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Stem Cell-mediated Delivery of SPIO-loaded Gold Nanoparticles for the Theranosis of Liver Injury and Hepatocellular Carcinoma

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Abstract

The treatment of liver injuries or hepatocellular carcinoma (HCC) with has been hindered by the lack of efficient drug delivery. Even with the help of nanoparticles or other synthetic delivering agents, a large portion of the dose is still sequestered in the reticuloendothelial system (RES). As an alternative, adipose-derived mesenchymal cells (AD-MSCs), which have the capability of homing to the injured liver, can be used as a unique carrier for theranostic agents. Theranostic agents must have the capacity for being non-toxic to host cells during transportation, and for timely activation once they arrive at the injury sites. In this study, we loaded AD-MSCs with superparamagnetic iron oxide-coated gold nanoparticles (SPIO@AuNPs) and tested their effects against liver injury and HCC in cells and in mice. SPIO@AuNP is a non-toxic MRIactive contrast agent that can generate heat when irradiated with near-infrared laser. Our results showed that SPIO@AuNPs were successfully transfected into AD-MSCs without compromising either cell viability ($P > 0.05$) or cell differentiability. *In vivo* MRI imaging and histologic analysis confirmed the active homing of AD-MSCs. Upon laser irradiation, the SPIO@AuNPloaded AD-MSCs could thermally ablate surrounding HCC tumor cells. SPIO@AuNP-loaded AD-MSCs proved a promising theranostic approach for injured liver and HCC.

Keywords

stem cell; SPIO; gold nanoparticle; MRI; liver

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1. Introduction

The liver is the largest gland in the human body. It carries out the chief metabolic functions of the body and mounts a biological defense against foreign toxins [1]. Chronic liver injuries, such as those due to hepatitis B/C or alcohol abuse, may progress to liver fibrosis, cirrhosis and even hepatocellular carcinoma (HCC) if the patient does not receive adequate treatment [2]. HCC is the fifth most common cancer worldwide, and its incidence and mortality are on the rise, particularly in younger patients [3]. Timely diagnosis of such liver injuries or HCC, followed by proper treatment, would significantly prolong the patients' survival and improve their quality of life.

Theranostic agents, which combine diagnostic and therapeutic modalities, offer enormous potential for the management of chronic liver injury or HCC. However, the efficient delivery of theranostic agents to injury sites in the liver is greatly hindered by the presence of Kupffer cells [4]. An important component of the reticuloendothelial system (RES), Kupffer cells effectively sequester nanoparticles for subsequent degradation or excretion. Indeed, a critical guideline for nanoparticle-assisted drug delivery is to minimize nanoparticle uptake in the RES organs [5]. Unfortunately, a significant portion of synthetic nanocarriers still end up in the RES organs along with the encapsulated drug, leading to undesirable systemic toxicity and reduced efficacy.

In addition to synthetic carriers, a variety of cells also have been used to deliver drugs, such as immunocytes, macrophages, lymphocytes, neutrophils and stem cells [6]. Among these, mesenchymal stem cells (MSC) exhibit some unique characteristics, including multi-potent differentiation, extensive self-renewal, hypo-immunogenicity, anti-inflammatory activity and intrinsic homing to injury sites or tumor tissues, that make them attractive for drug delivery [7]. MSCs have been used to deliver various anticancer agents, including pro-apoptotic proteins, oncolytic viruses, and chemotherapy drugs [7, 8]. Currently, there are many limitations on loading chemodrugs into MSCs. First, the chemodrugs have to be loaded at an amount sufficient for therapeutic effect and protected against degradative enzymes to preserve their antitumor toxicity. Second, the chemodrugs must not harm the host MSCs or impair their differentiation. Finally, the chemodrugs should be released into the surrounding tumor tissue once they arrive, although the timing of release may vary among different applications. The source and availability of MSCs are also important issues. A large number of cells is required. The MSCs should be harvested from the patient to minimize the risk of disease transmission [9].

