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Thermosensitive/magnetic poly(organophosphazene) hydrogel as a long-term magnetic resonance contrast platform

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ABSTRACT

A thermosensitive/magnetic poly(organophosphazene) hydrogel (a magnetic hydrogel) was designed and synthesized for long-term magnetic resonance (MR) imaging. To turn a thermosensitive poly(organophosphazene) hydrogel (an original hydrogel) into a long-term MR contrast platform, cobalt ferrite ($CoFe_2O_4$) nanoparticles, which have hydrophobic surfaces, were bound to the original hydrogel via interactions between the hydrophobic surfaces of the nanoparticles and the L-isoleucine ethyl esters of the polymer. The magnetic hydrogel showed extremely low cytotoxicity and adequate magnetic properties for use in long-term MR imaging, in addition to possessing the same properties of the original hydrogel, such as viscosity, thermosensitivity, biodegradability, biocompatibility, a reversible sol-to-gel phase transition near body temperature, and injectability. The magnetic hydrogel was injected into a rat brain using stereotactic surgery. After the injection, the applicable potentiality as a long-term MR contrast platform was successfully estimated over 4–5 weeks. Consequently, it was shown that a magnetic hydrogel as a long-term MR contrast platform has the potential to be applied in a long-term theranostic hydrogel system. Furthermore, it is expected that this platform can be useful in the clinical field of incurable diseases due to either surgical difficulties or lethality, such as with brain tumors, when the platform is combined with therapeutic drugs for long-term MR theragnosis in further studies.
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1. Introduction

In the last few decades, many kinds of contrast agents have been developed in tandem with advances in medical imaging modalities, such as computed tomography (CT), single photon emission computed tomography (SPECT), positron emission tomography (PET), and magnetic resonance imaging (MRI) [1–9]. Common keywords of those contrast agents have been 'intravenous (i.v.) administration' or 'intravenous injection' because the i.v. method is the clinically general way of passing these agents through a circulatory system [10], and thereby those contrast agents in the i.v. system can obtain advantages in the case of i.v. administration. However, medical care for i.v. administration of the contrast agents is needed for patients every time due to the short half-life of the agents *in vivo* whenever either a medical imaging diagnosis or a theragnosis is performed [4]. This means that i.v.-administered contrast agents cannot give us information regarding the long-term progress of medical treatments over a few weeks.

In other cases, several studies have been conducted on drug/cell delivery or scaffold systems using magnetic gels, such as injectable ferrogels and implantable ferrosponges [11–14]. Though these materials show remarkable properties for local drug/cell delivery systems, the results of such studies have shown that they do not adequately possess all the properties required of an ideal long-term imaging system, such as injectability, biodegradability, and sustainability.

To overcome the above limitations of contrast agents of an i.v. system and ferrogels/ferrosponges of the locally injectable/implantable system, we have designed a long-term magnetic

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resonance (MR) contrast platform system with the following remarkable properties: thermosensitivity, biocompatibility, biodegradability, injectability, localizability, sustainability, magnetic responsibility, and so on [15–18]. Our long-term MR contrast platform system satisfies all the above properties for use in medical applications requiring MRI. Furthermore, the long-term MR contrast platform system is not an *in situ* heterogeneous system but a homogenous magnetic hydrogel system using simple 'hydrophobic interactions' between hydrogels and magnetic materials. With these properties, our study is a highly creative demonstration, to the best of our knowledge, in the field of long-term MR imaging using a magnetic hydrogel and animal brain *in vivo*.

In this study, cobalt ferrite nanoparticles, which are ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles, were selected to form the long-term MR contrast platform with the thermosensitive/biodegradable poly(organophosphazene) hydrogel system. It is well known that MRI does not result in tissue damage because it is a non-radioactive system. Tissue damage due to localization, sustained release, and long-term monitoring may occur in radioactive medical imaging modalities, such as PET, SPECT, and CT [19–23]. Furthermore, since MRI has the highest spatial and temporal resolution among the medical imaging modalities [24,25], it is the most adequate candidate to monitor the progress of the environmental changes in and out of the long-term MR contrast platform *in vivo*.

