

The Assessment of Photo-Induced Toxicity of [NaYF₄:Yb³⁺, Er³⁺] Upconversion Nanoparticles on Model Normal and Cancer Cell Lines *in Vitro*

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Abstract. Upconversion nanoparticles (UCNPs) are promising alternatives to traditional fluorescent labels for cell imaging that have outstanding potential in biological and clinical applications. As we previously established, the co-incubation of synthesized in-house [NaYF₄:Yb³⁺, Er³⁺] UCNPs uncoated and coated by SiO₂ shell with the cell lines resulted in particles' uptake. Because of the tendency to particle internalization by cells, the assessment of the biocompatibility of UCNPs intended for biomedical applications and their ability to induce desirable biological effects under the appropriate wavelength irradiation is an important step in UCNPs development. The present work is focused on the assessment of [NaYF₄:Yb³⁺, Er³⁺] UCNPs' phototoxicity and changes in human lung-derived embryonic fibroblast (FLEH-104) and human laryngeal epidermoid carcinoma (Hep-2) cell lines' morphology in response to the influence of UCNPs. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: cell lines; upconversion nanoparticles; luminescence; phototoxicity; laser; NIR.

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1 Introduction

Cancer is the leading cause of mortality worldwide and a significant factor hindering life expectancy growth. According to the World Health Organization statistics from 2019, cancer ranks as the primary or secondary contributor to mortality under the age of 70 in 112 out of 183 countries [1, 2]. Therefore, the development of new methods for cancer diagnostics and therapy at early stages and the amendment of currently existing ones are top priorities for modern medicine.

Currently, conventional surgery remains one of the most commonly used approaches for tumor removal. However, tumor surgery is accompanied by a substantial risk of malignant cells remaining in the surgery site, which can later result in the disease recurrence [3]. Radiotherapy and chemotherapy are also commonly used approaches, however, the resistance of some tumors to these types of therapy can also result in the treatment failure or even disease relapsing [4, 5]. Besides, conventional approaches to cancer therapy possess multiple undesirable side effects including off-target

toxicity of chemotherapeutic agents affecting healthy organs, tissues, and cells. Targeted therapy, which aims to affect only tumor cells, represents an alternative direction in the development of anticancer remedies [6–10]. The application of monoclonal antibodies targeting tumor-specific antigens [11], and oligonucleotide aptamers targeting different tumor-specific structures including genes [12, 13] are illustrative examples of this therapy realm.

The early-stage cancer diagnostics is crucial for therapy success and facilitates the chances of long-term patient survival. The usage of optical methods for cancer diagnostics deserves close attention. For the last two decades, label-free optical methods have been capturing the interest of researchers [14–16]. Differences in fluorescence level of endogenous fluorophores and morphology of normal and malignant cells are pillars of label-free approaches. Whereas exogenous fluorophores' usage for cancer cell labeling is a more conventional approach and, currently, is widely used in clinic [17–19]. The selective labeling of cancer-specific structures within malignant cells or on their surface by fluorophores, conjugated with molecules providing cancer-targeting, is the grounds of this technique [8–9, 20, 21].

The successful experience of selective fluorescent labeling application in clinic encourages researchers to search for new natural fluorophores and develop new synthetic fluorophores and nanostructures possessing fluorescent properties [22–28].

Many types of luminophores are currently used in bioimaging (e.g. quantum dots, fluorescent proteins, organic dyes, dye-doped silica nanoparticles, metallic nanoparticles). However, the use of organic probes for luminescence imaging of living organisms has some limitations. Excitation of traditional bio-labels usually requires UV or VIS light, which results in low signal-to-noise ratio due to high biological autofluorescence, low light penetration depth and possible cell photodamage. Therefore, it is desirable to use fluorescent bio-labels that can be excited by near-infrared light. Compared with organic dyes and semiconductor quantum dots, upconversion nanoparticles have attractive chemical and optical properties, such as low toxicity, sharp emission bandwidths, large anti-Stokes shifts and high resistance to photobleaching and photoblinking. NIR light can non-invasively penetrate deep into living organisms because the excitation wavelength is within the optical transparency window of tissues (700–1000 nm).

From the viewpoint of the elaboration of exogenous fluorescence labels, nanomaterials have attracted great interest due to their unique physical and chemical properties. So, fluorescent semiconductor quantum dots possess larger photon action areas than organic fluorophores (>1,000 GM compared to 1,300 GM for organic fluorophores) ($1 \text{ GM} = 10^{-50} \text{ cm}^4 \text{ s photon}^{-1}$) [29]. However, quantum dots mostly comprise heavy metals characterized by substantial toxicity [30] which significantly obstruct their introduction to clinical practice. Gold nanoparticles also have a large photon

action area (greater than 2000 GM for gold nanorods), however, in contrast to quantum dots, they are biocompatible, which is crucial for their biomedical applications [27, 31]. The other promising class of nanoscale luminophores is rare-earth-doped upconversion nanoparticles (UCNPs) capable of converting longer wavelength near-infrared radiation into visible one via a nonlinear optical process [32]. The unique NIR-induced photoluminescence properties of UCNPs benefit bioimaging through the image contrast improvement via avoiding tissue autofluorescence, and through the increased labels' photostability, ensuring prolonged imaging even at the single nanoparticle level [16–21]. These make UCNPs attractive for application in biological sensing, biomedical imaging, and disease theranostics [33–38].

Thus, UCNPs are promising alternatives to traditional fluorescent biolabels for cell imaging and have outstanding potential in biological and clinical applications such as photodynamic therapy (PDT). In this therapy, reactive oxygen species (ROS) are used as a lethal factor. In the traditional case of PDT, a photosensitizer is activated by visible light to generate ROS; as described above, this type of light has limitations in tissue penetration. Materials with upconverting properties could be used to activate the photosensitizer directly in the tumor environment. UCNPs excited by NIR light transfer the absorbed energy to photosensitizer molecules and cause the generation of ROS. The most efficient host material for 980 nm upconversion is the hexagonal phase NaYF_4 , often doped with rare earth ions.