We reasoned that adipose-derived MSCs (AD-MSCs) could be used to effectively deliver superparamagnetic iron oxide (SPIO)-loaded gold nanoparticles (SPIO@AuNP) into liver injury sites or HCC tumors (Figure 1). SPIO@AuNP is intrinsically nontoxic. The SPIO makes it magnetic resonance imaging (MRI) active. It also mediates heat upon irradiation with a nearinfrared laser, potentially inducing thermal ablation on tumor tissue. Unlike conventional chemotherapy drugs, SPIO@AuNP does not harm host cells. Its capacity for thermally ablating surrounding tumor cells does not require its release from host cells. Another advantage of this system is that AD-MSCs can be harvested easily from a patient's adipose tissue in sufficient numbers [10]. We hypothesized that this multimodal approach

can accurately localize stem cells at the site of liver injury and effectively kill HCC cells. To test this, we monitored the distribution of SPIO@AuNP-loaded AD-MSCs using *in vivo* MRI and *ex vivo* histologic analysis, and evaluated their ability to mediate light-induced thermal ablation in an *in vitro* setting.

2. Materials and Methods

2.1. Materials

Methoxy-polyethylene glycol-sulfhydryl (PEG-SH, molecular weight [MW] 5000 Dalton) and phosphate-buffered saline solution (PBS; pH, 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). PD-10 columns were purchased from GE Healthcare (Piscataway, NJ). Trisodium citrate dihydrate (>99%) and chloroauric acid trihydrate (American Chemical Society reagent grade) were purchased from Fisher Scientific (Pittsburg, PA) and used without further purification. SPIO (EMG 304) was purchased from FerroTec (Bedford, NH).

2.2. Preparation and characterization of SPIO@AuNP

SPIO@AuNPs were prepared by using protocols described elsewhere with detailed characterizations [11]. Only relevant characterizations are presented in this manuscript. Briefly, 10-nm SPIO nanoparticles were first coated with a layer of amorphous silica via the sol-gel method. Next, gold nano-crystals of 2 to 3 nm in size were deposited on the amine function-modified silica surface of the nanoparticles. The gold nano-crystals acted as the seed to mediate nucleation and growth of a gold over-layer to form the SPIO@AuNP. The particles were subjected to centrifugation at 8,000 rpm for 15 min and washed with deionized water three times. The resulting particles were resuspended in PBS.

The SPIO@AuNPs were characterized in terms of size, zeta potential at different pH, morphology, composition, optical absorption, and magnetization. For the transmission electron microscopy study, SPIO@AuNPs were applied to a 100-mesh nickel grid coated with a polyvinyl formal resin and carbon (Sigma-Aldrich). The nanoparticles were allowed to attach to the grid for 1 hr, after which the grid was rinsed with deionized water and dried in air. The samples were examined using a transmission electron microscope (JEM 2100F, JEOL Ltd., Japan) at an accelerating voltage of 200 kV. The average size and thickness of the gold layer were calculated for at least 50 particles. The size and zeta potential at different pH (5.0, 7.4, and 10.0) was acquired using Zeta PALS (Brookhaven Instruments Corporation, Holtsville, NY). The ultraviolet-visible light spectroscopy was recorded on a Beckman Coulter DU800 UV-Vis spectrophotometer using a 1-cm optical path-length quartz cuvette. The concentrations of iron and gold in the SPIO@AuNP suspension were measured using an inductively coupled plasma mass spectrometer (ICP-MS) (Galbraith Laboratories Inc., Knoxville, TN).

2.3. Adipose-derived Mesenchymal Stem Cells

AD-MSC separation protocols—AD-MSCs were isolated from porcine subcutaneous adipose tissue. Approximately 10 grams of tissue was minced in a sterile petri dish into a fine, homogeneous preparation with scissors, and dissociated using automated cell recovery instrumentation and reagents (InGeneron, Inc., Houston, TX) according to the

