Multifunctional Eu$^{3+}$/Gd$^{3+}$ dual-doped calcium phosphate vesicle-like nanospheres for sustained drug release and imaging

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1. Introduction

Calcium phosphates (CaP) are the most important inorganic constituents of human hard tissues including bone and tooth [1–3]. Therefore, synthetic CaP materials have been recognized as promising biomaterials with a great value and significance, and have been investigated for applications in bone repair/tissue engineering, drug and gene delivery, and other biomedical areas [4–8]. CaP materials with different morphologies including nanorods, plate-like nanocrystals, nanoparticles, nanotubes and three-dimensional structures have been prepared [9–15]. The chemical composition, structure and properties of CaP materials are usually determined by their preparation process. The facile synthesis of CaP nanostructured materials with well-defined structure, size, morphology and multifunctions is of great significance and remains a great challenge. Recently, amphiphilic block polymers were used in the synthesis of CaP nanostructures which showed enhanced properties in the application of drug/gene delivery [16–18].

Luminescence imaging has become an important tool for biomedical applications, particularly in the region of near-infrared (NIR) spectrum [19,20], in which low absorptivity by tissue chromophores can be used in deep-tissue imaging [21]. However, many fluorophores of organic molecules show low fluorescence quantum yield, solvatochromic effects, photobleaching, short half-life and in vivo instability, which limit their applications in vivo [22–24]. It has been reported that nanostructured CaP significantly increased brightness and prolonged signal intensity when organic fluorophores were encapsulated compared with the free fluorophores [25,26].

Europium, with 4f intra orbital electronic transitions which span both the visible and near-infrared ranges, leads to long lifetimes of the excited states, and allows the use of time-resolved detection, a definitive asset for bioassays and biological luminescence imaging [27–29]. Meanwhile, gadolinium can be used as a contrast agent to provide brighter magnetic resonance (MR)
signal [30]. Furthermore, lanthanide ions including Eu³⁺ and Gd³⁺ are known functional mimics of Ca²⁺ ions and have been shown to affect the bone remodeling cycle, and have a potential for the treatment of bone density disorders such as osteoporosis [31–32]. Recently, the research on lanthanide ions-doped calcium phosphate for biomedical applications has become a hot topic [33–37].

Herein, we report a facile room-temperature solution method for the preparation of multifunctional Eu³⁺ and Gd³⁺ dual-doped CaP (Eu³⁺/Gd³⁺/CaP) vesicle-like nanospheres in the presence of an amphiphilic block copolymer polylactide–block–monomethoxy(polyethylene glycol) (PLA–mPEG). The multifunctions of photoluminescence (PL), magnetism and drug delivery are realized in Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres. The Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres are promising for applications in the biomedical fields such as multifunctional drug delivery systems and tissue engineering scaffolds with bio-imaging guidance.

2. Materials and methods

2.1. Preparation of Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres

The block copolymer PLA–mPEG (Mw = 8000) was purchased from Jinan Daigang Biomaterials Co. Ltd, and the molecular weight of the mPEG segment was 5000. Other chemicals were purchased from Sinopharm Chemical Reagent Co. and used as received without further purification. For the preparation of undoped CaP sample, 0.3548 g of Na₂HPO₄·12H₂O and 0.0250 g of PLA–mPEG were dissolved in 60 mL of deionized water to form Solution A. 0.1660 g of CaCl₂, 0.0250 g of PLA–mPEG were dissolved in 60 mL of deionized water to form Solution B. Then, Solution B was added into Solution A at an addition rate of 20 mL min⁻¹. The mixed solution was stirred at 1000 rpm and kept for 30 min at room-temperature. The pH value was maintained at 11 by slow addition of ammonia.

