Novel of Core-Shell AlOOH/Cu Nanostructures: Synthesis, Characterization, Antimicrobial Activity and In Vitro Toxicity in Neuro-2a Cells

O. V. Bakina[1,2,a)], A. N. Fomenko[1,2,b)], M. S. Korovin[1,2,c)], E. A. Glazkova[1,2,d)], and N. V. Svarovskaya[1,2,e)]

[1] *Institute of Strength Physics and Materials Sciences SB RAS, Akademicheskii Pr. 2/4, Tomsk, 634055 Russia*
[2] *National Research Tomsk Polytechnic University, Lenin Avenue 30, Tomsk, 634050 Russia*

[a)] Corresponding author: ovbakina@ispms.tsc.ru
[b)] alserova@ ispms.tsc.ru
[c)] msk@ ispms.tsc.ru
[d)] eagl@ ispms.tsc.ru
[e)] nvsv@ ispms.tsc.ru

Abstract. Core-shell micro/nanostructures were fabricated by the reaction of Al/Cu bimetallic nanoparticles with water. Al/Cu nanoparticles have been obtained using the method of simultaneous electrical explosion of a pair of the corresponding metal wires in an argon atmosphere. The nanoparticles are chemically active and interact with water at 60°C to form core-shell micro/nanostructures. The obtained products were characterized by means of X-ray diffraction, scanning electron microscopy, transmission electron microscopy and dynamic light scattering and the nitrogen adsorption method. The antibacterial activity of the synthesized structures was investigated against E. coli and St. aureus. The toxic effect of these nanostructures against the Neuro-2a neuroblastoma cell line was investigated**.** AlOOH/Cu nanostructures are shown to inhibit cell proliferation. The AlOOH/Cu nanostructures are good candidates for medical applications.

INTRODUCTION

Recent advances in nanotechnology have enabled progress in the development of novel nanostructures and nanocomposites. Nanoparticles with functional core and shell architecture have attracted tremendous attention due to their unique morphology and properties associated with promising applications, such as targeted drug delivery, catalysis, separation, and sensors [1]. In biological applications core-shell nanoparticles have major advantages over simple nanoparticles leading to the improvement of properties such as less cytotoxicity, increase in dispersibility, better conjugation with other bioactive molecules, thermal and chemical stability [2]. The use of core-shell structures in medicine is one of the most important research areas [3].

Core-shell nanoparticles have been prepared using a variety of techniques, such as sol-gel method [4], underpotential co-deposition method [5], hydrotermal and solvothermal method and a variety of organic and inorganic surfactants or templates are all widely used for the synthesis. Most of these methods involve complicated processes. High temperature or high vacuum conditions are often adopted, which are not favorable for large-scale production. Sol–gel methods and solvothermal (hydrothermal) processes can be performed at a relatively low temperature, but they are usually time-consuming. Furthermore, the extraction and purification of the produced structures for further applications are still important issues. However, the operation of most of these methods are relatively complex and the products need more elaborate cleaning procedure [6]. Thus, to develop a simple synthetic method for core-shell nanoparticles with in aqueous solution is highly desirable.

The newest generation of copper nanoparticles is the core–shell, which has potential applications in several areas [7]. The core–shell copper-containing composite has many advantages as antibacterial agent, such as high anti-bacterial activity, low toxicity, chemical stability and long lasting action period [8]. Authors [8] reported that Cu@SiO_2/,bacterial cellulose composite, is a good antibacterial material. Silica shells protected Cu nanoparticles from being oxidized. Meanwhile, the Cu cores would release copper ions slowly through the outer porous SiO_2 layer.

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Earlier, we have produced core-shell nanostructures having a high antimicrobial activity by the oxidation of the Al/Cu bimetallic nanoparticles with water [9]. In this report, core-shell AlOOH/Cu nanostructures were prepared via direct reaction of Al/Cu bimetallic nanopowders with water. Potential medical applications of the structures require an assessment of their toxicity. The aim of our study was to explore the physicochemical properties and toxicity assays of the structures. The Neuro-2A (N2A) mouse neuroblastoma cell line was used as a model to determine the potential toxicological effect of AlOOH/Cu structures [10, 11].

