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A $NaYbF_4$: Tm^{3+} nanoprobe for CT and NIR-to-NIR fluorescent bimodal imaging

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ABSTRACT

Early diagnosis that combines the high-resolutional CT and sensitive NIR-fluorescence bioimaging could provide more accurate information for cancerous tissues, which, however, remain a big challenge. Here we report a simple bimodal imaging platform based on PEGylated $NaYbF_4$: Tm^{3+} nanopaticles (NPs) of less than 20 nm in diameter for both CT and NIR-fluorescence bioimaging. The as-designed nanoprobes showed excellent *in vitro* and *in vivo* performances in the dual-bioimaging, very low cytotoxicity and no detectable tissue damge in one month. Remarkably, the Yb^{3+} in the lattice of $NaYbF_4$: Tm^{3+} NPs functions not only as a promising CT contrast medium due to its high X-ray absorption coefficiency, but also an excellent sensitizer contributing to the strong NIR-fluorescent emissions for its large NIR absorption cross-section. In addition, these NPs could be easily excreted mainly *via* feces without detectable remnant in the animal bodies.

1. Introduction

X-ray computed tomography (CT), an efficient non-invasive clinical diagnosis technique, could give high-resolution 3D structure details of tissues based on their differential X-ray absorption features. However, CT imaging could not offer clear images of the cancerous lesions and their surrounding soft tissues due to the low density differences between them. Therefore, the CT contrast media (CM) become necessary to distinguish cancerous lesions from their surroundings. The clinically used CT CM is based on the molecule iodinated compounds, not because of their high-performance in X-ray absorption, but their low toxicity and cost. Therefore, large doses of iodinated CM must be applied to get clear images of soft tissues, which would unfortunately cause very short circulation time *in vivo* (within a few minutes) by rapid clearance *via* kidney [1]. These drawbacks limit their applications for targeted tumor diagnostic imaging in CT modality and cause potential renal toxicity because of their rapid kidney's accumulation [2]. Over the past few years, nanoparticles (NPs) as alternative CM have cast new light on long-time circulation and targeted CT imaging due to their unique size effects and interactions with biomolecules both on the cell surfaces and inside cells [3–11]. In addition to many developed iodinated polymeric NPs, such as liposomes, micelles and dendrimers, heavy metal based NPs have emerged recently as new CT CM due to their excellent performance in X-ray attenuation and good biocompatibility [12–15]. Among them, Au NPs are the most investigated candidates for contrast-enhanced *in vivo* CT imaging because of their low toxicity and high X-ray absorption coefficiency [15,16]. Recently, Weissleder's group has developed an efficient CT contrast medium based on Bi_2S_3 NPs, which shows much better performance than iodine based CM [14]. Later, Lu's group has developed a new method to produce ultra-small Bi_2S_3 nanodots for CT imaging [17]. However, these CM can only be used for contrast-enhanced CT imaging, and multifunctional nano-particulate platforms as contrast agents for more imaging modalities are urgently required to combine the merits of each imaging modality.

Despite the advantages in anatomical delineation, the CT imaging is not sensitive to cells and could not provide cell-level information. Comparatively, fluorescent imaging possesses unique advantages in the sensitivity and cellular level imaging. Once the cellular-sensitive fluorescent imaging is combined with high-resolution CT technology, more accurate information for cancerous tissues could be anticipated. Especially in cancer surgery,

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CT imaging can provide anatomical information assisting surgeons to evaluate tumor infiltration and determine the macroscopic resection extent, while with the help of intraoperative fluorescent imaging, the accurate tumor margin and microscopic lesions could be precisely delineated by using the contrast media selectively accumulated in tumor tissues *via* the enhanced permeability and retention (EPR) effect [18–20]. Therefore, several researchers have investigated the clinical potentials of dual-modal imaging intended to combine the unique advantages of CT and fluorescent imaging. Previously, Mericle's group reported CdS: Mn/ZnS quantum dots (QDs) to combine optical imaging with CT [21]. However, the large usage of QDs for CT imaging is not practically possible due to their inherent toxicity caused by Cd ions [22]. Very recently, Choi and Hyeon's groups developed TaO_x NPs modified with Rhodamine-B-isothiocyanate (RITC) for CT and fluorescent bimodal imaging [23]. However, the fluorescent emissions from such organic dyes are easy to quench and have low tissue penetration, which limit their further application for *in vivo* dual-imaging. In contrast, near infrared (NIR) excited upconversion NPs (UCNPs) with NIR emission have been regarded as stable fluorescent probes with low toxicity and deep tissue penetration, because NIR-to-NIR fluorescences are just in the 'optical transmission window' of tissues (750 ~ 1000 nm) [24–30]. In our previous research, we have constructed UCNP@SiO_2-Au composite NPs for fluorescent, magnetic resonance and CT imaging, where Au NPs act as efficient contrast media for CT imaging [31]. Whereas the efficient fluorescent imaging could not be obtained under the presence of large amount of attached Au NPs. Recently, several researchers have explored the feasibility of $NaGdF_4$ NPs as CT CM *in vitro* and *in vivo* (*via* local injection), but these preliminary works are only limited to the visible-fluorescent imaging with very low tissue penetration and the systematic investigations (including the toxicity, biodistribution and excretion *in vivo*, *etc.*) have not been involved [32,33]. Moreover, $NaGdF_4$ is not an efficient host material for UCNPs' emission compared with $NaYF_4$ and the fluorescent emission of UCNPs has been demonstrated to quench gradually with the increasing gadolinium (Gd) content in UCNPs especially when the doped content is higher than 40% [34]. Therefore, a platform for CT & strong NIR-fluorescent imaging still remains a big challenge and the investigations of its performance, biocompatibility, biodistribution and excretion behavior both *in vitro* and especially *in vivo* are also indispensible.