Meanwhile, the best target of the long-term MR contrast platform is a brain tumor among the various cancerous diseases due to either surgical difficulty or high lethality [26]. In this study, all the properties needed for long-term MR imaging are assessed for the configuration of the rat brain by using the thermosensitive/magnetic poly(organophosphazene) hydrogel system as a long-term MR contrast platform. The characteristics of the system were determined by several measurements and estimation.

2. Materials and methods

2.1. Instruments and measurements

D/MAX-2500 (Rigaku international Corporation, Japan) was used as small angle X-ray spectroscopy. X-ray was 1.54056 Å of Cu $K_{\alpha 1}$/30 kV/100 mA and scan mode was $2\theta/\theta$. Scanning range was between 3 and 80 °. Scan step was 0.010 ° and scan speed was 4.000 °/min. All nanoparticle samples were powder type. Tecnai G2 (FEI Hong Kong Co., Ltd.) was used as TEM and EDX devices. Cobalt ferrite nanoparticle sample was dispersed in hexane with 1 wt% solution (except EDX samples (5 wt%)) and the magnetic hydrogel sample was dispersed in distilled water. For the measurement of mass magnetization, MPMS5 (Quantum Design Co., USA) was used as superconductor quantum interference device (SQUID) magnetometer at room temperature. The amount of each powder sample was between 80 and 100 mg. In the case of iron content confirmation, SOLAAR M (Thermo Fisher Scientific Inc., USA), a device of flame & flameless analysis, was used as AAS and samples were dispersed in distilled water. The viscosity measurements of the hydrogel and the magnetic hydrogel were performed by a Brookfield RVDV-III+ viscometer between 5 and 70 °C under a fixed shear rate of 1/0.08 using SC4-14 spindle. The measurements were carried out with a set spindle speed of 0.2 rpm and with a heating rate of 0.33 °C/min. In the case of MTT assay, Spectra MAX340 (Molecular Devices, Inc., USA) was used with softmax Pro v.5.3. The stereotactic surgery was performed on the stereotactic frame (David Kopf instruments, USA). BioSpec® 47/40 USR imaging system (4.7 Tesla, Bruker BioSpin, Germany) was used for *in vitro* MR phantom study, and BioSpec® 70/20 USR(7 Tesla, Bruker BioSpin, Germany) was used for the *in vivo* long-term MR imaging.

2.2. Synthesis of magnetic nanoparticles

Magnetite nanoparticles were synthesized using high temperature thermal decomposition method with reagents as follows [31]: iron(III) acetylacetonate (≥97.0%(RT)), 1,2-hexadecanediol (technical grade, 90%), *cis*-9-Octadecenoic acid (reagent grade, ~99% (GC)), cis-1-Amino-9-octadecene (technical grade, 70%), and dibenzyl ether (99%). All reagents were purchased from Aldrich Chemical Co..

$Fe(acac)_3$ (20 mmol), 1,2-hexadecanediol (100 mmol), *cis*-9-Octadecenoic acid (60 mmol), and cis-1-Amino-9-octadecene (60 mmol) were mixed and magnetically stirred in benzyl ether (250 ml) under an N_2 atmosphere. The mixture was heated to 200 °C for 3 h and additionally heated to reflux (300 °C) for 1 h. After refluxing, the black-brown mixture was cooled to room temperature. Excess amounts of absolute ethanol were added to the mixture which was magnetically stirred, and a black-colored product was precipitated and centrifuged. The precipitates were dissolved in hexane with adding of *cis*-9-Octadecenoic acid (0.5 mL) and cis-1-Amino-9-octadecene (0.5 mL). Undispersed residues were removed using centrifugation at 4000 rpm for 20 min. The mixture without undispersed residues was re-precipitated with absolute ethanol, and the products, iron oxide nanoparticles, were obtained after the solvent was removed using centrifugation. Previous refining steps were repeated several times and the final products were dispersed in hexane.

Cobalt ferrite nanoparticle was synthesized under the identical conditions using the reagents as follows: cobalt(II) acetylacetonate (97%) was used. Reagent was purchased from Aldrich Chemical Co. and the reagent (8 mmol) was reacted with 16 mmol of $Fe(acac)_3$, and product was dispersed in hexane.

