Preparation of amphiphilic superparamagnetic composite particles with tumor targeted MRI contrast agent

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Abstract

BACKGROUND: Superparamagnetic iron oxide nanoparticles (Fe_3O_4 NPs) have been widely used in MRI. It is vital to prepare the superparamagnetic MRI contrast agent with high stability, biocompatibility and tumor targeting in order to prevent the aggregation of Fe_3O_4 NPs and realize the high-precision diagnose of tumor.

OBJECTIVE: To prepare the amphiphilic superparamagnetic composite particles with tumor targeting mediated by folate receptor.

METHODS: The stable amphiphilic superparamagnetic composite particles with tumor targeting function were prepared by coating the Fe_3O_4 NPs with a Pluronic F127-folic acid conjugate, which was synthesized *via* an esterification reaction between the carboxyl group of the tumor targeting molecule, folic acid and the hydroxyl group of an amphiphilic triblock copolymer, Pluronic F127. The resultant Pluronic F127-folic acid-Fe_3O_4 composite particles were characterized by transmission electron microscopy, Fourier transform infrared-spectra, UV-vis absorption spectra, thermal gravimetric analysis, vibrating sample magnetometer and T2-weighted imaging. WST assay was used to characterize their cytotoxicity preliminarily.

RESULTS AND CONCLUSION: The Pluronic F127-folic acid conjugates were prepared *via* esterification reaction. Then Fe_3O_4 NPs were wrapped with Pluronic F127-folic acid to result in the superparamagnetic composite particles with well dispersion and biocompatibility. The size of most superparamagnetic composite particles was less than 200 nm and the size of Fe_3O_4 core was 10–20 nm from the observation of transmission electron microscopy. The results from the Fourier transform infrared-spectra and UV-vis absorption spectroscop confirmed that folic acid molecules were modified on the surface of the superparamagnetic composite particles successfully. The mass ratio of Pluronic F127-folic acid conjugate was determined by thermal gravimetric analysis as 27.2 wt% in the resultant Pluronic F127-folic acid-Fe_3O_4 composite particles. The saturated magnetic intensity of the superparamagnetic composite particles was 47.35 emu/g by vibrating sample magnetometer and the relaxation rate was 0.025×10^6 mol/s from MRI. The WST assay showed the negligible cell cytotoxicity of Pluronic F127-folic acid-Fe_3O_4. The superparamagnetic composite particles have potential application as the MRI contrast agents with tumor targeting, and the Pluronic F127-folic acid-Fe_3O_4 composite particles is expected to be used as a MRI contrast agent for tumor targeting.

Subject headings: tumor; molecular targeted therapy; metal nanoparticles; folic acid
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INTRODUCTION

Superparamagnetic Fe_3O_4 nanoparticles (Fe_3O_4 NPs) are emerging as a promising candidate for various biomedical applications, such as MRI[1], targeted drug delivery[2], hyperthermia treatment[3], the labeling and sorting of cells[4], and the separation of biochemical products[5]. For these applications, it is important for the superparamagnetic nanoparticles to be biocompatible, water soluble, and physically and chemically stable in a physiological environment. In general, nanoparticles tend to flocculate due to van der Waals forces, but magnetic nanoparticles can in addition be magnetically attracted between each other and agglomerated. Therefore, it is necessary to modify the superparamagnetic nanoparticles to improve their biocompatibility, solubility and stability in physiological environments for various biomedical applications. Coating materials for biological applications include organic molecules (*e.g.* citrate)[6], polymers (*e.g.* dextran[7-8], poly(ethylene glycol)[9], starch[10], proteins

(human serum albumin[11], protein corona[12-13]) and inorganic molecules and shells (*e.g*. silica[14], Au nanoparticles[15-16]). The research interest of surface functionality of Fe_3O_4 NPs with polymeric shell owes to the flexibility in the control of the chemical structure, composition, and function of the polymers. Poly(ethylene glycol) is the most widely used synthetic biocompatible polymer for coating of magnetic nanoparticles to extend their circulation time, improve their hydrophilicity and biocompatibility and reduce the non-specific uptake by macrophage cells[9, 17]. Moreover, magnetic nanoparticles covered with poly(ethylene glycol) are considered to be nonimmunogenic, nonantigenic, and protein resistant. Therefore, poly(ethylene glycol) has been one of the most popular polymers used for magnetic nanoparticles coating.

