JUNIOR ACADEMY OF SCIENCES OF UKRAINE

BIOSYNTHESIS OF NANOMATERIALS AND THEIR APPLICATIONS IN MEDICINE

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Keywords:

#nanomaterials #nanoparticles #quantum dots #biosynthesis #phytoncides #antibacterial drug #infusoria #yeast #bacteria #viburnum #plant extract #epithelium #fluorescence #microscopy

The subject of the research:

Biosynthesis of nanomaterials; the effect of the drug on the vital activity of the infusoria, of yeast and bacteria of the oral cavity; method of staining cells for fluorescence microscopy using quantum dots.

The goals of the research :

To synthesize selenium nanoparticles, quantum dots, create an antibacterial drug, investigate its activity and to stain cells with quantum dots.

The objects of the research:

Biosynthesized selenium nanoparticles, quantum dots, complex drug based on viburnum juice on selenium nanoparticles

Research objectives:

1. To get acquainted with some species of plants, bacteria, fungi, protozoa, features of their life information on nanomaterials and their properties.

2. To Synthesize nanomaterials, create an antibacterial drug, study the activity of the drug, use quantum dots to stain cells.

3. To analyze the results and determine the prospects of the research.

Nanoparticles and quantum dots

Nanoparticles (Fig. 1A) - particles of matter up to 100 nm. Quantum dots (Fig. 1B) are semiconductor nanoparticles up to 10 nm in size that are capable of fluorescence.

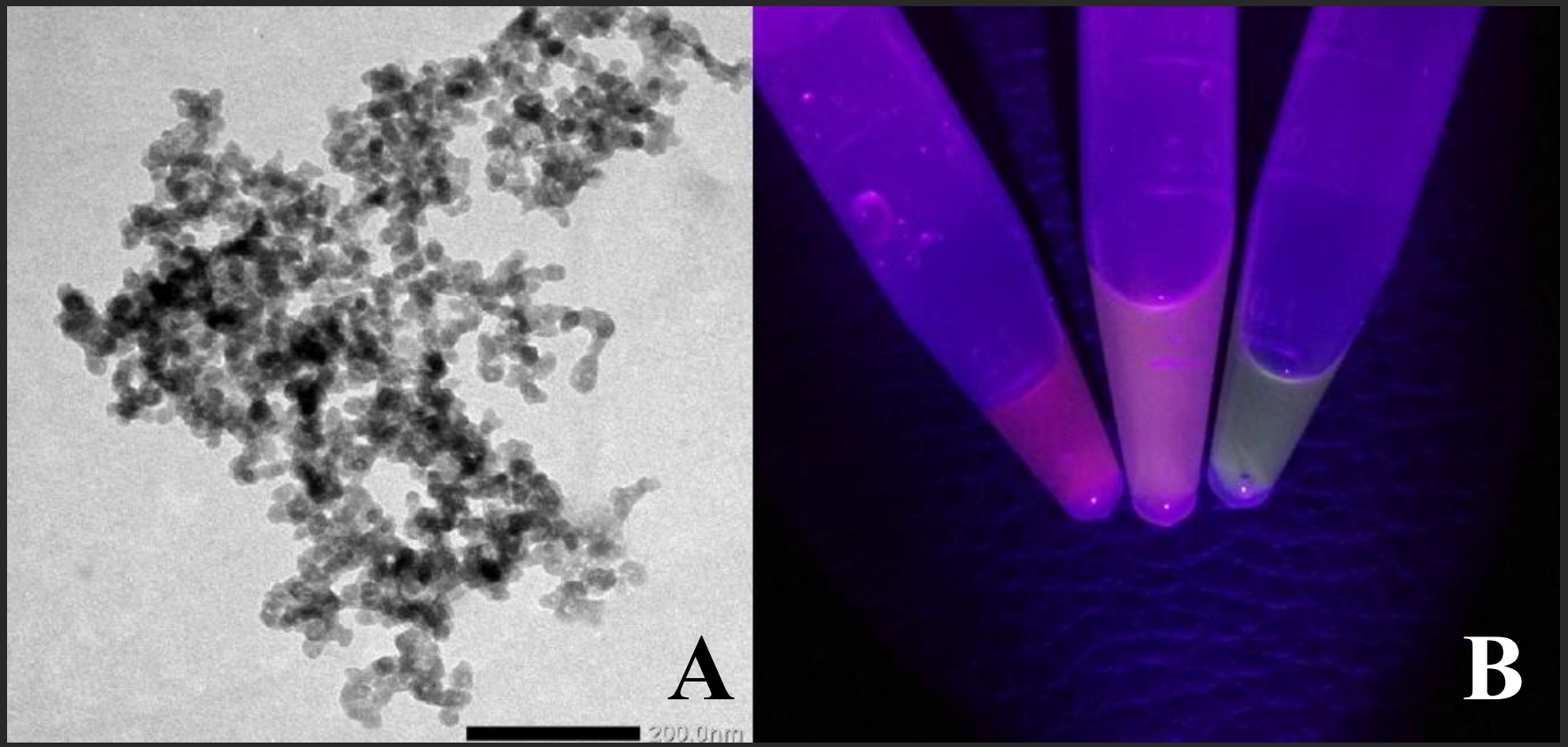


Fig. 1 Biosynthetic nanomaterials (source: author's development).

Selection of organisms for research

Gram-positive bacterium *Bacillus thuringiensis* was selected for the biosynthesis of selenium nanoparticles (Fig. 2C), and quantum dots were synthesized using yeast culture (Fig. 2A). The activity of the drug was determined in relation to infusoria (Fig. 2B), yeast and multiculture of oral cavity bacterial culture (Fig. 2D).

