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Aluminium hydroxide stabilised $MnFe_2O_4$ and Fe_3O_4 nanoparticles as dual-modality contrasts agent for MRI and PET imaging

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ABSTRACT

Magnetic nanoparticles (NPs) $MnFe_2O_4$ and Fe_3O_4 were stabilised by depositing an $Al(OH)_3$ layer *via* a hydrolysis process. The particles displayed excellent colloidal stability in water and a high affinity to [^{18}F]-fluoride and bisphosphonate groups. A high radiolabeling efficiency, 97% for ^{18}F-fluoride and 100% for ^{64}Cu-bisphosphonate conjugate, was achieved by simply incubating NPs with radioactivity solution at room temperature for 5 min. The properties of particles were strongly dependant on the thickness and hardness of the $Al(OH)_3$ layer which could in turn be controlled by the hydrolysis method. The application of these $Al(OH)_3$ coated magnetic NPs in molecular imaging has been further explored. The results demonstrated that these NPs are potential candidates as dual modal probes for MR and PET. *In vivo* PET imaging showed a slow release of ^{18}F from NPs, but no sign of efflux of ^{64}Cu.

1. Introduction

Superparamagnetic nanoparticles (NPs) have been intensively investigated due to their potential applications in biosensors [1–3], targeted drug delivery [4–7], MRI [8,9] and localised hyperthermia induction [10,11]. An obstacle to application of these NPs is that they tend to aggregate and form larger secondary particles, in order to minimise their surface energy. Moreover, magnetic NPs are most often synthesised in organic solvents and coated with an organic layer of oleylamine or oleic acid rendering them soluble only in non-polar solvents. On the other hand, medical or bio-applications require colloidal stability and dispersibility in water and biological environments. Many methods have been developed to obtain stable colloids of magnetic NPs, reviewed by Laurent et al. [12]. Amongst them, coating with polyethyleneglycol (PEG) [8] or Dextran [13] has been widely used, as these hydrophilic and biocompatible materials not only provide a steric barrier against aggregation, but also make them hardly recognised by the macrophage-monocytic system [14]. To avoid desorption of the polymeric coating by heating or dilution, one or more functional groups, such as carbonate or phosphonate, are necessary to bind with the NPs. Such polymers, however, involve a complicated multi-step synthesis approach [8,15]. Therefore the use of an inorganic shell material that introduces stability, functionality and water-solubility is desirable.

Herein, we report a simple approach to stabilise magnetic NPs by coating them with an $Al(OH)_3$ layer. The aluminium hydroxide

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coating was selected, due to its high affinity with fluoride anions [16] and bisphosphonate groups [17], which allow easy radiolabelling and functionalisation, and its biocompatibility as shown by its application in vaccine adjuvants [18].

2. Experimental section

2.1. Materials and general characterisation

All chemicals were used as purchased without further purification. Deionised water was obtained from an ELGA PureLab Option Q system. Bisphosphonate polyethyleneglycol (BP-PEG) polymers were synthesised in house according to published methods [8]. X-Ray powder diffraction (XRD) measurements were recorded at room temperature on a PANalytical X'Pert PRO diffractometer using Cu-$K_{\alpha 1}$ radiation ($\lambda = 1.540598$ Å) at 40 kV, 40 mA, a scan speed of 0.02°/s and a step size of 0.026° in 2θ, at Nottingham University. X-Ray photoelectron spectra were recorded using a Thermo Fisher ESCALAB 250 X-ray Photoelectron Spectrometer with a hemispherical sector energy analyser at Aston University. Monochromatic Al K_α X-ray source was used at excitation energy of 15 kV and an emission current of 6 mA. The analyser pass energy of 20 eV with step size of 0.1 eV was used throughout the experiment. Transmission electron microscope (TEM) images were taken on a Tecnai FEI T20 at Centre for Ultrastructural Imaging, King's College London. Attenuated total reflectance infrared (ATR-IR or IR) spectra were recorded on a Perkin Elmer spectrum 100. Dynamic light scattering (DLS) experiments were carried out on Zetasizer Nano ZS from Malvern Instruments with a measure angle 175° and a 632.8 nm laser. Zeta potential for all samples was measured in neutral aqueous solution with a pH value ≈7.