For the preparation of Eu³⁺/Gd³⁺/CaP–mPEG vesicle-like nanospheres, the experimental procedure was the same as the above except that certain amounts of europium nitrate and gadolinium nitrate were dissolved in Solution B. The total doping concentration of Eu³⁺ and Gd³⁺ was fixed at 5 mol% relative to Ca²⁺, and the molar ratio of Ca²⁺/Gd³⁺ was 1:0, 2:1, 1:1, 1:2 and 0:1. The product was washed with deionized water several times and drying to dry powder.

2.2. Characterization of samples

The dynamic light scattering (DLS) and Zeta potential measurements were performed using a Zeta potential analyzer (ZetaPlus, Brookhaven Instruments Corporation). X-ray powder diffraction (XRD) patterns were recorded using a Rigaku D/max 2500V X-ray diffractometer with a graphite monochromator (Ku Kα radiation, λ = 1.54178 Å). Transmission electron microscopy (TEM) micrographs and energy dispersive spectroscopy (EDS) were taken with a JEOL JEM 2100 field-emission transmission electron microscope. The transmission electron microscope (TEM) was operated at an accelerating voltage of 750 nm in flowing air. The photoluminescence (PL) measurements were carried out on a spectrofluorometer (Fluorolog-3, Jobin Yvon) at room-temperature. The BET specific surface areas were measured with an accelerated surface area and porosimetry system (Micromeritics ASAP2010, USA). A physical property measurement system (PPMS, Quantum Design, USA) was used to evaluate the magnetic properties at room-temperature.

2.3. Drug loading and in vitro drug release

The typical drug loading and in vitro drug release experiments were performed as follows: 0.5 g of dried powder of Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres was added into 50 mL hexane with an ibuprofen concentration of 40 mg mL⁻¹. The suspension in a sealed vessel was treated with ultrasonic for 1 h, and then was shaken in a sealed vessel for 24 h at 37 °C. The sample with loaded drug was removed from the solution by centrifugation, and the filtrate was analyzed by UV–vis absorption spectroscopy at a wavelength of 263 nm to evaluate the ibuprofen loading capacity. The drug loaded sample was rinsed with fresh hexane and dried to powder at 60 °C in air. Then, the powder was compacted into disks (0.3 g for each disk, diameter = 10 mm) at a pressure of 3 MPa. For the drug release experiments, each disk was immersed into 200 mL of simulated body fluid (SBF) at 37 °C and shaken at a certain rate using a desktop-type oscillator (THZ–D, China). 2 mL of the solution was withdrawn for UV–vis absorption analysis at 263 nm at given time intervals to measure the released amount of ibuprofen. This quantity of solution was replaced with the same volume of fresh SBF. Hydroxyapatite (HAp) nanorods used as a control sample in drug loading and release experiments were prepared according to our previous report [36].

2.4. Cell viability test

The human gastric carcinoma cells (MGC–803), which were cultured in an RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C for 48 h, were used for cell viability tests. Then, the cells were seeded in 96 well flat-bottom microassay plates at a concentration of 1 × 10⁴ cells mL⁻¹ and cultured for 24 h. The sterilized samples were added into wells at the concentrations from 10 to 100 μg mL⁻¹, and were co-cultured with cells for 48 h. The sample free tissue culture plate was used as a control. The cell viability was quantified by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and the data are representative as the mean value of five parallel experiments.

2.5. In vitro MR imaging

The as-prepared Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:2) were dispersed in aqueous solution at different concentrations for in vitro T1- and T2-weighted MR imaging and MR relaxometry. The T1- and T2-weighted images were acquired with a conventional spin echo acquisition (repetition time = 1000 ms) with an echo time of 60 ms, section thickness of 0.6 mm in 0.5 T MinMR systems (Shanghai Niumag Corporation, China). The MR relaxometry measurements including relaxation times T1 and T2 were recorded using an NMII0–Analyst (Shanghai Niumag Corporation, China).

