Phototoxicity and luminescence of the upconversion nanoparticles embedded in the cells

I.Yu. Yanina,[*1,2] E.A. Sagaidachnaya,[1] I. V. Vidyasheva,[3] N. A. Navolokin,[4]
V.I. Kochubey,[1,2] V. V. Tuchin[1,2,5]

[1] Department of Optics and Biophotonics, Saratov State University (National Research), Russian Federation
[2] Interdisciplinary Laboratory of Biophotonics, Tomsk State University (National Research), Russian Federation
[3]Education and Research Institution of Nanostructures and Biosystems, Saratov State University (National Research), Saratov, Russia
[4] Department of Pathological Anatomy, Saratov State Medical University, Saratov, Russia
[5]Institute of Precision Mechanics and Control RAS, Russian Federation

[*]e-mail: irina-yanina@list.ru

Abstract

The present work demonstrates the visualization of the intracellular distribution of upconversion nanoparticles (UCNPs) by microscopy with excitation in the NIR spectral range and detection of upconversion luminescence in the VIS range. The cell viability is scored for cytotoxic effects of UCNPs at dark and light exposed conditions. Non-functionalized UCNPs incubated with the cells are found to be endocytosed by cells. The obtained results confirm a high sensitivity of the luminescent UCNPs to the concentration variations within cells. UCNPs are promising alternatives to traditional fluorescent labels for cell imaging and possess prominent potentials in biological and clinical applications.

Keyword list: upconversion nanoparticles, cell culture, luminescence, phototoxicity

1. INTRODUCTION

Cancer is the second leading cause of death, after heart diseases.[1] It is well known that early detection of precancerous lesions can dramatically decrease morbidity and mortality.[2] So, there is an urgent need for new diagnostic tools in order to aid in early cancer detection. More than 85% of all cancers begin as precancerous lesions that are confined to the surface epithelium, which can be as thick as 500 μm in human tissue.[3]

One of the main methods of treatment of most tumors is their surgical removal, but there is a risk that the body will remain part of the transformed cells, which can later cause recurrence of the disease. Other traditional methods of treatment are radiation and chemotherapy, but some types of tumors for not always clear reasons, are resistant to these types of effects. Even after successful treatment cycles, often, the tumor does not disappear completely, becomes resistant to therapy, or “goes” into a state of rest, to return to a more aggressive form later. Traditional therapy has many undesirable side effects, the main of which is the undirected effect of drugs, as a result of which normal cells of the body are affected. An alternative direction in the development of anticancer drugs is targeted therapy, which should be effective only in tumor cells. The focus of therapy can be achieved by methods of genetic engineering, for example, by acting on defective genes that define "signs of a tumor," or by using monoclonal antibodies specific for markers characteristic of a given tumor.

Cell cultures were and remain the simplest *in vitro* technical models of tumors.

A recent study has demonstrated that morphological and fluorescence quantification from two-photon imaging, for example, of endogenous fluorophores can be used to distinguish cancerous and precancerous from normal tissue down to 40 μm deep.[4]

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By attaching a fluorescent contrast agent to a nonfluorescent target of interest, imaging also has the ability to monitor a variety of additional biomolecular signatures that are more strongly indicative of cancer. The promise of this technique has prompted researchers to investigate the use of traditional and engineered organic fluorophores[5] as well as new classes of luminescent contrast agents such as quantum dots[6] and metallic nanoparticles.[7-10] Though fluorescent semiconductor quantum dots offer much larger photon action cross-sections than organic fluorophores (>10000 GM compared to 1−300 GM for organic fluorophores) (1 GM = 10^{-50} cm^4 s $photon^{-1}$),[11] the most well-studied quantum dots have heavy metals that are cytotoxic, making them unsuitable for in vivo clinical applications. Gold nanoparticles, on the other hand, are biocompatible[12] and can have large photon action cross-sections (larger than 2000 GM for gold nanorods).[9]

However, in recent years, a new class of nanoscale luminophores is studying such as upconverting luminescent NPs. Rare earth doped upconversion nanoparticles, which can convert long wavelength near infrared radiation into short wavelength visible radiation via a non-linear optical process, are emerging as a new class of fluorescent biolables. The unique photoluminescence properties of upconversion nanoparticles (UCNPs) induced by NIR benefit bioimaging via enhanced image contrast, due to absence of tissue autofluorescence, and enhanced photostability, which enables prolonged imaging even on the single nanoparticle level.[13-15] In this way, it therefore allows for deep tissue penetration, making them attractive as promising contrast agents for biological sensing, biomedical imaging, and disease theranostics.[16]

The goal of the present study is the application the luminescent UCNPs ($NaYF_4$:Yb^{3+}, Er^{3+}) for visualization of the intracellular distribution of UCNPs by microscopy with excitation in the NIR spectral range and detection of upconversion luminescence in the VIS range.

