Research Article

Gum Arabic-Coated Magnetic Nanoparticles for Potential Application in Simultaneous Magnetic Targeting and Tumor Imaging

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Abstract. Magnetic iron oxide nanoparticles (MNP) coated with gum arabic (GA), a biocompatible phytochemical glycoprotein widely used in the food industry, were successfully synthesized and characterized. GA-coated MNP (GA-MNP) displayed a narrow hydrodynamic particle size distribution averaging about 100 nm; a GA content of 15.6% by dry weight; a saturation magnetization of 93.1 emu/g Fe; and a superparamagnetic behavior essential for most magnetic-mediated applications. The GA coating offers two major benefits: it both enhances colloidal stability and provides reactive functional groups suitable for coupling of bioactive compounds. *In vitro* results showed that GA-MNP possessed a superior stability upon storage in aqueous media when compared to commercial MNP products currently used in magnetic resonance imaging (MRI). In addition, significant cellular uptake of GA-MNP was evaluated in 9L glioma cells by electron spin resonance (ESR) spectroscopy, fluorescence microscopy, and MRI analyses. Based on these findings, it was hypothesized that GA-MNP might be utilized as a MRI-visible drug carrier in achieving both magnetic tumor targeting and intracellular drug delivery. Indeed, preliminary *in vivo* investigations validate this clinical potential. MRI visually confirmed the accumulation of GA-MNP at the tumor site following intravenous administration to rats harboring 9L glioma tumors under the application of an external magnetic field. ESR spectroscopy quantitatively revealed a 12-fold increase in GA-MNP accumulation in excised tumors when compared to contralateral normal brain. Overall, the results presented show promise that GA-MNP could potentially be employed to achieve simultaneous tumor imaging and targeted intra-tumoral drug delivery.

KEY WORDS: brain tumor; drug delivery; gum arabic; magnetic nanoparticle; magnetic targeting.

INTRODUCTION

Recent advancements in nanotechnology have shed light on utilizing magnetic iron oxide nanoparticles (MNP) as drug carriers. With their unique response to an external magnetic field, MNP can be both passively and actively targeted to tumors. Passively, the size of nano-carriers enables them to extravasate into tumor interstitium through the hyperpermeable vasculature characteristic of solid tumors, a phenomenon known as the enhanced permeability and retention (EPR) effect (1). Actively, the magnetic properties of MNP allow their targeting to and accumulation at the tumor site with the aid of an external magnetic field, a phenomenon typically referred to as magnetic targeting (2). In addition, MNP reduce both T_1 and T_2/T_2^* relaxation times, rendering them

“visible” in magnetic resonance imaging (MRI)—a non-invasive, high spatial resolution clinical modality that has been used extensively in tumor diagnosis (3).

Polymer-coated MNP, having core-shell structure, typically consists of a mono- or multi-crystalline magnetic, iron oxide (magnetite or maghemite) core coated with a biocompatible polymer shell. In one respect, the coating is important because it can provide functional groups for conjugation of drug molecules and/or targeting ligands. In another, the coating can alter the surface properties of “bare” MNP, altering *in vivo* stability and circulation half-life. With respect to both stability and half-life, nanoparticle aggregation is of greatest concern as aggregated MNP are no longer nano-sized. Larger particles are rapidly identified by the reticuloendothelial system (RES), cleared by the liver and spleen, and are unable to extravasate tumor vasculature, resulting in minimal tumor accumulation via the EPR effect. Normally, iron oxide cores alone aggregate quickly at physiological pH and in ionic environments due to van der Waals attraction between particles (4). Polymer coatings, however, create steric hindrance to such forces in addition to providing a resistance to RES clearance—the most common route of MNP elimination *in vivo*. Hydrophilic polymers are normally preferred as they provide a strong steric barrier to opsonin adsorption, thereby improving circulation half-life (5).

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Gum arabic (GA) is a nontoxic, hydrophilic, phytochemical glycoprotein polymer widely used as a stabilizer in the food and pharmaceutical industries. It is a heterogeneous polymer comprising three main components: low-protein content arabinogalactan (90%); high-protein content arabinogalactan (10%); and high-protein content glycoproteins (<1%) (6,7). Although not fully understood, the most widely accepted structure of GA can be described as a number of arabinogalactan units link to a polypeptide chain. Recently, GA has been used to functionalize and stabilize nanoparticles. GA molecules contain charged groups (amine and carboxyl) that can physically adsorb onto the surface of a nanoparticle (6,8). Through its highly branched polysaccharide structures, GA causes steric repulsion between nanoparticles to improve colloidal stability. Indeed, it has been demonstrated that GA-coated gold nanoparticles were stable in human serum albumin solution and strong ionic environments (6). GA has also enhanced nanoparticle stability *in vivo* (9). Furthermore, GA contains abundant carboxyl groups that can be easily activated and readily linked with other biomolecules (10). As an example, doxorubicin was conjugated to a GA-coated MNP via a pH-sensitive hydrazone bond (11).

