Ligand-free upconversion nanoparticles for cell labeling and their effects on stem cell differentiation

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Abstract
Recently, the wide application of upconversion nanoparticles (UCNPs) in the field of bioimaging has raised the requirement of biocompatibility. Current cytocompatibility studies on UCNPs mainly focus on cancer cells; however, their potential effects on normal cells are rarely addressed. Herein, the cellular effects of a trace amount of ligand-free NaYF₄:Yb/Er nanocrystals on the differentiation of rat bone mesenchymal stem cells (rBMSCs) were investigated. First, due to their excellent upconversion fluorescent properties, the cellular uptake of ligand-free NaYF₄:Yb/Er nanocrystals was confirmed by confocal laser scanning microscopy, and a homogeneous cytoplasmic distribution was imaged. Second, the viability of the rBMSCs cultured with a series of concentrations of nanoparticles (0, 30, 300, and 3000 ng ml⁻¹) was evaluated, and a dose threshold was determined. Third, the effects of ligand-free NaYF₄:Yb/Er nanocrystals on the osteogenesis of the rBMSCs were intensively characterized. The alkaline phosphatase activity assay, quantitative real time polymerase chain reaction for related osteogenic genes, and immunofluorescence staining of specific biomarkers and mineral deposits demonstrated that the ligand-free NaYF₄:Yb/Er nanocrystals at a proper concentration can enhance osteogenic differentiation. Finally, intracytoplasmic lipid detection showed that the adipogenic differentiation of rBMSCs might be inhibited in the presence of ligand-free NaYF₄:Yb/Er nanocrystals. Meanwhile, these results showed that the effects of ligand-free NaYF₄:Yb/Er nanocrystals on rBMSCs were concentration-dependent and reciprocal between osteogenic and adipogenic differentiation. This work provides new insights into the exploring the biocompatibility of UCNPs and will benefit the research community engaged in nanotechnology and biomedicine.

Keywords: upconversion nanoparticles, ligand-free, mesenchymal stem cells, cell differentiation, concentration-dependent

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

1. Introduction
Recently, upconversion nanoparticles (UCNPs) have received interest in bioimaging applications, owing to their superior physicochemical properties of converting weak near-infrared light to high-energy visible light. Because of the unique upconversion process, bioimaging using UCNPs display weak tissue damage, deep penetration, and no autofluorescence

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Liu et al. investigated the characteristics of UCNPs to track the retrograde transport neurons in individual-grain resolution [3]. UCNPs have also been explored for their potential applications in cancer diagnosis and treatment [4]. Recently, due to the increasing application of UCNPs in in vivo imaging experiments, the biosafety of these nanoparticles has become a growing concern [5, 6]. Currently, toxicity studies in vitro have mainly focused on the cell viability of targeted cancer cells; however, the potential effects on the functionalities of the surrounding normal cells have rarely been addressed.

Several studies have reported that nanoparticles play an important role in regulating cell activities, especially cell differentiation. It is well known that mesenchymal stem cells (MSCs) are pluripotent cells which exist in many tissues and possess multidirectional differentiation potentials [7, 8]. Thus, it is crucial to confirm the effects on MSCs by biocompatibility tests. Ionic iron oxide can promote cell growth and accelerate cell cycle progression and silver nanoparticles can increase cell stress and inhibit proliferation of MSCs in a time- and dose-dependent manner [9, 10]. Hou et al. demonstrated that TiO2 nanoparticles can negatively affect the viability and cycle of MSCs in a dose- and size-dependent manner. Further, Chung et al. showed that the surface charge of the mesoporous silica nanoparticles can influence the uptake and biological functions of MSCs [11, 12]. Gold nanoparticles have been intensively investigated and demonstrated to promote osteogenic differentiation of MSCs through the p38 mitogen-activated protein kinase pathway demonstrated to promote osteogenic differentiation of MSCs [11, 12]. For UCNPs, Zhao et al. demonstrated that the stem cells labeled by conjugated polyethylenimine (α-NaYF4:Yb3+,Tm3+)/CaF2 were less potent than the unlabeled ones [19]. Qu et al. reported that MSCs cultured on the upconversion substrate could be differentially guided to osteogenic or adipogenic differentiation with the control of an NIR laser [20]. Kang et al. used upconversion nanotransducers to distantly control intracellular calcium and differentiation of stem cells in vivo [21, 22]. Considering that ligand-modified nanoparticles having specific functions might degrade in the cytoplasm and be exposed to a complex cellular environment, the effects of bare nanoparticles on the MSCs could be different in the absence of surface coatings [23-25].

