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Biomedical applications of superparamagnetic iron oxide nanoparticles encapsulated within chitosan

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Abstract

Microspheres composed of superparamagnetic iron oxide (SPIO; Fe_3O_4; magnetite) nanoparticles and chitosan were developed as a novel MRI-detectable embolic material. Spherical SPIO nanoparticles were synthesized and embedded in polyglucosamine (chitosan) by sonochemical method. The ferrofluid, solution of SPIO-embedded chitosan, was sprayed on the surface of an alkali solution with a nozzle to produce SPIO-chitosan microspheres, from which 100–150 μm microspheres were sifted out. The sifted microspheres were injected into the blood vessel leading to the kidney of a New Zealand white rabbit via an angiographic catheter *in vivo*, and they appeared to be detected in MR images of the kidney.

Keywords: Superparamagnetic iron oxide; Nanoparticles; Sonochemical method; Ferrofluid; Embolic material

1. Introduction

Embolization, the process in which a blood vessel or organ is obstructed by the lodgment of a material mass, has been used in cancer treatment. Introduction of embolic materials into the blood vessels leading to the tumor would starve the tumor of its blood supply, and thus cause its death. One of the authors has been developing an embolic material made of chitosan, which is biocompatible and biodegradable [1]. The embolic material was composed of spherical chitosan particles having an average diameter of 50–1000 μm. This material was used for embolization of blood vessels by inserting a catheter into the blood vessel and injecting the material through the catheter. After injection, however, it was difficult to locate embolic material injected and to monitor the change in its appearance during a certain period of time.

Superparamagnetic iron oxide (SPIO; Fe_3O_4; magnetite) nanoparticles find clinical applications in MRI contrast enhancement [2–4]. The SPIO nanoparticles produce an enhanced proton relaxation in MRI, especially useful for T2-weighted images. We have used a sonochemical method to synthesize the SPIO nanoparticles having narrow size distribution and high magnetization [5,6]. Ferrofluids, or magnetic fluids, from these nanoparticles have shown good MRI image contrast similar to those of Resovist®, a commercially available superparamagnetic contrast agent for MRI from Schering [6]. The SPIO can be incorporated into embolic materials to enable MRI detection and thus find a practical application in embolotherapy.

The objective of this study was to develop a novel embolic material capable of MRI contrast enhancement. We prepared spherical SPIO nanoparticles about 15 nm in radius by sonochemistry, and embedded them in chitosan to synthesize a ferrofluid. To make an embolic material, the synthesized ferrofluid was sprayed on the surface of an alkali solution so that the ferrofluid in microsphere form was dispersed in the solution.

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) from Junsei. All the chemicals used were of 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 were irradiated (Ulsso Hitech Co. Ltd., Model ULH700S, 10 mm, Ti horn, 20 kHz) to the mixture at 665 W, we rapidly added NH_4OH (60.0 mM) solution to the mixture to obtain black particles (Fe_3O_4; magnetite) at room temperature. These black particles were washed free of anions with deionized water, and the particle size and morphology were examined with TEM (Philips-F20) and AFM. The wet particles were dried in a vacuum oven to characterize the crystal structure and

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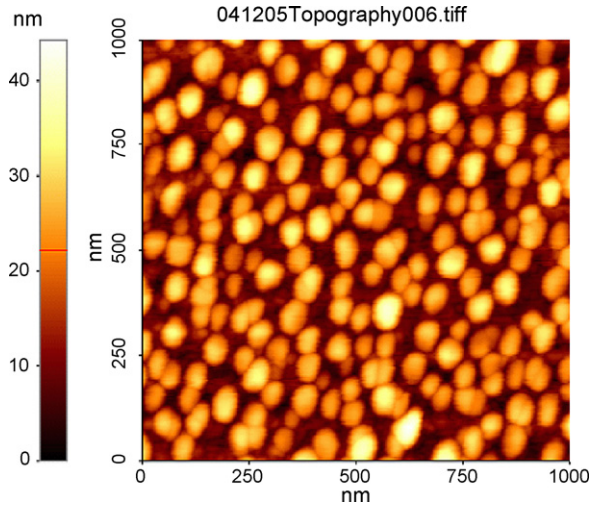

Fig. 1. AFM image of the SPIO nanoparticle synthesized by sonochemical method.

