^{188}Re labeled MPEG-modified superparamagnetic nanogels: preparation and preliminary application in mice

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Abstract Superparamagnetic poly(acrylamide) magnetic nanogels produced *via* photochemical method have been developed. After Hoffmann degradation of carbonyl, the nanogels with amino groups, or poly(acrylamide-vinyl amine) magnetic nanogels, were also obtained. And the magnetic nanogels were further modified by methoxy poly(ethylene glycol) (MPEG) for higher dispersibility and stability. The MPEG-modified magnetic nanogels were characterized by X-ray diffraction (XRD), photo correlation spectroscopy (PCS) and scanning electron microscopy (SEM), respectively. The MPEG-modified magnetic nanogels were labeled by ^{188}Re radiopharmaceuticals and intravenously injected into tails of mice in the presence and absence of a 0.5 T external magnetic field targeted on the bellies. The radioactivity distribution was monitored *in vivo*. In the absence of magnetic field, the radioactivity was mainly distributed in liver, spleen, kidney, stomach and lung. In the presence of the magnetic field, the radioactivity was mainly accumulated on the targeted point, verifying the magnetically targeted character.

Keywords Magnetic nanogels, Photochemical method, MPEG, Magnetic targeted radiopharmaceuticals carriers

CLC numbers O635, O644.1, O611.62

1 Introduction

Modified magnetic micro- and nanoparticles have recently been receiving growing attention because of their extensive applications in the fields of biology and medicine such as protein and enzyme immobilization,[1] bioseparation,[2] immunoassays[3] and so on. In recent years, a great deal of efforts have been devoted to developing targeted drug carriers with magnetic nanogels.[4]

Magnetic nanogels are nanoscale particles of inorganic/polymer core-shell composites, i.e., ferromagnetic magnetite (Fe_3O_4) core and polymer nanogel shell. The Fe_3O_4 core, which possesses strong magnetic property and superparamagnetic behavior, is of relatively low toxicity to human body when encapsulated in the protective polymer shell. The shell which is cross-linked polymer hydrogels prevents the Fe_3O_4 core from oxidation and aggregation. With good hydrophilicity and biocompatibility, the magnetic nanogels with hydrogel shell can be desirable for biomedical applications, such as targeted drug delivery system.

In this paper, we propose an alternative approach to synthesize polyacrylamide (PAM) superparamagnetic nanogels *via* photochemical reactions at room temperatures in an emulsion and initiator free aqueous system. Superparamagnetic nanogels with functional amino groups were obtained after Hoffmann elimination of the carbonyl groups. The magnetic nanogels with amino groups were further modified by methoxy poly (ethylene glycol) (MPEG) for higher dispersibility and stability, and covalently radiolabeled with ^{188}Re complex. The MPEG-modified magnetic nanogels radiolabeled with ^{188}Re complex were intravenously injected into tails of three mice to find out the

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radioactivity distribution *in vivo* in control group and into tails of three mice in another group in the presence of 0.5 T external magnetic field targeted on the bellies to investigate the magnetically targeting feature.

2 Experimental

2.1 Materials

All chemicals were of analytical grade, commercially available and used without further treatments, except that acrylamide (AM), from Shanghai Chemical Reagents Corporation (SCRC), had been recrystallized prior to use. Toluene and pyridine, from SCRC, had been refluxed for four hours to remove water thoroughly. N, N'-Methylene-bis-(acrylamide), serving as cross-linker in photochemical synthesis, was supplied by SCRC, too. NaClO and NaOH were supplied by Shanghai Hengxin Chemical Reagents Ltd. A 500W Xe lamp was used as UV light source. N_2 (99.99%) was used as protective gas. Triply distilled water was used in the experiment. Carrier-free ^{188}Re-perrhenate was freshly eluted with saline from an alumina-based ^{188}W/^{188}Re-generator (Shanghai Kexing Pharm. Co. Ltd.; ^{188}W supplied by Oak Ridge National Laboratory, USA).

2.2 Modification of poly(acrylamide-vinyl amine) magnetic nanogels with MPEG

Superparamagnetic Fe_3O_4 nanoparticles were prepared by partial reduction method, and coated with polyacrylamide by photochemical method. The magnetic nanogels with amino groups, namely poly(acrylamide-vinyl amine), were obtained with further Hoffmann degradation. The details can be seen from Ref.[5].

78 g of MPEG, 16 mL of pyridine and 50 mL of toluene, mole ratio 1:3:1, were added into the three-necked flask in turn. After dropping with 44 mL thionyl chloride, the solution was reflux for four hours. The filtrate of the solution at room temperature was distilled at reduced pressure to remove redundant toluene and the MPEG-Cl produced was of a productive rate of 91%. The prepared MPEG-Cl adjusted to pH=11 by 2.4% NaOH was mixed with the poly(acrylamide-vinyl amine) magnetic nanogels as prepared above also adjusted to pH=11 by 2.4% NaOH. After 12 hours incubation at room temperature, the MPEG-modified superparamagnetic nanogels were obtained successfully.