2.6. In vitro and in vivo imaging

In vitro and in vivo luminescence imaging and in vivo X-ray imaging were done with the Carestream FX PRO in vivo image system (Kodak, USA). The images were captured and analyzed using the Carestream Molecular Image SE software (Kodak, USA). All animal procedures were in accord with institutional animal use and care regulations. The nude mice weighing 20–22 g were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (China). The nude mice were randomized into two groups with three animals per group, including the control group and subcutaneous injection group. After the mice were anesthetized with 100 μL of 10% chloral hydrate, 20 μL of 0.9% NaCl solution containing Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres with a concentration of 1 mg mL⁻¹ was subcutaneously injected into the abdomen area of the mice. The excitation filter of 670 nm and emission filter of 750 nm were used for luminescence imaging.

3. Results and discussion

3.1. Characterization of Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres

PLA–mPEG, as an amphiphilic block copolymer, can form spherical micelles through self-assembly in aqueous solution, and has been used in drug delivery application [38]. It was reported that the negatively charged oxygen atoms of C–O–C groups in PEG molecules could interact with Ca²⁺ cations to form Ca²⁺–PEG complexes via electrostatic attraction [39,40]. PLA–mPEG exhibits favorable properties for hybrid nanomaterials and displays remarkable colloidal stability at high ionic strength [41]. Therefore, spherical micelles of PLA–mPEG in aqueous solution can be used as the excellent template for the deposition of CaP. The presence of Eu³⁺/Gd³⁺ ions in the same reaction system can dual-dope in CaP in the precipitation reaction, and realizes photoluminescent and magnetic multifunctions of CaP. The formation of Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres may follow a possible process depicted in Fig. 1. First, PLA–mPEG molecules self-assemble to form micelles in aqueous solution before the CaP precipitation reaction occurs, the micelles are constructed with the hydrophobic PLA section as the core and hydrophilic PEG section as the outer layer. The CaP precipitation reaction takes place on the hydrophilic PEG chains self-assembled shell of the PLA–mPEG micelle, forming the amorphous CaP layer. At the same time, Eu³⁺/Gd³⁺ dual-doping in CaP also proceeds during the CaP precipitation process. In this way, Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres form. The PLA–mPEG micelles play an important role in the formation of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres, acting as the soft template. The morphology of the as-prepared undoped CaP and Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:1) was investigated with TEM. Fig. 2 shows the TEM micrographs of undoped CaP
and Eu\(^{3+}/\text{Gd}^{3+}\)-CaP samples. One can see that both samples exhibited the morphology of vesicle-like nanospheres with diameters ranging from 10 to 30 nm. The size distribution of the as-prepared Eu\(^{3+}/\text{Gd}^{3+}\)-CaP vesicle-like nanospheres dispersed in ethanol was measured by dynamic light scattering (DLS), and the result is shown in Fig. 2c. The sizes of Eu\(^{3+}/\text{Gd}^{3+}\)-CaP vesicle-like nanospheres in solution were mainly in the range of 91–120 nm, and the average size was approximately 100 nm. The EDS measurement was performed for Eu\(^{3+}/\text{Gd}^{3+}\)-CaP vesicle-like nanospheres (Fig. 2d), which showed that the product consisted of

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**Fig. 1.** The proposed formation process of Eu\(^{3+}/\text{Gd}^{3+}\)-CaP vesicle-like nanospheres.

**Fig. 2.** (a) TEM micrographs of undoped CaP vesicle-like nanospheres; (b) TEM micrographs, (c) the size distribution in ethanol and (d) EDS pattern of Eu\(^{3+}/\text{Gd}^{3+}\)-CaP vesicle-like nanospheres.
Ca, P, Eu and Gd elements, implying that the product was Eu\(^{3+}\) and Gd\(^{3+}\) dual-doped calcium phosphate. The total content of Eu and Gd in Eu\(^{3+}\)/Gd\(^{3+}\)–CaP vesicle-like nanospheres was about 4.6 mol\% of Ca, which is close to the nominal doping ratio of 5 mol\%.