Mostly UCNPs are hydrophobic and their surface modification can provide the improvement of particles' dispersibility in the aqueous biological media. Charged or polar moieties such as amphiphilic (co)polymers, lipids and silica are therefore attached to the particle surface. The silica coating imparts many useful properties to the nanoparticles, including the capability for their additional functionalization and biocompatibility [44]. The implementation of a silica shell is based on its unique properties, including high thermal and chemical stability, optical transparency, and easy control of the coating shell. In addition, the biocompatibility of the SiO_2 shell and its further fictionalization with versatile organic and inorganic compounds are crucial for its biological applications as well as for enabling nanoparticle dispersion in polar and non-polar environments. By coating the $\text{NaYF}_4:\text{Yb}, \text{Tm}$ NCs with a uniform SiO_2 , the hydrophobic UCNs become water dispersible [45].

The surface modification of the nanoparticles by silica results in an increase in photoluminescence intensity of about 9% compared to the uncoated sample of $\text{NaYF}_4:(\text{Yb}^{3+}, \text{Er}^{3+})$ phosphor. This increase in photoluminescence intensity can be explained by the suppression of non-radiative recombination of electron-hole pairs via surface defects [46].

Furthermore, the use of SiO_2 for the external coating of lanthanide-doped UCNPs is attractive because silica is known to be relatively harmless when used in biological

systems. The nanomaterials are relatively non-toxic even at relatively high concentrations (50 mg/ml) for 48 h incubation in HeLa cells [47]. Also, the dependence of UNPs' cytotoxicity on their coating type was comprehensively considered previously in Ref [48].

Previously, we have demonstrated the possibility of Hep-2 cells labeling with uncoated and coated with SiO₂ shell [NaYF₄:Yb³⁺, Er³⁺] UCNP*s in vitro* and estimated their phototoxicity [49]. SiO₂-coated nanoparticles were found more toxic under NIR irradiation than uncoated ones and capable of inducing cell apoptosis (pycnosis stage). Also, we found a decrease in the number of cells containing autophagosomes, which can probably point to the blocking of resistance in tumor cells. Uncoated UCNP*s* did not substantially decrease Hep-2 cells' viability, however, the same effect of autophagy blockage as for covered nanoparticles was found. Therefore, our preliminary data point to the prospective of the elaborated SiO₂-coated UCNP*s* for application in cancer therapy.

Here, we compared the photocytotoxic effect of [NaYF₄:Yb³⁺, Er³⁺] UCNP*s* on human lung-derived

embryonic fibroblast (FLEH-104) and human laryngeal epidermoid carcinoma (Hep-2) cell lines under the NIR irradiation. Also, we assessed the effect of both types of nanoparticles in the presence of NIR irradiation on the morphology of normal and cancer cell lines.

2 Experimental Section

2.1 UCNP*s* Synthesis and Characterization

Uncoated and coated with SiO₂ shell [NaYF₄:Yb³⁺, Er³⁺] (fluoride matrix doped with ytterbium and erbium ions) UCNP*s* were synthesized by a hydrothermal method as it was described previously in Ref. [50]. The final UCNP concentration was 10 mg/mL. UCNP images (Fig. 1(a)) obtained using the field-emission scanning electron microscope (SEM)MIRA 2 LMU(TESCAN, Czech) were used for particles morphology and size assessment. The average size of UCNP*s* was measured using ImageJ software.