2.3 Radiolabeling of MPEG-modified magnetic nanogels with ^{188}Re complex

Glutaraldehyde was used as cross-linker for immobilizing histidine onto the nanogels with amino groups according to the method of Cao Jinquan et al[6] as shown in Fig.1.

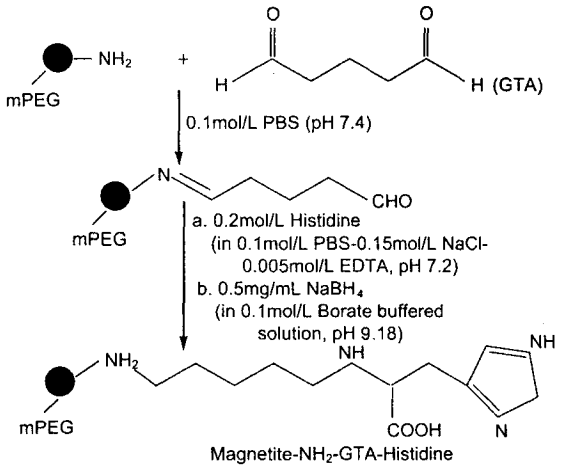

Fig.1 Procedure for covalently linking histidine onto the MPEG-modified magnetic nanogels.

The $[^{188}Re(CO)_3(H_2O)_3]^+$ was prepared according to Schibli et al[7] for labeling the histidine-immbolized superparamagnetic nanogels. Radioactivity of the labeled product was measured by a γ-ray counter. Labeling efficiency is the ratio of the measured radioactivity to the total radioactivity of the added ^{188}Re described as follows:

$$\text{Labeling efficiency}=(1-\text{radioactivity of supernatant/total radioactivity})\times 100\% \quad (1)$$

2.4 Stability in vitro

One milliliter of calf serum was added into the ^{188}Re labeled superparamagnetic nanogels modified by MPEG and the magnetic nanogels were incubated at 37℃. The treated magnetic nanogels were sampled and analyzed at 5 points (1,4,8,18,24h). The labeling rentention stability of the magnetic nanogels was calculated according to Eq.(1).

2.5 The magnetically targeted experiment on mice

Six experimental mice were divided into two

groups. In the control group, 4.4MBq of ^{188}Re labeled MPEG-modified superparamagnetic nanogels diluted by 0.1 mol/L phosphate buffer solution (PBS, pH 7.4) was intravenously injected into the tails of mice. And the mice were dissected to calculate the average radioactivity in various organs after 40 min. The same dose was treated on other three mice in a 0.5 T external magnetic field targeted on the bellies to find out the magnetically targeted character.

3 Results and discussion

3.1 Confirmation of the magnetic nanogel's formation

FTIR measurements were performed to confirm formation of the magnetic nanogels.

The sample was obtained with AM monomer concentration of 0.1 mol/L, irradiated time of 5 h, stirring speed of 400 r/min, reaction temperature of 25ºC and a distance of 20 cm between the UV lamp and the quartz flask. FTIR spectra of the magnetic nanogels had been obtained before and after Hoffmann degradation to eliminate carbonyl from the polyacrylamide shell[5]. The peaks at 1661.9 and 1618.2 cm^{-1} correlate stretching vibration of carbonyl group and the scissor vibration of $-NH_2$, respectively. After the Hoffmann degradation, the absorbance at 1661.9 cm^{-1} decreased significantly. The FTIR results show that magnetic nanogels with amino groups, or poly(acryiamide-vinyl amine) magnetic nanogels, were obtained successfully.

Fig.2 is fluorescence spectra of MPEG-modified superparamagnetic nanogels and poly (acrylamide-vinyl amine) magnetic nanogels. In contrast to poly (acrylamide-vinyl amine) magnetic nanogels, the maximal absorption spectra of MPEG-modified superparamagnetic nanogels have a large blue shift, which partially indicates that MPEG-modified superparamagnetic nanogels have been obtained successfully.

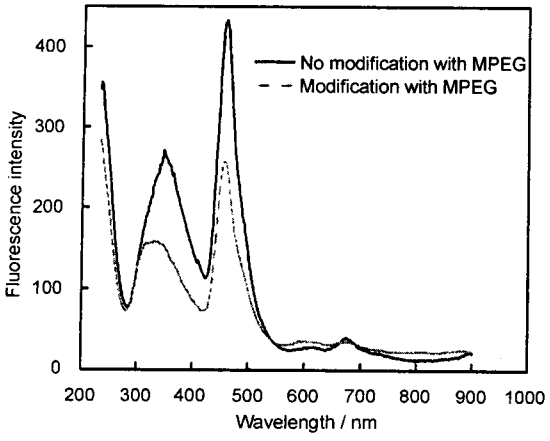

Fig.2 Fluorescence spectra of superparemagnetic nanogels before and after MPEG modification.