Magnetic nanoparticles composed of a magnetic core and a biocompatible polymeric shell can offer a potential method to get a targeted MRI reagent due to the EPR effect and the tumor targeting molecules conjugated with the polymeric shell. The methods of modification with a polymeric or silica shell over a magnetic core usually include *in situ* polymerization[18-19], grafting of polymer[20] or layer-by-layer self-assembly method[21], *etc*. However, all these techniques not only involve the complicated chemical reactions but also decrease the magnetization of magnetic cores. Interestingly, it is a challenge to develop a simple method for getting a biocompatible polymeric shell *via* chemical modification of magnetic cores and keeping the magnetization of the resultant polymer/magnetite hybrid particles. Pluronic F127 (PF127), a block copolymer of poly(ethylene glycol) and poly(propylene glycol) with triblock structures of poly(ethylene glycol-b-propylene glycol-b-ethylene glycol), is highly possible for such a requirement for its amphiphilic block components. It has been widely used in pharmaceutics[22], biomedical[23-24] and personal care products due to its favorable toxicity profile[25] and its action as ‘‘solubilizer’’. It can form ordered aggregates, most commonly as micelles in aqueous solutions, which show a good biocompatible character and prolong the circulation time *in vivo* due to its poly(ethylene glycol) shell. As a result, it behaves high biocompatiblity through effectively preventing aggregation, the adsorption of proteins and recognition by the reticulo-endothelial system *in vivo*. Further, it becomes possible and facile to coat the magnetic core nanoparticles with PF127 based on its unique self-assembly behaviors.

The development of tumor-targeted nanoparticles is essential for early detection of cancers. It is highly desirable that magnetic nanoparticles can provide sensitive and specific imaging information in cancer site. The magnetic nanoparticles conjugated with tumor-targeting ligands such as monoclonal antibodies[26-27], peptides[28-30], or small molecules, such as folic acid[31-34], can be engineered to provide a MRI reagent for tumor selected imaging.

In order to prepare the superparamagnetic MRI contrast agent with high stability, biocompatibility and to realize the high-precision diagnose of tumors, a facile method was developed to synthesize a tumor targeting and stable magnetic nanoparticles in this work. PF127 is conjugated with folic acid (FA) at first since the folate receptors (FRs) is overexpressed in many malignant tissues and it can bind and internalize folic acid conjugates. Then the PF127-FA conjugate is used to coat superparamagnetic Fe_3O_4 NPS *via* an efficient spontaneous interaction.

MATERIALS AND METHODS

Design

The design of material and its structures and properties characterization.

Time and setting

The work was completed in the Key Laboratory of Functional Polymer Materials, Ministry of Education, Institute of Polymer Chemistry, College of Chemistry, Nankai University, from February 2012 to December 2012.

Materials

Experimental reagents:

Reagent	Source
FA	Nanjing Boquan Technology Co., Nanjing, China
PF127 (Average Mn - 14600), 1,3-dicyclohexylcarbodiimide, 4-dimethylaminopyridine	Aldrich
Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), sodium hydroxide	Tianjin No. 3 Chemical Plant, Tianjin, China
Sephadex G-15	Shanghai Seebio Biotechnology Inc., Shanghai, China
Dimethyl sulfoxide	Tianjin Fengchuan Chemical Reagent Science and Technology Co., Ltd., China
Plasmid DNA (pDNA), HeLa cells	School of Pharmacy, Tianjin Medical University, China

Experimental instruments:

Instrument	Source
Transmission electron microscopy	Philips, FEI
Superconducting quantum interference device magnetometer	LDJ
Laser scattering spectrometer	Beckman
Confocal fluorescence microscopy	Olympus
Gel electrophoresis apparatus	BIO-RAD
Centrifuge	Eppendorf
Ultrasonic Cleaner	Kun Shan Ultrasonic Instruments Co., Ltd., Bruker

Methods

Superparamagnetic Fe_3O_4 NPs were prepared by chemical co-precipitation method

Fe_3O_4 NPs were prepared by chemical co-precipitation of Fe^{3+} and Fe^{2+} under a basic condition. $FeCl_3•6H_2O$ (270.0 mg, 1 mmol) and $FeCl_2•4H_2O$ (99.5 mg, 0.5 mmol) were dissolved in 50 mL of deionized water with a mechanical stirring under argon for 30 minutes, then 2 mL (8 mol/L) of ammonium hydroxide was added. The reaction solution was then heated at 60 ℃ and stirred for 2 hours.

The resultant magnetite nanoparticles were washed with deionized water with the aid of a magnet. Deionized water was then added to disperse the resultant Fe_3O_4 NPs. The solid content of the magnetic fluid was adjusted to 4 mg/mL for further preparation.

