A rheumatoid arthritis magnetic resonance imaging contrast agent based on folic acid conjugated PEG-b-PAA@SPION‡

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Superparamagnetic iron oxide nanoparticles (SPIONs) offer unique properties for magnetic resonance imaging (MRI). Targeting imaging of rheumatoid arthritis in vivo requires a specific, magnetic sensitive and ultra-stable MRI contrast agent. In this study, SPIONs with a preferable colloid stability and optimized size were obtained by using an in situ polyol method with the diblock copolymer PEG-b-PAA acting as a surface ligand. Increasing the degree of polymerization (DP) of PAA from 18 to 36 to 57 led to the decreasing size of the iron oxide nanoparticles from 52 nm to 17 nm to 9 nm, respectively. Folic acid was conjugated onto PEG-PAA@SPION as a specific targeting molecule for activated macrophages in a rheumatoid arthritis joint. To evaluate the stability and magnetic properties of the particle, a series of tests were conducted to evaluate and optimize the nanoparticles. In vitro endocytosis experiments confirmed the better performance of the folic acid conjugated SPIONs than the non-folic acid modified SPIONs. In vivo MRI clearly demonstrated the significant diminishment of the arthritis joint in antigen induced arthritis (AIA) rats by intravenous injection of the optimized nanoparticles FA–PEG-b-PAA@SPION. These results indicated that FA–PEG-b-PAA@SPION could serve as a promising candidate for the MRI of rheumatoid arthritis.

1 Introduction

Rheumatoid arthritis (RA) is a permanent and incurable disease which causes a high degree of morbidity.1 Therefore, diagnosing the disease and assessing its severity is crucial for preventing the exacerbation of the disease. Among the clinical imaging methods, magnetic resonance imaging (MRI) harbors a number of advantages including a high spatial resolution and soft-tissue resolution over conventional radiography, which enable the characterization of the structural damage of the joint in unprecedented detail and depth.2 However, as one of the most commonly used T₁ weighted MR contrast agents, gadodiamide dimeglumine (Magnevist) shows some limitations in nosing the disease and assessing its severity is crucial for pre-

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stability and the magnetic properties of the particle, we have specifically designed a series of SPIONs with different core sizes. Various tests were conducted to evaluate and optimize the nanoparticles. With the selected nanoparticle PEG-b-PAA@SPION, the MRI experiment exhibited an enhanced performance in the diagnosis of RA in vivo.

2 Experimental section

2.1 Materials

HO–PEG–OH \((M_w=2000)\), iron\([\text{II}]\) acetylacetonate \((\text{Fe(acac})_3)\), tert-butyl acrylate, folic acid (FA), dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), triethylene glycol (TREG) and trifluoroacetic acid (TFA) were purchased from Aladdin Reagent (Shanghai, China). 2-Bromoisobutyryl bromide (98%), copper bromide \((\text{CuBr}, 98\%)\), N\(_2\)N\(_3\)N\(_3\)N\(_3\)-pentamethyldiethylenetriamine \((\text{PMDETA}, 99\%)\) and triethylamine \((\text{TEA}, \geq 99\%)\) were purchased from Sigma-Aldrich (St. Louis, Missouri). All the reagents were of analytical grade and used without further purification. High-purity water (Milli-Q Integral) with a conductivity of 18 MΩ cm\(^{-1}\) was used for the preparation of all aqueous solutions.

2.2 The synthesis of HO–PEG–Br

PEG-b-PAA\(_x\) diblock copolymers were synthesized by atom transfer radical polymerization (ATRP). Macronitiator HO–PEG–Br was first synthesized by the reaction of HO–PEG–OH and 2-bromoisobutyryl bromide. In brief, 10 g HO–PEG–OH and 0.505 g triethylamine were dissolved in 20 mL anhydrous dichloromethane, and then 1.15 g 2-bromoisobutyryl bromide dissolved in anhydrous dichloromethane was added dropwise with gentle stirring at 0 °C. The solution was warmed to room temperature and stirred for 48 h. The mixture was washed three times with the saturated sodium chloride solution containing a few drops of concentrated hydrochloric acid and then precipitated in cold diethyl ether.