3.2 Magnetic property of MPEG-modified superparamagnetic nanogels

Magnetic property of Fe_3O_4 and MPEG-modified superparamagnetic nanogels were characterized by XRD patterns (Fig.3 and Fig.4), which were consistent with crystal characteristic of magnetite (JCPDS No. 19-0629). The results show also that MPEG-modified superparamagnetic nanogels retain high crystallization of Fe_3O_4 after coating and modification. The saturation magnetization and the coercivity of MPEG-modified superparamagnetic nanogels stored in N_2 atmosphere for three months remained almost constant.

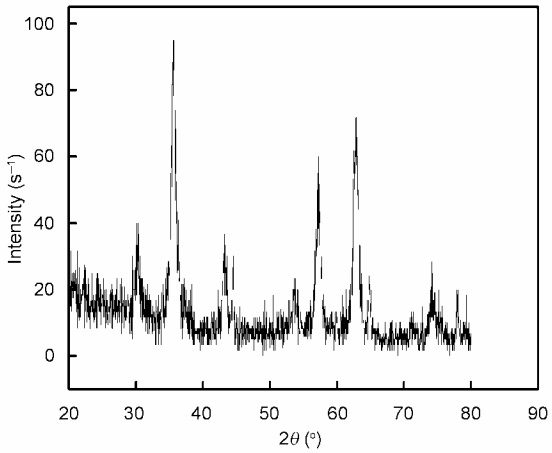

Fig.3 XRD pattern of Fe_3O_4 nanoparticles.

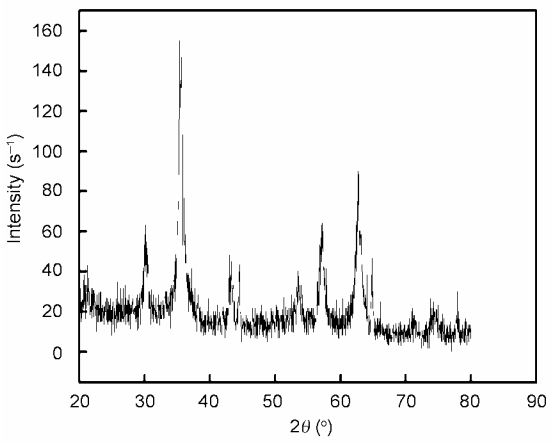

Fig.4 XRD pattern of MPEG-modified superparamagnetic nanogels.

3.3 Surface property and morphology of MPEG-modified superparamagnetic nanogels

Small negative charge can be desirable for delivery of magnetic nanogels *in vivo* as it helps to reduce combination with haemoglobin. The value of MPEG-modified superparamagnetic nanogels was of -18.5mV at pH 7 (Fig.5), which is suitable for delivery of magnetic nanogels, and this might in turn increase the cycle time of the magnetic nanogels in bood. As a result, more magnetic nanogels might concentrate on the targeted point. Moreover, the magnetic nanogels show better hydrophilicity and biocompatibility after modification with MPEG.

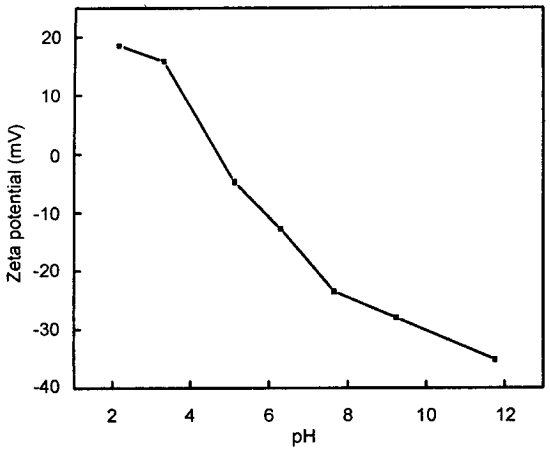

Fig.5 Dependence of zeta potential range of MPEG-modified superparamagnetic nanogels on pH.

Morphology of MPEG-modified superparamagnetic nanogels were studied by SEM. As shown in Fig.6, the magnetic nanogels are of regular morphology and the particle size of the magnetic nanogels is around 25 nm in diameter in dry state.