The conjugates of PF127 and FA were prepared via an esterification reaction

88 mg (0.20 mmol) of FA was dissolved in 5 mL of dried dimethyl sulfoxide, then 45.3 mg (0.22 mmol) of 1,3-dicyclohexylcarbodiimide with catalytic amount of 4-dimethylaminopyridine was added. Finally 620 mg (0.05 mmol) of PF127 was added to the above solution. The reaction was allowed to proceed for 48 hours at room temperature. After reaction, the solution was condensed by a rotary evaporating. The condensed solution was precipitated in acetone and the precipitation was dissolved in water. The obtained solution was centrifuged at 5 000 r/min and the supernatant was placed into the dialysis chamber, which was dialyzed in PBS with pH 7.4 in order to remove the free FA since it can be dissolved under this condition. The yellow solution in dialysis chamber was lyophilization to afford PF127-FA conjugate.

Superparamagnetic composite particles

PF127-FA-Fe_3O_4 with tumor targeting were prepared The above 5 mL of magnetic fluid was diluted into 195 mL of deionized water and stirred with a mechanical stirring under argon. Then PF127-FA (35 mg) dissolved in 5 mL of deionized water was dropped into the magnetic fluid and stirred for 6 hours continuously. Finally, the resulting product with 50% yield was washed with deionized water by magnetic separation and dried in a vacuum oven.

The structures and properties of PF127-FA-Fe_3O_4 composite particles were characterized

Transmission electron microscopy (FEI, TECNAI-20) was used to characterize the size and morphology of the samples. Fourier transform infrared spectra were obtained using a Bruker Tensor 27 spectrometer. UV spectra were determined on an Ultra-visible-near IR spectrophotometer (JASCO, V-570).

The composition of PF127-FA-Fe_3O_4 was determined with thermal gravimetric analysis using a Netzsch TG209 with a heating rate of 10 ℃/min from room temperature to 800 ℃ under N_2. The magnetization curves of Fe_3O_4 and PF127-FA-Fe_3O_4 were measured as a function of the applied magnetic field H with a 9 600 VSM (LDJ Co.) superconducting quantum interference device magnetometer. The hysteresis of the magnetization was obtained by changing H between +6 000 and −6 000 Oe at 300 K. The iron content of PF127-FA-Fe_3O_4 was determined with atomic absorption spectrophotometer analysis (HITACHI, 180-80). T2-weighted signal intensities were measured with a clinical 3.0 T magnetic resonance scanner (Siemens, TrioTim) using iron concentrations ranging from 0 to 0.168 mmol/L at room temperature. The T2-weighted images were acquired using a fast gradient echo pulse sequence (repetition time/echo time/flip angle: 1 000/41.4/5.5). Relaxivity values were calculated through the curve fitting of 1/T2 relaxation time (/ms) versus the iron concentrations (mmol/L).

The cytotoxicity of PF127-FA-Fe_3O_4 composite particles against Hela cells was determined by a standard WST-1 assay using the WST-1 cell proliferation and cytotoxicity assay kit. In brief, Hela cells were seeded in 96-well plates at a density of 5 000 cells/well and cultivated with Dulbecco's modified Eagle's medium plus 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin (200 μL) and incubated at 37 ℃ for 24 hours. The wells were then replaced in a culture medium (200 μL) containing PF127-FA-Fe_3O_4 composite particles with the concentration of 0.1, 1, 10, 50, 100, 200, 500 μg/mL. After the incubation for 48 hours, the medium in each well was washed with PBS for five times and replaced with fresh Dulbecco's modified Eagle's medium without fetal bovine serum (100 μL) and WST-1 solution (10 μL). The plate was incubated for a further 1 hour at 37 ℃, which allowed viable cells to reduce WST-1 into the orange formazan crystal.

The plate was read at 450 nm on a Bio-Rad microplate reader. Data are presented as mean±SD, n=3. The relative cellular viabilities were obtained according to the equation (1).

$$\text{Relative cellular viability} = 1-A/A_0 \quad (1)$$

Where, A is the absorbance value of experimental groups under different concentration of PF127-FA-Fe_3O_4 composite particles, and A_0 is the absorbance value of control groups.

Main outcome measures

Relative cellular viability of Hela cells after treatment with PF127-FA-Fe_3O_4 composite particles with different concentrations.

