Magnetic Iron Sulfide Nanoparticles as Thrombolytic Agents for Magnetocaloric Therapy and Photothermal Therapy of Thrombosis

Dapeng Fu, Junle Liu, Qilong Ren, Jinhui Ding, Heyi Ding, Xuan Chen and Xiaohu Ge

Non-invasive removal of thrombosis is a difficult problem in clinical vascular disease. Herein, we reported magnetic hyperthermia combined photothermal therapy for celiac vein thrombosis using Fe₃S₄ nanoparticles as thrombolytic agents under the stimulation of a near infrared (NIR) laser and an external alternating magnetic field (AMF). Fe₃S₄ nanoparticles showed excellent magnetothermal conversion performance under the continuous stimulation of an AMF and very good photothermal conversion performance under the irradiation of a NIR laser. Moreover, Fe₃S₄ nanoparticles exhibited a synergistic thermal conversion effect under the co-stimulation of NIR and AMF. In addition, the Fe₃S₄ nanoparticles possess the ability for magnetic resonance (MR) imaging with the transverse relaxivity (r₂) is up to 53.1 mM⁻¹ s⁻¹. Finally, we, for the first time, proved the Fe₃S₄ nanoparticles as a promising thrombolytic agent for both photothermal thrombolytic capacity and magnetothermal thrombolytic ability. Our work provides the insight of hyperthermia for removal of the thrombosis.

Keywords: photothermal therapy, magnetocaloric therapy, thrombosis, iron sulfide, magnetic resonance imaging

INTRODUCTION

Cardiovascular disease is one of the main causes of harm to human health (Mackman, 2008; Engelmann and Massberg, 2013). Because the blood vessels are aging and the blood vessel wall is damaged, it is easy to form the thromboses in the blood vessel, and it is also more likely to suffer from diseases such as hypertension and arteriosclerosis. Surgery and chemotherapy are still the main treatment options for thrombosis. Surgical treatment causes great pain to the patient’s body; chemotherapy is non-targeting and belongs to systemic administration, which has great side effects (Voros et al., 2015). Traditional treatments often treat known thrombi, but may cause new problems for blood vessels. Older people with thrombosis often do not dare to use thrombolytic drugs. Therefore, it is of great significance to develop an efficient and accurate thrombosis treatment system for non-invasive and precise treatment of thrombosis.

Clinically approved thrombolytic agents are characterized by short half-life, short blood flow life and damage to local bleeding (Hacke et al., 2008; Derex and Nighoghossian, 2009). Nanoparticles have proven to be promising diagnostic agents for the treatment of a
variety of diseases and have relatively long blood circulation agents (Vogel and Venugopalan, 2003; Mahmoudi et al., 2011; Li et al., 2017). At present, it has been reported that hyperthermia can achieve a good thrombolytic effect (Voros et al., 2015; Wang X. et al., 2017). Hyperthermia methods include photothermal therapy and magnetic hyperthermia (Dai et al., 2019). Photothermal therapy is the treatment through heat generated by near-infrared (NIR) light-induced photothermal reagents (Fang et al., 2012; Zhang et al., 2013; Chen et al., 2014; Li et al., 2014, 2015, 2017). Wang et al. prove that heat generated by the photothermal effect of gold can achieve the purpose of thrombolysis (Wang X. et al., 2017). Magnetic hyperthermia is a way in which a magnetothermal conversion reagent generates heat to treat a disease under the action of an external alternating magnetic field (AMF) (Wang F. et al., 2017; Li et al., 2018; Dai et al., 2019). The magnetocaloric effect of iron oxide has been shown to accelerate the dissolution of blood clots in Voros et al.’ report (Voros et al., 2015). However, there are more or less defects in the way of thrombolysis. In the photothermal treatment, due to the poor penetration of light, it is impossible to treat deep thrombus, such as celiac vein thrombosis. In the case of iron oxide magnetothermal treatment of thrombus, it causes certain side effects due to the long-term presence of iron oxide in the body. Therefore, there is a great of necessity and importance to adjust the current strategy for treating thrombosis to treat thrombosis.