Interestingly, as a representative heavy metal, ytterbium (Yb) is a promising candidate as CT CM for its excellent biocompatibility and high X-ray absorption coefficient (*e.g.,* Yb: 3.88 $cm^2\ g^{-1}$; Gd: 3.11 $cm^2\ g^{-1}$ and I: 1.94 $cm^2\ g^{-1}$ at 100 keV) which is higher than gadolinium and double of iodine [35]. Coincidentally, Yb^{3+} is a sensitizer with a large absorption cross-section in the NIR region and its existence is very beneficial for the fluorescent emission of UCNPs [36]. It has been demonstrated that the fluorescent intensity of UCNPs can be enhanced by up to 43 times along with the increase of Yb^{3+} contents from 20 to 100% [37]. With the doping of 2% Tm^{3+} in UCNPs, the strong NIR emission (~800 nm) could be obtained and attributed to the Tm^{3+} transition ($^3H_4 \rightarrow {}^3H_6$) [37–39], having a deep fluorescent penetration in tissues. Therefore, due to the special dual-function of Yb^{3+} ions, the dual-bioimaging (CT & fluorescence) could be anticipated.

In this study, we developed a multifunctional contrast-enhanced imaging platform based on PEGylated upcoversion NPs-$NaYbF_4$: 2% Tm^{3+} nanoparticles as efficient contrast media for dual-modal CT and NIR-fluorescent imaging. These sub-20 nm NPs were explored their feasibilities in CT and NIR-fluorescent imaging both *in vitro* and *in vivo*. Moreover, the cytotoxicity, histological assessment, biodistribution and excretion of these nanoparticles have been systematically investigated in this report.

2. Experimental

2.1. Materials

Rare earth chlorides (99.9%) and 1-octadecene (90%) were purchased from Sigma-Aldrich. Oleic acid (OA), NaOH, NH_4F and CH_3OH were obtained from Shanghai Lingfeng Chemical Reagent Co., LTD. Methoxy PEG thiol (MW 5000, PEG_{5000}-SH) was purchased from JenKem Technology Co., Ltd. All reagents were of analytical grade and used without any purification.

2.2. Synthesis of UCM

$NaYbF_4$: 2% Tm^{3+} NPs were prepared according to the literature with slight modification [40]. In a typical procedure, 1 mmol of $RECl_3$ (RE = 98% Yb^{3+} and 2% Tm^{3+}) powder was dissolved in 100 mL flask containing 4 mL of water, 6 mL of oleic acid and 15 mL of 1-octadecene. The solution was heated to 160 °C for 1 h to remove the excess water and then cooled down to room temperature. Then, methanol solution of NH_4F (148 mg) and NaOH (100 mg) was added into the solution (pH value has been changed from 5.8 to 7.2) and stirred for 2 h. After the evaporation of methanol, the solution was slowly heated to 280 °C and maintained for 1 h under the protection of argon. The obtained product was washed with ethanol several times and finally dispersed in cyclohexane. After stirring with PEG_{5000}-SH water solution for 24 h, the hydrophobic NPs were easily changed to be hydrophilic with PEG modification. After washing with water for 5 times, the water-soluble UCM were obtained.

2.3. Characterization

X-ray powder diffraction (XRD) measurements were performed on a Rigaku D/MAX-2250 V diffractometer with graphite-monochromatized Cu Kα radiation. The morphology and energy-dispersive X-ray analysis (EDXA) of the nanocrystals were performed on a JEOL 200CX microscope operated at 200 kV. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Nicolet 7000-C

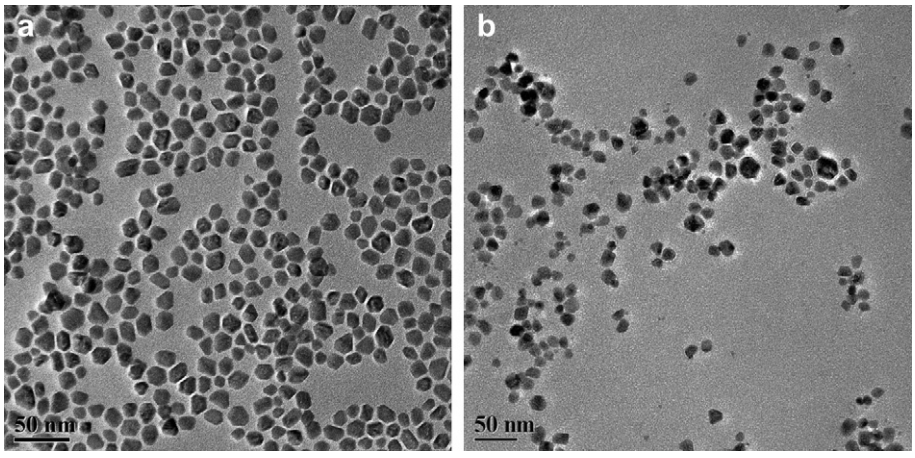

Fig. 1. TEM images of (a) OA-$NaYbF_4$: 2% Tm^{3+} in cyclohexane, (b) UCM in water.