2.3 The synthesis of PEG-b-PAA\(_x\)

HO–PEG–Br \((1 \text{ equiv.})\) was dissolved in a mixed solvent of 2-butanone and isopropanol \((7:3)\), and then PMDETA \((1.1 \text{ equiv.})\), Cu(i)Br \((1.1 \text{ equiv.)}\) and the monomers \((5–100 \text{ equiv.)}\) were added. The mixture was degassed with nitrogen for 30 minutes and reacted at 50 °C for 24 hours. By controlling the different ratios of the tBA monomer and the macronitiator HO–PEG–Br, PEG-b-PAA\(_x\) with different tBA chain lengths were obtained. Specifically, PEG-b-PtBA\(_{37}\), PEG-b-PtBA\(_{36}\) and PEG-b-PtBA\(_{38}\) were polymerized by the initiator/monomer ratios of 100 : 1, 60 : 1 and 30 : 1, respectively. The mixture was filtered through an alumina column to remove copper and was dialyzed against deionized water to remove the residual tBA monomer and then lyophilized to obtain a white powder. The obtained PEG-b-PAA\(_x\) was hydrolyzed in the solution of trifluoroacetic acid and dichloromethane \((1:1)\) for 24 h. Residual TFA and dichloromethane were removed by rotary evaporation and a light yellow sticky product was obtained.

2.4 The synthesis of PEG-b-PAA\(_x@\)SPION

The synthesis was conducted as follows: 1.0 g PEG-b-PAA\(_x\) was dissolved in 25 mL triethylene glycol in 100 °C. 1.0 g Fe(acac)_3 was added after the polymer was completely dissolved. The mixture was heated to 190 °C for 30 minutes. Afterwards the solution was refluxed at 290 °C under a blanket of nitrogen for 30 minutes. The black product was then dialyzed against deionized water for 2 days and separated via centrifugation of 15 000 rpm.

2.5 The conjugation of folic acid

The conjugation of folic acid was achieved via a DCC/DMAP reaction. Folic acid \((1 \text{ equiv.)}\) and DCC \((1 \text{ equiv.)}\) were added to anhydrous DMSO and stirred overnight. PEG-PAA@SPION \((1 \text{ equiv. of HO-PEG-PAA}_x)\) and DMAP \((0.1 \text{ equiv.)}\) were then added. The solution was stirred for 24 h and the nanoparticles were separated via centrifugation of 15 000 rpm.

2.6 The stability of PEG-PAA\(_x@\)SPION and FA–PEG-PAA\(_x@\)SPION

The stability test of PEG-b-PAA\(_x@\)SPION and FA–PEG-b-PAA\(_x@\)SPION was conducted by measuring the hydrodynamic diameter of the nanoparticles dispersed into DMEM cell culture medium \((10\% \text{ FBS})\) for different periods of time \((0, 12, 24, 48 \text{ and } 72 \text{ hours})\). The hydrodynamic diameter was measured by dynamic light scattering (DLS) following a previous report. Triple measurements of the number-weighted mean size were performed.

2.7 Characterizations

The structure of PEG-b-PAA\(_x\) was examined by NMR (Bruker Avance 600 MHz, Bruker AXS Inc., Madison, Wisconsin). The hydrodynamic size and size distribution of the particles were measured by dynamic light scattering using a Malvern Zetasizer nano ZS apparatus (Malvern Instruments, Malvern, United Kingdom). The morphology of PEG-b-PAA\(_x@\)SPION was observed by transmission electron microscopy (TEM, JEOL 2100F) at 120 kV. Thermogravimetric analysis (TGA) was performed using a TG-209-F3 thermogravimetric analyzer (Netzsch Instruments, Germany) with a heating rate of 10 °C min\(^{-1}\) from 30 to 800 °C in an N\(_2\) atmosphere. Magnetic measurements were carried out at room temperature using a MPMS XL-7 Quantum Design SQUID magnetometer (Quantum Design, America, San Diego) with the H magnetic intensity ranging from \(-1 \times 10^4\) to \(1 \times 10^3\). UV vis spectrophotometry measurements were performed on a TU-1810 ultraviolet and visible spectrophotometer (Persee, China) with a wavelength range from 250 nm to 800 nm. The surface compositions of the nanoparticles were analyzed via X-ray photoelectron spectroscopy (XPS) on a Thermo Escalab 250Xi (Thermo, America) with a monochromatized Al K\(_\alpha\) X-ray source. The samples were mounted on the standard sample studs using double-sided adhesive tapes. The reference for the binding energies (BEs) was 284.8 eV [C 1s hydrocarbon peak]. The NMR relaxometry of PEG-b-PAA\(_x@\)SPION was measured by a NMR spectrometer (Minispec, mq60,
to previous literature.