Fe₃S₄ nanoparticles have similar properties to iron oxide (Liu et al., 2014). It has been revealed that Fe₃S₄ nanoparticles have a very good magnetocaloric effect which could be used in magnetic hyperthermia for deep thrombosis (Guan et al., 2018; Moore et al., 2019). Moreover, Fe₃S₄ nanoparticles have been shown to degrade rapidly in vivo (Guan et al., 2018). Therefore, Fe₃S₄ nanoparticles show great potential for the magnetic hyperthermia for deep thrombosis. Herein, we reported hyperthermia for celiac vein thrombosis using Fe₃S₄ nanoparticles as a thrombolytic reagent under the stimulation of a NIR laser and an AMF. Fe₃S₄ nanoparticles show a synergistic thermal transition effect under the co-action of a NIR laser and an AMF. In addition, the Fe₃S₄ nanoparticles possess the ability for magnetic resonance (MR) imaging with the transverse relaxivity (r₂) is up to 53.1 mM⁻¹ s⁻¹. Finally, we, for the first time, proved the Fe₃S₄ nanoparticles as a promising thrombolytic agent for magnetic hyperthermia for celiac vein thrombosis.

RESULTS AND DISCUSSION

Synthesis and Characterization of Fe₃S₄ Nanoparticles

Hydrophilic Fe₃S₄ nanoparticles were synthesized via a simple hydrothermal method by reaction the FeSO₄ with L-Cysteine in water at 220°C for 20h. During the preparation, the polyvinylpyrrolidone (PVP) was added into the reaction as surface ligand to improve their biocompatibility. Thus, the Fe₃S₄ nanoparticles are capped by PVP (demonstrated by FTIR, Figure S1) and hydrophilic, and can be used as thrombolytic agents without any further modification. As shown in Figure 1A, transmission electron microscopy (TEM) image demonstrated that the as-prepared products were highly dispersible nanoparticles. The size of NCs was found to be 17.7 nm based on the TEM images (Figure S2). More microstructure information can be achieved from high resolution TEM (Figure 1B). It shows an interplanar spacing of 0.298 nm, which can be indexed to (220) planes of greigite structured Fe₃S₄. As shown in Figure 1C, X-ray diffraction (XRD) pattern of the products could be well-matched with that of greigite structured Fe₃S₄ (JCPDS file no.: 16-0713), indicating that we obtained pure greigite structured Fe₃S₄ with high crystallinity. X-ray photoelectron spectroscopy (XPS) revealed the composition and element state of the as-prepared products (Figure S3). It showed that the products mainly contain Fe and S elements with no other impurities. We analyzed the valency state of Fe in Fe₃S₄ nanoparticles. Figure 1D shows Fe 2p spectrum for the Fe₃S₄ nanoparticles. It was demonstrated that there was a mixed Fe oxidation state, i.e., Fe²⁺ and Fe³⁺ (Guan et al., 2018), indicating a defect structure in Fe₃S₄ nanoparticles. According to the above results, it can be concluded that the pure Fe₃S₄ nanoparticles with high crystallinity was successfully formed.

Magnetocaloric Conversion Performance of Fe₃S₄ Nanoparticles

Figures 2A,B shows the magnetocaloric conversion performance of Fe₃S₄ nanoparticles. Under the continuous simulation of AMF (4.2 × 10⁹ A m⁻¹ s⁻¹), the temperature of Fe₃S₄ nanoparticles with a concentration of 0.5 mg/mL can increase by 12.8°C, while the temperature change of pure water is not obvious. When the concentration is increased to 1.0 mg/mL, the temperature of Fe₃S₄ nanoparticle dispersion can be raised by 20°C which is high enough to dissolve thrombus. Figure 2C shows the thermal imaging of pure water and Fe₃S₄ nanoparticles (1.0 mg/mL) under the action of an AMF for 5 min, respectively. As we can see from Figure 2D, a high contrast image was obtained. These results indicated that Fe₃S₄ nanoparticles showed an excellent magnetocaloric conversion performance.

