Supporting Information for

Fluoridated HAp:Ln^{3+} (Ln=Eu or Tb) nanoparticles for cell-imaging

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Experimental Section

Reagents

$Ca(NO_3)_2 \cdot 4H_2O$, $Na_3PO_4 \cdot 12H_2O$, NaF, octadecylamine, oleic acid, ethanol, $Eu(NO_3)_3 \cdot 6H_2O$, $Tb(NO_3)_3 \cdot 6H_2O$, and cyclohexane were obtained from Beijing Chemical Reagents Company, China. Pluronic F127 was purchased from Sigma Chemical (St. Louis, MO, USA). Other chemical agents were obtained from commercial routes were of at least analytical grade and used without further purification.

1. Synthesis of hydrophobic fluoridated HAp:Eu^{3+}(or Tb^{3+}) nanoparticles:

In a 40 mL Teflon-lined autoclave, 0.5 g octadecylamine were dissolved in 4mL oleic acid by heating, which were mixed with ethanol (16 mL) and aqueous solution of $Ca(NO_3)_2$ (0.28 M, 7 mL) under agitation. Then aqueous solutions of $Eu(NO_3)_3$ or $Tb(NO_3)_3$ (0.28 M, 0.35-0.7 mL), NaF (0.28M, 0.7-1.4 mL) and Na_3PO_4 (0.168 M, 7 mL) were added to the solution. The mixture was agitated for about 5 min and sealed and hydrothermally treated at a controlled temperature of 180 °C for 12 h. The obtained nanoparticles were collected by centrifugation, washed with cyclohexane and ethanol several times, and finally re-dispersed in cyclohexane.

2. Preparation of hydrophilic fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles:

The transformation of hydrophobic fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles into hydrophilic ones was carried out as follows. Approximately 18 mg of fluoridated HAp:Eu^{3+}(or Tb^{3+}) nanoparticles in 2 mL of cyclohexane were mixed with 20 mg of Pluronic F127 in 10 mL of H_2O in a 50 mL vial. The mixture formed two layers, the upper layer contained the fluoridated HAp:Eu^{3+}(or Tb^{3+}) nanoparticles and the lower

layer contained the Pluronic F127. And 6 mL of tetrahydrofuran (THF) was subsequently added into the mixture and well mixed with stir to obtain a turbid suspension. Then the organic solvents were removed with a rotatory evaporator at reduced pressure. To remove the excess Pluronic F127, the Pluronic F127 dispersed fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles were treated by repeated centrifugal washing process for thrice.

The sizes and morphologies of the fluoridated HAp: Eu^{3+}(or Tb^{3+}) nanoparticles were observed with HITACHI H-7650B transmission electron microscope at 80 kV and Tecnai G2 F20 S-Twin highresolution transmission electron microscope operated at 200 kV. Luminescence spectra were recorded using a Hitachi F-4500 fluorescence spectrophotometer. Photographs of the samples dispersed in cyclohexane or water were obtained by camera.

3. Cytotoxicity of fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles:

Cell morphology was observed to examine the effects of fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles to A549 and HeLa cells. Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells mL^{-1} in 2 mL of respective media containing 10% FBS. After cell attachment, plates were washed with PBS and the cells were treated with complete cell culture medium, or 100 μg mL^{-1} of fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized nanoparticles. The morphology of cells was observed by using an optical microscopy (Leica, Germany).

The cell viability of fluoridated FHAp:Eu^{3+} (or Tb^{3+}) nanoparticles on A549 and HeLa cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 20, 40, 80, 160, 320 μg mL^{-1} fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles for 24 h. Then nanoparticles were removed and cells were washed with PBS three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to controls (cells not exposure to fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated

controls. Results are presented as mean ± standard deviation (SD).

4. Confocal microscopic imaging of cells using fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles:

A549 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 µg mL^{-1} of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture medium was changed every three days for maintaining the exponential growth of the cells. On the day prior to treatment, cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with fluoridated HAp:Eu^{3+} (or Tb^{3+}) nanoparticles at a final concentration of 150 µg mL^{-1} for 4 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the fluoridated HAP:Eu^{3+} (or Tb^{3+}) nanoparticles and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken using a Laser Scanning Confocal Microscope (LCSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelengths of 405 nm and 488 nm.