formaldehyde (4%) for 30 minutes, and then incubated for 30 minutes from 0 to 60 mg mL\(^{-1}\) for 30 minutes. The activation of the RAW 264.7 cells was conducted as described previously.\(^\text{17}\) RAW 264.7 cells were plated in a 24-well plate at density of 2 \times 10^4 per well, and cytokines IFN-\(\gamma\) and TNF-\(\alpha\) were added to the cell culture medium with the final concentration of 200 U mL\(^{-1}\) and 100 U mL\(^{-1}\) for 24 hours. The concentration and time dependent RAW 264.7 cell uptake of FA-PEG-b-PAA,\(_{17}\)@SPION were conducted by co-culturing SPION with the RAW 264.7 cells at different times or concentrations. Once the incubation was ended, the cells were washed with PBS three times, fixed with 500 \(\mu\)L paraformaldehyde (4%) for 30 minutes, and then incubated for 30 minutes at 37 \(^{\circ}\)C with a 500 \(\mu\)L Prussian blue solution containing 10% hydrochloride and 20% potassium ferrocyanide(n) trihydrate.

### 2.9 Ferrozine assay

The iron concentrations were measured using the ferrozine assay. The cells were washed three times with PBS after incubation. 250 \(\mu\)L reagent A (4.5% KMnO\(_4\) and 1.2 M HCl mixed at equal volumes) was added and mixed for 1 hour. After that 50 \(\mu\)L reagent B (6.5 mM ferrozine, 13.1 mM neocuproine, 2 M ascorbic acid and 5 M ammonium acetate) was added. The absorbance of the samples was tested at 570 nm using a Bio-Rad microplate reader and the standard curve was prepared with ferrous ethylenediammonium sulphate in 0.01 M HCl, ranging from 0 to 60 mg mL\(^{-1}\).

### 2.10 The SPION enhanced MRI of antigen-induced arthritis

All procedures involving experimental animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23, revised 1985). Lewis rats were sensitized by an intradermal injection in the back feet with 100 \(\mu\)L complete Freund’s adjuvant mixed and completely emulsified with physiological saline. Joint inflammation was assessed by measuring the diameter of the ankle joint. The inflammation of the four paws was graded from 0 to 4 according to previous literature.\(^\text{18}\) After 14 days, the animals were all graded to validate the AIA model for further use. 31 days after the injection with complete Freund’s adjuvant, the animals were intravenous injected with PEG-b-PAA\(_{18}\)@SPION or FA-PEG-b-PAA\(_{16}\)@SPION at a dose of 5 mg kg\(^{-1}\). The infected right hind ankle was imaged with a 7-T Bruker (Bruker Medical Systems, Germany). The \(T_2\)-weighted imaging sequence was applied and a transverse section orientation was selected. The signal intensity was quantified through the signal intensity ratio of the surrounding water proton/the arrowhead indicated area. The parameters were as follows: TR = 3000, TE = 45, matrix = 256 \times 256, field of view (FOV) = 3.0 \times 3.0 cm\(^2\), slice thickness = 1.00 mm and the number of excitation (NEX) = 1.

### 2.11 Histopathology assessment

After the MRI, the animals were sacrificed. The main organs and ankle joints were harvested and fixed in 10% neutral buffered formalin for 24 hours. After that they were decalcified over night, and processed through a gradient of alcohols for paraffin embedding. For hematoxylin–eosin staining, the slides were immersed in the stain, washed, and mounted in p-xylene-bis-pyridiniumbromide (VWR, Dorset, England) with obtained tissue section of 5 \(\mu\)m. For Prussian blue staining, the slides were incubated with an equal mixture of fresh 2% hydrochloric acid and 2% potassium ferrocyanide for 30 minutes at room temperature. Afterwards, the slides were washed and counterstained with nuclear fast red.

