Biomaterials 35 (2014) 3348–3355

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Long-term biodistribution *in vivo* and toxicity of radioactive/magnetic hydroxyapatite nanorods

Ying Liu [a], Yun Sun [a,b], Cong Cao [a], Yang Yang [a], Yongquan Wu [a], Dianwen Ju [b,**], Fuyou Li [a,*]

[a] Department of Chemistry, The State Key Laboratory of Molecular Engineering of Polymers, Fudan University, 220 Handan Road, Shanghai 200433, China
[b] Department of Biosynthesis, School of Pharmacy, Fudan University, Shanghai 201203, China

A R T I C L E I N F O

Article history:
Received 21 October 2013
Accepted 20 December 2013
Available online 15 January 2014

Keywords:
Hydroxyapatite nanorods
Magnetic resonance imaging (MRI)
Single photon emission computed tomography (SPECT)
Biodistribution
Biosafety

A B S T R A C T

Although nanoscale hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$; HA] has been widely investigated as a carrier in the delivery of drugs, genes, or siRNA, the *in vivo* toxicity of nanoscale HA is not clear and the long-term dynamic distribution *in vivo* has not hitherto been visualized. In this work, gadolinium-doped HA nanorods (HA:Gd) with an r_1 value of 5.49 s^{-1} $(mM)^{-1}$ have been prepared by a hydrothermal method. Samarium-153 (^{153}Sm) was then effectively post-labeled onto the HA:Gd (^{153}Sm-HA:Gd) with a labeling rate of ~100% and a radio-labeling stability *in vitro* of ~100% over 48 h. The product could serve as a new dual-modality probe for SPECT and MR imaging *in vivo*. By means of SPECT and MRI, the HA:Gd nanorods were found to be quickly taken up by the mononuclear phagocyte system, especially the liver and spleen. The nanorods in the liver and lung tended to be eliminated within 24 h, but nanorods in the spleen behaved differently and proved difficult to excrete. *In vitro* studies by cell transmission electron microscopy (TEM) and methyl thiazolyl tetrazolium (MTT) assay showed good biocompatibility of the HA:Gd nanorods with HeLa cells, even at a high concentration. The indicators of body weight, histology, and serology demonstrated that the HA:Gd nanorods exhibited excellent biocompatibility *in vivo* for at least 61 days. Therefore, ^{153}Sm-HA:Gd nanorods with excellent relaxivity, γ-emission, and biosafety offer clear advantages and potential for bioapplications.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$; HA] is a well-known biomaterial constituent of biological hard tissues such as bone and tooth [1,2]. Owing to their excellent bioactivity and biocompatibility, hydroxyapatites with various surface properties have been investigated for applications in bone repair and tissue engineering [3–7]. Recently, nanoscale hydroxyapatites have been synthesized and further used as carriers for the delivery of drugs, genes, or siRNA [8–12]. For example, Lin and co-workers developed hydroxyapatite nanoparticles as carriers for the storage and release of ibuprofen [9]. Moreover, because of the high stability and flexibility of the apatite structure [13,14], some transition metal or lanthanide cations may be doped in the apatite lattice [14–18]. In particular, composites resulting from doping with lanthanides have potential applications in the fields of bioceramics, bioimaging, and therapeutics [5,19,20].

In view of the fact that the employment of hydroxyapatite nanomaterials in the biomedical field shows great promise, rigorous assessment of their potential toxicity is urgently required as data are as yet scarce. To date, the cytotoxicity of hydroxyapatite nanoparticles has been investigated by *in vitro* assays with a number of different cell lines [21–24]. However, reports on the *in vivo* toxicity of hydroxyapatite nanomaterials have been absent, except for a study using zebra fish as a model [24]. Because the *in vivo* distribution determines the organs or cells that interact with materials, a thorough investigation of the biodistribution of hydroxyapatite nanomaterials is required for *in vivo* biosafety assessment. Due to a lack of appropriate markers and tools, however, visualization of the *in vivo* distribution of hydroxyapatite nanomaterials has not been reported to date.

Single-photon emission computed tomography (SPECT) allows noninvasive determination of the *in vivo* biodistribution of radiotracers at picomolar concentrations [25]. SPECT images, however, have limited spatial resolution and lack anatomical details for referencing, making the precise location of lesions difficult. Magnetic resonance imaging (MRI) offers specific advantages, including a lack of ionizing radiation and high soft-tissue contrast [26–28]. Dual-modality imaging by SPECT and MRI thus facilitates simultaneous dynamic monitoring of structure and function, and provides direct information on the pharmacokinetics and metabolism of drugs [29]. Unfortunately, however, to the best of our knowledge,

* Corresponding author. Fax: +86 21 55664185.
** Corresponding author. Fax: +86 21 51980036.
E-mail addresses: dianwenju@fudan.edu.cn (D. Ju), fyli@fudan.edu.cn (F. Li).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.12.064

no example of a hydroxyapatite nanomaterial with dual radioactive and magnetic properties has hitherto been reported.