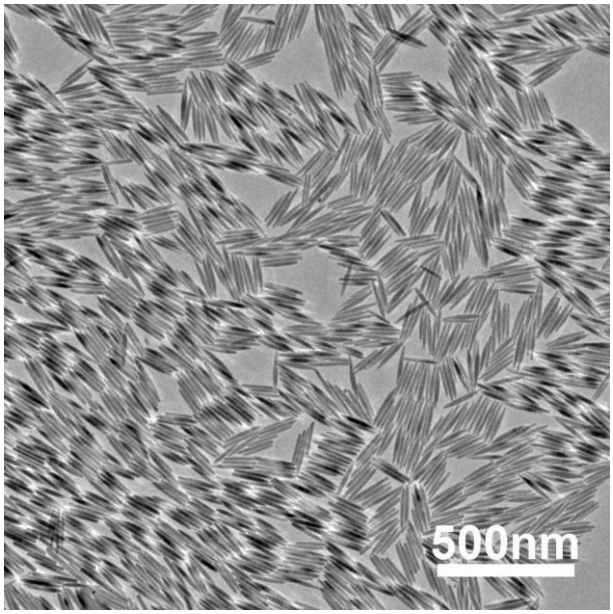

Fig. S1 TEM image of fusiform FHAp:5% Eu^{3+} nanoparticles dispersed in cyclohexane

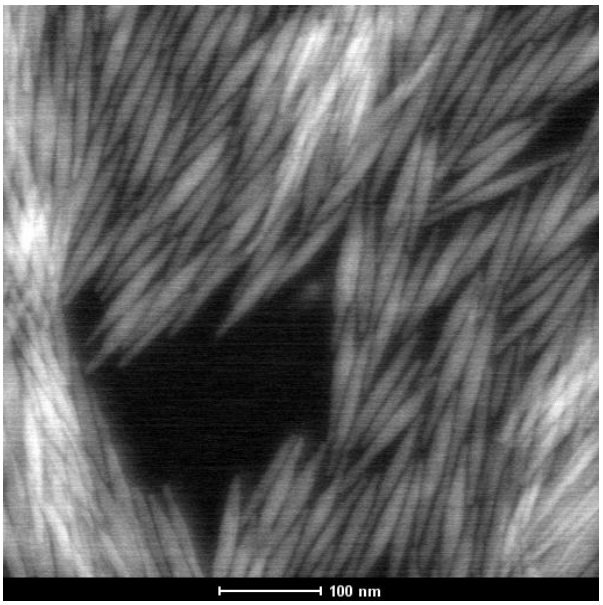

Fig. S2 STEM image of fusiform FAp:5% Eu^{3+} nanoparticles dispersed in cyclohexane.

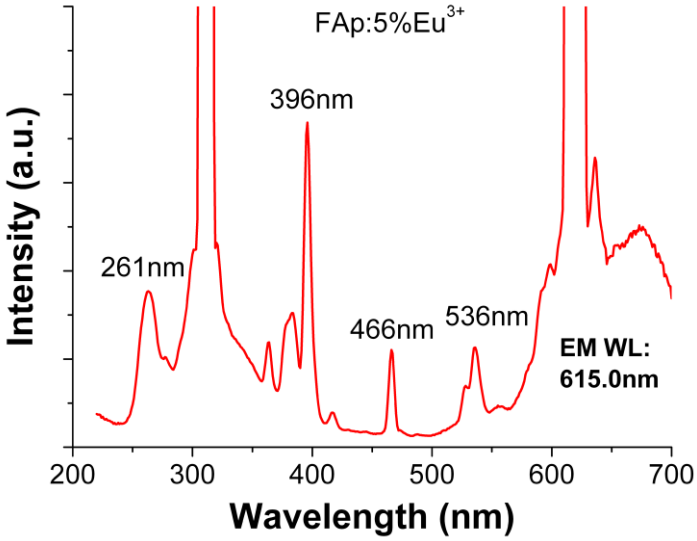

Fig. S3 Luminescent excitation spectrum of fusiform FAp:5%Eu^{3+} nanoparticles dispersed in cyclohexane.

