Surface Modification on Rare-earth Doped Ceramic Nanophosphors via Ligand Exchange Method for Near-infrared Biophotonics

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1. Introduction
RARE-earTH doped ceramic nanophosphors (RED-CNPs) are promising materials for fluorescence bioimaging probes owing to their unique optical properties [1-3]. RED-CNPs are known to show over-1000 nm near-infrared (OTN-NIR) fluorescence under NIR excitation [1-5]. The OTN-NIR wavelength region (1000 - 1700 nm) is also called as “second biological window” and has much deeper tissue penetration compared to the below-1000 nm region for current fluorescence bioimaging [5]. Recently, one of the RED-CNPs, hexagonal-phase NaYF₄ nanoparticles (β-NaYF₄ NPs) have been demonstrated to be the best NIR phosphors [1, 6, 7]. However, most of synthesized β-NaYF₄ NPs are coated with a surface layer of hydrophobic oleic acid (OA) and thus is difficult to disperse in aqueous media. In this study, we investigated the surface modification of the β-NaYF₄ NPs via a facile ligand exchange route. The OA molecules on the NPs surface was at first replaced by BF₄⁻ anions, followed by displacement to anionic polymer, poly(acrylic acid) (PAAc). Additionally, poly(ethylene glycol) (PEG)/polycation block copolymer, PEG-b-polym(2-N,N-dimethylamino)ethyl methacrylate) (PEG-b-PAMA) was adsorbed on the negatively charged surface of the PAAc-capped β-NaYF₄ NPs (PAAc-NaYF₄ NPs) by electrostatic interactions. The PEG is well known as a biocompatible polymer to improve the dispersion stability and biocompatibility of NPs under physiological conditions [4, 8-10]. The preparation method, dispersion stability and cell toxicology are discussed in detail below.

2. Methods
The OA-capped β-NaYF₄ NPs co-doped with 20 mol % Yb³⁺ and 2 mol % Er³⁺ (OA-NaYF₄ NPs) were synthesized by thermal decomposition method as previously described in the literature [6]. The surface of the OA-NaYF₄ NPs was modified by a ligand exchange procedure [7]. Briefly, nitrosyl tetrafluoroborate (NOBF₄) (50 mg) was weighted into a vial container and dissolved in 5 mL of DMF. A solution of OA-NaYF₄ NPs (5 mg) in 1 mL of cyclohexane was added dropwise into the mixture and stirred for 2 h at room temperature. The obtained NOBF₄-capped NaYF₄ (NOBF₄-NaYF₄) NPs were purified by centrifugation (2 × 10⁴ G, 20 min, 3 times) with toluene, and the NOBF₄-NaYF₄ NPs were redispersed in DMF. Subsequently, the obtained NOBF₄-NaYF₄ NPs (5 mg) in 5 mL of DMF were added into the solution of PAAc (30 mg) in 5 mL
of DMF and stirred for 12 h at room temperature. The obtained PAAc-NaYF₄ NPs were purified by centrifugation (2 × 10⁴ G, 20 min, 3 times) with ethanol and pure water (1:1 v/v), and the solvent was changed to pure water. The PEG-b-PAMA (25 mg, Mn=5000/3000, Polymer source Inc., Canada) was dissolved in 5 mL of pure water and the solution was added into the PAAc-NaYF₄ NPs dispersion (5 mg/mL) and pH value was adjusted to 7.0 by adding NaOH and HCl. The mixture was stirred for 12 h at room temperature. Finally, free excess polymers in the solutions were removed by centrifugation (2 × 10⁴ G, 20 min, 3 times) and the obtained PEGylated NaYF₄ NPs were redispersed in water.

Figure 2. Change in the $\zeta$-potential of $\beta$-NaYF₄ NP samples with pH ranging from 3 to 12 (open squares) PAAc-NaYF₄ NPs and (filled circles) PEGylated NaYF₄ NPs ($\zeta$-potential measurement: room temperature; 10 mM NaCl solution).

Figure 3. Particle size distribution of $\beta$-NaYF₄ NPs in HEPES buffer solution (pH 7.4, 20 mM HEPES, 150 mM NaCl) PAAc-NaYF₄ NPs (dashed line) and PEGylated NaYF₄ NPs (solid line).

Figure 4. Viability of HLE cells after 24 h incubation with PEGylated NaYF₄ NPs at various concentrations.

3. Results and discussion

The OA-NaYF₄ NPs were synthesized by the thermal decomposition method [6]. Surface modification of the OA-NaYF₄ NPs was then carried out via ligand exchange method [7]. A Fourier transform infrared (FT-IR) spectroscopy suggested that the surface capping agent, OA on the surface of NaYF₄ NPs was replaced by BF₄– anions and followed by displaced by PAAc (data not shown). Then, PEG-b-PAMA was adsorbed on the PAAc-NaYF₄ surface by electrostatic interactions. The adsorption of polymer on the NaYF₄ NPs surface was confirmed by $\zeta$-potential measurement. Figure 2 shows the change in the $\zeta$-potential of surface modified NaYF₄ NPs with pH ranging from 3 to 12. The $\zeta$-potential of the PAAc-NaYF₄ NPs was about -35 mV at pH 7.4 and increased with decreasing pH. This results indicated the PAAc was adsorbed on the NaYF₄ NPs surface. In contrast, the $\zeta$-potential of the PEGylated NaYF₄ NPs were close to zero regardless of the environmental pH. This result suggest that the surface charge of the PAAc-NaYF₄ was effectively shielded by the PEGylation. Therefore, the PEGylated NaYF₄ was successfully obtained via stepwise surface modification, ligand exchange procedure and electrostatically adsorption. The dispersion stability of the obtained polymer modified NaYF₄ NPs were evaluated by dynamic light scattering (DLS) measurement. Figure 3 shows the particle size distribution of PAAc-NaYF₄ NPs and PEG-NaYF₄ NPs under physiological conditions. The average particle size of PAAc-NaYF₄ NPs was about 2000 nm. This result shows the PAAc-NaYF₄ NPs were difficult to disperse in high ionic solution environment by
electrostatic repulsion. On the other hand, the average particle size of PEGylated NaYF₄ NPs was about 150 nm. This result suggests that the PEG chains on the NPs surface prevents agglomeration of the β-NaYF₄ NPs and improves dispersion stability under physiological conditions. Furthermore, to confirm the potential toxicity of the PEGylated NaYF₄ NPs, trypan blue-exclusion test was carried out by using human hepatoma HLE cells [9]. As shown in Figure 4, the PEGylated NaYF₄ NPs have little effect on cell viability even at high concentrations. The amount of the PEG-NaYF₄ NPs at the highest concentration was 10 mg mL⁻¹, which was higher than that in previous reports on typical live cell imaging by using NaYF₄ NPs (1 - 5 mg mL⁻¹) [9, 10]. Therefore, we concluded that the PEG-NaYF₄ NPs have minimal cell toxicity. From these results, it is anticipated that the PEG-NaYF₄ NPs is suitable to be used as NIR bioimaging probes.

4. Conclusions
The surface of NaYF₄ NPs were successfully modified via stepwise modification procedure, ligand exchange and electrostatic adsorption reactions. The obtained PEGylated NaYF₄ NPs showed high dispersion stability under physiological conditions. Furthermore, the PEGylated NaYF₄ NPs displayed minimal cell cytotoxicity in the range of general criteria of RED-CNPs concentration for live cell imaging. Therefore, the obtained PEGylated NaYF₄ NPs is a promising candidate of NIR imaging probes.

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References