Modification of $NaYF_4$:Yb_{3+},Er_{3+} Up-Converting Nanoparticles with Phospholipid Coating for Cell Imaging

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Introduction. Various organic dyes and nanoparticles are widely investigated due to their potential application in sensing, imaging, and therapy. Fluorescent nanoparticles (quantum dots, noble metal nanoclusters) have many advantages (excelent photostability, size dependant photoluminescence band, easy surface modification, high two-photon absorbtion cross section) over organic dyes. However these nanomaterials still have some drawbacks which restrict using new methods up to their full potential. Quantum dots (QD) are made up of toxic elements. Noble metal nanoclusters have relatively low photoluminescence quantum yield. These nanoparticles usually absorb and fluoresce in visible spectral region, thus limiting their diagnostics and therapy applications to superficial layers since tissues have high absorption in visible region. Deeper layers of tissue can be achieved by exciting fluorophores in tissue optical window (near infrared (NIR) region), however in this case two photon excitation is needed, which requires very powerful pulsed laser systems (pulse power ~10^8 W), which are very expensive and hard to operate. These disadvantages can be overcome by using upconverting nanoparticles (UCNPs). Upconversion is a process when two or more photons are absorbed in order to generate visible light from NIR excitation. In order to observe upconversion, inexpensive, continuous wave lasers can be used (average power ≤1W or less). Due to their advantages over other fluorophores, such as low toxicity, chemical stability, narrow emission peaks, deeper NIR penetration into biological tissue and tunable optical properties depending on lanthanide dopants in host matrix, UCNPs are promising nanomaterials for imaging cells and tissues [1].

In this study, we have investigated the photostability and spectral properties of water soluble $NaYF_4$:Yb^{3+}, Er^{3+} UCNP's after modification with different phospholipids and their mixtures. We have also studied cytotoxicity and accumulation of UCNP's in NIH3T3 cell line.

Materials and methods. Oleate-capped $NaYF_4$ doped with Yb^{3+} (20%), Er^{3+} (2%) upconverting nanoparticles were synthesized according to previously reported procedure [2] with slight modifications. We tested two different phospholipids (1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), polyethylene glycol 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (PEG-DOPE)) and their

mixture as UCNPs surface coating agents. Phospholipids were added to UCNPs solution in chloroform (UCNPs: phospholipids molar ratio 1:3,4×10^5) in one necked flask. Chloroform was evaporated in inert gas (argon) atmosphere. After complete evaporation of chloroform phosphate buffer solution (PBS) pH7 was added, and the flask was heated to 60 °C under vigorous stirring. The obtained suspension contained lipid coated UCNPs.

Immortalized mouse embryonic fibroblast cell line NIH3T3 was cultured in Dulbecco's Modified Eagle's medium (Gibco, USA). To evaluate DOPC:PEG-DOPE-UCNPs accumulation in cells, NIH3T3 cells were plated (3 x 10^4 cells per well) in 8-well chambered cover-slips (Nunc, USA) and cultured in a CO_2 incubator for 24 hours. Next day cells were washed three times with phosphate-buffered saline (pH 7.4) (PBS) and treated with DOPC:PEG-DOPE-UCNPs for 3 hours. After treatment cells were rinsed with PBS and fixed with paraformaldehyde. Nuclei were stained with DAPI. To evaluate UCNP impact on viability of NIH3T3 cells, standard XTT viability assay was used.

Upconversion spectra were measured using FLS920 fluorimeter (Edinburgh Instruments Inc. UK). Semiconductor CW laser emitting 980nm light was used for UCNPs excitation. Cells were imaged using custom modified Nikon Eclipse E400 microscope, equipped with 980 nm laser for UCNPs excitation and band-pass filter for UCNPs emission in green (500nm-560nm) spectral range. The images were further processed using ImageJ 1.41 software (NIH, USA).

Results. Upconversion spectrum of $NaYF_4$:Yb^{3+}, Er^{3+} UCNPs dispersed in chloroform has emission bands at 522 nm, 541 nm and 653 nm (see Fig. 1).

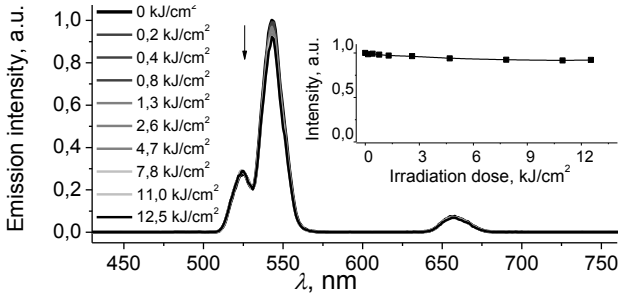

Fig. 1. Upconversion spectrum of UCNPs dispersed in chloroform (λ_{ex}= 980 nm) after irradiation with NIR laser (irradiation doses indicated in legend). Inset - dependance of emission band intensity (at 541nm) on irradiation dose.

