Poly(amino acid)s micelle-mediated assembly of magnetite nanoparticles for ultra-sensitive long-term MR imaging of tumors†‡

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Micelles composed of a novel weakly cationic poly(amino acid)s shell and clustered magnetite nanoparticles as a core were prepared and evaluated as a potent MR imaging contrast agent based on their superior physicochemical and magnetic properties and the effective MR imaging of cancer in vitro and in vivo.

Fabrication of novel organic and inorganic nanomaterials as well as hybrids thereof in conjunction with biomedical applications such as drug delivery and medical imaging is one of the most topical issues in nanobiomaterial science.1 Such studies covering a wide experimental scope, from nanochemistry to biomedicine, have not only advanced the development of multifunctional nanobiomaterials but also have innovated biomedical investigations.1 One noticeable trend in this area is sensitivity-improved magnetic resonance imaging (MRI) with superparamagnetic nanoparticulates,1–3 and the research has been rapidly accelerated over the past several years with the reports of thermal decomposition methods producing highly size-controlled monodisperse magnetic nanoparticles coated with a hydrocarbon monolayer.4 The majority of in vivo works have been reported from ultrasmall superparamagnetic iron oxide nanoparticle (USPION, less than 20 nm in size) based systems which offer the advantages of longer circulation and less clearance by organs;11–2 although superparamagnetic iron oxide nanoparticle (SPION, several tens of nm in diameter by gathering of USPION) can show considerably enhanced MR imaging contrast by the aggregated state of magnetic nanoparticles.5 Very recently, a few studies utilizing SPION demonstrated effective in vivo MR imaging of the target site by employing appropriate delivery strategies such as receptor-mediated endocytosis or magnetic targeting by an additionally applied local magnetic field.1b–d

Here we show a very successful in vivo MR imaging of tumor by the use of simple SPION-type micellar nanoparticles from novel poly(amino acid)s without the help of any targeting ligands or magnetic field guidance. We report on the aggregation of magnetite (Fe3O4) nanoparticles enclosed by synthetic poly(amino acid)s micelles and the application as an effective MR imaging contrast agent. The appropriate size of micelles enabled targeted accumulation at tumor by the enhanced permeability and retention (EPR) effect and the clustered magnetite nanoparticles greatly enhanced MRI sensitivity. Furthermore, weakly cationic poly(amino acid)s shell not only assures high colloidal stability and biocompatibility but also assists targeted delivery by enhancing localization near tumor tissue and binding with cancer cells.

Water-soluble amphiphilic poly(amino acid)s, poly-2,β-(N-2-dimethylaminoethyl t-aspartamide) modified hydrophobically with octadecyl chains (PDMAEA-g-C18), was prepared from poly(succinimidyl) (PSI) by simple aminolysis with octadecylamine and N,N-dimethylethyleneamine sequentially (Fig. S1 in ESI†). Dimethylaminoethyl group has been used as a functional moiety in many polymethacrylate-based drug delivery systems such as polycations for gene delivery and stimuli-sensitive drug delivery. However, it has rarely been introduced to PSI, which can be easily converted to various useful poly(amino acid) derivatives for delivery carriers, as shown in our previous studies.2–5

PDMAEA-g-C18 micelles were loaded with as-synthesized 6 nm hydrophobic magnetite nanoparticles (MNP6s)3d–g via emulsion formation to prepare magnetic micelles named as P-SPION which consists of the PDMAEA shell and aggregated MNP6s as the core as shown in Scheme 1 (for further details, see ESI†). Characteristics of the PDMAEA-g-C18, MNP6, and P-SPION are listed in Table 1.

The mean effective hydrodynamic diameter (Dh) of P-SPION in pH 7.4 phosphate buffered saline (PBS) solution was determined by dynamic light scattering (DLS) as 95.7 nm, which is an adequate size for passive tumor targeting by the EPR effect. Additionally, it seems that the PDMAEA shell offers sufficient aqueous colloidal stability upon variation of the external conditions, for the Dh value was maintained at a similar level over two weeks as well as under variation of the pH of the media (Fig. S3 in ESI†). Also no significant change was found through lyophilization and reconstitution.