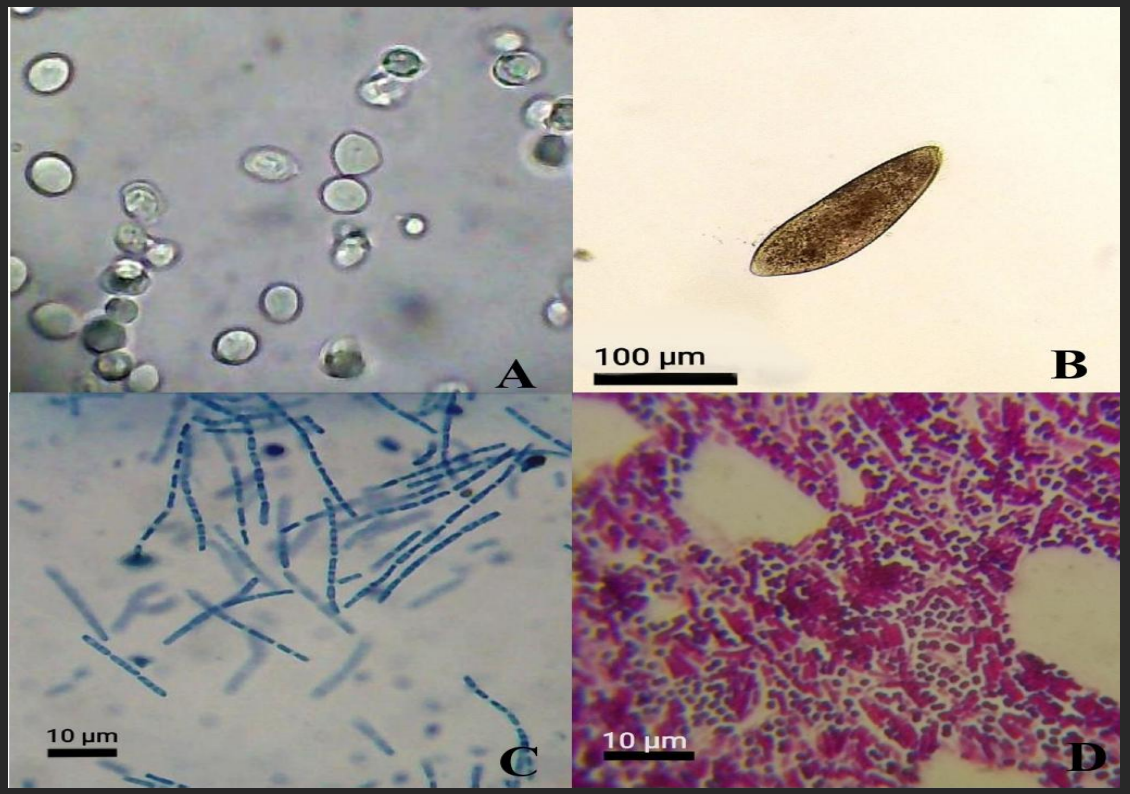


Fig. 2 Model organisms (source: author's development)

Methods of biosynthesis of nanoparticles and quantum dots

1. For the biosynthesis of selenium nanoparticles, the biomass of the bacterium Bacillus thuringiensis was first accumulated during 24 h, then potassium selenite was added. Two days later, the nanoparticles were centrifuged at 10,000 rpm (Fig. 3) and characterized by electron microscopy.

2. For the biosynthesis of quantum dots (Fig. 4), the yeast culture was incubated for 24 h with potassium selenite and 24 h with cadmium chloride. Nanomaterials sere isolated from cells using sulfuric acid.

Properties of extracts of various plants

Property / plant	Viburnum, Viburnum opulus L. (juice)	Beetroot, Beta vulgaris L. (juice)	Carrots, Daucus carota L. (essential oil)
Chelating agents	High concentration	High concentration	-
Effects on bacteria	Inhibits	Increase the CFU	Inhibits
Effects on yeast in high concentrations	Low	Increase the CFU	Medium
Effect on infusoria in high concentrations (cells were applied into the native solution)	Kill into 5,6 seconds	Kill into 18,5 seconds	Kill into 137,7 seconds

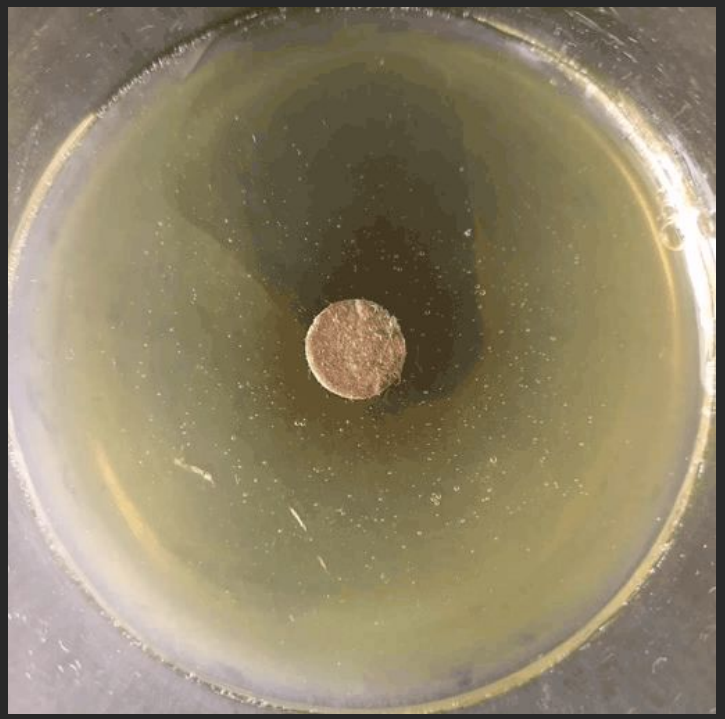
Table 1 Properties of extracts of various plants (source: author's development)