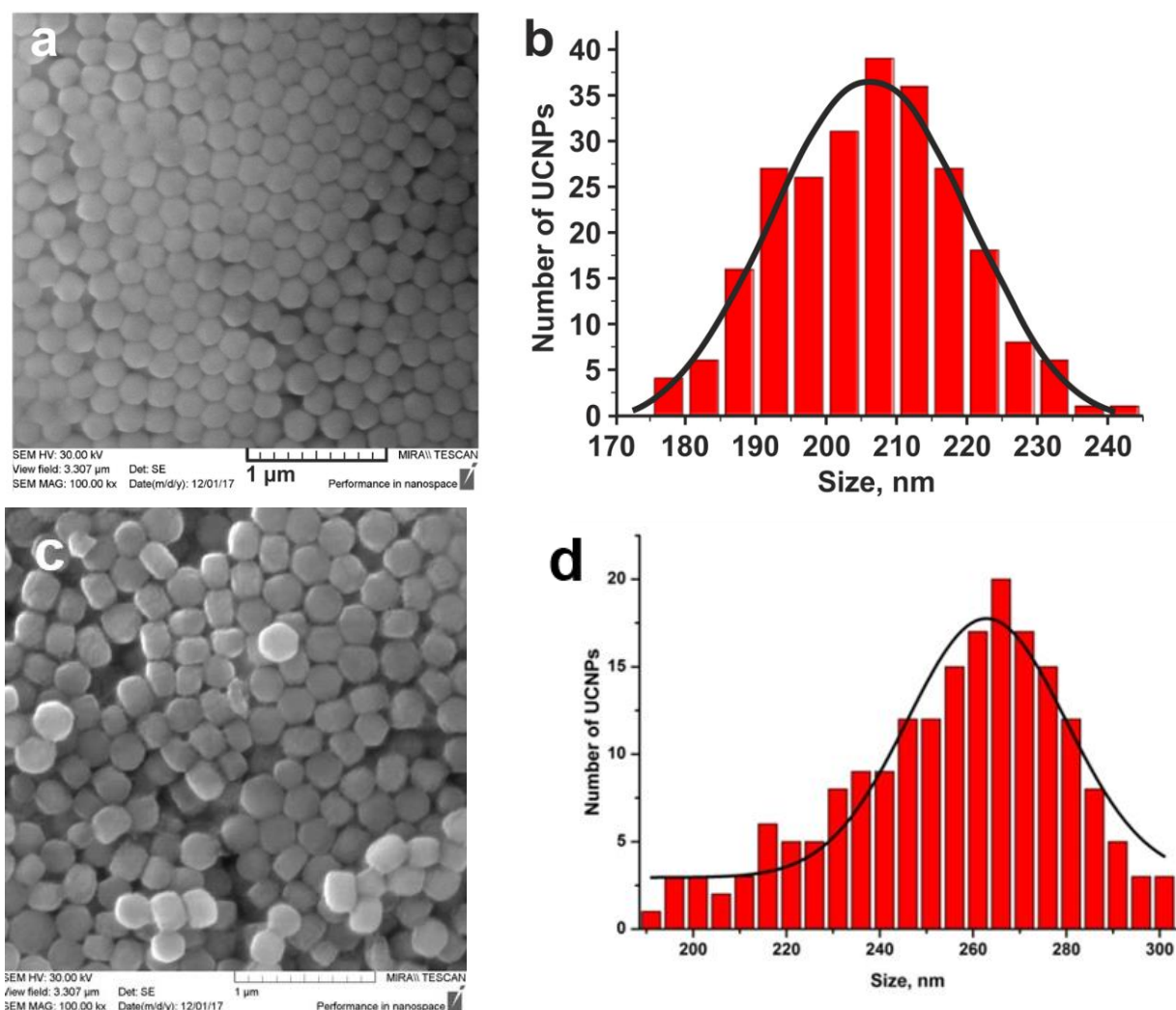


Fig. 1 (a) SEM image of the uncoated [NaYF₄:Yb³⁺, Er³⁺] UCNP*s*, (b) size distribution of uncoated UCNP*s*, (c) SEM image of the coated [NaYF₄:Yb³⁺, Er³⁺] UCNP*s*, (d) size distribution of coated UCNP*s*.

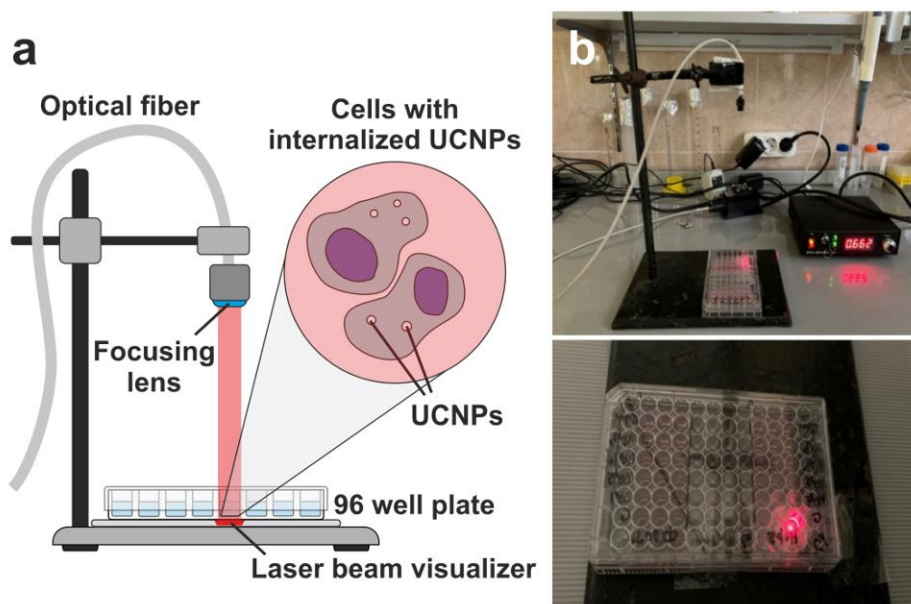


Fig. 2 (a) Scheme of the experimental setup and (b) photos of the setup with the 96 well plate under NIR light exposure.

2.2 Cell Cultures

Hep-2 and FLEH-104 cell lines were provided by the Department of Cell Engineering, Education and Research Institute of Nanostructures and Biosystems, Saratov State University, Russia. All cell lines were plated separately in tissue culture flasks and grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin-streptomycin antibiotic antifungal cocktail, 2 mM of L-glutamine, and 10% fetal bovine serum (FBS), in the humidified atmosphere of 5% CO₂ in air at 37 °C. The media were replaced every three days. Cell cultures with 75–85% confluence were harvested using 0.25% trypsin and counted using a Countess automated cell counter (Thermo Fisher Scientific, USA).

2.3 Experimental Procedures

The scheme and photo of the experimental setup used for cells exposure are presented in Fig. 2(a, b). The cells were plated in a 96-well plate with a transparent bottom at 10⁴ cells per well density and incubated overnight. After the incubation, uncoated and coated UCNP were added to cells at a concentration of 10 mg/mL and incubated for 24 h. Then, each well except the control ones was irradiated by the 980 nm light source (0.5 W/cm² power density). The cell culture media was replaced by Dulbecco's Phosphate Buffered Saline (DPBS) for the duration of the light exposure. The 980 nm diode laser (laser's power: 108 mW) (LSR980NL-1000, Lasever, China) equipped with 600 μm optic fiber was used for UCNP excitation. The exposure time varied in the range from 5 to 45 min. IR imager IRISYS 4010 (InfraRed Integrated System Ltd, UK) located at a distance of 30 cm from the sample was used for non-contact measurement of the samples' surface temperature. The temperature of the cages did not increase during the experiment. Finally, cell viability was

evaluated using resazurin-based Alamar Blue cell viability reagent. For this dye was added to each well at a concentration of 10% v/v and incubated for 4 h. The fluorescence intensity (Ex/Em: 560/590 nm) of the reduced form of dye (resorufin) was measured using the Synergy H1 multimode reader (BioTekInstruments, Inc., USA).

Acridine orange dye was used for the cell morphology assessment. Cells were stained by 400 nM final concentration dye solution for 10 min, then triply washed with PBS and investigated using the inverted IX 73 fluorescent microscope (Olympus America Inc., USA).

3 Results and Discussion

Synthesized [NaYF₄:Yb³⁺, Er³⁺] UCNP possessed hexagonal-like shape with the average size 205 ± 10 nm (Fig. 1(a)). The size distribution of synthesized UCNP is presented in Fig. 1(b). The synthesized particles are characterized by a narrow size distribution, indicating their monodispersity. UCNP coated with a layer of SiO₂ (Fig. 1(c)) have a wider size distribution due to the heterogeneity of the shell thickness. The size of the coated UCNP is 262 ± 21 nm.

Cells exposure with NIR light was performed using the setup depicted in Fig. 2(a, b). The laser beam size was adjusted to cover only a single well of the 96-well plate to prevent the overexposure of cells in the adjacent wells by direct laser light. It enabled us to control the exposure time for all experimental groups. The guidance of the NIR laser spot on the well center was performed using the laser beam visualizer fixed under the bottom of the transparent 96-well plate.

