Toxicity of laser irradiated photoactive fluoride PrF_3 nanoparticles toward bacteria

2014 J. Phys.: Conf. Ser. 560 012011

(http://iopscience.iop.org/1742-6596/560/1/012011)

Toxicity of laser irradiated photoactive fluoride PrF_3 nanoparticles toward bacteria

M S Pudovkin, S L Korableva, A O Krasheninnicova, A S Nizamutdinov, V V Semashko, P V Zelenihin, E. M. Alakshin, T A Nevzorova

Kazan Federal University, 420008, Kremlevskaja str., 18, Kazan, Russian Federation

E-mail: jaz7778@list.ru

Abstract. The article is devoted to exploration of biological effects of crystalline PrF_3 nanoparticles toward *Salmonella typhimurium* TA 98 bacteria under the laser irradiation. Obtained results show bactericidal activity of PrF_3 nanoparticles and optimal parameters of laser irradiation (power of laser irradiation, wavelength, diameter of the laser spoil, and exposure time) have been found under which the effects of bactericidal activity become the most significant. Survival of bacterial cells under laser irradiation with wavelength 532 nm in colloidal solution of PrF_3 nanoparticles was 39%, 34%, 20% for exposure times 5 minutes, 15 minutes and 30 minutes, correspondingly.

1. Introduction

Nanosized materials are used in different application of industry. Because of the unique physical and chemical properties of nanoparticles they can be used in a broad range of biological applications. Several last researches [1-4] have reported that photoactive nanoparticles could cause oxidative stress and they can be activated by UV and visible irradiation generating reactive oxygen species (ROS), such as hydroxyl radical $OH\cdot$ and superoxide anion O_2^-. In consequence, the photoactive nanoparticles can be applied in waste water treatments as bactericidal medium, in environmental remediation, organic contaminants mineralization, and cancer treatment [5, 6]. There are three traditional methods of cancer treatment: chemotherapy, surgery, and radiotherapy. Undoubtedly there are alternative methods of cancer treatment that can be used as independent methods or with traditional methods jointly. Therapy with photoactive nanoparticles is one of those alternative methods. The ROS, produced by photoactive nanoparticles, can damage cellular components like proteins and DNA and cause the death of treated cells. Selective oxidative stress is sometimes desirable and can be used therapeutically also. Semiconductor nanostructures TiO_2, ZnO with photocatalytic activity have already found a wide range of applications [1-4]**.** But these semiconductor nanostructures have relatively low photocatalytic activity. Besides, they could be activated by UV irradiation only. On the other hand there are very few reports devoted to biological effects of photoactive fluoride nanoparticles. In spite of this they are discussed as very promising nanosized materials for different biological applications because of their high absorption capacity in the wavelength range of 450 nm - 550 nm and high photoenergy conversion efficiency and they can be used in different biological applications, including cancer treatment [7]. Tests on procariotic sells is the first step of toxicity investigation according to traditional methodics.

The main aim of present investigation was to test hypothesis of toxicity of photoactive fluoride nanoparticles PrF_3 towards procariotic cells under laser irradiation.

2. Materials and methods

Team of Laboratory of Quantum Electronics and Radiospectroscopy named after S. A. Altshuller at Kazan Federal University has utilized a technology of synthesizing fluoride crystalline nanoparticles PrF_3 by chemical methods. The method is based on deposition from colloidal solution and hydrothermal reaction and is described in [8-12]. The HRTEM images of nanoparticles output are shown in the figure 1. The material used in present experiments consisted of crystalline nanoparticles with size about 30 nm. In the experiments we used CW lasers with wavelength 532 mn (green light) and 473 nm (blue light) with power 30 mW as activation irradiation sources. We have investigated toxicity of floride nanoparticles PrF_3 under the laser irradiation towards *S. typhimurium*. *S. typhimurium* TA 98 was cultivated in LB medium with 50 μg/ml ampicillin sodium for 18 h at 30^0C, then centrifuged (3000 g, 20 m) and the cell pellet was resuspended in physiological saline solution to the final concentration 10^6 cell/ml. 200 μl per well of bacterial cell suspension together with colloidal solution of PrF_3 nanoparticles was placed into white opaque 96-well microplates (PerkinElmer) and irradiated with lasers. Concentration of PrF_3 nanoparticles was kept at the level 0,1 mg/l in the well. Also control wells were kept without PrF_3 nanoparticles solution. After the irradiation cell suspension was diluted with physiological saline solution in 10^5 times and plated onto agarised LB medium with 50 μg/ml ampicillin sodium. After 2 days of incubation at 30^0C the number of colony-forming units was counted.

