Influence of Physicochemical Properties and PEG Modification of Magnetic Liposomes on Their Interaction with Intestinal Epithelial Caco-2 Cells

Yusuke Kono,*a,b Hitomi Jinzai,a Yota Kotera,a and Takuya Fujitaa,b,c

a Laboratory of Molecular Pharmacokinetics, College of Pharmaceutical Sciences, Ritsumeikan University; 1–1–1 Noji-Higashi, Kusatsu, Shiga 525–8577, Japan; b Ritsumeikan-Global Innovation Research Organization, Ritsumeikan University; 1–1–1 Noji-Higashi, Kusatsu, Shiga 525–8577, Japan; and c Research Center for Drug Discovery and Development, Ritsumeikan University; 1–1–1 Noji-Higashi, Kusatsu, Shiga 525–8577, Japan.

Received July 11, 2017; accepted September 20, 2017; advance publication released online September 30, 2017

The present study aimed to investigate the effect of particle size (100, 500 nm), surface charge (cationic, neutral and anionic) and polyethylene glycol (PEG) modification of magnetic liposomes on their interaction with the human intestinal epithelial cell line, Caco-2. The cellular associated amount of all the magnetic liposomes was significantly increased by the presence of a magnetic field. The highest association and internalization into Caco-2 cells was observed with magnetic cationic liposomes. Moreover, small magnetic liposomes were more efficiently associated and taken up into the cells, than large ones. In contrast, PEG modification significantly attenuated the enhancing effect of the magnetic field on the cellular association of magnetic liposomes. We also found that magnetic cationic liposomes had the highest retention properties to Caco-2 cells. Moreover, the retention of large magnetic liposomes to the cells was much longer than that of small ones. In addition, magnetic cationic and neutral liposomes had relatively high stability in Caco-2 cells, whereas magnetic anionic liposomes rapidly degraded. These results indicate that the physicochemical properties and PEG modification of magnetic liposomes greatly influences their intestinal epithelial transport.

Key words magnetic liposome; particle size; surface charge; polyethylene glycol; Caco-2 cell

External stimuli, such as magnetic field, ultrasound, electrical stimulation, and mechanical pressure, have been widely used for site-specific drug delivery. These stimulation-based drug delivery systems are combinatorial methods of systemic or local administration of stimulus-responsive nano or microparticulate drug carriers with specific exposure of the external stimulus to the targeted region. External stimuli provide conventional passive drug carriers with several benefits, such as selective and efficient accumulation and retention at the targeted site, and spatiotemporally controlled release of the loaded drug or gene.

Among these external stimuli, the magnetic field is considered the most promising external stimulus for targeted drug delivery. An external magnetic field permits the drug-loaded magnetic nanoparticles not only to be guided to the exposed site, but also to be retained there. In addition, the magnetic field has been reported to enhance the binding of magnetic nanoparticles on cell surface, resulting in the increase of their cellular uptake in the targeted region. These effects of an external magnetic field contribute to both increasing the therapeutic efficacy of drugs loaded on magnetic nanoparticles and diminishing the occurrence of adverse effects by them. However, it is necessary to provide magnetic nanoparticles with several properties, including monodispersity, stability, biocompatibility, safety and drug loading capacity, to exploit magnetic nanoparticles as a drug carrier. Many research groups have succeeded in adding these properties to superparamagnetic iron oxide nanoparticles (SPIONs) by direct functionalization, such as surface modification, addition of biocompatible polymers, and covalent linkage of targeting molecules or therapeutics to SPIONs. However, liposomalization of SPIONs is also a promising approach for adding these properties to SPIONs. Liposomes possess high monodispersity, biocompatibility and stability. In addition, it is easy to control their physicochemical properties, such as particle size and surface charge, and to modify their surface with polymers or targeting molecules. Furthermore, liposomes are capable of entrapping various therapeutic agents, including not only hydrophilic and hydrophobic drugs, but also proteins and genes.

To achieve efficient magnetic drug targeting by SPION-incorporated liposomes (magnetic liposomes), it is important to understand the intracellular transport properties of magnetic liposomes as well as their biodistribution characteristics. Similarly to conventional nanoparticulate drug carriers, cellular internalization of magnetic nanoparticles has been reported to be dominantly mediated by endocytosis even under a magnetic field, without accompanying cellular poration like other external stimulation-based drug targeting systems including ultrasound and electrical stimulation. Therefore, it appears that the physicochemical properties of magnetic liposomes greatly affect their transport behaviour into cells and their subsequent fate.

