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Optic bionanospherical probe from Gd_2O_3: Yb, Er upconverting nanosphere and mAbˆCD133 antibody for precise imaging label of cancer stem cell NTERA-2

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Abstract. *Rare earth photonic nanomaterials are increasingly prominently applied in various fields of biomedicine. Currently, there is greater focus on the investigation to control the size and shape of nanomaterials, including the nanospherical form, which allows for precise labeling by only one nanoparticle. This paper demonstrates, for the first time, the construction of a biological nanospherical probe (BNSP) Gd_2O_3: Yb^{3+}, Er^{3+}/Silica/NH/mAbˆCD133 for diagnostic labeling of cancer stem cells (CSCs) NTERA-2. The BNSP was constructed using highly monodisperse spheres with around 200nm uniform size of Gd_2O_3: 7.6% Yb^{3+}, 1.6% Er^{3+}. They were functionalized by an amine group-contained shell coating and conjugated with CD133 monoclonal antibody.*

The functionalized nanosphere Gd_2O_3: Yb, Er/silica/NH_2 showed strong upconversion luminescence in red color upon laser excitation in the near-infrared region at 975 nm. The Gd_2O_3: Yb^{3+}, Er^{3+}/silica/NH_2 was carefully implemented to conjugate mAbˆCD133 via a linker, glutaraldehyde, to obtain the predictable probe Gd_2O_3: Yb^{3+}, Er^{3+}/Silica/NH/mAbˆCD133. Then, this BNSP was tested in vitro for its capacity to label NTERA-2 cancer stem cells. The efficient labeling based on the fluorescent immunoassay method was detected by incorporating a nanophotometer, Field Energy Scan Electron Microscopy (FESEM), and precisely determined by fluorescent microscopy. The study shows that the BNSP is highly efficient with targeting capacity and specificity in the labeling of cancer stem cells. These advanced results open up promising avenues for the development of precise imaging diagnostics in cancer cellular biomedicine, and beyond.

Keywords: bionanoprobe; upconversion Gd_2O_3: Yb, Er; label; cancer stem cells NTERA-2.

Classification numbers: 78.55.Kz; 85.60.-q..

