The Effect of Silver and Zinc Nanoparticles on The Structural Characteristics of Bacterial Cells

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Abstract*: *The paper presents the results of studying the influence of nanoparticles of silver and zinc on the culture of Madin-Darby Bovine Kidney (MDBK) and bacterial cells of E. coli at various dilution rates and various incubation times. The interaction of MDBK cells with the nanoparticles of zinc has a strong deleterious effect on the cells and causes morphological changes in them. At the same time, no toxic effects were observed after the interaction of silver nanoparticles and the cells. During incubation of E. coli bacterial cells with zinc nanoparticles, not only changes in the morphology of the cell membrane surface but also the destruction of bacterial cells were observed. During incubation with silver nanoparticles at various dilution rates for various times, changes in the cell shape from rod-shaped to rounded, without symptoms of destruction were noted.

Keywords*: *Silver Nanoparticles, Zinc Nanoparticles, Mdbk, Escherichia Coli, Incubation, Atomic Force Microscopy, Damaging Effect.

I. INTRODUCTION

Over the past decade, nanomaterials and nanotechnology have gained importance as factors of great potential for further development of science and technology [1-4]. Their large-scale implementation is promoted by the discovery of unique properties of metal nanoparticles, and by their influence on the quality of the human habitat, agricultural products, and flora and fauna [5-7]. Due to their small size (100 nm), nanoparticles easily get into the human and animal organisms through the protective barriers (epithelium, mucosa, etc.), the respiratory system and the gastrointestinal tract [1, 8-10]. Common preparations converted to nanopowder (aspirin, calcium gluconate) have higher activity than in the usual form. The absorbing properties of nanoparticles are significantly higher than those of other molecules [1, 9, 11-13]. Earlier studies of metal nanoparticles' biological activity with the use of experimental animals showed that nanocrystalline iron and zinc in biotic dosages accelerated the growth of animals and birds, enhanced liver regeneration after partial hepatectomy, and accelerated tissue healing [14-16].

The relevance of this work in the context of nanotechnology is in the base of certain properties of the nanoparticles of powdered metals, such as silver, copper, iron, and zinc [17]. It is known that the physical properties of many substances depend on the size of the sample, while nanoparticles of the materials often have properties not observed in the samples of the same substances with usual dimensions [9, 18-21]. For example, silver is not involved in most chemical reactions. However, silver nanoparticles not only catalyze chemical reactions but are also directly involved in chemical reactions. The high reactive ability of silver nanoparticles explains the fact that they have strong antibacterial [22-25], and zinc nanoparticles – immune-stimulating action [26-29].

Nanoparticles of various materials are an effective means of delivering substances to the desired cells. The use of nanoparticles as carriers of various substances of known preparations has many advantages – they can get to the most remote cells, penetrating through various biochemical or immunological barriers. They can be used for optimizing the distribution of substances in the organism, for improving the efficiency and the selectivity of their action, and for reducing toxicity [30-32].

Given the aforesaid, it seems quite reasonable to use nanoparticles of bioelements for activating the immune system and the metabolic processes in the organism in case of infectious diseases in animals. This research was aimed at studying the effect of silver and zinc nanoparticles on the structural features of the cells using the method of atomic force microscopy.

II. MATERIALS AND METHODS

The work was performed at the laboratory of the Institute of Experimental Veterinary Medicine n.a. S. N. Vyshelessky. For the study, nanoparticles of silver (Ag) and zinc oxide (ZnO) were chosen, and for studying their effect on the cells of animals — the MDBK (Madin-Darby Bovine Kidney) culture, since kidneys play an important role in maintaining the acid-base balance of the blood plasma, and ensure constant concentration of the osmotically active substances in the blood in various water conditions for maintaining the water-and-salt balance.

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Kidneys remove the end products of nitrogen metabolism from the organism, alien and toxic compounds (including the active substances of many preparations), the excess of organic and inorganic substances; they are involved in the carbohydrates and proteins metabolism, and in the formation of biologically active substances [33, 34].

The surface of nanoparticles of biometals was visualized by the methods of atomic force microscopy. The procedure of sample preparation for atomic force microscopy involved immobilization on a flat substrate. The substrates were atomically smooth substrates made of mica and other layered materials. For applying a suspension of metal onto the surface of the substrate, the powder was transferred to distilled water, and a drop of water (4 µl) was placed on the substrate surface [35, 36].