manufacturer's instructions [12]. Briefly, the minced adipose tissue was transferred to a 50-mL conical tube containing 20 mL of lactated Ringer's solution; 2.5 mL of Matrase™ enzyme (InGeneron) was added, and the tube was inverted several times to mix thoroughly. Tubes were then placed into the Transpose RT tissue processing unit and subjected to automated enzymatic dissociation for 45 minutes. Dissociated tissue was next filtered and washed, and the resultant cells were pelleted and washed twice with PBS in the processing unit to yield the stromal vascular fraction (SVF). The SVF was resuspended in 10 mL of minimum essential medium (MEM) containing 20% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells ($1.5 \sim 2.0 \times 10^6$) were seeded into a T-75 tissue culture flask (BD Falcon, NJ, USA) and incubated at 37°C in a humidified chamber containing 5% CO₂ for 24 hr, after which the non-adherent cells and debris were removed by aspiration. The remaining attached cells were designated AD-MSCs and were maintained with medium changes at 3-day intervals. Cells at less than passage seven were used for all experiments.

GFP-expressing AD-MSCs—A nonviral piggyBac transposon system was used to mediate stable gene transfer and persistent expression of a bi-color fluorescent protein-labeling cassette H2B-EGFP-2A-mCherry-GPI in AD-MSCs [13]. Cells were harvested using 0.25% trypsin and incubated at 37°C in 5% CO₂ for 5 min, followed by addition of trypsin inhibitor-containing medium and repeated gentle pipetting to yield a single cell suspension. Cells were counted and resuspended at a density of 1.1×10^7 cells/mL. For each electroporation, 2 µg helper plasmid (pCMV-mPB-pA) and 8 µg donor plasmid (both at 1 µg DNA/µL) were mixed with 90 µL cell suspension (about 10^6 cells). Electroporation was performed using a Neon Transfection System (Invitrogen, Inc., Carlsbad, CA) in a 100-µL tip chamber using two 1200-V pulses of 20 ms duration. Following electroporation, cells were immediately seeded into a T-25 flask without antibiotics. On days 3–13 after electroporation, cells were cultured in a selection medium containing antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin) and G418 at a concentration of 100 µg/mL and transferred to T-75 flasks as required. On day 14, and thereafter, cells were cultured in medium without G418. Surviving cells were verified to be tri-lineage differentiation-capable mesenchymal stem cells.

2.4. In vitro transfection of AD-MSCs with SPIO@AuNPs

Low-passage AD-MSCs were seeded in a monolayer and maintained at 37°C in the medium until reaching 80% confluence. The transfecting medium was prepared by suspending SPIO@AuNPs in a serum-free, antibiotics-free MEM and co-incubating with lipofectamine (Invitrogen) for 30 min at room temperature. The culture medium was aspirated and the cells were washed with prewarmed PBS buffer. The transfecting medium was added and co-incubated with the cells for 6 hr at 37°C. At the end of transfection, the transfecting medium was removed and cells were trypsinized for further studies.

Cell survival during transfection was evaluated by using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MTS) assay. Upon completion of transfection, cells were maintained in the MEM medium containing 20% MTS reagent at 37°C for 2 to 4 hr. The absorbance at 490 nm was recorded (N = 6).

Untransfected cells were used as the negative control. A response curve of cell survival vs. SPIO@AuNP dose was plotted.

2.5. Confirmation of SPIO@AuNP uptake in AD-MSCs

Prussian-blue staining and ICP-MS analysis—Slides were reacted with unactivated or activated (containing 0.03% hydrogen peroxide) 0.014% diaminobenzide for 15 min each, washed three times with Prussian blue stain, and then counterstained with nuclear fast red [14]. Samples of SPIO@AuNP-labeled AD-MSCs were sent to Galbraith Laboratories (Knoxville, TN) for ICP-MS analysis to quantify iron and gold contents.