In this work, we developed a facile method to prepare a GA-coated MNP (GA-MNP). Results showed that GA-MNP exhibits good stability in a physiologically simulated environment. In addition, we showed that GA can be readily conjugated with bioactive agents using the fluorophore rhodamine B as a model compound. *In vitro* studies revealed a significant cellular uptake of rhodamine-linked GA-MNP in 9L glioma cells. Preliminary *in vivo* investigations in rats bearing 9L glioma tumors showed, for the first time, that GA-MNP could potentially be used as a MRI-visible drug carrier, magnetically targetable to tumors.

MATERIALS AND METHODS

Materials

Unless otherwise stated, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (>99%), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (>99%), $\text{NH}_3 \cdot \text{H}_2\text{O}$ (NH_3 content, 28~30%), GA, 2-[*N*-morpholino] ethane sulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and all the other chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO). Reagents for cell culture were purchased from Gibco Invitrogen (Carlsbad, CA). Dialysis bags were obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Water was deionized (dH_2O) on a Milli-Q water purification system (Millipore, Billerica, MA). Commercial MNP products used for *in vitro* stability comparisons, including fluidMAG-D (starch coating), fluidMAG-CMX (carboxymethyl dextran coating), fluidMAG-Heparin (heparin coating), and fluidMAG-DEAE (dextran diethylaminoethyl coating) were provided by Chemicell® (Berlin, Germany). These MNP products all possess a hydrodynamic diameter of approximately 100 nm.

Preparation of GA-MNP

Magnetite (Fe_3O_4) MNP were synthesized using a previously reported co-precipitation method (12). In brief, a solution containing ferric chloride and ferrous chloride was

added dropwise to a 1.5 M NaOH solution under vigorous mechanical stirring and nitrogen gas protection at room temperature. The reaction temperature was gradually increased to 75°C and held for 1 h under stirring and nitrogen gas protection. The synthesized MNP product were separated using a magnetic separator and washed five times with dH_2O .

GA-MNP were prepared by adding 1 ml of the above MNP (10 mg Fe/ml) to 5 ml of 10% GA solution. The GA/MNP mixture was vortexed for 1 min and sonicated for 10 min to form a transparent colloidal solution. GA-MNP were then purified using a magnetic separator and washed five times with dH_2O .

Characterization of GA-MNP

Phase composition of lyophilized magnetic nanoparticles powder was analyzed with a Rigaku Rotoflex 200B 12KW rotating anode X-ray diffractometer (RIGAKU, Inc., Tokyo, Japan) equipped with Cu-K α radiation ($\lambda=1.54056 \text{ \AA}$). Morphology of GA-MNP was obtained on a JEOL 3010 high-resolution transmission electron microscope (HR-TEM, JEOL, Ltd., Tokyo, Japan). Samples were prepared by dropping diluted particle suspensions on copper grids coated with formvar film (Ted Pella, Inc., Redding, CA) followed by drying at room temperature. Hydrodynamic particle size was measured by photon correlation spectroscopy using a PSS Nicomp 380 ZLS particle sizing system (Nicomp, Inc., Santa Barbara, CA). Iron concentration of all MNP samples was determined by inductively coupled plasma optical emission spectroscopy using an Optima 2000 DV spectrometer (Perkin-Elmer, Inc., Boston, MA). Magnetization measurements were performed using an MPMS-XL superconducting quantum interference device (SQUID) magnetometer (Quantum Design Inc., San Diego, CA). To measure relative GA content, GA-MNP were lyophilized and analyzed by thermogravimetric analysis (TGA) using a TGA-7 instrument (Perkin-Elmer, Inc.).