The XRD patterns (Fig. 3) of both undoped CaP and Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres (Eu\(^{3+}\)/Gd\(^{3+}\) = 1:1) exhibited no discernable peaks of crystalline calcium phosphate but a characteristic hump of the amorphous CaP phase at around 2\(\theta\) = 30\(^{\circ}\), indicating that both samples consisted of the amorphous CaP phase [42]. Amorphous calcium phosphate (ACP) is an important kind of calcium phosphates and exists in the natural bone, thus ACP is promising for applications in many biomedical fields. Compared with HAp, ACP is bioactive with a better bio-degradability, and can promote osteoblast adhesion and osteoconductivity [43,44]. However, the development of multifunctional nanostructured biosystems based on ACP is still less reported.

TG analysis was employed to determine the content of PLA–mPEG in as-prepared undoped CaP vesicle-like nanospheres. The TG curves of the samples of undoped CaP vesicle-like nanospheres and the CaP control sample which was prepared in the absence of PLA–mPEG are shown in Fig. 4. On the basis of the TG analysis, the total weight loss was about 25.7\% and 16.9\% for the undoped CaP vesicle-like nanospheres and CaP control sample, respectively. The weight percentage of PLA–mPEG in the undoped CaP vesicle-like nanospheres was estimated to be about 8.8\%.

3.2. Photoluminescence properties of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres

PL excitation and emission spectra of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres measured at room-temperature are shown in Fig. 5a,b. The excitation wavelength was chosen according to the excitation spectrum in Fig. 5a, from which one can see that two intense excitation peaks were located at 393 and 464 nm. In the PL emission spectra (Fig. 5b), four intense emission peaks appeared at about 590, 616, 650 and 700 nm at an excitation wavelength of 393 nm, and they can be attributed to the \(5\mathrm{D}_0 \rightarrow 7\mathrm{F}_1\), \(5\mathrm{D}_0 \rightarrow 7\mathrm{F}_2\), \(5\mathrm{D}_0 \rightarrow 7\mathrm{F}_3\) and \(5\mathrm{D}_0 \rightarrow 7\mathrm{F}_4\) transitions within Eu\(^{3+}\) ions, respectively. Our experiments indicated that the PL intensity varied considerably by varying Eu\(^{3+}\) and Gd\(^{3+}\) concentrations. The PL emission intensities of all samples of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres were lower than that of the sample prepared at 5 mol\% Eu\(^{3+}\) doping without Gd\(^{3+}\). The PL emission intensity decreased with reducing content of Eu\(^{3+}\) dopant, and no obvious PL emission was observed at 5 mol\% Gd\(^{3+}\) doping without Eu\(^{3+}\). However, when the doping molar ratio of Eu\(^{3+}\) to Gd\(^{3+}\) was 1:2, the PL emission intensities at 616 and 700 nm were higher than those at the ratio of 2:1 and 1:1, indicating that the PL emission intensity reached a peak at a certain concentration of Gd\(^{3+}\) doping in this system. The PL spectrum of a control sample prepared with the same Eu\(^{3+}\) doping concentration but in the absence of Gd\(^{3+}\) under the same conditions was also measured (Fig. S1, supporting information), from which one can see that the PL intensity was obviously lower than that of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres (Eu\(^{3+}/\)Gd\(^{3+}\) = 1:2). A similar result was reported in the Eu-dibenzoylemethane-cetylpyridinium-chloride system, in which the fluorescence intensity of the system significantly increased by Gd\(^{3+}\) doping in the presence of triethanolamine [45]. Under the irradiation at a wavelength of 365 nm by an UV lamp, the powder of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres exhibited visible PL emission, as shown in Fig. 5c. The powders of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres with various doping concentrations could be well re-dispersed in ethanol, and the resulting colloidal solutions were stable for a relative long period of time (Fig. 5d).