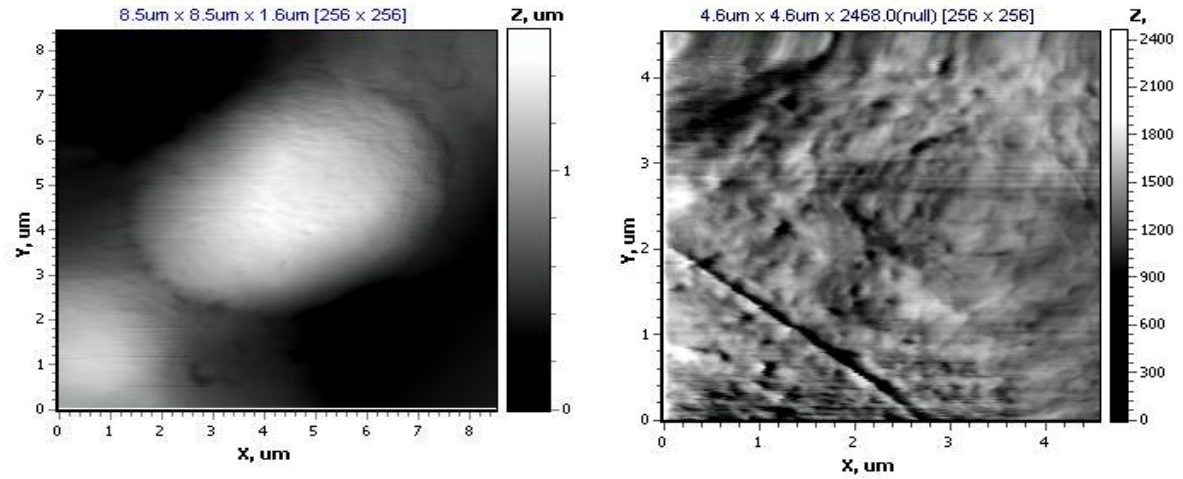
Visualization was performed in various conditions of atomic force microscopy. Due to the high stiffness of biometal nanoparticles, the observations were performed by contact, without taking special measures for minimizing the impact force of the tip on the surface of the particle.

In studying the effect of nanoparticles on the adhesion properties, MDBK cells were used. The work of the adhesion was determined by the energy used for breaking the link upon contact between the phases as a function of the area unit.

III. RESULTS AND DISCUSSION

To study the effect of nanoparticles on the cells of animals, silver (Ag) and zinc oxide (ZnO) nanoparticles were chosen. It is known that nanoparticles of silver derivatives have a devastating effect on viruses, while nanoparticles of zinc oxide do not have antibacterial properties.

Figure 1 shows an individual MDBK cell and part of the membrane surface under lateral forces.



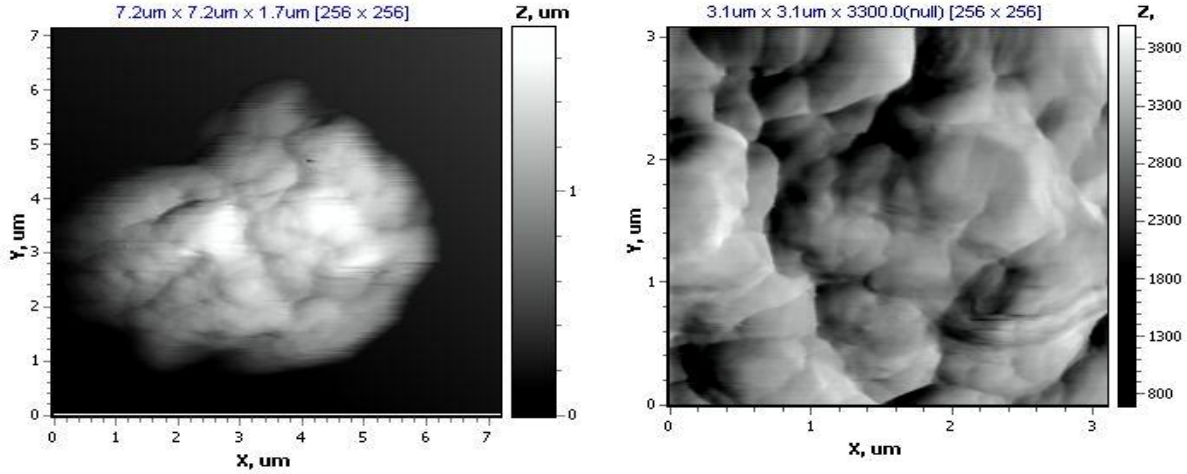
a – topography of the surface, the scan area is 8.5x8.5 µm^2

b – the image under lateral forces, the scan area is 4.6x4.6 mm^2

Fig. 1: AFM images of the surface of MDBK cells

Studying topography showed that the surface of MDBK cells was smooth and flat, without distinct structural elements.

The results of studying the topography of the surface after incubation with a solution of Ag nanoparticles at 37 °C are shown in Figure 2.



a – topography of the surface, the scan area is 7.2x7.2 µm^2

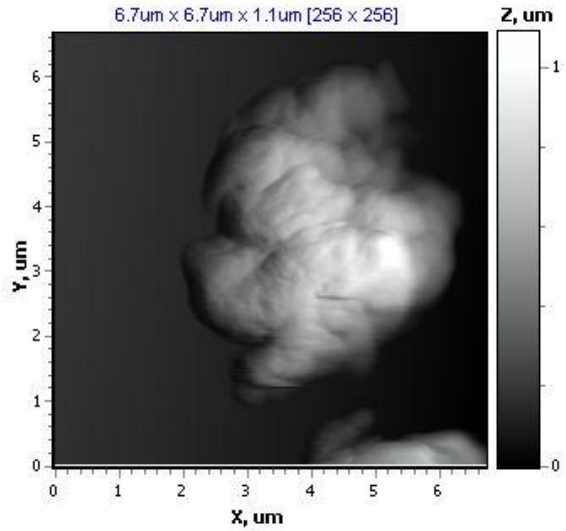
b – the image under lateral forces, the scan area is 3.1x3.1 mm^2