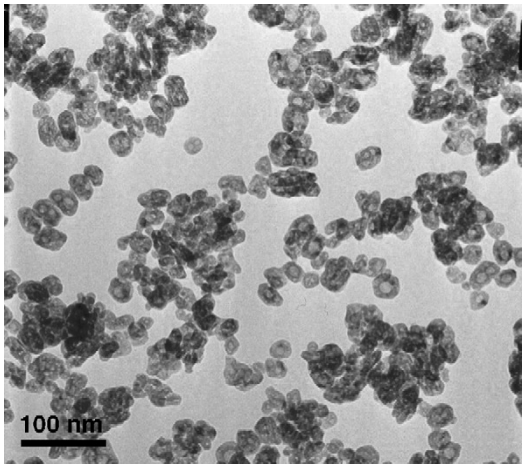

Figure 1. The HRTEM images of fluoride photoactive nanoparticles PrF_3 synthesized by means of hydrothermal reaction according to method described in [8].

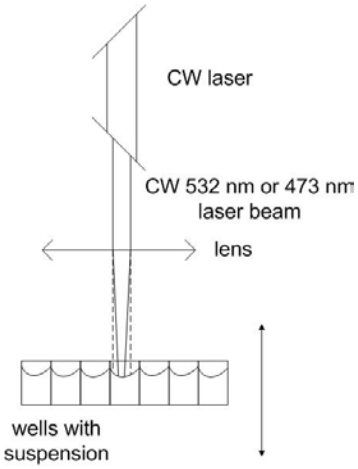

Figure 2. The experimental set up for photoactivated toxicity studies of PrF_3 nanoparticles.

The toxicity test consisted in exposure of the well with cells and nanoparticles to the laser radiation as it is illustrated at the experimental set-up scheme in the figure 2. We have made experiments for laser wavelengths 473 nm and 532 nm. Laser spot at the cell sample was formed by the lens with 7,5 cm focal length. We have investigated the influence of laser irradiation on the cells using three configuration of experimental set-up: well exposure under the laser irradiation without the lens (irradiation power density 637 mW/cm^2), the well in the visual focus of the lens (irradiation power density 1,3 W/cm^2), and the well behind the visual focus of the lens (irradiation power density 285 mW/cm^2). We have also investigated the influence of nanoparticles without the laser irradiation and the influence of laser irradiation without the nanoparticles.

3. Results and discussion

The first experiments at 473 nm and 532 nm wavelengths of irradiation have shown higher photoenergy conversion efficiency for the wells behind the visual focus of the lens under laser irradiation with wavelength 532 nm. Results of the toxicity tests have shown in Figure 3. Laser irradiation at 473 nm and 532 nm appears to be toxic itself with the higher effect of green light (survival index is 81 % for 473 nm light and 57 % for 532 nm light). Increasing the power density leads to increase of the toxic effect. Also the toxicity of PrF_3 nanoparticles is well pronounced. Presence of nanoparticles increases toxic effect in about 20 %. The toxic effect of irradiated nanoparticles showed some saturation as it slightly decreased with increasing the power density. The most significant toxic effect appeared to be for cases of 637 mW/cm2 of 532 nm laser light with 22 % of survival index and 285 mW/cm2 of 473 nm laser light with 62 % of survival index.

