The ROS-generating photosensitizer-free NaYF₄:Yb,Tm@SiO₂ upconverting nanoparticles for photodynamic therapy application

P Kowalik¹,* P Kamińska¹, K Fronc¹, A Borodziuk¹, M Duda¹, T Wojciechowski¹, K Sobczak¹, D Kalinowska¹, M T Klepka¹ and B Sikora¹,*

¹ Institute of Physics, Polish Academy of Sciences, Warsaw, Poland
² Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

E-mail: pkowalik@ifpan.edu.pl and sikorab@ifpan.edu.pl

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Abstract

In this work we adapt rare-earth-ion-doped NaYF₄ nanoparticles coated with a silicon oxide shell (NaYF₄:20%Yb,0.2%Tm@SiO₂) for biological and medical applications (for example, imaging of cancer cells and therapy at the nano level). The wide upconversion emission range under 980 nm excitation allows one to use the nanoparticles for cancer cell (4T1) photodynamic therapy (PDT) without a photosensitizer. The reactive oxygen species (ROS) are generated by Tm/Yb ion upconversion emission (blue and UV light). The in vitro PDT was tested on 4T1 cells incubated with NaYF₄:20%Yb,0.2%Tm@SiO₂ nanoparticles and irradiated with NIR light. After 24 h, cell viability decreased to below 10%, demonstrating very good treatment efficiency. High modification susceptibility of the SiO₂ shell allows for attachment of biological molecules (specific antibodies). In this work we attached the anti-human IgG antibody to silane-PEG-NHS-modified NaYF₄:20%Yb,0.2%Tm@SiO₂ nanoparticles and a specifically marked membrane model by bio-conjugation. Thus, it was possible to perform a selective search (a high-quality optical method with a very low-level organic background) and eventually damage the targeted cancer cells. The study focuses on therapeutic properties of NaYF₄:20%Yb,0.2%Tm@SiO₂ nanoparticles and demonstrates, upon biological functionalization, their potential for targeted therapy.

Supplementary material for this article is available online

Keywords: nanoparticles, upconversion, photodynamic therapy, cancer disease, reactive oxygen species, bio-functionalization

(Some figures may appear in colour only in the online journal)

1. Introduction

In recent years, the number of people affected by cancer has continued to grow. Only in 2018, 9.6 million people died due to different kinds of cancer [1]. Speculation concerning worldwide health has predicted an increase in cancer cases—up to around 20 million new patients in 2025 [2, 3]. At the same
time, previously known cancers are evolving genetically [4]. Modern cancer treatment based on nano-sized objects allows increasing the efficiency of therapy. This happens thanks to the reduction of side effects through precise treatment within neoplastic changes. This action reduces the negative impact on healthy tissue and modifies the character of the therapy to become local and at the molecular level [5]. PDT is based on photosensitizer molecules as a source of free radicals, under specific wavelength light excitation. At present, PDT is developing on many levels and over many application areas [6–9].

Hybrid nanomaterials, connecting nanoparticles (NPs) with optical properties and biological molecules, allow for advanced treatment. Well known upconverting nanoparticles (UCNPs) can generate visible and ultraviolet light inside human tissues under near infrared (NIR) excitation. This process takes place as a multiphoton absorption and energy transfer between doping atoms. One of the most efficient host material is yttrium sodium fluoride (NaYF₄) doped with rare-earth ions with the hexagonal crystal structure [10–13]. One of the most important branch of development in the UCNPs-based PDT is a new reactive oxygen species (ROS) sources exploration. The most common system is based on photosensitizer (PS) molecules attached to the UCNPs surface. After NIR excitation of UCNPs, the upconversion emission is transferred to the PS and it can produce ROS and destroy cancer [14]. Unfortunately, some PS molecules can lose their properties because of photobleaching process and chemical instability [15–17]. For this reason the other way of modern PDT developing are ROS-generating NPs without organic components. An another advantage of PS-free materials is the simpler construction. The PS loading can be troublesome with closing the expected PS quantity. To eliminate this limitation, the TiO₂ layer on NP surface was reported. This solution allows for performing an effectively in vitro PDT using cancer cells (OSCC cell line viability decreased to 30%). In other studies, UCNPs@TiO₂ nanoparticles were used in connection with a drug (doxorubicin) and tested on cancer cells (MCF-7) as a PDT and chemotherapy hybrid. This research shows similar treatment results. Another PS-free NP design is to use a ZnO shell. The PDT treatment using UCNPs@ZnO nanoparticles leads to cell (MDA-MB-231) viability to decease to 50% [18–23]. The extra layers influence at final NPs of a larger size. Thus, we decided to propose a less complicated solution. Recently, we showed for the first time the possibility of using β-NaYF₄:20%Yb,0.2%M@SiO₂ NPs to generate ROS without any additional organic molecules on their surface. We observed a 70% increase in the EPR signal of ROS trapping after 20 min of 15 mg ml⁻¹ NP suspension irradiated at 980 nm with 1 W cm⁻² of power density. The ROS generation from Tm-doped nanoparticles appeared to be promising for future PDT therapy [24].