### 3 Results and discussion

#### 3.1 The preparation and characterization of PEG-b-PAA,\(_n\)@SPION

The synthesis of the magnetite nanoparticles was carried out by reacting the iron precursor, iron(m) acetylacetonate (Fe(acac)\(_3\)), with PEG-b-PAA,\(_n\) at an elevated temperature. The block copolymer of PEG-b-PAA,\(_n\) in this reaction played a dual role in controlling the core size of the iron oxide particles efficiently and enhancing the colloidal stability of the iron oxide particles in the physiological environment. Learning from the previous studies that an exceedingly long chain of PEG might have an adverse impact on the \(T_2\) relaxation, a moderate length of PEG (\(M_\text{w} = 2000\)) was adopted in our study.\(^\text{19,20}\) Three block copolymers of PEG-b-PAA,\(_n\) with different chain lengths of PAA were synthesized via ATRP. The synthesis route for the PEG-b-PAA,\(_n\) coated SPIONs is shown in Scheme 1.

Fig. 1 shows the \(^1\)H NMR spectra of the HO–PEG–Br, PEG-b-PtBA, and PEG-b-PtBA,\(_n\)@SPION. In the macroinitiator HO–PEG–Br, feature signals of \(-\text{CH}_2\text{–OH}\) (\(\delta = 3.68\)) and \(-\text{C(C}_2\text{H}_1)_3\text{Br}\) (\(\delta = 1.98\)) appeared, which indicated the formation of OH–PEG–Br. The structure of PEG-b-PtBA,\(_n\) was confirmed by the feature peaks of \(-\text{C(CH}_3)_3\) (\(\delta = 1.45\)), \(-\text{CH–CH}_2\text{–}\) (\(\delta = 2.23\)) and \(-\text{CH–CH}_2\text{–}\) (\(\delta = 1.86\)), which belonged to the PtBA block. After the copolymers were hydrolyzed by trifluoroacetic acid (TFA), the peak of \(-\text{C(CH}_3)_3\) disappeared and the two peaks of the main chain \(-\text{CH–CH}_2\text{–}\) (\(\delta = 2.31\)) and \(-\text{CH–CH}_2\text{–}\) (\(\delta = 1.51\)) were slightly moved.\(^\text{21,22}\) The DP (degree of polymerization) was calculated according to the integral area ratio. For instance, the DP of PtBA was calculated to be 36 based on the integral area ratio of \(-\text{CH}_2\text{–CH}_2\text{–}\) and \(-\text{C(CH}_3)_3\). Fig. S1† shows the \(^1\)H NMR spectra of the three kinds of PEG-b-PtBA,\(_n\). The DP of PtBA could be calculated from the integral area ratio of \(-\text{CH}_2\text{–CH}_2\text{–}\) and \(-\text{C(CH}_3)_3\). By controlling the amount of the \(t\)BA monomer added, PEG-b-PtBA\(_{18}\), PEG-b-PtBA\(_{36}\) and PEG-b-PtBA\(_{57}\) were synthesized.\(^\text{23,24}\)
3.2 The size and colloid stability of PEG-PAAx@SPION

The size of the iron oxide nanoparticles was confirmed by transmission electron microscopy and dynamic light scattering analysis in aqueous medium (Fig. 2 and Table 1). The TEM observation result indicated that PEG-b-PAA57@SPION and PEG-b-PAA36@SPION presented similar mono-dispersed and spherical-like structures. The average core sizes of these two particles were 6.6 ± 0.7 nm and 12.4 ± 1.3 nm, respectively.

Scheme 1 A schematic representation of the PEG-b-PAAx@SPION synthesis with different core sizes and an evaluation of the optimized nanoparticles for enhanced magnetic resonance imaging of rheumatoid arthritis. The iron oxide cores are imaged to be red small balls in the scheme. The PEG chains (yellow colored lines) are coated on the outer shell of the nanoparticle while the PAA chains (blue colored lines) are firmly attached to the surface of the core. The folic acid molecules are represented as red small balls which are conjugated on the end of the PEG molecules.