3.3. Specific surface area and drug loading and release

Fig. 6 shows the N\(_2\) adsorption–desorption isotherms of the undoped CaP and Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres (Eu\(^{3+}/\)Gd\(^{3+}\) = 1:2). According to the International Union of Pure and Applied Chemistry (IUPAC), they can be classified as a type IV isotherm loop which is characteristic of the porous structure [46]. The Brunauer–Emmett–Teller (BET) specific surface area of undoped CaP and Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres (Eu\(^{3+}/\)Gd\(^{3+}\) = 1:2) were 86.7 and 85.4 m\(^2\) g\(^{-1}\), respectively. A control sample of HAp nanorods was used for comparison, and the TEM micrograph of this sample is shown in Fig. S2 (supporting information). The BET specific surface area of HAp nanorods was measured to be 47.2 m\(^2\) g\(^{-1}\) [36]. The Zeta potential tests were employed to investigate the surface charge property of as-prepared Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres. The Zeta potential of Eu\(^{3+}/\)Gd\(^{3+}\)–CaP vesicle-like nanospheres was measured to be about +10.7 mV, which is much higher than those of undoped CaP vesicle-like nanospheres (–6.9 mV) and the control sample of HAp nanorods (+0.71 mV). The zeta potential of ibuprofen aqueous solution was as low as –271 mV [36].

The drug loading capacity of the as-prepared samples was evaluated from the absorbance values of ibuprofen hexane solution before and after drug loading. The drug loading capacity of HAp nanorods, undoped CaP and Eu\(^{3+}/\)Gd\(^{3+}\)–CaP (Eu\(^{3+}/\)Gd\(^{3+}\) = 1:2) vesicle-like nanospheres reached 653.5, 761.9 and 833.8 mg per
Fig. 5. (a) Room-temperature excitation spectrum and (b) PL emission spectra of Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres; (c) the photograph of the powders of Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres under the irradiation at a wavelength of 365 nm by an UV lamp; and (d) the photograph of the solutions of Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres dispersed in ethanol after storage for 24 h. The doping concentration of Eu$^{3+}$/Gd$^{3+}$ is at 5 mol% relative to Ca$^{2+}$, and the molar ratios of Eu$^{3+}$ to Gd$^{3+}$ in (c) and (d) from 1 to 5 are 1:0, 2:1, 1:1, 1:2 and 0:1, respectively.

Fig. 6. N$_2$ adsorption–desorption isotherms: (a) undoped CaP vesicle-like nanospheres; (b) Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres (Eu$^{3+}$/Gd$^{3+}$ = 1:2).

Fig. 7. (a) The drug release curves of the control sample of HAp nanorods, undoped CaP vesicle-like nanospheres and Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres (Eu$^{3+}$/Gd$^{3+}$ = 1:2) in simulated body fluid (SBF); (b) the cumulative drug release percentage versus square root of release time for the three drug delivery systems in SBF.
gram carrier, respectively. The drug loading capacity of the as-synthesized Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres was much higher than those of HAp nanorods and undoped CaP vesicle-like nanospheres. This result may be explained by the factors such as BET specific surface area and zeta potential of the drug nanocarriers. The higher zeta potential led to enhanced electric charge interaction between Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres and ibuprofen molecules. Therefore, Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres are favorable for the application as the drug nanocarriers.