In this work, oleate-capped NaYF4:Yb/Er nanocrystals were synthesized by the thermal co-precipitation method and then oleate ligands were removed from the surface by an acid treatment [26, 27]. Afterwards, we used the obtained ligand-free UCNPs to label rat bone mesenchymal stem cells (rBMSCs) and studied the effects of trace amounts of ligand-free UCNPs on the activities of the rBMSCs. This study could open new paths for assessing the toxicity of nanoparticles and could also provide insights into developing tissue engineering materials to a wider scope.

2. Materials and methods

2.1. Materials

YCl3 · 6H2O, YbCl3 · 6H2O, ErCl3 · 6H2O, NaOH, NH4F, 1-octadecene, oleic acid, β-glycerophosphate, dexamethasone, ascorbic acid, 3-isobutyl-1-methyloxanthine (IBMX), indomethacin, insulin, Alizarin Red S (ARS), and Oil Red O (ORO), and Triton X-100 were purchased from Sigma-Aldrich. Dulbecco modified Eagle’s medium (DMEM, low glucose), fetal bovine serum (FBS), penicillin-streptomycin, and trypsin were purchased from Thermo Fisher Scientific (USA).

2.2. Synthesis of ligand-free NaYF4:Yb/Er nanocrystals

Oleate-capped NaYF4:Yb/Er nanocrystals were prepared according to the method described in Haase et al. [26]. In a typical procedure, YCl3 (78%), YbCl3 (20%), and ErCl3 (2%) with a total lanthanide amount of 0.4 mmol were added into a 20 ml mixture of 1-octadecene and oleic acid, and then heated to 160 °C for 30 min under argon protection. Then, a 5 ml methanol solution containing NH4F (1.6 mmol) and NaOH (1 mmol) was added with stirring. When the methanol volatilized completely, the solution was heated at 310 °C under argon protection for 1 h. After that, the nanoparticles were washed with water and ethanol repeatedly.

The ligands capped on the NaYF4:Yb/Er nanocrystals were removed via the acid method [27]. Accordingly, the above nanoparticles were dispersed in an acidic ethanol solution (pH 1) and ultrasonicated for 5 min. After centrifugation at 16 500 rpm for 20 min, the nanoparticles were re-dispersed in another acid solution (pH 4). Finally, the nanoparticles were rinsed with ultrapure water repeatedly, and finally, dissolved in ultrapure water.

2.3. Characterization

The crystallographic and morphological information were obtained using an X-ray diffractometer (XRD; Bruker D8 Advance) and a transmission electron microscopy (TEM; JEOL JEM-1400), respectively. Inductively coupled plasma-optical emission spectrometry (ICP-OES; ICP8000, PE) was used to quantify the Y3+, Yb3+, and Er3+ concentrations in the NaYF4:Yb/Er nanocrystals. Fourier transform infrared (FTIR) spectra of the oleate-capped and ligand-free NaYF4:Er3+/Yb3+ nanoparticles were recorded on a VERTEX 70 spectrometer (Bruker, Germany) in a wavenumber range of 4000–450 cm−1. The zeta potential of the ligand-free nanoparticles in phosphate buffer solution (PBS; pH 7.2) was determined by dynamic light scattering measurement (SZ-100Z, Horiba, Japan) at room temperature. The upconversion luminescence (UCL) spectra were obtained at room temperature using an Edinburgh F920 fluorescence spectrometer with a continuous wave (CW) diode 980 nm laser.
2.4. RBMSC isolation and culture

RBMSCs were extracted from the femurs and tibias of 30-day-old Wista rats [7]. The cells were cultured using DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C, with an atmosphere of 5% CO2 and 95% humidity. The RBMSCs showed high purity through flow cytometry [28]. The RBMSCs extracted from the third passage were used for the subsequent experiments.