Fig. 2: AFM images of the surface of MDBK cells after their incubation with Ag

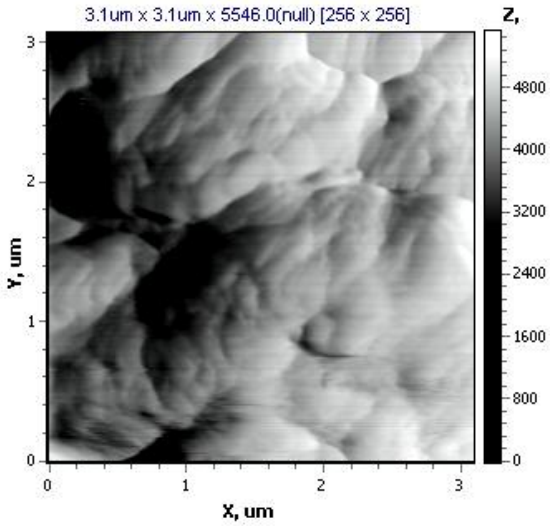
After the interaction of MDBK line cells with Ag nanoparticles, changes in the shape of the cell surface were observed, compared to the reference sample. The surface of the cells became more prominent, with pronounced rounded ridges.

Studying the effect of zinc oxide (ZnO) nanoparticles on the MDBK cells is shown in Figure 3.

As a result of MDBK cells interaction with nanoparticles, it was found that the shape of the cells after incubation with ZnO nanoparticles had strongly changed compared to the shape of the cells in the reference sample; also the surface morphology was different from the surface morphology of the cells incubated with Ag nanoparticles. The non-uniform surface of cells with pronounced structural formations was observed. It may be assumed that due to the effect of ZnO nanoparticles, the internal structure of the cells gets damaged.



a – topography of the surface, the scan area is 6.7x6.7 μm^2



b – the image under lateral forces, the scan area is 3.1x3.1 mm^2

Fig. 3: AFM image of the surface of MDBK cells after incubation with ZnO nanoparticles at 37°C

The next phase of the study involved determining the effect of nanoparticles on the adhesion properties of MDBK cells. The authors calculated the strength of cell surface adhesion, the work of cell adhesion, and the values of cell surface energy. The results are shown in Table 1.

Table 1: Characteristics of MDBK cells adhesion

Sample	F, H	A, J	ω_a, J/m^2
Reference	48.91±3.26	0.26±0.01	0.13±0.01
$AgNO_3$	41.95±3.89	0.22±0.02	0.11±0.01
ZnO	36.80±6.71	0.19±0.03	0.09±0.01

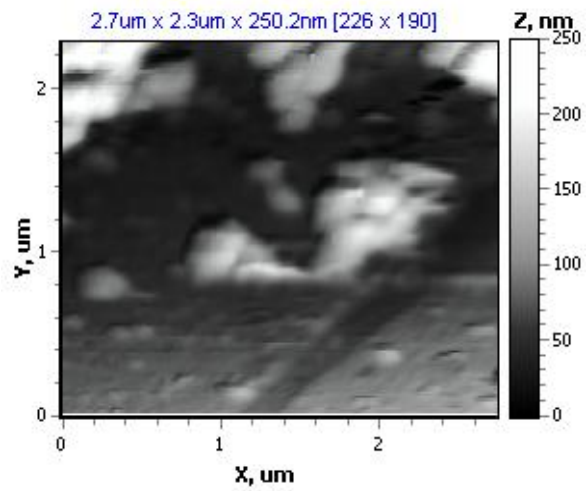
It was found that ZnO nanoparticles increased the adhesion force. Earlier studies showed that these particles decreased the modulus of elasticity, also caused morphological changes in the cells. Thus, these nanoparticles have a strong deleterious effect on MDBK cells, unlike AgNO3, which did not have toxic effect.

During the research, the authors studied the effect of silver and zinc nanoparticles on bacterial cells.

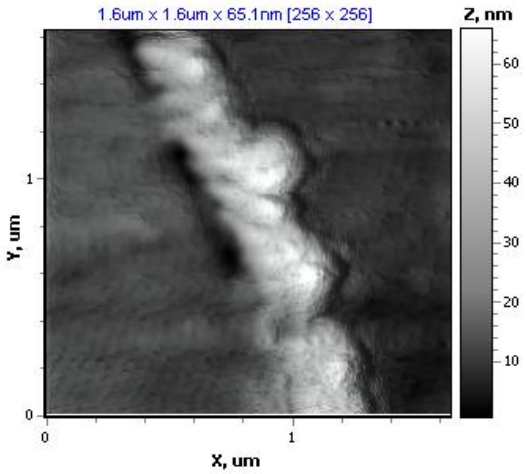
The authors studied the effect of the Zn (zinc) nanoparticles-based preparation on the bacterial cells Escherichia coli at various dilution rates of the preparation and various incubation times.