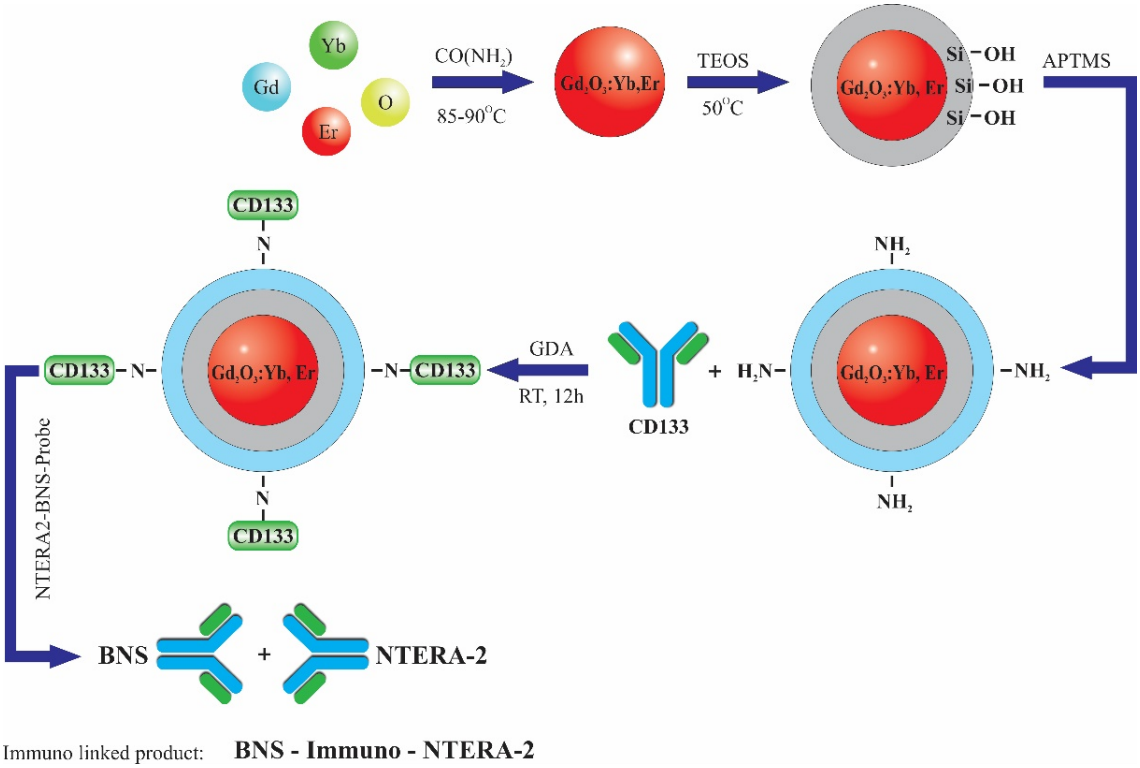
1. Introduction

Rare earth (RE)-based luminescent nanoparticles have received great attention in prominent applications in photonic, electronic, many technologies and especially in biomedicine [1–3]. Recently, among RE nanoluminophors, the spherical nanophosphors have been intensively investigated to develop a bio probe and drug carrier in biomedicine [4–6]. Moreover, RE up conversion (UC) nanophosphors in comparison to conventional fluorophores, such as organic dyes or semiconductor quantum dots, are promising optical contrast agents for biomedical applications due to their photo stability, sharp emission peaks, and long emission lifetime [7–10]. The infrared excitation allows deep tissue penetration of light beam, avoiding the background signal interference, and hindering bio cell damage. Furthermore, the nanoparticles from polymer or inorganic compounds as drug carrier are generally in the size range of 100 nm and 500 nm, but preffered size around 200–300 nm for medical application [11, 12]. Moreover, RE spherical nanoparticles with diameters around 200 nm are very useful to develop simultaneously cellular bio internalization labeling and targeted drug delivery in tumor cell medicine. Thus, for extensive use of UC nanoparticles as probes for biological applications, optimizing ideal conditions and new synthetic approaches are essential and need to be developed. Furthermore, the exploratory research of RE Nano spheres with intense UC luminescence built in unique nanostructure are intensively undertaken [13, 14]. In optimizing UC nanoparticles brightness and responsiveness for dynamic sensing, detection of stimuli with a nanoscale spatial resolution approaching that of individual nanoparticles remains an outstanding challenge. Here, there are highlight trend the existing capabilities and outstanding challenges of RE nanoparticles sensors, en-route to nanometer scale, single particle sensor resolution. In our laboratory, the research direction to achieve great monodispersity of Gd_2O_3: Yb^{3+}, Er^{3+} with the extremely low standard deviation, strong UC luminescence intensity, which meet simultaneous the requirements of the single sphere labeling probe and also the preferential size range around 200nm for drug efficient delivery [6, 15]. Recently we have started to investigate the construction way of a biomedical probe based on the high uniform nano sphere Gd_2O_3: RE^{3+} for precise detection of cancer stem cell via internalization label.

For the first time we present the construction research of a Biological Nano Spherical Probe (BNSP) Gd_2O_3: Yb^{3+}, Er^{3+}/silica/NH/mAbCD133 for labeling cancer stem cell NTERA-2. For

the whole process, the XRD, FESEM, FT-IR, Upconversion (UC) Luminescence is utilized to determine the structure, size, shape and optical properties. The nanosphere Gd_2O_3: Yb^{3+}, Er^{3+} was obtained by the synergic synthesis method and functionalized by using a solgel process. The functioned Gd_2O_3: Yb^{3+}, Er^{3+}/silica/NH_2 was studied to connect monoclonal antibody mAbˆCD133 via linker glutaral aldehyde for obtaining a BNSP probe. The BNSP used internalization labeling tumor stem cell NTERA-2 was implemented in vitro. The estimation of the labeling effect was observed by a nanophotometer, FESEM and especially determined by fluorescent mode of a Stochastic Optical Reconstruction Microscopy (STORM) system. The STORM measurement results were analyzed to obtain the images with fine resolution and contrast clearly shown the precise labeling capacity of the BNSP bio probe to cancer stem cell NTERA-2.

