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Preparation of ferrofluids in chitosan for clinical applications

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1. Introduction

Superparamagnetic iron oxide nanoparticles (SPIO) are of great interest for several clinical applications: they were recently introduced as a suitable material for drug-targeting [1], hyperthermia [2], embolotherapy [3], and magnetic resonance imaging agent [4]. Especialiy for magnetic resonance imaging (MRI) contrast agents, superparamagnetic contrast agents have an advantage of producing an enhanced proton relaxation in MRI in comparison with paramagnetic ones. Consequently, less amount of SPIO agents was needed to dose into the human body than paramagnetic.

In this study, we synthesized a magnetite, SPIO by a sonochemical method and synthesized ferrofluids for MRI contrast agent by coating them with oleic acid and then dispersed them in the chitosan, which is a suitable carrier for bioapplications. in addition, we made micospheres with this ferrofluid to be used in embolotherapy. We characterized the nanoparticles by using an x-ray diffraction (XRD), a superconducting quantum interference device (SQUID), and a transmission electron microscope (TEM). The coated particles were analyzed by photon correlation spectroscopy (PCS) to measure the particle size. The MR images of the ferrofluids were obtained and compared with the images of Resovist. Resovist is a commercial MRI contrast agent from Schering company and this is consists of SPIO nanoparticles coated with carboxydextrane.

2. Experimental

All the chemicals were of reagent grade used without further purification. 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 result in black nanoparticles. These black nanoparticles were washed free of anions with deionized water and dried at 80 ℃. The nanoparticles were characterize the crystal structure and magnetic properties by using an XRD (Rigaku D/Max II) and a SQUID (Quantum design-MPMS5). The particle size and morphology were examined with TEM (Philips-F20).

Washed magnetite nanoparticles (1.73 g) were decanted and coated with 0.87ml of oleic acid at 70 ℃ for 30 min. To prepare ferrofluids, the coated SPIO nanoparticles were dispersed in 112.5 ml of chitosan by ultrasonic irradiation for 30 min. The synthesized ferrofluids were purified by a centrifuge at 3000 rpm for 20 min. Also, we sprayed this ferrofluid in alkali solution (NaOH/ethanol/water:4/30/66(w/v/v%), we could make microspheres for embolotherapy. The hydrodynamic diameters of the coated nanoparticles were calculated by PCS (MASTERSIZER 2000). The T1- and T2- weighted MR images of these ferrofluids were obtained. All MR imaging examinations were performed with a 1.0 T imaging system (Medius Co. Korea, Model Magnum 1.0 T of Medius Co.) by using a spin echo technique. The MR images of the ferrofluids were compared to those of Resovist.

3. Results and Discussion

The result of XRD showed that all resulted ferrite nanoparticles had spinel magnetite crystal structure. The magnetite nanoparticles by the sonochemical method showed, however, higher crystallinity than the particles by the coprecipitation (figure is not shown). The magnetite by the

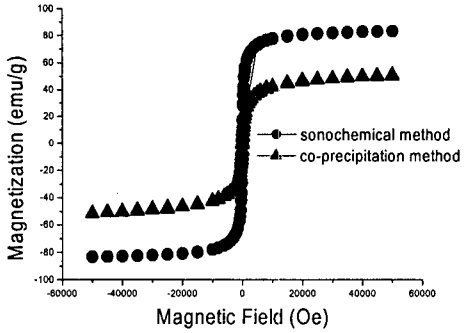

Fig. 1. Magnetic hysteresis curves of the SPIO nanoparticles by the sonochemical and coprecipitation method.

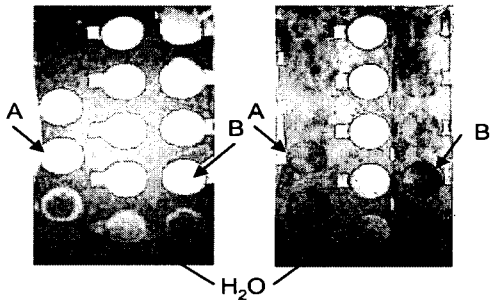

Fig. 2 (left) T1 (left)- and (right) T2 (right)-weighted images of Resovist (A), synthetic ferrofluid (B) and water (H_2O) in the same Fe concentration of, 0.2 mM. of Fe.

sonochemical method were spherical and had the average diameter of about 15 nm. Considering their size, shape and crystallinity, the SPIO by sonochemistry were suitable to synthesize ferrofluids for MRI contrast agents. The superparamagnetic relaxation is an essential requirement for the SPIO nanoparticles to be used for MRI contrast enhancement. The magnetic hysteresis curves of magnetite nanoparticles by the sonochemical and coprecipitation are shown in figure Fig. 1. The hysteresis curves of these nanoparticles had no coercive force, all magnetite nanoparticles from two different methods behaved superparamagnetic. The saturation magnetization of magnetite particles by the sonochemical (83 emu/g) was higher than the that of nanoparticles by coprecipitation (51emu/g).

The mean particle size of the coated SPIO nanoparticles was measured to be 65 nm by using PCS, which was close to that of the SPIO (61 nm) in Resovist. Resovist would be injected to an adult to result in the Fe concentration of Fe of 0.2 mM in the liver. To cover the Fe concentration of Resovist, we first prepared the ferrofluids with Fe concentration of 0.2 M, and then diluted the ferrofluid with varying Fe concentrations from 0.02 mM to 0.02 mM with 5% dextrose solution. These ferrofluid of various concentrations were stable for 30 days without precipitation. We obtained both T1- and T2-weighted images of Resovist and the ferrofluids prepared with the same concentration as Resovist. The images of pure water were also taken as standard MR images (Fig. 2). The T1-weighted images of Resovist and the ferrofluids were brighter than that of the water while T2-weighted images darker. Both the T1- and T2-weighted images of the ferrofluids were similar to those of Resovist.

4. Conclusion

Superparamagnetic iron oxide nanoparticles (SPIO) were synthesized by the sonochemical method. These spherical particles of about 15 nm in diameter showed superparamagnetic behavior. We confirmed these SPIO could be well dispersed in chitosan to make ferrofluids. And the ferrofluids exhibited the enhancement of MRI contrasts comparable to Resovist in vitro. Still much work remains to be done to make the ferrofluids reliable for the clinical applications.

References

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