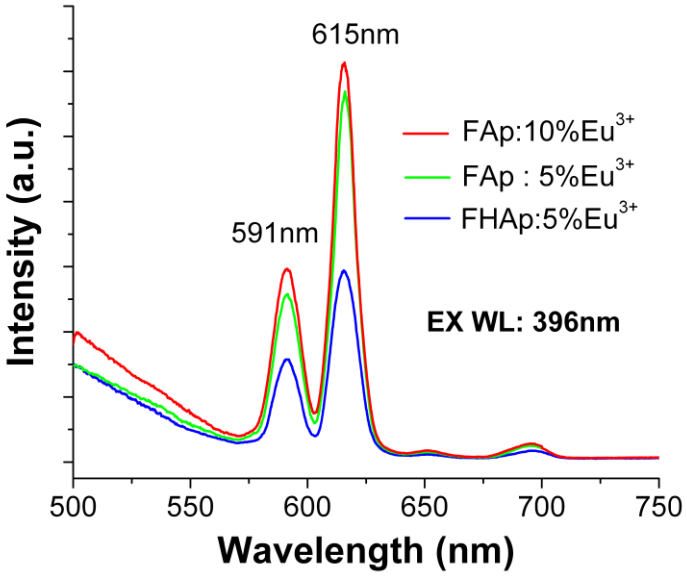

Fig. S4 Luminescent spectra of fusiform FAp:5%Eu^{3+}, FHAp:5%Eu^{3+}, FAp:10%Eu^{3+} nanoparticles dispersed in cyclohexane under excitation at 396 nm.

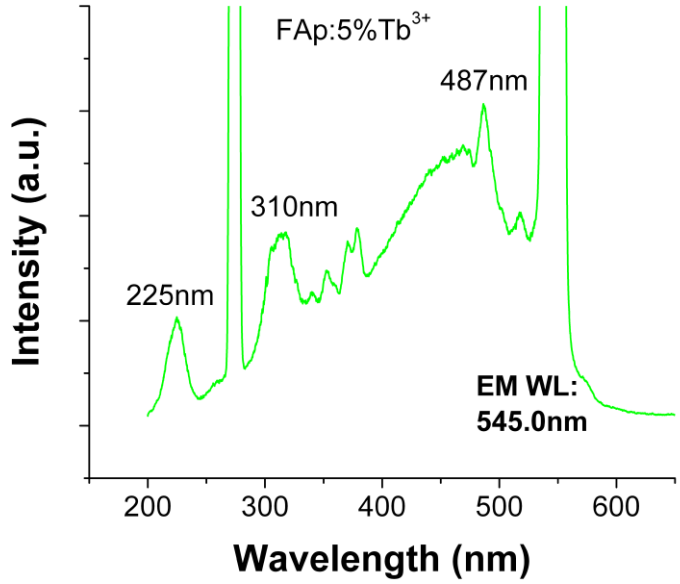

Fig. S5 Luminescent excitation spectrum of fusiform FAp:5%Tb^{3+} nanoparticles dispersed in cyclohexane.

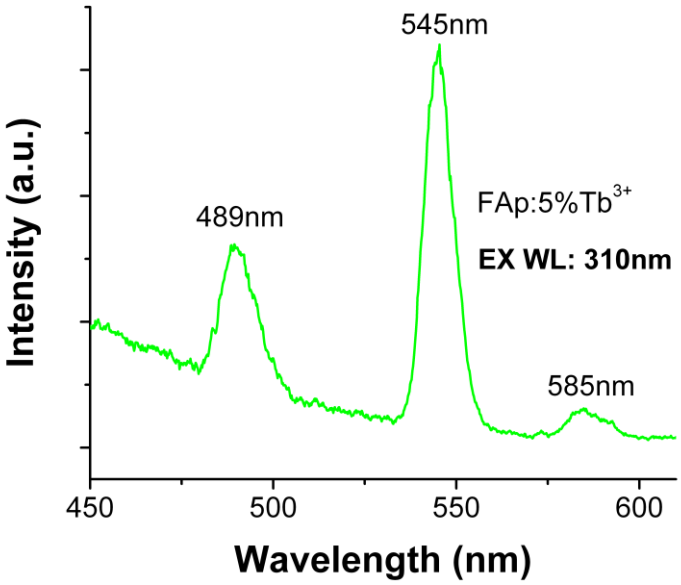

Fig. S6 Luminescent spectrum of fusiform FAp:5%Tb^{3+} nanoparticles dispersed in cyclohexane under excitation at 310 nm.

