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Synthesis of polyacrylamide modified magnetic nanoparticles and radiolabeling with ^{188}Re for magnetically targeted radiotherapy

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Abstract

Magnetic nanoparticles were synthesized, modified with polyacrylamide, and then characterized by TEM, FTIR, VSM and PCS. Rhenium-188 (^{188}Re) was bound to the nanoparticles by imidazolyl groups of histidine immobilized on the surface. The labeling yield was about 90% with good in vitro stability. Such nanoparticles might be useful for magnetically targeted radiotherapy.

Keywords: Nanoparticles; Rhenium-188; Re-188; Radiotherapy; Radiolabeling; Polyacrylamide; Histidine

1. Introduction

One major hurdle that underlies the use of nanoparticle therapy is how to get the nanoparticles to a particular site in the body. Magnetic nanoparticles have been proposed for use as biomedical purposes for several years, for which a potential benefit is the use of localized magnetic field gradients to attract the particles to a targeted site until the therapy is complete and then to remove them.

Magnetic carriers were first used to target cytotoxic drugs (doxorubicin) to sarcoma tumors implanted in rat tail [1]. The possibility of targeting radionuclides to specific site was also evaluated [2].

For in vivo applications the magnetic particles must be coated with a biocompatible polymer to prevent aggregation during or after the synthesis process and biodegradation when exposed to the biological system. On the other hand, the polymer should also allow binding, adsorption and entrapment of drugs on the particles.

In this paper, we synthesized magnetic nanoparticles and modified the particles with

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polyacrylamide. The modified particles were radiolabeled for magnetically targeted therapy.

2. Experimental

2-*N*-morpholinoethanesulfonic acid (MES) and BH_3NH_3 were purchased from Fluka. Carbon monoxide gas was purchased from Shanghai Ruifang Gas Co., Ltd. All other chemicals were analytical reagents and purchased from Shanghai Chemical Regents Company and used without further purification. Carrier-free ^{188}Re-perrhenate was freshly eluted with saline from an alumina-based $^{188}W/^{188}$Re-generator (Amersham-Kexing RadioPharm. Co., Ltd. PR China). Particle size distribution and morphology were characterized with Photo Correlation Spectrum (PCS) (Zetasizer 3000HS, Malvern Instruments Ltd.) and TEM (Hitachi 600). Magnetic properties were characterized in a vibrating sample magnetometer (VSM) (Model 155, EG&G Princeton. Research, USA). The chelating efficiency was determined by thin layer chromatography (TLC) and the radioactivity was monitored in a TLC scanner (AR2000, Bioscan) and γ-counter (SN-697, Shanghai Rihuan Photoelectronic Instrument Co., Ltd. Shanghai, PR China).

2.1. Synthesis of magnetic nanoparticles

The preparation consisted of two steps: the preparation of Fe_3O_4 nanoparticles and the following hydrothermal process. For the first step the partial reduction method [3] in nitrogen atmosphere was used.

$$6Fe^{3+} + SO_3^{2-} + 18NH_4OH \rightarrow 2Fe_3O_4 + SO_4^{2-} + 18NH_4^+ + 9H_2O.$$

In brief, 6 ml of 1 M ferric chloride solution was added into a 100 ml three-necked flask and then diluted into 16 ml. Pure N_2 was bubbled throughout the preparation procedure to prevent oxidation. One milliliter of 1 M freshly-prepared sodium sulfite solution was diluted into 7 ml and then added dropwise to the above solution. After the color of the reaction solution turned to light yellow, 12 ml of concentrated ammonia was added quickly and the dark Fe_3O_4 precipitated from the solution. The mixture was taken out and dropped slowly into a 50-ml teflon-lined stainless autoclave, which was put into oven and kept at 180 °C for 6 h, then naturally cooled to room temperature. The black magnetic precipitate was recovered in an external magnetic field and washed several times with distilled water and water–ethanol (2:1), respectively.

2.2. Surface modification of the particles with polyacrylamide

Light irradiation polymerization on line was used for the modification of the particles with acrylamide as monomer, diacrylamide as cross-linking agent and distilled water as solvent. Typically, after deoxgenation with nitrogen gas for 25 min, the acrylamide and diacrylamide were added until the concentrations reached 0.1 M and 0.25 mM, respectively. A 500 W Xe lamp at a wavelength of 230–280 nm was used to irradiate for 10 h under mechanical stirring (400 r/min). The modification was performed at room temperature and the distance from the light source to the sample was 20 cm. During the irradiation process nitrogen was bubbled as the protection gas. The sample was collected with a magnet and washed with distilled water three times and finally dispersed in water using ultrasound.