The scheme of studying of activity of drug concerning microorganisms

1. To study the activity of the drug to the protozoa, a test with the infusoria was performed.

2. The Activity against yeast was studied by the culturing for one and two days in the presence (experiment) and absence of the drug (control). CFU/cm3 were determined by direct counting of cells, their morphology was evaluated. Statistical data processing was performed using the computer program statsoft STATISTICA 12.

3. The activity of the drug to a multiculture of oral cavity bacteria was determined by the disk method.



Animation 1 The disk method (source: author's development)

Method of staining epithelial cells with quantum dots

1. First, the epithelium of the oral cavity was collected with a cotton swab and applied to the slide.

2. To prevent the smear from drying out, a few drops of native solution of quantum dots were applied.

3. To prevent evaporation, the mixture was covered with a cover glass.

4. After 5-10 minutes, the drug can be examined using a fluorescence microscope in a wide range of initiating waves (~ from 200 to 450 nm).

Methods of studying of nanoparticles and quantum dots

1. To study the properties of selenium nanoparticles (Fig. 3) and quantum dots (Fig. 4) a transmission electron microscopy on a microscope JEOL JEM-1400 was used.

2. To determine the fluorescence spectrum of quantum dots, a self-made spectrograph calibrated along the Fraunhofer lines of the solar spectrum was used. The initiating wave was 390 nm (Fig. 4G).

3. To confirm the presence of quantum dots inside the yeast cells, fluorescence microscopy was performed (Fig. 4B).

Biosynthesis of selenium nanoparticles

1. According to the method, selenium nanoparticles were isolated, for further studies some nanoparticles were chelated with gelatin and polysorbate-20.

2. During the TEM (next slide), the presence of nanosized particles was detected and it was determined that their diameter ranged from 10 nm to 60 nm, with an average diameter of 30 nm.

3. It was determined that nanoparticles with a diameter of 30 to 60 nm are released in the form of elemental selenium (Fig. 3B, 3C), and with a diameter of less than 30 nm in vesicles (Fig. 3D, 3E, 3F).