Photothermal Conversion Performance of Fe₃S₄ Nanoparticles

We also measured the UV-vis absorbance spectrum of the Fe₃S₄ nanoparticles, which is important for photothermal performance. From Figure 3A, we can see that the Fe₃S₄ nanoparticles showed a strong absorption in the NIR region from 700 to 1,000 nm, resulted from the defect structure in Fe₃S₄ nanoparticles. Then varied concentrations (0–0.5 mg/mL) of Fe₃S₄ nanoparticles were exposed to an 808 nm (0.33 W cm⁻²) laser to evaluate the photothermal effect. As expected, the Fe₃S₄ nanoparticles showed a good photothermal conversion performance with concentration-dependent photothermal effect (Figure 3B). Fe₃S₄ nanoparticles with a concentration at 0.5 mg/mL, the temperature can increase by about 30°C, while the temperature of pure water showed little change. The photothermal performance of Fe₃S₄ nanoparticles ensured the potential of Fe₃S₄ nanoparticles as a thrombolytic reagent.
Synergistic Thermal Conversion Effect
To test the synergistic thermal conversion effect of Fe₃S₄ nanoparticles, Fe₃S₄ nanoparticles (500 ppm, 100 μL) was assessed under the simultaneous stimulation including a NIR (808 nm, 0.33 W cm⁻²) laser and an AMF (4.2 × 10⁹ A m⁻¹ s⁻¹) for 5 min. Temperature change was recorded by a thermal imaging camera. It was found that the temperature elevation can reach 37.1°C under the co-stimulation of NIR and AMF (Figure 4A). From Figure 4B, we can see that the temperature change from the co-stimulation of NIR and AMF was much higher than that from the single stimulation of NIR or AMF, indicating a synergistic thermal conversion effect.

Combined Therapy of Thrombotic in vitro
Due to the excellent thermal conversion performance of Fe₃S₄ nanoparticles, we measured the in vitro thrombolytic capacity of Fe₃S₄ nanoparticles under an 808 nm (0.33 W cm⁻²) laser and/or an AMF (4.2 × 10⁹ A m⁻¹ s⁻¹). Thrombosis was obtained 1 week after ligation of the abdominal vena cava in mice using the surgical suture. A thrombus block was placed in a 20 mL glass vial followed by a 5 mL Fe₃S₄ nanoparticle solution. Finally, it was irradiated by an 808 nm (0.33 W cm⁻²) laser and/or an AMF (4.2 × 10⁹ A m⁻¹ s⁻¹) for in vitro thrombolysis. As a control, the thrombus block in another bottle containing nanoparticle solution wasn’t stimulated by NIR laser. It showed that the thrombus can be partially dissolved under the stimulation of NIR or AMF combined with Fe₃S₄ nanoparticles. Moreover, when co-stimulated by NIR and AMF, the thrombus was almost disappeared (Figure S4). However, the thrombus in the control showed little change. Therefore, Fe₃S₄ nanoparticles can be used as a thrombolytic agent under the stimulation of NIR or/and AMF.

