

The Influence of Silver-Containing Bionanomaterials Based on Humic Substances on Biofilm Formation in Opportunistic Pathogens

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Keywords: humic substances; silver nanoparticles; opportunistic pathogens; antibacterial activity; biofilm formation

Introduction. The uncontrolled use of antibiotics has led to a global problem of antimicrobial resistance. One of the main mechanisms of bacterial resistance is the formation of biofilms. In order to prevent the growth of antimicrobial resistance, it is crucial to develop new antibacterial agents that are capable of inhibiting the formation of biofilms. Promising candidates for these antibacterial agents are new bionanomaterials made from natural humic substances and silver nanoparticles. These substances have the potential to not only directly kill microorganisms but also penetrate biofilms and inhibit their formation.

The goal of this study is to research influence of silver-containing bionanomaterials based on humic ligands on biofilm formation in the most dangerous opportunistic pathogens.

Materials and Methods. To study the antibacterial effect, 12 samples of the active pharmaceutical substances (HS-AgNPs) were taken, which are bionanomaterials based on silver nanoparticles ultradispersed in a matrix of humic substances. These bionanomaterials were synthesized in the Laboratory of Natural Humic Systems of the Faculty of Chemistry, Lomonosov MSU.

For the study, there were used standard strains of opportunistic microorganisms: *Escherichia coli*, *Staphylococcus aureus*, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and clinical isolates of following types of bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, isolated in the bacteriological laboratory of SSMU clinics from various patient materials (urine, sputum, wounds, the uterine cavity, and blood). A panel of microorganisms relevant to clinical practice and capable of forming biofilms was selected. Then, the studied substances were screened for antibacterial properties. We also studied the effect of bionanomaterials on the bacterial cell wall, biofilms, and the viability of bacteria inside them.

Results. As a result of screening the antimicrobial activity of bionanomaterials, an antibacterial effect was discovered for 11 of the 12 substances studied. The substances with the highest antimicrobial activity are sample CHP-pHQ-FE-AgNPs (p-hydroquinone derivative of HS-AgNP synthesized by Fenton reaction) against gram-negative bacteria and sample CHP-AgNPs-MW (microwave-synthesized HS-AgNP) against gram-positive bacteria. Sample CHP-oHQ-FE-AgNPs (o-hydroquinone derivative of HS-AgNP synthesized by Fenton reaction), being a sample with the broadest spectrum of antimicrobial activity, damages the cell wall of bacteria such as MRSA, *K. pneumoniae* and *P. aeruginosa*. Sample CHP-pHQ-FE-AgNPs suppresses the biofilms formation and destroys biofilms of all studied gram-negative microorganisms. Sample CHP-AgNPs-MW suppresses biofilm formation and destroys biofilms of all studied gram-positive microorganisms. Sample CHP-pHQ-FE-AgNPs reduces the viability of gram-negative bacteria in biofilms. Bionanomaterial CHP-AgNPs-MW reduces the viability of gram-positive bacteria in biofilms.

Conclusion. Thus, bionanomaterials based on silver nanoparticles ultradispersed in the matrix of humic substances demonstrated antibacterial properties against all selected opportunistic microorganisms capable of forming biofilms.