MATERIAL AND METHODS

Nanopowders of Al/Cu bimetallic nanoparticles were produced by the method of simultaneous electric explosion of aluminum and copper twisted wires in the argon atmosphere [12]. Two twisted wires were exposed to a high-density current pulse (10^7 A/cm^2) produced using a high voltage source. The current pulse passed through the wires, and explosive destruction of the metals was observed; it was accompanied by bright flash, blast wave, metal dispersion and fast expansion of explosion products into surrounding gas. The explosion products were cooled, and nanoparticles were formed. The nanoparticle samples were passivated in air to decrease their pyrophorcity. The mass ratio of aluminum (w_{Al}) and copper (w_{Cu}) in the nanopowder was 50 : 50 wt. %.

Core-shell AlOOH/Cu nanostructures were synthesized by a one-step route via the facile reaction of Al/Cu bimetallic nanoparticles with water [9]. In a typical procedure, 0.05 g of precursor was added to 50 ml of deionized water. The suspension was then put into a water bath reactor with stirring at 60°C for 1 h. The reaction products were separated by centrifugation, washed with deionized water several times, and finally dried at 100°C for 3 h.

The morphology of the nanoparticles and nanostructures was characterized by transmission electron microscopy (JEOL-2100, JEM, Japan, operated at 200 kV) and scanning electron microscopy (LEO EVO 50, operated at 30 kV). The phase composition of the samples was determined by a method of 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 (Catakon, Russia) nitrogen adsorption apparatus. The specific surface area was calculated using the BET method in the relative pressure range of 0.05–0.35. The zeta potential was determined using a Zetasizer Nano ZSP (Malvern Instruments Ltd, UK).

The mouse neuroblastoma cell line N2A was purchased from Vector, Koltsovo (Russian Federation). The cells were grown in Minimum Essential Medium Eagle supplemented with 10% fetal bovine serum and 5% penicillin streptomycin glutamine. The cells were cultured at 37°C in a 5% CO_2 humidified atmosphere until grown to the desired density in 75 cm^2 flasks. All the materials were sterilized in an autoclave at 121°C for 20 min. 1 mg · ml^{-1} AlOOH/Cu nanostructures were incubated with cells in 24-well plates at a density of 160 000 cells per well at 37°C in a 5% CO_2 humidified atmosphere for specified time periods of 24, 48 and 72 h. An aliquot of suspended single cells was incubated for 5 minutes at room temperature with trypan blue vital stain in an appropriate dilution and the total viable single cells count was estimated using a hemocytometer chamber. For control samples the nanoparticles were not added.

The antimicrobial activity of the nanostructures was measured using a method of radial diffusion in agar [13] against tested bacteria *E. coli* ATCC 25922 and *St. aureus* 209. The bacteria for the study were taken from the Russian National Collection of Industrial Microorganisms, Moscow, Russia. 20 ml of liquid Nutrient agar (pH 7.3) was poured onto disposable sterilized Petri dishes and allowed to solidify. A 0.5 ml McFarland (1×10^7 CFU · ml^{-1}) concentration of bacteria was inoculated on solidified agar. The 1 mg · ml^{-1} nanostructures were suspended in sterilized deionized water. Filter-paper disks according to the number of samples were placed on the surface of the medium and 40 μl of the samples were dropped over disks. After 24 h incubation at 37°C, the inhibition zone (IZ) diameter was measured. All the measurements were performed in triplicate, and the results were expressed as the mean ± standard deviation.