2.5. Uptake of NaYF4:Yb/Er nanoparticles

The uptake of NaYF4:Yb/Er by rBMSCs was characterized by confocal laser scanning microscopy (CLSM). First, rBMSCs with an initial seeding density of $1 \times 10^5$ cells/well were seeded in 48-well culture plates. After incubation with 3000 ng ml$^{-1}$ of NaYF4:Yb/Er for 4 h, the cells were pretreated, washed with PBS several times, fixed with 4% paraformaldehyde solution for 10 min, and permeabilized using 0.1% Triton X-100, blocked with 5% BSA. Subsequently, actin filaments (F-actin) and nuclei were stained with rhodamine phalloidin and 4'-6 diamidino-2-phenolindole (DAPI; Invitrogen) and re-washed repeatedly. Finally, the cells were examined using CLSM (FV3000, Olympus) under excitation at CW 980 nm.

2.6. Cell viability

For the viability assay, rBMSCs were seeded in a 48-well plate at a density of $1 \times 10^4$ cells/well. After culturing for 24 h, the Cell Counting Kit-8 (CCK-8) was used to quantitatively evaluate the proliferation of the rBMSCs co-cultured with 0, 30, 300, and 3000 ng ml$^{-1}$ of NaYF4:Yb/Er nanoparticles for 1, 3, and 5 days, by recording the optical density (OD) at 450 nm using a microplate reader (Synergy™ H1, Biotek, America).

2.7. Osteogenic differentiation

2.7.1. Alkaline phosphatase (ALP) activity. A cell suspension of $1 \times 10^4$ rBMSCs was added to 48-well plates and cultured in the osteogenic medium (10 mM β-glycerol phosphate, 10 nM of dexamethasone and 50 μg ml$^{-1}$ of L-ascorbic acid) containing 0, 30, 300, and 3000 ng ml$^{-1}$ of NaYF4:Yb/Er nanoparticles. After 10 and 14 days, the rBMSCs were lysed by 0.1% Triton X-100 to extract the total protein content, which was evaluated by a BCA protein assay kit (KeyGEN BioTECH). The ALP activity was estimated by an ALP activity assay kit (Wako Pure Chemical Industries, Ltd.) and normalized for the protein amount.

2.7.2. Quantitative real time polymerase chain reaction (Q-PCR) assay. With an initial seeding density of $1 \times 10^5$ cells/well, rBMSCs were cultured in 6-well plates. After 14 days, the RNAeasy Plus Mini Kit (Qiagen) was used to extract the total RNA from the rBMSCs (control, 30, 300, and 3000 ng ml$^{-1}$). Q-PCR analysis was performed with glyceraldehyde-3-phosphate (GAPDH) as the reference gene, and osteopontin (OPN) and osteocalcin (OCN) as the osteogenic genes. The sequences of the forward and reverse primers were obtained from a previous study [29]. The relative mRNA expression was normalized to that of GAPDH.

2.7.3. Immunofluorescence staining. At a density of $1 \times 10^4$ cells/well, the rBMSCs were seeded on confocal dishes. After culturing with different concentrations of NaYF4:Yb/Er nanoparticles for 2 weeks in an osteogenic medium, the rBMSCs were first pretreated according to the method described in section 2.5, and then incubated with the primary antibodies against OPN and OCN [30]. After being washed with PBS several times, the cells were incubated with secondary antibodies labeled with fluorescein for 1 h and DAPI for 5 min. Images were observed by CLSM under 405 and 488 excitation wavelengths.

2.7.4. Mineralized matrix formation assay. For 14 days, the rBMSCs with an initial seeding density of $1 \times 10^4$ cells/well in 48-well culture plates were fixed in 4% paraformaldehyde, washed with PBS, stained with ARS solution, and observed under a microscope. To quantify the calcium nodules, 10% (w/v) cetylpyridium chloride was added to dissolve the stained ARS, and the OD value was monitored at 570 nm. The relative mineralization rate was expressed as a percentage: (ODsample/ODcontrol) × 100%.

2.8. Adipogenic differentiation

Following treatment with NaYF4:Yb/Er nanoparticles in the adipogenic medium (10 μg ml$^{-1}$ of insulin, 100 nM of dexamethasone, 200 μM of IBMX, and 200 μM of indomethacin) for 14 days, rBMSCs with an initial seeding density of $1 \times 10^4$ cells/well in 48-well culture plates were stained with ORO solution and observed under a microscope. To quantify the cytoplasmic oil content, isopropl alcohol was used to elute the stained ORO and the OD was recorded at 490 nm.

2.9. Statistical analysis

Statistics were analyzed using ANOVA in PASW Statistics 18.0 (SPSS, Inc.). Data were expressed as a mean-standard error, and the significance was determined at $^{*}p < 0.01$.