2.3. Elimination of carbonyl on the modification layer

In order to make the surface more suitable for activation the carbonyl from polyacrylamide was taken off by the Hofmann degradation reaction. Sodium hypochlorite (1 M) in 2.4 wt% sodium hydroxide aqueous solution was added to the above purified sample slowly under stirring. The carbonyl was completely eliminated in 0.5 h, as confirmed by FTIR.

2.4. Immobilization of histidine

Glutaraldehyde was used as a cross-linker for the immobilization of histidine to the modified

nanoparticles. For this purpose, 10 mg of the modified particles was dispersed in 1 ml of 5% (v/v) glutaraldehyde in 0.1 M PBS (pH = 7.4). After stirring for 3 h at room temperature, the particles were recovered and washed with 0.1 M PBS for three times. Then the particles were redispersed in 1 ml of 0.2 M histidine aqueous solution in 0.1 M PBS–0.15 M NaCl–0.005 M EDTA (pH 7.2) and incubated for 12 h at room temperature. The particles were retrieved and washed with 0.1 M borate buffer solution (pH = 9.4), and redispersed in 0.5 mg/ml $NaBH_4$ and stirred for 30 min at 4 °C. Finally, the particles were recovered and washed with 0.1 M PBS (pH = 7.4) for radiolabeling.

2.5. Labeling of particles with ^{188}Re

Carbonyl rhenium was prepared according to Schibli [4]. The histidine-immobilized particles were then labeled as follows. Five milligram of boron amine complex was weighed into 10 ml glass vial sealed with aluminum-backed rubber stopper. Carbon monoxide gas was introduced into the vial brimming for about 20 min, followed by adding 1 ml of freshly diluted $^{188}ReO_4^-$ solution with weak acidity and incubating at 75 °C for 20 min. TLC with glass-backed silica gel as stationary phase (GF_{254}) and 99% (v/v) methanol 1%(v/v) concentrated HCl as mobile phase was used to determine the level of labeling efficiency of ^{188}Re.

Freshly prepared carbonyl ^{188}Re (0.1 mCi) was added to 0.5 M MES solution in a volume containing 10 mg magnetic particles for labeling. Reaction time, temperature and reaction volume were varied for optimization of the labeling conditions. The radioactivity was measured by γ-counter. The labeling efficiency refers to the ratio of activity bound to the particles over the total activity added.

3. Result and discussion

3.1. Synthesis of magnetic nanoparticles

By partial reduction and the following hydrothermal process, we prepared Fe_3O_4 nanoparticles with high crystallization and good magnetic properties. The mean particle size was 10 nm (8–13 nm: 85.4% analyzed with IPP 4.5 software) and the saturation magnetization was 71 emu/g with a small coercivity value of $\sigma = 17$ Oe (Fig. 1).

3.2. Surface modification of the particles with polyacrylamide

The modified magnetic particles were characterized by PCS as shown in Fig. 2. The diameter of the particle was about 78 nm with narrow size distribution. The polydispersity index was 0.217. It was found that the thickness of modified shell can be adjusted in the range of 10–150 nm with distribution of ± 0.02 depending on the monomer concentration, irradiation doses, reaction temperature and stirring speed. The modification layer can be made thinner and harder when small amounts of cross-linking agent (0.25 M in this experiment) was added under otherwise identical conditions.

The carbonyl group of polyacrylamide in the modified shell was eliminated successfully through Hofmann degradation. The FT-IR spectrum of the modified nanoparticles is shown in Fig. 3. The peaks at 1661.9 cm^{-1} and 1618.2 cm^{-1} are the absorbance of stretching vibration of carbonyl group and scissor vibration of $-NH_2$, respectively. After carbonyl elimination, absorbance at

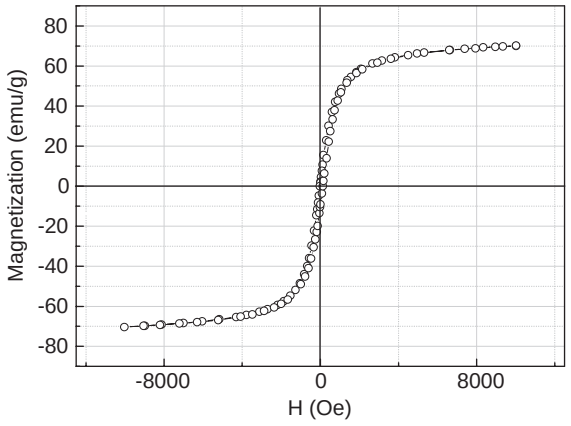

Fig. 1. Magnetic hysteresis curve for the magnetic naoparticles measured at room temperature.