The drug release behaviors of drug loaded HAp nanorods, undoped CaP and Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres (Eu\(^{3+}\)/Gd\(^{3+}\) = 1:2) in SBF were also investigated, and the drug release curves of three drug delivery systems (tablet samples) are shown in Fig. 7a. From Fig. 7a, one can see that both drug delivery systems of undoped CaP and Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres had an ultralong sustained drug release process. The cumulative drug release percentages from undoped CaP vesicle-like nanospheres were 13.6, 20.4, 61.7 and 89% at a release time of 24, 180, 1004 and 2016 h, respectively. In comparison, the drug delivery system of Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres had a similar drug release rate to that of undoped CaP vesicle-like nanospheres, and the cumulative drug release percentages from Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres were 14.8, 20.4, 60.2 and 85% at a release time of 24, 180, 1004 and 2016 h, respectively. In contrast, the cumulative drug release percentages of ibuprofen from HAp nanorods were 75% and 82% at 24 and 60 h, respectively, that is to say, the drug release from HAp nanorods was much faster than those from both drug delivery systems of undoped CaP and Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres. Both drug delivery systems of undoped CaP and Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres showed an ultralong and sustained drug release (more than 80 days), which avoided the explosive drug release and significantly prolonged the drug effect. Compared with HAp nanorods, the significantly prolonged drug release from Eu\(^{3+}\)/Gd\(^{3+}\)/CaP vesicle-like nanospheres were 14.8, 20.4, 60.2 and 85% at a release time of 24, 180, 1004 and 2016 h, respectively.
like nanospheres may be explained by the combined effects of several factors such as relatively high specific surface area, surface chemical properties, the presence of amphiphilic block copolymer of PLA–mPEG and the interaction between drug molecules and the nanocarrier. Fig. 7b shows a relationship between the cumulative amount of released drug and the square root of release time for the three drug delivery systems. According the Higuchi model, the drug release from three drug delivery systems may be governed by a diffusion process.

3.4. Cytotoxicity tests

The cytotoxicity tests of the as-prepared control sample of HAp nanorods, undoped CaP and Eu³⁺/Gd³⁺/CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:2) were performed using human gastric carcinoma cells (MGC-803). The MTT assays revealed that there was essentially no toxicity when the cells were subjected to the three samples at the concentrations in the range of 10–100 µg mL⁻¹ (Fig. 8).

3.5. In vitro magnetic resonance imaging (MRI)

Due to the isotropic electronic ground state ⁸S₇/₂ and half-filled f-orbital with seven electrons, Gd³⁺ ions possess a high magnetic moment, leading to obvious effects on both longitudinal and transverse proton relaxation even at low applied magnetic fields. Gd-containing materials have a potential as MRI contrast agents because of their positive signal enhancement ability. Fig. 9 shows the room-temperature magnetization curves of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres with different doping concentrations. Obviously, the samples of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres exhibited a paramagnetic behavior, and the magnetization increased with increasing doping concentration of Gd³⁺.

Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres were evaluated for their relaxation time T₁- and T₂-weighted images. In order to further investigate the MRI contrast effect, T₁ and T₂ relaxation times of the aqueous solutions containing Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:2) at different concentrations were measured. The longitudinal and transverse relaxation rates (1/T₁ and 1/T₂) as a function of the concentration of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres exhibited a well-correlated linear relationship (Fig. 10). Fig. 11 shows the T₁- and T₂-weighted images in the concentrations of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres ranging from 0.5 to 10 mg/mL. As the concentration of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres increased, the T₁-weighted MRI signal intensity continuously increased, resulting in brighter images, and on the other hand, T₂-weighted MRI signal intensity continuously decreased, resulting in darker images. These results suggest that Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres have a potential application as a positive MRI contrast agent.

Fig. 12. The in vitro fluorescence imaging of phosphate buffer saline (PBS) solution containing as-prepared Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:2) at different concentrations overlaid on visible light image of the same samples. The concentrations of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres from 1 to 4 were 0, 0.5, 1 and 2 mg mL⁻¹.