In addition to the physicochemical properties, surface chemistry is considered as an important determinant of the cellular uptake efficiency of magnetic liposomes. Polyethylene glycol (PEG) is one of the most widely used polymers for surface coating of nanoparticulate drug carriers. PEG provides nanoparticles with several advantages, such as water solubility, increased stability, resistance to enzymatic degradation and opsonization. However, PEGylation of nanoparticles has been reported to reduce the uptake efficiency of nanoparticles into cells because of steric hindrance, assuming that PEG modification strongly influences the cellular internalization process of magnetic liposomes.
In the present study, we evaluated the effects of particle size, surface charge and PEG modification of magnetic liposomes on their interaction with Caco-2 human intestinal epithelial cells, including cellular uptake, retention and stability. In addition to the physicochemical properties, we investigated the influence of surface modification of magnetic liposomes with PEG.

**MATERIALS AND METHODS**

**Cell Line** Caco-2 human epithelial colorectal adenocarcinoma cells were purchased from DS Pharm Biomedical Co., Ltd. (Osaka, Japan). Caco-2 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum, penicillin G (100U/mL) and streptomycin (100µg/mL) at 37°C in 5% CO2/95% air.

**Preparation of Magnetic Liposomes** Magnetic cationic, neutral and anionic liposomes were composed of 1,2-distearoyl-3-trimethylammonium propane (DSTAP) (Avanti Polar Lipids Inc., Alabaster, AL, U.S.A.): cholesterol=1:1 (mol), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) (Avanti Polar Lipids): cholesterol=1:1 (mol), and 1,2-distearoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DSPG) (Avanti Polar Lipids): cholesterol=1:1 (mol), respectively. For the preparation of PEGylated magnetic liposomes, 5mol% N-(carboxyl-methoxy PEG2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (NOF Co., Tokyo, Japan) was added to magnetic neutral liposomes. Magnetic cationic, neutral and PEGylated magnetic liposomes were labelled with 1% (V-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Thermo Fisher Scientific K.K., Kanagawa, Japan). Magnetic anionic liposomes were labelled with DiIC18(3) (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The lipids were mixed in chloroform, and the mixture was dried on a rotary evaporator. After vacuum desiccation, each large magnetic liposome was obtained by hydration of the resultant lipid film with a sterile 5% glucose solution containing 0.1mg/mL iron oxide(II, III) magnetic nanoparticles (Sigma-Aldrich, St. Louis, MO, U.S.A.) for 30 min at 70°C under mechanical agitation. Small magnetic liposomes were produced by sonication of each large magnetic liposome in a bath sonicator for 15 min, followed by a probe-type sonicator for 3 min. Non-encapsulated magnetic nanoparticles were removed by centrifugal filtration using Amicon® Ultra Centrifugal Filters (MWCO: 100000; Merck Millipore, Tokyo, Japan). The particle sizes and z-potentials of magnetic liposomes were measured using the Zetasizer Nano ZS Instrument (Malvern Instrument, Worcestershire, U.K.). The magnetic nanoparticle content in magnetic liposomes was measured with hexacyanoferrate (II), according to the method reported by Dandamudi and Campbell.28

**Cell Viability Assay** Caco-2 cells were plated in 24-well culture plates at a density of 5×10² cells/cm², and cultured for 14d. The cultured cells were placed on a magnetic plate (OZ Sciences, Ibaraki, Japan). The results are expressed as viability (%).

**Preparation of Magnetic Liposomes**

**RESULTS**

**Evaluation of the Retention of Magnetic Liposomes to Caco-2 Cells** Caco-2 cells were seeded in 24-well Transwell® plates with 0.4-µm Pore Polycarbonate Membrane inserts (Costar, Bedford, MA, U.S.A.) at a density of 5×10⁴ cells/cm², and cultured for 14d. The culture media in the apical and basolateral sides of the inserts were replaced with HBSS. Then, 20µg lipid of magnetic liposomes were added to the apical side, and incubated for 1h under a magnetic field. After the culture media in both sides were replaced with fresh DMEM containing 10% fetal bovine serum (FBS), the cells were collected at predetermined time points, and the fluorescence intensity of each sample was measured.