MR Imaging Guided Thrombotic in vivo
Fe₃S₄ nanoparticles can be served for imaging guided magnetic hyperthermia of thrombosis. We established a model of deep vein thrombosis using black C57 mice, and the penetration depth of the laser did not reach the lesions. Moreover, the temperature of the hair of the C57 mice increased after laser irradiation to cause burntness. So we use magnetic hyperthermia to dissolve the thrombus. First, Fe₃S₄ nanoparticle phantom images and proton T₂ relaxation test at varied Fe concentrations were measured. As shown in Figure 5A, T₂-weighted MR imaging signal intensity was increased with the increase of the concentration of Fe₃S₄ nanoparticle. The transverse relaxivity (r₂) value of the CMO NCs was calculated to be 53.1 mM⁻¹ s⁻¹, indicating an efficient MRI contrast agent (Figure 5B). We then evaluated animal experiments on the T₂-weighted MR imaging guided magnetic hyperthermia of thrombosis. From Figure 5C, we can see that the signal in thrombosis sites (left) was light white. Under the action of Fe₃S₄ nanoparticles combined with AMF, light white region in thrombosis sites (right) obviously decreased. Therefore, Fe₃S₄
nanoparticles can be served as an efficient thrombolytic agent in vivo.

Biomaterials must have good biocompatibility in clinical applications. Then in vivo long-term toxicity of the as-prepared Fe₃S₄ nanoparticles was evaluated by blood bioanalysis and hematoxylin and Eosin analysis, respectively. There was no obvious difference detected in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Figures 6A,B), which indicated that Fe₃S₄ nanoparticles have good biocompatibility to the liver and heart (Zhang et al., 2018). Then histological examination analysis for main organs was performed to observe the shape and the size of cells after the intravenous injection of Fe₃S₄ nanoparticles at a dosage of 12 mg/kg. The organs included the heart, kidney, liver, lung, and spleen. As shown in Figure 6C, there is no tissue damage or adverse effect compared with those of control groups. This suggests
that these Fe$_3$S$_4$ nanoparticles at the given dose are not obviously toxic. It was reported that inorganic nanoparticles (quantum dots used as a model system) with relatively large sizes would accumulate in reticuloendothelial systems (RES) such as liver and spleen for long periods of time (Choi et al., 2007). Ideally, it would be the best to make Fe$_3$S$_4$ nanoparticles quickly excreted from the normal organs of the body, while being able to effectively accumulate and retain in lesions. A study from guan reported that the clearance of Fe$_3$S$_4$ nanoparticles was quickly post intravenous injection (Guan et al., 2018). We also studied the distribution and metabolism of the Fe$_3$S$_4$ nanoparticles, mice were intravenously injected with 12 mg·kg$^{-1}$ of the Fe$_3$S$_4$ nanoparticles. At different intervals of time (i.e., 1, 3, 7, and 10 days, $n = 3$ at each time point), mice were sacrificed to obtain major organs including kidney, spleen, heart, liver, and lung. These organs were digested and solubilized. An ICP-MS analysis was used to determine Fe content in each organ. It was (Figure S5) found that the Fe$_3$S$_4$ nanoparticles mainly accumulate at spleen and liver, indicating that Fe$_3$S$_4$ nanoparticles was mainly degraded in these two organs.

CONCLUSION

In conclusion, Fe$_3$S$_4$ nanoparticles, as a new imaging-guided thrombolytic agent, have been successfully prepared by a simple hydrothermal route. The as-prepared Fe$_3$S$_4$ nanoparticles have a good dispersity and show an excellent magnetothermal conversion performance and photothermal effect, and exhibited a synergistic thermal conversion effect under the co-stimulation of NIR and AMF. They also possess an effective MR imaging in vivo. Furthermore, the in vivo toxicity results indicate their excellent biocompatibility. With the stimulation of an external AMF and
a NIR laser, the Fe$_3$S$_4$ nanoparticles can be used as thrombolytic agents with MR imaging guided hyperthermia of thrombosis.

**EXPERIMENTAL SECTIONS**

**Synthesis of Fe$_3$S$_4$ Nanoparticles**

One millimole of FeSO$_4$ and 1 mmol L-cysteine were consecutively dissolved in 40 mL water. Seven hundred milligram of poly (vinyl pyrrolidone) (PVP) was then added. Then the reaction was kept at 220°C for 24 h in a stainless steel autoclave. The products were collected through centrifugation and finally washed with ethanol and deionized water for three times.