Fig. 1 The 1H NMR spectra of (1) HO-PEG-Br dissolved in DMSO-d6; (2) PEG-b-PtBA36 dissolved in CDCl3 and (3) PEG-b-PAA36 dissolved in DMSO-d6.
tested by DLS at di
medium containing 10% FBS and the hydrodynamic sizes were
SPIONs, the nanoparticles were incubated in DMEM cell culture
PEG-PAA18@SPION were 9.2
PAA18 and the structure of PEG-
PAA36@SPION showed a larger core size of 36.6
whereas PEG-b-PAA18@SPION showed a larger core size of 36.6 ± 9.3 nm. This might result from the inefficiency in controlling the iron oxide crystal growth of the shorter chain length of PAA18 and the structure of PEG-b-PAA18@SPION was largely consistent with a recent report on the polyl process synthesis of iron oxide nanoparticles. The hydrodynamic diameters of PEG-b-PAA57@SPION, PEG-b-PAA36@SPION and PEG-b-PAA18@SPION were 9.2 ± 1.2 nm, 17.7 ± 1.4 nm and 52.0 ± 5.2 nm, respectively, which are consistent with the TEM results (Table 1).

The colloid stability was very important for the design of the SPIONs as MRI contrast agents. To assess the stability of the SPIONs, the nanoparticles were incubated in DMEM cell culture medium containing 10% FBS and the hydrodynamic sizes were tested by DLS at different times (Fig. 3). PEG-b-PAA36@SPION and PEG-b-PAA57@SPION were stable and did not have any significant change for 72 hours, whereas PEG-b-PAA18@SPION tended to aggregate and gradually precipitate in the solution. The optimized magnetic particles PEG-b-PAA18@SPION and PEG-b-PAA57@SPION were selected for further arthritis MRI study.

### Table 1 The hydrodynamic size and core size of PEG-b-PAA57@SPION, PEG-b-PAA36@SPION and PEG-b-PAA18@SPION

<table>
<thead>
<tr>
<th>Polymer coated nanoparticles</th>
<th>Core size (nm)</th>
<th>Hydrodynamic size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-b-PAA57@SPION</td>
<td>6.6 ± 0.7</td>
<td>9.2 ± 1.2</td>
</tr>
<tr>
<td>PEG-b-PAA36@SPION</td>
<td>12.4 ± 1.3</td>
<td>17.7 ± 1.4</td>
</tr>
<tr>
<td>PEG-b-PAA18@SPION</td>
<td>36.6 ± 9.3</td>
<td>52.0 ± 5.2</td>
</tr>
</tbody>
</table>

3.3 The synthesis of the folic acid conjugated PEG-b-PAA36@SPION

Macrophages play a major role in various stages of RA, as previous research has shown that the MRI signal of normal synovium remained constant after the intravenous administration of SPIONs since there were no activated macrophages in the normal synovium. Macrophages have been proven to be a biomarker for the inflamed lesions assessment of RA. SPION MRI contrast agent targeting macrophages might have a high sensitivity and specificity for the evaluation of RA. In this section, folic acid conjugated PEG-b-PAA36@SPION for targeting MRI of rheumatoid arthritis was obtained by an esterification reaction. The conjugation of folic acid and SPION was confirmed by XPS as seen in Fig. 4a and b. The N1s peak was shown in the wild-scan spectra of FA-PEG-b-PAA57@SPION and FA-PEG-b-PAA18@SPION, whereas no significant N1s peaks were found in the spectra of PEG-b-PAA36@SPION and PEG-b-PAA18@SPION. The C1s core-level spectra of PEG-b-PAA57@SPION and PEG-b-PAA18@SPION (Fig. 4c and d) could both be curve-fitted with three peaks at the binding energies (BEs) of 284.6, 286.2 and 288.6 eV, which were attributed to the C–C/C–H, C–O, and O═C–O bonds, respectively. In comparison, two new peaks at 285.6 and 287.8 eV in FA-PEG-b-PAA57@SPION and FA-PEG-b-PAA18@SPION (Fig. 4e and f) were attributed to the peaks of the C–N and O═C–N bonds, which belong to the conjugated folic acid. A UV spectrophotometry method (Fig. 5) was also used to verify the existence of folic acid. The characteristic absorption peak of folic acid located at 280 nm was exhibited in the spectra of FA-PEG-b-PAA57@SPION and FA-PEG-b-PAA18@SPION, whereas it was absent in the spectra of PEG-b-PAA36@SPION and PEG-b-PAA18@SPION. The UV-vis spectrophotometry feature of the folic acid conjugated SPIONs was consistent with a previous report on the chemical conjugation of FA. The conjugation of FA onto the particles could be quantified by using a calibration curve of free FA (Fig. S3†). The amount of the conjugated FA was calculated to be 40.7 and 20.5