In course of studying with the use of the method of atomic force microscopy, images of bacterial cells E. coli were obtained before and after incubation with the zinc nanoparticles-based preparation with the following dilution rates – 1:5, 1:10, 1:20. Also, the incubation was performed for two hours and within one day. The obtained images are shown in Figures 4 and 5.

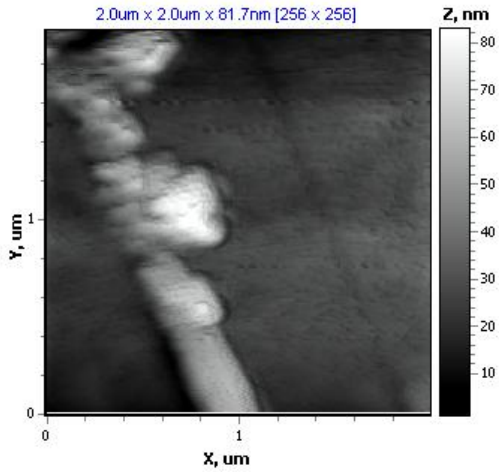
The AFM images of the bacterial cells before (reference sample) and after the interaction with the preparation based on zinc nanoparticles clearly show the changes in their morphology. This process becomes more pronounced with decreasing the preparation dilution rate.



a – Reference (before incubation)



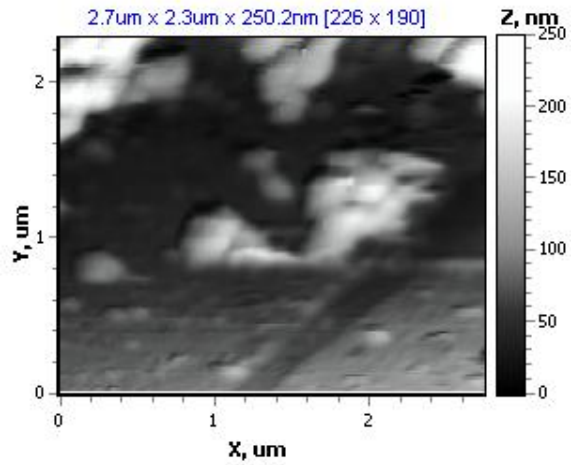
b – After incubation (two hours) at the dilution rate of 1:10



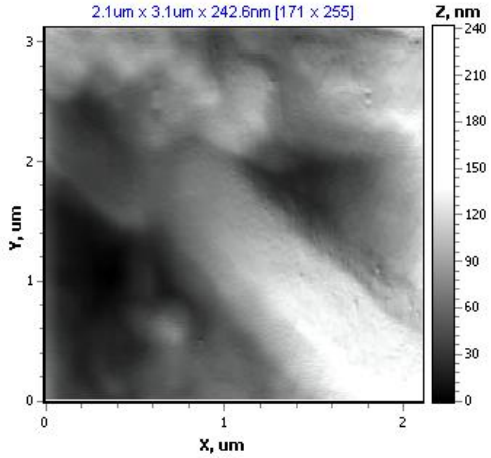
c – After incubation (two hours) at the dilution rate of 1:20

Fig. 4: AFM images of the E. coli cells before and after incubation for two hours with the preparation based on zinc nanoparticles

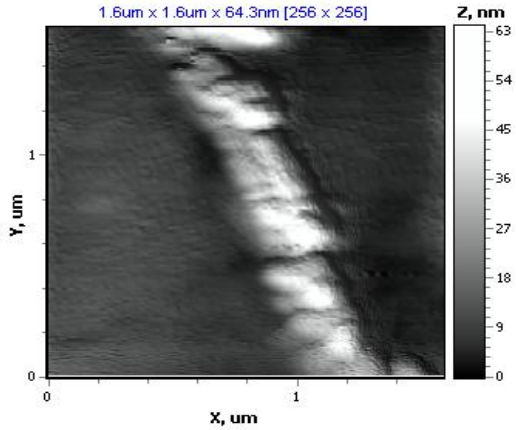
Figure 5 demonstrates the bacterial cells with Zn nanoparticles on the surface with the preparation dilution rate of 1:20, which shows the process of the preparation interaction with the membrane of the bacterial cell. In the case of the preparation dilution rate of 1:5, complete destruction of the bacterial cells is observed.