**Stability of Magnetic Liposomes in Caco-2 Cells**

Caco-2 cells cultured in 100-nm culture dishes for 14d were collected in HBSS, and homogenized. The resultant homogenate (final concentration of 1mg/mL protein) was incubated with 10µg lipid of magnetic liposomes for 30 min at 37°C. The mixture was loaded onto a Sephadex G-25 column (PD-10, GE Healthcare, Buckinghamshire, U.K.), and eluted with HBSS (pH 7.4), followed by fractional collection. The fluorescence intensity of the collected fractions was measured.

**Statistical Analysis** Results are presented as the mean±standard deviation (S.D.) of four experiments. ANOVA was used to test the statistical significance of differences between groups. Two-group comparisons were performed with Student’s t-test. Multiple comparisons among control groups and other groups were performed with Dunnett’s test.

**Effect of Particle Size and Surface Charge of Magnetic Liposomes on Their Association and/or Uptake by Caco-2 Cells** In this study, we prepared several magnetic liposomes with two different particle sizes (small and large) and three different surface charges (cationic, neutral and anionic; Table 1). Since we have confirmed that the absolute value of the zeta potential of charged liposomes is increased as the composition ratio of charged lipid is increased, here we prepared magnetic charged (cationic and anionic) liposomes by mixing charged lipid and cholesterol at a molar ratio of 1:1 to clearly evaluate the effect of the surface charge. The particle size of the small and large magnetic liposomes was approximately 100 and 500 nm, respectively. The zeta potential of the magnetic cationic, neutral and anionic liposomes was approximately 60, 0 and −50mV, respectively. The encapsulation efficacy of iron oxide magnetic nanoparticles into liposomes was approximately 15%, regardless of the physicochemical properties or magnetic field. Then, the cells were washed twice with ice-cold HBSS and lysed with lysis buffer (0.05% Triton X-100, 2mM ethylenediaminetetraacetic acid (EDTA), 0.1m Tris, pH 7.8). After centrifugation at 10000×g for 10min at 4°C, the fluorescence intensity of the supernatant was measured using an SH-8100 microplate reader (Corona Electric Co., Ltd., Ibaraki, Japan).
Table 1. Particle Sizes and $\zeta$-Potentials of Magnetic Liposomes

<table>
<thead>
<tr>
<th></th>
<th>Small</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (nm)</td>
<td>$101.8 \pm 4.1$</td>
<td>$510.3 \pm 4.5$</td>
</tr>
<tr>
<td>$\zeta$-Potential (mV)</td>
<td>$56.3 \pm 2.8$</td>
<td>$54.0 \pm 5.9$</td>
</tr>
<tr>
<td>Cationic liposomes</td>
<td>$99.4 \pm 3.1$</td>
<td>$506.7 \pm 9.3$</td>
</tr>
<tr>
<td>Neutral liposomes</td>
<td>$-3.8 \pm 4.9$</td>
<td>$3.8 \pm 2.3$</td>
</tr>
<tr>
<td>Anionic liposomes</td>
<td>$99.6 \pm 4.6$</td>
<td>$489.2 \pm 10.2$</td>
</tr>
<tr>
<td>$-54.8 \pm 5.4$</td>
<td>$-48.8 \pm 4.7$</td>
<td></td>
</tr>
<tr>
<td>PEGylated liposomes</td>
<td>$102.2 \pm 2.6$</td>
<td>$497.3 \pm 17.2$</td>
</tr>
<tr>
<td>$-5.3 \pm 6.0$</td>
<td>$-4.6 \pm 2.9$</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. ($n=4$).

Fig. 1. Cell Viability of Caco-2 Cells Incubated with Magnetic Liposomes

Twenty micrograms lipid of small (A) or large (B) magnetic cationic liposomes, small (C) or large (D) magnetic neutral liposomes, and small (E) or large (F) magnetic anionic liposomes were incubated for 60 min at 37°C with or without a magnetic field. Cell viability was measured by the WST-8 assay. Each value represents the mean±S.D. ($n=4$).
PEG modification of the liposomes.