![Fig. 2](image) The TEM images of (a) PEG-b-PAA57@SPION; (b) PEG-b-PAA36@SPION and (c) PEG-b-PAA18@SPION.

![Fig. 3](image) The serum stability of PEG-b-PAA36@SPION.
mg per g per nanoparticles for FA–PEG-b-PAA_{36}@SPION and FA–PEG-b-PAA_{57}@SPION, respectively.

### 3.4 The magnetic properties of PEG-PAA_{x}@SPION and FA–PEG-PAA_{x}@SPION

Hysteresis loops measured by a vibrating sample magnetometer exhibited similar superparamagnetic characteristics with no remnant magnetization and coercivity of PEG-b-PAA_{36}@SPION and PEG-b-PAA_{57}@SPION as well as folic acid conjugated FA–PEG-b-PAA_{36}@SPION and FA–PEG-b-PAA_{57}@SPION at room temperature (Fig. 6). Based on the TGA results (Fig. S2†), the saturation magnetizations (M_s) of PEG-b-PAA_{36}@SPION and PEG-b-PAA_{57}@SPION were calculated to be 54.3 Fe emu g^{-1} and 17.0 Fe emu g^{-1}, respectively, and the M_s for FA–PEG-b-PAA_{36}@SPION and FA–PEG-b-PAA_{57}@SPION were 53.1 Fe emu g^{-1} and 13.7 Fe emu g^{-1}, respectively, which indicated no obvious loss of magnetization per Fe unit after the conjugation of folic acid.

To further assess their magnetic properties for MRI applications, the r_2 relaxivities of the nanoparticles were calculated by measuring the change of the spin–spin relaxation rate (T_2^{-1}) per unit Fe concentration. The r_2 of PEG-b-PAA_{36}@SPION and FA–PEG-b-PAA_{36}@SPION were significantly larger than those of PEG-b-PAA_{57}@SPION and FA–PEG-b-PAA_{57}@SPION, whereas the conjugation of folic acid possess little impact on the r_2 value of the nanoparticle. The hysteresis loops and r_2 relaxivity results indicated that PEG-b-PAA_{36}@SPION was much more magnetic.
sensitive than PEG-b-PAA57@SPION. This might be attributed to the larger core size and therefore the smaller spin canting effect of PEG-b-PAA36@SPION. The r² value of FA–PEG-b-PAA36@SPION was close to that of Resovist, a commercial SPION for lymph node imaging which has a hydrodynamic size of about 40 nm. The reason for the similarity might lie in the fact that commercial Resovist was obtained by the co-precipitation method, while the polyl method produced PEG-b-PAA@SPION were prepared under high temperature conditions. Due to the strong Ostwald ripening procedure, the crystallinity and magnetic performance of the iron oxide was improved (Fig. 7).

