against the growth of fungi and algae

Target fungi	Biological test	Nanomaterial	Ref.
Saccharomyces cerevisiae	Contact with culture broth	CuNPs/fluoropolymer films	45
	Contact with culture broth	CuNPs/polymer composites	32
	Contact with culture broth	CuNPs/polymer composites	30
	Contact with culture broth	CuNPs/polymer composites	31
Aspergillus niger	Contact with inoculum	Cu doped amorphous hydrogenated carbon	46
Chaetomium globosum	Contact with inoculum	Cu doped amorphous hydrogenated carbon	46
Cladosporium cladosporioides	Contact with inoculum	Cu doped amorphous hydrogenated carbon	46
Epicoccum nigrum	Contact with inoculum	Cu doped amorphous hydrogenated carbon	46
Pestalotia heteromorpha	Contact with inoculum	Cu doped amorphous hydrogenated carbon	46
Candida albicans	Agar diffusion assay	Cu-SiO_2 nanocomposites	42
Penicillium citrinum	Agar diffusion assay	Cu-SiO_2 nanocomposites	42
Issatchenkia orientalis	Plate counting	n-CuO doped glass	44
Target algae	**Biological test**	**Nanomaterial**	**Ref.**
Chlamydomonas sp. strain CD1	Contact with culture broth	CuNPs/polymer composites	34
Phaeodactylum tricornutum CCMP 1327	Contact with culture broth	CuNPs/polymer composites	34

co-sputtering technique to deposit different fluoropolymer/CuNPs hybrid materials in an extremely controlled fashion, just by adjusting the sputtering deposition experimental conditions [45]. The analytical characterization of these layers revealed that inorganic nanoparticles composed of $Cu^{(II)}$ species could be evenly dispersed in a branched fluoropolymer matrix at different copper/fluoropolymer concentration ratios, ranging from 0 to 35% [45]. However, ion release quantifications showed that these unstabilized CuNPs give rise to extremely fast and intense releases that are not compatible with long term usage or re-usage needs.

To the best of our knowledge, the first work aiming at depositing nano-antimicrobials by ion beam sputtering was published by V.I. Ivanov-Omskii *el al.* in 2000. The Authors reported on the modification of hydrogenated amorphous carbon (a-C:H) films with nano-sized copper clusters by ion co-sputtering of copper and graphite targets in argon-hydrogen DC magnetron plasma. Copper trapped into the a-C:H matrix was shown to deeply modify the structure and chemical characteristics of the layer, and interesting applications to the prevention of fungi growth were innovatively provided already in that year [46].

In a series of more recent studies, copper inclusions were implanted into medical-grade polyethylene (PE) specimens by means of a plasma immersion ion implanter (PIII) equipped with a copper cathodic arc plasma source [47-49]. Compared with single plasma implantation processes, this dual PIII process provided a better control over the copper release rate and improved the long-term antibacterial properties of the Cu-PE nanomaterials [47].

Applications and potentialities of bioactive copper nanomaterials

In the next years Cu-containing nanomaterials will surely find a lot of real-world applications due to their high bioactivity, as well as to the relatively low cost of copper and to its widespread actual use in several technological fields.

As outlined in the introduction section, copper has itself an excellent antimicrobial activity against a wide range of microorganisms (such as bacteria, fungi, algae and viruses), and such a property is greatly improved when copper is properly nano-dispersed.

All papers so far discussed dealt with the growth inhibition or killing effects of the novel copper nanostructures towards several microorganisms. The different studies on bioactivity of each nanomaterial are itemized in Tab. 1 and 2, and classified as a function of the target microorganism. Bacterial sensitivity to nanostructures was found to vary depending on the microbial species and on the experimental set-up. Actually, a quantitative comparison of these bioactivity effects is not possible, since in all cases the antimicrobial effectiveness was studied using different experimental parameters, such as methods, time of contact, microorganism strain as well as its initial concentration. So far, in the future the use of the so-called “minimum inhibition concentration” method is highly desirable, thus allowing biological comparative studies.

Despite these difficulties, the results mentioned in Tab. 1 and 2 demonstrate the great potentialities of functionalized CuNPs as efficient biocide agents with a wide action. The large active surface area offered by the NPs makes it possible to reduce the active metal loading at $1\%_{weight/weight}$ or even less. The use of stabilized and/or polymer-dispersed CuNPs provides better leaching control, especially when NPs are chemically attached to the polymer backbone or they are further stabilized, due to a core-shell structure.

Biological activity is not compromised by the use of controlled releasing Cu-nanomaterials; on the contrary, we have recently demonstrated that these materials may have a great value for the development of antibacterial paints and coatings for household materials, textiles, biomedical devices, hospital and food storage equipments, etc. [50, 51].

The porous nature of agarose polymer films coupled with CuNPs can find application in antimicrobial membrane filters and coatings [29]. This kind of nanomaterial can be utilized in food packaging, sanitation and fabrics, because of the ability of agarose to form transparent gels and films [29].