RESULTS

Preparation of PF127-FA-Fe_3O_4 composite particles and characterization of their structures

Since the magnetic nanoparticles without surface modification are prone to aggregate in the solvent due to

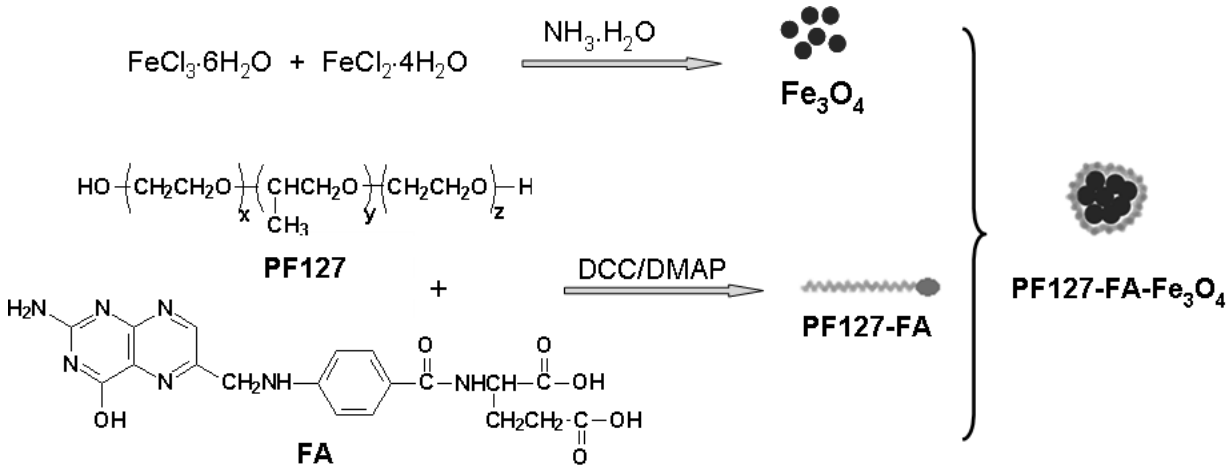

Figure 1 Amphiphilic superparamagnetic Pluronic F127 (PF127)-folic acid (FA)-Fe_3O_4 composite particles for MRI contrast agent were prepared

their high specific surface area. Coating of magnetic nanoparticles with a polymer layer may prevent such aggregation, which also improves the chemical stability of the composite nanoparticles under rigid condition. In this work, magnetic Fe_3O_4 NPs were first prepared by the chemical co-precipitation of Fe^{3+}/Fe^{2+} (2/1 in molar ratio) salts in an NH_4OH solution at 60 ℃ via a well-known sol-gel process. Then PF127 was conjugated with FA by ester bonding between the hydroxyl groups of PF127 and the carboxylic acid groups of FA to give PF127-FA. Finally, superparamagnetic Fe_3O_4 NPs were coated with PF127-FA spontaneously to endow a possible tumor targeting and prevent the aggregation, as shown in **Figure 1**.

The morphologies of superparamagnetic Fe_3O_4 NPs before and after coating with PF127-FA were characterized with transmission electron microscopy (**Figure 2**), which indicated that the size of Fe_3O_4 NPs was 10−20 nm (**Figure 2A**) and most of PF127-FA-Fe_3O_4 composite particles had the slightly aggregated size of below 200 nm (**Figure 2B**). Further, Fe_3O_4 NPs aggregation coated with a thin film of PF127-FA conjugates is clearly observed in **Figure 2C** with a higher magnification.

PF127-FA conjugate was synthesized *via* using the excessive molar ratios of FA to PF127 to ensure that at least one or two hydroxyl groups of PF127 were conjugated with the carboxylic acid groups of FA. The successful syntheses of PF127-FA conjugate and its coated superparamagnetic composite particles (PF127-FA-Fe_3O_4) were confirmed by Fourier transform infrared-spectra and Ultra-visible-near IR spectrophotometer in **Figure 3**.

Fourier transform infrared spectrum of the PF127-FA conjugate in **Figure 3A** indicated the presence of the characteristics peaks at 1 691, 1 648 and 1 605/cm corresponding to the stretching vibrations from FA component together with the peaks at 2 929, 2 866 and 1 105/cm assigning to the stretching vibrations of CH_2-bonds from PF127 chains. The Fourier transform infrared spectrum of PF127-FA-Fe_3O_4 composite particles in **Figure 3A** had a new peak at 577 /cm attributing to the stretching vibration of Fe-O bond in Fe_3O_4 core together with all the characteristic peaks from PF127-FA conjugate. The UV-vis spectrum of PF127-FA in **Figure 3B** had a peak at 280 nm attributing to the characteristic absorbance of FA component, which was also clearly observed in the UV-vis spectrum of PF127-FA-Fe_3O_4 composite particles. The mass ratio of PF127-FA conjugate was determined by thermal gravimetric analysis as 27.2 wt% in the resultant PF127-FA-Fe_3O_4 composite particles. All these results indicated that PF127-FA conjugate was successfully coated onto the Fe_3O_4 NPs.