a – Reference (before incubation)



b – After incubation (one day) at the dilution rate of 1:10

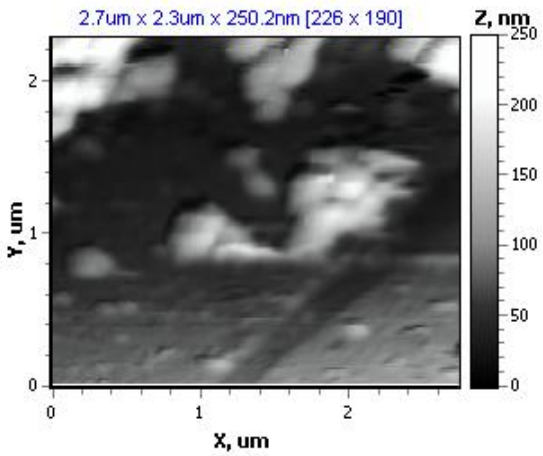


c – After incubation (one day) at the dilution rate of 1:20

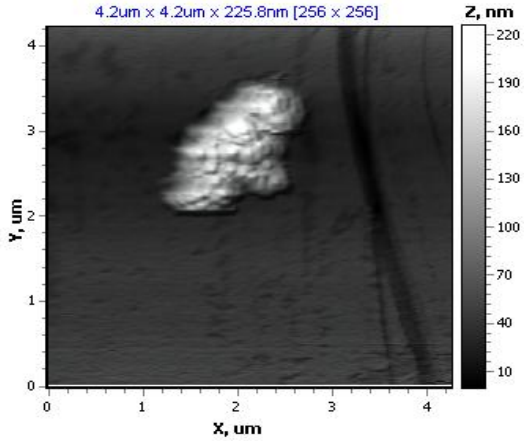
Fig. 5: AFM images of the E. coli cells before and after incubation for one day with the preparation based on zinc nanoparticles

The authors also studied the effect of a complex preparation based on Ag (silver) nanoparticles on the E. coli bacterial cells at various dilution rates of the preparation and various incubation times.

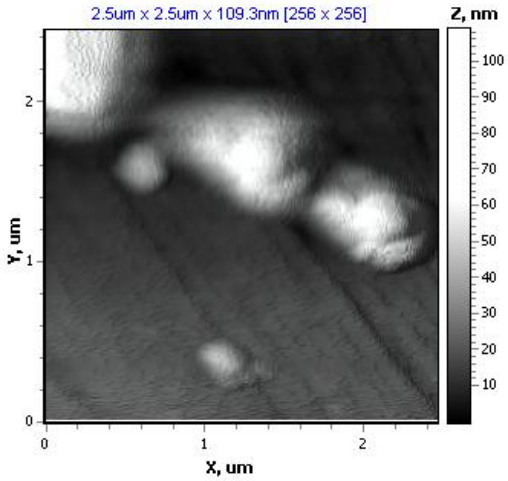
In the course of studying using the atomic force microscopy, images of E. coli bacterial cells were obtained before and after incubation with the preparation based on silver nanoparticles. Also, the incubation was performed for two hours, and for one day. The obtained images are shown below (Figure 6, 7).



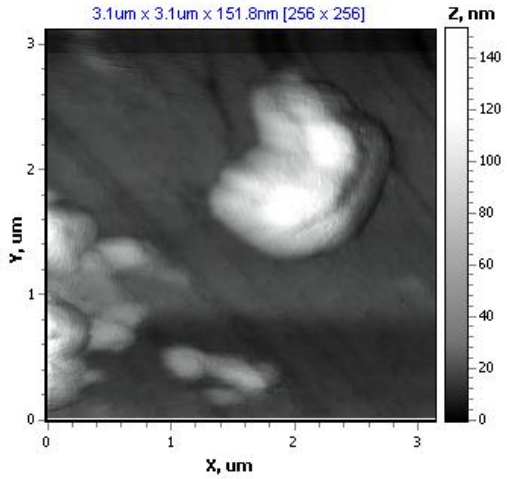
a – Reference (before incubation)



b – After incubation (two hours) at the dilution rate of 1:5



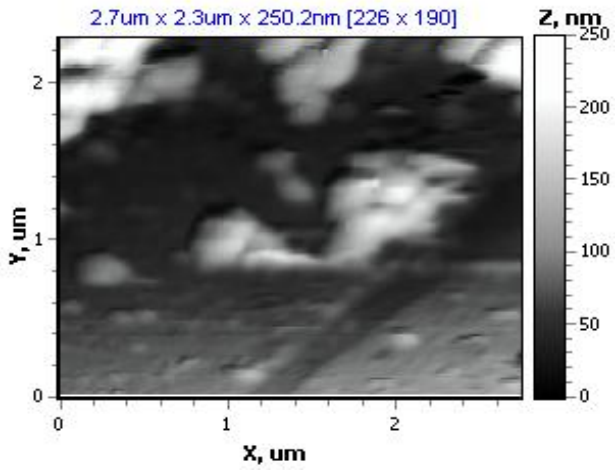
c – After incubation (two hours) at the dilution rate of 1:10



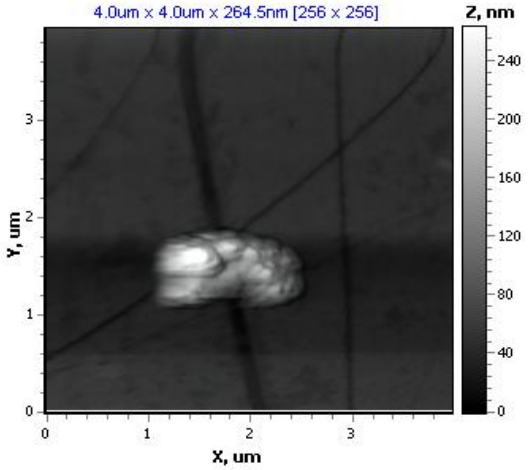
d – After incubation (two hours) at the dilution rate of 1:20