2. EXPERIMENTAL PROCEDURES

We used the in-house synthesized upconversion NPs $NaYF_4$: Yb^{3+}, Er^{3+} (fluoride matrix doped with ions of ytterbium and erbium), uncoated and coated by SiO_2 shell. UCNP concentrations were 1, 5, and 10 mg/ml.

The UCNPs (ca. 220 nm in size, see Figure 1 (a)) were synthesized by a hydrothermal method. Field emission scanning electron microscope (MIRA 2 LMU, TESCAN) was used to obtain images of particles.

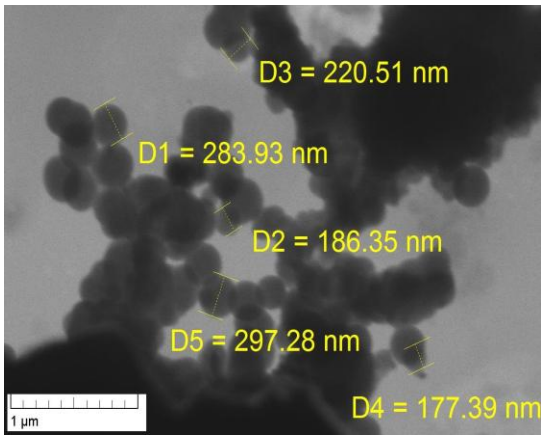

Figure 1. SEM (MIRA 2 LMU, TESCAN) image of the [$NaYF_4$: Er^{3+}, Yb^{3+}] UCNPs

Figure 2 shows a schematic diagram of the experimental setup.

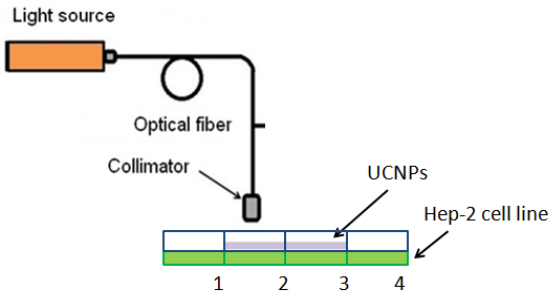

Figure 2. Experimental setup

UCNP luminescence was excited by a diode laser (980 nm, 0.5 W/cm^2) (LSR980NL-1000, Lasever, China).

Hep-2 (HeLa derivative) cell line human was used in passages 2–6. All the cells were plated separately in tissue culture flasks and cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin–streptomycin antibiotic antifungal cocktail of 2 mM L-glutamine and containing 10% fetal bovine serum, in a humidified incubator containing 5% CO_2 at 37 °C. The media were replaced every 2 days. Cell cultures with 75–85% confluence were harvested using 0.25% trypsin and counted with a hemocytometer.

Cells were put in 48 plates at the density 4*10^4 cells per well. Next day the UCNPs were added in concentration 1, 5 and 10 mg/ml NPs in four repetitionswells. Fresh medium was added to each of wells. Subsequently, cells were incubated (Innova CO-170, New Brunswick Scientific) at 37 °C overnight, together with the added UCNPs. At the last step, to each well fluorescence dye 10% of the total volume of the medium in the wellwas added and intensity was measured by spectrophotometer (Gemini XPS Microplate Reader, Molecular Devices). The experiment showed the ability of metabolically active cells to convert the Alamar Blue reagent into a fluorescent and colorimetric indicator.[17] In the experiment with irradiation we used concentration 10 mg/ml and the dye was added immediately after irradiation.

Hep-2 cell was obtained from Biolot. Dulbecco's Modified Eagle Medium (DMEM), 1% penicillin–streptomycin antibiotic antifungal cocktail, 2 mM L-glutamine, trypsin, tetramethylrhodamine (TRITC), dispase, 2%, Alamar Blue cell viability reagent were purchased from Sigma-Aldrich. 10% fetal bovine serum (FBS) was obtained from Hyclone.

