SPIO-Loaded Nanostructured Lipid Carriers for T2-weighted Magnetic Resonance Imaging of Hepatocarcinoma

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Research

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Abstract

Background

The development of sensitive and specific MRI contrast agents for early diagnosis of hepatocellular carcinoma is highly demanded. Here, a peptide A54 (sequence: AGKGTPSLETPP) functionalized superparamagnetic iron oxide nanoparticles (SPIONs)-based magnetic nanostructured lipid carrier(A54-MNLC)as a hepatoma targeting negative magnetic resonance imaging (MRI) contrast agent was developed.

Results

Firstly, the A54 peptide functionalized PEGylated SA (A54-PEG-SA) was synthesized by the coupling reaction between A54 and NH₂-PEG-SA. Then SPIONs-loaded MNLC(MNLC and A54-MNLC)were prepared by the solvent diffusion method. The A54-MNLC with a diameter of about 50 nm, and *in vitro* cell viability study revealed that the prepared A54-MNLC was cytocompatible in the given particles' concentration ranges. The cellular uptake results indicated that A54-MNLC was able to be uptaken specifically by human hepatoma cell line (Bel-7402 cells) and also can be used as efficient probe for targeted MR imaging of cancer cells *in vitro*. The *in vivo* MR imaging experiments using an orthotopic implantation model further validated the hepatoma-targeting ability of A54-MNLC with a more remarkable MR imaging contrast effect compared to MNLC.

Conclusions

These results demonstrate that the A54-MNLC is a promising nanoplatform as a targeted MR imaging contrast agent for the diagnosis of hepatocellular carcinoma.

Background

Liver cancer is the most common malignant tumor in the world, and it is also the third most common cause of cancer-related death worldwide, which plays a great threat to human health [1, 2]. Hepatocellular carcinoma (HCC) represents approximately 80–90% of all liver cancer cases [3]. In China, around 383000 people die of HCC every year, accounting 51% of all liver cancer-related deaths in the world [4]. Because the traditional diagnostic methods can only detect lesions at an advanced stage of the disease, many patients lose the best chance of surgical resection, resulting in significantly shortened patient survival and severely reduced quality of life. In recent years, with the advent of nanomedicine and the introduction of the concept of precision medicine, higher requirements have been placed on the diagnosis and treatment of tumors. Therefore, how to achieve the early diagnosis of small lesion of HCC and achieve timely and efficient treatment of tumors, and then improve the survival rate of HCC patients is of great significance.

Magnetic resonance imaging (MRI) can perform multi-parameter, multi-sequence imaging, has high soft tissue resolution and spatial resolution, and because of imaging by protons, there is no ionizing radiation [5, 6]. Therefore, MRI can provide multi-functional imaging modes and perform quantitative and semi-quantitative analysis of imaging, so it has emerged as one of the most accurate and promising non-invasive means in the diagnosis and evaluation of diseases. However, in the diagnosis of HCC, plain MRI scans often have great difficulty in accurately assessing the lesions, so enhanced scans are often required. There are two types of contrast agents commonly used in MRI-enhanced scanning, positive contrast in T1-weighted images and negative contrast in T2-weighted images, which are widely utilized and enable improvements in detection and characterization of HCC. Among them, the T2 contrast agent can shorten the T2 relaxation of the neighboring tissues, which results in a decrease in the signal intensity in MR images. Superparamagnetic iron oxide nanoparticles (SPIONs) are the most commonly used T2 contrast agent, which have been used clinically to detect HCC in patients [7, 8]. SPIONs possess unique properties with strong shortening effects under longitudinal relaxation and transverse relaxation pathways [9]. However, the naked SPIONs undergo non-specific interactions with the serum proteins and they tend to agglomerate *in vivo* when exposed to a physiological environment [10]. Moreover, the SPIONs are also rapidly eliminated from the circulation or circulated inside the body without any specific interaction with the organs or tissues [11]. These barriers significantly hinder its application in targeting imaging and dynamic MRI. Therefore, the development of a SPIONs-based drug delivery system to improve the stability, enhanced contrast imaging, and the tumor targeting is important for the early and precise diagnosis of HCC.

In recent years, nanostructured lipid carrier (NLC) is a potential drug delivery system with complex architecture under development, which are composed of a solid lipid and an oil phase confers them higher stability and drug loading capacity as compared to solid lipid nanoparticles [12–14]. Previous studies have demonstrated that the NLC-based drug delivery system can be served as a nanocarrier for biomedical imaging and treatment of tumor [15–18]. However, NLC in many previous studies lacked the targeting ability *in vivo*, making them nonspecifically uptake by phagocytic cells and reticuloendothelial system (RES) or easily opsonized as foreign intruders. Therefore, the development of a tumor-targeting capability NLC-based drug delivery system for diagnosis of tumor is highly demanded.