The TEM of selenium nanoparticles

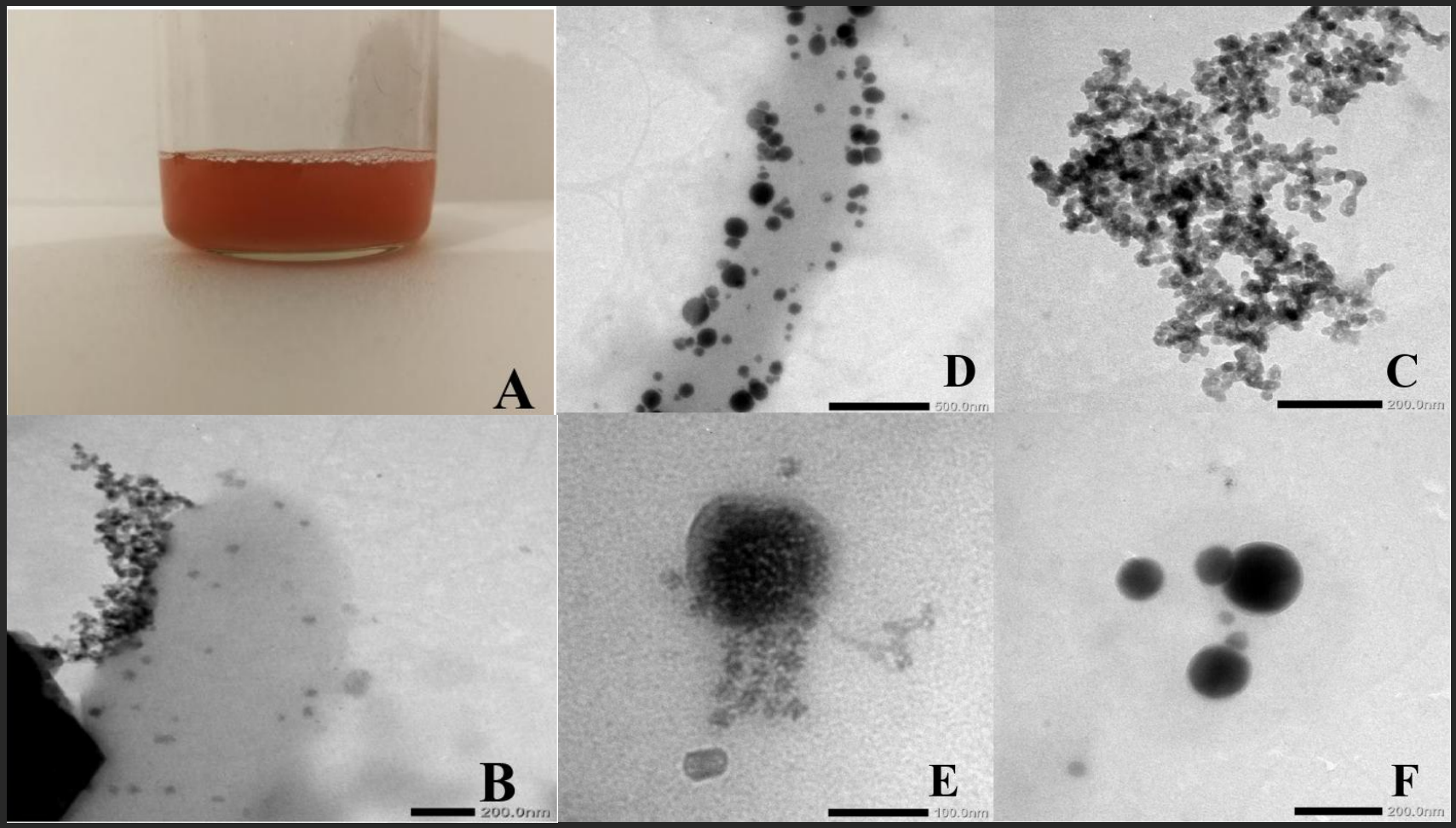


Fig. 3 Properties of selenium nanoparticles A) Stabilized suspension of selenium nanoparticles; B), C) TEM of nanoparticles in the form of elemental selenium; D), E), F) TEM jf nanoparticles in vesicles (source: author's development)

Biosynthesis of quantum dots and study of their properties

1. After incubation, a large number of yeast cells were detected (Fig. 4A).

2. Fluorescence microscopy of these cells confirmed the presence of quantum dots in the cytoplasm (Fig. 4B).

3. These quantum dots are capable of fluorescence at a wavelength of 390 nm (Fig. 4F).

4. The main emission peaks of quantum dots at this wavelength were 600 and 670 nm (Fig. 4G).

5. The presence of nanoscale structures close in shape to spheres was confirmed by the TEM (Figs. 4C, 4D, 4E). There were enough quantum points in the field of view for visible fluorescence. Thus, the presence of quantum dots was confirmed by several methods.

The properties of quantum dots

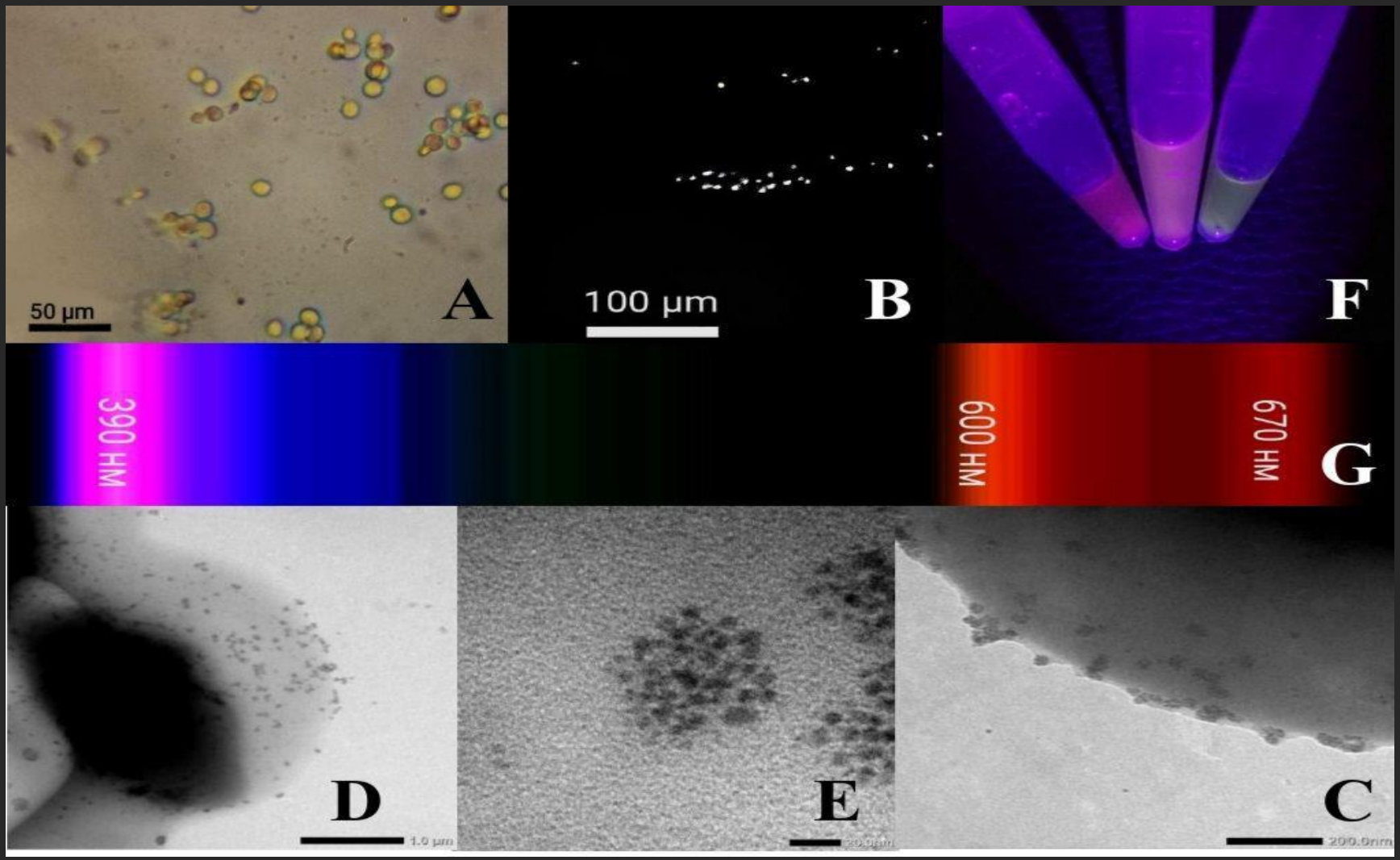


Fig. 4 Properties of biosynthetic quantum dots (source: author's development)

The effect of the drug on the infusoria

1. When tested with protozoa (Paramecium caudatum) the drug showed very high activity. The average time of cell death could not be determined. The cells died immediately after entering the drug solution. After death from the drug, most cells become a typical form (Fig. 5D).

