Effect of long-wavelength coherent radiation on a biological object (unicellular organism Paramecium Caudatum) in presence of silicon nanoparticles

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Abstract: We report an effect of continuous and pulsed near infrared (808 nm) laser radiation on cells (Paramecium Caudatum) incubated with silicon (Si) nanoparticles (NPs), which were obtained by laser ablation of crystalline Si wafer in water. The data obtained indicate the possibility of enhancing the destructive effect on cells by using pulsed laser irradiation and Si NPs.

Keywords: Si nanoparticles, coherent emission, IR laser, Paramecium Caudatum

1. Introduction
Silicon (Si) nanoparticles (NPs), including Si nanocrystals, are widely investigated for applications in biomedicine, in particular, for theranostics (simultaneous diagnostics and therapy) of oncological and other socially significant diseases [1-4]. It was previously found that porous Si-NPs can act as photosensitizers for the singlet oxygen generation and also as agents that enhance photohyperthermia effect [5].

2. Materials and methods
During this work in order to obtain Si-NPs we used the method of femtosecond laser ablation that appears to be the promising method of solid state surfaces procession due to energy dissipation features mostly flowing after the laser pulse interruption. Laser pulses power management allows us to control the process of target material vaporization before it is thrown out into surrounding media and was formed as NPs [2]. This method allows to control the size of NPs by radiation properties variation and replacement of the surrounding media. Different pulsed laser radiation sources can also be used but its power demand is obligatory – it should be high enough to reach the threshold point of local melting. Using appropriate optical devices, the laser beam is focused on the target surface. The amount of ablated substance corresponds to the number of pulses and is determined by the size of the laser spot. Since the laser, as an
energy source, is placed outside the deposition area, thus the deposition geometry can be varied widely (the possibility of using several targets, the possibility of changing the distance and angle between the target and the substrate). The main advantage of heating from such source is the absence of mechanical contact between heating element and substrate that significantly simplifies the rotation of the substrate, if it is necessary.

In the experiments we used standard optical polished wafers of monocrystalline Si with a resistivity of 10-20 Ohm-cm and a surface orientation (100) and (110). Before laser irradiation, the plates were subjected to short-term (1-2 s) etching in an aqueous solution of HF (48%) to remove natural oxide. For laser irradiation, a femtosecond laser system composed of a femtosecond solid-state laser with a diode pump Teta 10 (Avesta, Russia) was used. The laser radiation had a wavelength of $\lambda_0 = 1030$ nm and was linearly polarized. Pulses with a duration of 300 fs with an energy of about 400 $\mu$J followed at a frequency of 25 kHz. The radiation was focused into a beam with a diameter of 3.3 mm with a normal incidence on the surface of the sample. The irradiation was carried out at room temperature.

For the analyze of the Si NPS particles toxicity and to study the effect of continuous and pulsed long-wave laser radiation (808 nm) on living infusoria of Paramecium Caudatum was selected. Paramecium caudatum infusoria, despite the simplicity of the organization, combines the features of a single cell and the whole body. In the analytical aspect, they are interesting because they can be considered as simple receptor-effector systems that have the ability to respond to the chemical effects of a whole complex of biological, physiological and biochemical changes.

Purpose of research: invest the effect of continuous and pulsed laser radiation (808 nm) on cells in a medium with the addition of silicon in vitro - Figure 1.

In the experiments, a semiconductor laser with a wavelength of 808 nm and a power of about 3 W in continuous mode and with a similar average power density in the pulse mode was used [5]. An aqueous suspension of Si-NPs with a concentration of 0.4 mg / ml in the volume of 1 ml was added to a plastic cuvette to 2 ml of the cell suspension, incubated for 30 min, and then exposed to laser radiation for 5 min. the concentration of cells in each sample at the beginning of the experiment was 7÷8 individuals in 1 ml.