The properties of PF127-FA-Fe3O4 composite particles were characterized

The presence of magnetite on PF127-FA-Fe_3O_4 was proven by a superconducting quantum interference device magnetometer. The magnetization curves of Fe_3O_4 and PF127-FA-Fe_3O_4 were measured at room temperature as shown in **Figure 3A**. The magnetic hysteresis loops were S-like curves, while the saturation magnetization of PF127-FA-Fe_3O_4 was 47.35 emu/g with near zero of magnetic remanence. This value was smaller than that of bulk Fe_3O_4 (53.9 emu/g), which was due to the coating of PF127-FA on Fe_3O_4. The results indicated that there was almost no remaining magnetization when the external magnetic field was removed, suggesting that PF127-FA-Fe_3O_4 composite particles exhibited a superparamagnetic behavior. These superparamagnetic PF127-FA-Fe_3O_4 composite particles could be dispersed stably as shown in the inserted photograph in **Figure 4A**.

To check the MRI efficiency of PF127-FA-Fe_3O_4 as a tumor targeting contrast agent, T2-weighted signal intensities were measured with a clinical 3.0 T magnetic resonance scanner using iron concentrations ranging from 0 to 0.14 mmol/L at room temperature. Relaxivity values are calculated through the curve fitting of 1/T2 relaxation time (/ms) versus the iron concentrations (mmol/L). The iron content of PF127-FA-Fe_3O_4

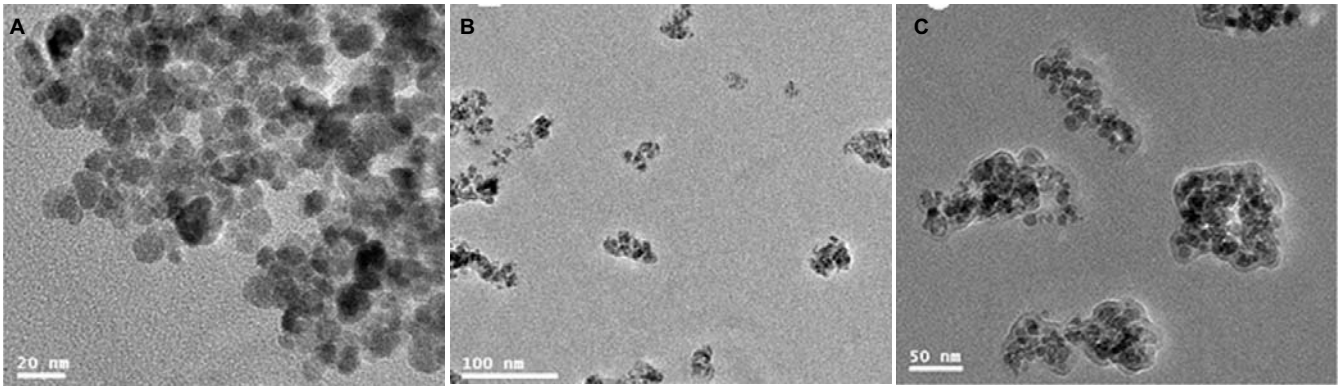

Figure 2 Transmission electron microscopy images of Fe_3O_4 nanoparticles and PF127-FA-Fe_3O_4 composite particles
Note: (A) Fe_3O_4 nanoparticles, bar: 20 nm; (B) PF127-FA-Fe_3O_4 composite particles, bar: 100 nm; (C) PF127-FA-Fe_3O_4 composite particles with higher magnification, bar: 50 nm. PF127: Pluronic F127; FA: folic acid.