Fig. 6: AFM images of the E. coli cells before and after incubation for two hours with the preparation based on silver nanoparticles

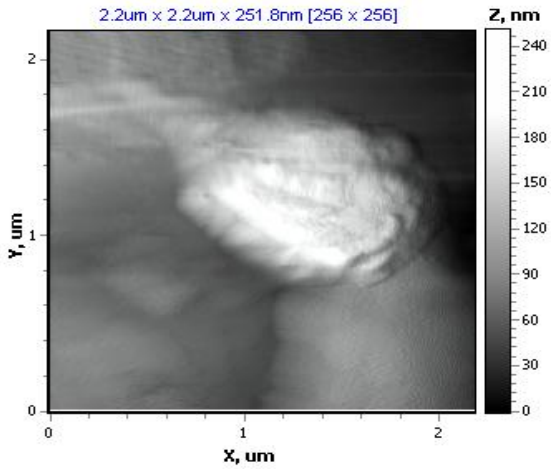
The obtained AFM images show changes in the morphology of the bacterial cells. Figures 7b and 7c show the changes in the cell shape from rod-shaped to rounded, compared to the reference sample.



a – Reference (before incubation)



b – After incubation (one day) at the dilution rate of 1:5



c – After incubation (one day) at the dilution rate of 1:10

Fig. 7: AFM images of the E. coli cells before and after incubation for one day with the preparation based on silver nanoparticles

Thus, studying the morphology of changes in the E. coli bacterial cells exposed to various dilutions of the preparation with various incubation times showed that there was a change in the morphology of the cell membrane surface of the E. coli bacterial cells. Moreover, these processes are the most pronounced in case of incubation with zinc preparation at the dilution rate of 1:5, when complete destruction of the bacterial cells occurs. This effect is observed regardless of the cells with the preparation incubation time. With decreasing the time of incubation and increasing the preparation dilution rate, the intensity of these processes reduces, which reflects less significant changes in the morphology in these conditions.

IV. CONCLUSION

The interaction of MDBK cells with ZnO nanoparticles strongly changes their shape; the surface of the cells becomes uneven, with pronounced structural formations. Besides, the effect of ZnO nanoparticles increases the adhesion strength, decreases the elasticity modulus, and causes morphological changes in the cells. These nanoparticles have a strong deleterious effect on MDBK cells, unlike AgNO3, which did not have a toxic effect.

Changes in the surface morphology of the E. coli bacterial cell membrane in the case of incubation with zinc nanoparticles are observed regardless of the incubation time. In the case of a dilution rate of 1:5, complete destruction of bacterial cells occurs. In the case of E. coli bacterial cells incubation with silver nanoparticles at various dilutions rates and various incubation times, the cell shape changes from rod-shaped to rounded.

REFERENCES

1. A.G. Koshchaev, I.V. Shchukina, A.V. Garkovenko, E.V. Ilnitskaya, V.V. Radchenko, A.A. Bakharev, L.A. Khrabrova, “Allelic variation of marker genes of hereditary diseases and economically important traits in dairy breeding cattle population”. Journal of Pharmaceutical Sciences and Research, 10(6), 2018, pp. 1566-1572.
2. L.N. Skvortsova, A.G. Koshchaev, V.I. Shcherbatov, Y.A. Lysenko, V.I. Fisinin, I.P. Saleeva, S.F. Sukhanova, “The use of probiotics for improving the biological potential of broiler chickens”. International Journal of Pharmaceutical Research, 10(4), 2018, pp. 760-765.