3. EXPERIMENTAL RESULTS AND DISCUSSION

In this experiments we show the absence of toxicity of our UCNPs using qualitative (proliferation, adhesion and morphology of cell line) and quantitative (Alamar blue assay) methods (Fig. 3). Hep-2 cells with NPs at 1, 5 and 10 mg/ml culturing has a well-spread morphology typical of attached cells, with the morphology typical of spreading Hep-2 cell line. The quantity assay related to percent of survived cells is presented in Figure 3. The concentration 1, 5 and 10 mg/ml is not statistically different from the control one.

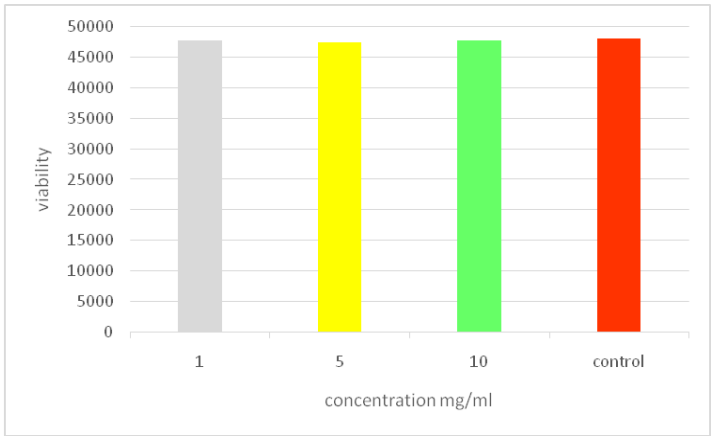

Figure 3. UCNP cytotoxicity test for Hep-2 Cell Line

In experiments Hep-2 cells with UCNPs and after irradiation we show the absence of toxicity of irradiation on Hep-2 cells line using Alamar blue assay methods (Fig. 4). The concentration 10 mg/ml with irradiation is not statistically different from the Hep-2 with irradiation and Hep-2 without irradiation.

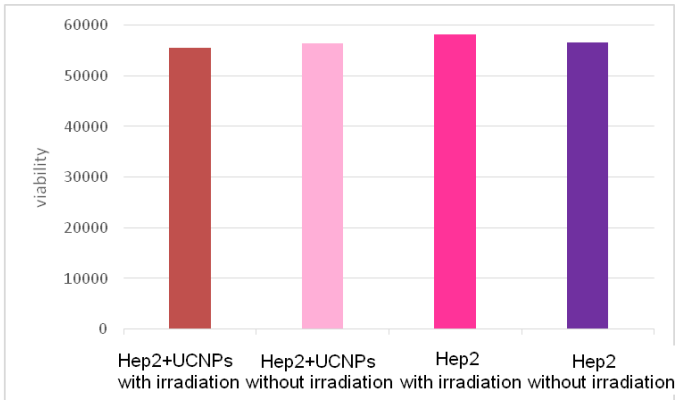

Figure 4. UCNP cytotoxicity test for Hep-2 Cell Line after NIR irradiation

The control (only the cell line without adding NPs) corresponds to the morphology of the Hep-2 line, polymorphism is extremely rare, and the monolayer is dense. When cells without NPs are irradiated, there are observed detached separate cells (round shape) in the sample. Necrotic zones and signs of apoptosis are not observed, which indicates less sensitivity to cell radiation without NPs (see Table 1).

In samples with UCNPs (but without irradiation) the cell line is denser. There are no apoptotic bodies. The cell morphology corresponds to this cell line, although polymorphism is often observed. The monolayer is not broken. Cytotoxic zones are absent. There are single detached cells (dead cells).

In the samples with UCNPs and after irradiation zones with a pronounced cytotoxic (necrotic) effect were observed. This is due, most likely, to the sensitivity of cells to local hyperthermia (due to asymmetric distribution / subsidence of particles on the surface of the cell layer). Mass cell death caused by necrotic damage is accompanied by cell lysis, due to membrane damage. Also in the cytoplasm of Hep-2 cells after irradiation, granular structures similar to apoptotic bodies were identified. In these samples one can observe morphological signs of apoptosis: a change in cell contours and fragmentation with the formation of apoptotic bodies [see Fig.5 (b)]. Samples contain polymorphic cells of an atypical form: cells with a round or oval shape. Intercellular contact loss is observed. Cells without staining of the cytoplasm are present. More zones of lysis were observed in samples with UCNPs coated SiO_2.

