области в частности – ОАО «Птицефабрика Оршанская», ОАО «Птицефабрика «Городок», ОАО ПУ «Глубокский ККЗ» и др. Наиболее значительно торговые прилавки заняты яйцом диетическим, характеризующимся по категориям: С-0, С-1, Д-0, Д-1.

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## THE CYTOTOXIC EFFECT OF OXIDIZED GRAPHENE NANOPARTICLES ON THE MORPHIC-TINCTORIAL PROPERTIES OF BACTERIA

Over the past 50 years, researchers have been actively studying the use of nanoparticles and nanostructured materials in various sectors of biomedicine and veterinary medicine. The term "nanoparticle" is usually applied to the smallest particles of some substance having a physical size (diameter) from 1 to 100 nm. Nanotechnology has caused a new technological revolution in science as nanosubstances have found widespread use as antibacterial substances.

In medicine and veterinary medicine, nanoparticles of allotropic forms of carbon, in particular graphene, have recently found application. They have a wide arsenal of biomodulating effects on the body. The positive aspects should be attributed to their antibacterial action. Among them, oxidized graphene is considered one of the promising materials in biomedical research. In particular, it is known as an antimicrobial nanocomponent with satisfactory biocompatibility and a nanomaterial with acceptable properties valuable for biomedical applications.

The aim of the research was to study the effect of oxidized graphene nanoparticles on bacterial cells of the main representatives of opportunistic microbiota (*Escherichia coli* and *Staphylococcus aureus*) using atomic force and classical light microscopy.

Materials and research methods. As a test nanomaterial with a supposed cytotoxic effect, we used a sample of a colloidal solution of oxidized graphene with stable physicochemical parameters. The initial concentration of nanoparticles in the sample was 600 µg/ml, the average diameter of the nanoparticles was in the range of 100–120 nm.

The studied microorganisms were 18-hour bacterial cultures of two microorganisms: *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538. The test microorganisms were cultivated in Mueller-Hinton broth. At the same time, microorganisms were cultivated on Mueller-Hinton agar with the addition of oxidized graphene nanoparticles introduced into wells cut out in the thickness of the agar. At the margin of the growth inhibition zone, bacterial cultures were selected for microscopic examination in two ways: by classical light microscopy with Gram staining and atomic force microscopy (after additional contact with nanoparticles).

Research results. The bacterial culture was selected at the border of the zone of bacterial growth inhibition, where a para-inhibitory concentration of nanoparticles was expected. As a control, colonies of microorganisms were selected on the same medium without the addition of nanoparticles.

Comparison of the morpho-tinctorial characteristics of microbial cultures made it possible to evaluate the nature of the cytotoxic effect of nanoparticles with a subinhibitory concentration of oxidized graphene.

Light microscopy of the smears revealed the preservation of the typical morphological properties of *Escherichia coli* and *Staphylococcus aureus* (short rods and cocci, respectively). However, with Gram staining, an unusual phenomenon of tinctorialtransversion of the staphylococcus culture was noted, in which the cocci partially changed their tinctorial identity from gram-positive to gram-negative. This phenomenon appeared to have a cluster nature, but was noted only in smears of cultures taken at the demarcation line of the zone of inhibition of bacterial growth, where a para-lethal concentration of nanoparticles was expected. A similar phenomenon was not observed in the culture of *Escherichia coli.*

Atomic force microscopy made it possible to visually assess the nature of morphological changes in the bacterial cells and the entire bacterial population as a whole, caused by the toxic effect of the oxidized graphene nanoparticles. Control samples of bacterial cultures during AFM visually corresponded to the typical morphology and size of test microorganisms.

In smears of bacterial cultures, both treated with oxidized graphene nanoparticles and selected at the border of growth inhibition, morphological changes in the bacterial cells as well as the composition of the entire microbial culture were noted. The nature of the changes was generally the same, and their presence was detected after 30 minutes of treatment with the oxidized graphene nanoparticles. The initial changes were characterized by a destruction of the bacterial cells contours compared to the control samples: the sharpness of the outlines of bacterial cells was drastically reduced, the intercellular space was diminished, the contours of scanned objects lost their spatial contrast, and there was a partial exit of the cytoplasm beyond the bacterial cells.

Conclusion. Oxidized graphene nanoparticles have antibacterial properties, which are manifested by obvious cytotoxic effects against prokaryotic cells.

When exposed to toxic concentrations of oxidized graphene nanoparticles on individual Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538), tinctorialtransversion phenomenon has been observed with a change of their Gram identity, which indicates a possible toxic effect on the structure or composition of the bacterial cell wall. The effect of toxic concentrations of oxidized graphene nanoparticles for 30 minutes on the main morphological types of bacteria (cocci, rods) is accompanied by morphological degradation of the bacterial cells.