In this work we tested the aforementioned kind of UCNPs on living cancer cells as an alternative to PS-modified UCNPs for PDT. In this publication, such an experiment with PDT based on Tm/Yb-doped NPs, without any PS, was performed for the first time. The availability of numerous methods for silicon oxide shell modification in combination with constantly expanding knowledge about cancer cells changes makes fabrication of individually targeted cancer treatment possible. Cancerous tissues can be recognized by bio-functionalized NPs. The presence of specific receptors on the cancer cells surface allowed us to mark and destroy them [25–27]. The multi-functional NP system gives hope for individually designed cancer treatment; therapy which can recognize tumor cells located in hard to reach places (not available for surgery), adaptable therapy treatment which is safe for healthy tissue, and the reduction of therapeutic invasiveness (NIR light activation). At the same time, upconverting properties and specifically the ‘fingerprint’ emission spectrum can help with cancer imaging with an unnoticeable biological background. The research presented in this paper was based on the study of physical properties and modifications of NaYF₄:20%Yb,0.2%M@SiO₂ NPs. The NP physical properties (such as upconversion from NIR to UV and blue light) allows for ROS generation and cancer cell destruction after NIR excitation without a PS on the NP surface. At the same time, the NP surface was modified by a selected antibody for potential application as a targeted cancer treatment.

2. Experimental

2.1. Materials

Dulbecco’s Modified Eagle Medium (DMEM) (Gibco Life Technologies); fetal bovine serum (FBS) (Gibco Life Technologies); phosphate-buffered saline (PBS) (Invitrogen); PrestoBlue (Molecular Probes); LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes); formaldehyde (37%; Sigma-Aldrich); Anti-EEA1 antibody - Early Endosome Marker (Abcam); Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (Abcam); Hoechst 33258 (Sigma-Aldrich); 2,7-dichlorodihydrofluorescein (DCF) (Cayman Chemical Company); triethoxysilane poly(ethylene glycol) 2000 succinimidyl ester (Nanocs); IgG human serum (Sigma-Aldrich); anti-human IgG (whole molecule) antibody produced in goat (Sigma-Aldrich); ethanol (99.8%, Stanlab); 2-propanol (Chempur).

2.2. Formation of NaYF₄:20%Yb,0.2%M@SiO₂ (UCNPs@SiO₂) core@shell NPs

The core@shell NPs were synthesized in two steps using homogenous solution co-precipitation and sol-gel methods as described previously [24]. After synthesis the NPs were suspended in ethanol or water, depending on the further application.

2.3. Characterization of UCNPs@SiO₂

The morphology of UCNPs and UCNPs@SiO₂ core@shell NPs were evaluated by scanning electron microscopy (SEM; Zeiss Auriga Neo 40). The received images were used to calculate the NP size distributions. For both materials, the microscope worked at an acceleration voltage of 5 kV.
Additionally, silicon oxide coated NPs were analyzed by transmission electron microscope (TEM). TEM investigations were carried out on an FEI Talos F200X microscope operated at 200 kV. Observations were performed in scanning transmission electron microscopy (STEM) mode using high-angle annular dark field (HAADF) imaging. High-resolution mode was used to confirm the presence of the silicon oxide layer and separation of the NPs. Energy dispersive x-ray spectroscopy (EDX) using a Super-X system with four silicon drift detectors (SDDS) was applied to detect differences in local chemical composition.