The homing peptides were extensively studied for active targeting with low molecular weight and noncytotoxicity. The peptide A54, screened from a phase-display random peptide library, has the highest affinity with the human hepatoma cell line, which can navigate the specific and improved accumulation of the drug loaded nanoparticles into the liver tumor position [19]. And many previous reports indicated that the nanocarriers modified with A54 peptide displayed higher specificity and cellular internalization to cells compared with those without A54 modification [20–23]. Thus, using hepatoma-targeting MRI contrast agent can provide a more useful information for evaluating hepatic tumor.

In this study, a hepatoma-targeting nanostructured lipid carrier (A54-NLC) was designed as an MRI probe to deliver magnetic SPIONs (A54-MNLC) for noninvasive diagnosis of hepatic tumor. The

physicochemical properties and evaluated its cytotoxicity on liver cells were investigated. Finally, *in vitro* and *in vivo* tumor targeting ability of A54-MNLC was also evaluated.

Materials And Methods

Materials

A54 was purchased from Guangzhou Sinoasis Pharmaceuticals Inc. (Guangzhou, China). Di-tert-butyl dicarbonate ((Boc)₂O), N-Hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbo-diimide (EDC) were purchased from Shanghai Medpep Co., Ltd. (Shanghai, China). SA was purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Superparamagnetic iron oxide (II, III) (SPION, 5 nm), octadecylamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), hoechst 33342 and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich Chemical. Amino-terminated polyethylene glycol (NH₂-PEG-NH₂, MW = 2000) was purchased from Yare Biotech, Inc. (Shanghai, China). Poloxamer 188 was from Shenyang Jiqi Pharmaceutical Co, Ltd. (Shenyang, China). Oleic acid was obtained from Hangzhou Shuanglin Chemical Industry Co. Ltd. (Hangzhou, China). Indocyanine green (ICG) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Octadecylamine (ODA, 95%) was purchased from Fluka, USA. All other chemicals were of analytical reagent grade.

The human hepatocellular carcinoma cell line (Bel-7402 cells), human hepatocellular cell line (HepG2 cells) and normal liver cell line (LO2) were purchased from the Institute of Biochemistry and Cell Biology of the China Academy of Sciences. These cells were cultured in DMEM medium containing 10% fetal bovine serum and penicillin/streptomycin (100 U·mL⁻¹, 100 U·mL⁻¹) at 37°C with a humidified atmosphere of 5% CO₂.

Female BALB/c nude mice (4–5 weeks, body weight: 18 ± 2 g) were purchased from the Shanghai Silaike Laboratory Animal Limited Liability Company. All the animal studies were in accordance with the guidelines of Ethical Committee of Zhejiang University. The experiment protocols were approved by the Committee for Animal Care of Zhejiang University.

Preparation of SPIONs loaded A54-NLC (A54-MNLC)

A54-PEG-SA was firstly synthesized according to the previous study [22]. A54-MNLC was prepared by emulsion-diffusion method. Briefly, 100 µl Fe₃O₄ dispersion was added into 4.7 mL Poloxamer 188 (0.1%) and then dispersed in DI water by a probe sonicator (Sonicator JY92-II DN, China) for 30 minutes to obtain a water dispersed magnetic nanoparticles. 100 µl of OA in ethanol solution (10 mg/mL) was further added and followed by ultrasound waterbath for 10 minutes (solution A). Subsequently, monostearin (32 mg) and A54-PEG-SA (3 mg) were dissolved in 2 mL ethanol solution at 70 °C (solution B), 200 µl of the mixture was then immediately injected into the solution A and followed by ultrasound waterbath for 10 minutes to yield the SPIONs loaded NLC nanoparticles. MNLC as a control was also fabricated using the same method as described above.

Characterization

The ^1H NMR spectra of the synthesized A54-PEG-SA was obtained by NMR spectrometer (AC-80; Bruker Biospin, Germany). The average particle size, polydispersity, and zeta potential of the MNLC, A54-MNLC were determined by dynamic laser scattering (DLS) measurement using a Zetasizer (3000HS, Malvern Instruments Ltd, UK). The morphology of the nanoparticles was examined by transmission electron microscopy (TEM, JEM-1200EX, JEOL, Ltd., Tokyo, Japan).