3. Results and discussion

3.1. Characterization of ligand-free NaYF4:Yb/Er nanoparticles

Figure 1(a) shows a typical TEM image of the NaYF4:Yb/Er nanoparticles after ligand removal. The nanoparticles are well dispersed and exhibited a regular hexagonal shape with a diameter of 15 ~ 20 nm. The XRD results shown in figure 1(b) demonstrate the typical peaks of the nanocrystals assigned to the hexagonal NaYF4:Yb/Er crystal (PDF 28-1192), which also confirm the hexagonal structure. The Y$^{3+}$/Yb$^{3+}$/Er$^{3+}$ molar ratio of the NaYF4:Yb/Er crystals quantified by ICP-OES is 79.3:18.6:2.1, approximate the
stoichiometric ratio of the rare-earth precursors added into the synthetic reaction system.

FTIR spectra of the oleate-capped and ligand-free UCNPs were characterized and shown in figure 1(c). Compared with the FTIR spectra of oleate-capped UCNPs, the intensity of the peaks centered at 2924 and 2854 cm\(^{-1}\) attributed to the asymmetric and symmetric stretches of methylene were significantly weakened in the spectra of ligand-free UCNPs [31]. Peaks at 1465 and 1564 cm\(^{-1}\) belonging to the asymmetric and symmetric stretching vibrations of carboxyl groups, respectively, disappeared [27]. The results confirmed the ligands were successfully removed from the surface of the oleate-capped NaYF\(_4\):Yb/Er nanocrystals.

Zeta potential is an important indicator of a stabilized colloidal system [27]. Herein, we estimated the zeta potential of ligand-free NaYF\(_4\):Yb/Er nanocrystals in PBS (pH 7.2) and determined the value as \(-38.6\) mV. According to Zhao et al the colloidal system with a zeta potential of \(\pm 25\) mV is assumed stable [32]. Thus, the PBS solution system at a pH value containing ligand-free UCNPs was stable, thereby signifying the water-solubility of the nanoparticles after the removal of surface ligands.

Owing to the excellent resonances between the \(^{2}\text{F}_{5/2}\) state of the Yb\(^{3+}\) and the \(^{4}\text{I}_{11/2}\) excited state of Er\(^{3+}\), the ligand-free NaYF\(_4\):Yb/Er nanoparticles displayed UCL properties [33]. The room temperature UCL spectrum in water under CW excitation at 980 nm is shown in figure 1(d). From the figure, we can see three characteristic emission peaks at 520, 540, and 654 nm, originating from the f-f transitions of Er\(^{3+}\), \(^{2}\text{H}_{11/2} \rightarrow ^{4}\text{I}_{15/2}\), \(^{4}\text{S}_{3/2} \rightarrow ^{4}\text{I}_{15/2}\), and \(^{4}\text{F}_{9/2} \rightarrow ^{4}\text{I}_{15/2}\), respectively [34]. During the process, Yb\(^{3+}\) are used as the sensitizers, which can absorb the excitation energy and transfer it to \(^{4}\text{I}_{11/2}\) and then the \(^{2}\text{H}_{11/2}\), \(^{4}\text{S}_{3/2}\), \(^{4}\text{F}_{9/2}\) state of Er\(^{3+}\) by a two-photon process [35].

### 3.2. Cell proliferation

To elucidate the UCL properties of these nanoparticles, CLSM equipped with a CW 980 nm laser was used to track their uptake by rBMSCs. After incubation for 24 h, the cells were completely labeled with green emission (figure 2(a)) emitted by ligand-free UCNPs under the excitation of a CW 980 nm laser. The F-actin was stained in red (figure 2(b)) and the cell nucleus in blue (figure 2(c)). The rBMSCs were spread in a spindle-shaped form, the typical form of
fibroblasts. The merged image (figure 2(d)) demonstrates that UCNPs were distributed homogeneously throughout the cytoplasm.

Figure 2(e) shows the proliferation of rBMSCs co-cultured in the presence of different concentrations of UCNPs (0, 30, 300, and 3000 ng ml\(^{-1}\)) for 1, 3, and 5 days. On day 1, there was no evident cytotoxicity regardless of the concentration of the nanoparticles in the medium. After days 3 and 5, there were significant negative effects on rBMSC proliferation with concentrations of 30 and 300 ng ml\(^{-1}\) as compared with the control group. Surprisingly, a little enhancement of cell proliferation was found in the medium with 300 ng ml\(^{-1}\) of UCNPs. However, at a higher concentration (3000 ng ml\(^{-1}\)), NaYF\(_4\):Yb/Er nanoparticles inhibited the proliferation of rBMSCs after 5 days. These results demonstrated that 3000 ng ml\(^{-1}\) of UCNPs could slightly inhibit the growth of rBMSCs.