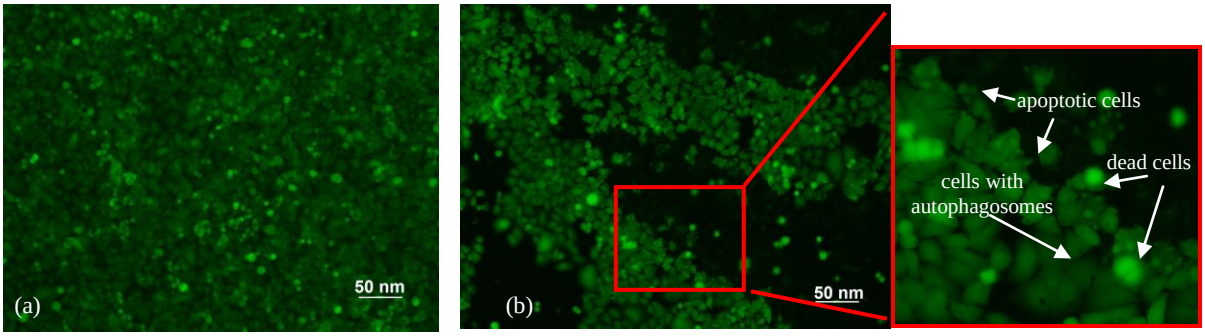

Figure 5. (a) Hep-2 cells at NIR irradiation; (b) Hep-2 cells labeled by UCNPs coated by SiO2 at NIR irradiation

Table 1. Effect of UCNPs without and with irradiation on Hep-2 Cell Culture

Group	Without irradiation			With irradiation		
	Control	Coated UCNPs	Uncoated UCNPs	Control	Coated UCNPs	Uncoated UCNPs
Average number of cells in the field of view	1000±35	1000±35	1000±35	1000±35	466±65	800±25
dead cells, %	8.4±2	4.9±2.4	11.5± 9.2	8.5+3	12.45±1.6	10±3
apoptotic cells, %	0	0	0	0	19.3±2.5	0
cells with autophagosomes from the living cell, %	0	1.5±0.5	2.15±0.6	5.1±2	2.15±0.7	3.125±0.6
swelling cells, %	0	2±0.3	1.6±0.6	0	1.72±0.9	2.25±0.6
living cells, %	91.6±4	91.6±2.1	84.75±12.4	86.4±5	64.38±2.4	84.625±5

According to results *in vitro* on Hep-2 cell culture, coated UCNPs with irradiation showed the highest activity, which caused more than other cell death compared to control, but also activated apoptosis into the cell (pycnosis stage). In addition, the number of cells with autophagosomes decreased with these particles, which indicates blocking of resistance in tumor cells.[18,19]

Uncoated UCNPs did not show pronounced cytostatic and cytotoxic activity, also inactivate apoptosis in cells, but it is showed the ability to block the development of autophagy in cells and most of all this ability manifested itself in coated UCNPs, but without irradiation.

Thus, according to preliminary data in experiments *in vitro* coated UCNPs are the most promising particles for the development of cancer treatment methods.

4. CONCLUSIONS

It has been shown the possibility of applying of the luminescent UCNPs [$NaYF_4$:Yb^{3+}, Er^{3+}] for visualization of the intracellular distribution of UCNPs by microscopy with excitation in the NIR spectral range and detection of upconversion luminescence in the VIS range.

The cell viability is scored for cytotoxic effects of UCNPs at dark and light exposed (to demonstrate the direct phototoxic effects) conditions. Non-functionalized UCNPs (ca. 220 nm in size) incubated with the cells are found to be endocytosed by cells.

The obtained results confirm a high activety of the luminescent coated UCNPs to the cancer cells Hep-2 and show a potential for the theranostics, i.e. for visualization and controlled thermal effects in pathological cells. The particles showed cytotoxic, cytostatic properties. Due to the activation of the photodynamic reaction, apoptosis is triggered in

tumor cells and preventing resistance in tumor cells by blocking autophagy. This makes these types of NPs the most promising for studying in experimental oncology. Therefore, UCNPs are promising alternatives to traditional fluorescent labels for cell imaging and possess prominent potentials in biological and clinical applications.

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