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Synthesis of SPIO-chitosan microspheres for MRI-detectable embolotherapy

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Abstract

Spherical SPIO nanoparticles were synthesized and embedded in polyglucosamine (chitosan) by a sonochemical method. The embedded microspheres were shifted out in the range of 100–150 μm. The microspheres were injected into the kidney of a New Zealand white rabbit via an angiographic catheter, and detected in magnetic resonance images of the kidney.

Keywords: Superparamagnetic iron oxide; Sonochemical method; Ferrofluid; MRI; Embolic material; Chitosan; Microspheres; Rabbit; Kidney

1. Introduction

Embolization, the process in which a blood vessel or organ is obstructed by the lodgement of a material mass, has been used in cancer treatment [1]. Introduction of embolic materials into the blood vessels leading to a tumor diminishes its blood supply, thus starving the tumor. Spherical chitosan particles, having an average diameter of 50–1000 μm, have been used for embolization of blood vessels by inserting a catheter into the blood vessel and injecting the particles through the catheter. After injection, however, it was difficult to locate the injected embolic material and to monitor changes in its structure over time.

Superparamagnetic iron oxide (SPIO) nanoparticles were developed for clinical applications in magnetic resonance imaging (MRI) contrast enhancement [2–4]. The SPIO nanoparticles have the advantage of producing an enhanced proton relaxation in MRI, especially useful for T2-weighted images. We used a sonochemical method to synthesize SPIO nanoparticles with narrow size distribution and high magnetization [5]. In our previous research, magnetic fluids containing these nanoparticles have shown good MRI image contrast, similar to those of Resovist®, a commercially available, superparamagnetic, MRI contrast agent (Schering AG). The SPIO nanoparticles can

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be incorporated into embolic materials to enable MRI detection, thus finding a practical application in embolotherapy.

The objective of this study was to develop a novel embolic material with increased contrast for MRI. We prepared spherical SPIO nanoparticles about 15 nm in diameter by sonochemistry and embedded them in chitosan to prepare a ferrofluid. To make an embolic material, the synthesized ferrofluid was sprayed on the surface of an alkali solution so that a microsphere form of the ferrofluid was dispersed in the solution. These microspheres were injected into the renal artery of New Zealand white rabbits using an angiographic catheter. Finally, MRI measurements were performed to locate the microspheres in the kidney.

2. Experimental

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) and polyglucosamine (chitosan) were purchased from Aldrich, while ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH), and ethanol (C_2H_5OH) were purchased from from Junsei. All chemicals used were reagent grade.

A mixed solution of 0.15 M $FeCl_2$ (50 ml, 7.5 mM) and 0.30 M $FeCl_3$ (50 ml, 15.0 mM) was prepared. As soon as ultrasonic waves irradiated (ULSSO HITECH Co. LTD, Model ULH700S, 10 mm, Ti horn, 20 kHz) the mixture at 665 W, 60.0 mM NH_4OH was added rapidly, resulting in black particles at room temperature. These black particles were washed free of anions with deionized water, and the particle size and morphology were examined by transmission electron microscopy (TEM, Philips-F20). The wet particles were dried at 80 °C in a vacuum oven, and the crystal structure and magnetic properties were characterized using an XRD (Rigaku D/Max II) and a SQUID (Quantum design-MPMS5).

To prepare the ferrofluid, washed nanoparticles (1.73 g) were decanted and dispersed in 112.5 ml of a solution containing 1% chitosan and 1% acetic acid, by ultrasonic irradiation for 30 min. This ferrofluid was purified by centrifugation at 3000 rpm for 20 min at room temperature. The iron (Fe) concentration of this stock solution was 0.20 M and the stock solution was diluted to 0.2 mM.

The ferrofluid made of SPIO and chitosan was sprayed by a nozzle on the surface of the alkali solution (NaOH/ethanol/water, 4/30/66, w/v/v) to prepare embolic materials in the form of microspheres. Microspheres small enough to be injected into blood vessels (100–150 μm) were shifted out. The shifted microspheres were washed several times with deionized water. Embolization of a renal artery was performed in three New Zealand white rabbits weighing 2.0–3.5 kg. Blood laboratory results, including CBC with differential count, blood chemistry (BUN, Creatinine, AST, ALT), and electrolytes (Na, K, Cl), were normal before initiation of the study. A renal artery was selected and a 4 F angiography cobra catheter (Terumo, Tokyo, Japan) was inserted into the artery. Embolic material was injected into the selected renal artery using a 5 ml syringe. After embolization, the catheter was removed and the artery was tied with 3-0 silk. T2-weighted magnetic resonance (MR) images of the kidney were obtained to confirm detection of the injected microspheres. All MR imaging examinations were performed with a 1.0 T imaging system (Medius Co. Korea, Model Magnum 1.0 T) using a spin echo technique.

