Synthesis of iron oxide based nanostructures with antimicrobial activity

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Abstract. This paper explores the antimicrobial activity of AlOOH and FeOOH nanostructures, and FeOOH/AlOOH composite nanostructures. The synthesized nanostructures have been examined by transmission electron microscopy, X-ray diffraction, low-temperature nitrogen adsorption, and microelectrophoresis. Antimicrobial activity is assessed by a 96-well plate method. It is shown that the synthesized nanoparticles have varying degrees of antibacterial effect on bacterial strains of E.coli and methicillin-resistant Staphylococcus aureus (MRSA). The FeOOH/AlOOH nanostructures possess the highest antibacterial activity, which indicates a synergistic antimicrobial effect of composite nanostructures.

1. Introduction

Today, the problems associated with the emergence of resistant strains of microorganisms and uncontrolled use of antibiotics stimulate the search for new approaches to prevent microbial contamination and its consequences [1]. In view of the pronounced geno- and cytotoxicity of metal nanoparticles, more attention is given to metal oxide nanostructures with low toxicity, such as iron, aluminum, magnesium, and others [2]. Owing to the developed surface and unusual crystalline morphologies [3], such structures can efficiently eliminate pathogens.

The use of iron oxide nanostructures as an antibacterial material is attractive due to their non-toxicity, biocompatibility, and unique magnetic properties. Irshad et al. [4] showed that the inhibitory activity of iron oxides is based on their electrostatic interaction with the bacterial cell membrane, which causes the cell damage in the contact region and induces toxic oxidative stress due to the production of intracellular reactive oxygen species. However, a persistent antimicrobial effect can be achieved at high concentrations of iron oxide nanostructures (> 10 mg/mL), which limits their use. Low antimicrobial activity is associated, first of all, with the agglomeration of nanostructures owing to their high surface energy and magnetization. There are two approaches to enhance the antibacterial activity of iron oxide nanostructures:

1. Surface modification by highly effective antibacterial drugs, e.g., antibiotics [5];
2. Development of antibacterial nanocomposites that would stabilize and preserve the original structure of iron oxide nanostructures [6].

The use of alumina as a component of antibacterial nanocomposites is attractive due to its high adhesion to cell membranes [7]. It adsorbs well both microorganisms and endotoxins [8]. Therefore, we can expect a synergistic antimicrobial effect of the composite, which contains alumina and iron oxide nanostructures. Earlier, we obtained effective antibacterial composite nanostructures AlOOH/Zn [9] and AlOOH/Cuby electric explosion of conductors [10]. The aim of this work is to synthesize FeOOH/AlOOH composite nanostructures and to study their physicochemical characteristics and antimicrobial activity against gram-positive and gram-negative microorganisms, including the antibiotic resistant strain MRSA.

2. Materials and methods

2.1 Synthesis of AlOOH nanostructures

AlOOH nanostructures were synthesized by a one-step route via the facile reaction of AlN/Al composite nanoparticles with water using the procedure detailed in Ref. [11]. AlN/Al nanoparticles were produced by electric explosion of aluminum wire in a nitrogen atmosphere [12].
2.2 Synthesis of FeOOH nanostructures
FeOOH nanostructures were synthesized by the chemical deposition method, which is easily scaled up and does not require the presence of additional toxic solvents, which is crucial for biomedical applications [13]. CP grade reagents were used. The FeOOH synthesis procedure was the following: 90 mL of 1 M sodium sulfate (Na₂SO₄) solution was mixed with 20 mL of 0.5 M FeCl₃ solution with constant stirring and stirred for 20 minutes; then 80 mL of distilled water was added. The resulting suspension was placed into an autoclave with a Teflon insert and heated to 140 °C for 6 hours. The resulting precipitate was washed with deionized water and dried at 120 °C.

2.3 Synthesis of FeOOH/AlOOH composite nanostructures
FeOOH/AlOOH composite nanostructures were synthesized by adding 6 g of AlOOH at the stage of mixing Na₂SO₄ and FeCl₃, produced according to Paragraph 2.1. After that, the synthesis was carried out according to Paragraph 2.2.