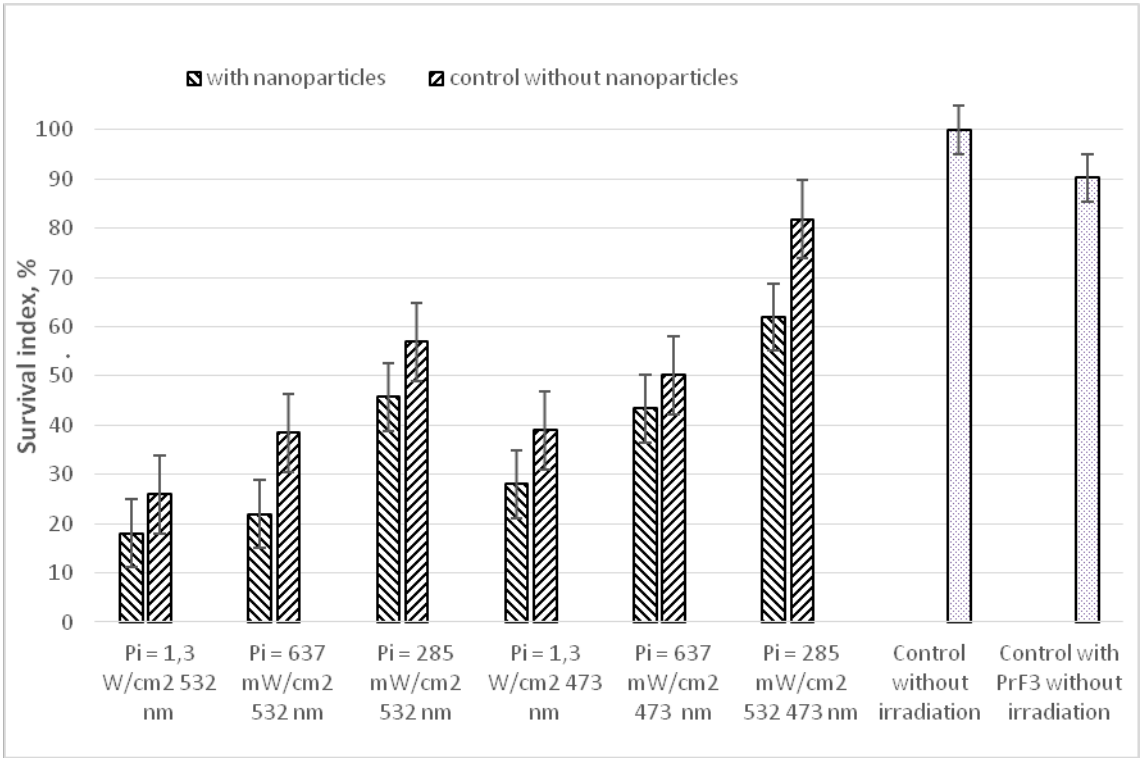

Figure 3. Survival of the *S. typhimurium* TA 98 cells with PrF_3 nanoparticles under various power density of irradiation.

In the second series of experiments we have investigated *S. typhimurium* TA 98 cells survival treated with nanoparticles at various exposure times of the laser irradiation. Three duration of exposure time: 5 minutes, 15 minutes, 30 minutes were chosen. It is seen from the diagram in Figure 4 that cells survival index decreases with increase of exposure. The number of CFU was 39%, 34%, 20% for exposure time 5 minutes, 15 minutes and 30 minutes compared with the control, correspondingly, under laser irradiation with wavelength 532 nm. The result of almost complete bacterial inactivation is apparently due to both effects of nanoparticles photoactivation and 532 nm irradiation itself. Behavior of survival for cells irradiated at wavelength 473 nm also speaks for bactericidal effect and appears to be exposure dependent but laser irradiation at this wavelength does not show good conversion efficiency. And treatment by PrF_3 nanoparticles increases the bactericidal effect.

