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Copper nanoparticles with high antimicrobial activity

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ABSTRACT

Copper (Cu) hydrosol containing small nanoparticles (NPs) (~5 nm) with narrow size distribution were used to investigate antimicrobial activity toward representative microorganisms of public concern (*Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*). Microbial reduction measurements were performed as a function of CuNPs concentration, after 2 h of their contact. Surface and morphological alterations of the strains exposed to the Cu NPs were studied on the cellular level using an atomic force microscope (AFM).

1. Introduction

The current interest for exploring metal NPs for antimicrobial applications, is due to the growing microbial resistances to multiple antimicrobial agents and the development of resistant strains. Their high surface area to volume ratio compared to bulk material, provides a large active surface with large number of the low-coordinate atoms, available to interact with microbial membranes or to release metal ions [1].

Among the others metal NPs, the recent interest in the CuNPs is propelled by both, the advances of these NPs as a cheap alternatives for expensive metal NPs in the area of micro-electronics applications as well as the possibility of exploring them as ultimate antimicrobial agent [2–4]. Even today, the exact mechanism of antimicrobial action of the CuNPs remains unknown. The general view seems to be a combination of several factors: releasing Cu^{2+} ions, their penetration and disruption cell membrane and biochemical pathway by chelating cellular enzymes and DNA damage [2–4]. Based on such studies, leading to the ability that NPs in the range of 1–10 nm showed greater interaction with bacteria [5], which depends on as much as free surface to liberate ions, it becomes desirable to synthesize small and bare NPs. Therefore, we prepared CuNPs (~5 nm) in the absence of polymers/surfactants, and used them to investigate antimicrobial activity and provide direct visual evidence of changes created in cell membrane morphology of tested strains.

2. Experimental

Synthesis and characterization of CuNPs. The CuNPs were prepared by reduction of copper (II) chloride hydrate solution (0.5 mM) with sodium borohydride (13.2 mM) in the presence of ascorbic acid (1.25 mM) as an antioxidant. To avoid fabricating metal oxide NPs and intermediate copper (I) oxide/hydroxide, the synthesis was performed in an inert atmosphere in weakly acidic medium (pH 3–4). Particles characterizations were performed with transmission electron microscopy (TEM, JEOL-1200EX) and scanning electron microscopy (SEM, JEOL JSM-6610LV) with energy dispersive X-ray spectroscopy (EDS). UV–vis absorption of Cu hydrosol was carried out on a Termoscientific Evolution 600 spectrophotometer.

Antimicrobial assays. The antimicrobial activity of CuNPs was quantitatively assessed against Gram-negative bacteria *Escherichia coli* (ATCC 25922), Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and fungus *Candida albicans* (ATCC 10259) (see experimental details in ESI). The percentage of microbial growth reduction (R, %) was calculated using the follow equation:

$$R = \frac{C_0}{C}100 \quad (1)$$

where C_0 and C are the number of colony forming units (CFU) from control and treated sample, respectively.