The optical properties of the upconverting core were detected by a spectral system with a 980 nm laser (Lumics LU0980D300-DNA014) as an excitation source and a spectrophotometer (Horiba Jobin Yvon Fluorolog3) as a spectrum detector. Measurements were performed with 12.14 W cm\(^{-2}\) of excitation light power density.

### 2.4. Cytotoxicity of UCNPs@SiO\(_2\) for living cells

The experiments with living cells (cytotoxicity of UCNPs@SiO\(_2\) and PDT test) were carried out with a mouse’s mammary gland cells—4T1 (ATCC\(^\circ\) CRL2539\(^\circ\)). The cells were cultured in DMEM containing 10% FBS at 37 °C and 5% CO\(_2\). For cytotoxicity assay, cells were seeded to a 96-well plate (10 000 cells per well). The plates were left in the dark (37 °C, 5% CO\(_2\)) overnight. Then the cells were washed two times with PBS and flooded with fresh medium including UCNPs@SiO\(_2\) at a concentration of 100 μg ml\(^{-1}\) (200 μl well\(^{-1}\)). The plates were incubated in the dark for different lengths of time: for cytotoxicity assays in the dark: 12 and 24 h; for PDT with laser light: 12 h. Cell viability was detected by using a PrestoBlue assay. The medium including UCNPs@SiO\(_2\) was replaced with a fresh medium including UCNPs@SiO\(_2\) at a concentration of 100 μg ml\(^{-1}\) (200 μl well\(^{-1}\)) and incubated for 1 h. The plates were read by a microplate reader (Promega GLOMAX Discover GM3000) working in fluorescence mode: excitation filter: 520 nm; emission filter: 580–640 nm; temperature control: 37 °C.

### 2.5. PDT test with cancer cells

PDT potential of UCNPs@SiO\(_2\) was studied using 4T1 cells. Similarly to the cytotoxicity assay, cells were grown in a 96-well plate (10 000 cells per well). The following day, cells were washed by PBS and incubated with fresh medium included UCNPs@SiO\(_2\) (concentration: 100 μg ml\(^{-1}\)). Cells were incubated for 12 h in the dark, then irradiated by a 980 nm wavelength laser (2 W cm\(^{-2}\) of power density) for 9.5 min: 1.5 min of irradiation to 0.5 min break as the exposure cycle. The efficiency was measured by the cell viability: PrestoBlue Viability Assay and LIVE/DEAD Viability/Cytotoxicity Kit (supplementary information). The PrestoBlue assay was prepared like described for cytotoxicity measurements. The cell viability test results are present as means compared with the control (untreated) cells ± percentage standard deviation. Statistical significance was determined by Student’s t-test. For statistical significance, we considered p-values < 0.05.

### 2.6. ROS generation by UCNPs@SiO\(_2\) inside living cells during PDT

The experiment of intracellular ROS detection was performed with 4T1 cells. Cells were grown in a 96-well plate (10 000 cells per well). After overnight incubation, the cells were washed by PBS and incubated with fresh medium including UCNPs@SiO\(_2\) (concentration: 100 μg ml\(^{-1}\)). After incubation with UCNPs@SiO\(_2\) (12 h), the cells were washed twice by PBS and stained by 25 μM DCF in PBS solution for 30 min. Then the cells were irradiated by a 980 nm wavelength laser (2 W cm\(^{-2}\) of power density) for 9.5 min: 1.5 min of irradiation to a 0.5 min break as the exposure cycle. The plates were read by a microplate reader (Promega GLOMAX Discover GM3000) working in fluorescence mode: excitation wavelength: 485 nm; emission wavelength, 535 nm. The intracellular ROS level results are present as means compared with normalized values of the control (untreated) cells ± standard deviation. Statistical significance was determined by Student’s t-test. For statistical significance, we considered p-values < 0.05.