2. Experiment



Scheme 1. Construction proceed of Gd_2O_3: Yb^{3+}, Er^{3+}/silica/NH/mAbˆCD133 probe (BNSP).

The construction line from monodisperse nanosphere Gd_2O_3: Yb, Er to build the optic bionanospherical probe Gd_2O_3: Yb^{3+}, Er^{3+}/silica/NH/mAbˆCD133 (BNSP) and labeling immunoassay interaction is shown in Scheme 1. It can divide the line into 5 big steps: (1) synthesis of monodisperse nanosphere (2) functionalization by using solgel technique (3) conjugation between functioned nanosphere and antibody (4) labeling cancer stem cell by using immunoassay interaction (5) the observation and tracking by optical spectra and high resolution optical microscopy. To

fully implement the entire construction process, the first and second steps were carried out at the Photochemistry, Imaging, and Photonics Laboratory of Institute of Materials Science (IMS). The third and fourth steps were conducted at Prof. Do Thi Thao Lab, Institute of Biotechnology (IBT), while the final step was completed at the Nanobiophotonic Team Lab, Dr. Nguyen Trong Nghia, at the Institute of Physics (IOP).

2.1. Materials synthesis and construction of bionanosphere probe Gd_2O_3: Yb^{3+}, Er^{3+}/silica/NH/mAbˆCD133 (BNSP)

Synthesis of Gd_2O_3:Yb^{3+}, Er^{3+} nanospheres was modified the synergic chemical method reported previously elsewhere in [15]. In general, a mixture of RE salts of gadolinium, ytterbium, and erbium nitrate and urea in DI water was precipitated at temperature from 85˚C and 90˚C for 2-3 hours upon magnetic stir. The resulted powder was separated by a centrifugation around 8000 rpm, dried at 70˚C for 24 hours and then at 105˚C for 5 hours. The annealing treatment step by step at 650, 900, 1000 ˚C each step for 2 hours. To enhance the standard derivation of the diameter size were modified the synthesis conditions such as reaction temperature, urea/rare earth ratio and reaction proceed for more reasonable. Based on this synergic reaction procedure, the typical composition Gd_2O_3:7.6%Yb^{3+}, 1.6%Er^{3+} with the strongest upconversion intensity in the red color with maximum at 662 nm has been used for construction of BNSP.

Functionalization was prepared as follow: A mixture of isopropanol (Sigma-Aldrich, 30%) and DI water was added in suspension of Gd_2O_3:Yb^{3+}, Er^{3+}. After that ammonium hydroxide (Sigma-Aldrich, 28%-30%) was added and the stirring was maintained for another 1 hour. Then tetraethylorthosilicate (TEOS, Sigma-Aldrich, 97%) was added, and the solution was stirred overnight. The resulted particles were precipitated with acetone and then collected by centrifugation at 8000 rpm, DI water was used to redisperse particles, furthered 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich, 99%) in ethanol was added to the Gd_2O_3: Yb, Er/silica from the previous step, and then the solution was refluxed at 80ºC for 1 hour. Functionalized particles were centrifuged out at 8000 rpm and were further washed with a mixture 50/50 of DI water and ethanol. Finally, the obtained particles were dispersed in 2 mL ethanol.

The accomplishment of the conjugation and labeling step (the third and fourth step) was partly similar protocol for biological cellular label for cancer stem cell of the institute IBT as follow:

Conjugation was accomplished: Nanosphere Gd_2O_3: 7.6% Yb^{3+}, 1.6% Er^{3+}/silica/NH_2 in Phosphate Buffer Saline (PBS, pH 7.4) was gently vortexes before adding 0.5% glutaraldehyde solution in a ratio of 1:0.5 (*v*/*v*) and mixed for 1 hour at room temperature (RT) to disperse completely. The mixture was centrifuged and washed three times with PBS solution to remove glutaraldehyde. Then, CD133 monoclonal antibody (Thermo Fisher, Invitrogen, Carlsbad, CA, USA) was added into the glutaraldehyde pre-activated Gd_2O_3: 7.6% Yb^{3+}, 1.6% Er^{3+}/silica/NH_2 and incubated at 37°C for 30 min with gentle shaking. After incubation, the suspension was centrifuged at 6000 rpm for 5 min at 4 °C; the supernatant was retained to determine the amount of unconjugated antibodies in the combined efficiency study. The PBS washing residue Gd_2O_3: Yb^{3+}, Er^{3+}/silica/Ami/mAbˆCD133 (BNSP) was reconstituted in PBS and stored at 4°C. Conjugation efficiency was measured through the indirect determination of unbound IgG in the supernatant after combining mAb with nanomaterials using a NANOPHOTOMETER P300 system (IMPLEN.INC., USA).

2.2. BNPS used Labeling of Tumor Stem Cell NTERA-2

In this investigation, the NTERA-2 cell line, which is a pluripotent human embryonic carcinoma cell line, served as CSCs and CCD-18Co cells (the human colon normal) were used as healthy cells. Cells were maintained in DMEM medium supplemented with fetal bovine serum and antibiotics (antibiotic-antimycotic solution, Invitrogen, Carlsbad, CA, USA) in incubator at 37°C, 5% CO_2, and 100% humidity. Cells at log phase were seeded into 96-well plates with a concentration of 10,000 cells/well and incubated at 37°C, 5% CO_2 for 24 hours. The culture medium was removed, then cells were fixed with formaldehyde for 10 min at RT. The BNSP Gd_2O_3:Yb^{3+}, Er^{3+}/silica/NH/mAbˆCD133 was diluted in PBS before it was added into each well and incubated at 4°C for 1 hour. The unbound BNSP were washed away with PBS three times. At the end of process PBS was added to the wells before the cells were measured by the fluorescent mode of STORM. This system was built on the Ti2-E inverted microscope (Nikon). The excitation lasers used were near infrared at 975 nm with powers from 100 to 1000 mW and illuminated at the angle of 40 degrees on the top of samples. The camera used was EMCCD iXon Ultra 897 (Andor). The pixel dimension was 1.34 μm/pixel when capturing images with a 10x objective lens. All samples were immersed in just plain PBS and exposed to just their respective excitation lasers. Images of the samples BNS Probe + NTERA-2 cells and NTERA-2 cells were collected and the samples immersed in PBS were exposed to 975 nm near infrared diode laser excitation. Fluorescent images and corresponding transmission images were overlaid by Fiji software.

3. Results and discussion

In this work, high monodisperse nanospheres Gd_2O_3:Yb, Er, monoclonal antibody CD133 and cancer stem cell NTERA-2 were chosen for the investigations due to the following reasons: (1) Gadolinium oxide Gd_2O_3 are always used as a good host material for Ln^{3+} ions contained phosphors due to its low phonon cutoff energy (600 cm^{-1}) and chemical resistance, which can promote upconversion luminescence by inhibiting quenching effects both inside and on the surface of crystal particles. Additionally, Gd_2O_3 is a good matrix material with excellent biocompatibility features. (2) The 200 nm diameter nanosphere with very low standard deviation (SD) enable simultaneous cellular labeling and target drug carrying due to high flow mobility in physiological environment and chemical containing capacity. (3) Photonic bio nano probe Gd_2O_3: Yb, Er 200 nm nanosphere and mAbˆCD133 antibody were chosen for precise and high efficient label and possible target nanodrug due to mAbˆCD133 antibody possessing the ability to detect and treat cancers. (4) Moreover, cancer stem cell NTERA-2 was also chosen in this investigation because of their self-renewal, differentiation, and tumorigenicity abilities and the identification of CSCs is based on typical cellular surface markers, such as Cluster of Differentiation 133 (CD133), CD44, CD24, of which CD133 appears in various types of cancer cells in solid tumors. Thus, the investigation of internalization label by the BNPS would be implemented precisely in high efficacy [16, 17].