![Figure 1. Schematic view (a) and photographic image of the experimental setup (b). 1- laser, 2 - temperature sensor.](image)

The results are shown in Table 1.
Table 1. Data on the investigated (or studied) groups and exposure modes (microscopy after 60 min, 90 min, 120 min)

| Test | Total number of viable cells in 1 ml | Number of damaged cells in 1 ml |
|------|--------------------------------------|---------------------------------|
|      | 60 min / 90 min / 120 min | 60 min / 90 min / 120 min |
| №1. Intact (unicell Paramecium caudatum in culture medium) | 7/7/7 | 0/0/0 |
| №2. R₁ (Paramecium + nc-Si) | 8/7/7 | 2/3/3 |
| №3. RA (Paramecium + Laser cw) | 4/4/3 | 3/3/4 |
| №4. RB (Paramecium + Laser puls) | 1/1/1 | 7/7/8 |
| №5 A (Paramecium + nc-Si + Laser cw) | 3/3/2 | 3/3/4 |
| №6 B (Paramecium + nc-Si + Laser puls) | 0/0/0 | 8/8/8 |

Figure 2. Histogram of the number of living cells before irradiation and 24 hours after irradiation.

Numbers of living cells before irradiation and 24 hours after irradiation are showed in Figure 2.
3. Results and discussion
The analysis of the obtained data allows to draw (drawing) a conclusion about stimulation of cytotoxic effect of nanoparticles under pulsed laser irradiation. In particular, in the sample №6 100% the cell death was noted in one hour after exposure to laser radiation. In the sample № 4 the number of viable cells, both two hours after irradiation and after four days, decreased sharply, and after 24 hours the cell death was 100%. At the same time, in samples № 2 and № 7, as well as in the sample with intact cells, an increase in the number of cells was observed on the fourth day, which is due to the absence of adverse factors for the processes of division in the environment.

It should be noted that the conclusion about the possible toxic effect of the investigated NPs gives the first approximate assessment and serves as the basis for the choice of the strategy for further analysis. It is known that Paramecium caudatum, which is used in this work, can live in both aerobic and anaerobic conditions. This high adaptive capacity provides a huge number of genes, most of which in everyday life do not need infusoria, but included when conditions change. As the protozoa adapt to the environment, all their vital functions are rebuilt, the speed of movement, the rate of reproduction and the ability to absorb food, as well as the shape and size of the body. But if the environment does not change, the properties of the infusorians remain stable, and this allows them to be used as model organisms. Of course, this applies only to infusorians cultivated in the laboratory. In biotests on the ciliates is the easiest way to record the change of mobility, loss of life and reproduction rate. The analysis of only the last parameter in the performed study does not allow to judge the details of the mechanism of action of laser radiation and LF at the genomic and epigenetic level.

4. Conclusion
At the same time, considering the infusoria as biological test objects, it is assumed that the performance of complex functions of these single-celled organisms provides the so-called cytoskeleton (which in addition to everything else creates the outer shape of the cell). The surface of the infusoria is covered with thin villi, or cilia, which are used for swimming and consist mainly of small tubular structures, called microtubules. Cytoskeleton (outer shell) is also formed by these microtubules, as well as protein-actin and intermediate fibers. Apparently, it is the effect of Si-NPs activated by laser radiation on the cytoskeleton of infusoria that can explain the observed suppression of their reproduction rate and, ultimately, death after a certain period of time after exposure.

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References
[1] Kabashin A V and Timoshenko V Yu 2016 What theranostic applications could ultra-pure laser-synthesized Si nanoparticles have in cancer? Nanomedicine 11 (17) 2247-2250
[2] Osminkina L A, Timoshenko V Yu 2016 Porous Silicon as a Sensitizer for Bio-medical Applications: Mini-review Mesoporous Biomaterials 3 39–48
[3] Gongalsky M B, Pereira A, Manankov A A, Fedorenko A A, Vasiliev A N, Soloviev V V, Kudryavtsev A A, Sentis M, Kabashin A V and Timoshenko V Yu 2016 Laser-synthesized oxide-passivated bright Si quantum dots for bioimaging Scientific Reports 6 24732
[4] Timoshenko V Yu 2014 Porous Silicon in Photodynamic and Photothermal Therapy (Springer Publ), ed L Canham (Switzerland: Handbook of Porous Silicon) pp 929–936
[5] Bezotosny V V, Bondarev V Yu, Krokhin O N, Mikaelyan G T, Oleschenko V A, Pevtsov V F, Popov Yu M, Cheshev E A 2009 Laser diodes emitting up to 25 W at 808 nm Quantum electronics 39 (3) 241
[6] Spivak Yu M, Bespalova K A, Belorus A O, Panevin A A, Somov P A, Grigoryeva N Yu, Chistyakova L V, Zhuravsky S G and Moshnikov V A 2017 The obtaining method and example of drug functionalization of porous silicon nanoparticles surface Clinical medicine 3(51) 69