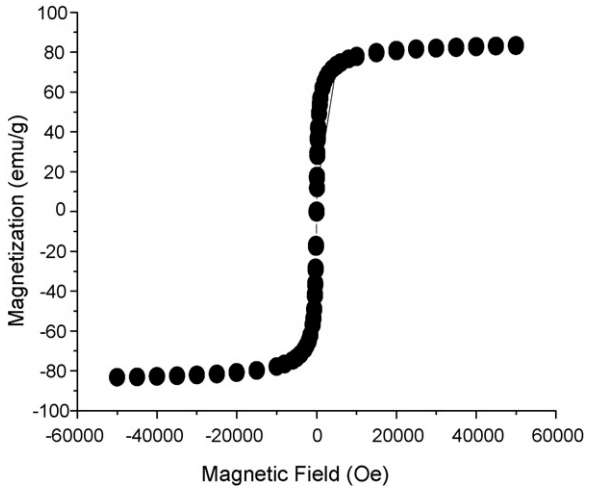

Fig. 2. Magnetic hysteresis curve of the SPIO nanoparticles synthesized by sonochemical method.

magnetic properties using 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 chitosan-acetic acid solution with concentration of 1% by ultrasonic irradiation for 30 min. This ferrofluid was purified with a centrifuge at 3000 rpm for 20 min at room temperature. Iron (Fe) concentration of this stock solution was 0.20 M and the stock solution was diluted to 0.2 mM, because Resovist® composed of SPIO nanoparticles coated with carboxydextrane would be injected to an adult to result in the Fe concentration of 0.2 mM in the liver. We obtained both T1- and T2-weighted images of Resovist® and our synthesized ferrofluid with the same Fe concentration.

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. The, sifted out 100–150 μm microspheres were small enough to be injected to blood vessels. The sifted microspheres were washed with deionized water several times, before they were injected into a blood vessel leading to the kidney artery of a New Zealand white rabbit via an angiographic catheter. After injection, T2-weighted MR images of the kidney were obtained to confirm whether the injected microspheres could be detected or not. All MR imaging examinations were performed with a 1.0 T imaging system (Medius Co. Korea, Model Magnum 1.0 T) by using a spin echo technique. Finally, we have checked the blood of left kidney of the rabbit by pathological anatomy.

3. Results and discussion

The SPIO nanoparticles synthesized by the sonochemistry were spherical and had an average radius of about 15 nm as shown in Fig. 1. Considering their uniform size and shape, these SPIO particles were suitable to prepare 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 with the same concentration comparing to pure water images as standard MR images. Both the T1- and T2-weighted images of the ferrofluids were similar to those of Resovist®. At the Fe concentration of 0.2 mM, the MRI contrast was quite enhanced in comparison with that of the pure water. Therefore, the ferrofluid has a potential to be used in developing an MR-detectable embolic material.

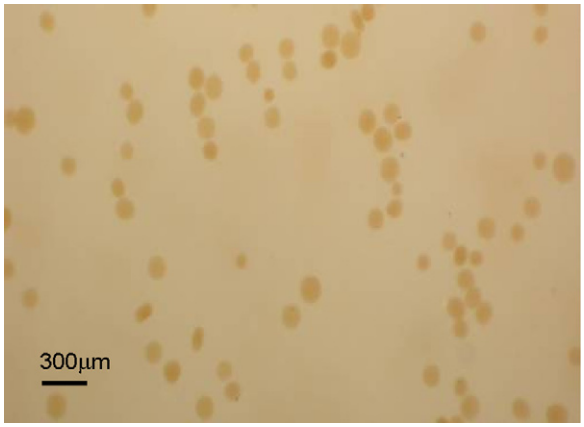

Fig. 3. A photograph of microspheres made up of SPIO and chitosan. The particle size ranged from 100 to 150 μm.