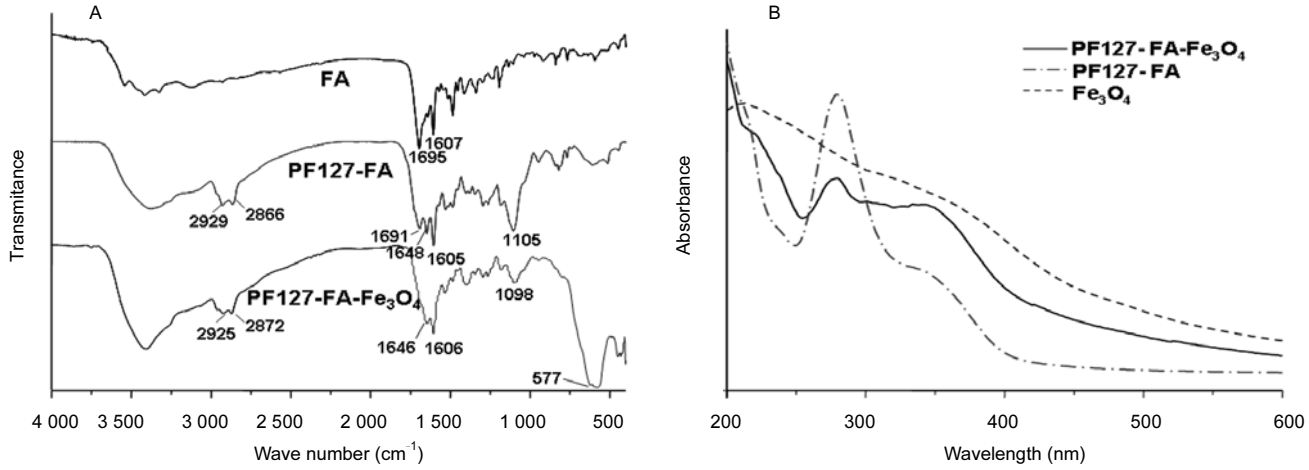

Figure 3 The superparamagnetic PF127-FA-Fe_3O_4 composite particles were confirmed by FT-IR spectra and UV-vis spectra
Note: (A) FT-IR spectra of FA, PF127-FA and PF127-FA-Fe_3O_4; (B) UV-vis spectra of PF127-FA-Fe_3O_4, PF127-FA and Fe_3O_4. FT-IR: Fourier transform infrared-spectra; UV-vis: ultra-visible-near IR spectrophotometer; PF127: Pluronic F127; FA: folic acid.

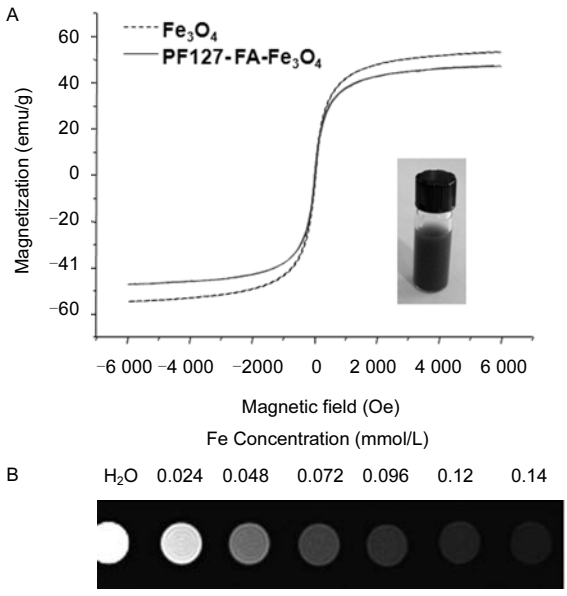

Figure 4 Properties of PF127-FA-Fe_3O_4 composite particles
Note: (A) Magnetization curves of Fe_3O_4 nanoparticles and PF127-FA- Fe_3O_4 composite particles and the insert is the photos of stable suspension of PF127-FA- Fe_3O_4 composite particles in water; and (b) T2-weighted MRI images of PF127-FA- Fe_3O_4 with the various concentrations. PF127: Pluronic F127; FA: folic acid.

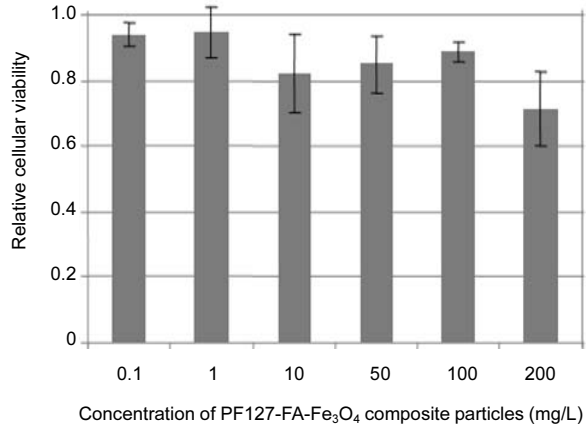

Figure 5 Relative cellular viability of Hela cells after treatment with PF127-FA-Fe_3O_4 composite particles with different concentrations
Note: The absorbance value was read at 450 nm on a Bio-Rad microplate reader and the relative cellular viability was calculated according to the equation (1). Data are presented as mean±SD (n=3). The result of WST-1 assay indicated that the PF127-FA-Fe_3O_4 composite particles showed no obvious toxicity at 200 mg/L to Hela cells for the cytotoxicity evaluation. PF127: Pluronic F127; FA: folic acid.