Since previous reports performed cellular association and uptake studies of liposomes at a liposome concentration of 10–40 µg lipid/mL, we used 20 µg lipid/mL of magnetic liposomes (as approximately 120 ng/mL of incorporated magnetic nanoparticles) for cytotoxicity and cellular association and uptake study. Prior to evaluating the cellular association of the magnetic liposomes, we assessed the cytotoxic effect of magnetic liposomes on Caco-2 cells. All the magnetic liposomes showed no cytotoxicity with or without a magnetic field (Fig. 1). Then, we investigated the effect of the physicochemical properties of the magnetic liposomes on their association and internalization into Caco-2 cells. As shown in Fig. 2, the associated and/or taken up amount of magnetic liposomes by Caco-2 cells was significantly increased by the presence of a magnetic field, regardless of the particle size and surface charge. When comparing surface charge, the highest cellular association was observed with magnetic cationic liposomes. With regard to particle size, the cellular associated and/or taken up amount of small magnetic liposomes was relatively higher than that of large ones.

We also evaluated the cellular association of magnetic liposomes to Caco-2 cells at 4°C; at this temperature there was a significant reduction in the energy-dependent cellular uptake. The cellular association of all the magnetic liposomes was significantly lower at 4°C than that at 37°C (Fig. 3). In particular, the highest quantitative change in cellular association between 4 and 37°C was observed with magnetic cationic liposomes.

**Effect of Surface Modification of Magnetic Liposomes with PEG Molecules on Their Association and/or Uptake by Caco-2 Cells**

We also evaluated the influence of PEG modification of magnetic liposomes on their cellular association and/or uptake by Caco-2 cells at 37°C. PEG modification of the liposomes significantly increased the cellular association and uptake. The highest cellular association was observed with magnetic cationic liposomes, followed by neutral and anionic liposomes (Fig. 4).

**Fig. 2. Cellular Association and/or Uptake of Magnetic Liposomes under Magnetic Field by Caco-2 Cells**

Twenty micrograms lipid of small (A) or large (B) magnetic cationic liposomes, small (C) or large (D) magnetic neutral liposomes, and small (E) or large (F) magnetic anionic liposomes were incubated for 10, 30 and 60 min at 37°C with or without a magnetic field. Each value represents the mean±S.D. (n=4). **p<0.01, compared with no magnet.
PEGylated magnetic liposomes were prepared by the addition of 5 mol% PEG<sub>2000</sub>-distearoylphosphatidylethanolamine (DSPE) to magnetic neutral liposomes. As shown in Figs. 4A and B, PEGylated magnetic liposomes did not show any cytotoxicity against Caco-2 cells. While the cellular association of PEGylated magnetic liposomes was slightly increased by the presence of a magnetic field (Figs. 4C, D), their increased ratio by a magnetic field was much lower than that of magnetic neutral liposomes shown in Figs. 2C and D.

**Retention Properties of Magnetic Liposomes in Caco-2 Cells** In addition to the cellular association properties of magnetic liposomes, we investigated their retention properties to Caco-2 cells. Approximately 55–70% of magnetic cationic liposomes were retained to Caco-2 cells for 24 h, whereas the retained ratio of magnetic neutral and anionic liposomes at 24 h was less than 45% (Fig. 5). With respect to particle size, the retained ratio of small magnetic liposomes was much lower even at 3 h than that of large magnetic liposomes.

**Stability of Magnetic Liposomes in Caco-2 Cell Homogenate** Similarly to the transport properties, liposome stability greatly affects its drug targeting efficiency and therapeutic effect. Therefore, we evaluated the magnetic liposomes’ stability in Caco-2 cell homogenate by gel filtration. When incubated with Caco-2 cell homogenate, the peaks corresponding to magnetic cationic or neutral liposomes shifted to the earlier fraction and these peak areas were slightly lower (Figs. 6A–D). In contrast, the peaks corresponding to magnetic anionic liposomes were dramatically diminished by incubation with the Caco-2 cell homogenate (Figs. 6E, F). A significant peak difference between small and large magnetic liposomes was not observed.

**Fig. 3. Energy-Dependent Uptake of Magnetic Liposomes by Caco-2 Cells**

Twenty micrograms lipid of small or large magnetic cationic, neutral or anionic liposomes were incubated for 60 min at 4 or 37°C with a magnetic field. Each value represents the mean±S.D. (n=4). *p<0.05; **p<0.01, compared with 37°C.