3.1. Structure, morphology, optical properties of nanosphere Gd_2O_3: Yb^{3+}, Er^{3+} and probe Gd_2O_3: Yb^{3+}, Er^{3+}/silica/Ami/mAbCD133

The SEM images of the typical products from the synergic synthesis and functionalization are presented in Figs. 1a and 1b, which indicate that one could control the size uniform around 200 nm diameter and the morphology of the nanosphere mostly monodisperse feature. The sphere with

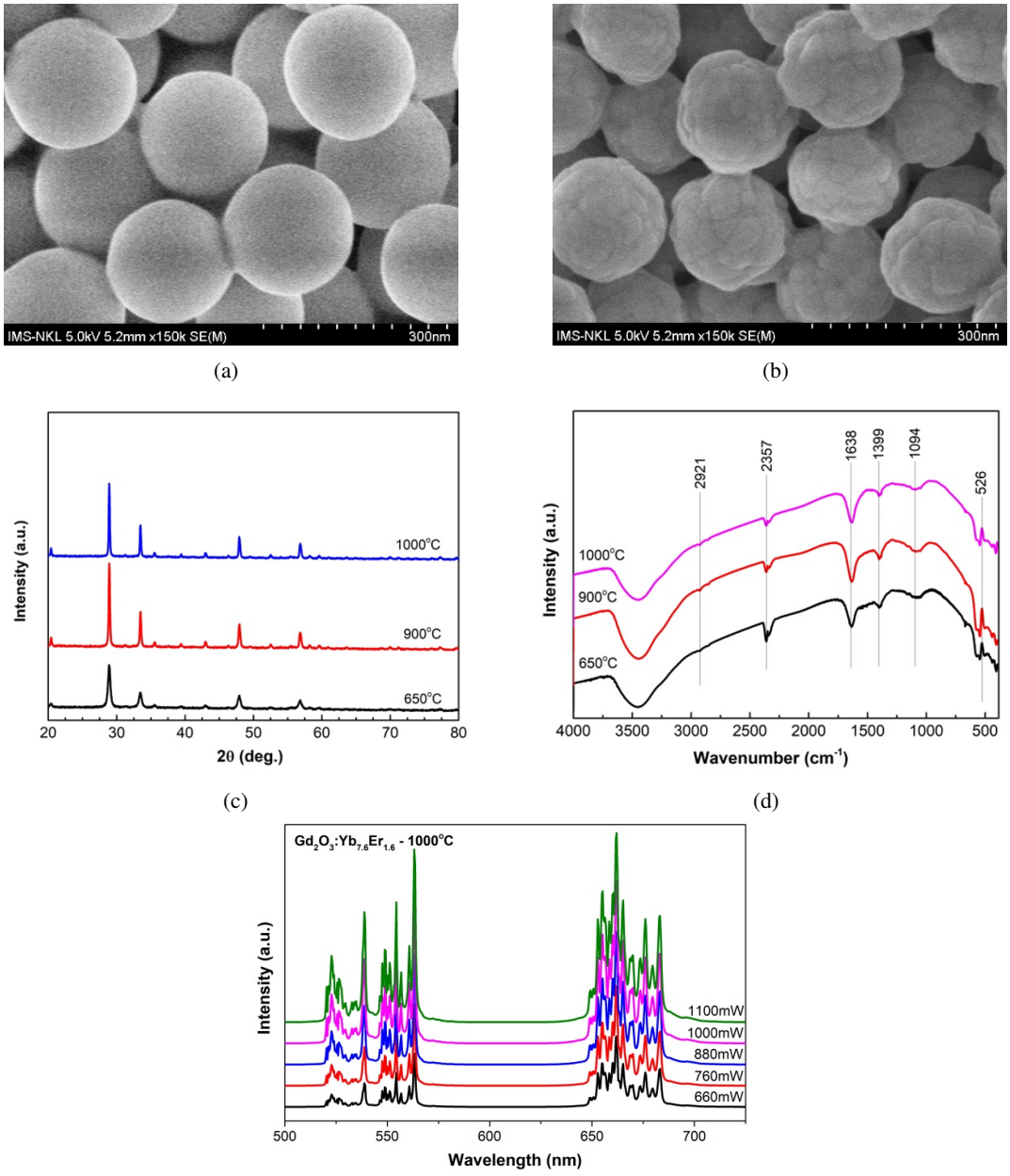


Fig. 1. SEM images of Gd_2O_3: Yb^{3+}, Er^{3+} (a) and Gd_2O_3:Yb^{3+},Er^{3+}@silica-NH_2 (b), XRD pattern (c), FT-IR (d), and UCL spectra of Gd_2O_3: Yb^{3+}, Er^{3+} (e).