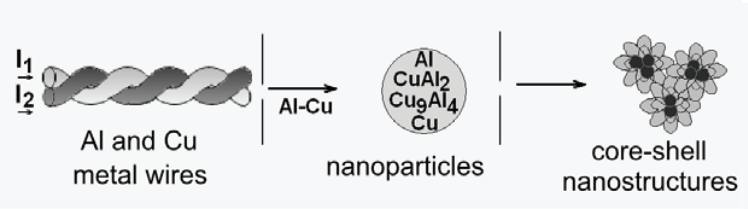


FIGURE 1. Conceptual synthesis scheme of AlOOH/Cu nanostructures

TABLE 1. Sample characteristics

Sample	The average particle size	Surface area, (m^2/g)	Crystalline structure	Zeta potential, mV
Al/Cu	112 nm	6	Al, Cu, $CuAl_2$, Cu_9Al_4	14 ± 2
AlOOH/Cu	1–2 μm	90	AlOOH, Cu, $CuAl_2$, Cu_9Al_4	25 ± 2

RESULT AND DISCUSSION

The results from characterization of Al/Cu nanoparticles and AlOOH/Cu nanostructures were summarized in Table 1.

In the case of a simultaneous electric explosion of aluminum and copper wires in argon atmosphere, spherical 80–120 nm particles are formed (Fig. 2).

The surfaces of the particles are covered with a thin film, most likely composed of oxide. On the diffractogram of the Al/Cu nanoparticles, the main reflexes correspond to intermetallic Cu9Al4 compounds. The samples also contain phases of Al, Cu and CuAl2 solid solution, which correspond to the diagram of Cu-Al system state [14]. The specific surface area of nanoparticles is 6 $m^2 \cdot g^{-1}$. The average particle size is 112 nm. The EDX analysis of the nanoparticles represents the weight percentage (%) of the elements Al : Cu : O in the ratio of approximately 52 : 44 : 4. Aluminum and copper are uniformly distributed over the volume of the particles. The surface of particles is covered with thin passivating film, most likely oxide.

Al/Cu bimetallic nanoparticles are chemically active and interact with water at 60°C. The core-shell nanostructures produced via oxidation of bimetallic nanoparticles are particularly interesting. SEM image of the AlOOH/Cu composite structures (Fig. 3) show the multiple nanostructures with diameters of 1–2 μm.

Core-shell AlOOH/Cu nanostructures are structurally non-uniform. The study of substructural characteristics by EDX-TEM analysis data revealed that aluminum and oxygen are uniformly distributed over the volume of the AlOOH/Cu particles, while copper are detected as electron-dense inclusions. Electron-dense copper-containing spherical inclusions are surrounded with crumpled alumina nanosheets [15–17].

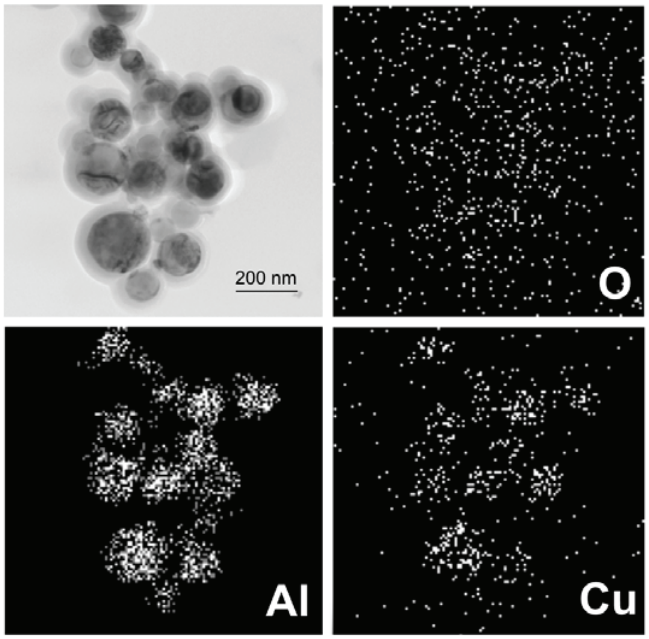


FIGURE 2. TEM image of Al/Cu nanoparticles

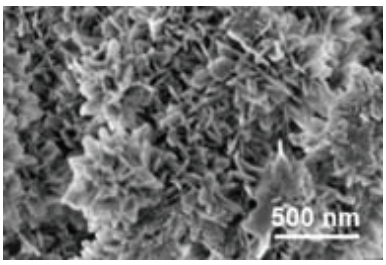


FIGURE 3. SEM image of AlOOH/Cu nanostructures

A TEM image of AlOOH/Cu nanostructure is shown in (Fig. 4) and indicates that the exterior of the core-shell nanostructure was of multiple randomly assembled crumpled nanosheets with a thickness of approximately 5 nm and irregular- composed shaped inclusions with size less than 10 nm. According to X-ray phase analysis data, AlOOH/Cu structures involve poorly crystallized pseudoboehmite and bayerite. These are the reaction products of electro-explosive aluminum with water. Furthermore, intermetallic compounds 1–2 μm were detected.

