Antifungal activity of polymer-based copper nanocomposite coatings

Nicola Cioffi, Luisa Torsi,[a] Nicoletta Ditaranto,
Luigia Sabbatini, and Pier Giorgio Zambonin
Dipartimento di Chimica, Università degli Studi di Bari, 4, via Orabona, 70126 Bari, Italy

Giuseppina Tantillo
Dipartimento di Sanità e Benessere degli Animali, Università degli Studi di Bari, Strada Provinciale per Casamassima Km 3, 70010 Valenzano, Bari, Italy

Lina Ghibelli and Maria D'Alessio
Dipartimento di Biologia, Università di Roma Tor Vergata, 1, Via della Ricerca Scientifica, 00133 Roma, Italy

Teresa Bleve-Zacheo
Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., 165/A, via Amendola 70126 Bari, Italy

Enrico Traversa
Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma Tor Vergata, 1, Via della Ricerca Scientifica, 00133 Roma, Italy

Eukaryotes, such as fungi, can be harmful pathogen agents, and the control of their bioactivity is critical as humans are eukaryote organisms, too. Here, copper/polymer nanocomposites are proposed as antifungal spinnable coatings with controlled copper-releasing properties. The tests of the bioactivity show that fungal growth is inhibited on the nanocomposite-coated plates, and the antifungal activity can be modulated by controlling the Cu nanoparticle loading. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1794381]

The antimicrobial properties of metal-containing composite materials are promising for biotechnological applications, such as safe food packaging or sterile coatings for biomedical devices, and it is widely recognized that important advancements can be reached with nanocomposites. However, while a great deal of work has been recently performed on nanoscale materials[1] and also on copper-based nanocomposites,[2,3] little is yet known concerning their antimicrobial properties.[4] Besides, papers published so far deal only with the study of the antibacterial properties of composites, mainly loading silver[4–8] which exerts a stronger toxic action against prokaryotes (i.e., all types of bacteria) than eukaryotes (i.e., all other organisms, from fungi to humans).[9,10]

Eukaryotes such as fungi can be dangerous pathogenic agents, and effective antifungal aqueous solutions based on copper ions[11] as well as complex copper species[12–15] or copper-containing polymers[16,17] have often been proposed and used. This recommends exploring copper-based composites for effective antifungal coatings. The main concern is the similarities existing among eukaryotes, in terms of metabolism, that would require a material capable of controlling the release of copper, in order to minimize human toxicity. Recently, some reports have appeared on the antimicrobial properties of technologically appealing materials such as fabrics, paints, or coatings containing copper-based active powders or pigments.[18–20] However, the development of materials capable of releasing, in a controlled fashion, antifungal agents such as copper ions, is still an open issue.

In this work, polymers loading copper nanoparticles are proposed as spinnable coatings capable of releasing a quantifiable amount of copper species to a broth of Saccharomyces *cerevisiae* yeast, selected as a model for eukaryotic microorganisms.

The composites have been prepared following an easy-to-perform two-step procedure. CuNPs were electrochemically synthesized in an alkyl ammonium micellar environment,[21] following a procedure previously used with other metals.[22–24] The resulting nanoparticles have a mean core diameter as small as 3.2+1.6 nm, as shown in Fig. 1(a), and are constituted of a metallic inner core covered by a stabilizing surfactant shell.[24,25] X-ray photoelectron spectroscopy (XPS) analysis shows that the metallic core of air exposed CuNPs is rapidly converted into CuO. The as-synthesized CuNPs colloid was ultrasonically mixed to three different water insoluble polymers, namely, polyvinylmethylketone (PVMK), polyvinylchloride (PVC), and polyvinylidenefluoride (PVDF), used as embedding matrices.[26] Each of the polymer solutions was mixed (9:1 ratio) with the CuNPs colloidal dispersion, and the resultant solutions were
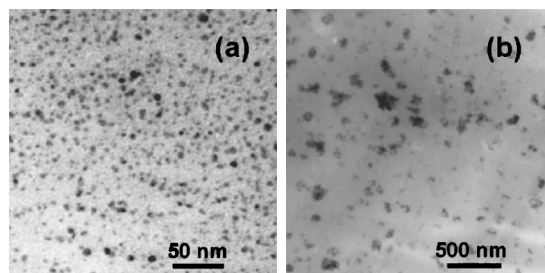

FIG. 1. Transmission electron microscope images of the tetrabutylammoni-umperchlorate (TBAP)-stabilized Cu nanoparticles (a) and of the NC#1 nanocomposite, showing the clusters of nanoparticles (b). All the images have been obtained at 100 KV.

[a]Phone: +390805442092; fax +390805442026; electronic mail: torsi@chimica.uniba.it

spin coated on sterile and inert glass plates. For "blank" experiments, polymers containing only the alkyl ammonium surfactant were obtained by substituting the CuNPs colloid with a solution of bare alkyl ammonium salt.[27]

A surface metal loading of 1–2 at. %, evaluated by means of XPS elemental analysis, resulted for a nominal bulk copper loading of 5 wt % in the case of the CuNP-PVMK and CuNP-PVC nanocomposites (called NC#1 and NC#2, respectively, in the following). For the same nominal bulk loading, Cu surface percentages lower than one order of magnitude were obtained in CuNP-PVDF (NC#3) films; this finding is ascribable to the slightly different preparation conditions necessarily adopted for this nanocomposite because of the insolubility of PVDF in the colloid solvent.

The morphology of a typical NC#1 film is shown in Fig. 1(b). Aggregates with an average diameter of about 50 nm are evenly dispersed into the polymer matrix; the clusters contain several hundreds of nanoparticles with a mean diameter of 4.6 ± 1.8 nm. The other nanocomposites showed a similar nanostructured morphology.

The screening of the antifungal activity of the three nanocomposites was performed on Saccharomyces *cerevisiae* yeast using a two-step protocol. In the first step, properly diluted cultures containing the living cells were let in contact, in sterile conditions, with the nanostructured layers (deposited onto the glass plate) for 4 h. The same procedure was performed on specimens prepared for control and "blank" experiments performed to exclude that any antifungal effect had to be ascribed either to the bare polymer matrices or to the alkyl ammonium salt. In the second step, a plate counter agar solid culture medium was poured into the glass plates that were subsequently incubated so that the *vital* yeast cells, eventually present, could grow into colonies. The striking results are reported in Figs. 2(a) and 2(b), where the microscope pictures of the PVMK specimens plates are reported. The plate of the PVMK added with the surfactant [Fig. 2(a)] exhibits a number of colony forming units (CFU per ml of yeast culture per plate: CFU/ml) which is comparable, within the experimental error, to that of the bare PVMK film and to the control experiment of colony growth on the culture medium. The strong antifungal activity of the nanocomposite is apparent as no CFU can be observed on the NC#1 plate [Fig. 2(b)]. Antifungal activity was also observed with the other nanocomposites but not all the yeasts were completely inactive in these cases [see Fig. 2(c)]. Although a slight run-to-run variation of the number of CFU/ml developed was observed, the NC#1 films always exhibited the strongest antifungal effect, while the least effective were the NC#3 ones. The results reported in Fig. 2(d) (left *Y* axis) are relevant to the study of the antifungal activity of seven NC#1 nanocomposites loaded with different amounts of CuNPs. The results clearly show that the higher the nanoparticle loading, the lower the number of CFU; i.e., the stronger the antifungal effect.

The results of the antifungal activity correlate very well with the electrothermal atomic absorption spectroscopy (ETAAS) analysis of the copper released by the nanocomposites in a yeast-free culture broth.[28] Figure 2(d) (right *Y* axis) shows that the extent of the copper release increases with the metal loading in the films; this result demonstrates that the releasing properties of such nanocomposites can also be controlled by a proper modulation of the CuNPs loading. It was also found that comparable amounts of Cu are re-
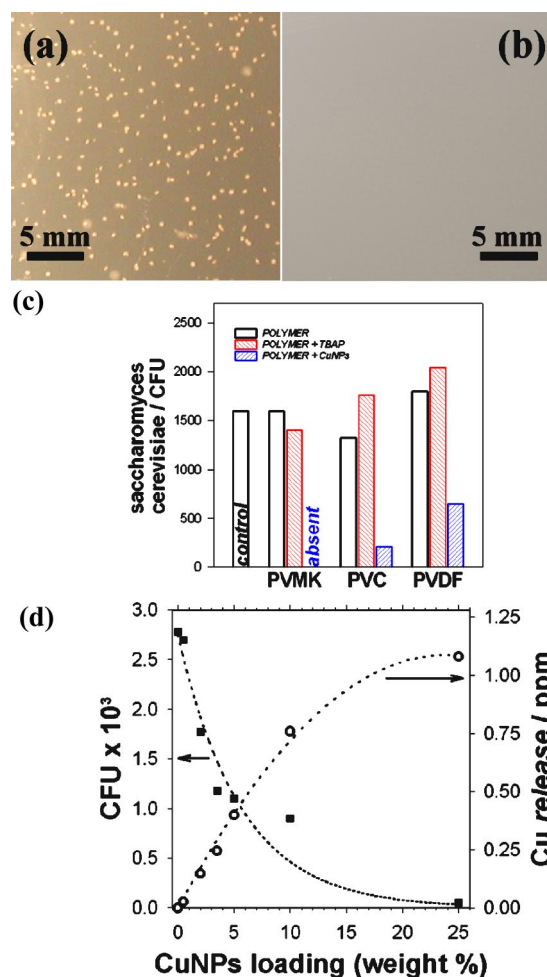

FIG. 2. (a) Microscope pictures of the TBAP-containing PVMK films and (b) NC#1 plates, after yeast incubation. (c) Histogram of the number of CFU/ml on the plates of the three different nanocomposites and of all the control specimens. (d) The left *Y* axis reports the amount of the CFU/ml for seven different PVMK nanocomposites loaded with different bulk concentrations of CuNPs. The right *Y* axis reports the copper concentration released by the nanocomposites in the culture broth after 4 h exposure. Dotted curves between the experimental points are just a guide for the eyes. CFU/ml data of (c) and (d) are relevant to two different experiments in which a different colony growth occurred also on the control test in the culture medium.

leased by NC#1 and NC#2, while much lower quantities are released by NC#3, as expected, given the lower copper content effectively embedded in this composite.

To obtain insights into the interesting and controlled releasing properties of the nanocomposites, the kinetic copper release curves from the nanocomposites loaded with different amounts of CuNPs were studied. The coatings were let in contact with the yeast-free culture broth for 4 h and the solutions were sampled several times and subjected to ETAAS analysis. The curves were satisfactorily modeled as first-order kinetic processes. On the basis of XPS and ETAAS results, copper release is ascribed to the dissolution of the CuO, present on the NPs, to soluble Cu(II). Further work is in progress to assess the role of the NPs stabilizing surfactant shell in controlling the release process. Once the NPs oxide layer dissolves, the copper ions diffuse through the polymer matrix to the solution. To exclude matrix-related retardant effects, a composite formed by a PVMK film embedding CuCl$_2$, in an amount comparable to that of the nanocomposite, was studied. The diffusion of Cu(II) through the polymer

matrix being the slower step could be ruled out as the release in the case of the copper chloride PVMK film was much faster than in the nanocomposites. The maximum copper release by the salt-containing composite was also much higher and comparable to the Cu(II) solubility in the aqueous culture broth, meaning that this material exerts no control over the copper release as the process is controlled by the Cu(II) solubility in the broth.

In conclusion, polymer-based copper nanocomposites are proposed as spinnable bioactive coatings. Their biostatic activity has been demonstrated on Saccharomyces *cerevisiae*, chosen as a model for eukaryote microorganisms. A correlation between the biological tests and the material's release properties has been established and a rationale has been proposed for the metal dissolution process. Coatings, also containing silver nanoparticles, will be tested against pathogen microorganisms such as *Escherichia Coli*, and *Staphylococcus Aureus*, and *Lysteria*.

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[21] CuNPs were prepared employing 0.1 M tetrabutylammonium perchlorate in acetonitrile (ACN)/tetrahydrofurane (THF): 1/3 in a three-electrode cell, under a nitrogen atmosphere. Anode and cathode electrodes were twin copper sheets. The potential applied to the anode was +4 V vs an Ag/AgNO$_3$ (0.1 M in ACN) reference electrode.

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[26] PVMK and PVC dispersing polymers were ultrasonically dissolved in ACN/THF: 1/3 solution at a concentration of 25 gL^{-1}. Due to its insolubility in ACN/THF, PVDF was dissolved in dimethylformamide at the same concentration.

[27] 0.1 M tetrabutylammonium perchlorate in ACN/THF: 1/3.

[28] The analysis was carried out using a hollow cathode Perkin Elmer spectrophotometer and the following temperature program. Step 1: up to 110 °C in 30 s, hold time 30 s; step 2: up to 1000 °C in 30 s, hold time 30 s; step 3: up to 2000 °C immediately, hold time 10 s; step 4: up to 2600 °C in 2 s, hold time 2 s.