The assessment of the metabolic activity of model cell lines after their 24 h co-incubation with elaborated uncoated/coated UCNP without NIR exposure did not reveal a statistically significant decrease in cell viability.

The irradiation of cells after the period of co-incubation with all types of particles, in turn, negatively affected the cell viability (Fig. 3). So, an increase in the irradiation duration resulted in the mitigation of cell viability. Also, we found that the susceptibility of the Hep-2 cell line to the uncoated/coated UCNPs action under the NIR exposure was lower than for FLEH-104. The probable reason for this is the difference in cell line morphology. Fibroblasts are characterized by larger surface area if compared with epidermoid carcinoma cells. Therefore, the probability of malignant cell interaction with added uncoated/coated UCNPs is lower if compared with such for fibroblasts, which may be a reason for lower particle internalization efficiency. The laser irradiation dose obtained for 5 min did not induce a decrease in cells' metabolic activity for both cell lines, while the cells' irradiation for 10 min caused a statistically significant decrease in their viability.

Fig. 4 shows images of Hep-2 and FLEH-104 cell cultures after different types of exposure under fluorescence microscopy, where living cells were stained with acridine orange. Autophagy and apoptosis are observed in some cases.

In control samples of Hep-2, cells form contacts and connections. The cells are round or polygonal. They vary

widely in size. The nucleus is rounded. Nucleoli are present. Mitotic figures are present. There are no signs of apoptosis and individual autophagosomes are seen in the cytoplasm (Fig. 4(a)). When uncoated with SiO₂ shell UCNPs are added to the cells, a decrease in cell density is observed. However, the cells remain in contact.

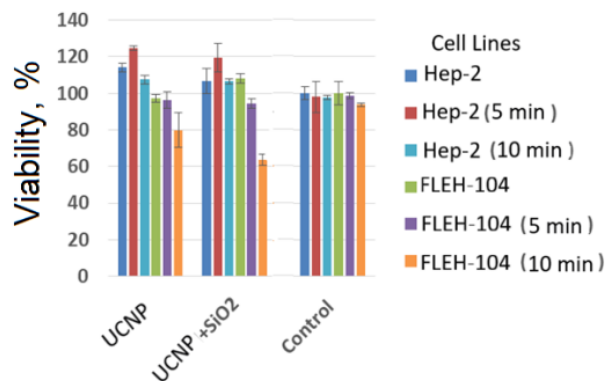


Fig. 3 The viability of Hep-2 and FLEH-104 cell lines after 24 h of incubation with uncoated/coated UCNPs at 10 mg/mL particle concentration, depending on the particles' type and the exposure time (5 and 10 min).

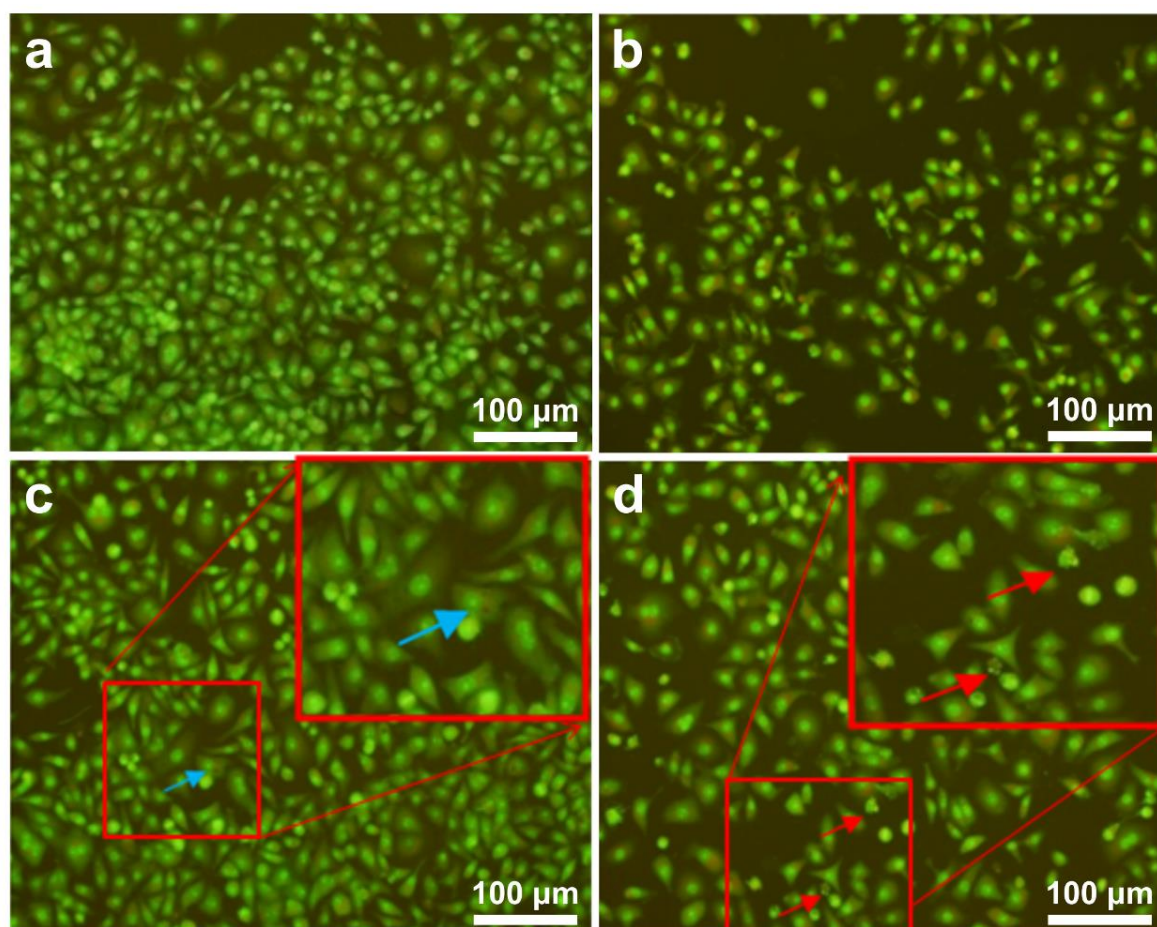


Fig. 4 Fluorescent images of Hep-2 cells stained with acridine orange: (a) control – cells incubated in the absence of uncoated with SiO₂ shell UCNPs, (b–d) cells incubated with uncoated UCNPs for 24 h and exposed by NIR-irradiation for (b) 5 min, (c) 15 min, and (d) 45 min. Cells containing autophagosomes are marked by the blue arrows; apoptotic cells are indicated by red arrows.