2.2. Synthesis

2.2.1. Synthesis of $MnFe_2O_4$ and Fe_3O_4

Magnetic NPs were obtained *via* a method reported previously [19,20]. Typically, 6 mmol 1,2-hexadecanediol was added to a 100 ml flask containing 20 ml phenyl ether, 5 ml oleylamine and 5 ml oleic acid at 120 °C, and the resultant solution was kept at this temperature under vacuum for over 30 min to remove water in the solvent. To this light yellow solution, 1 mmol $Mn(acac)_2$ and 2 mmol $Fe(acac)_3$ (or 2 mmol $Fe(acac)_3$ for Fe_3O_4), was added under N_2, and then temperature was increased to 270 °C at a rate of 10 °C/min with magnetic stirring. After 30 min, the flask was cooled to room temperature by removal from the hotplate. To precipitate out the NPs, 40 ml ethanol was added. The particles were collected by centrifugation (Jouan CR312, at a speed of 3000 rpm for 30 min) and washed with ethanol/hexane twice.

2.2.2. Synthesis of $MnFe_2O_4$@$Al(OH)_3$ (**1**)

$MnFe_2O_4$ (80 mg, 0.33 mmol) was dissolved in 30 ml diethyl ether by sonication for 20 min to form a dark brown solution, and then 10 ml of a diethyl ether solution containing $AlCl_3$ (144 mg, 1 mmol) was added dropwise. The mixture was sonicated for 2 min before the addition of 500 µl water (27.8 mmol). The subsequent addition of 10 ml acetone led to a brown suspension. The product was collected by centrifugation and then dried in a stream of N_2 to remove ether and acetone, and redispersed in water.

2.2.3. Synthesis of Fe_3O_4@$Al(OH)_3$ samples (**2**–**4**)

In the case of Fe_3O_4@$Al(OH)_3$ (with a precursor molar ratio of Fe_3O_4 to $AlCl_3$ of 1:3) (**4**), a faster uncontrolled hydrolysis method was used. Fe_3O_4 (82 mg, 0.33 mmol) was dissolved in 30 ml diethyl ether after sonication for 20 min to form a dark brown solution, and then 10 ml diethyl ether solution containing $AlCl_3$ (144 mg, 1 mmol) was added dropwise. The mixture was sonicated for 2 min before the addition of 10 ml acetone leading to a brown suspension. The product was collected by centrifugation and then dried with a stream of N_2 to remove ether and acetone, and re-dispersed in water. Corresponding amounts of $AlCl_3$ were used with the same volume of Et_2O to obtain Fe_3O_4@$Al(OH)_3$ (1:1) (**2**) and Fe_3O_4@$Al(OH)_3$ (1:2) (**3**) samples with various core–shell ratios.

2.2.4. Filtration of MFe_2O_4@$Al(OH)_3$ (M = Mn or Fe)

The $Al(OH)_3$@MFe_2O_4 solution prepared as described in Section 2.2.2 (200 µl) was diluted with water (1 ml) to form a transparent brown solution, and then transferred to a 1 ml centrifuge tube with a filter inside (NanoSep, cut-off-molecular size, 30 K). Brown NPs were obtained on the filter by centrifugation at 5000 rpm for 20 min.

2.2.5. Preparation of Fe_3O_4@$Al(OH)_3$-BP-PEG(5K)

Bisphosphonate polyethyleneglycol (prepared as described elsewhere [8]) (5 mg) was added to the aqueous solution of Fe_3O_4@$Al(OH)_3$ (5 ml, *ca.* 4 mg/ml), followed by a sonication treatment for 10 min.

2.3. Radiolabelling with ^{18}F and radiochemical stability in water

^{18}F labelling of MFe_2O_4@$Al(OH)_3$ (M = Mn, or Fe, **1**–**4**) was measured in triplicate at different concentrations. Typically, 50 µl aqueous [^{18}F]sodium fluoride solution containing *ca.* 5 MBq radioactivity was added to a 450 µl solution of varying concentrations of $MnFe_2O_4$@$Al(OH)_3$ in NanoSep with a cutoff size of 30k. After 10 min incubation with continuous shaking at room temperature, labelled NPs were separated by centrifugation at 5000 rpm (Eppendorf centrifuge 5424) for 20 min. The radioactivity of the supernatant and particles (on the filter) was measured separately using a gamma counter. The labelling efficiency was given by the following Equation (1):

$$\text{Labelling efficiency (\%)} = \frac{\text{Activity of NPs}}{\text{Activity of NPs} + \text{Activity of supernatant}} \times 100\% \quad (1)$$