2. According to the data obtained, nanoparticles disrupt the osmotic balance of the cell. When mixing the culture and the solution of the drug protists immediately stop moving, although the lashes are still moving. Cells contract and die. Figure 5 compares the activity of the drug, selenium nanoparticles and viburnum juice.

The activity of various substances against infusoria

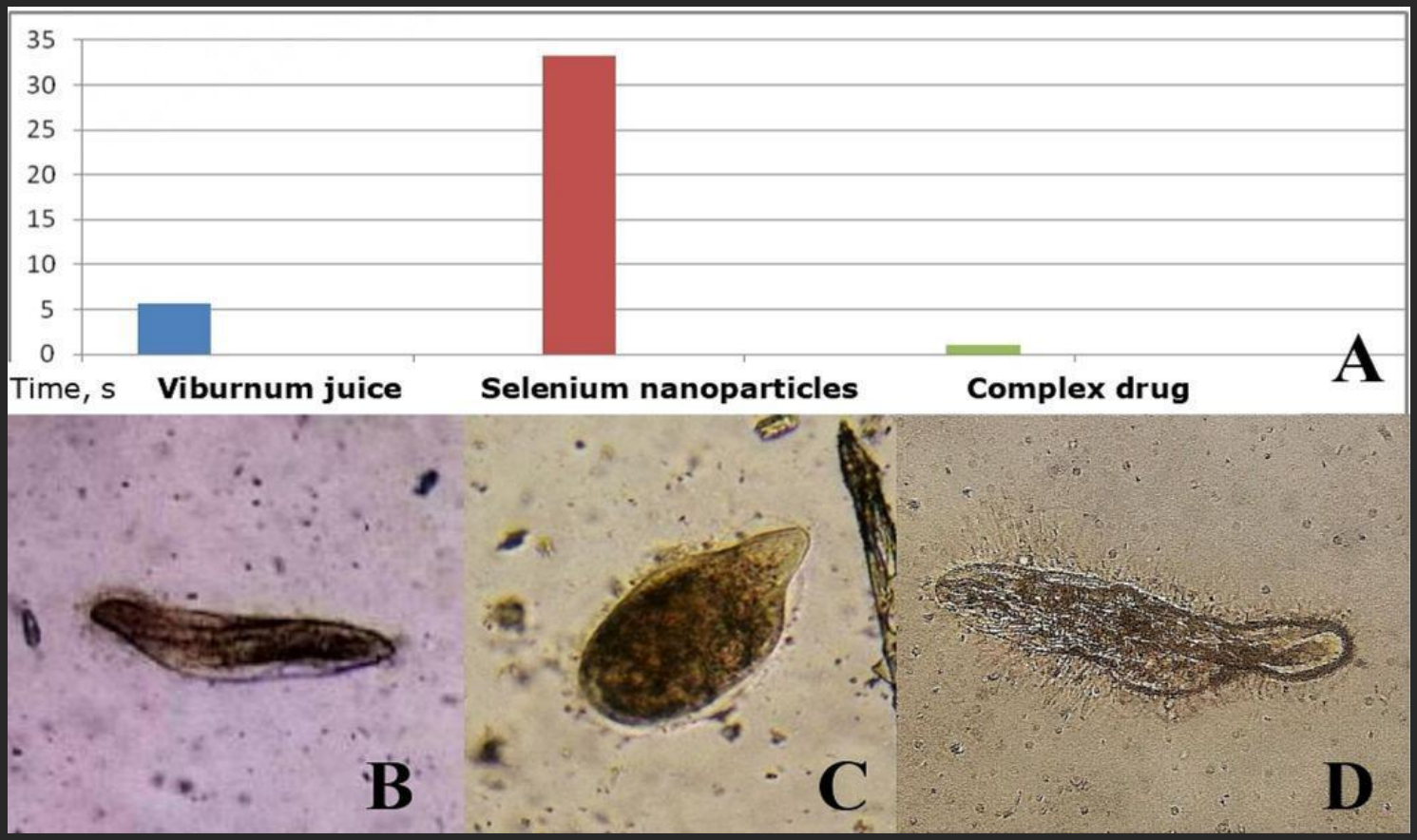


Fig. 5 Toxicity of viburnum juice, selenium nanoparticles and complex drug to *Paramecium caudatum* A) Table with time of death in seconds; B) Infusoria cell after death in the native solution of viburnum juice; C) after death in the solution of selenium nanoparticle; D) after death in the native solution of complex drug (source: author's development).

The effect of the drug on yeast cells

The drug shows low fungistatic activity against Saccharomyces cerevisiae. When cultivated for 24 hours, it inhibits yeast growth by 1.12 times. The size of yeast cells decreases under the influence of the drug (Fig. 6). The drug also affects the change of haploid and diploid yeast cells by reducing the number of diploid cells. After 48 hours, the drug inhibits yeast growth by 1.19 times. The effect on the change of cell shapes increased, in some fields of view no diploid cells were identified. Figure 6 compares the activity of different substances.