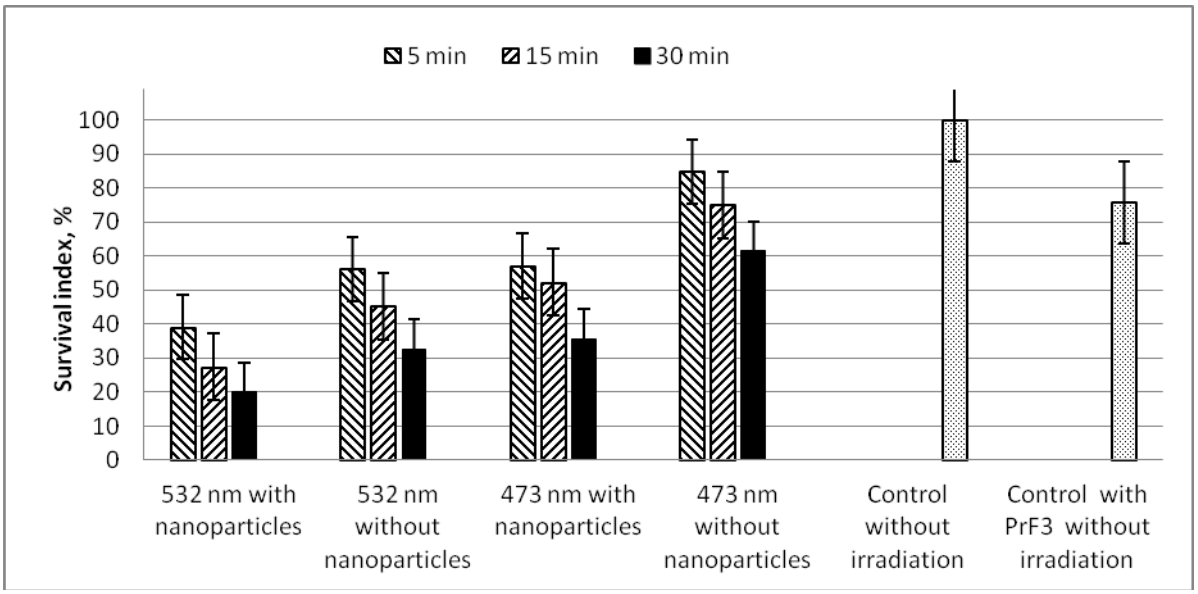

Figure 4. The influence of exposure time of laser irradiation at 532 nm and 473 nm on survival of *S. typhimurium* TA 98 cells treated with the PrF_3 nanoparticles for exposures 5, 15 and 30 min.

We have confirmed the hypothesis toxicity of photoactive fluoride nanoparticles PrF_3 toward *S. typhimurium* TA 98. These nanoparticles show photoactivated bactericidal activity of the same level as widely investigated materials on the basis of semiconductor nanoparticles (ZnO, TiO_2 [1-3,6]) which is about 18 % at 1,3 mW/cm^2 of 532 nm laser irradiation. Hypothetical mechanism of photoactivity could be also the same. It is known that H_2O inevitably appear at the surface and in the pores of fluoride nanoparticle prepared by hydrothermal method of growth [13-15]. When light is absorbed the photoexcited nanoparticle stores energy by charge separation and creating electron−hole pairs. These free charge carriers react with the adsorbed H_2O and thus formed hydroxyl radicals and holes (named as ROS) are responsible for bactericidal activity during irradiation of nanoparticle. Pure semiconductors usually exhibit low photoenergy conversion efficiency probably because of their relatively low charge separation efficiency and faster recombination of charge carriers [6]. It might be supposed that fluoride nanoparticles have the same mechanism of generating ROS. Laser-induced ROS production by photoactive nanoparticles may be one of the reasons for their toxicity. This effect is shown for titanium dioxide nanoparticles, which have bactericidal [16] and virucidal [17] activity because of ROS production induced by radiation. We suppose a similar mechanism of activation for PrF_3 nanoparticles.