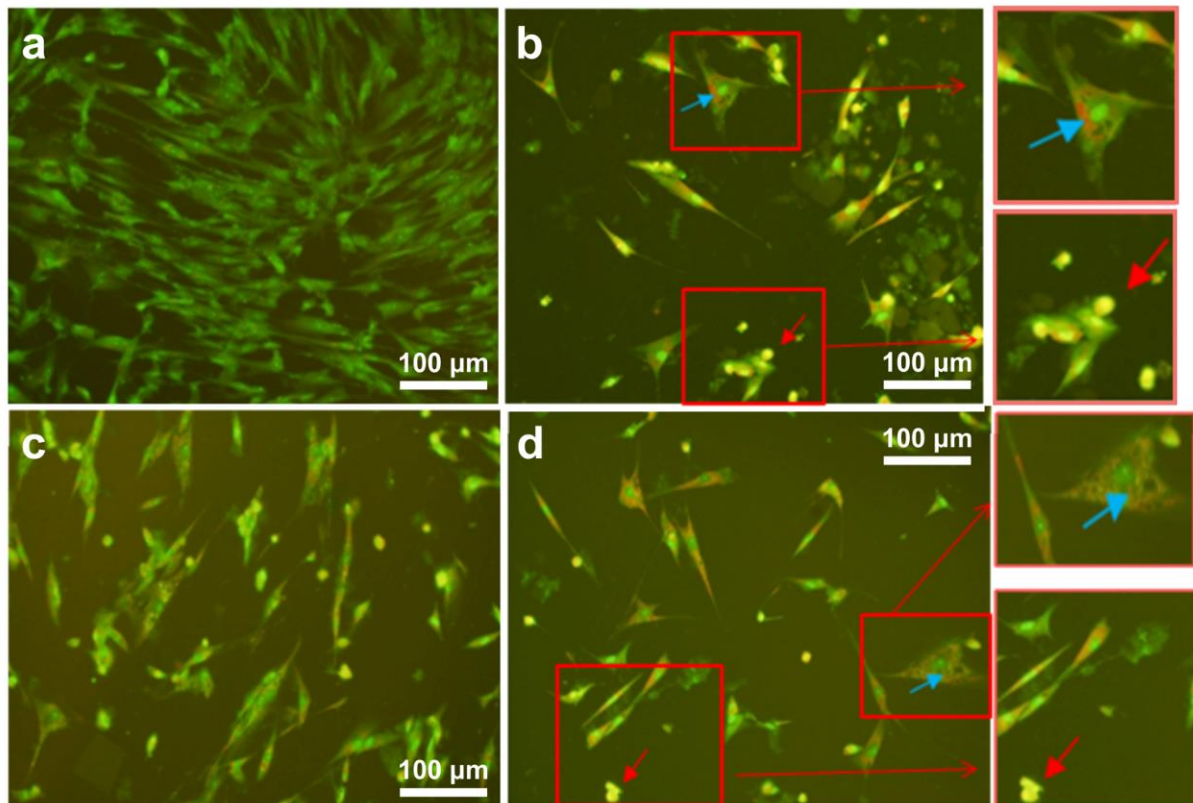


Fig. 5 Fluorescent images of FLEH-104 cells stained with acridine orange: (a) control – cells incubated in the absence of uncoated with SiO₂ shell UCNPs, (b–d) cells incubated with uncoated UCNPs for 24 h and exposed by NIR-irradiation for (b) 5 min, (c) 15 min, and (d) 45 min. Cells containing autophagosomes are marked by the blue arrows; apoptotic cells are indicated by red arrows.

A single pycnosis of the nuclei is observed. Apoptotic bodies are also detected. And in some cells there are single microautophages. The cell density is greatly reduced when irradiated for 5 min. Cells are more scattered. All nuclei are pyknotic and condensed. Many lysosomes appear, but autophagosomes are absent. However, despite the pronounced pycnosis, there are figures of mitosis, activation of proliferative activity, which explains the higher survival rate of this culture according to the previous test at 5 minutes of irradiation (Fig. 4(b)). After 15 min of irradiation, the cells maintained their contacts. Individual pycnosis and microautophagosomes, mitotic figures are present (Fig. 4(c)). At 30 min irradiation, pycnosis was observed with disappearance of cell cytoplasm, which is a morphological sign of the initial stage of apoptosis. At 45 min of irradiation, the cell density is greatly reduced. Cells are more elongated. Nuclei are hyperchromic and condensed. Many lysosomes and individual microautophagosomes appear. Nuclear pycnosis with disappearance of cytoplasm and apoptotic bodies were observed (Fig. 4(d)). After 60 min of irradiation, the cell density is reduced. Cells are more elongated. The nuclei are small, hyperchromic and condensed. Pycnosis of nuclei was registered - a sign of apoptosis. There are no autophagosomes in the cells.