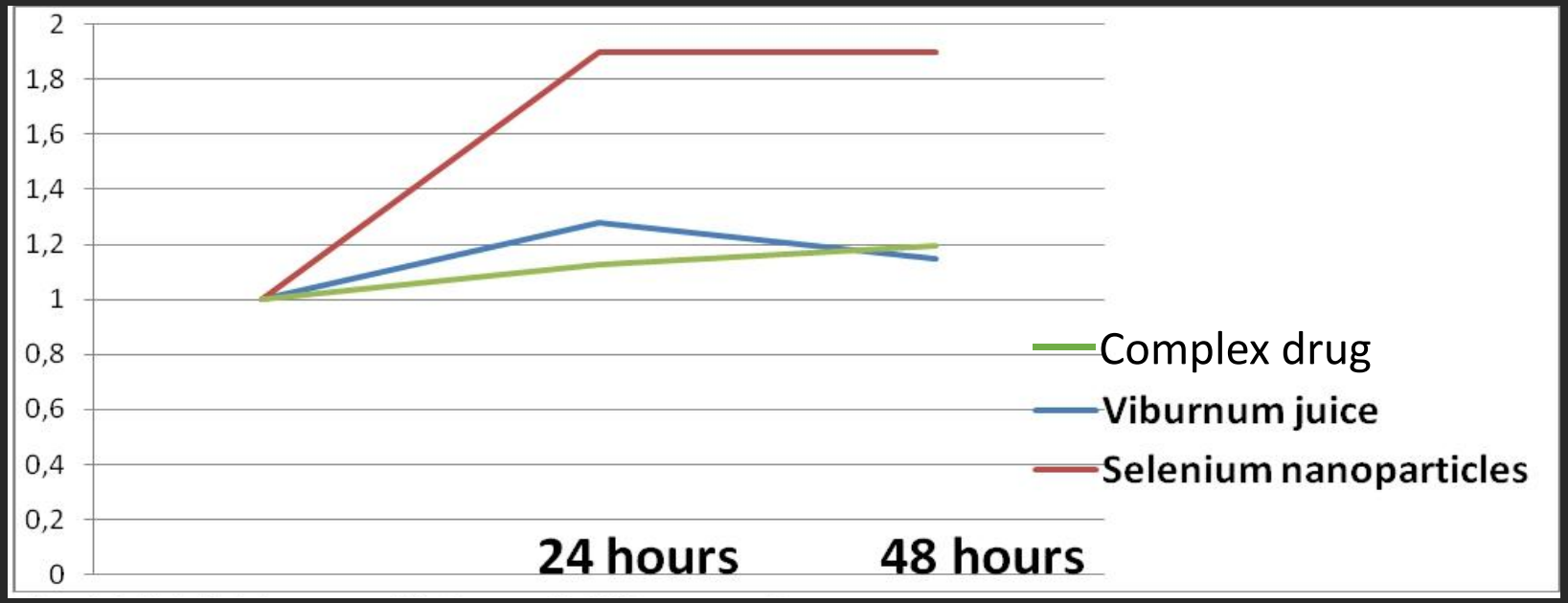


Fig. 6 Inhibition coefficient of different substances (source: author's development)