4. Conclusion

In this work the photoactivated toxicity of crystalline PrF_3 nanoparticles towards *Salmonella typhimurium* TA 98 bacteria has been tested. The fluoride photoactive PrF_3 nanoparticles were synthesized in the laboratory by chemical method consisting in deposition from colloidal solution and hydrothermal reaction. The nanoparticles used were monocrystals with size of about 30 nm. We have confirmed the bactericidal effect of photoactive fluoride nanoparticles PrF_3 for *S. typhimurium* TA 98 under 532 nm laser light irradiation and it appeared to be significant (about 18 % of survival index) compared to semiconductor nanoparticles but the laser light have shown small bactericidal effect itself.

Acknowledgements
This work was funded by the subsidy allocated to Kazan Federal University for the project part of the state assignment in the sphere of scientific activities.

References

[1] Melissa F, José M, Amadeu M, Barata S 2014 *Science of the Total Environment* **470** 379-389
[2] Apalangya V, Rangari V, Tiimob B, Jeelani S 2014 *Applied Surface Science* **295** 108– 114
[3] Socorro O, Rodolfo Z, Blanca P 2013 *Journal of Hazardous Materials* **263** 28– 35
[4] Behnajady M, Hamed E 2013 *Chemical Engineering Journal* **228** 1207–1213
[5] Raghavendra A, Gunveen W, Niranjan D, Bonamali P 2013 *Environ Sci Pollut Res* **20** 6521–6530
[6] Weiwei H, Hyun-Kyung K, Wayne W, David M, John C, Jun-Jie Y 2014 *J. Am. Chem. Soc.* **136** 750−757
[7] Shizhu Chen, Cuimiao Zhang, Guang Jia, Jianlei Duan, ShuxiangWang, Jinchao Zhang, Size-dependent cytotoxicity of europium doped NaYF4 nanoparticles in endothelial cells 2014 *Materials Science and Engineering* **43** 330–342
[8] Lin Ma, Wei-Xiang Chen, Yi-Fan Zheng, Jie Zhao, Zhude Xu, Microwave-assisted hydrothermal synthesis and characterizations of PrF3 hollow nanoparticles 2007 *Materials Letters* **61** 2765–2768
[9] Alakshin E, Gabidullin B, Gubaidullin A, Klochkov A, Korableva S, Neklyudovac M, Sabitova A, Tagirov M 2011 *arXiv*.1104 0208
[10] Alakshin E M et al Spin kinetics of He-3 in contact with synthesized PrF3 nanoparticles 2011 *Journal of Low Temperature Physics* **162** 645-652
[11] Wang X, Li Y Rare-Earth-Compound Nanowires, Nanotubes, and Fullerene-Like Nanoparticles: Synthesis, Characterization, and Properties 2003 *Chemistry - A European Journal* **9 (22)** 5627-5635
[12] Alakshin E M et al Annealing of PrF3 Nanoparticles by Microwave Irradiation 2014 *Optics and Spectroscopy* **116** 721 -723
[13] Qibing Chang, Jian-er Zhou, Yongqing Wang, Guangyao Meng 2010 *Advanced Powder Technology* **21** 425–430
[14] Popov A.V., Orlovskii Yu.V., Vanetsev A.S., Gaitko O.M., Orlovskaya E.O., Sildos I. 2014 *Journal of Luminescence* **145** 774–778
[15] Alakshin E M, Blokhin D S , A M Sabitova, A V Klochkov, V V Klochkov, K Kono, Korableva S L , Tagirov M S 2012 *JETP Letters* **96** 181-183
[16] Melian J.A. et al The photocatalytic disinfection of urba wastewaters 2000 *Chemosphere* **41** 323–327
[17] Watts R J et al Photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent 1995 *Water Res* **29** 95–100