The metallic copper and intermetallic compounds were found in the same ratios as in the Al/Cu bimetallic nanoparticles. They do not react with water under the given conditions. When aluminum reacts with water and alumina is formed as crumpled nanosheets, the reacting particle grows in size. At the same time, the copper-containing inclusions are usually located in the center of the composite particles. The specific surface area of the core-shell AlOOH/Cu nanostructures is approximately 90 $m^2 \cdot g^{-1}$, and the zeta potential is 25 ± 3 mV.

The synthesized nanostructures exhibited antimicrobial activity against *E. coli* and *St. aureus*. The inhibition zones were 17.4 ± 0.2 and 18.3 ± 0.1 mm, respectively. The results of the present study are in agreement with other reports that indicate the greater activity of copper [18] nanoparticles against Gram-negative and Gram-positive microorganisms. After 24 h of exposure of the N2A cells to AlOOH/Cu nanostructures the cell viability decreased significantly. Compared to untreated cells (100%), the cell viability was decreased to 35.2 ± 3.2 %. After 72 h of exposure of the cells to the AlOOH/Cu micro/nanostructures the cell viability was 28.4 ± 2.0 %, Thus, AlOOH/Cu structures inhibit the cell proliferation.

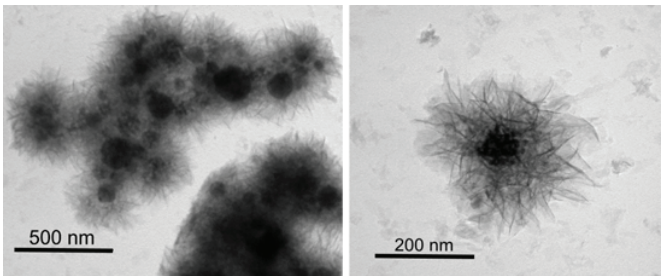
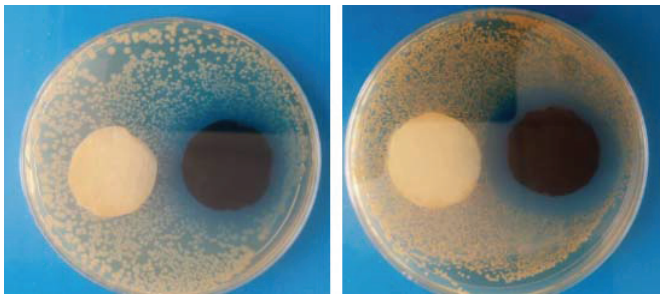


FIGURE 4. TEM image of AlOOH/Cu nanostructures



(a) (b)

FIGURE 5. Antibacterial activity of AlOOH/Cu structures against *E. coli* (a) and *St. aureus* (b) shown by the paper disk diffusion method

CONCLUSION

Core-shell structures containing adsorbent and antimicrobial agent within the same particle are a new generation of materials with considerable potential in the field of biomedicine. Therefore, it is concluded that the micro/nanostructures showed biocidal activity against Gram-negative and Gram-positive bacteria. These properties make the nanostructures AlOOH/Cu good candidates for medical applications such as antimicrobial and healing dressing components and research.

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The study reported in this article was conducted according to accepted ethical guidelines involving research in humans and/or animals and was approved by an appropriate institution or national research organization. The study is compliant with the ethical standards as currently outlined in the Declaration of Helsinki. All individual participants discussed in this study, or for whom any identifying information or image has been presented, have freely given their informed written consent for such information and/or image to be included in the published article.

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