### 2.7. Confocal microscopy imaging after a PDT test

During the time of cell viability measurements, the cells were observed using confocal microscopy before and after exposure for NIR light—with and without UCNPs@SiO\(_2\) presence. The samples were prepared by using the immunofluorescence technique. The cells were fixed using a 3.7% solution of formaldehyde. Early endosomes were marked by a rabbit polyclonal antibody to EEA1 and Goat polyclonal Secondary Antibody to Rabbit IgG H&L (Alexa Fluor® 488). Cell nuclei were marked by Hoechst 33258. The presented pictures are digital imposition of images from the following channels: (1) for early endosomes (Alexa Fluor 488 dye), the excitation wavelength was 488 nm and the detection range was 495–572 nm; (2) for cell nuclei (Hoechst 33258 dye), the excitation wavelength was 705 nm and the detection range was 425–475 nm, (3) for UCNPs@SiO\(_2\), the excitation wavelength was 980 nm and the detection range was 415–726 nm.

### 2.8. Bio-functionalization of UCNPs@SiO\(_2\)

The first stage of bio-functionalization, the triethoxysilane poly(ethylene glycol) 2000 succinimidyl ester (silane-PEG NHS) was attached to UCNPs@SiO\(_2\) [28]. 3 mg of core@shell NPs was suspended in 200 μl of ethanol and connected with 500 μl of silane-PEG-NHS ethanol solution (concentration 20 mg ml\(^{-1}\)). The mixture was magnetically stirred for 2 h (20 °C). The UCNPs@SiO\(_2\)-PEG-NHS were collected by centrifugation and washed using cold distilled water. For prolonged storage, the NPs were suspended in a 2-propanol solution at 4 °C.

In the next step of bio-functionalization, the PEG-NHS-free ends of NPs were modified [29]. The entirety of the core@shell NPs connected with silane-PEG-NHS were washed using a borate buffer (0.1 M, pH = 7.5) and after centrifugation were suspended in 1 ml of buffer. The fresh solution of anti-human IgG (anti-h-IgG) antibody (200 μl,
2 mg ml⁻¹ at borate buffer, 0.1 M, pH-7.5) was mixed with the NP solution and incubated at 4 ºC for 1 h. The final UCNPs@SiO₂-PEG-NHS-anti-h-IgG were collected by centrifugation and suspended in 1 ml of TRIS-HCl buffer (0.01 M, pH = 7).

2.8.1. Fourier-transform infrared spectroscopy and dot-blot techniques for bio-functionalization characterization. The poly(ethylene glycol) conjugation to the core@shell NPs was controlled by Fourier-transform infrared spectroscopy (FTIR). Measurements were performed using FTIR spectrometer (Thermo Scientific Nicolet iS5) using the attenuated total reflection technique and the spectral range was 4000-400 cm⁻¹.

The UCNPs@SiO₂-PEG-NHS bio-functionalized by the anti-h-IgG antibody was controlled by the dot-blot method. In the case of the dot-blot technique, the nitrocellulose membrane was locally covered by a solution of human IgG (h-IgG) serum at increasing concentrations (0.1, 1 and 10 µg ml⁻¹; control area was uncovered). After covering, the membrane was blocked by 1 h incubation in a buffer (Tris-HCl, 0.01 M, pH = 7, 0.5% Tween, 1% BSA). Then the 400 µl of UCNPs@SiO₂-PEG-NHS-anti-h-IgG solution was mixed with 1.6 ml of 0.01 M, pH = 7, Tris-HCl with 0.5% Tween buffer. The membrane was placed on the top of NP solution to eliminate the gravitational fall of NPs onto the membrane surface. After 2 h, the membrane was washed using buffer (Tris-HCl, 0.01 M, pH = 7, 0.5% Tween). The membrane surface was analyzed with confocal microscopy at 980 nm excitation to observe signal from core of the NPs. The luminescence spectra of UCNPs on the membrane were measured using a confocal microscope with 980 nm of excitation using lambda scan mode as we described previously [13]. The procedure created a cumulative image of the registered confocal images for several emission wavelengths simultaneously. After this, the luminescence spectra were generated for the selecting point on the image with suitable software.

