

Synthesis and Characterization of Electro-Explosive Magnetic Nanoparticles for Biomedical Applications

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Abstract. Nowadays there are new magnetic nanostructures based on bioactive metals with low toxicity and high efficiency for a wide range of biomedical applications including drugs delivery, antimicrobial drugs design, cells' separation and contrasting. For such applications it is necessary to develop highly magnetic particles with less than 100 nm in size. In the present study magnetic nanoparticles Fe, Fe₃O₄ and bimetallic Cu/Fe with the average size of 60–90 nm have been synthesized by electrical explosion of wire in an oxygen or argon atmosphere. The produced nanoparticles have been characterized with transmission electron microscopy, X-ray phase analysis, and nitrogen thermal desorption. The synthesized particles have shown antibacterial activity to gram-positive (*S. aureus*, MRSA) and gram-negative (*E. coli*, *P. aeruginosa*) bacteria. According to the cytological data Fe, Fe₃O₄ and Cu/Fe nanoparticles have effectively inhibited viability of cancer cell lines Neuro-2a and J774. The obtained nanoparticles are promising for new antimicrobial drugs and antitumor agents' development.

INTRODUCTION

Nowadays there has been a rising interest to the nanoparticles based on iron and its compounds for biomedical applications. Iron is a bioactive metal with magnetic properties and low toxicity to mammalian cells. Despite numerous reports devoted to iron compounds their antimicrobial and anticancer activity data is ambiguous. Using iron nanoparticles as antimicrobial agents against *E. coli* was shown in 2008 by Lee et al. [1]. At the same time, it was noted that iron nanoparticles' activity is higher in anaerobic conditions. After saturation of nanoparticles' suspension their oxidation in the air oxygen takes place. The oxide film formed on the surface of nanoparticles inhibits ion migration to suspension and results in low antimicrobial activity. The antimicrobial activity of iron nanoparticles was also shown in [2, 3] etc. The mechanism of iron nanoparticles action on microbial cell is based on physical disturbance of cell membrane and on reactive oxygen species (ROS) generation that are formed after interaction of iron ions with oxygen or hydrogenium peroxide. However, it was shown that only fresh produced iron nanoparticles had bactericidal effect. The influence of aging processes on antimicrobial activity of nanoparticles was shown in [4]. It was shown that iron oxide nanoparticles have a little antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* [5]. Only the coating with chitosan molecule resulted in a significant increase in antimicrobial activity of iron oxide nanoparticles. Using nanoparticles containing iron as the anti-cancer agent [6] and potential drug carrier in cancer therapy has attracted much attention recently [7]. Such particles can also kill cancer cells because of generating ROS [8].

Iron containing nanoparticles can be synthesized by various methods, mainly by colloidal chemistry methods and chemical precipitation. However, these processes include several toxic chemicals as reducing agents. In this regard development of pure synthesizing processes with electric explosion of the wire (EEW) is needed.

In the present work, we concentrated on producing Fe, Fe₃O₄ and bimetallic Cu/Fe nanoparticles by EEW method and on the comparison of nanoparticles' antimicrobial activity and their anticancer effect.

MATERIALS AND METHODS

Fe and Fe₃O₄ nanoparticles were produced by the method of electric explosion (EEW) of iron wire in an argon and oxygen atmosphere respectively. Fe/Cu bimetallic nanoparticles were produced by EEW of simultaneous electric explosion of iron and copper twisted wires in an argon atmosphere [9]. The nanoparticle samples were passivated in air to decrease their pyrophoricity. The mass ratio of iron (w_{Fe}) and copper (w_{Cu}) in the nanopowder was 50 : 50 wt %.

The morphology of the nanoparticles and nanostructures was characterized by transmission electron microscopy (JEOL-2100, JEM, Japan, operated at 200 kV). The phase composition of the samples was determined by X-ray phase analysis with CuK α -emission (XRD-6000, Shimadzu, Japan). The specific surface areas of the micro/nanostructures were determined by the nitrogen adsorption method using a Sorbtometer M (Catakron, Russia). The specific surface area was calculated using the BET method in the relative pressure range of 0.05–0.35.

Bacterial strains used for investigations were obtained from the Russian National Collection of Industrial Microorganisms: *Staphylococcus aureus* (Gram-positive) ATCC 29213 strain; *Pseudomonas aeruginosa* (Gram-negative) ATCC 9027 strain; *Escherichia coli* (Gram-negative) K-12 strain. We also used clinical strain of methicillin-resistant *Staphylococcus aureus* (MRSA) (Gram-positive). Antimicrobial activity was determined with the standard serial microdilution method [3]. This method is widely used to determine growth kinetics of microorganisms in the presence of nanoparticles. Water dispersion of nanoparticles was prepared for this method. 150 μ l of Muller-Hinton Broth, 30 μ l of bacterial suspension with 10⁶ CFU/ml concentration and 20 μ l of nanoparticles' suspensions were added to 96-well microplate. Microorganisms' growth assessment was performed after 3, 6, 9, 12, 15, 18, 24 hours of incubation at 37°C with plate spectrophotometer Thermo Scientific Multiskan FC (Thermo Fisher Scientific, USA). There were not less than five measurements in parallel for each concentration of nanoparticles. Wells with bacterial suspension and broth were used as a control. The absorbance of the samples was determined before and after incubation to eliminate nanoparticles' interference.