In the control FLEH-104 cells, the cells form multiple contacts and connections, are sharply elongated, the nucleus is not expressed, the nucleoli are present. There

are no signs of apoptosis and autophagy (Fig. 5(a)). When uncoated with SiO₂ shell UCNPs are added to the cells, a decrease in cellular activity is observed, but the cells retain contacts, a single pycnosis, increased granularity in the cells, and microautophages are observed. The viability of the fibroblasts was not affected by uncoated UCNPs. The data obtained are consistent with the results of the authors who showed that the use of particles in concentrations of 62.5–125 μg/mL does not lead to a violation of fibroblast viability [51]. After irradiation for 5 min, the cellular activity is greatly reduced, the cells are more elongated, the nuclei are hyperchromic, condensed, many lysosomes appear, there is cellular debris (remnants of destroyed cells). Morphological signs of autophagosomes (appearance of vacuoles surrounded by lysosomes) are present in cell cultures. Pycnosis of nuclei was registered – possibly apoptosis (Fig. 5(b)). At 15 min of irradiation, the cell activity of the culture is reduced, but the cells maintain contacts, there is a single pycnosis and microautophages, and the granularity in the cells is increased (Fig. 5(c)). After 30 min of irradiation, pycnosis predominates and signs of apoptosis are possible. At 45 min irradiation, cellular activity is greatly reduced, cells are more elongated, nuclei are hyperchromic and condensed, many lysosomes appear, cellular debris and autophagosomes are present. Pycnosis of nuclei was registered - signs of apoptosis are possible (Fig. 5(d)). After 60 min of irradiation, the activity of the cells is greatly reduced, the

cells are more elongated, the nuclei are hyperchromic and condensed, there are many lysosomes and autophagosomes, and there is cellular debris. Pycnosis of nuclei was registered – signs of apoptosis are possible.

4 Conclusion

UCNPs have been approved as a promising material for cancer diagnostics and treatment. Here we demonstrated the capability of [NaYF₄:Yb³⁺, Er³⁺] UCNPs to induce cell death through apoptosis under the NIR irradiation. The irradiation of cells, preliminary co-incubated with elaborated UCNPs (10 mg/mL) for 24 h, by 980 nm laser (power density: 0.5 W/cm²) for 10 min was found sufficient to significantly decrease the viability of both model cell lines. The phototoxic effect of UCNPs on the fibroblast cell line was more pronounced than on the epidermoid carcinoma cells, possibly due to differences in particle internalization efficiency by these cell lines.

It has been shown the photocytotoxic and morphological comparison of FLEH-104 and Hep-2 cell lines responses to influence of uncoated and coated by SiO₂ shell UCNPs.

Cell viability is assessed for cytotoxic effects of UCNPs at light exposure (to demonstrate the direct phototoxic effects).

The addition of UCNPs has a negative effect on FLEH-104. This result may be due to the fact that fibroblasts have a different morphology (they are larger and elongated) than Hep-2, as a consequence their cell surface area is larger and when particles are added, they capture a greater amount. This means that after 5 min no toxic effect is observed, but after 10 min the fibroblasts begin to die (this number of UCNPs and the duration of

exposure are sufficient to heat the cell and cause its death).

Morphological signs of autophagy (appearance of vacuoles surrounded by lysosomes) and apoptosis (pronounced pycnosis of the nucleus) are observed in some cases during irradiation of Hep-2 and FLEH-104 cells.

The results confirm the high activity of luminescent-uncoated UCNPs against Hep-2 cancer cells and show potential for theranostics, i.e. imaging and controlled thermal effects on pathological cells. The particles exhibited cytotoxic, cytostatic properties. By activating the photodynamic reaction in tumor cells, apoptosis is induced and tumor cell resistance is prevented by extending the irradiation time to 60 min by blocking autophagy. At the same time, maximum laser irradiation of FLEH-104 culture treated with luminescent coating UCNPs induces not only apoptosis but also autophagy, indicating that normal cells are less sensitive to the action of the particles. This makes these types of nanoparticles the most promising to study in experimental oncology. Thus, UCNPs are a promising alternative to conventional fluorescent labels for cell imaging and have great potential in biological and clinical applications.

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Disclosures

All authors declare that there is no conflict of interests in this paper.

References

1. J. Ferlay, M. Colombet, I. Soerjomataram, D. M. Parkin, M. Piñeros, A. Znaor, and F. Bray, “[Cancer statistics for the year 2020: An overview](#),” *International Journal of Cancer* 149(4), 778–789 (2021).
2. Cancer, World Health Organization (accessed 15 September 2022). [<https://www.who.int/news-room/fact-sheets/detail/cancer>].
3. J. G. Hiller, N. J. Perry, G. Poulgiannis, B. Riedel, and E. K. Sloan, “[Perioperative events influence cancer recurrence risk after surgery](#),” *Nature Reviews Clinical Oncology* 15(4), 205–218 (2018).
4. Y. P. Liu, C. C. Zheng, Y. N. Huang, M. L. He, W. W. Xu, and B. Li, “[Molecular mechanisms of chemo- and radiotherapy resistance and the potential implications for cancer treatment](#),” *MedComm* 2(3), 315–340 (2021).
5. Y. Wu, Y. Song, R. Wang, and T. Wang, “[Molecular mechanisms of tumor resistance to radiotherapy](#),” *Molecular Cancer* 22(1), 96 (2023).
6. N. V. Polukonova, M. A. Baryshnikova, D. A. Khochankov, E. V. Stepanova, E. S. Solomko, A. V. Polukonova, D. A. Mudrak, A. M. Mylnikov, A. B. Bucharskaya, G. N. Maslyakova, and N. A. Navolokin, “[Activation of Apoptosis and Autophagy by Gratiola Officinalis Extract in Human Tumor Cell Lines](#),” *Journal of Biomedical Photonics & Engineering* 7(4), 040307 (2021).
7. H. Modjtahedi, S. Ali, and S. Essapen, “[Therapeutic application of monoclonal antibodies in cancer: advances and challenges](#),” *British Medical Bulletin* 104, 41–59 (2012).
8. V. M. Tolmachev, V. I. Chernov, and S. M. Deyev, “[Targeted nuclear medicine. Seek and destroy](#),” *Russian Chemical Reviews* 91(3), RCR5034 (2022).
9. N. Widmer, C. Bardin, E. Chatelut, A. Paci, J. Beijnen, D. Levêque, G. Veal, and A. Astier, “[Review of therapeutic drug monitoring of anticancer drugs part two – Targeted therapies](#),” *European Journal of Cancer* 50(12), 2020–2036 (2014).