3. Results and discussion

3.1. Characterization of UCNPs@SiO₂

The first step in preparation of UCNPs for PDT and cellular targeting was the synthesis and characterization of NaYF₄:20%Yb,0.2%Tm NPs. The morphology and crystal structure size were determined by the SEM measurements. The UCNPs average size was 21.9 +/- 1.2 nm and had a very narrow size distribution (figure 1(a)).

In the next step, the UCNPs were coated by a thin silicon oxide shell. UCNPs@SiO₂ size increased to 28.6 +/- 1.3 nm (figure 1(b)), indicating a shell thickness of about 3.3 nm.

An analysis of the EDX mapping data showed an homogenous distribution of the main elements creating NaYF₄ NPs (Na, Y and F) (figure 2). Si and O elements formed a shell on the UCNP surface. These studies showed successful covering with SiO₂.

The upconversion spectra suspension of UCNPs@SiO₂ in water (2 mg ml⁻¹) under 980 nm laser excitation (power density 12.14 W cm⁻²) is shown in figure 3. The UV–vis emission bands, under 980 nm excitation, are assigned to 4f-4f levels of Tm⁴⁺ ions. An observed emission is caused by cascading phenomena: 980 nm light absorption by ytterbium ions, energy transfer from Yb⁴⁺ ions (²F₅/₂ state) to the Tm⁴⁺ ions (to ³H₅, ³H₆, ²F₂ and ²G₄ states) and after non-radiative transitions, the emission from the states ¹G₄ → ³H₂, ¹G₄ → ⁵F₄, ⁵F₃ → ⁵H₆, and ³H₄ → ³H₂ can be observed.

The emission from the following states correspond to maxima spectrum peaks: 475, 646, 697 and 803 nm [24]. The energy level diagram is presented in the supplementary information (figure S.1. (available online at stacks.iop.org/NANO/32/475101/mmedia)).

The upconversion luminescence of UCNPs@SiO₂ can be used in medical applications: (1) the 803 nm luminescence in bioimaging (NIR window [30]), (2) the 475 nm to ROS generation in PDT. We previously reported [24] on ROS generation from UCNPs@SiO₂ after 980 nm excitation. We showed, for the first time, ROS generation from NaYF₄ NPs doped with Tm³⁺ and Yb³⁺ ions in water without PS. We observed a 70% increase in the EPR signal of ROS trapping after 20 min of irradiation. We showed ROS generation from
Tm-doped NPs as a potential compound for PDT therapy [24].

3.2. Intracellular ROS generation and photodynamic therapy for cancer cells

Compared to the traditional PDT [6, 31, 32] and the approach based on the NP modifications by PS [8, 33–35], NaYF₄ NPs doped with Tm/Yb ions can generate ROS without any other components attached to their surface [24]. The ROS generation is possible thanks to the UV/blue range emission. The ROS generation mechanism by Tm³⁺-doped NPs is not well understood at present. The R. Lubart research group studied the influence of visible light for ROS generation on bacteria [36]. They showed that the best phototoxicity with S. aureus and E. coli was measured at 415 and 455 nm blue light (100 mW cm⁻²). The 415 nm light induced more ROS. A similar effect was obtained by M Eichler et al. They demonstrated much greater amounts of ROS in mammalian cells excited by blue light than other visible wavelengths [37]. In our study we used a similar light wavelength (blue range) for ROS production.

Therapeutic properties of UCNPs@SiO₂ were studied using various methods. First, after UCNPs@SiO₂ treatment (100 μg of UCNPs@SiO₂ per 50 000 cells) and 980 nm laser irradiation (2 W cm⁻²; 10 min in cycle: 1.5 min irradiation and 0.5 min break), cell viability was measured using a PrestoBlue assay (figure 4(a)). The next experiments were based on the cell morphology observations after the therapy using immunofluorescence confocal microscopy (figures 4(b)–(e)).