3. Results and discussion

The SPIO nanoparticles synthesized by sonochemistry were spherical and had an average diameter of about 15 nm as shown in Fig. 1 [5]. Because of their uniform size and shape, these SPIO particles were suitable for preparing ferrofluids for medical applications. In addition, the hysteresis curve of the nanoparticles had no coercive force showing superparamagnetic behavior (Fig. 2).

We obtained both T1- and T2-weighted images of Resovist® and the ferrofluids at the same concentrations and compared them to pure water as a standard (Fig. 3). Both the T1- and T2-weighted images of the ferrofluids were similar to those of Resovist®. At a Fe concentration of 0.2 mM, the MRI contrast was much higher than

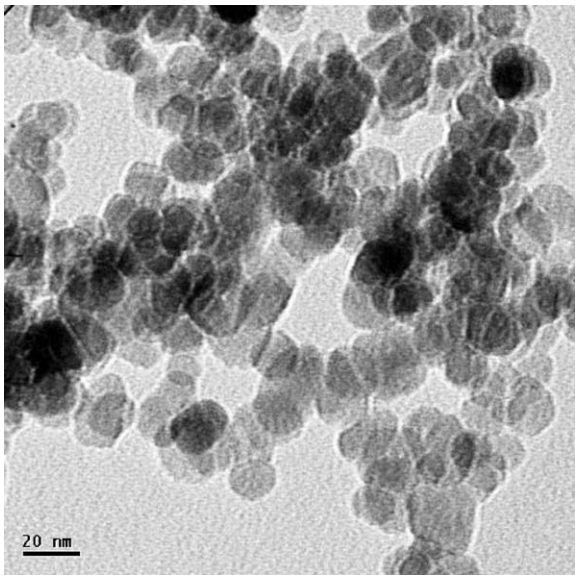

Fig. 1. TEM micrographs of SPIO nanoparticles synthesized by a sonochemical method.

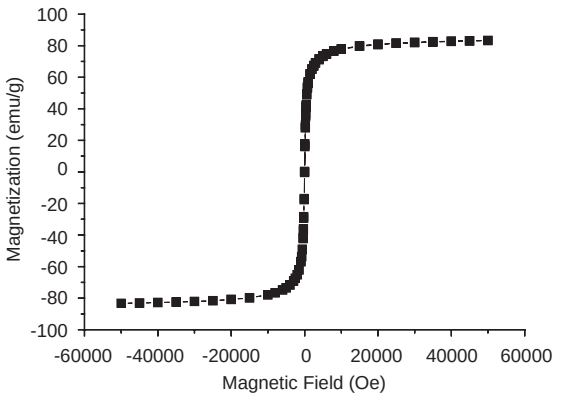

Fig. 2. Magnetic hysteresis curve of the synthesized SPIO nanoparticles.

that of pure water. Therefore, the ferrofluid may potentially be useful in developing an MR-detectable embolic material.

Microspheres with a narrow size distribution and small enough to be easily injected through the blood vessel via angiographic catheters would be an ideal embolic material. It was necessary to narrow the size distribution of the sprayed microspheres so that microspheres of 100–150 μm in diameter were sifted out and selected (Fig. 4).

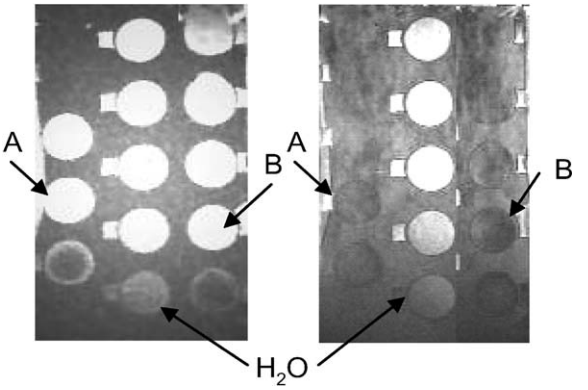

Fig. 3. T1- (left) and T2-weighted images (right) of Resovist® (A) and synthesized ferrofluid (B) at the same iron concentration of 0.2 mM. Images of water were used as a standard for comparison.

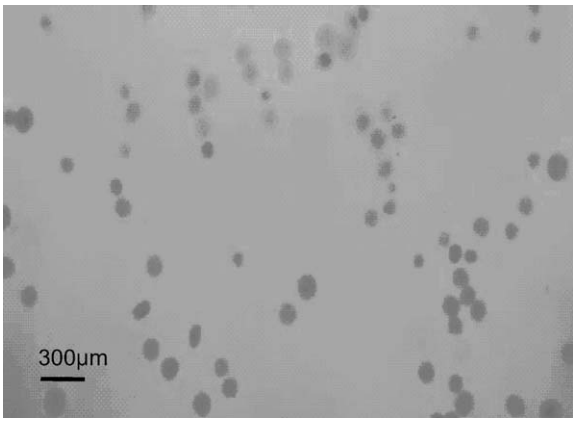

Fig. 4. A photograph of microspheres made up of SPIO and chitosan. The particle size ranged from 100 to 150 μm.