In the present study, hydroxyapatite nanorods doped with gadolinium (Gd^{3+}) have been synthesized and then post-labeled with radioactive samarium ($^{153}Sm^{3+}$) by cation exchange. The product thus obtained has been assessed as a multimodal probe for MR and SPECT imaging. Herein, the Gd^{3+}-doped hydroxyapatite nanorods and those labeled with radioactive $^{153}Sm^{3+}$ are abbreviated as HA:Gd and ^{153}Sm-HA:Gd, respectively. The relaxivity parameters (r_1) of the HA:Gd nanorods and the labeling rate and labeling stability have been carefully investigated. Furthermore, the *in vivo* distribution of ^{153}Sm-HA:Gd nanorods has been visualized by MRI and SPECT imaging. Finally, the *in vivo* biosafety of HA:Gd has been carefully explored, including indicators of mouse weight, histology, and serology.

2. Materials and methods

2.1. Materials

Gd_2O_3 (99.999%) were purchased from Beijing Chemical Company. $GdCl_3$ were prepared by dissolving the corresponding Gd_2O_3 in hydrochloric solution at elevated temperature and then evaporating the water completely [30]. $CaCl_2$, $Na_2HPO_4 \cdot 12H_2O$, NaOH were purchased from Shanghai Qiangshun Chemical Reagent Co, Ltd., 2-aminoethyl dihydrogen phosphate (AEP) were purchased from Acros. All the chemical reagents used were of analytical grade without further purification. Deionized water was used throughout the experiment.

2.2. Characterization

Sizes and morphologies of the sample were determined at 200 kV using a JEOL JEM-2010F low- and high-resolution transmission electron microscope (HR-TEM). Energy-dispersive X-ray analysis (EDXA) of the sample was performed during HR-TEM measurements. Powder X-ray diffraction (XRD) measurement was performed with a Bruker D4 X-ray diffractometer at a scanning rate of 4°/min in the 2θ range from 20° to 60° (Cu Ka radiation, $\lambda = 0.15406$ nm).

2.3. Synthesis of hydrophilic Gd^{3+}-doped HA nanorods

In a typical experiment, 0.053 g $GdCl_3$, 0.111 g of $CaCl_2$ and 0.141 g of AEP were dissolved in mixed solvents with 5 mL anhydrous ethanol and 15 mL deionized water. After vigorous stirring for 30 min, 20 mL 0.03 mol/L Na_2HPO_4 aqueous solution was dropwise added, then the pH value was adjusted to 10 using 6 M NaOH after continuous stirring 30 min. The mixture was transferred to a 50 mL autoclave, sealed and hydrothermally treated at 140 °C for 8 h, cooled to room temperature naturally. Subsequently, the obtained precipitate was centrifuged and washed 3 times with deionized water and anhydrous ethanol. The resulting powder was dried in a vacuum for further characterization. In this way, the Gd^{3+} doped hydroxyapatite nanorods were obtained.

2.4. Synthesis of ^{153}Sm-HA:Gd nanorods

$^{153}Sm^{3+}$ (~15 mCi) in 1 mL saline and 1 mL distilled water were added to 5 mg as-prepared HA:Gd nanorods. The reaction mixture was incubated for 10 min at room temperature and centrifuged to obtain ^{153}Sm-HA:Gd nanorods. The ^{153}Sm-HA:Gd nanorods were washed three times in distilled water by sonication and centrifugation. Radio-TLC analysis demonstrated ~100% radiochemical purity of ^{153}Sm-HA:Gd nanorods.

2.5. Relaxivity measurement in vitro

The T_1-weighted MR images were obtained using a 3 T S Magnetom Trio running on Siemens' Syngo software version B15 (Siemens Medical Systems, Shanghai Key Lab of MR, Shanghai, China), in conjunction with an 8 array Loop coil (Siemens Medical Systems). Dilutions of HA:Gd nanorods in deionized water were placed in a series of 1.5 mL tubes for T_1-weighted MRI and T_1-weighted contrast enhancement. The following parameters were adopted: a standard inversion recovery (IR) spin-echo sequence: a repetition time (TR) of 5000 ms, an echo time (TE) of 7.2 ms, and ten inversion recovery times (TI = 23, 100, 200, 400, 600, 800, 1000, 1200, 1500 and 2000 ms). Flip angle = 120 deg, slice thickness = 2.0 mm, FOV read = 190 mm, base resolution = 320. The MR signal intensity in tubes was ascertained by the average intensity in the defined regions of interests (ROIs). T_1 values of each tube were calculated using the following formula: $S(TI) = S0 \times [1-2\exp(-TI/T_1)]$ to fit the T_1 recovery curve in the circular regions of interest for the sample. The resulting T_1 values were averaged over the region of interest and plotted as $1/T_1$ (R_1) vs molar concentration of HA:Gd nanorods. The slope of the line provides the molar relaxivity r_1.