In vitro relaxivity of AD-MSCs labeled with SPIO@AuNPs—After transfection, AD-MSCs were washed with PBS to remove unbound SPIO, then scraped from the bottom of the petri dish and dispersed in PBS (500 μ L) and subjected to centrifugation at 1100 rpm for 5 min. The supernatant was removed, and the cell pellet was resuspended in 1.5% agarose gel (volume total $\sim$ 0.5 mL) and then placed inside a 5-mm nuclear magnetic resonance tube (height of tube $\sim$ 3 cm). The samples were allowed to solidify at 5°C and then were subjected to direct MRI. MRI of cell suspensions was performed using fast-spinecho sequence with 24 TE values ranging from 15 to 360 ms. Other scan parameters were TR = 1000 ms, FOV = 3.2 cm, slice thickness = 1 mm, and acquisition matrix = 64 $\times$ 64. T2 values were calculated as the slopes from a linear least-square line fit to the log of the mean measured MR signal in a region of interest versus TE.

Measurement of tumor necrosis factor (TNF)- α and interferon (IFN)- β secretion—Secretion of TNF- α and IFN- β were measured in the supernatant using the ELISA method. Specifically, human hepatoblastoma, HepG2 (ATCC, Manassas, VA), cells were seeded at 1×10^4 cells per well in 96-well plate overnight. The culture medium was replaced with different concentration of SPIOAu NP (0, 2.5, 5, 10, 20, 40 μ g/mL) in media and was incubated for 3 hrs or 24 hrs (n=3). PBS and LPS at 10 μ g/mL (Sigma, St. Louis, MO) were used as controls. The supernatant in the well was collected and centrifuged at 13,000 RPM for 15 min at 4 °C. The precipitates were discarded. The clear supernatant was stored at -80 °C until ELISA test. ELISA kits for TNF- α and IFN- β were purchased from Thermo Fisher Scientific Inc (Rockford, IL) and was performed in accordance to the manufacturer's instructions. Results were expressed as pg/ml.

2.6. Differentiation of AD-MSCs after transfection

Three AD-MSC differentiation lineages were tested *in vitro*: adipogenic, osteogenic, and chondrogenic. The AD-MSCs were seeded at 1×10^4 , 1.6×10^7 and 5×10^3 cells/cm², respectively. The cells were allowed to attach for 2 hr, the medium was replaced with prewarmed differentiation medium and the cells were cultured at 37°C. The medium was refreshed every 3 days; the cell culture was stopped after 14 days for adipogenic and chondrogenic cells and after 21 days for osteogenic cells.

The cell monolayers were washed with PBS and fixed with 4% formaldehyde for 30 min. The adipogenic cells were then rinsed with PBS and 60% isopropanol, stained with oil red O (which stains lipid vesicles) for 15 min, and rinsed again with 60% isopropanol and

deionized water. The osteogenic cells were rinsed with deionized water, stained with 2% alizarin red (which stains basic calcium phosphate crystals) for 3 min, and rinsed with deionized water. The chondrogenic cells were rinsed with PBS, stained with 1% alcian blue (which stains mucopolysaccharides and glycosaminoglycans) for 30 min, and rinsed with 0.1 N HCl and deionized water. Cells with and without SPIO@AuNP transfection were compared in pairs.

2.7. In vivo MR imaging and histology studies

Liver damage was induced in 6-week-old nude mice by implanting a pellet releasing 70 mg 2-acetylaminofluorene (2-AAF) at 2.5 mg/day in the neck of each animal. On day 7 after implantation, each mouse received a single dose of CCl₄ (0.66 mL/kg of body weight) diluted 1:1 (v/v) in corn oil. Two weeks after the CCl₄ injection, SPIO@AuNP-loaded AD-MSCs were injected into each mouse's spleen. The mice were then prepared for MRI at predetermined time points (N = 3 for each time point) [15]. For the *in vivo* feasibility investigation, the fate of SPIO-loaded AD-MSCs was monitored using a respiratory gated T₂* mapping sequence (TE_{min} = 4 ms, TE = 5 ms, FA = 30, FOV = 4 × 3 cm).

All mice were sacrificed after imaging and their livers harvested for histologic examination. The livers were fixed with 4% paraformaldehyde and sectioned into 5-μm slices. Prussian blue staining and fluorescence immunohistochemistry were performed to reveal the SPIO@AuNPs and AD-MSCs, respectively. The other parts of liver were stained with hematoxylin & eosin (H&E) to reveal the extent of liver fibrosis.