Fig. 13. (a) The optical image, (b) in vitro near-infrared fluorescence image, and (c) X-ray and fluorescent overlaid image of the nude mice after subcutaneous injection without (left) and with (right) Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:2); (d) X-ray image of the mice after subcutaneous injection of Eu³⁺/Gd³⁺–CaP vesicle-like nanospheres (Eu³⁺:Gd³⁺ = 1:2); (e) Pixel intensities obtained from X-ray images at different sites of the mouse. The labels from 1 to 4 stand for the positions of subcutaneous injection, abdomen, chest, and spine.
3.6. In vivo near-infrared fluorescence imaging

The applicability of as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres (Eu$^{3+}$/Gd$^{3+}$ = 1:2) as a NIF fluorescence imaging contrast agent was investigated in vitro. The excitation spectrum was scanned from 410 to 610 nm, and the result indicated that the emission wavelength of 700 nm was specific when the emission wavelength was fixed at 700 nm (Fig. S3, supporting information). Under the excitation at the wavelength of 700 nm, the as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres exhibited a strong near-infrared emission at 700 nm, as shown in Fig. 12.

To demonstrate the feasibility of as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres as the agent for in vivo NIF fluorescence imaging, the as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres (Eu$^{3+}$/Gd$^{3+}$ = 1:2) were subcutaneously injected into the mice (20 μL with a concentration of 1 mg mL$^{-1}$ per animal). The mice were imaged using an excitation wavelength of 700 nm, as shown in Fig. 13. The corresponding subcutaneous injection site of the mice in the Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres administrated group displayed a clearly distinguished NIF fluorescence signal, whereas no obvious NIF fluorescence signal was observed in the mice as the control experiment, indicating that the Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres are effective for NIF fluorescence imaging in vivo.

The X-ray contrast property of as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres (Eu$^{3+}$/Gd$^{3+}$ = 1:2) was also studied. Fig. 13d shows the in vivo whole-body X-ray imaging of the nude mouse with the injection of Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres. Compared with the positions of abdomen, chest and spine, the pixel intensity obtained from the X-ray image of subcutaneous injection position was similar to the data from the position of spine (Fig. 13e). The obvious signal of Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres under X-ray irradiation may be explained by the similar chemical nature to bone tissue. The above results indicate that the as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres are promising for the application in the fluorescence and X-ray dual-modal imaging in vivo.

4. Conclusions

A facile room-temperature solution method for the preparation of multifunctional Eu$^{3+}$/Gd$^{3+}$ dual-doped CaP vesicle-like nanospheres in the presence of an amphiphilic block copolymer PLA–mPEG has been developed. The photoluminescent and magnetic multifunctions of CaP vesicle-like nanospheres are realized by dual-doping with Eu$^{3+}$/Gd$^{3+}$ ions. The as-prepared Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres exhibit essentially an inappreciable toxicity to the cells in vitro. Under the excitation of a wavelength at 393 nm, Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres exhibit a strong near-infrared emission at 700 nm in the NIF spectrum, and the PL intensity can be adjusted by varying Eu$^{3+}$ and Gd$^{3+}$ concentrations. Furthermore, Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres can be used as the drug nanocarriers and have a high drug loading capacity and ultralong sustained drug release using ibuprofen as a model drug, and the drug release from the drug delivery system of Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres can sustain for a very long period of time (more than 80 days). More importantly, the noninvasive visualization of nude mice with subcutaneous injection indicates that the Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres are suitable for the application in in vivo bio-imaging. Therefore, the Eu$^{3+}$/Gd$^{3+}$-CaP vesicle-like nanospheres are promising for applications in the biomedical fields such as multifunctional drug delivery systems and tissue engineering scaffolds with bioimaging guidance.

Acknowledgements

The financial support from the National Basic Research Program of China (973 Program, No. 2012CB933600, No. 2010CB933901), the National Natural Science Foundation of China (51172260, 51102258, 51121064), the Science and Technology Commission of Shanghai (11nm0506600, 1052nm06200, 11ZR1441800) and CAS/SSAFEA International Partnership Program for Creative Research Teams is gratefully acknowledged.

Appendix A. Supplementary material

Supplementary data associated with this paper can be found, in the online version, at doi:10.1016/j.biomaterials.2012.05.059.

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