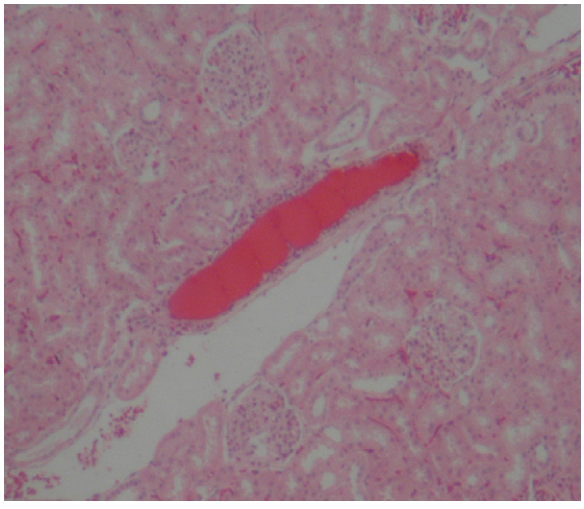

Fig. 4. Pathological anatomical result of the blood vessel of the left kidney of the rabbit.

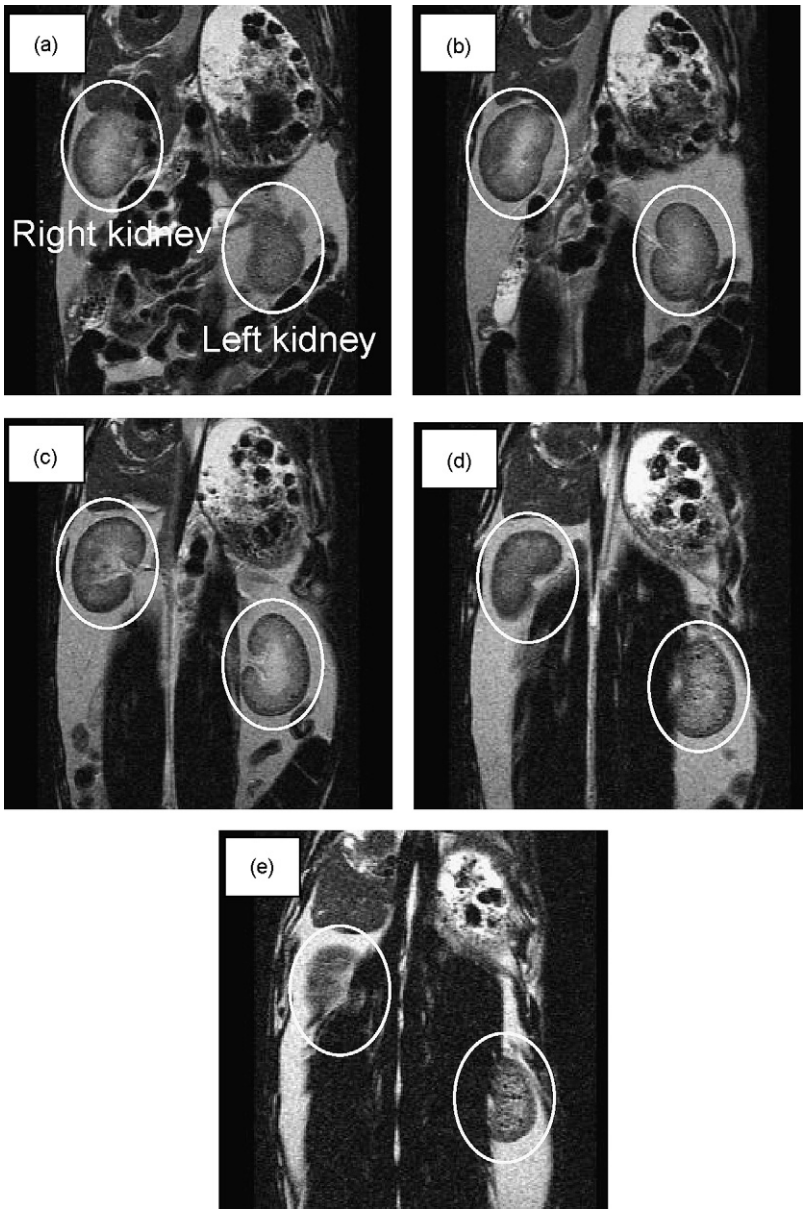

Fig. 5. T2-weighted MR images for five slices of the kidneys: (a) upper, (b) upper intermediate, (c) middle, (d) lower intermediate, and (e) lower. (a)–(e) were taken as a series of succeeding slices. A slice thickness was 2.8 mm and a slice gap 0.4 mm. The microspheres were injected into the left kidney, but not injected into the right one.

An ideal embolic material might be microspheres with a narrow size distribution and should be small enough to be easily injected through the blood vessel via angiographic catheters. It was thus required to narrow down the size distribution of the sprayed microspheres so that microspheres of 100–150 μm in diameter were sifted out (Fig. 3). Sieved microspheres were nearly spherical and maintained their shape in water for more than 60 days. Fig. 4 shows T2-weighted images of chitosan solution, ferrofluid, and SPIO-chitosan microspheres. The image of the ferrofluid could not be distinguished from the surrounding so that a circle with broken lines indicated its position. The chitosan had no enhancement of the contrast while the ferrofluid and microspheres showed a significant enhancement as expected from the previous MRI examination.

These microspheres were injected to the blood vessel leading to the left kidney of the rabbit. Then, we obtained only T2-weighted MR images of the kidney (Fig. 5), because the SPIO has been known as a good agent in enhancing the contrast of the T2-weighted MR images. Fig. 5 shows MR images for five slices of the kidneys. A slice thickness was 2.8 mm and a slice gap 0.4 mm. Fig. 5c corresponds to the middle position of the kidney of the lying rabbit. Fig. 5a–e were taken as a series of succeeding slices. The microspheres were injected only into the left kidney, but not injected into the right kidney, enabling to compare the respective MR images. In general, the MRI contrast agent would appear to be darker than the surrounding in T2-weighted images. Comparing the left and right kidneys, it is difficult to observe noticeable differences in Fig. 5a–c