Magnetic properties of A54-MNLC

Saturated magnetic intensity was also performed to evaluate the magnetic properties of A54-MNLC. The solutions of free SPIO, MNLC and A54-MNLC were lyophilized, then the magnetic properties of the nanoparticles were measured by a vibrating sample magnetometer (PPMS, Quantum Design, MPMS-XL-5, CA, USA) at room temperature.

In addition, the relaxivity properties were performed using a 3.0 T MRI scanner (GE, Discovery MR 750, USA). The T2-weighted MR images of naked SPIONs, MNLC and A54-MNLC solutions with different Fe_3O_4 concentrations (0, 1, 10, 15, 25, 50, 100 $\mu\text{g/mL}$) were acquired. The R2 relaxation times of the free SPIONs, MNLC and A54-MNLC were measured with the following parameters: Field of View, 180×180 mm; TR, 2000 ms; TE, 12 ms; slice thickness, 3.0 mm; number of slices, 8.

In vitro cytotoxicity

In vitro cytotoxicities of MNLC and A54-MNLC were performed on LO2 and Bel-7402 cells by MTT assay. Briefly, LO2 cells and Bel-7402 cells were seeded in 96-well plates and cultured for 24 h. Then, the medium was replaced by MNLC and A54-MNLC solution with different concentrations. The cells were incubated for another 48 h afterwards. Then the cell viability of each groups was measured by MTT assay: 20 μL of MTT solution (5 mg/mL) was added to each well and the plates were cultured for an additional 4 h. Then, the medium containing MTT solution was removed and the insoluble formazan-containing crystal was dissolved after addition of DMSO, the absorbance was recorded at 570 nm using an automatic microplate reader (Bio-Rad, model 680, USA). The untreated cells were taken as a control group with 100% cell viability. The relative cell viability was measured by the absorbance values of the treatment group divided by the absorbance values of the control group. All experiments were performed in triplicate.

In vitro Cellular uptake

The cellular uptake studies of A54-MNLC and MNLC nanoparticles were conducted on Bel-7402 and LO2 cells. Fluorescence microscopy was used to observe the time dependent cellular uptake behavior, FITC was used as a fluorescent probe. ODA-FITC was synthesized according to our previous study [24]. The FITC labeled A54-MNLC and MNLC nanoparticles were fabricated by the method as mentioned above. LO2 and Bel-7402 cells were cultured on six-well plates at a density of 3×10^4 cells per well and incubated overnight afterwards. The FITC labeled A54-MNLC and MNLC nanoparticles were added and incubated

with cells for 1, 3, 6 and 12 h. To study the competitive inhibition, free A54 peptide (20 µg) was added to the Bel-7402 cells for 1 h before adding FITC labeled A54-MNLC nanoparticles. Then, 20 µL of Hoechst was added to stain the cell nuclei for 30 min. After incubation, the cells were washed with PBS for three times and the fixed cells were examined under a fluorescence microscopy. The quantitative analysis of the above cellular uptake was further evaluated using a flow cytometry (FC 500 MCL; Beckman Coulter, USA). All experiments were repeated three times.

In vivo distribution of A54-MNLC in extopic tumor model

The xenografted tumor models were built by subcutaneous injection of Bel-7402 cells or HepG2 cells into the hind legs of female BALB/c nude mice (4–5 weeks, 18 ± 2 g). Two weeks after the xenografted hepatoma models of nude mice were constructed, the real-time biodistribution of A54-MNLC was investigated by the Maestro system (Cambridge Research & Instrumentation, Inc., Woburn, MA, USA). Xenografted Bel-7402 and HepG2 tumor bearing mice were injected intravenously with 0.2 mL ICG labeled A54-MNLC (SPIONs content was 100 µg/mL) at a dose of 5.0 mg/kg. Biodistribution of the nanoparticles in the tumor bearing mice were visualized at the predetermined time (1, 3, 6, 12, 24, 48 h). At 48 h, the major tissues of sacrificed mice were collected and imaged to observe the fluorescence signals. Fluorescence intensity associated with the amounts of A54-MNLC nanoparticles in various organs and tumors was semi-quantified using the imaging system. The distribution and accumulation of A54-MNLC in various tissues were calculated as %ID/g.