was estimated as 32 wt% by atomic absorption spectrometry. The T2 relaxivity was increased dramatically with the increasing of the iron concentrations in PF127-FA-Fe_3O_4. The linear function equation of Y=0.124 667 $\times10^{-5}$ + 0.02494 X (*R*=0.9974, *n*=6) was obtained with iron concentration as x-axis and 1/T2 as y-axis. The transverse r2 relaxivities was obtained as 0.025×10^6/mol/s from the slope of linear. **Figure 4B** showed the T2-weighted MRI images of PF127-FA-Fe_3O_4 with the various iron concentrations. PF127-FA-Fe_3O_4 had a significant change of light intensity with the changes of iron concentration. These superparamagnetic composite particles with FA conjugating may find a potential application for special detection of cancers.

The cytotoxicity of PF127-FA-Fe_3O_4 composite particles was valued by WST assay

As a MRI contrast agent, the potential toxicity PF127-FA-Fe_3O_4 composite particles should be concerned for its further application in biomedical fields. In order to examine the cytotoxicity of PF127-FA-Fe_3O_4 composite particles, Hela cells were incubated 24 hours with PF127-FA-Fe_3O_4 composite particles in the concentration range from 0.1 to 200 mg/L. The result of WST-1 assay indicated that the PF127-FA-Fe_3O_4 composite particles showed no obvious toxicity at 200 mg/L to Hela cells for the cytotoxicity evaluation as shown in **Figure 5**. This confirmed their low levels of cytotoxicity.

DISCUSSION

Fe_3O_4 NPs can be prepared by chemical co-precipitation. The reaction conditions, including the ratio of Fe^{3+} and Fe^{2+}, the concentration of reaction solution and the reaction temperature, will have effects on their size and morphology. Therefore, it should be optimized to obtain the Fe_3O_4 NPs with advisable size.

Superparamagnetic Fe_3O_4 NPs are unstable when they are placed in the process of *in vitro* due to van der Waals forces and magnetic dipole-dipole interaction between each others. When they enter *in vivo*, they will easily absorb numerous biomacromolecules such as protein. This results in their enlarged size and they will be phagocytized by the reticuloendothelial system which shortens their half-life.

To improve their stability in solutions and biocompatibility and target the tumor site actively, in this study, amphiphilic block polymer PF127 with high biocompatibility was conjugated with FA molecules which can target most of the tumor cells via an esterification reaction. The PF127-FA conjugates were coated on the surface of Fe_3O_4 NPs and the superparamagnetic PF127-FA-Fe_3O_4 composite particles with well dispersion in solution were obtained.

The surface morphology and size of the resultant products were characterized by transmission electron microscopy. The size of Fe_3O_4 NPs are about 10−20 nm and most of PF127-FA-Fe_3O_4 composite particles are below 200 nanometers from the result of transmission electron microscopy. The structure of PF127-FA-Fe_3O_4 composite particles was confirmed by Fourier transform infrared-spectra and UV-vis absorption spectroscopy. The mass ratio of PF127-FA conjugate in the resultant PF127-FA-Fe_3O_4 composite particles was further determined as 27.2 wt% by thermal gravimetric analysis.

An S-like magnetic hysteresis loop in the magnetization curve of the resultant PF127-FA-Fe_3O_4 composite particles suggested that they were superparamagnetics. Their saturation magnetization was 47.35 emu/g, which met the demand them as the MRI contrast agent. The result of their T2-weighted MRI indicated that the T2 relaxivity was increased dramatically with the increasing of the iron concentrations in PF127-FA-Fe_3O_4. The transverse r2 relaxivities was obtained as 0.025×10^6/mol/s from the linear relation of the change of T2-weighted light intensity with the various iron concentrations.The result shows that these superparamagnetic composite particles with FA conjugating may be used in the special detection of cancer potentially. The cytotoxicity PF127-FA-Fe_3O_4 composite particles were further valued by WST-1 assay preliminarily. The result showed they had low cytotoxicity. This indicates that it is possible to use them as an MRI contrast reagent mediated by folate receptor.