3. I.N. Tuzov, V.G. Ryadchikov, A.N. Ratoshniy, N.I. Kulikova, A.G. Koshchaev, "Using Holstein Cattle in Conditions of the Krasnodar Territory". Journal of Pharmaceutical Sciences and Research, 10(12), 2018, pp. 3160-3163.
4. P. Chauhan, M. Sharma, R. Puri, S. Puri, "Phytochemical Screening of Acorus calamus Linn and Cuscuta reflexa Roxb". Annals of Agri-bio Research, 24(1), pp. 115-118.
5. A.G. Koshchaev, Y.A. Lysenko, A.A. Nesterenko, A.V. Luneva, A.N. Gneush, "Development of feed additives for poultry farming". Dusunen Adam, 10(1), 2019, pp. 1567-1572.
6. E.I. Anisimova, A.G. Koshchaev, O.N. Eremenko, A.S. Krivonogova, K.V. Plemyashov, T.V. Kalashnikova, S.V. Shabunin, "Economic efficiency of productive features of various dairy cattle genotypes". International Journal of Innovative Technology and Exploring Engineering, 8(8), 2019, pp. 3207-3211.
7. A.G. Koshchaev, T.A. Inyukina, N.N. Guguchvili, Y.A. Makarov, A.M. Gulyukin, O.P. Neverova, V.N. Shevkopljas, "The influence of metabolic products of Echinococcus granulosus on the oxidation processes in the organism of pigs". Journal of Pharmaceutical Sciences and Research, 10(9), 2018, pp. 2317-2325.
8. E.V. Yausheva, "Issledovanie biologicheskogo deystviya nanochastits metallov" [Studying the biological effect of metal nanoparticles]. Issues of biological, medical, and pharmaceutical chemistry, 9, 2013, pp. 54-60.
9. N.V. Rukosuev, "Preparaty na osnove nanochastits v klinicheskoy praktike: dostizheniya i perspektivy" [Preparations based on nanoparticles in clinical practice: achievements and perspectives]. Issues of biological, medical, and pharmaceutical chemistry, 10, 2014, pp. 3-22.
10. I.P. Saleeva, V.S. Lukashenko, A.G. Koshchaev, V.G. Volik, D.Y. Ismailova, "Quality of Broiler Chicken Meat with the Use of Various Methods of Growing". Journal of Pharmaceutical Sciences and Research, 10(11), 2018, pp. 2979-2984.
11. I.V. Serdyuchenko, A.G. Koshchaev, N.N. Guguchvili, I.S. Zholobova, I.M. Donnik, A.M. Smirnov, B.V. Usha, "Microbiocenosis of the intestinal tract of honey bees and its correction". OnLine Journal of Biological Sciences, 18(1), 2018, pp. 74-83.
12. A.N. Ratoshny, A.A. Soldatov, S.I. Kononenko, I.N. Tuzov, A.G. Koshchaev, "Organization of feeding dairy cows for preventing metabolic disorders". Journal of Pharmaceutical Sciences and Research, 10(12), 2018, pp. 3273-3276.
13. A.N. Troshin, P.D. Onischuk, A.G. Koshchaev, S.P. Kudinova, O.V. Koshchaeva, V.Y. Nikitin, A.S. Krivonogova, "Parameters of acute toxicity of the Ferro-Quin iron-sorbitol-protein complex". International Journal of Pharmaceutical Research, 10(4), 2018, pp. 784-790.
14. A.A. Rakhmetova, "Antibakterialnaya i ranozazhivlyayuschaya aktivnosty mazey s nanochastitsami medi na osnove proizvodnyh metiltsellyulozy" [Antibacterial and wound-healing activity of ointments containing copper nanoparticles based on methylcellulose derivatives]. Issues of biological, medical, and pharmaceutical chemistry, 3, 2014, pp. 22-27.
15. I.G. Kiselev, I.A. Rodin, A.N. Bezin, A.A. Stekolnikov, A.G. Koshchaev, M.G. Yakovets, A.S. Krivonogova, "Clinical aspects of the use of smooth and full-threaded rods of the VOSIS veterinary orthopedic set in cats". International Journal of Innovative Technology and Exploring Engineering, 8(8), 2019, pp. 3212-3215.
16. S.V. Svistunov, N.N. Bondarenko, A.G. Koshchaev, D.A. Normov, V.N. Shevkopljas, O.P. Neverova, A.M. Smirnov, "Productive qualities of gray mountain caucasian bees of type Krasnopolyansky". International Journal of Innovative Technology and Exploring Engineeringm 8(7), 2019, pp. 631-635.
17. V.G. Tyurin, V.G. Semenov, D.A. Nikitin, A.V. Lopatnikov, I.N. Madebeikin, A.G. Koshchaev, O.V. Koshchaeva, "Stimulation of adaptogenesis in aberdeen-angus calves for improving productive qualities". International Journal of Engineering and Advanced Technology, 8(5), 2019, pp. 440-444.
18. A.A. Bakharev, O.M. Sheveleva, K.A. Fomintsev, K.N. Grigoryev, A.G. Koshchaev, K.A. Amerkhanov, I.M. Dunin, "Biotechnological characteristics of meat cattle breeds in the Tyumen region". Journal of Pharmaceutical Sciences and Research, 10(9), 2018, pp. 2383-2390.
19. A.G. Koshchaev, Y.A. Lysenko, A.V. Luneva, A.N. Gneush, M.V. Aniskina, V.I. Fisinin, I.P. Saleeva, "Studying Biological Activity of Lactobacillus Hydrolysates". Journal of Pharmaceutical Sciences and Research, 10(10), 2018, pp. 2475-2479.