1661.9 cm^{-1} was significantly decreased, as was the vibrational absorbance of the hydrogen bonds between amine and carbonyl at 3344.14 cm^{-1} and 3319.97 cm^{-1}.

3.3. Labeling of particles with ^{188}Re

According to Schibli, the R_f value of $[^{188}Re(H_2O)_3(CO)_3]^+$ was 0.4 and that for free $^{188}ReO_4^-$ was 0.7 [4]. In our experimental TLC results, however, the carbonyl rhenium prepared by the above method was a mixture of $[^{188}Re(H_2O)_{6-\chi}(CO)_\chi]$ and the R_f for it was between 0.4–0.6. Level of carbonylization of ^{188}Re was about 95%. The ^{188}Re-carbonyl complex used to label the nanoparticles was unstable and always had to be prepared freshly.

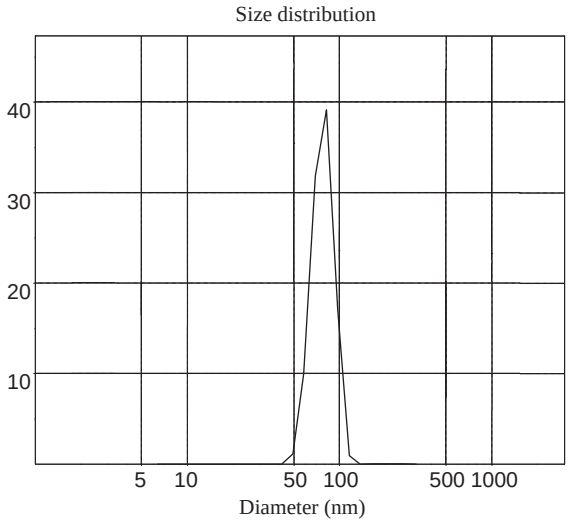

Fig. 2. The size distribution of modified Fe_3O_4 characterized by PCS.

The radiolabeling results show that the carbonyl complexes were chelated well by histidine on the surface of the magnetic nanoparticles. Fig. 4 shows the relationship between radiolabeling efficiency and reaction time. The effect of reaction time on the radiolabeling efficiency was similar between 30 and 70 min. However, the release of ^{188}Re at 37 °C in bovine serum albumin from the particles labeled in 30 min was about 25% at 24 h and 10.7% labeled in 40 min, respectively. The labeling efficiency was above 90% at 50 min with less than 5% release of radioactivity (Fig. 7). For this reason, we used 50 min as the time for further reaction optimizations.

The radiolabeling efficiency increased with temperature when 0.1 mCi of carbonyl ^{188}Re and 10 mg magnetic nanoparticles were incubated in 50 μl reaction bath for 50 min (Fig. 5). The labeling efficiency approached about 90% at 50 °C and with further temperature increase, it began to decrease. Perhaps the carbonyl rhenium was not stable at higher temperature under the reaction condition.

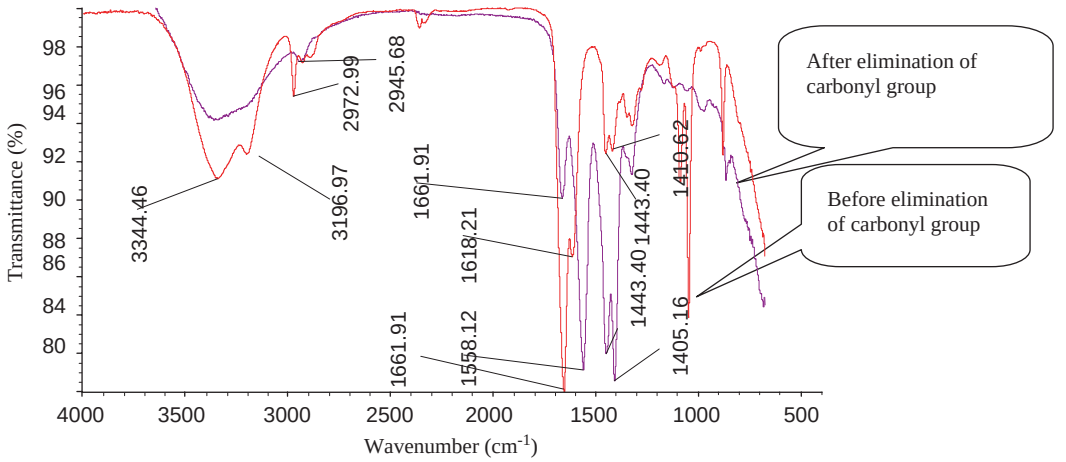

Fig. 3. FT-IR spectra of modified particle before and after elimination of carbonyl group.