Superparamagnetic Fe_3O_4 nanopartiles with tumor targeting function were prepared by coating the Fe_3O_4 NPs with a PF127-FA conjugate *via* an esterification reaction between the hydroxyl group of amphiphilic triblock copolymer PF127 copolymer and the carboxyl group of a tumor targeting FA molecule. The size of Fe_3O_4 NPs was 10−20 nm and most of PF127-FA-Fe_3O_4 composite particles had the size of below 200 nm. Fourier transform infrared-spectra and UV-vis spectra were used to confirm the formation of PF127-FA-Fe_3O_4.

The saturation magnetization of the tumor targeting superparamagnetic PF127-FA-Fe_3O_4 composite particles was 47.35 emu/g with near zero magnetic remanence. The T2 relaxivity value of PF127-FA-Fe_3O_4 was 0.025×10^6/mol/s, which illustrated the potential application for a MRI contrast agent that specifically targets folate receptor overexpressing tumor cells.

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用于肿瘤靶向性 MRI 对比剂双亲性超顺磁复合物的制备

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文章亮点：

为提高 Fe_3O_4 NPs 的溶液稳定性和生物相容性，同时能将其主动靶向到肿瘤部位，实验将具有生物相容性的双亲性高分子 Pluronic-F127 与能靶向多数肿瘤细胞的叶酸分子通过酯化反应连接，制备 PF127-FA 偶联物，再用该偶联物自发包覆 Fe_3O_4 纳米粒子，最终制得能够在溶液中稳定分散的超顺磁 PF127-FA-Fe_3O_4 复合粒子，对其进行结构和性能表征，并初步评价其作为肿瘤靶向性 MRI 对比剂的可能。

关键词：

生物材料；纳米材料；双亲性材料；肿瘤靶向性；超顺磁性；Fe_3O_4 纳米粒子；MRI 对比剂；Pluronic；叶酸；国家自然科学基金

主题词：

肿瘤；分子靶向治疗；金属纳米粒子；叶酸

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摘要

背景：超顺磁四氧化三铁纳米粒子(Fe_3O_4 NPs)被广泛应用于 MRI 成像，为防止其聚集和实现高精度肿瘤诊断，制备高度稳定性、生物相容性和肿瘤靶向性的超顺磁 MRI 对比剂至关重要。

目的：合成具有基于叶酸受体靶向的肿瘤靶向性双亲性超顺磁复合粒子。

方法：首先通过化学共沉淀法制备出 Fe_3O_4 NPs，再用 N，N'-二环已基碳二亚胺作脱水剂，通过酯键将双亲性高分子 Pluronic-F127(PF127)与叶酸(FA)分子连接，从而形成 PF127-FA 偶联物，最后用 PF127-FA 包裹 Fe_3O_4 纳米粒子，形成稳定的具有肿瘤靶向功能的双亲性超顺磁复合粒子。分别采用透射电镜、傅里叶红外光谱、紫外可见吸收光谱、热重分析、振动样品磁强计和 T2 加权成像对其进行表征，通过细胞毒性实验初步表征其细胞毒性。

结果与结论：通过酯化反应制备了 Pluronic-F127 与 FA 偶联物，再用其包裹 Fe_3O_4 纳米粒子，成功制备出具有良好水溶性和生物相容性的超顺磁性复合粒子。该 PF127-FA-Fe_3O_4 复合粒子透射电镜观察到该复合粒子大部分粒径小于 200 nm，Fe_3O_4 核心大小为 10-20 nm，傅里叶红外光谱和紫外可见吸收光谱结果证明了叶酸被成功修饰到超顺磁性复合粒子表面。热重分析结果表明 PF127-FA 占 PF127-FA-Fe_3O_4 复合粒子总量的 27.2 wt%。磁性检测结果表明该复合粒子饱和磁强度 Ms 为 47.35 emu/g，核磁共振仪成像测得其弛豫率为 0.025×10^6 mol/s。细胞毒性实验表明显示了可以忽略的毒性。因此，实验成功制备了可用于肿瘤靶向性 MRI 对比剂的双亲性超顺磁复合物，实验所制备的 PF127-FA-Fe_3O_4 复合粒子有望用于肿瘤靶向性 MRI 对比剂。

作者贡献：第一、四作者进行实验设计与成文，第二、三作者进行实验操作与评估。第一、四作者对文章负责。

利益冲突：文章及内容不涉及相关利益冲突。

学术术语：纳米粒子-是指粒度在 1-100 nm 之间的粒子(纳米粒子又称超细微粒)。属于胶体粒子大小的范畴。它们处于原子簇和宏观物体之间的过度区，处于微观体系和宏观体系之间，是由数目不多的原子或分子组成的集团，因此它们既非典型的微观系统亦非典型的宏观系统。

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