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Supporting Information for

Synthesis of Iron Oxide coated Fluoridated HAp/Ln^{3+} (Ln=Eu or Tb) nanocomposites for Biological Applications

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Materials and methods

1. Materials

$Ca(NO_3)_2 \cdot 4H_2$，$Na_3PO_4 \cdot 12H_2O$，NaF，NaOH，octadecylamine，oleic acid, ethanol, cyclohexane，polyvinylpyrrolidone (PVP)，triethylene glycol (TEG) and Iron(III) acetylacetonate ($Fe(acac)_3$) were obtained from Beijing Chemical Reagents Company, China. $Eu(NO_3)_3 \cdot 6H_2O$, $Tb(NO_3)_3 \cdot 6H_2O$ and Pluronic F127 was purchased from Sigma Chemical (St. Louis, MO, USA). All of the chemicals were used without further purification.

2. Preparation of hydrophilic FHAp/Eu^{3+} (or Tb^{3+}) nanorods

Based on a procedure described by Hui e*t al*, hydrophobic FHAp/Eu^{3+}(or Tb^{3+}) nanorods were fabricated.[1] In a brief, in a teflon-lined autoclave (50ml), octadecylamine (0.5 g) were dissolved in oleic acid (4 mL) by heating. This solution was mixed with ethanol (16 mL) and an aqueous solution of $Ca(NO_3)_2$ (0.28M, 7 mL) under agitation. Next, aqueous solutions of $Eu(NO_3)_3$ or $Tb(NO_3)_3$ (0.28M, 0.7 mL), NaF (0.28M, 0.35 mL)，and Na_3PO_4,(0.16M, 7 mL) were added to the solution. The mixture was agitated for about 5 min. Furthermore, this mixture was sealed and hydrothermally treated at a controlled temperature of 180°C for about 12 h. After centrifugation, the obtained nanorods were further washed with cyclohexane and ethanol several times. Finally, these nanorods were re-dispersed in cyclohexane.

To enable hydrophobic FHAp/Eu^{3+} (or Tb^{3+}) nanorods dissolve in aqueous solutions, the following approach was exploited.[1] Briefly, 60 mg FHAp/Eu^{3+} (or Tb^{3+}) nanorods in 5 mL of cyclohexane were mixed with 80 mg of Pluronic F127 in a 100 mL vial with 30 mL H_2O. Subsequently, 10 mL of tetrahydrofuran (THF) was added and stirred to obtain a turbid suspension. A rotatory evaporator was used to remove organic solvents at reduced pressure. And the Pluronic F127 dispersed FHAp/Eu^{3+} (or Tb^{3+}) nanorods were washed by centrifugation many times in order to remove excess Pluronic F127. Finally, hydrophilic FHAp/Eu^{3+} (or Tb^{3+}) nanorods were collected with centrifugation for further use.

3. Preparation of iron oxide coated FHAp/Eu^{3+} (or Tb^{3+}) nanocomposites

Fluorescent-magnetic nanocomposites of iron oxide coated FHAp/Eu^{3+} (or Tb^{3+})

nanorods were fabricated through thermal decomposition of Iron (III) acetylacetonate ($Fe(acac)_3$) in triethylene glycol (TEG) on the surface of FHAp/Eu^{3+} (or Tb^{3+}) nanorods. In detailed, iron oxide coated FHAp/ Eu^{3+} (or Tb^{3+}) nanocomposites (abbreviation as IO-Eu-FHAp or IO-Tb-FHAp) were prepared by thermal decomposition of $Fe(acac)_3$ in triethylene glycol (TEG) in the presence of hydrophilic FHAp:Eu^{3+}(or Tb^{3+}) nanorods. 2 mg (or 5 mg or 10 mg) of $Fe(acac)_3$ and 50 mg of PVP (90k) were firstly dissolved in 6 mL TEG. Furthermore, 10 mg hydrophilic FHAp/ Eu^{3+} (or Tb^{3+}) nanorods was added in above solution and dispersed through vigorous stirring for about 5 h. After transferred into a Teflon-lined stainless steel autoclave (10 mL in total volume), the suspension was sealed and maintained at 180 °C for 15 h. Finally, the suspension was cooled to room temperature naturally followed by centrifugation and washing several times with water.

4. Characterization of nanocomposites

The sizes and morphologies of nanocomposites were investigated with HITACHI H-7650B transmission electron microscope at 100 kV and Tecnai G2 F20 S-Twin high resolution transmission electron microscope operated at 200 kV. The phase purity of the products was examined by XRD on a Rigaku RU-200b X-ray powder diffractometer by using a nickel-filtered Cu_{Ka} radiation in the range 10–80° with a scan rate of 10°/min. Luminescence spectra were determined with a Hitachi F-4500 fluorescence spectrophotometer at an excitation wavelength of 405 and 488 nm for IO-Eu-FHAp (FHAp/ Eu^{3+}: IO = 2:1) and IO-Tb-FHAp (FHAp/ Tb^{3+}: IO = 2:1), respectively. A vibrating sample magnetometer (VSM) was exploited to investigate the hysteresis curve of IO-Eu-FHAp (FHAp/ Eu^{3+}: IO = 2:1). Dried sample of known mass was taken in non-magnetic aluminum sheet. The sample was subjected to varying magnetic field at room temperature and the magnetization was measured.

5. *Cytotoxicity of nanocomposites*

A549 cells were cultured in the Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin as the antibiotics in humidified environment of 5% CO_2 at 37°C. The cells were pre-cultured until 80% confluence was reached. Cell counting kit-8 (CCK-8) assay was

used to evaluate the cytotoxicity of the nanocomposites. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells/mL in medium. After 24 h seeding, the cells were further treated with DMEM at 0, 20, 40, 80, 150 and 300 μg mL^{-1} nanocomposites concentration for 8 and 24 h, respectively. Next, old medium containing nanocomposites was removed, and cells were washed with PBS three times. Furthermore, 10 μL of CCK-8 dye and 100 μL of fresh DMEM were added to each sample well followed by incubation for 2 h at 37 °C. The absorbance intensity of each sample well was determined with a microplate reader (VictorIII, Perkin-Elmer). The absorbance wavelength of formazan dye was set at 450 nm and reference wavelength at 620 nm. Cell viability was expressed as absorbance relative to that of untreated controls.

6. In vitro cell imaging

Cells were cultured in chamber (LAB-TEK, Chambered Coverglass System) until reach 80% confluence. And the medium was removed and the adherent cells were washed twice with PBS buffer. Next, the cells were incubated in the medium with IO-Eu-FHAp (FHAp/ Eu^{3+}: IO = 2:1) and IO-Tb-FHAp (FHAp/ Tb^{3+}: IO = 2:1) at 250μg mL^{-1} nanocomposites concentration. After incubation for 4 h, the cells were washed three times with PBS and then fixed with 4% paraformaldehyde for 10 min at room temperature. The cells were further washed twice with PBS and observed using a confocal laser scanning microscope (CLSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelengths of 405 nm and 488 nm, respectively.

Reference

1. J. Hui, X. Zhang, Z. Zhang, S. Wang, L. Tao, Y. Wei and X. Wang, *Nanoscale*, 2012, **4**, 6967-6970.