2.8. Proof of concept: photothermal therapy

SPIO@AuNP-loaded AD-MSCs was suspended in PBS buffer at 4×10⁶/mL. Liver carcinoma HepG2 cells were suspended in PBS buffer at 4×10⁶/mL. The two cell suspensions were mixed together at a 1:1 ratio and subjected to laser irradiation (λ = 808 nm) at 3 W for 5 min. The temperature of the cell suspension was monitored using a Luxtron Fluoroptic Temperature Probe in conjunction with a m3300 Fluoroptic Thermometer. A HepG2 cell suspension without AD-MSCs was used as control.

2.9. Statistical analysis

Results were analyzed using a two-tailed unpaired Student t-test or one-way ANOVA. A P < 0.05 was considered statistically significant.

3. Results and Discussion

Our findings showed that AD-MSCs could effectively deliver SPIO@AuNPs into sites of liver injury or HCC tumors (Figure 1). SPIO@AuNPs were synthesized according to methods previously reported (scheme shown in Figure 2A) [11]. The size and morphology of nanoparticles was monitored at every step of the synthesis using transmission electron microscopy. The successful embedment of SPIO in silica matrix was demonstrated by the presence of a grey silica layer around the black SPIO (Figure 2B). The gold over-layer was not continuous, but rather composed of discrete spheres grown from the individual gold seeds (Figure 2C). Calculation of the size results to a diameter of 82 ± 4 nm. The zeta

potential was 6.7 ± 0.6 , 6.7 ± 2.0 , and 14.4 ± 2.4 for acidic (pH=5), neutral (pH=7.4), and basic (pH=10) pH, respectively. This result indicated that the SPIO@AuNP remained positive at these different pH ranges. The SPIO@AuNPs had strong absorbance between 600 and 1000 nm and thus this preparation was suitable for a wide spectrum of near-infrared laser irradiation (Figure 2D). SPIO@AuNPs rapidly mediated heating upon irradiation with an 808-nm laser. The temperature of the SPIO@AuNP suspension in double-distilled water increased to about 55°C within 2 min of laser treatment, reached a plateau during laser treatment and then rapidly decreased when the laser was turned off (Figure 2E). When different concentrations of SPIO@AuNP were subjected to MRI, there was a concentration-dependent decrease in T2*-weighted signals (Figure 2F). The magnetic relaxivity (R_2^*) of SPIO@AuNP was measured as $369 \text{ mM}^{-1}\text{s}^{-1}$.

After transfection, the presence of SPIO@AuNPs inside AD-MSCs was confirmed by Prussian blue staining (Figure 3A). The average iron and gold contents per cell were quantified using ICP-MS as 94 and 5.4 pg, respectively. The iron-to-gold ratio was consistent with the chemical composition of SPIO@AuNPs. The viability of AD-MSCs was not significantly altered after transfection in the medium containing 10 µg/mL SPIO@AuNPs (Figure 3B). The accelerated cell growth (viability >100%) may arise from the growth-stimulating effect of SPIO to mesenchymal stem cells [21]. All further transfection studies were performed using 5 µg/mL SPIO@AuNPs in order to minimize the adverse effect to cells. AD-MSCs transfected with SPIO@AuNPs showed a darkening effect on T2-weighted MRI, which is characteristic for SPIO. Representative MR images of cell suspensions are shown at the top of the graph in Figure 3C.

The ability of AD-MSCs to differentiate after transfection with SPIO@AuNPs was tested in appropriate media. Adipogenic differentiation of SPIO@AuNP-loaded AD-MSCs was confirmed by the presence of lipid compartments (stained with oil red O, Figure 3D). Osteogenic differentiation was proven by calcific deposition (stained by alizarin red, Figure 3E). Chondrogenic differentiation was demonstrated by the abundance of glycoaminoglycan, a component of cartilage (stained by alcian blue, Figure 3F). In contrast to the nontoxic SPIO@AuNPs, chemodrugs may cause the death of the host cells that are used for drug delivery. For example, when Steinfeld and colleagues used T lymphocytes to deliver micelle-encapsulated doxorubicin [16], doxorubicin was encapsulated in polymeric micelles and then loaded into the lymphocytes. Despite the micellar formulation, doxorubicin still caused significant cell death 15 hr after loading, probably because of leaching of the cytotoxic agent out of the micelles.