2.6. Cells culture

A human cervical epidermal carcinoma cell line (HeLa cell) was provided by Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS, China). Cells were grown in RPMI 1640 (Roswell Park Memorial Institute's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C and 5% CO_2.

2.7. Animal

Four-week-old BALB/c mice were purchased from the Second Military Medical University (Shanghai, China). Animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee.

2.8. Cell TEM

Cell uptake experiments were performed by incubating HA:Gd nanorods with HeLa cells. HeLa cells were incubated with 150 μg/mL HA:Gd nanorods in a serum-free medium for 5 h at 37 °C under 5% CO_2 and washed with phosphate buffered saline to remove excess of HA:Gd nanorods. Then cells were fixed in 0.25% glutaraldehyde in saline and postfixed in 1% osmium tetraoxide, dehydrated in ascending concentration of ethanol and embedded in Epon 812. Ultrathin sections prepared with a LKB-1 Ultracut were lightly stained with 1% uranyl acetate and lead citrate and observed with Philips CM120 electron microscope.

2.9. Methyl thiazolyl tetrazolium (MTT) assay

In vitro cytotoxicity was measured by performing MTT (Sigma–Aldrich) assays in the HeLa cell line. The cells were seeded into a 96-well cell culture plate at 1×10^4/well, under 100% humidity, and were cultured at 37 °C and 5% CO_2 for 24 h; then different concentration of HA:Gd nanorods (0, 200, 400, 600 and 800 μg/mL, diluted in RPMI 1640) were added to the wells. The cells were subsequently incubated for 24 h and 48 h at 37 °C under 5% CO_2. Thereafter, MTT (10 μL; 5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO_2. An enzyme-linked immunosorbent assay (ELISA) reader (infinite M200, Tecan, Austria) was used to measure the OD570 (Abs. value) of each well referenced at 690 nm. The following formula was used to calculate the inhibition of cell growth:

$$\text{Cell viability (\%)} = (\text{mean of Abs. value of treatment group}/\text{mean Abs. value of control}) \times 100\%$$

2.10. MRI in vivo

Animal procedures were in agreement with the guidelines of the regional ethic committee for animal experiments. *In vivo* experiments were performed on anesthetized mice (20 g) with 10% chloral hydrate 100 μL. MRI was conducted on a 3 T S Magnetom Trio, using a T_1-mapping sequence (TR = 200 ms, TE = 13 ms, slice thickness = 1.5 mm, 256 × 256 matrix, FOV read = 120 mm). The mice were scanned before and after the administration of contrast agent. The saline solution of HA:Gd nanorods (150 μL, 2 mg/mL) was injected intravenously and MRI images were obtained for animal. Image J software was used to measure signal intensities of the liver and spleen area.

2.11. SPECT/CT imaging in vivo and biodistribution studies ex vivo

For SPECT imaging, ^{153}Sm-HA:Gd nanorods (~3 mCi) in 0.2 mL saline were injected into an anesthetized mouse (20 g) through the tail vein under authorization of the regional ethic committee for animal experiments. SPECT images were recorded on a Bioscan NanoSPECT/CT scanner at 1 h, 24 h, 48 h and 72 h. The experimental results were analyzed by HiSPECT software. For biodistribution studies, ^{153}Sm-HA:Gd nanorods (~1 mCi) in 0.1 mL of saline were injected into mice. The major organs were collected and weighed at 1 h, 24 h 48 h and 72 h post-injection. Radioactivity in the organs was counted in γ-counter (WIZARD 1470, Perkin–Elmer Wallac, USA) and calibrated against a known aliquot of the injectate. The percentage of added dose per gram (% ID/g) for each organ was calculated by comparing its activity with appropriate standard of injected dose (ID), the values expressed as mean ± standard deviation for 6 mice per group. All data have been processed with attenuation correction.

2.12. Body weight monitoring

HA:Gd nanorods at a total dose of 15 mg/kg were injected into mice ($n = 6$) through the tail vein; this group of mice acted as the test group. Mice ($n = 6$) without injection of nanorods were acted as the control group. The body weight of the mice in both groups was recorded every other day for 61 days.

2.13. Histology and serology studies

HA:Gd nanorods at a total dose of 15 mg/kg were injected into mice through the tail vein; this group of mice acted as the test group. Mice with no injection of nanorods were acted as the control group. Blood samples and tissues were harvested from mice injected with HA:Gd nanorods and from mice receiving no injection, respectively. Blood

Download English Version:

https://daneshyari.com/en/article/10227949

Download Persian Version:

https://daneshyari.com/article/10227949

Daneshyari.com