GA-MNP Stability and Comparison with Other Commercial MNP Products

In vitro stability of GA-MNP and other MNP products was assessed in a physiological simulated buffer of Dulbecco's phosphate-buffered saline (PBS, with 0.901 mM Ca^{2+} and 0.493 mM Mg^{2+}) containing 10% fetal bovine serum (FBS). Briefly, MNP samples were added to the above solution and shaken to homogeneity (final iron concentration, 50 $\mu\text{g/ml}$). Immediately afterwards, aliquots containing 0.2 ml of the sample were loaded onto a 96-well plate, and the turbidity in the wells was measured at 400 nm using a micro-plate reader (Powerwave X340, BioTek Instruments, Inc., Winooski, VT) at 5-min intervals for 1 h (13). In addition, 3 ml of each test sample was added to a 20-ml glass scintillation vial for visual observation up through 24 h.

Cell Viability Evaluation

Rat 9L glioma cells (Brain Tumor Research Center, University of California, San Francisco) were cultured at 37°C under a humidified atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 IU/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, and

0.29 mg of L-glutamine. The cells were seeded on a 96-well plate at $\sim 10^4$ cells/well and incubated with fresh media for 24 h. Following incubation, the culture media were replaced with fresh media containing GA-MNP at various concentrations (0.01–20 mg/ml), and cells were further incubated at 37°C for 4 h. The GA-MNP solution was then replaced with fresh media, and cell viability was measured after 24 h using a standard (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (10).

Cellular Uptake of GA-MNP

RhB-GA was prepared by conjugating rhodamine B (RhB) with GA through the formation of an amide bond as previously described (10). Briefly, the GA solution was prepared by dissolving 2 g GA in 20 ml 0.1 M MES, whereas a RhB solution was prepared by dissolving 40 mg rhodamine B in 1 ml 0.1 M MES. The two solutions were then mixed, followed by the addition of 25 mg/ml EDC solution. The mixture was stirred for 2 h at room temperature. The RhB-GA conjugates were then purified by dialysis using dH₂O for 3 days. The RhB-GA-MNP product were then prepared analogously to GA-MNP as described above.

To carry out cell uptake studies, rat 9L glioma cells were cultured in a 12-well plate and incubated with 80 μ l of either GA-MNP or the RhB-GA-MNP (5 mg Fe per milliliter) in serum-free DMEM or DMEM with 10% FBS (120 μ l) at 37°C for 2 h. After incubation, the cells were washed five times with PBS and subjected to fluorescence and *in vitro* MR imaging.

To quantitatively compare the degree of cellular uptake between GA-MNP and a commercial starch-coated MNP (i.e., fluidMAG-D), rat 9L glioma cells were cultured in a 12-well plate and incubated with the two different MNP samples (at the same iron concentration ranging from 0.02 to 2.0 mg Fe per milliliter) in 1 ml DMEM containing 10% FBS at 37°C for 2 h. Following incubation, the cells were washed five times with PBS, and cellular iron was measured by ESR spectroscopy according to a previously established protocol (2). ESR spectra of samples were acquired using an EMX ESR spectrometer (Bruker Instruments Inc., Billerica, MA). Cells cultured without MNP were used as a control.

In Vivo Study

All animal experiments were conducted according to protocols approved by the University of Michigan Committee on Use and Care of Animals.

9L glioma cells were grown to confluency, harvested, and resuspended in serum-free DMEM at a concentration of $\sim 10^4$ cells per microliter. Ten microliters of cell suspension was implanted in the right forebrains of Fisher 344 rats (body weight, 200 g) at a depth of 3–4 mm beneath the skull through a 1-mm diameter burr hole. The surgical field was cleaned with 70% ethanol, and the burr hole was sealed with bone wax (Ethicon Inc., Summerfield, NJ) to prevent extracerebral extension of the tumor (13,14).

MRI experiments were performed using a 12-cm horizontal bore, 7 Tesla Varian Unity Inova imaging system (Varian, PaloAlto, CA) according to a previously established procedure (2). Animals were anesthetized with 1.5% isoflur-

ane/air mixture and imaged using a 35-mm diameter quadrature RF head coil (USA Instruments Inc., OH). MRI of the rat brain was initiated 10 days after tumor cell implantation. Axial sections of the rat brain were acquired with a T₂-weighted fast spin echo sequence using the following parameters: repetition time (TR)=4 s, echo time (TE) = 60 ms, field of view = 30×30 over 128×128 matrix, slice thickness = 1 mm, slice separation = 1.5 mm, four signal averages per phase encoding step. T₂-weighted images were inspected to determine which slice corresponded to the best cross-sectional visualization of the tumor. A single gradient echo (GE) scan was acquired at this optimized position to provide qualitative information on GA-MNP accumulation at the tumor site. GE images were acquired with the following parameters: TR=275.13 ms, TE=15 ms, field of view = 30×30 over 128×128 matrix, slice thickness = 1 mm.