### 3.3. Osteogenic differentiation

#### 3.3.1. ALP activity

ALP is the phenotypic early marker of osteogenic differentiation [36]. Therefore, the ALP activity was monitored in the rBMSCs. After the addition of 300 ng ml\(^{-1}\) of UCNPs, the ALP activity increased from day 10 to day 14 (figure 3(a)). At both time points, there were nearly no differences in the ALP activities of rBMSCs following the addition of 30 ng ml\(^{-1}\) of UCNPs as compared with the control group. After incubation in a medium containing 300 ng ml\(^{-1}\) of nanocrystals, the rBMSCs showed significantly increased ALP activity, which demonstrated the enhanced osteogenic differentiation of rBMSCs at this concentration. However, the ALP activity significantly decreased when the concentration of the UCNPs was increased to 3000 ng ml\(^{-1}\). The results demonstrated that the effects of NaYF\(_4\):Yb/Er on the ALP activity were concentration-dependent and enhanced at a proper concentration.

#### 3.3.2. Q-PCR

Both OPN and OCN play important roles in the bone remodeling process and are usually used as biomarkers of osteogenesis [30, 37]. Figure 3(b) shows the relative mRNA expression of both OPN and OCN by Q-PCR, demonstrating that the gene levels of rBMSCs co-cultured with NaYF\(_4\):Yb/Er nanoparticles were concentration-dependent. Particularly, the OCN and OPN expression of rBMSCs exposed to 300 μg ml\(^{-1}\) of nanocrystals was up-regulated by 1.7- and 2.2-fold as compared to the control group, respectively. Meanwhile, in rBMSCs incubated with 30 ng ml\(^{-1}\) of UCNPs, both OCN and OPN mRNA expression was similar to that of the control groups. In the cells treated by 3000 ng ml\(^{-1}\) of UCNPs, the level of OCN transcript was significantly inhibited. These results confirmed that the effects of NaYF\(_4\):Yb/Er nanocrystals were concentration-dependent and that 300 ng ml\(^{-1}\) of UCNPs could efficiently promote osteogenic differentiation.

#### 3.3.3. Immunofluorescence staining

Besides the mRNA transcript levels of the bone-specific protein characterized by Q-PCR above, the OPN and OCN protein expressions was further detected by immunofluorescence staining in rBMSCs co-cultured with different concentrations of NaYF\(_4\):Yb/Er nanoparticles (0, 30, 300, and 3000 ng ml\(^{-1}\)) for 14 days (figure 4). The fluorescence intensity indicating OPN was enhanced in the rBMSCs treated with 300 ng ml\(^{-1}\) of NaYF\(_4\):Yb/Er nanoparticles as compared to the control.
group. In contrast, in rBMSCs incubated in 3000 ng ml\(^{-1}\) of UCNPs, both the cell number and immunofluorescence intensity significantly decreased. Thus, the immunostaining results of OPN agreed well with the Q-PCR results. Similar results were also found with the expression of OCN. Therefore, the immunofluorescence staining results of OPN and OCN further confirmed the dose-dependent effects of NaYF\(_4\):Yb/Er nanoparticles on the osteogenic differentiation of rBMSCs.

3.3.4. Evidence of mineralization activity. The formation of mineralized deposits was observed during osteogenic differentiation. An ARS staining assay was used to evaluate

Figure 3. Analysis of osteogenic markers of ALP activity (a); relative transcript level of OPN and OCN (b), normalized to GAPDH after 14 days and expressed as mean ± S.D. (n = 3, \(^*\)p < 0.01).