the diameter from 100 nm to over 300 nm with SD0 below 5% could be produced. It is noted that the shape a spherical form remained after high temperature annealing from 200˚C to over 1000˚C.

However, the sphere surface became rougher as the annealing temperature increased. And porosity of the annealed sphere was increased strongly due to the release process of H_2O and CO_2 at high temperature in converting hydroxycarbonate $GdOHCO_3$: Yb, Er to oxide Gd_2O_3: Yb, Er. The precursor spheres $GdOHCO_3$: Yb, Er was amorphous, but Gd_2O_3: Yb, Er sphere was crystaline in cubic phase, which XRD pattern in comparison with the standard JCPDS card No. 11-0604 can be seen in Fig.1c. The controlled size and shape value are a little better compared to those from the previously reported our research in [15] and much better in [18]. The SD was below 5%. Furthermore, the FTIR spectra of Gd_2O_3: Yb,Er are shown in Fig.1d. The UCL spectra (Fig.1e) upon the excitation at 975 nm of Gd_2O_3: 7,6%Yb, 1,6%Er is shown red color with strongest band around 662 nm. The Upconversion fluorescence intensity strongly increased while the heating temperature increased from 650˚C to 1000˚C. It notes that the red UC emission ranging from 650 nm to 1000 nm "biological windows" could escape from the deeper tissue and be detected efficiently with higher signal-to-noise ratios [15]. These enable the development of a very effective photonic sensor to detect a disease cell.

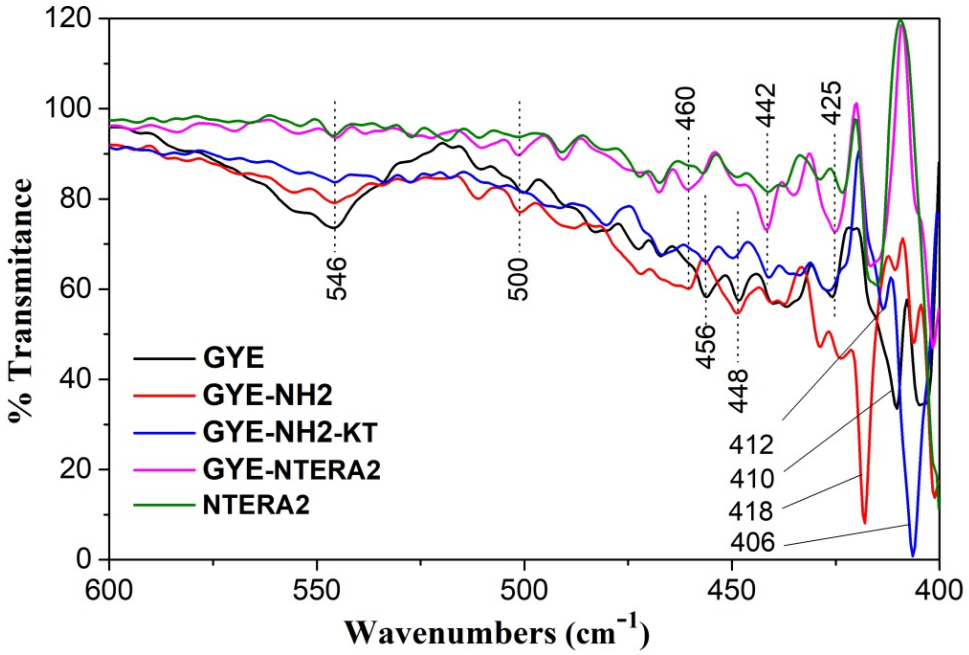


Fig. 2. FT-IR spectra of a) GYE (black curve), b) GYE-NH_2 (red curve), c) GYE-NH_2-KT (blue curve), d) GYE-NTERA2 (pink curve) and e) NTERA-2 as reference (paint green curve).