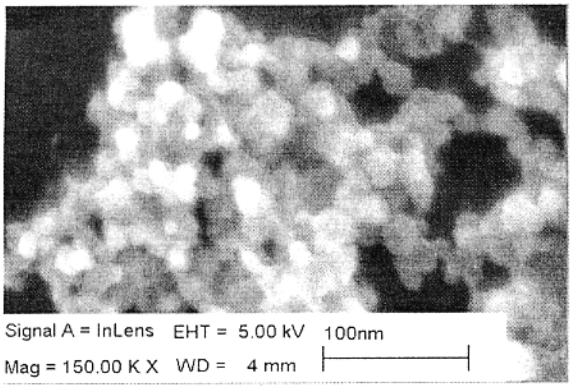

Fig.6 SEM image of MPEG-modified superparamagnetic nanogels.

3.4 Stability of MPEG-modified superparamagnetic nanogels *in vitro* and magnetically targeted character *in vivo*

In calf serum, more than 98% of radioactivity was retained by MPEG-modified superparamagnetic nanogels at 37℃ over 24 hours (Fig.7), which shows good stability *in vitro* and is of importance for targeted delivery *in vivo*.

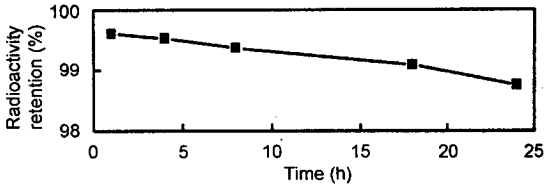

Fig.7 Stability of MPEG-modified superparamagnetic nanogels labeled by ^{188}Re complex.

The experimental results *in vivo* (Fig.8) showed that in the absence of magnetic field, the radioactivity was mainly distributed in liver, spleen, kidney, stomach and lung, less radioactivity was distributed in blood, thighbone and muscle, and a tiny radioactivity was distributed in brain (0.00041%). In the presence of a 0.5 T external magnetic field, the radioactivity was mainly accumulated on the targeted point (the right point of Fig.9) and decreased gradually at the fringe. The result indicated the magnetically targeted character of the magnetic nanogels (Fig.9). In addition, more radioactivity was distributed in lung (the left point of Fig.9). ^{188}Re labeled magnetic nanogels were intravenously injected into tail of mice, went through lung then into heart at last. So more radioactivity was accumulated on lung of mice. Further studies will be conducted in magnetically targeted delivery.

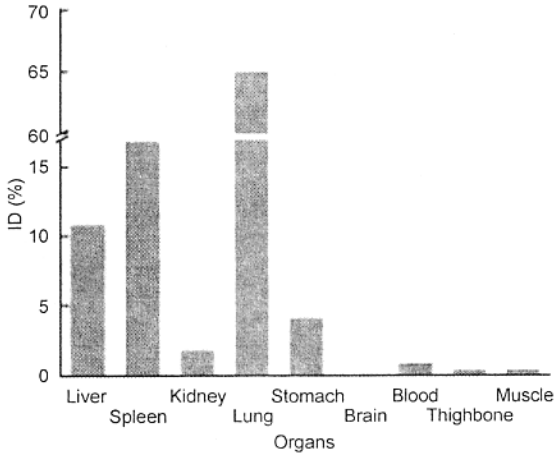

Fig.8 Radioactivity distribution of MPEG-modified superparamagnetic nanogels labeled by ^{188}Re in control group.

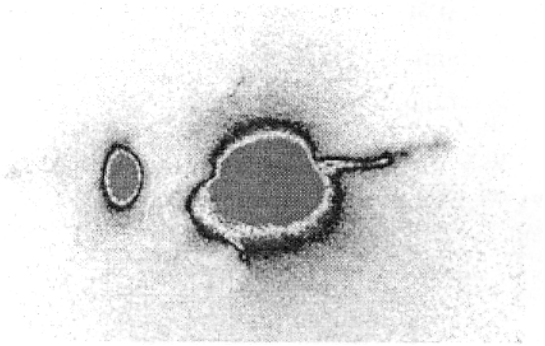

Fig.9 Radioactivity distribution of MPEG-modified superparamagnetic nanogels labeled by ^{188}Re in the presence of a 0.5 T external magnetic field targeted on the belly (the centre of the magnet was located on the belly, i.e., the right point of the image, and the ^{188}Re labeled magnetic nanogels was intravenously injected into the mice). The image above was obtained 40 min later.

4 Conclusions

The magnetic nanogels with amino groups, or poly(acrylamide-vinyl amine) magnetic nanogels can be prepared by photochemical method in emulsion-free aqueous system together with superparamagnetic Fe_3O_4 nanoparticles, and by further Hoffmann elimination. MPEG-modified superparamagnetic nanogels obtained by MPEG treatment were of well magnetic property, small negative charge and regular morphology. In the presence of a 0.5 T external magnetic field, the MPEG-modified super paramagnetic nanogels showed intense magnetically targeted character.

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