Sieved microspheres were nearly spherical and maintained their shape in water for more than 60 days.

These microspheres were injected into the renal artery leading to the left kidney of the rabbit as shown in Fig. 5. We obtained only T2-weighted MR images of the kidney (Fig. 6), because the SPIO has been known as a good agent for enhancing the contrast of T2-weighted MR images. Fig. 6 shows MR images for five kidney slices. The slice thickness was 2.8 mm and the slice gap was 0.4 mm. Fig. 6c corresponds to the middle position of the kidney of the supine rabbit.

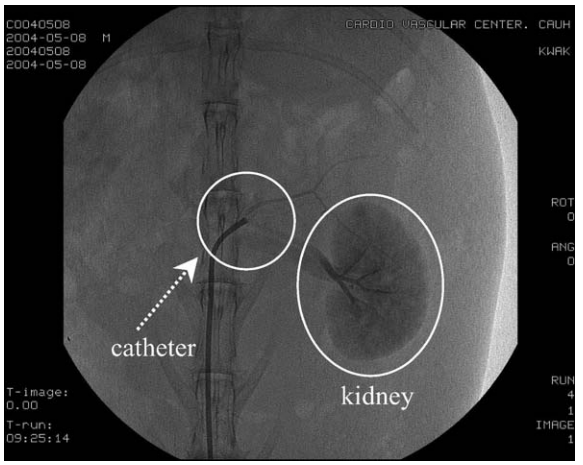

Fig. 5. Angiography of a catheter inserted into the renal artery of the left kidney of a New Zealand white rabbit.

Figs. 6a–e were taken as a series of successive slices. The microspheres were injected only into the left kidney, enabling comparison of the MR images with the right (control) kidney. In general, the MRI contrast agent appears darker than the surrounding tissue in T2-weighted images. Comparing the left and right kidneys, many dark spots were observed in the left kidney, but not the right kidney (Fig. 6d). In Fig. 6e, the T2-weighted images of the left kidney had distinct spots which were easily distinguished from background, demonstrated by the right kidney. These dark spots corresponded to microspheres deposited in the renal artery.

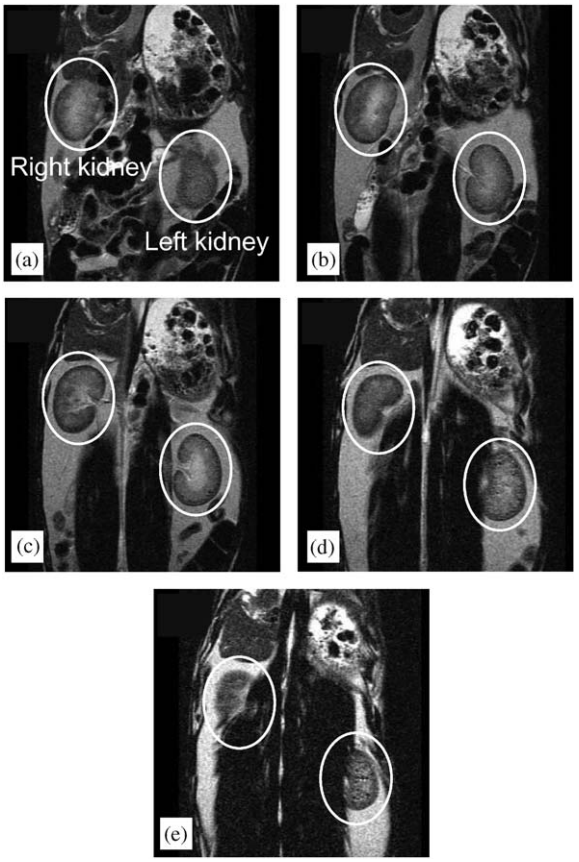

Fig. 6. T2-weighted MR images for five slices of the kidneys: (a) upper, (b) upper intermediate, (c) middle, (d) lower intermediate, and (e) lower. (a)–(e) were taken as a series of successive slices. The slice thickness was 2.8 mm and the slice gap was 0.4 mm. Microspheres were injected only into the left kidney. The right kidney served as a non-injected control.

4. Conclusion

We developed microspheres composed of SPIO nanoparticles and chitosan as a novel MRI-detectable embolic material. The SPIO-chitosan microspheres showed a strong enhancement of MR image contrast similar to the ferrofluid in vitro. When injected into the renal artery of a New Zealand rabbit, the microspheres appeared as distinct dark spots in T2-weighted images, demonstrating that the microspheres occupied the renal artery. The presence of microspheres in the left kidney was easily distinguished from the absence of microspheres in the right (control) kidney in vivo.

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