**Fig. 4. Effect of PEG Modification of Magnetic Liposomes on the Cell Viability of Caco-2 Cells and Cellular Association**

Cell viability of Caco-2 cells incubated with small (A) or large (B) PEGylated magnetic liposomes. PEGylated magnetic liposomes (20 µg lipid) were incubated for 60 min at 37°C with or without a magnetic field. Cellular associated and/or uptake amount of small (C) or large (D) PEGylated magnetic liposomes were measured after incubation of 20 µg lipid of PEGylated magnetic liposomes with Caco-2 cells for 10, 30 and 60 min at 37°C. Each value represents the mean±S.D. (n=4).
has been reported to be quite low. Therefore, we evaluated the transcytosis efficacy of nanoparticles in epithelial cells, rather than by the transcytosis with liposomes, because from liposomes, which bind on cell surface or are taken up by the basolateral side at intestine would occur by their diffusion port of drugs encapsulated in liposomes from the apical side to the cell. We also observed that the cellular association of magnetic liposomes was much higher than that of magnetic neutral or anionic liposomes. This concentration is sufficiently low with respect to the loading efficacy of magnetic nanoparticles in liposomes. We confirmed that the cellular association of magnetic liposomes was much higher than that of magnetic neutral or anionic liposomes. This is consistent with another report demonstrating that HeLa cells endocytose positively charged liposomes to a greater extent than neutral or negatively charged liposomes. With regard to particle size, cellular association of small magnetic liposomes was relatively higher than that of large ones (Fig. 2). Moreover, the quantitative change in cellular association of small magnetic liposomes between 4°C and 37°C was more significant than that of large ones (Fig. 3). It has been reported that smaller nanoparticles undergo endocytic uptake by cells more easily and rapidly than larger ones. These previous observations are in accordance with our present results. 

Along with the physicochemical properties, we investigated the effect of surface modification of magnetic liposomes with PEG molecules on their cellular association. Our results demonstrated that PEG modification significantly reduced the interaction of magnetic liposomes with Caco-2 cells (Fig. 4). This observation is similar to previous reports, showing that the cellular uptake of SPIONs was reduced by PEG modification. Furthermore, the enhancement of cellular association of magnetic liposomes by a magnetic field was significantly attenuated by PEG modification (Fig. 4). This

**DISCUSSION**

Magnetic nanoparticles have been successfully applied to disease diagnosis and treatment, and much effort has been made to achieve efficient magnetic drug targeting in the field of cancer therapy. This magnetic nanoparticle-based drug delivery system is also expected to improve the bioavailability of oral drugs. However, there are few reports on the potential of magnetic nanoparticles as an oral drug carrier. In this study, we used Caco-2 cells, generally utilized as a standard model for the evaluation of intestinal drug absorption, and conducted a systematic study on the role of physicochemical properties and PEG modification of magnetic liposomes in their intestinal epithelial transport characteristics.

As it has been reported that excess accumulation of magnetic nanoparticles in cells induces cell death via oxidative stress, we first evaluated the cytotoxicity of magnetic liposomes against Caco-2 cells. Our results showed that the magnetic liposomes tested had no significant cytotoxic effect on Caco-2 cells (Fig. 1). We suppose that this is because of the low concentration of magnetic nanoparticles in liposomes. We confirmed that the loading efficacy of magnetic nanoparticles into each liposome used in this study is approximately 0.6% (w/w), hence Caco-2 cells were only exposed to approximately 120 ng/mL magnetic nanoparticles in all the present experiments. This concentration is sufficiently low with respect to cytotoxicity, compared with previous reports that demonstrated the cytotoxicity and mutagenicity of magnetic nanoparticles (10–50 µg/mL). We also confirmed that the magnetic nanoparticles used in this study exhibited no cytotoxicity against Caco-2 cells at concentrations up to 1 µg/mL (Fig. S1).

