Bionanomaterials with antibacterial properties based on humic substances

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For today, infectious diseases occupy a leading position in the world. The deficiency of effective therapeutic methods in the treatment of chronic non-healing wounds induces to infection, and against the background of the existing problem of antibiotic resistance - to sepsis and death. That's why, any potentially perspective molecules with antimicrobial and wound-healing properties have to be detailed studied and introduced into medical practice.

In the Natural Humic System Lab MSU by Professor I.V. Perminova the methodology that includes targeted synthesis of bionanomaterials based on humic substances (HS) by polyelectrolyte assembly and template synthesis of nanoparticles was developed. The original humic substances samples (HS) and the samples silver nanoparticles ultradispersed in humic substances (HS-AgNPs) were tested in this study: HS of high-moor peats (Russia), and also commercial HS by Humintech (Germany), Genesis (Russia), etc. There were a total of 16 tested samples. All experiments were performed in comparison with the colloidal silver preparation Protargol®.

The study of the cytotoxicity of the tested substances (HS/HS-AgNPs) on two lines of cell cultures was done and the limits of toxic effects in the concentration range were established. The study of the cell membranes permeability on the lines of cell cultures in relation to the HS/HS-AgNPs was done and the parameters of their intracellular distribution were established, and also their ability to penetrate through the cell membranes into the cytosol of the cell and the nucleus. The study of the prooxidant and antioxidant / antiradical activity of the HS/HS-AgNPs on the cell culture line was done and their redox reactivity were established, and also their ability to reduce the intracellular level of reactive oxygen species in the prooxidants introduced into the incubation medium (H₂O₂, tert-butyl hydroperoxide, Fe²⁺). The study of immunomodulatory properties (activity balance of NO-synthase / arginase) of the HS/HS-AgNPs in the cell culture of peritoneal macrophages, including relative to LPS-stimulated control was done.

The antibacterial activity of the samples according to the development of periodic bacterial culture response to the samples was studied. Also, we determined the minimum inhibitory concentrations (MIC) for substances with established antibacterial activity. In this study the control test-strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* was used. The study of changes in the permeability of the cell wall and cytoplasmic membrane (example by *Staphylococcus aureus*) was done and the changes in the activity of resistance genes under the conditions of the tested substances action were determined. The study of the antibacterial and wound-healing properties of the HS-AgNPs to *in vivo* experiments on a model of purulent-inflammatory soft tissues in experimental animals was done.

Based on research, the pharmacological properties base for the HS-AgNPs was

formed and the perspective samples with antibacterial and wound-healing properties were selected.

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