2.4 Nanoparticle characteristics
The synthesized nanostructures were examined using X-ray diffraction methods in Cu Kα radiation (XRD-6000, Shimadzu, Japan), microelectrophoretic analysis (Zetasizer Nano ZSP, UK), low-temperature nitrogen adsorption (Sorbometer-M, Katakon, Russia), and transmission electron microscopy (JEM-100, JEOL, Japan).

2.5 Antibacterial activity assay
The antibacterial activity of the synthesized nanostructures was studied using the 96-well plate method [14]. The bacterial strains used were E. coli ATCC 25922 and clinical strain MRSA, purchased at the Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center Kurchatov Institute (Russia). Microorganisms were grown on meat-peptone agar and Mueller–Hinton broth at a temperature of 37±1 °C. The concentration of bacterial suspensions was approximately 10⁷ CFU/mL. Each well of a 96-well plate was inoculated with 100 µL of Mueller–Hinton broth and 20 µL of the bacterial suspension. Suspensions of the investigated nanostructures in deionized sterile water in concentrations of 0.1, 0.075, 0.05, 0.025, and 0.0125 mg/mL were prepared in advance. The resulting suspensions were treated by a HielscherUP-100M ultrasonic homogenizer at a frequency of 30 kHz for 1 minute. The powder suspensions were added to wells (50 µL/well). Wells without nanostructures were used as controls. The plates were incubated at 37±1 °C for 12 hours. The growth kinetics of bacteria was estimated by the change in the optical density of the medium in wells relative to the control wells. The optical density was measured at 600 nm. Statistical processing of the results was carried out using parametric methods at a significance level of p ≤0.05.

3. Results and discussion
Figure 1 shows the electron microscopic images and the main physicochemical characteristics of the synthesized nanostructures. TEM images of nanostructures (figure 1) demonstrate that the nanostructures synthesized by precipitation from FeCl₃ are porous agglomerates with a developed surface consisting of 2–5 nm iron oxide flakes (Sample 2). The precipitation of FeOOH nanostructures in the presence of AlOOH preserves the structure of AlOOH nanosheets, and FeOOH is evenly distributed over the nanosheet surface (Sample 3). The study of the samples by the thermal nitrogen desorption method showed that Samples 1 and 3 mostly have mesopores, whereas Sample 2 is microporous. The precipitation of FeOOH on AlOOH sheets increases the specific surface area (SSA) of the sample to 343 m²/g.
Figure 1. TEM images and characterization of nanostructures: 1 – AlOOH nanostructures; 2 – FeOOH nanostructures; 3 – FeOOH/AlOOH nanostructures.

The antibacterial activity of the AlOOH and FeOOH nanostructures has been separately investigated in a large number of works. It can be expected that composite nanostructures will exhibit higher activity. Our studies revealed that all samples in concentrations up to 0.075 mg/mL have weak antimicrobial effect on MRSA (figure 2). The activity of the samples increases with increasing concentration, and a pronounced bactericidal effect is achieved at a concentration of 0.1 mg/mL of FeOOH/AlOOH composite nanostructures (figure 2 c).

Figure 2. Effect of nanostructures on MRSA: a - AlOOH nanostructures; b - FeOOH nanostructures; c - FeOOH/AlOOH nanostructures.

The E. coli culture was more sensitive and less selective with respect to all three types of nanostructures (figure 3).

Figure 3. Effect of nanostructures on E. coli: a - AlOOH nanostructures; b - FeOOH nanostructures; c - AlOOH/FeOOH nanostructures.

AlOOH nanostructures at concentrations of 0.0125 and 0.025 mg/mL slightly inhibit the growth of living E. coli cells, but this effect is enhanced with increasing concentrations to 0.1 mg/mL (figure 3.
a). The antibacterial effect of FeOOH and FeOOH/AlOOH nanostructures is almost independent of the concentration. After 6 hours of strain incubation with nanostructures, the growth of *E. coli* stops.

4. Conclusions

FeOOH/AlOOH composite nanostructures with high antibacterial activity against gram-positive (*MRSA*) and gram-negative (*E. coli*) bacterial strains were synthesized. Low toxicity and antibacterial efficacy makes them promising candidates for biomedical applications.

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