2.3. Conversion processes to the magnetic hydrogel

The thermosensitive/biodegradable poly(organophosphazene) hydrogel was synthesized using the previous method [15]. To give the hydrogel the magnetic property, two types of solutions were prepared. One was polymer solution in distilled water and another was an organic solution of cobalt ferrite nanoparticles in n-hexane. The nanoparticle solution was added to the polymer solution. The double layered solutions were sonicated at 4 °C until they were merged into a single layered solution. After sufficient sonication, the merged solution was kept under the ice condition until they were separated into double layer of n-hexane and product. After the complete separation, the spent n-hexane layer was removed under the gelation condition at T_{max} of the hydrogel and the fresh n-hexane was added to the product. These processes were repeated several times until the spent n-hexane layer was transparent. The product was kept under 4 °C for 24 h. Finally, the thermosensitive/magnetic poly(organophosphazene) hydrogel was obtained via collecting and freeze-drying processes to remove the residual solvents. The obtained magnetic hydrogel was kept under −25 °C after syringe filtering of 0.2 μm.

2.4. Assessment of weight loss of hydrogels under a physiological condition

The hydrogel (10 wt%) and the magnetic hydrogel were prepared, which was fully dissolved in PBS buffer of pH 7.4 by magnetic stirring at 4 °C. Totally 0.2 mL of the each polymer solution ($n = 5$) was loaded into a millicell of which pore size was 12 μm (Millipore, USA). The solution within the millicell was gelated in a dried incubator at 37 °C for 10 min. After the gelation was over, the millicell was incubated in PBS buffer (4 mL) of pH 7.4 with shaking (50 rpm) at 37 °C. Each millicell was collected and freeze-dried at each pre-determined time point of sampling. After freeze-drying was finished, each millicell was weighed and the remained amounts of the sample were calculated.

2.5. In vitro MTT assays

NIH3T3 mouse embryo fibroblast cells (1.5×10^4 cells/well, $n = 10$) and U-87 MG human glioblastoma and astrocytoma cells (1.5×10^4 cells/well, $n = 10$) were seeded in 96-well tissue culture plate (SPL, Korea). The cells were incubated for 24 h with the hydrogel and the magnetic hydrogel, respectively. After the incubation, the spent media were cleared out and the cells were washed once with a fresh DPBS. After washing and suction, solution (0.2 mL/well) of MTT assay reagent (thiazoly blue tetrazolium bromide (98%), Alfa Aesar) was added to the cells of which the concentration was 5 mg/mL (reagent in DPBS) in 10% culture media. The cells were incubated for 3 h at 37 °C in a humidified atmosphere of 5% CO_2. After the suction, DMSO (0.1 mL/well) was added to the cells and the plate was shaken gently for 15 min. The absorbance of the solution was determined at the excitation wavelength of 570 nm, using Spectra MAX 340.

2.6. Stereotactic surgery

All protocols used in the animal studies were approved by the Institutional Animal Care and Use Committee in National Cancer Center. 7-week-old female F344 rats were purchased from Orient-Bio (Charles River Korea, Seoul, Korea). Rats were anesthetized with ketamine (90 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal) and placed in a stereotactic frame. A burr hole was drilled in the skull to expose the dura: the anterior of 3 mm and the lateral of 1.5 mm from the bregma. A micro-syringe (Hamilton, Reno, NV, USA) was fitted with a 22-gauge needle and connected to the manipulating arm of the stereotactic frame. Totally 10 μL of the magnetic hydrogel was injected into the right striatum area of rat brain at the depth of 4 mm from the dura for a 5 min period. The needle was kept in the place for 10 min and then withdrawn slowly over a further 10 min. After the injection, the scalp wound was closed with 4-0 black silk sutures.

2.7. In vitro & in vivo MR imaging

4.7 Tesla BioSpec® 47/40 USR (Bruker BioSpin, Germany) was used for *in vitro* MR phantom study. The echo times (TE) were 15, 35, 55, 76, 96, 116, and 137 ms. The repetition time (TR) was 2000 ms and the field of view (FOV) was 9 cm × 5 cm. The slice thickness was 1.5 mm and the scan time was 12–13 min. The spatial resolution

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