To better understand insight of the construction step it can be tracked the biochemical connection line illustrated in scheme 1, the FT-IR transmission spectra could be useful due to many sharp picks in the spectral region from 600 cm^{-1} to 400 cm^{-1} shown in Fig.2. It can be observed that the typical peaks around 546, 456 and 440 cm^{-1} are assigned to the Gd-O vibration of cubic Gd_2O_3 [19, 20]. The next step as functionalization with amine group the bands at 418 and

410 cm^{-1} appear, which were from the N-H vibrations of Amine group on the surface of GdYb, Er/silica nanospheres Gd_2O_3:Yb^{3+},Er^{3+}@Silica-NH_2 (GYE-NH_2) (red curve). In the third step of the conjugation, it could be indicated the appearances of the two strong and sharp bands at 412 and 406 cm^{-1} from C-H or C-C vibrations of the monoclonal antibody CD133. The last one of the connection between photonic bionanosphere probe BNSP and cancer stem cells could be tracked by the three significant sharp peaks 460 cm^{-1}, 442 cm^{-1} 425 cm^{-1} from the NTERA-2 cell. These typical bands enable exactly to determine the labeling efficiency of BNSP probe and NTERA-2 cell (the green curve). These results could indicate that the constructed stimuli probe can accurately label cancer stem cells.

3.2. Determination of label capacity of BNSP probe for precise labeling tumor stem cell NTERA-2

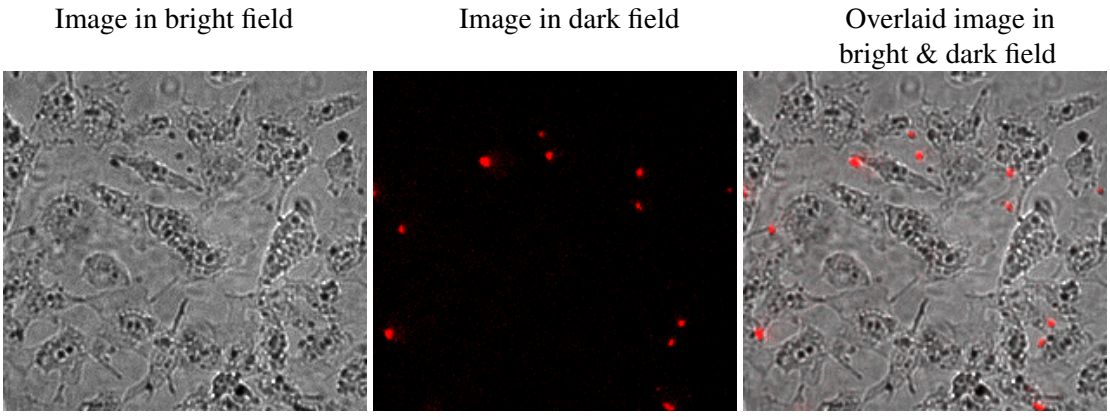


Fig. 3. Microscopic Images of Gd_2O_3:7.6% Yb^{3+},1.6% Er^{3+}@silica-NH_2-mAbˆCD133-NTERA2.

Fig. 4. Microscopic images of a cancer stem cell NTERA-2 as reference.