The mouse neuroblastoma cell line Neuro-2a and mouse histiocytic sarcoma cell line J774 were purchased from Vector, Koltsovo (Russian Federation). The cells were grown in Minimum Essential Eagle's Medium supplemented with 10% fetal bovine serum and 5% penicillin streptomycin glutamine. The cells were cultured at 37°C in a 5% CO₂ humidified atmosphere until grown to the desired density in 75 cm² flasks. All the materials were sterilized in an autoclave at 121°C for 20 min. 0.01 mg·ml⁻¹ of AlOOH/Cu nanostructures were incubated with the cells in 24-well plates at a density of 160 000 cells per well at 37°C in a 5% CO₂ humidified atmosphere for specified time periods of 24 h. An aliquot of suspended single cells was incubated for 5 min at room temperature with trypan blue vital stain in an appropriate dilution and the total viable single cells count was estimated using a hemocytometer. Nanoparticles were not added to the control samples.

RESULTS AND DISCUSSION

The powders were provided by the Advanced Powder Technologies Company, Tomsk, Russia (<http://www.nanosized-powders.com/en/>). Electrical explosion of wire (EEW) was used to obtain nanoparticles. Fe nanoparticles are formed after EEW in an argon atmosphere, electrical explosion of wire was performed in an oxygen atmosphere to produce Fe₃O₄ nanoparticles. Cu/Fe nanoparticles were produced in combined electrical explosion of iron and copper wire in an argon atmosphere. Nanoparticles' composition was adjusted by diameter of wires. To produce nanoparticles with mass ratio 50:50 copper and iron wires with diameter 0.2 mm were used.

TABLE. Sample characteristics

Nanoparticle	The average particle size, nm	Specific surface area, m ² /g	Phase composition
Fe	87 nm	3.6	Fe
Fe ₃ O ₄	77 nm	5.4	Fe ₂ O ₃ , FeO
Cu/Fe	63 nm	7.8	Fe, Cu

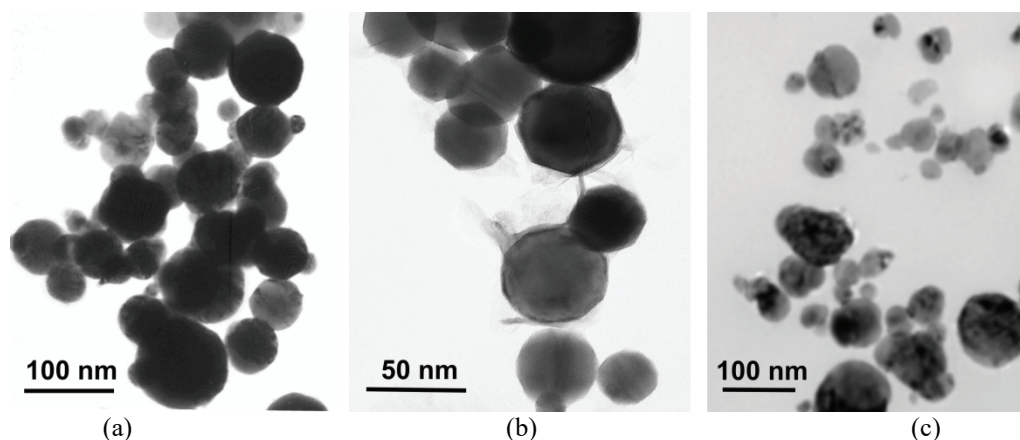


FIGURE 1. TEM-image of nanoparticles: (a) Fe, (b) Fe_3O_4 , (c) Fe/Cu

The results from characterization of nanoparticles were summarized in the Table. After EEW spherical particles were formed (Fig. 1). This shape was conducted by mechanism of nanoparticles formation [9]. All these nanoparticles were formed in liquid phase. The surface of nanoparticles was coated with a thin oxide film formed after passivation of the nanoparticles in air.

Mean Fe particles' size is 87 nm, Fe_3O_4 —77 nm, Cu/Fe—63 nm. The phase composition of all nanoparticles is presented in the Table. After the detailed investigation of Cu/Fe nanoparticles with EDAX-TEM analysis it can be noted that the particles have a complex composition (Fig. 2). All particles contain copper and iron. There are sites enriched with one of the components with definite phase composition.