Triplicate samples of ^{18}F labelled NPs were separated as described above. The NPs retained on the filter were re-suspended in deionised water in the inner NanoSep tube and then centrifuged at 5000 rpm for 20 min. This step was repeated three times. The percent binding retained after each washing step was calculated using equation (1). The correction for cumulative loss of label for the second and third washing steps was performed as exemplified by the following equation (2):

$$\text{Cumulative Binding} = \text{Activity \% in NPs} \times \text{Activity \% in NPs prewash} \quad (2)$$

2.4. Radiochemical stability of ^{18}F-labelled **1**, **2**, **3**, **4** in serum

Triplicate samples of labelled NPs were prepared on a NanoSep membrane as described above. The NPs retained in the filtrate were re-suspended in 25% serum in water (v/v), incubated at 37 °C for a period of up to 6 h, and then centrifuged at 10,000 rpm (Eppendorf centrifuge 5424) for 30 min. The cumulative binding was calculated using equation (2) as described previously.

2.5. Adsorption of non-radioactive ^{19}F

5 mg NP **1** were dissolved in 5 ml freshly prepared NaF solution with concentrations of 0.01 mmol/L, 0.1 mmol/L, 1 mmol/L and 10 mmol/L. The suspensions of NPs were sonicated with the laboratory sonicator bath for 1 h, and then left overnight. The samples were centrifuged for 30 min at 3000 rpm (Jouan CR312) and 4 ml of supernatant was then withdrawn from each sample. The concentrations of fluoride anions in supernatant and corresponding particle-free NaF solution were measured with an Orion Star 214 bench-top meter with a fluoride combination electrode (from Fisher Scientific). Duplicate samples were prepared for each concentration. Adsorption percentage was obtained by dividing the concentration difference between the supernatant and the initial particle-free solution by the initial concentration.

2.6. [^{18}F]-fluoride radiolabelling of washed Fe_3O_4@$Al(OH)_3$ samples

500 µl of 1.34 mg/ml suspension of **2** in water (or 2 mg/ml **3** NPs, or 2.35 mg/ml **4** NPs) was placed in a NanoSep tube with omega membrane (molecular weight cutoff, 30 kDa). The tubes were centrifuged at 5000 rpm (Eppendorf centrifuge 5424) for 20 min, and then these NPs were re-dissolved in 450 µl water. 50 µl [^{18}F]sodium fluoride (*ca.* 5 MBq) was added to these NPs solutions in the NanoSep tubes. After 10 min incubation by continuous shaking at room temperature, the tubes were centrifuged at 5000 rpm for 20 min. As described before, the activities in the filtrate and remaining on NPs (on the filter) were separately measured with a gamma counter, to produce a labelling efficiency for the 1st washed Fe_3O_4@$Al(OH)_3$ samples. To measure the labelling efficiency for 2nd washed NPs, the washing step was repeat twice before incubation with ^{18}F-fluoride radioactivity.

2.7. Radiolabelling of **1** with ^{64}Cu

1 mg bis(dithiocarbamate) bisphosphonate (DTCBP) [15] was dissolved in 100 mM Na_2CO_3 buffer (pH 9). 200 µl of the above solution was added to 200 µl $^{64}CuCl_2$ radioactivity (*ca.* 20 MBq) solution that was buffered to pH 5 with sodium acetate. It is essential to maintain the solution at neutral pH, since $Al(OH)_3$ is not stable either in acidic or in basic solution. After 5 min, 200 µl 0.5 mg/ml $MnFe_2O_4$@$Al(OH)_3$ solution containing 0.2 mg/ml PEG-5K was added and the mixture was incubated at room temperature for another 5 min. The radiolabelled NPs were isolated by filter centrifugation at speed of 5000 rpm for 15 min, using a Nanosep with a cutoff size of 30 K. There was no radioactivity observed in the filtrate, and all radioactivity remained on NPs in the filter. The ^{64}Cu radiolabelled NPs were re-dissolved in 100 µl saline for injection.

2.8. T_1, T_2 and T_2^* relaxivity measurement

MR imaging of all particles was performed with a standard extremity flex coil on a clinical 3T Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands). T_1 mapping was obtained by using a 2D sequence that employed two non-selective inversion pulses with inversion times ranging from 20 to 2000 ms, followed by eight segmented readouts for eight individual images [21]. The two imaging trains resulted in a set of 16 images per slice with increasing inversion times (FOV = 200*200 mm, matrix = 200*179 mm, in-plane resolution = 1*1.12 mm, measured slice thickness = 3 mm, slices = 16, TR/TE = 3.2/1.6 ms, FA = 10°). T_2 was