20. N.I. Kryukov, V.O. Yurchenko, A.G. Koshchaev, N.E. Gorkovenko, D.P. Vinokurova, A.A. Bogosyan, S.F. Sukhanova, "The Derivative Of Prussian Blue Paint – Khzh-90 Cesium Isotopes' Sorbent At Mycotoxicoses", International Journal of Pharmaceutical Research, 10(4), 2018, pp. 669-674.
21. A.G. Koshchaev, T.A. Inyukina, N.N. Guguchvili, A.V. Uspensky, S.V. Larionov, F.I. Vasilevich, S.V. Engashev, "The effect of metabolites Echinococcus granulosus on the amino acid composition of the cattle slaughter products". International Journal of Innovative Technology and Exploring Engineering, 8(7), 2019, pp. 589-596.
22. N.A. Samoilov, "Issledovanie antimikrobnyh svoystv nanochastits serebra, stabilizirovannyh sopolimerami maleinovoy kisloty" [Studying the antimicrobial properties of silver nanoparticles stabilized with copolymers of maleic acid]. Biotechnology, 1, 2015, pp. 75-84.
23. P.A. Krasochko, A.E. Stankut, "Protivovirusnye i antibakterialnye svojstva nanochastits serebra" [Antiviral and antibacterial properties of silver nanoparticles]. Our agriculture. Veterinary science and animal breeding, 6, 2013, pp. 64 – 67.
24. A.G. Koshchaev, Y.A. Lysenko, A.A. Lysenko, A.V. Luneva, I.P. Saleeva, V.I. Fisinin, "Screening of microorganism symbiont strains as a base of probiotics for poultry industry". Journal of Pharmaceutical Sciences and Research, 9(8), 2017, pp. 1373-1379.
25. S.V. Svistunov, A.G. Koshchaev, N.N. Bondarenko, O.V. Koshchaeva, A.M. Smirnov, Y.A. Yuldashbayev, O.G. Lorets, "Selection of Bees of the Gray Mountain Caucasian Breed: Apis mellifera caucasica L. of the Krasnaya Polyana Type". Journal of Pharmaceutical Sciences and Research, 10(12), 2018, pp. 3185-3188.
26. P.A. Krasochko, V.A. Mashero, "Immunostimulyatory i sovremennye sposoby korrektsii immunnogo otveta" [Immunostimulants and modern methods of correcting the immune response]. Epidemiology, immunobiology, pharmacology, and sanitation, 1, 2004, pp. 32-36.
27. A.G. Koshchaev, Y.A. Lysenko, M.P. Semenenko, E.V. Kuzminova, I.A. Egorov, E.J. Javadov, "Engineering and development of probiotics for poultry industry". Asian Journal of Pharmaceutics, 12(4), 2018, pp. S1179-S1185.
28. M.I. Zverzhanovskiy, S.N. Zabashta, T.S. Kataeva, A.G. Koshchaev, M.V. Nazarov, "Epizootic trichinellosis situation and consortive links in jackals (Canis aureus L.) in North-western Region of Russia". Indian Veterinary Journal, 94(10), 2017, pp. 29-32.
29. A.N. Troshin, A.N. Turchenko, P.D. Onischuk, A.G. Koshchaev, S.P. Kudinova, A.Y. Shantyz, O.V. Koshchaeva, "Long-term use of iron-mineral and iron-organic drugs". International Journal of Pharmaceutical Research, 10(4), 2018, pp. 791-797.
30. I.A. Khmel, O.A. Koksharova, M.A. Radtsig, "Antibakterialnye effekty ionov serebra: vliyanie na rost gramotritsatelynyh bakteriy i obrazovanie bioplyonok" [Antibacterial effects of silver ions: the effect on the growth of gram-negative bacteria and biofilm formation]. Molecular Genetics, Microbiology, and Virology, 4, 2009, pp. 27-31.
31. A.V. Garkovenko, V. V. Radchenko, E.V. Ilnitskaya, A. G. Koshchaev, I.V. Shchukina, A.A. Bakharev, S.F. Sukhanova, "Polymorphism of cattle microsatellite complexes". Journal of Pharmaceutical Sciences and Research, 10(6), 2018, pp. 1545-1551.
32. V.G. Semenov, D.A. Nikitin, A.V. Volkov, V.G. Tyurin, A.G. Koshchaev, A.A. Nesterenko, S.V. Shabunin, "Preventing Shipping Stress in Imported Heifers with the Use of Immunocorrection". International Journal of Innovative Technology and Exploring Engineering, 8(5), 2019, pp. 1591-1595.
33. E.I. Anisimova, A.G. Koshchaev, A.A. Nesterenko, A.A. Bakharev, A.G. Isaeva, T.M. Shuvaeva, T.V. Kalashnikova, "Comparative Assessment Of The Relationship Between Intrabreed Types Of Simmental Cows And Sectionized Traits". International Journal of Pharmaceutical Research, 10(4), 2018, pp. 604-610.
34. I.S. Koba, A.A. Lysenko, A.G. Koshchaev, A.K. Shantyz, I.M. Donnik, V.I. Dorozhkin, S.V. Shabunin, "Prevention of Mastitis in Dairy Cows on Industrial Farms". Journal of Pharmaceutical Sciences and Research, 10(10), 2018, pp. 2582-2585.
35. E.V. Kuzminova, M.P. Semenenko, A.G. Koshchaev, O.Y. Chernyh, A.N. Turchenko, "Pharmacological prevention of obstetric and gynecological diseases in cows". Dusunen Adam, 10(1), 2019, pp. 608-612.
36. N.I. Kulikova, O.N. Eremenko, A.G. Koshchaev, T.V. Kalashnikova, N.V. Evdokimov, K.A. Amerkhanov, I.M. Dunin, "Productive longevity of holstein bulls' female offspring". International Journal of Engineering and Advanced Technology, 8(5), 2019, pp. 435-439.