For magnetic targeting, animals were anesthetized through inhalation of a 1.5% isoflurane/air mixture. Rats were then placed ventrally on a platform with the head positioned between the poles of an electromagnet. GA-MNP (in PBS) were injected at a dose of 12 mg Fe per kilogram through the tail vein and retained in the magnetic field for 30 min. Animals dosed with GA-MNP, but not subject to magnetic targeting, were used as controls. Animals were imaged with MRI before the administration of GA-MNP and after magnetic targeting (30 min after GA-MNP administration). To quantitatively examine the accumulation of GA-MNP in the tumor and normal brain tissue, animals were killed immediately following targeting. Brain tumors were carefully dissected from the right hemisphere. Tumor and left hemisphere tissues were analyzed by ESR as previously described (2).

RESULTS AND DISCUSSION

GA-MNP Characterization

Figure 1 showed the X-ray diffraction (XRD) pattern of GA-MNP with characteristic peaks of 2θ at 30.1°, 35.4°, 43.0°, 53.5°, 56.9°, and 62.6°, corresponding to the indices (220), (311), (400), (422), (511), and (440), respectively, of the magnetite crystal. In agreement with the results reported in

Fig. 1. X-ray diffraction pattern of GA-MNP lyophilized powder. The XRD pattern confirmed that GA-MNP contained a magnetite core

the literature, XRD analysis confirmed that GA-MNP were composed of pure magnetite with spinal crystal structure (16).

The morphology of GA-MNP was examined using transmission electron microscopy (TEM). TEM image in Fig. 2 visually showed the core-shell structure of GA-MNP. The cores consisted of a number of spherical magnetite nanocrystals with an average size of 14 ± 3.8 nm coated with a shell of GA polymer. The multi-nanocrystal cores were formed probably because the GA molecules, much larger than the nanocrystals, could adsorb onto several nanocrystals through their carboxyl groups and hold them together. The relative GA content of GA-MNP by mass was found to be 15.6% by TGA.

For *in vivo* applications of magnetic nanoparticles, such as magnetic targeting and MRI, superparamagnetic behavior is of paramount importance. Superparamagnetic particles do not remain magnetized in the absence of an external magnetic field. Thus, they do not agglomerate without exposure to a field. MNP only exhibit superparamagnetic behavior, though, below a certain size threshold, namely the size of a single magnetic domain. The domain size for GA-MNP has been determined to be below the apparent limit of 25 nm (17). Indeed, negligible hysteresis was observed in magnetization experiments (Fig. 3), confirming that GA-MNP possessed superparamagnetic behavior. Overall, the synthesized GA-MNP showed negligible coercivity (H_c) and remnant magnetization (M_r), as well as a saturation magnetization value of 93.1 emu/g Fe. These findings were all comparable to those of the commercial products obtained from Chemicell® (94 emu/g Fe) (15). Saturation magnetization was slightly lower than that of the bulk magnetite (127 emu/g Fe), probably due to the fact that the surface nonmagnetic layer (also known as “dead layer”) of GA-MNP accounted for a higher composition fraction when the magnetite crystal size was reduced to nanosize (18). On the other hand, the saturation magnetization of GA-MNP was greater than that of the FDA-approved contrast agent Feridex® (70 emu/g Fe), possibly attributed to the smaller magnetite crystal size of Feridex® (~4.8 nm) (19). It has been demonstrated that larger magnetite crystals yield stronger saturation magnetization (20).

Fig. 2. TEM image of GA-coated MNP (scale bar, 100 nm). The image showed the core-shell structure of GA-MNP. The cores consisted of magnetite nanocrystals coated by a GA polymer shell (indicated by arrows)

Fig. 3. Magnetization of GA-MNP measured by SQUID. The figure showed the induced magnetization of GA-MNP corresponding to both increasing and decreasing external magnetic field. The lack of hysteresis loop confirmed that the prepared GA-MNP possessed a superparamagnetic behavior