The experiments described above were performed using several control groups. First, the 980 nm laser light effect on the cancer cells was studied. We did not observe any changes of cell viability and morphology after irradiation (figures 4(a) and (d)). The morphology of the cells treated only with the UCNPs@SiO₂ (figure 4(c)) did not show any changes compared to the main control group (figures 4(a) and (b)). The viability of main experimental group—the cells treated by UCNPs@SiO₂ and irradiated by 980 nm laser—decreased in the subsequent stages of therapy. Directly after 980 nm laser light irradiation, cell viability drastically decreased to 16%. After the next 24 h, cell viability decreased to 6% (figure 4(a)). Statistical analysis (using Student’s t-test) confirmed the high relevance of results for this cell group.
Fluctuation of cell viability levels in all control groups was in the statistical error range. A similar viability decreasing effect was observed using the LIVE/DEAD cell viability assay. The live/dead marked cells were differentiated by calcein AM (live cells, green fluorescence) and ethidium homodimer-1 (dead cells, red fluorescence) containing after PDT treatment. Cell viability decreased directly after exposure to 20% and after the next 24 h decreased to 13%. The dead cell amount increased to 80% and 87% respectively. The live/dead assay results are presented in the supplementary information (figure S.2). This implies that the UCNPs@SiO2 exhibited enough ROS for cancer cell damage.

The morphology of the cells was carefully analyzed by confocal microscopy. The morphology of the main experimental group (cells treated with UCNPs@SiO2 and irradiated by 980 nm laser) was different compared to all controls. After treatment, the characteristic shape of the adherent cells was not observed. The cells were smaller, with a weak cytoplasmic area, and the confluence of grooving was significantly lower (figure 4(e)).

In the next step, we confirmed that only $^1$O$_2$ molecules are responsible for the cellularly damaged organs and caused the death of tumor cells after 980 nm of excitation.

The generation of intracellular ROS was detected by monitoring the fluorescence of dichlorofluorescein (DCF). The presence of ROS caused the reaction of nonfluorescent 2,7-dichlorofluorescein diacetate (DCFH-DA) to form DCF fluorescence. The fluorescent DCF signal intensity for each of cell control groups and the experimental group is shown in figure 5. When the 4T1 cells were incubated with UCNPs@SiO2 in the dark, the DCF green fluorescence was at the same level as the control group. The statistically significant changes at the DCF fluorescence levels were observed for two groups of
cells (cells irradiated by the laser without NPs and experimental group—cells irradiated by the laser with NPs). The cells exhibited negligible green fluorescence under 980 nm irradiation (similar results were obtained by Yong Il Park et al and other groups [38–40]). This is probably caused by the increase of the temperature in the cell environment. However, the cells that were incubated with UCNP@SiO2 and irradiated at 980 nm (2 W cm−2) showed bright green fluorescence (the fluorescence signal was 2.5 times higher than the control). These results clearly proved ROS generation by UCNP@SiO2 under NIR light irradiation.

The experiments with PDT based on Tm3+/Yb3+-doped NPs, without any PS, were performed, to the best of our knowledge, for the first time. This could be a breakthrough in the PDT treatment of cancer cells—lack of photobleaching of the PS, deeper penetration because of NIR excitation, better tumor localization because of the emission in the NIR region.

**3.3. Bio-functionalization of UCNP@SiO2**

In the next step, a silane reactive PEG2000, activated as an N-hydroxysuccinimide (NHS) ester, was covalently bounded to the UCNP@SiO2 surface as a spacer [21]. The PEG spacer prevents aggregation, reduces unspecific binding, and improves the solubility of NPs in water. The NHS ester groups are highly reactive towards proteins [27]. The PEG connection to the UCNPs@SiO2 surface was confirmed by FTIR spectroscopy (UCNPs@SiO2-PEG-NHS) (figure 6).

Comparison of the so-called fingerprint region of both absorption spectra (UCNPs@SiO2 and UCNPs@SiO2-PEG-NHS) shows that the substitution was successful.