TNF- α is a central regulator of inflammation. Suppression of TNF- α expression has been previously shown to be an essential sign of downregulation of pro-inflammatory cytokine cascade in macrophages and non-macrophage cells [17]. On the other hand, IFN- β activates the Janus kinase and signal transducer of transcription (JAK-STAT) pathway, and induces the IFN regulated genes responsible for anti-viral and anti-inflammatory response [18]. In this study, secretions of TNF- α and IFN- β when penetrating into HepG2 cells were probed using the ELISA method. Figure 4 show that there is no significant increase in TNF- α (A) and IFN- β (B) release when incubated with different concentration of SPIO@AuNP ranging from 2.5 – 40 µg/mL (p-values > 0.05). Results also show that there is no significant change

in the levels of TNF- α and IFN- β when comparing 3 hr versus 24 hr incubation. Consistent with previously published literature, LPS, which is a positive control, failed to alter the TNF- α concentrations in HepG2 cells when compared with untreated control at any time point indicated [19]. Thus, SPIO@AuNP does not induce pro-inflammatory response when penetrating into cells.

MRI and histology confirmed the preferential homing of the SPIO@AuNP-loaded AD-MSCs to the diseased area. GFP-expressing AD-MSCs were used so that they could be identified during histologic analysis. After direct injection into the mouse spleen, the cells gradually moved to the site of induced liver injury. T₂-weighted MR images showed darkening of the liver 3 hr after injection (Figure 5). This darkening effect persisted until 24 hr after injection, but had returned to baseline 7 days after treatment. Quantification of the T₂* values indicated that AD-MSC accumulation in the liver was at the highest 3 hr after injection ($R2^*=8.86 \pm 0.57$), which is faster than many synthetic delivery carriers. This darkening effect remained significant at 24 hr ($R2^*=9.51 \pm 0.36$) and had gradually waned by day 7 ($R2^*=14.84 \pm 0.10$). This attenuation of MR signals can be attributed to the excretion of SPIO@AuNPs.

The liver tissues were then excised for histologic evaluation; representative images are shown in Figure 6. Liver fibrosis was evidenced by the prevalence of fibrotic septa arising from the portal area and hydropic degeneration after H&E staining (Figure 6A). The detailed structures are illustrated in Figure 6C. The presence of SPIO@AuNPs in the AD-MSCs was confirmed by Prussian blue staining (Figure 6B). The blue-colored SPIO@AuNPs localized well in the fibrotic regions. Fluorescence imaging confirmed that the fibrotic regions were populated with the incoming AD-MSCs (Figure 6D). Figure 6E reveals the excretion process captured by histologic analysis, showing that multinucleated liver macrophages engulfed the MSC debris along with the SPIO@AuNPs.

A common problem associated with cell-mediated drug delivery is that the drug has to be released out of the host cell in order to be functional. At the start of this study, we postulated that SPIO@AuNP does not need to be released from AD-MSCs to be functional, assuming that SPIO@AuNP-loaded AD-MSCs, when irradiated by laser, could generate heat sufficient for tumor ablation. As a proof-of-concept study, we irradiated a cell suspension consisting of a 1:1 mixture of SPIO@AuNP-loaded AD-MSCs and HepG2 liver carcinoma cells in PBS buffer with a 808-nm near-infrared laser. The laser power setting used is the one used routinely in our laboratory for photothermal therapy [20]. Upon irradiation, the control group (HepG2 cells alone, no SPIO@AuNP) did not exhibit significant temperature increase, as evinced by a buffer temperature below 30°C throughout the experiment. When the mixed cell suspension was irradiated, however, the buffer temperature rapidly increased, to >45°C within 20 s. The temperature peaked at 90°C after 5 min of laser treatment (Figure 7). This temperature is greater than 54°C, which is enough to cause spontaneous killing effects. These results also prove that the SPIO@AuNPs not only heated the host AD-MSCs but also nearby tumor cells that did not contain SPIO@AuNPs. Considering that the *in vivo* cell packing would be much denser than the cell suspensions, and that the space within a real tumor would be much more confined than the experimental conditions, we concluded that SPIO@AuNP-labeled AD-MSCs could potentially effectively kill the surrounding tumor

cells *in vivo*. The detailed antitumor efficacy studies, which exceed the scope of the current study, are currently under way. We also proved that the laser-induced thermal ablation did not require the release of SPIO@AuNPs from the AD-MSCs.