Particle size measurements (Fig. 4) showed that the synthesized GA-MNP possessed a mean hydrodynamic diameter of 118 ± 12 nm in PBS buffer. It is important to note that the hydrodynamic size of MNP is a key factor in governing the success of magnetic targeting and reduced *in vivo* clearance. It has been reported that attractive magnetic forces on MNP smaller than 50 nm are not sufficient to overcome forces from Brownian motion, resulting in poor MNP accumulation at the target site when subject to magnetic targeting (21). Thus, from a targeting standpoint, MNP with large core structures and hydrodynamic sizes are preferred. MNP over a certain size (e.g., >300 nm), however, are rapidly cleared from the body via the RES, resulting in a significantly shortened circulation half-life (22). Moreover, the size cutoff of tumor vasculature permeation is on the order of several hundred nanometers (1). Larger MNP and aggregates may not be able to extravasate into the interstitial space of the tumor, resulting in minimal tumor accumulation. In our previous studies, we demonstrated that starch-coated MNP with a mean size of 100 nm were capable of extravasation and accumulation in tumor with the aid of magnetic targeting (2). It is of little doubt that a 100 nm GA-MNP would yield similar results. Further studies, though, are needed to validate this assumption.

Fig. 4. Hydrodynamic particle size of GA-MNP in PBS buffer. GA-MNP showed a narrow particle size distribution with mean of 118 ± 12 nm. The observed size was similar to commercial MNP products tested

Stability Evaluation and Comparison

MNP are typically administrated intravenously. Therefore, stability prior to, during, and after administration is of great importance in determining the fate of MNP. Among various methods, turbidity is a sensitive and straightforward means to monitor the stability of a colloidal suspension (4). Through this method, we discovered that all MNP samples investigated, including our GA-MNP product, remained stable in water for a period of several months. When mixed with physiologically simulated buffer, however, fluidMAG-CMX, fluidMAG-Heparin, and fluidMAG-DEAE samples became turbid immediately after suspension, suggesting rapid agglomeration (Fig. 5). In contrast, the turbidity of GA-MNP and fluidMAG-D samples remained unchanged over the course of 1 h. Indeed, GA-MNP suspension was clear even after 24 h of storage. The good stability of the GA-coated MNP could be due to dynamic motion of the highly branched polysaccharide chains in GA that promote strong steric resistance between individual particles.

Cellular Uptake of GA-MNP

MNP and GA have been shown to be nontoxic and biocompatible by other researchers (6,23). Consistent with their findings, our MTT assay results indicated that GA-MNP were not cytotoxic to 9L glioma cells in DMEM with and without FBS even at an iron concentration as high as 20 mg Fe per milliliter (data not shown). Fluorescence microscopy demonstrated internalization of RhB-GA-MNP into 9L glioma cells 2 h after incubation (Fig. 6a). It is speculated that cellular uptake is attributed to endocytosis due to the small size of GA-MNP, as observed by other investigators studying other nanoparticles (24). No fluorescence signal was found inside the nucleus, suggesting that RhB-GA-MNP remains in the cytosol or endosome. This result was somewhat anticipated because MNP of ~ 100 nm would have difficulty penetrating through the narrow channels of nuclear pores (diameter <40 nm) (10).

Consistent with results reported in the literature, *in vitro* GE MR images further confirmed cellular uptake of GA-

Fig. 5. Physiologically simulated stability of GA-MNP and several commercial MNP products including: heparin- (*Heparine*); GA- (*GAM*); DEAE- (*DEAE*); CMDX- (*CMDX*); and starch-coated (*Starch*) MNP in PBS (containing 10% FBS) medium. Turbidity changes were measured as a function of time

Fig. 6. a Fluorescence microscopy of cellular uptake of RhB-GA-MNP (scale bar, 10 μ m). b *In vitro* gradient echo MR images of 9L glioma cells, from top: 1 control cells, 2 cells treated by GA-MNP in DMEM with 10% FBS, and 3 cells treated by GA-MNP in DMEM without serum. c Gradient echo MR images of GA-MNP at iron concentrations of 0, 5, 10, 20, and 50 ppm (from top to bottom)

MNP (19). Pronounced hypointensity was observed in cells treated with GA-MNP when compared to the control, indicating the presence of GA-MNP inside the cells (see Fig. 6b). Furthermore, Fig. 6c revealed an iron concentration-dependent drop in signal intensity on GE images for GA-MNP, implicating higher cellular uptake of GA-MNP at higher concentrations.