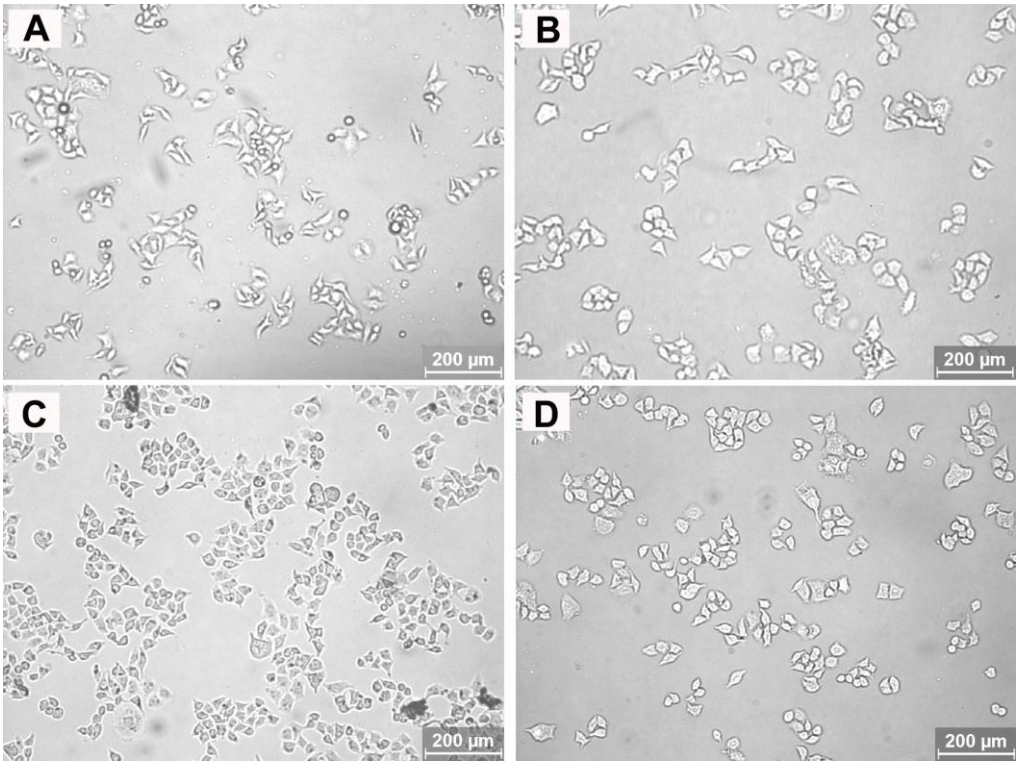

Fig. S7 The morphology of HeLa cells incubated for 48 h at 37 °C in media containing 80 μg mL^{-1} of FAp:5%Eu^{3+} (A), FHAp:5%Eu^{3+} (B), FAp:10% Eu^{3+} (C) nanoparticles, and nothing (D), respectively.

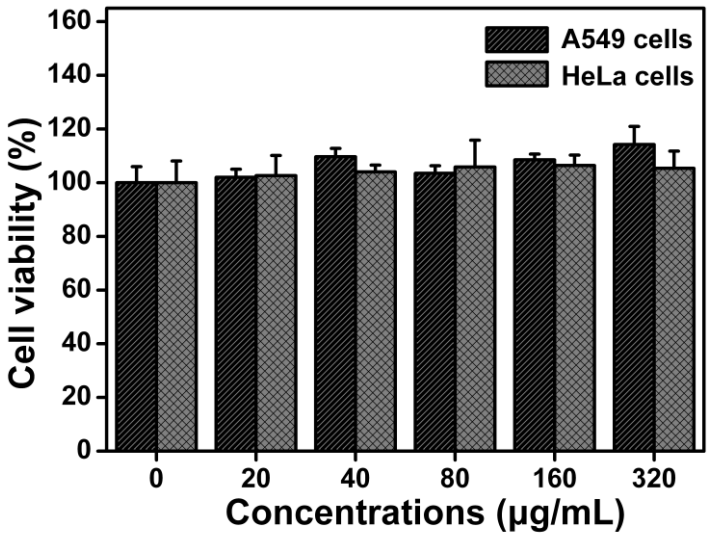

Fig. S8 CCK-8 assay of A549 cells and HeLa cells cultured for 24 h at 37 °C in media containing FAp:5%Tb^{3+} nanoparticles.