**Characterization**

The morphology as well as the size of nanoparticles was achieved by TEM (JEOL JEM-2010F, Japan). The crystal phase of Fe$_3$S$_4$ nanoparticles was measured by XRD (Bruker D4). The oxidation state analysis of Fe$_3$S$_4$ nanoparticles was measured by X-ray photoelectron spectra (XPS, ESCA-Lab 250Xi). Concentration of Fe ions released from Fe$_3$S$_4$ nanoparticles was tested by ICP-AES (Leeman Laboratories Prodigy).

**Measurement of Magnetocaloric Conversion Performance of Fe$_3$S$_4$ Nanoparticles**

For the evaluation of the magnetocaloric conversion performance of Fe$_3$S$_4$ nanoparticles, 100 µL of Fe$_3$S$_4$ nanoparticles dispersed in deionized water at varied concentrations was simulated under the external AMF ($4.2 \times 10^{9}$ A m$^{-1}$ s$^{-1}$). Temperature change was recorded by a thermal imaging camera.

**Measurement of Photothermal Effect of Fe$_3$S$_4$ Nanoparticles**

For the evaluation of the photothermal performance of Fe$_3$S$_4$ nanoparticles, 100 µL of Fe$_3$S$_4$ nanoparticles dispersed in deionized water at varied concentrations was exposed upon the irradiation of an 808 nm laser. Temperature change was recorded by a thermal imaging camera.

**Thrombotic Animal Model**

All animal experiments were approved by the Animal Ethics Committee of Karamay central hospital. 8-week-old C57 mice were anesthetized and laparotomy, the inferior vena cava was separated, and the inferior vena cava was ligated under the left renal vein with a surgical line (Kyogashima et al., 1999).

**Hyperthermia of Thrombosis in vitro**

To evaluate in vitro thrombolytic capacity of Fe$_3$S$_4$ nanoparticles under the irradiation of a NIR laser or/and AMF, thromboses was obtained 1 week after ligation of the abdominal vena cava in mice using a surgical line. A thrombus block was placed in a 20 mL glass vial followed by a 5 mL Fe$_3$S$_4$ nanoparticle solution. Finally, it was irradiated by an 808 nm laser or/and AMF for in vitro thrombolysis. As a control, the thrombus block in another
bottle containing nanoparticle solution wasn’t stimulated by the 808 nm laser or/and AMF.

**MR Imaging Guided Hyperthermia of Thrombosis in vivo**

The Fe$_3$S$_4$ nanoparticle dispersions with varied Fe concentrations (0–0.28 mM) were scanned at room temperature via the animal MR imagine scanner under a 0.5 T MRI scanner at room temperature. Before in vivo MR imaging guided magnetic hyperthermia of thrombosis, thrombosis model mouse were scanned with the same MR scanner with the same parameters to be a control. After intravenous injection with the Fe$_3$S$_4$ nanoparticle dispersion (100 µL, 12 mg/kg) followed by stimulation under an AMF, the thrombosis model mouse were scanned again with the MR scanner.

**Histological Examination Analysis and Blood Analysis**

As for the histological examination, one mouse in each group was killed under anesthesia after the indicated treatment. Then the organs included the heart, kidney, liver, lung, and spleen were harvested, and then sectioned into 4 µm slices using a conventional microtome, finally stained with H&E. The slices were examined via a microscope. Also, the blood from mice in control group and Fe$_3$S$_4$ group was collected to test the biocompatibility of Fe$_3$S$_4$ nanoparticle to major organs.

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**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the article/Supplementary Material.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the Animal Ethics Committee of Karamay Central Hospital.

**AUTHOR CONTRIBUTIONS**

DF, JD, and XG designed the project. DF, QR, JL, and HD carried out the experiment and performed the experimental data analysis. DF and XC wrote the paper. All the authors contributed to discussion of the results.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmats.2019.00316/full#supplementary-material
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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