10. E. Raschi, V. Vasina, M. G. Ursino, G. Boriani, A. Martoni, and F. De Ponti, “[Anticancer drugs and cardiotoxicity: Insights and perspectives in the era of targeted therapy](#),” *Pharmacology & Therapeutics* 125(2), 196–218 (2010).
11. T. A. Baudino, “[Targeted Cancer Therapy: The Next Generation of Cancer Treatment](#),” *Current Drug Discovery Technologies* 12(1), 3–20 (2015).
12. M. Kim, D. M. Kim, K. S. Kim, W. Jung, and D. E. Kim, “[Applications of Cancer Cell-Specific Aptamers in Targeted Delivery of Anticancer Therapeutic Agents](#),” *Molecules* 23(4), 830 (2018).
13. H. Sun, X. Zhu, P. Y. Lu, R. R. Rosato, W. Tan, and Y. Zu, “[Oligonucleotide aptamers: new tools for targeted cancer therapy](#),” *Molecular Therapy-Nucleic Acids* 3(8), e182 (2014).
14. M. C. Skala, J. M. Squirrell, K. M. Vrotsos, J. C. Eickhoff, A. Gendron-Fitzpatrick, K. W. Eliceiri, and N. Ramanujam, “[Multiphoton microscopy of endogenous fluorescence differentiates normal, precancerous, and cancerous squamous epithelial tissues](#),” *Cancer Research* 65(4), 1180–1186 (2005).
15. S. Maryam, M. S. Nogueira, R. Gautam, S. Krishnamoorthy, S. K. V. Sekar, K. W. Kho, H. Lu, R. N. Riordain, L. Feeley, P. Sheahan, R. Burke, and S. Andersson-Engels, “[Label-Free Optical Spectroscopy for Early Detection of Oral Cancer](#),” *Diagnostics* 12(12), 2896 (2022).
16. L. Yang, J. Park, M. Marjanovic, E. J. Chaney, D. R. Spillman Jr, H. Phillips, and S. A. Boppart, “[Intraoperative Label-Free Multimodal Nonlinear Optical Imaging for Point-of-Procedure Cancer Diagnostics](#),” *IEEE Journal of Selected Topics in Quantum Electronics* 27(4), 1–12 (2021).
17. S. Hernot, L. van Manen, P. Debie, J. S. D. Mieog, and A. L. Vahrmeijer, “[Latest developments in molecular tracers for fluorescence image-guided cancer surgery](#),” *Lancet Oncology* 20(7), e354–e367 (2019).
18. D. Musumeci, C. Platella, C. Riccardi, F. Moccia, and D. Montesarchio, “[Fluorescence Sensing Using DNA Aptamers in Cancer Research and Clinical Diagnostics](#),” *Cancers* 9(12), 174 (2017).
19. M. Gao, F. Yu, C. Lv, J. Choo, and L. Chen, “[Fluorescent chemical probes for accurate tumor diagnosis and targeting therapy](#),” *Chemical Society Reviews* 46(8), 2237–2271 (2017).
20. V. Shupletsov, K. Kandurova, V. Dremine, E. Potapova, M. Apanaykin, U. Legchenko, and A. Dunaev, “[Fluorescence imaging system for biological tissues diagnosis: phantom and animal studies](#),” *Journal of Biomedical Photonics & Engineering* 6(1), 010303 (2020).
21. V. Maryakhina, Y. Korneva, I. Chekurov, and O. Shisterova, “[Fluorescent diagnostics of benign breast diseases and breast cancer](#),” *Journal of Biomedical Photonics & Engineering* 3(4), 040306 (2017).
22. A. D. Mironova, Y. V. Kargina, Olga S. Pavlova, A. M. Perepukhov, I. O. Sobina, and V. Yu. Timoshenko, “[Porous Silicon Nanoparticles with Rare Earth as Potential Contrast Agents for MRI and Luminescent Probes for Bioimaging](#),” *Journal of Biomedical Photonics & Engineering* 8(2), 020304 (2022).
23. M. Albota, D. Beljonne, J. L. Brédas, J. E. Ehrlich, J.-Y. Fu, A. A. Heikal, S. E. Hess, T. Kogej, M. D. Levin, S. R. Marder, D. Mccord-Maughon, J. W. Perry, H. Röckel, M. Rumi, G. Subramaniam, W. W. Webb, X.-L. Wu, and C. Xu, “[Design of organic molecules with large two-photon absorption cross sections](#),” *Science* 281(5383), 1653–1656 (1998).
24. D. R. Larson, W. R. Zipfel, R. M. Williams, S. W. Clark, M. P. Bruchez, F. W. Wise, and W. W. Webb, “[Water-soluble quantum dots for multiphoton fluorescence imaging in vivo](#),” *Science* 300(5624), 1434–1436 (2003).
25. R. A. Farrer, F. L. Butterfield, V. W. Chen, and J. T. Fourkas, “[Highly efficient multiphoton-absorption-induced luminescence from gold nanoparticles](#),” *Nano Letters* 5(6), 1139–1142 (2005).
26. C. Sönnichsen, A. P. Alivisatos, “[Gold nanorods as novel nonbleaching plasmon-based orientation sensors for polarized single-particle microscopy](#),” *Nano Letters* 5(2), 301–304 (2005).
27. H. Wang, T. B. Huff, D. A. Zweifel, W. He, P. S. Low, A. Wei, and J. X. Cheng, “[In vitro and in vivo two-photon luminescence imaging of single gold nanorods](#),” *Proceedings of the National Academy of Sciences* 102(44), 15752–15756 (2005).
28. D. Yelin, D. Oron, S. Thiberge, E. Moses, and Y. Silberberg, “[Multiphoton plasmon-resonance microscopy](#),” *Optics Express* 11(12), 1385–1391 (2003).
29. W. R. Zipfel, R. M. Williams, and W. W. Webb, “[Nonlinear magic: multiphoton microscopy in the biosciences](#),” *Nature Biotechnology* 21(11), 1369–1377 (2003).
30. B. Gidwani, V. Sahu, S. S. Shukla, R. Pandey, V. Joshi, V. K. Jain, and A. Vyas, “[Quantum dots: Prospectives, toxicity, advances and applications](#),” *Journal of Drug Delivery Science and Technology* 61, 102308 (2021).
31. M. J. Abrams, B. A. Murrer, “[Metal compounds in therapy and diagnosis](#),” *Science* 261(5122), 725–730 (1993).
32. Q. Fan, X. Cui, H. Guo, Y. Xu, G. Zhang, and B. Peng, “[Application of rare earth-doped nanoparticles in biological imaging and tumor treatment](#),” *Journal of Biomaterials Applications* 35(2), 237–263 (2020).
33. N. Zhou, J. Ni, and R. He, “[Advances of Upconversion Nanoparticles for Molecular Imaging](#),” *Nano Biomedicine & Engineering* 5(3), 131 (2013).
34. S. A. Hilderbrand, F. Shao, C. Salthouse, U. Mahmood, and R. Weissleder, “[Upconverting luminescent nanomaterials: application to in vivo bioimaging](#),” *Chemical Communications* (28), 4188–4190 (2009).
35. S. Andersson-Engels, H. Liu, C. T. Xu, P. Svenmarker, A. Gisselsson, P. Kjellman, L. Andersson, R. in’t Zandt, F. Olsson, and S. Fredriksson, “[In vivo luminescence imaging and tomography using upconverting nanoparticles as contrast agents](#),” In 2012 Asia Communications and Photonics Conference (ACP), AS3E.2 (2012).