4. Conclusions

We demonstrated that AD-MSC is an efficient carrier for the specific delivery of theranostic agents to liver injuries or HCC. SPIO@AuNP is a host-compatible cargo that enables both MRI enhancement and laser-induced thermal ablation. We conclude that combining AD-MSC with SPIO@AuNPs is a treatment strategy that may be advantageous for several reasons. The *in vivo* distribution of SPIO@AuNPs can be monitored in real time using non-invasive MR imaging. The timing and dose of thermal ablation can be tuned by changing the power and duration of laser irradiation. The ablation can be spatially defined by focusing the area of laser irradiation so that any collateral damage to non-tumor organs is minimized. The immediate therapeutic response also can be evaluated using MRI. In conclusion, SPIO@AuNP-loaded AD-MSCs proved a promising theranostic agent for liver injury and HCC.

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Figure 1. Schematic illustration of delivery of SPIO@AuNPs via AD-MSCs to sites of liver injury. The SPIO@AuNPs are loaded in AD-MSCs to evade uptake by Kupffer cells. The homing of AD-MSCs to injured liver enables targeted MR imaging of the injury. Upon laser irradiation, the SPIO@AuNPs generate heat to eradicate the surrounding tumor cells.

Figure 2. Synthesis and characterization of SPIO@AuNPs. (A) Schematic illustration of SPIO@AuNP structure. (B) Transmission electron microscopic image of SPIO coated with silica. (C) Transmission electron microscopic image of SPIO@AuNPs. (D) Ultraviolet-visible light spectrum of SPIO@AuNPs. (E) laser-induced heating and (F) MR relaxivity of SPIO@AuNP suspensions.

Figure 3.

Characterization of AD-MSCs transfected with SPIO@AuNPs. (A) Bright-field micrograph of AD-MSCs loaded with SPIO@AuNPs after Prussian blue staining. (B) Viability of AD-MSCs after loading with different doses of SPIO@AuNPs. Shown are means $\pm$ standard deviations (error bars). (C) Magnetization of AD-MSCs by SPIO@AuNPs is shown; representative MR images are shown at the top. (D–F) Staining revealed differentiation of ADMSCs toward adipogenic (D), osteogenic (E), and chondrogenic (F) lineages.

Figure 4.

TNF- α (A) and IFN- β (B) concentrations were measured in incubation media of isolated cells in cell cultures at 0, 2.5, 5, 10, 20, and 40 $\mu\text{g/mL}$ of SPIO@AuNP at 3 and 24 hr incubation. PBS and LPS were used as controls. Values represent the mean $\pm$ standard deviation of three cell preparations for each group.

Figure 5. T₂-weighted MR images of liver regions at various times after injection of SPIO@AuNP-loaded AD-MSCs.

Figure 6. Histologic and immunohistochemical analysis showing SPIO@AuNP-loaded AD-MSCs homing to sites of liver injury. (A) H&E staining of liver fibrotic regions; detail shown in (C). (B) Prussian blue staining of SPIO@AuNPs; the presence of SPIO@AuNP-loaded AD-MSCs was confirmed by GFP-enabled fluorescence microscopy, shown in (D). (E) Possible route of SPIOAu excretion. Scale bar = 100 μ m in panels A and B, 50 μ m in panels C, D, and E.

Figure 7.
Photo-thermal ablation effect mediated by SPIO@AuNP-loaded AD-MSCs upon laser irradiation. The start and end of laser treatment were marked by arrows.