Metal nanoparticle-loaded fibers (particularly cotton cellulose fibers) could be used in a number of biomedical and textile applications such as in medical devices, burn/wound dressings, healthcare (including disposables), personal care products, veterinary, military and bio-defense, protective suits, clothing, etc. [35, 37]. Among the fibrous products, alginate-based products are currently the most popular ones used in developing antimicrobial agents releasing systems or textile materials. In particular, copper nanoparticles-loaded composite fibers show fair mechanical strength, excellent Cu(II)-releasing capacity due to ion-exchange property and a high degree of antibacterial activity against model bacterium *E. coli* [36]. Similarly, films based on other biopolymers, such as chitosan, modified with CuNPs were demonstrated to be effective in reducing the bulk fluid concentration of two microorganisms affecting food quality and could be therefore used to improve food quality and extend its shelf life [38].

An inorganic antimicrobial agent incorporated into an organic biomedical polymer can significantly increase its usefulness in medicine [47] and even inorganic nanocomposites such as mesoporous copper-doped silica xerogels have been proposed as biomedical materials (for instance in the treatment of traumatic wounds), because of their excellent *in vitro* and *in vivo* biocompatibility [43].

CuO nanoparticles were demonstrated to be effective in killing a range of bacterial pathogens involved in hospital infections. Noteworthy, hospitals and transport are two particular areas that offer great opportunities for the use of nanocopper antimicrobials to prevent infections and pandemic diseases. Wall coverings, equipment, clothing, sits and bedding are all potential risk areas for the spread of infections, and this could be particularly important in case of microorganisms that have developed a resistance towards conventional disinfecting agents and antibiotics [27].

Finally, amorphous hydrogenated carbon films doped with copper were used as a protective media against biodeterioration related to fungi [46].

The number of potential applications is evidently enormous, and it is expected to grow further, considering that other metals - such as silver and zinc - can be used for similar purposes, and multi-metal nanomaterials could be developed, too.

The main limitations to the development of this research field reside in the need of carefully assessing any possible nanotoxicology issue related to the use of brand new nanomaterials with unknown properties. Again, considering this important aspect, we outline the great simplifications and the clear advantages provided by stabilized and/or polymer embedded CuNPs, acting as tunable nano-reservoirs for ionic release, more than behaving as free and unknown nano-bio-killers themselves.

Conclusions

A selection of recent studies dealing with the development of novel bioactive copper nanomaterials and their applications has been critically reviewed in the present paper. Thanks to the large number of possible approaches to these nanostructures and to the perspective growth of their real-life applications, it is reasonable to envisage a great development of this research field. Our acquaintance with bioactivity and nano-toxicology issues related to these materials needs surely to be cultivated and it will be further improved in the next years, as well.

Acknowledgements: Professors Luisa Torsi and Luigia Sabbatini are gratefully acknowledged for helpful scientific discussions.

List of acronyms

Cyt Ox	Cytochrome c oxidase
Fet3	FErrous Transport 3
Fet5	FErrous Transport 5
Fre1	Ferric/Cupric REductase 1
Fre2	Ferric/Cupric REductase 2
Ctr1	Copper Transport 1
Ctr3	Copper Transport 3
CopA	Copper resistance Protein A
CopB	Copper resistance Protein B
CutE	Copper transport E
Cu Res	Cu-Responsive cis-acting Elements
Mac1	Metal binding Activator 1
Cup1	Copper ion binding Protein 1
Sod1	Copper/zinc superoxide dismutase 1
Crs5	Copper Resistant Suppressor 5
Ace1	Activation of Cup1 Expression
MREs	Metal Responsive Elements
Atx1	Antioxidant 1
Cox17	Cytochrome c oxidase copper chaperone 17
Lys7 or Ccs1	Copper Chaperone for Sod1
Ccc2	Cross-complements Ca^{2+} phenotype of csg1

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RIASSUNTO

Nanomateriali bioattivi a base di rame - *Negli ultimi anni è cresciuto l'interesse accademico, ma anche industriale verso lo sviluppo di agenti antimicrobici innovativi. In questo contesto, sempre più spesso vengono proposti nuovi materiali nanostrutturati a base di metalli quali rame, zinco ed argento. In questa rassegna si vogliono descrivere i principali studi che riguardano la sintesi e la caratterizzazione di nanomateriali a base di rame in grado di garantire la prevenzione della bio-contaminazione in diversi contesti applicativi.*

Nicoletta Ditaranto accomplished her PhD studies in Chemistry of Innovative Materials at the University of Bari. In 2006 she received the "Ambrogio Mazzucotelli" Award from the Analytical Spectroscopy Group of the Italian Chemical Society (SCI). At present, she has a post-doc fellowship at the Chemistry Department of the University of Bari. Her main research interests are in the field of the synthesis, characterisation and analytical chemistry application of nanomaterials. Her scientific production: 18 papers on journals and textbooks, 2 European patents, 5 extended abstracts, and 56 conference communications.

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