36. M. A. Syroeshkin, F. Kuriakose, E. A. Saverina, V. A. Timofeeva, M. P. Egorov, and I. V. Alabugin, “[Upconversion of Reductants](#),” *Angewandte Chemie International Edition* 58(17), 5532–5550 (2019).
37. S. Han, R. Deng, X. Xie, and X. Liu, “[Enhancing luminescence in lanthanide-doped upconversion nanoparticles](#),” *Angewandte Chemie International Edition* 53(44), 11702–11715 (2014).
38. P. P. Nampi, A. Vakurov, L. E. Mackenzie, N. S. Scrutton, P. A. Millner, G. Jose, and S. Saha, “[Selective cellular imaging with lanthanide-based upconversion nanoparticles](#),” *Journal of Biophotonics* 12, e201800256 (2019).
39. H. Li, Q. Chen, J. Zhao, and K. Urmila, “[Fabricating upconversion fluorescent nanoparticles modified substrate for dynamical control of cancer cells and pathogenic bacteria](#),” *Journal of Biophotonics* 10(8), 1034–1042 (2017).
40. A. K. S. Braz, D. S. Moura, A. S. L. Gomes, T. Y. Ohulchanskyy, G. Chen, M. Liu, J. Damasco, R. E. de Araujo, and P. N. Prasad, “[TiO₂ -coated fluoride nanoparticles for dental multimodal optical imaging](#),” *Journal of Biophotonics* 11(4), e201700029 (2018).
41. Y. Yang, T. Zhang, and D. Xing, “[Single 808 nm near-infrared-triggered multifunctional upconverting phototheranostic nanocomposite for imaging-guided high-efficiency treatment of tumors](#),” *Journal of Biophotonics* 14 (9), e202100134 (2021).
42. Y. Hu, J. F. Honek, B. C. Wilson, and Q-B. Lu, “[Design, synthesis and photocytotoxicity of upconversion nanoparticles: Potential applications for near-infrared photodynamic and photothermal therapy](#),” *Journal of Biophotonics* 12, e201900129 (2019).
43. R. Liang, M. Wei, D. G. Evans, and X. Duan, “[Inorganic nanomaterials for bioimaging, targeted drug delivery and therapeutics](#),” *Chemical Communications* 50(91), 14071 (2014).
44. U. Kostiv, M. Šlouf, H. Macková, A. Zhigunov, H. Engstová, K. Smolková, P. Ježek, and D. Horák, “[Silica-coated upconversion lanthanide nanoparticles: The influence of crystal design on morphology, structure and optical properties](#),” *Beilstein Journal of Nanotechnology* 6, 2290–2299 (2015).
45. M. Tou, Z. Luo, S. Bai, F. Liu, Q. Chai, S. Li, and Z. Li, “[Sequentially coating upconversion NaYF₄:Yb,Tm nanocrystals with SiO₂ and ZnO layers for NIR-driven photocatalytic and antibacterial applications](#),” *Materials Science and Engineering: C* 70, 1141–1148 (2017).
46. T. Jang, M. J. Kim, and S. H. Sohn, “[Silica nanoparticle coating of NaYF₄:\(Yb³⁺, Er³⁺\) upconversion phosphor](#),” *Journal of Nanomaterials* 2022, 8961362 (2022).
47. P. Kowalik, D. Elbaum, J. Mikulski, et al., “[Upconversion fluorescence imaging of HeLa cells using ROS-generating SiO₂-coated lanthanide-doped NaYF₄ nanoconstructs](#),” *RSC Advances* 7(48), 30262–30273 (2017).
48. R. A. Verkhovskii, R. A. Anisimov, M. V. Lomova, D. K. Tuchina, E. N. Lazareva, A. A. Doronkina, A. M. Mylnikov, N. A. Navolokin, V. I. Kochubey, and I. Yu. Yanina, “[Cytotoxicity of various types of coated upconversion nanoparticles. Overview](#),” *Izvestiya of Saratov University. Physics* 22(4), 357–373 (2022). [in Russian].
49. I. Yu. Yanina, E. A. Sagaidachnaya, I. V. Vidyasheva, N. A. Navolokin, V. I. Kochubey, and V. V. Tuchin, “[Phototoxicity and luminescence of the upconversion nanoparticles embedded in the cells](#),” *Proceedings of SPIE* 10877, 108770Y (2019).
50. E. A. Sagaidachnaya, J. G. Konyukhova, N. I. Kazadaeva, A. A. Doronkina, I. Yu. Yanina, A. A. Skaptsov, A. B. Pravdin, and V. I. Kochubey, “[Effect of hydrothermal synthesis conditions on up-conversion luminescence intensity of β-NaYF₄: Er³⁺, Yb³⁺ particles](#),” *Quantum Electronics* 50(2), 109–113 (2020).
51. A. E. Guller, A. N. Generalova, E. V. Petersen, A. V. Nechaev, I. A. Trusova, N. N. Landyshev, A. Nadort, E. A. Grebenik, S. M. Deyev, A. B. Shekhter, and A. V. Zvyagin, “[Cytotoxicity and non-specific cellular uptake of bare and surface-modified upconversion nanoparticles in human skin cells](#),” *Nano Research* 8, 1546–1562 (2015).