These emission bands are specific to $NaYF_4$:Yb^{3+},Er^{3+} nanoparticles and bands can be assigned to transitions of Er^{3+} ions: $^2H_{11/2}\rightarrow{}^4I_{15/2}$, $^4S_{3/2}\rightarrow{}^4I_{15/2}$ and $^4F_{9/2}\rightarrow{}^4I_{15/2}$. The most intense upconversion band is at 541nm. Irradiation of UCNPs with 980 nm light (I=877 mW/cm^2) had very little effect on emission intensity even when accumulated dose was more than 12 kJ/cm^2. Emission intensity at 541 nm decreased by less than 8% during irradiation. This indicates that UCNPs are photostable and do not degrade even when exposed to high

irradiation doses. Decrease of upconversion efficiency could be explained by increased non-radiative (thermal) relaxation rate at higher temperatures.

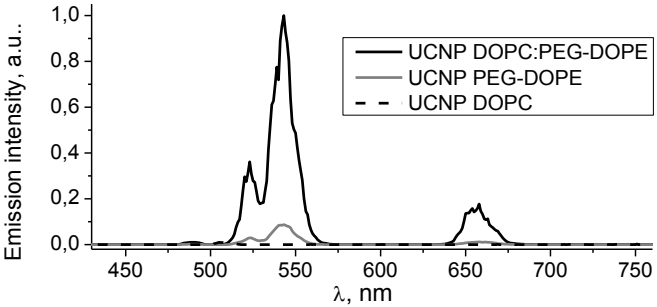

Fig. 2. Upconversion spectra of UCNPs coated with different phospholipid layers: DOPC, PEG-DOPE and 1:1 molar mixture of these phospholipids.

We modified surface of hydrophobic UCNPs with two types of phospholipids (DOPC and PEG-DOPE) and their mixture (see fig. 2). Upconversion signal was close to zero after coating UCNPs with DOPC phospholipids. This indicates that DOPC coated UCNPs were poorly soluble in PBS. Some studies have previously reported that PEG-free phospholipids tend to form large liposomal aggregates in aqueous media [3]. This could be the reason for poor UCNPs encapsulation within DOPC phospholipids. UCNPs were successfully dissolved in water after coating with PEG-DOPE or mixture (1:1) of DOPC:PEG-DOPE phospholipids. However upconversion emission intensity was approximately 10 times larger when mixture of phospholipids was used.

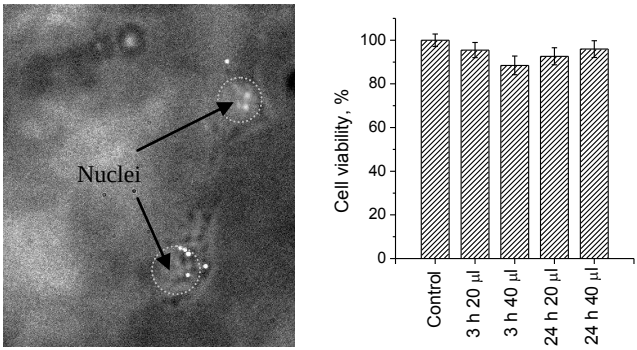

Fig. 3. Fluorescence microscopy image of NIH3T3 cells after 3h incubation with DOPC: PEG-DOPE-UCNPs. Nanoparticle accumulation around nuclei visible as white dots (left). NIH3T3 cell viability after incubation with DOPC: PEG-DOPE-UCNPs for 3 and 24 hours (right).

Cellular internalization behaviors of DOPC:PEG-DOPE-UCNPs were visualized by fluorescence microscopy imaging. Numerous bright green luminescent spots illuminating the NIH3T3 cell cytoplasm were observed after 3h of incubation (see Fig. 3 left). Accumulation of UCNPs was observed in cells, usually surrounding the nucleus region. UCNPs were packed into small clusters inside the cell, which indicates that UCNPs are taken into cells by endocytosis and accumulates in vesicles. We also tested the toxicity of UCNPs to NIH3T3 mouse embryonic fibroblast cells using the same concentration of UCNPs as in cell imaging experiments and twofold increased concentration. The decrease of cell viability was very small compared to control group (see Fig. 3 right). This indicates that DOPC:PEG-DOPE-UCNPs at used concentrations cause little to none toxic effects in NIH3T3 cells.

Conclusions UCNPs are very promising nanomaterial for biomedical imaging applications. They are photostable and do not degrade under NIR irradiation. UCNPs coated with DOPC:PEG-DOPE lipid mixture are hydrophylic and accumulate in NIH3T3 cells while being barely toxic at all.

Acknowledgement. This study was supported by the Research Council of Lithuania, Grant No. MIP-14185 and No. VP1-3.1-ŠMM-01-V-02-003.

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UCNPs are widely studied due to possible applications in biomedical imaging and therapy. In this study we investigated $NaYF_4$:Yb^{3+},Er^{3+} UCNPs as a potential nanomaterial for biomedical applications. We showed that UCNPs are photostable and do not degrade under prolonged exposure to NIR irradiation. Several surface modifications were tested using DOPC and PEG-DOPE phospholipids. Highest solubility in water was achieved when UCNPs were coated with the mixture of these lipids. NIH3T3 cells accumulated DOPC:PEG-DOPE-UCNPs and showed little to none viability decrease. UCNPs show potential as fluorescent nanomaterial but this relatively new field requires further research to utilize upconverting nanoparticles in medical diagnostics and therapy.