The zeta potential value of P-SPION measured at pH 7.4 and pH 5.0 is +7.14 mV and +11.3 mV, respectively.
This slightly charged surface is favorable to colloidal stability and its pH-responsive cationic property is contributable for tumor-specific delivery both at the cellular and body level owing to the low pH at the tumor tissue and the stronger negative charge of the cancer cell surface compared to normal cells. As observed with the zeta potential measurement, the positive surface charge of P-SPION is anticipated to be further heightened when it reaches the acidic tumor site so that the intracellular uptake into the cancer cells would be enhanced by a strong electrostatic interaction.

The structure of P-SPION was visualized. In the transmission electron microscope (TEM) image (Fig. 1), the core parts filled with tightly packed MNP6s are clearly shown by their high electron density. Also, by scanning electron microscope (SEM) imaging along with an energy dispersive spectrometer (EDS) analysis, the existence of smooth polymeric layer covering MNP6s underneath without any impurities was confirmed (Fig. S4 in ESI†). Fe content in P-SPION, analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES), is 13.5 wt% and the superparamagnetic magnetization was observed using superconducting quantum interference device (SQUID) (Fig. S5 in ESI†).

The $T_2$ contrast enhancing capability of P-SPION was examined and compared with that of Feridex I.V., which is a representative SPION-type commercial MRI contrast agent with a diameter of 80-150 nm. $T_2$ relaxation times ($T_2$) of solutions of P-SPION and Feridex I.V. in DDI water of various concentrations were measured with a 4.7 T clinical MRI instrument (for further details, see ESI†). Fe content in P-SPION, analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES), is 13.5 wt% and the superparamagnetic magnetization was observed using superconducting quantum interference device (SQUID) (Fig. S5 in ESI†).

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Timeframe of the experiment. At 30-minute postinjection, the SI of the tumor already decreased exceedingly as P-SPION had penetrated the tumor region. The decreased SI at the tumor was maintained long enough for detection at 72-hour postinjection. The MR image obtained at 72-hour postinjection still clearly visualizes the dark SI of the tumor so that expansion of the rapidly growing CT26 tumor is easily noticed. Therefore, the highly tumor accumulative P-SPION can be used not only for diagnosis of tumors at a given point of time but also for further investigations for a significant period of time. Those may include monitoring the change of rapidly growing tumors or tracking the cell migration. To analyze the relative signal drop at the tumor compared to preinjection, the changes in tumor-to-muscle SI ratio as a function of time were determined (Fig. S7 in ESI†). The relative signal drop was very high, ranging from 60–67%, which is even higher than the value obtained at the liver and has rarely been reported so far. The tumor accumulation of P-SPION was verified by *ex vivo* Prussian blue staining of CT26 tumor tissue extracted from the mouse immediately after *in vivo* MR imaging at 72-hour postinjection (Fig. S8 in ESI†). The blue color diffused across the tumor tissue indicates the uptake of P-SPION.

Since EPR effect is based on physiology of tumor tissue as well as nanoparticulate size, a clearer uptake mechanism is currently being investigated using various cancer models also with the effect to minimize the occasional small micelles with minimal amounts of MNPs. For the present results, we anticipate that high tumor accumulation *via* EPR effect is mainly attributed to P-SPION with diameter in 50-100 nm rather than smaller micelles which would show higher extravasation and lower capture in tumor as recently reported.3

In the present study, we demonstrated magnetic micelle P-SPION which has very concise structure but shows advanced functions as a *T*₂ contrast agent for MR imaging both *in vitro* and *in vivo*. Tumor-selective high signal drop and great MR sensitivity were achieved with the appropriate constitution of P-SPION. Suitable size and PDMAEA outlayer as a stable and functional outfit enabled effective uptake to tumor tissue and cells. Tightly packed magnetite particles greatly enhanced imaging contrast by accelerating *T*₂ relaxation. This potent contrast agent can be easily advanced multifunctionally by introduction of other bioactive agents such as anticancer drugs, genes, labels for optical imaging, or active targeting moieties for further biomedical explorations.

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**Notes and references**