In vivo MR imaging of A54-MNLC in orthotopic tumor model

The orthotopic hepatoma tumor models were established by transplanting with Bel-7402 tumors into the liver of female BALB/c nude mice (4–5 weeks, 18 ± 2 g). The normal liver tissues were exposed by cutting longitudinally to the abdominal cavity, then the abdominal wall and skin were sutured using biodegradable stitches after implanting 1 mm³ tumor tissue into the liver parenchyma.

After 2 weeks of transplantation, the tumor bearing mice were imaged by 3.0 T MRI. For T2-weighted MR imaging, Bel-7402 tumor-bearing mice were divided into two groups (n = 5 per group). 0.2 mL A54-MNLC and MNLC nanoparticles solution at an equivalent SPIONs dose (SPIONs, 100 µg/mL) were injected into the mice through tail vein, respectively. Mice were imaged both before and after injection at the predetermined time (1, 2, 3, 6, 12, 24 h) by 3.0 T MRI. The parameters were as follows: FOV = 60 × 60 mm; TR = 3000 ms; TE = 78.6 ms; slice thickness = 2.0 mm; number of slice = 10.

Histological analysis

The orthotopic hepatoma tumor bearing mice were sacrificed to obtain the liver, washed with PBS, fixed with 4% formaldehyde, then embedded in paraffin. The sections were stained using haematoxylin-eosin

(H&E) and immunohistochemistry based on the standard procedures. The microscopic images were observed under Leica fluorescence microscope.

Statistical analysis.

All the results were calculated by Student’s t test and one-way analysis of variance (ANOVA) and p-value < 0.05 was considered statistically significant. All the data were reported as the mean ± standard deviation (SD) of three separate experiments.

Results And Discussion

Synthesis and Characterization of A54-PEG-SA

A54-PEG-SA was synthesized via acylation reaction. NH₂-PEG-SA was firstly synthesized acylation between the carboxyl group of SA and the amino group of H₂N-PEG- H₂N. Then the A54-PEG-SA was further synthesized through acylation reaction between the carboxyl group of A54 peptide and the amino group of NH₂-PEG-SA. The successful synthesis of A54-PEG-SA was confirmed by analyzing its chemical structure via ¹H NMR spectra, which was shown in our previous report [24].

Preparation of SPIONs loaded A54-NLC (A54-MNLC)

In this study, SPIONs loaded NLC nanoparticles were prepared by using emulsion-diffusion method. The characteristics of A54-MNLC and MNLC nanoparticles were shown in Table 1. The size distribution and the morphology of A54-MNLC and MNLC were presented in Figs. 1A and 1B. The diameters of A54-MNLC and MNLC nanoparticles were 46.42 ± 3.44 nm and 45.77 ± 3.96 nm, respectively. Transmission electron microscopy (TEM) images indicated that the anionic MNLC and A54-MNLC were uniform distribution of spherical particles. The surface charges of A54-MNLC and MNLC were - 20.91 ± 1.05 mV and - 21.47 ± 1.13 mV, respectively. Thus, the modification of peptide A54 had little influence on the particle size and zeta potential of NLC

Table 1. Physicochemical characteristics of A54-MNLC.

sample	Size (nm)	PDI (-)	Zeta potential [mv]
A54-MNLC	46.42±3.44	0.26±0.05	-20.91±1.05
MNLC	45.77±3.96	0.23±0.07	-21.47±1.13

The transverse relaxation time (T2) of water protons in an aqueous solution of the prepared A54-MNLC was measured to explore its MR imaging potentials. As shown in Fig. 1C, with the increase of Fe concentration, A54-MNLC showed a gradual decrease of the MR signal intensity in the T2-weighted MR

images. The T2 relaxation rate ($1/T_2$), linearly proportional to the Fe concentration, and this feature demonstrated that the A54-MNLC had a great potential for the MR imaging application.

The magnetic properties of the A54-MNLC was investigated by the field-dependent magnetization measurement (Fig. 1D). The absence of a hysteresis loop indicated the superparamagnetic nature of A54-MNLC.

In vitro cytotoxicity

The cytotoxicity of A54-SNLC was tested on both Bel-7402 and LO2 cells through a standard MTT assay. The results shown in Fig. 2A and 2B, after 48 h of incubation, the cell viabilities of Bel-7402 and LO2 were higher than 80% at the experimental dose, which manifested the prepared A54-MNLC had a relatively high biocompatibility and low toxicity for both liver tumor cells and liver normal cells.