The effect of the drug on yeast cells

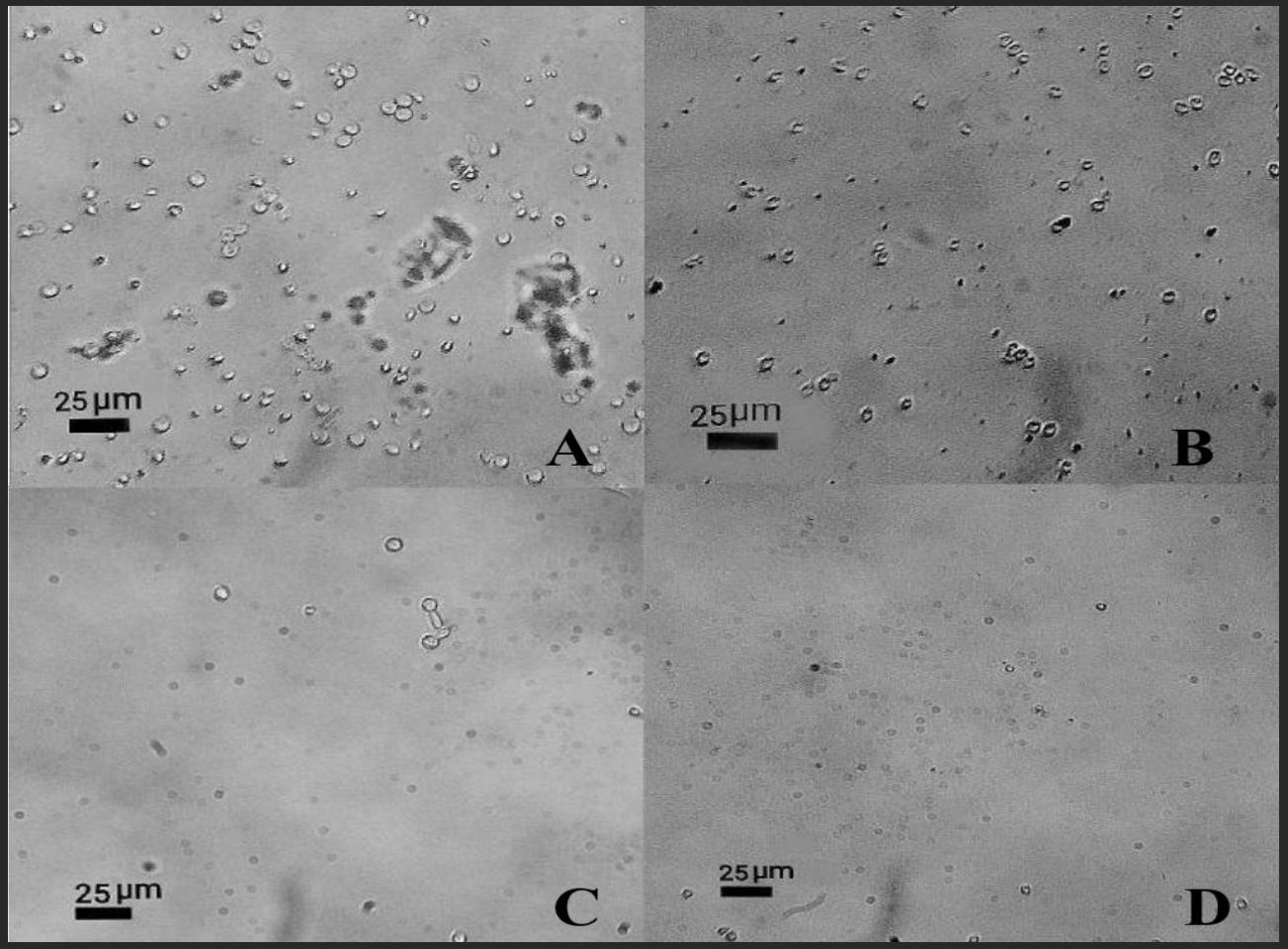


Fig. 7 Effect of complex drug on yeast cells A) Control yeast culture, 24 hours of incubation; B) Yeast culture containing complex drug, 24 hours of incubation; C) Control yeast culture, 48 hours of incubation; D) Yeast culture with drug, 48 hours of incubation (source: author's development)

The effect of the drug on the activity of yeast

Complex drug inhibits the growth of oral cavity bacteria culture. The diameter of the area of inhibition after 12 hours was 11 mm (Fig. 8A). According to this experiment activity is low. But after 120 hours the same areas were the same diameter and there were no bacterial colonies in this area (Fig. 8B). It means that bacteria do not become resistant to the drug. Therefore, antibacterial activity is high. Low activity in the first experiment could be explained by properties of nanoparticles and agar. Agar stops diffusion of nanoparticles as sorbent.

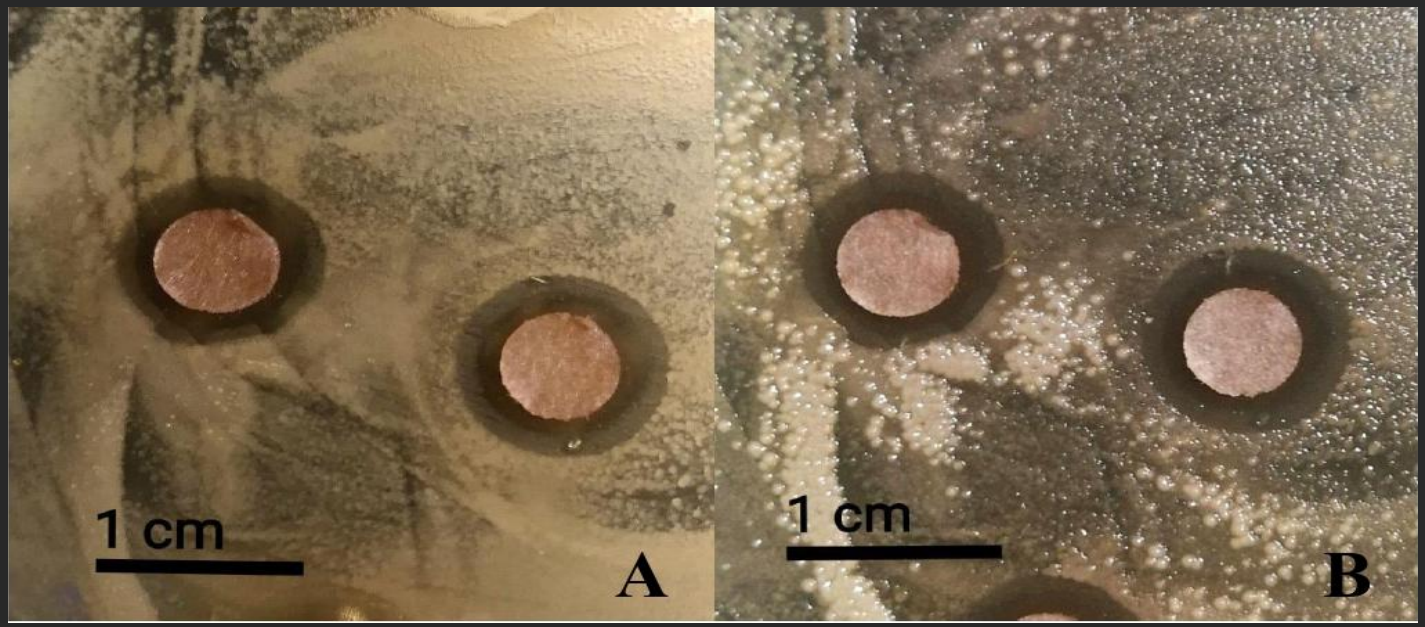


Fig. 8 Inhibition of growth of bacteria of an oral cavity by a complex preparation (source: author's development)

Staining epithelial cells with quantum dots

Epithelial cells were stained according to the procedure (Fig. 9). Cell nuclei can be distinguished from the cytoplasm, so quantum dots can be used as pH markers.