while many darker spots inside the left kidney were observed in Fig. 5d. In Fig. 5e, more distinct spots were observed in the left kidney while the right kidney became unclear compared with those in the other images. We considered that these darker spots corresponded to the microspheres existing in the blood vessel. We could confirm this by pathological anatomy in Fig. 5, the injected SPIO-chitosan microspheres which compactly filling the vessels.

4. Conclusion

We have 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*. The microspheres injected into the blood vessel of the rabbit appeared to be detected *in vivo* in the shape of dark spots, considered portion of the microspheres occupying the slice of the blood vessel. This is our first experiment carried out *in vivo*. We are currently carrying out more experiments *in vivo* to elucidate the effectiveness of the SPIO nanoparticles for the MRI contrast enhancement in embolotherapy. These microspheres may apply to the magnetic-field assisted cancer thermo therapy.

Acknowledgment

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References

[1] S.M. Han, B.K. Kwak, H.J. Shim, Embolic Materials Comprising of Chitin and/or Chitosan and Method for Preparing Thereof, Application No. 0201443, July 30, 2002 (Patent pending).
[2] A. Moore, J.P. Basilion, E.A. Chiocca, R. Weissleder, Biochim. Biophys. Acta 1402 (1998) 239.
[3] D.K. Kim, Y. Zhang, J. Kehr, T. Klason, B. Bjelke, M. Muhammed, J. Magn. Magn. Mater. 225 (2001) 256.
[4] M.P. Morales, O. Bomati-Miguel, R. Pérez de Alejo, J. Ruiz-Cabello, S. Veintemillas-Verdaguer, K. O'Grady, J. Magn. Magn. Mater. 266 (2003) 102.
[5] E.H. Kim, H.S. Lee, H. Soe, Key Eng. Mater. 277-9 (2005) 1044.
[6] E.H. Kim, H.S. Lee, B.K. Kwak, B.K. Kim, J. Magn. Magn. Mater. 289 (2005) 328.