In vitro cellular uptake

To investigate the targeting ability of the prepared A54-MNLC *in vitro*, the cellular uptake of A54-MNLC and MNLC nanoparticle on liver tumor cells Bel-7402 and normal cells LO2 were studied by labeling the nanoparticle with FITC. The fluorescence microscopy images were presented in Figs. 3A and 3B. It was found that the uptake of the A54-MNLC and MNLC by those two cells was in a time-dependent manner during 12 h of incubation. The results also indicated that the A54-MNLC had no specific recognition to HepG2 cells, as the FITC fluorescence signals of HepG2 cells incubated with A54-MNLC and MNLC were non-significantly at the same time point. As a contrast, the FITC fluorescence intensity of Bel-7402 cells cultured with A54-MNLC was much stronger than A54-blocking and MNLC groups. This phenomenon could be due to the strong affinity of A54 peptide to Bel-7402 cells. Therefore, the *in vitro* cellular uptake study confirmed the specific targeting ability of the A54-MNLC to liver tumor cell Bel-7402.

Furthermore, the T2-weighted images of Bel-7402 and HepG2 cells incubated with A54-MNLC and MNLC nanoparticles for 3 h and their MR signal intensity were shown in Figs. 4A and 4B. The MR signal intensities of Bel-7402 and HepG2 cells incubated with A54-MNLC and MNLC were decreased obviously. It should be noted that the decrease of the MR signal intensity of Bel-7402 cell incubated with A54-MNLC was significantly lower than that of the other groups ($P < 0.01$, $n = 3$). This result confirmed the specific targeting ability of A54-MNLC to Bel-7402 cell.

In vivo biodistribution

The *in vivo* targeting efficiency of A54-MNLC was investigated in a Bel-7402 tumor and HepG2 tumor bearing Balb/c nude mouse model. As shown in Fig. 5A, the fluorescent signal intensities of the tumors increased after the injection of ICG-labeled A54-MNLC nanoparticles and peaked at 36 h. Obviously, a significantly stronger fluorescent signal was observed in BEL-7402 tumor at every time points, reflecting more accumulation of A54-MNLC nanoparticles in Bel-7402 tumor tissues. The mouse was sacrificed at

48 h after injection, and the fluorescent images of the major organs and tumors were presented in Fig. 5B, the results indicated that the selective accumulation of A54-MNLC nanoparticles in the Bel-7402 tumor. The fluorescent signal intensity of Bel-7402 tumor was stronger than that of HepG2 tumor, which confirmed the targeting ability of A54-MNLC nanoparticles to Bel-7402 cells. Therefore, with modification of A54 peptide, the A54-MNLC nanoparticles had a highly specific targeting ability toward the Bel-7402 tumor *in vivo*.

In vivo MR imaging of A54-MNLC in orthotopic tumor model

To investigate the MRI contrast efficiency and targeting capability of A54-MNLC, the potential performance of A54-MNLC as an excellent MRI contrast agent was evaluated in the orthotopic Bel-7402 tumor model. Firstly, the orthotopic Bel-7402 tumor was established by transplanting with Bel-7402 tumors into the liver of female BALB/c nude mice (4–5 weeks, 18 ± 2 g). After 2 weeks of establishment, the tumor bearing mice were divided into two groups and imaged by a 3.0 T clinical MR scanner. Prior to the experiment, the successful of the orthotopic tumor model needed to be confirmed by histological analysis. As shown in Fig. 6, the tumor cells became pleomorphic with bigger nucleus and abundant cytoplasm, this result indicated the orthotopic tumor model was successfully transplanted.

The results of *in vivo* MRI of A54-MNLC in orthotopic tumor models were showed in Fig. 6. In this study, T2-weighted images of mice' liver were obtained before and after intravenously injected with A54-MNLC and MNLC (Fig. 6A). Before intravenous injection of nanoparticles, the tumor appeared homogeneous and bright in the MRI image. After administration of A54-MNLC, the tumor was significantly attenuated and the tumor boundary was much more clearly delineated than MNLC group. The MRI contrast efficiency of the A54-MNLC could also be clearly observed from the quantitatively analysis by plotting the T2-weighted images signal intensity of tumor, as shown in Fig. 6B. At 2 h post injection of A54-MNLC, the average MRI signal intensity of tumor tissues was decreased to 57.58% when compared with that before injection. However, the MRI signal intensity of tumor in MNLC treated group was just dropped to 76.8% under the same condition. The decreased MRI signal intensity of tumor in MNLC group could be associated with the passive enhanced permeability and retention (EPR) effect. The A54 peptide-directed targeting role is dominant to enable specific delivery of the particles to the Bel-7402 tumor site, enabling specific MR imaging of tumor. In addition, as shown in Fig. 6C, the T2-weighted MR images and the quantitative results of the T2 signal intensity of liver tissue showed that the signal intensity of the two groups of the experimental nude mice began to decrease after 1 h of administration and continued until 24 h, indicating that A54-MNLC and MNLC have no specific recognition effect on normal liver tissue. The decrease in the signal intensity of normal liver tissue may be caused by the passive phagocytosis of macrophages.