Quantitative measurements of cellular uptake showed that both GA-MNP and starch-MNP exhibit a concentration-dependent uptake of nanoparticles by 9L glioma cells (Fig. 7). The GA-MNP product, however, consistently displayed a two to threefold higher degree of uptake than starch-MNP when compared at the same iron concentrations. This result suggested that a GA-coated surface might possess a higher affinity toward tumor cells than that of starch.

Brain Tumor Targeting and Imaging

Similar to findings recently reported by our laboratory (2), GE MR images qualitatively revealed accumulation GA-MNP in the brains of animals subjected to magnetic targeting.

Fig. 7. Cellular uptake of GA-MNP and starch-MNP by 9L glioma cells after 2 h of incubation ($n=3$). *At all concentrations tested (from 0.02 to 2 mg/ml), cellular uptake of GA-MNP was higher than starch-MNP (unpaired t test, $p<0.05$)

Fig. 8. *In vivo* magnetic targeting of 9L glioma bearing rats. **a–c** Representative MR images of the targeted animals. **d–f** Representative MR images of the non-targeted animals. The arrows in **a–f** showed the position of tumors. **g** GA-MNP content in excised brain tumors and normal brain tissues of targeted ($n=4$) and non-targeted rats ($n=3$) in the ESR studies. *The plot showed a statistically significant difference comparing the targeted tumors to the targeted normal brain tissues and non-targeted tumors and normal brain tissues (unpaired t test, $p<0.001$)

As shown in Fig. 8a, tumor was clearly visible on a T₂-weighted image. When compared with the GE baseline image shown in Fig. 8b, the pronounced hypointensity of the tumor observed in Fig. 8c clearly demonstrated the accumulation of GA-MNP at the tumor site. In contrast, no detectable hypointensity was found in other parts of the brain. Similar MRI studies were also conducted on brain tumor-harboring animals not subjected to magnetic targeting, as shown in Fig. 8d–f; no obvious signal change was observed in the GE image (Fig. 8f). The MR images qualitatively proved that magnetic targeting improved brain tumor accumulation.

Quantitative tissue analysis by ESR further confirmed the selective accumulation of GA-MNP in tumor after magnetic targeting. For targeted animals (Fig. 8g), GA-MNP accumulation in the tumor tissue (63.8 ± 14.6 nmol Fe per gram tissue, $n=4$) was 12-fold higher than that found in the contralateral brain tissue (5.3 ± 3.5 nmol Fe per gram tissue, $n=4$). For animals not targeted, GA-MNP accumulation in tumors (8.4 ± 5 nmol Fe per gram tissue, $n=3$) was threefold higher than that found in the contralateral brain (2.9 ± 2.1 nmol Fe per gram tissue, $n=3$). Results from the control group indicated that the enhanced permeability of the tumor vasculature alone resulted in nanoparticle accumulation in tumors. With the application of the external magnetic field, though, tumor levels of MNP increased eightfold, suggesting that magnetic targeting further enhanced nanoparticle accumulation. Based on our recent findings (15), enhanced tumor selectivity might be related to pathological alteration of blood flow dynamics in the brain tumor. For instance, the linear blood flow rate (0.31 cm/s) in normal brain vessels is estimated to be about fourfold higher than that (0.074 cm/s) in brain tumors (15). A slower flow rate gives GA-MNP longer residence time in the microvasculature of the tumor. Furthermore, attraction of GA-MNP to an external magnetic field further enhances retention and accumulation of GA-MNP at the tumor. Overall, the selectivity of GA-MNP to the tumor confirms its *in vivo* stability. Since GA-MNP were able to extravasate the tumor, as evidenced visually by the clear hypointense MR images, it appeared that GA-MNP did not aggregate during *in vivo* application.

CONCLUSIONS

MNP were successfully stabilized and functionalized with nontoxic, phytochemical GA polymer via a facile procedure. Results showed that GA-MNP product possessed several major advantages over existing commercial MNP products: (1) improved stability under physiological conditions; (2) reactive functional groups for easy linking with biofunctional compounds or other molecules to improve their performance; and (3) a high level of cellular uptake by tumor cells. *In vivo* magnetic targeting studies revealed that GA-MNP accumulated in the brain tumor 12-fold higher than the normal brain. The observed selectivity in tumors was visibly monitored by MRI. In conclusion, GA-MNP showed great promise as a tool for achieving simultaneous imaging of and magnetically targeted drug delivery to virtually all types of solid tumors, including the brain model presented. To that end, further animal investigations are currently underway in our laboratory.

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