Next to strong absorption bands around 1100 cm−1 associated with asymmetric Si-O-Si vibrations, the spectrum of UCNPs@SiO2-PEG-NHS (blue line in figure 6) shows a number of absorption bands derived from the PEG molecule (similar with the free-PEG, dashed gray line in figure 6). Strong absorption bands around 840 cm−1 and 959 cm−1 are derived from the stretching vibrations of C−C bonds and bending vibrations of C−H and C−O bonds. Absorption from stretching C−N and C−O vibrations corresponds to bands at around 1237 cm−1 and 1279 cm−1 respectively. In the high-frequency part of spectrum there are strong absorption bands associated with stretching symmetric and asymmetric vibrations of methyl groups and absorption from hydrogen bonds. Moreover, a decrease in band intensity around 1784 cm−1 (C=O stretching vibrations) in comparison with PEG spectrum indicates a change in the atomic environment.

Analysis of PEG-conjugated NPs compared with previously described spectra shows changes in the following ranges: a decrease in the intensity of the peak at 700 cm−1 which shows the commitment of Si-O-Si bonds at the silicon shell to the PEG attachment; a decrease of the band at 650 cm−1 is caused by the involvement of the C-O bond from the PEG molecules to attach to the NP silicon shell.

In the next stage, the anti-h-IgG antibody was attached to the PEG-functionalized NP surface. This step was done to check the potential application of UCNPs@SiO2 for cancer cell targeting.

The efficiency of anti-h-IgG-functionalization of UCNPs@SiO2 was checked by the dot-blot method (figure 7). A secondary antibody (anti-h-IgG) attached to NP surface is characterized by high binding specificity to the primary antibody (h-IgG). The surface of the nitrocellulose membrane was covered by h-IgG to perform the function of model target tissue for the molecular targeted therapy. The UCNPs@SiO2-PEG anti-h-IgG successfully detected the h-IgG proteins covering the membrane at concentrations of 10 μg ml−1, 1 μg ml−1 and 0.1 μg ml−1. The nitrocellulose membrane surface was analyzed using a confocal microscope with a 980 nm laser as an excitation light source (figure 7). At the control area (uncovered by h-IgG, covered only by FBS), no upconversion signal was observed (figure 7(b)). A specific emission signal was noticed only in the experimental areas—covered with h-IgG. The signal intensified with an increase of the h-IgG concentration. The highest signal was noticed for the area covered by the highest concentration of h-IgG (10 μg ml−1, figure 7(e)). These experiments illustrate that the UCNPs@SiO2 can be used not only for the PDT but for various bio-labeling applications.

**4. Conclusions**

The basic material studied in this work is NaYF4 NPs doped with Yb3+ and Tm3+ ions with a diameter of around 22 nm and a very narrow size distribution, coated by a thin SiO2 shell (~3 nm). These NPs, because of their upconverting properties (NIR light to visible light conversion), can be used for cell detection (especially because of the 808 nm emission) without an undesirable organic background. Due to the upconversion to UV and blue light, the UCNPs can efficiently generate ROS (without any PS on their surface), which allows for a new path in PDT. The obtained therapeutic test results show a high cell viability decrease after treatment. This has been demonstrated for the first time in this report. This could be crucial for PDT because it avoids the standard problems...
with organic dyes such as visible excitation or photobleaching. The lack of attached chemical molecules allows for easy modification of surfaces by biological molecules. This property may allow for selective cancer cell treatment. The specific bio-functionalization of UCNPs@SiO₂-PEG-NHS-anti-h-IgG by the anti-h-IgG antibody makes them a promising material for individually targeted therapy designed for a given type of cancer. The presented model experiments confirm the possibility of controlling NP targeting by using biological molecules.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

ORCID iDs

P Kowalik https://orcid.org/0000-0003-4273-4670
I Kamińska https://orcid.org/0000-0002-3386-6017
A Borodziuk https://orcid.org/0000-0001-6943-8316
B Sikora https://orcid.org/0000-0001-5902-9682

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Figure 7. Comparison of nitrocellulose membrane areas after dot-blotting with bio-functionalized UCNPs@SiO₂-PEG-NHS-anti-h-IgG: the membrane surface was analyzed by a confocal microscope under 980 nm excitation. The characteristic spectrum of NPs measured under 980 nm excitation (a), control area, uncovered by a h-IgG antibody (b), areas covered by a h-IgG antibody with increasing concentration of UCNPs@SiO₂-PEG-NHS-anti-h-IgG: 0.1 (c), 1 (d) and 10 μg ml⁻¹ (e).
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