In addition, the accumulation of iron in the tumor tissue were further verified. As shown in Fig. 6D, more iron could be detected in the tumor tissue of the A54-MNLC group. This results further illustrated that the

A54-MNLC has targeting ability to the tumor cells Bel-7402.

Conclusion

In summary, a novel A54 peptide modified and SPIONs loaded nanostructured lipid carrier (NLC) was fabricated for targeted MR imaging of hepatocellular carcinoma. The prepared A54-MNLC nanoparticles were water dispersible and cytocompatible. The results of *in vitro* cellular uptake study revealed that the A54-MNLC was able to specifically uptaken by Bel-7402 cells. Furthermore, the developed A54-MNLC nanoparticles exhibited a great potential in specific MR imaging of orthotopic model *in vivo* through A54-mediated active targeting pathway. Therefore, the SPIONs-based and A54 modified NLC developed herein is a promising nanoplatform as a targeted MR imaging contrast agent for the diagnosis of hepatocellular carcinoma.

Declarations

Author's contribution

Ri-Sheng Yu, Yong-Zhong and Du Jian-Song Ji designed experiments. Chen-ying Lu and Nan-Nan Zhang finished the preparation, characterization, *in vitro* experiments and *in vivo* experiments. Gao-feng Shu, Min-jiang Chen and Xiu-liang Zhu established orthotopic model. Qiao-you Weng, Xian-yao Lin assisted with the intravenous injection during *in vivo* experiment.

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Acknowledgments

Not applicable.

Competing Interests

The authors declare no competing interest.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All animal procedures were carried out in accordance with National Institutes of Health (NIH, USA) protocols, approved by the local animal experiments ethical committee of Animal Experiments at Zhejiang University in China.

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Figures

Figure 1

Characterization of A54-MNLC nanoparticles. (A) TEM images and size distribution of MNLC and A54-MNLC. (B) Surface charges (zeta-potential) of MNLC and A54-MNLC. (C) T2-weighted MR images and linear fitting of T2 relaxation rates (1/T2 values, S-1) of A54-MNLC nanoparticles at various Fe concentration. (D) Magnetic hysteresis loops of A54-MNLC and MNLC nanoparticles.

Figure 2

Assessment on cytotoxicity of A54-MNLC and MNLC nanoparticles on normal liver cells LO2(A) and tumor cells Bel-7402 at various concentrations. (n = 3).

Figure 3

Evaluation on targeting ability of A54-MNLC in vitro. (A) Fluorescence images of Bel-7402 cells and HepG2 cells incubated with A54-MNLC and MNLC for 1 h, 3 h, 6 h and 12 h, respectively. Fluorescence intensity of Bel-7402 (B) and HepG2 cells (C) measured by flow cytometry when cells incubated with A54-MNLC, A54-MNLC pre-treated with A54 blocking and MNLC for 1 h, 3 h, 6 h and 12 h, respectively.

Figure 4

The T2-weighted images (A) and MR signal intensity (B) of Bel-7402 cells and HepG2 cells incubated with A54-MNLC for 3 h, respectively. n=3, **P<0.01.

Figure 5

Evaluation on targeting ability of A54-MNLC in vivo. (A) Fluorescence images of tumor-bearing nude mice treated with A54-MNLC at different point in-times. (B) Fluorescence images of tumor and main organs in the tumor-bearing mice injected intravenously with A54-MNLC at 48 h. (C) Semi-quantitative analysis of the fluorescence signal of the tumors and organs in (B).

Figure 6

Assessment on enhanced ability of A54-MNLC ternary nanoparticles in MR imaging in vivo. (A) In vivo T2-weighted mages of Bel-7402 tumor-bearing livers before and after intravenous administration of A54-MNLC and MNLC nanoparticles. The yellow dashed circles indicate the tumor region. T2-weighted signal

intensity of the tumor (B) and liver (C). (D) Microscopic view of Prussian blue-stained tumor-liver tissue section of nude mice treated with A54-MNLC and MNLC, respectively (magnification: $\times 400$).

Supplementary Files

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