Figure 4. Immunofluorescence staining of osteogenic markers: OPN (a)–(i) and OCN (m)–(x) in rBMSCs treated with 0, 30, 300, and 3000 ng ml\(^{-1}\) of NaYF\(_4\):Yb/Er nanoparticles after 14 days of culture. OPN and OCN were stained green, and the cell nuclei blue. Scale bar: 100 \(\mu\)m.
the mineralized matrix formation [38]. After 14 days, the rBMSCs in the osteogenic medium began to form minerals, which were stained red (figures 5(a)–(d)). The formation of calcium nodules was also affected by the treatment with NaYF4:Yb/Er nanocrystals in a concentration-dependent manner, according to the biomarker analysis. As shown in figure 5(e), the stained ARS was extracted by cetylpyridium chloride from the rBMSCs to quantify the mineral content. As illustrated in figure 5(e), the mineralization of rBMSCs in the presence of 300 ng ml\(^{-1}\) of NaYF4:Yb/Er nanoparticles was significantly higher than that in the control group, which further confirmed that NaYF4:Yb/Er nanoparticles in this concentration promoted mineralization. Meanwhile, the mineralization in the rBMSCs incubated in 3000 ng ml\(^{-1}\) of UCNPs was similar to that in the control group, which was lower than that in the cells incubated with 30 ng ml\(^{-1}\). On the other hand, mineralization in the cells incubated with 30 ng ml\(^{-1}\) of UCNPs was similar to that in the control group. Therefore, the effect of UCNPs on the mineralization of the rBMSCs was also concentration-dependent and consistent with the above results.

3.4. Adipogenic differentiation

The osteogenic differentiation of the rBMSCs was characterized afterwards. Generally, except for osteoblasts, the rBMSCs possessed other differentiation potentials, for example, adipocytes. To assess the adipogenesis, ORO was used to specifically stain the intracytoplasmic lipids of the rBMSCs [39]. As shown in figures 6(a)–(d), the rBMSCs treated with different concentrations of NaYF4:Yb/Er nanoparticles were stained by ORO, indicating that rBMSCs could differentiate into adipocytes. From microscopic images, we could also see that the morphology of the cells changed from spindle shape to spherical shape, indicating the formation of adipocytes. To quantify the degree of adipogenic differentiation, the cytoplasmic lipid stained by ORO was dissolved by isopropanol, the absorbance of which was measured at 510 nm. The lipid content of the cells cultured with NaYF4:Yb/Er nanoparticles reduced, especially at 300 ng ml\(^{-1}\), compared to the control group (figure 6(e)). Thus, NaYF4:Yb/Er nanoparticles inhibited the adipogenic differentiation of rBMSCs, which should be attributed to the improved level of osteogenic differentiation.

MSCs are helpful for the treatment of complicated diseases in the field of regenerative medicine. Certain disturbances can influence cell functions and behaviors [40]. According to previous studies, nanoparticles can affect the osteogenic differentiation of rBMSCs through the Smad-dependent bone morphogenetic protein signaling pathway [18, 41, 42]. The above experimental results suggest that NaYF4:Yb/Er nanoparticles can regulate cell proliferation, and osteogenic and adipogenic differentiation of rBMSCs, and exert influences in a concentration-dependent manner. Less than 300 ng ml\(^{-1}\) of NaYF4:Yb/Er nanoparticles can promote osteogenic differentiation of rBMSCs with a negligible influence on cell viability, which was relative to the effects on the signaling pathways [43]. Particularly, 300 ng ml\(^{-1}\) of NaYF4:Yb/Er nanoparticles could efficiently promote the osteogenic differentiation of the rBMSCs, and simultaneously inhibit adipogenesis differentiation, which is related to the reciprocal relationship between osteogenesis and adipogenesis. Therefore, ligand-free UCNPs can regulate osteogenic and adipogenic differentiation by changing the
concentrations, and this will could widely benefit the research community involved in nanotechnology and biomedicine.

4. Conclusions

In this work, the effects of ligand-free NaYF₄:Yb/Er nanoparticles on the functions and differentiation of rBMSCs were studied. Ligand-free NaYF₄:Yb/Er nanoparticles with upconversion fluorescence were taken up by rBMSCs and they exerted significant effects on cell behavior in a concentration-dependent manner. At an appropriate concentration (300 ng ml⁻¹), NaYF₄:Yb/Er nanocrystals could efficiently promote the proliferation and osteogenic differentiation of rBMSCs with negligible effects on cell viability. Such a concentration of NaYF₄:Yb/Er nanoparticles could inhibit the adipogenic differentiation of rBMSCs in accordance with the reciprocal relationship of the two directed differentiations. Consequently, NaYF₄:Yb/Er nanocrystals at a proper concentration are beneficial to the turnover of bone cells, which is suggestive for the novel design of functional nanomaterials in the biomedical field.

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Declarations of interest

None.

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