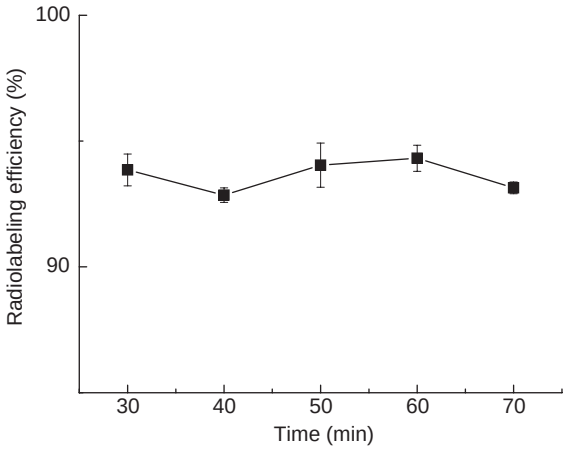

Fig. 4. Relationship between radio labeling efficiency and reaction time (with 50 °C reaction temperature and 50 μl reaction volume).

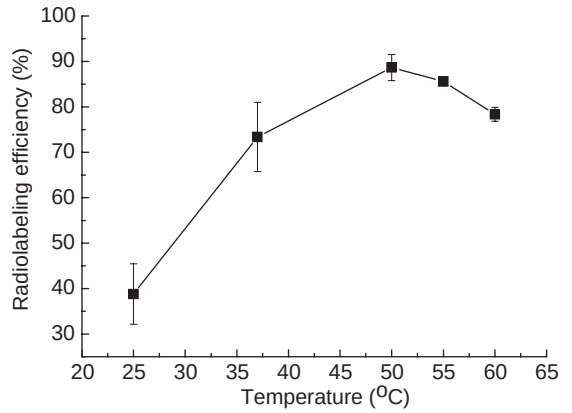

Fig. 5. Relationship between radiolabeling efficiency and temperature (with 50 μl reaction volume and 50 min reaction time).

The volume of reaction bath affected the radiolabeling efficiency significantly (Fig. 6). With increasing volume from 50 to 400 μl, it almost linearly decreased. The preferred volume for labeling was therefore 50 μl.

We also investigated the labeling stability over 72 h at 37 °C in bovine serum albumin (Fig. 7). After 24 h, there was almost no ^{188}Re released from the particles and about 90% of ^{188}Re was still bound after 48 h.

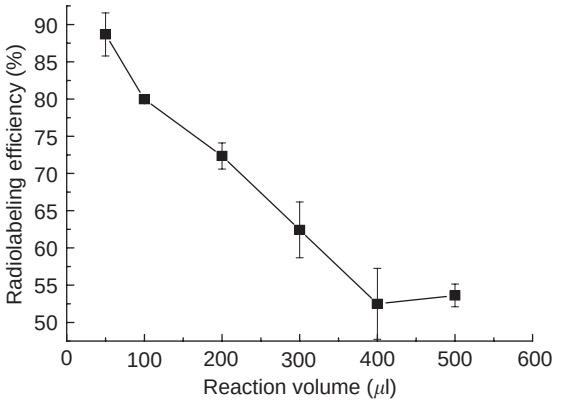

Fig. 6. Relationship between radiolabeling efficiency and reaction volume (with 50 °C reaction temperature and 50 min reaction time).

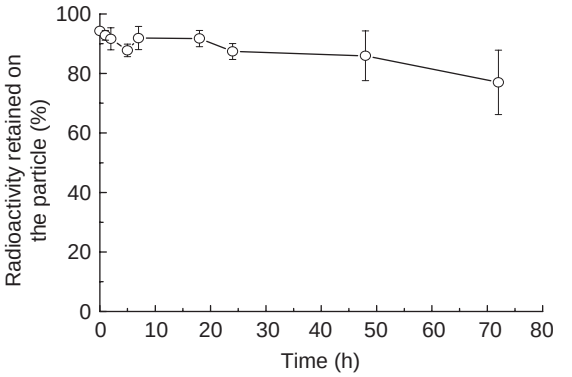

Fig. 7. Stability of radiolabeled particles.

4. Conclusion

We prepared magnetic nanoparticles and modified the particles with polyacrylamide by an irradiation chemical method. The diameter of the modified particles was about 78 nm with narrow size distribution. The polydispersity index was 0.217. In order to radiolabel the particles with ^{188}Re, tricarbonyl ^{188}Re was synthesized as a radiolabeling precursor and histidine, as a chelator, was immobilized on the particle surface in a modification layer. For 0.1 mCi carbonyl ^{188}Re and 10 mg magnetic nanoparticles, the best labeling condition was to incubate the particles in a

volume of 50 μl for 50 min at 50 °C. The labeling efficiency was about 90% and the stability in serum albumin at 37 °C was 90% after 48 h.

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