3.5 The specific endocytosis of FA–PEG-b-PAAx@SPION

The folate receptor expression of the RAW 264.7 cells could be largely up-regulated by the activation of IFN-γ/TNF-α. The specificity targeting ability of FA–PEG-PAA@SPION to the folate receptor was tested through the Prussian blue staining of activated RAW 264.7 cells which have endocytosed PEG-b-PAA@SPION or FA–PEG-b-PAA@SPION. The conditions of the cellular endocytosis were optimized by changing the time and the concentration of the nanoparticles used (Fig. S4 and S5†). After that, the experiment was conducted to investigate the specific endocytosis of FA–PEG-b-PAA@SPION and PEG-b-PAA@SPION. Fig. 8 indicates that the cellular uptake amount of FA–PEG-b-PAA@SPION (blue granules) was more than that of PEG-b-
PAA@SPION, which proved the specific targeting ability of folic acid. Moreover, the amount of cellular uptake of FA–PEG-b-PAA57@SPION was obviously larger than that of FA–PEG-b-PAA36@SPION. The iron concentrations of the cellular uptake amount were measured by a ferrozine assay (Fig. S6†) and the results were consistent with the Prussia staining. The cellular uptake amounts of FA–PEG-b-PAA36@SPION were two times larger than those of PEG-b-PAA@SPION, while the cellular uptake amount of FA–PEG-b-PAA36@SPION was nearly four times larger than that of FA–PEG-b-PAA57@SPION. These results indicated that FA–PEG-b-PAA36@SPION was a preferable SPION for active targeting compared to FA–PEG-b-PAA57@SPION.

3.6 The SPION enhanced MRI of antigen-induced arthritis

The AIA model was successfully built by the intradermal injection of complete Freund’s adjuvant in the back feet of the rats. Inflammatory cells (arrow) like macrophages could be found in the synovium and the peripheral area of the arthritic joint in the light micrographs of the hematoxylin–eosin stain sample (Fig. S7†). Based on the magnetic properties test and endocytosis experiment, FA–PEG-b-PAA36@SPION was chosen as the preferable nanoparticle for the MRI application of the RA joint.

Representative T₂-weighted images of the SPION groups using 7T MRI equipment are presented in Fig. 9. The synovium of the animals appeared to have a moderate signal decline on the T₂-weighted MR images after intravenous injection of PEG-b-PAA36@SPION (Fig. 9a). This finding indicated that the non-specific SPIONs could passively target the RA joint. In comparison, the FA–PEG-b-PAA36@SPION contrasted MRI demonstrated a more intact and clearer structure of the synovium (Fig. 9b), suggesting that the SPIONs were preferentially endocytosed by macrophages in the inflamed synovium. For FA–PEG-b-PAA36@SPION, the signal intensity ratio of the surrounding water proton/the arrowhead indicated area was 1.410 times larger than that of PEG-b-PAA36@SPION, which indicated that the signal decrease of the arrow indicated area contrasted by FA–PEG-b-PAA36@SPION was larger than that of PEG-b-PAA36@SPION. These results suggested that the FA–PEG-b-PAA36@SPION might be useful as a target MRI contrast to qualify the arthritic joint.

To better understand the targeting ability and biodistribution of the nanoparticles, the ankle joint and major organs including the liver, spleen, kidney, heart and lung were harvested and stained by Prussian blue after MRI (Fig. 10). The results indicated that PEG-b-PAA36@SPION could passively accumulate in the ankle joint, whereas FA–PEG-b-PAA36@SPION has shown a qualitative better accumulation in the joint, which might be due to the active targeting of folic acid. Except for the joint, the spleen and lung were found to have a small amount of SPION accumulation, which might be attributed to the macrophage endocytosis of the nanoparticles by reticulo-endothelial systems, while the liver, heart and kidney showed little accumulation of the nanoparticle.

4 Conclusion

In summary, a novel PEG-b-PAA@SPION contrast agent was synthesized by using an in situ polyol method. Nanoparticles of
different sizes were obtained by controlling the chain length of the PAA block of the copolymer PEG-b-PAA. By measuring the magnetic sensitivity, colloid stability and specific targeting ability, FA-PEG-b-PAA@SPION was chosen as a contrast agent for the MRI of an RA joint. The signal of synovium was significantly enhanced in the 7T MRI after the administration of FA-PEG-b-PAA@SPION in AIA model rats. The correlated histological outcome of the Prussia blue staining also confirmed the excellent permeability efficiency of the folic acid conjugated PEG-b-PAA@SPION in the joint. The enhanced MR imaging results proved significant opportunity for the FA-PEG-PAA@SPION contrast agent for targeted human diagnosis of rheumatoid arthritis.

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