In this investigation, the observation by using a fluorescent mode of the home made STORM system was implemented by reference procedure, in which image of NTERA-2 cancer cells + BNSP probe in comparison with NTERA-2 cancer stem cells. The obtained microscopic images

shown in figures 3 and 4. The spatial position of the photonic bionanosphere probe on the cell's surface in the internalization field was demonstrated clearly by a fluorescence microscope under 975 nm laser diode excitation. Three fluorescence images of the NTERA-2 were obtained without/with BNSP probe in the cases of bright field, dark field, and overlaid modes as shown in figures 3 and 4, respectively. This indicates that we can only observe a typical NTERA cancer stem cell with/without BNSP probes in the bright field mode, whereas in the dark field mode, the BNSP probes are significantly enhanced as shown by the spatial position image in the outside cells due to the upconversion caner emission effect [21]. The overlaid images show clearly that the BNSPs have cleaved on the surface of NTERA-2 cancer stem cells. The presence of the BNSP probe in the internalization field of the NTERA-2 cancer stem cells is examined accurately. Therefore, this investigation of internalization labels by the BNSP probe would be implemented precisely in high efficacy, via whose microscopic images it can recognize the precise localization of BNSP probes on the surface of cancer stem cells. Based on its development for construction of a single particle sensing simultaneous target drug delivery to treat cancer cell will be possible.

There are quite different shapes of nanomaterials such as particle, rod, wire, tube, sheet and sphere etc. Among them, nanospheres such as Gd_2O_3: RE^{3+} due to intrinsic features of optics and magnetism properties are one of the most interesting photonic-plasmonic materials and have very promising application in cancer cellular biomedicine. Currently, research synthesis focused on the more precise controlling morphology, desired nano-size range, enhancing UCL intensity, and tuning color of RE-based nanoparticles [22]. Indeed, the nanospheres doped with rare earth, especially Gd_2O_3: Ln^{3+} nanospheres with solid or hollow structure, which could be a great candidate for drug delivery [23]. Besides there are $TbPO_4$ nanorods, fluorescent core–shell aluminosilicate 10nm nanoparticles, $NaYF_4$: Yb, Er for applied in the label, inhibition and thermometry of cancer cells reported [17, 24, 25]. Development of a fluorescent label tool based on lanthanide nanophosphors for viral biomedical application has been developed for estimation of the vaccine quality in industrial production lines [26]. The quite different significant utilization of the downconversion and upconversion emission RE luminescence nanomaterials from fundamentals to frontier applications suggested or developed [27–30]. The optimization of Tb^{3+}/Gd^{3+}ratio in nanorods for rapid detection of naja atra cobra venom was indicated [31]. The critical review of stochastic optical reconstruction microscopy (STORM), $NaYF_4$:Yb^{3+}/Er^{3+}@silica-TPGS Complexes, for theranostics issued [21, 32–34]. The core–shell structured nanoparticles and cathodoluminescent nanoprobes for temperature sensing and cathodoluminescent nanophosphor for electron microscopy could be examined [35, 36]. In short the bionanospherical probe BNSPs will be remained the most promising position as nanosize and nanosphere structure to develop a stimuli probe for single particle nanosensor, which could be enable applied in versatile branches of modern technology, breakthrough engineering, life science and theranostic medicine.

4. Conclusion

In summary, we have successfully fabricated a photonic biological nanosphere probe and applied it for precise labeling of cancer stem cells. We synthesized highly monodisperse nanosphere Gd_2O_3:7.6%Yb^{3+}, 1.6% Er^{3+} with a size of around 200nm using the modified synergic method. The nanospheres were then coated with a silica thin shell and functionalized with an amine group. The resulting functionalized monodisperse nanosphere Gd_2O_3:7.6% Yb^{3+}, 1.6% Er^{3+}/Silica/Amine exhibited strong upconversion luminescence intensity excited by a diode laser at 975nm which can

be used for precise label cancer cell in vitro. We successfully fabricated the biological nanosphere probe Gd_2O_3:Yb^{3+}, Er^{3+}/silica/NH/mAbˆCD133 using a cellular incubation hub, which was then tested for internalization labeling of cancer stem cells NTERA-2. Microscopic determination by STORM microscopy in fluorescent mode revealed that this bionanoprobe was highly effective, targetable, and specific, with the capacity for precise detection and identification of cancer stem cell NTERA-2. In conclusion, our experimental study demonstrates that the bionanospherical probe is a useful tool for developing precise labeling with multi-modal imaging in the diagnostics of cancer cellular biomedicine. It can also serve as a photonic nanostimuli structure platform for developing single particle sensing in the near future.

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