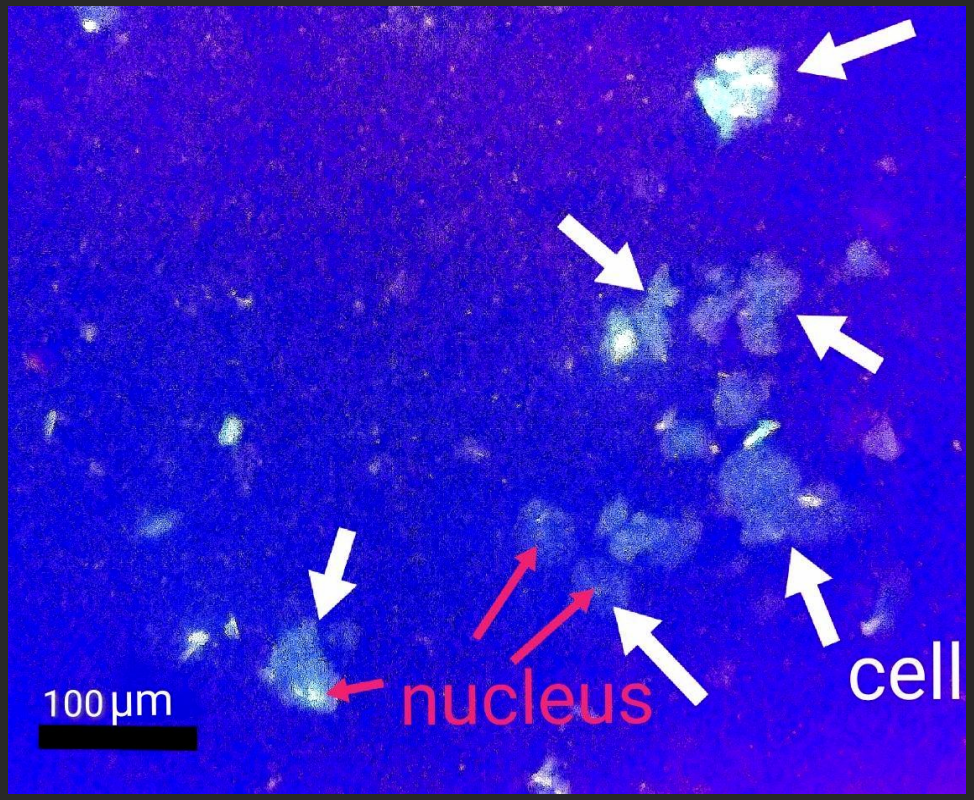


Fig. 9 Fluorescence microscopy of epithelial cells stained with biosynthetic quantum dots (source: author's development).

Significance

1. Most hypotheses were confirmed during the study. For the first time a complex antibacterial drug based on plant extract (viburnum) and nanoparticles (selenium) was created.

2. This drug shows medium antibacterial activity, but bacteria do not become resistant to it. In therapeutic concentrations, the drug is harmless for eukaryotes. After further tests, the possibility of using the drug in dental and cosmetic products will be considered. The drug can also be used as an antiprotozoal substance.

3. Biosynthetic quantum dots are capable of fluorescence, the intensity of fluorescence depends on the pH of the environment (at low increases). The possibility of using such quantum dots in fluorescence microscopy has been shown. Thus, it is possible to use such quantum dots to visualize cancer cells and tissues (their pH is different from normal cells).

Research prospects:

1. The prospect of the study is to conduct X-ray diffraction analysis to more accurately determine the characteristics of nanoparticles.

2. To determine the toxicity of the drug will be tested on human cell lines.

3. With the help of biochemical studies, the mechanism of toxicity will be studied in more detail, and especially the effect of the drug on specific metabolic reactions and physiological processes in bacterial and protist cells.

4. It is planned to study quantum dots using X-ray diffraction, spectral, electron diffraction methods.

5. Quantum dots of different composition and characteristics will be synthesized.

6. A method for visualizing cancer tumors and cells using biosynthetic quantum dots will be developed.

Personal contribution

1. Defining the topic and scheme of the study.
2. Conducting most of the experiments (except for electron microscopy).
3. Analysis, disclosure, visualization and explanation of research results.
4. Writing a scientific paper.
5. Execution of work in accordance with the standards of international competitions and adaptation in English.
6. Graphic design (video + animation, poster and presentation).

Research awards

1. The work was included in the top 100 works in the world according to the results of Google Science Fair 2018.
2. The work was to be presented at the final stage of the Genius Olympiad 2020 (USA) (the event was postponed to 2021 due to a pandemic).
3. The work was to be presented in the finals of Think Science 2020 (Dubai), but the trip of the Ukrainian (from MAN) delegation was canceled after the Boeing 737-800 crash over Iraq).
4. The work took 1st place at the largest Canadian event for inventors iCAN 2020.
5. The work took 2nd place at the Polish event International Warsaw Invention Show 2020.
6. In 2020, the work received the highest scores among all sections in the competition-defense in the Chernihiv region (97.7).
7. The work took 1st place at the ITEX 2020.

Conclusions

1. Selenium nanoparticles and quantum dots were synthesized.

2. Two ways of export of nanoparticles by *Bacillus thuringiensis* cells were observed. Particles up to 30 nm in diameter are exported in vesicles, and from 30 to 60 nm - in the form of elemental selenium.

3. The complex drug shows intermediate antibacterial activity, bacteria do not have resistance to it. The drug shows high antiprotozoal activity.

4. Biosynthetic quantum dots were used to stain epithelial cells.

5. The research helps to minimize the carbon footprint, as a result, complies with the UN environmental course.

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