With regard to oral drug delivery by liposomes, the transport of drugs encapsulated in liposomes from the apical side to the basolateral side at intestine would occur by their diffusion from liposomes, which bind on cell surface or are taken up by cells, rather than by the transcytosis with liposomes, because the transcytosis efficacy of nanoparticles in epithelial cells has been reported to be quite low. Therefore, we evaluated the effect of particle size and surface charge of magnetic liposomes, focusing on their surface binding and uptake in Caco-2 cells under a magnetic field. The cellular associated amount of all the magnetic liposomes increased by 3–10 times by the presence of the magnetic field (Fig. 2). In particular, we observed that the cellular association of magnetic cationic liposomes was much higher than that of magnetic neutral or anionic liposomes. This concentration is sufficiently low with respect to the loading efficacy of magnetic nanoparticles in liposomes. With regard to particle size, cellular association of small magnetic liposomes was relatively higher than that of large ones (Fig. 2). Moreover, the quantitative change in cellular association of small magnetic liposomes between 4°C and 37°C was more significant than that of large ones (Fig. 3). It has been reported that smaller nanoparticles undergo endocytic uptake by cells more easily and rapidly than larger ones. These previous observations are in accordance with our present results.
inhibition of the interaction between the magnetic liposomes and Caco-2 cells by PEG modification could be attributed to the steric hindrance of the PEG molecules, which may also be responsible for eliminating the enhancing effect of the magnetic field on cellular association of magnetic liposomes. Taken together, PEG modification is not beneficial for surface functionalization of magnetic liposomes.

The therapeutic efficiency of liposomal drugs depends not only on how liposomes are efficiently taken up by the cells, but also on how long they are retained there. We found that magnetic cationic liposomes showed relatively higher retention to Caco-2 cells than magnetic neutral or anionic liposomes (Fig. 5). Bannunah et al. have demonstrated that positively charged nanoparticles tend to stay inside Caco-2 cells, whereas negatively charged nanoparticles are more efficiently exported from Caco-2 cells. Our results are similar to this observation. In addition, with respect to particle size, we observed that large magnetic liposomes were better retained in Caco-2 cells than small ones (Fig. 5). This may be because of the difference in surface curvature between small and large magnetic liposomes. Ghitescu and Bendayan have demonstrated that the higher surface curvature of smaller colloidal gold nanoparticles restricted protein adsorption on the surface of gold nanoparticles, resulting in large background areas without protein coverage. Based on this report, we assume that large magnetic liposomes with lower surface curvature are capable of binding the cell surface more tightly than small ones. In addition to the strength of the interaction between magnetic liposomes and the cell surface, we consider that exocytosis may contribute to the higher retention of large magnetic liposomes to Caco-2 cells. Hu et al. have shown that 60 or 180 nm silica nanoparticles were more efficiently excreted...
from HepG2 cells, than 600 nm nanoparticles. This observation strongly supports our present results. Taking the present findings into consideration, magnetic cationic liposomes, particularly large ones, are likely to be suitable for local and/or sustained drug delivery to the intestine.

In addition to the cellular uptake and retention properties, the stability of magnetic liposomes in the cells is one of the major determinants of their therapeutic efficacy. Our results showed that magnetic cationic and neutral liposomes were relatively stable in Caco-2 cells, whereas magnetic anionic liposomes were significantly degraded (Fig. 6). It has been reported that liposomes are enzymatically degraded by phospholipase (PLA), particularly PLA2, after in vivo administration. PLA2 cleaves the sn-2 position of phospholipids to generate 1-acyl-lysophospholipids and free fatty acids, resulting in induction of a liposome burst. This PLA2 activity greatly depends on physical properties of the liposomes, including lipid composition. The negative charges of the phospholipid have been reported to enhance PLA2 activity via electrostatic attraction. Considering these observations, it seems that the rapid degradation of the magnetic anionic liposomes was attributed to the activation of PLA2 in the Caco-2 cell homogenate.

In conclusion, we showed that the physicochemical properties of magnetic liposomes are important determinants of their interaction with Caco-2 cells. Magnetic cationic liposomes were efficiently taken up by Caco-2 cells and retained there, compared with magnetic neutral or anionic liposomes. Magnetic cationic liposomes, as well as magnetic neutral liposomes, also had high stability in the Caco-2 cell homogenate. With respect to particle size, large magnetic liposomes showed higher retention to Caco-2 cells than small ones. We also demonstrated that PEG modification of magnetic liposomes attenuated the magnetic field-induced enhancement of cellular association of magnetic liposomes. These findings provide useful information for the application of magnetic liposomes to oral drug delivery.

Acknowledgments This work was supported in part by a Grant from the Strategic Research Foundation Grant-in-Aided Project for Private Universities, and a Grant-in-Aid for Young Scientists (B) (Grant Number 17K15438) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Ritsumeikan Global Innovation Research Organization (R-GIRO) project at Ritsumeikan University. We thank Michal Bell, Ph.D., from Edanz Group for editing a draft of this manuscript.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES