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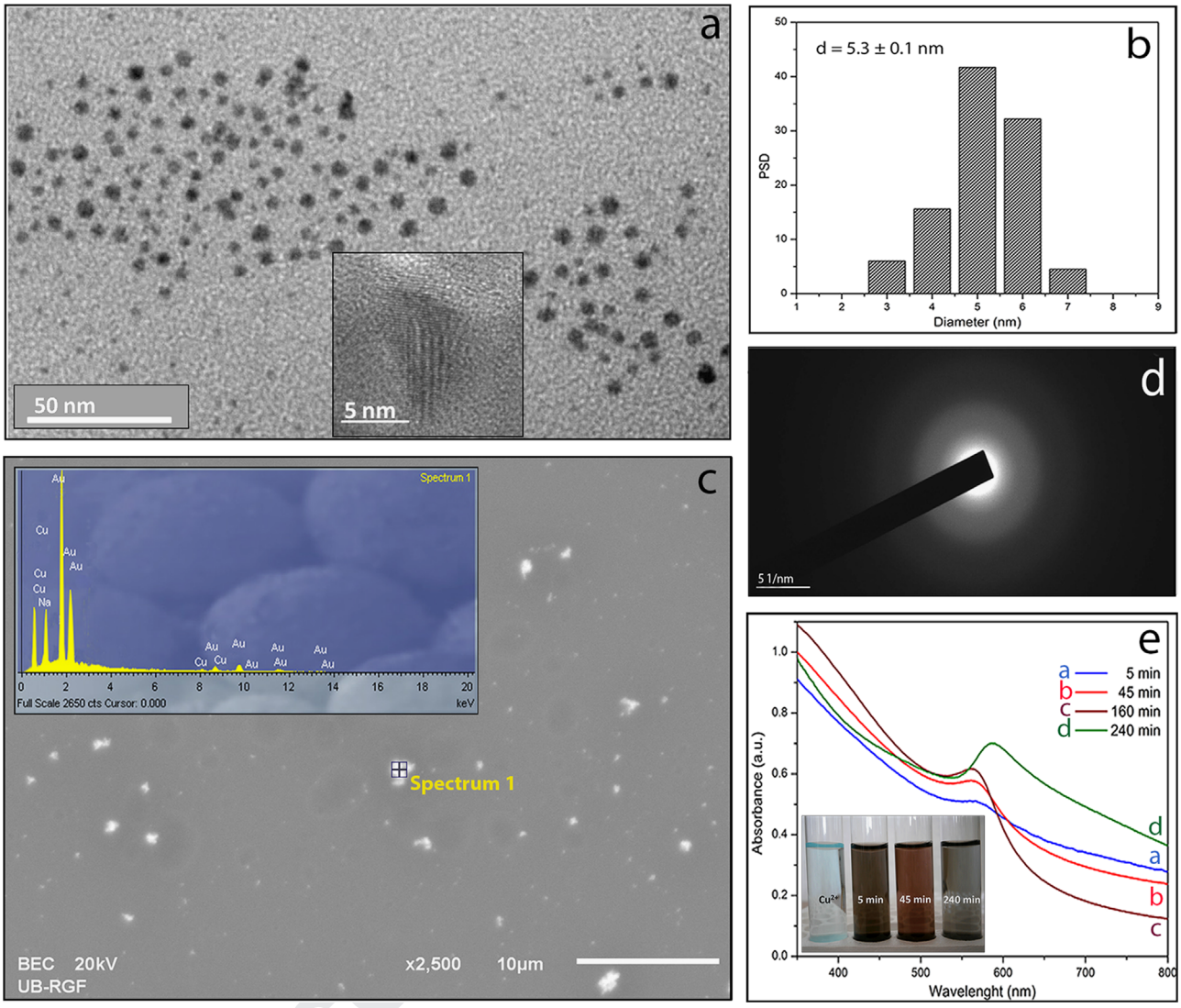

Fig. 1. (a) TEM image of the Cu NPs (inset: HRTEM image of the individual CuNP) with (b) corresponding particle size distribution (PSD); (c) SEM image with EDS spectrum; (d) SAED pattern; (e) Absorption spectra of the CuNPs as a function of aging time in the inert atmosphere with time evolution of the dispersion photographs.

Cells morphology study. Microstructure and morphological changes of microbial cell deposited on mica substrate were recorded by AFM (Quesant-Scope Universal Scanning, USA), operating in tapping mode (see experimental details in ESI).

3. Results and discussions

CuNPs characterization. To access the size and morphology of the CuNPs, TEM and SEM measurements were performed. The dark spots on the TEM image reveal that particles have almost spherical shape with an average diameter of 5.3 ± 0.1 nm (Fig. 1a and b). The bright spots on the backscattered electron image (BEI) obtained by SEM, support the morphological fact established by TEM, while EDS spectrum (Fig. 1c) confirms that these NPs are Cu (the additional Au signals are contribution from the Au coated grid). The value of interplanar spacing, was found to be 0.2041 nm, corresponds to highly reactive facets, (111). The diffusive rings assigned to (111) and (220) planes shown in the SAED pattern are simple manifestation of the crystal shape effect nanoscale-size particles [6].

The formation of CuNPs was primarily confirmed by the color change of the reaction mixture (Fig. 1e, inset) due to surface plasmon resonance (SPR), the frequency at which conduction electrons, mainly within the metal surface, oscillate in response to the alternating electric field of incident electromagnetic radiation. The evolution of the SPR peak position follows the nucleation-growth mechanism associated with the different stages, first, formation of small cluster (< 5 nm) with absorption from a delocalized band like electron distribution with SPR peak at 568 nm and second, growth of nuclei toward final stable NPs with SPR peak at 562 nm (Fig. 1e). As shown earlier [7] during this time, metal-catalyzed hydrolysis of BH_4^- ions and association of negative charges (BH_4^- and BO_3^{3-}) with the CuNPs, took place. These ions can temporarily stabilize the CuNPs by adsorption onto their surface, providing Coulomb repulsion between them and increasing effective concentration of conductive electrons in the NPs. The similar behavior was observed when the CuNPs left to grow under ambient conditions (Fig. S1). The onset of particles oxidation was detected after 2 h of aging in the air, revealing the formation of thin copper oxide shell, since the residual absorption peak at 800 nm was not observed [8].

Antimicrobial properties. Results of the biological activity trials on the microorganisms summarized in Table 1, clearly demonstrate that after just 2 h of contact, these NPs were able to reduce more than 98% of all tested strains at high CuNPs concentration

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