The mechanism of antibacterial activity of Fe and Fe_3O_4 nanoparticles Fe and Fe_3O_4 , has been already detailed by many authors, for example in [1, 9]. There are no data for Cu/Fe nanoparticles. The antimicrobial activity of nanoparticles has been determined in aerobic conditions by serial micro dilutions method in microplate. The water suspensions of Fe nanoparticles had no inhibiting effect on the growth of *P. aeruginosa* even in the highest concentration (500 $\mu\text{g/ml}$), as well as Fe_3O_4 nanoparticles. For gram-positive bacteria growth of *S. aureus* has stopped at 150 $\mu\text{g/ml}$ concentration of Fe nanoparticles, and 100 $\mu\text{g/ml}$ for Fe_3O_4 nanoparticles. For MRSA 250 and 150 $\mu\text{g/ml}$ respectively. In case of Cu/Fe nanoparticles a significant growth inhibition of all investigated microorganisms was observed. Cu/Fe nanoparticles inhibited the growth of all investigated microorganisms in the concentration not more than 125 $\mu\text{g/ml}$. Thus, Cu/Fe nanoparticles had the highest antimicrobial activity. Such particles can be perspective antimicrobial agents for new bacteriological protection technologies.

The preliminary results of nanoparticles' anticancer activity have shown that viability of Neuro-2a cells treated with all nanoparticles for 24 hours decreases to 39.5% for Fe nanoparticles, lowers to 29.7% for Cu/Fe nanoparticles, and to 25.7% for Fe_3O_4 nanoparticles (Fig. 3a).

The nanoparticles also have demonstrated anticancer activity to J774 cell line (Fig. 3b). The cells viability was 61.9% for Fe nanoparticles, 23.3% for Cu/Fe nanoparticles, and 60.9% for Fe_3O_4 nanoparticles. Thus, all nanoparticles inhibited the growth of cancer cells.

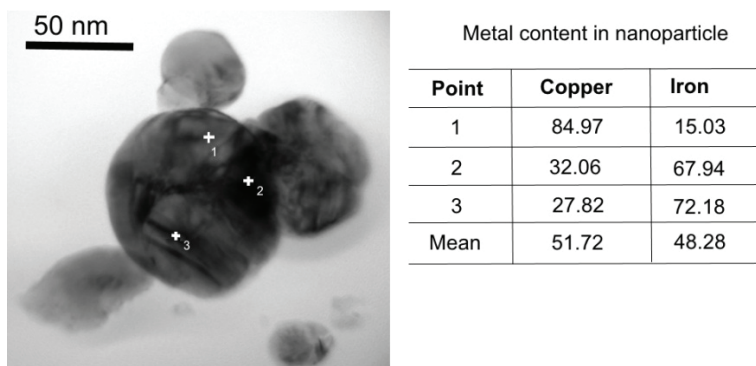


FIGURE 2. EDAX-TEM analysis of Fe/Cu nanoparticle

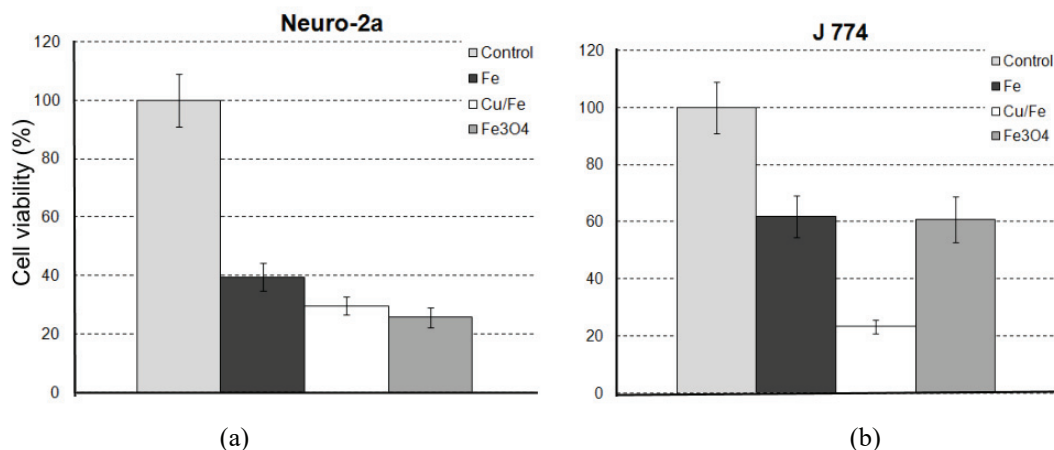


FIGURE 3. Cytotoxic assays

CONCLUSION

Magnetic nanoparticles synthesized by the EEW method have antimicrobial and anticancer activity. Such particles can be successfully used for a wide range of therapeutic applications.

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