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Controlled Drug Releasing of Doxorubicin Loaded Magnetic Nanoliposome using NIR Irradiation

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Abstract - The effective delivery of therapeutic agent to target tissue is a central challenge in drug delivery. Therefore, externally stimuli controlled drug release has emerged as a promising method to localized delivery. In addition, the design of a suitable drug loaded nanocarrier to target lesion and response to a certain stimulus is also a main issue. In this study, we fabricate superparamagnetic iron oxide (SPIO) and doxorubicin (DOX) loaded magnetic nanoliposomes (MNL) as a thermal sensitive drug loaded nanocarrier. Moreover, near-infer red (NIR) light, as a non-invasive and remote control approach, is introduced to control release of the entrapped DOX. Based on the DOX releasing rate results, an irreversible release under NIR irradiation is proposed. In addition, a tumor cell killing effect of MNL combined with NIR light irradiation is evaluated. As a result, we achieve a significantly enhanced tumor cell killing effect. Therefore, we expect that an enhanced localized drug accumulation and an anti-tumor therapy can be achieved through NIR irradiation.

Keywords - Superparamagnetic iron oxide (SPIO) nanoparticles, nanocarrier, magnetic nanoliposome (MNL), Near-infra red (NIR), drug delivery.

1. Introduction

Photo-thermal therapy (PTT) uses a generated heat through the absorption of near infrared light (NIR) to kill cancer cells without affecting healthy tissues. Compared with conventional thermal conducting methods, such as radiofrequency, microwave and focused ultrasound [1-3]. PTT is less invasive, easy controllable and highly efficient [4]. So far, a large number of nanomaterials have been developed as PTT agents, such as gold nanoparticles, carbon nanotubes, and graphene [5-7], all of which show strong optical absorbance in the NIR region. Current research led to promising results in cancer cell light ablation with these agents, both in vitro and in vivo. Even so, the potential toxicity is still an unresolved problem. Especially, carbon nanotubes, graphene and gold nanoparticles are slowly degradable, non-degradable or potentially toxic, which will unavoidably limit the future applications of PTT [8]. Furthermore, a complete tumor treatment with PTT alone is difficult because the distribution of photo-thermal agents is often uneven, which induces a heterogeneous heat distribution and sub-lethal thermal dose in some tumor area.

Liposomes currently represent one of the best drug carriers and are already being used clinically, and also can be one of the most promising thermosensitive drug carriers for controlled release because the transition temperature of liposomes is a few degrees above body temperature [9]. The temperature triggered nanocarriers with even higher phase transition temperature is not practical, since the elevated heat would itself destroy the normal tissue in the NIR irradiation area and could cause stasis within tumor vessels that could disturb drug delivery. In addition, liposome based drug carriers could meet the requirement of drug controlled release that can stably retain drug in the physiological conditions without an external stimulus but release it with stimulus presence.

Superparamagnetic iron oxide (SPIO) nanoparticles with a diameter of 5 to 50 nm have excellent biodegradability and biocompatibility and have been already widely used as magnetic resonance imaging contrast agents. SPIO offer the ideal characteristics of clinically suitable and can meet all the criteria desired for PTT agents and could be sufficiently entrapped in liposomes [4, 10].

Here, we provided a novel NIR light sensitive magnetic nanoliposomes (MNL) containing DOX and SPIO. The liposomes were designed using a thermal sensitive 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposome and SPIO were entrapped into the liposomes. The temperature increase of MNL media by NIR irradiation was measured with a thermal camera. Then, the controlled release of entrapped drug in MNL combined with NIR irradiation was confirmed. Finally, the tumor cell killing effect of MNL combined NIR light irradiation was evaluated. We expect that the drug loaded MNL combined with NIR light irradiation will be a promising method to improve the cancer treatment efficiency.

2. Methods

2.1 Synthesis of Superparamagnetic Iron oxide (SPIO) Nanoparticles

Hydrophilic SPIO were synthesized using a chemical co-precipitation method of ferrous and ferric salts in alkaline medium [11]. Briefly, 10mM iron(III) chloride and 5mM iron(II) chloride were dissolved in 24ml of an hydrochloride aqueous solution (HCl 1M). The solution was added dropwise to an aqueous solution of NaOH 1M containing 5g of polyvinyl alcohol (PVA) with vigorous stirring for 60min under the protection of dry nitrogen at 60°C and additional 30min at 95°C. The black precipitate

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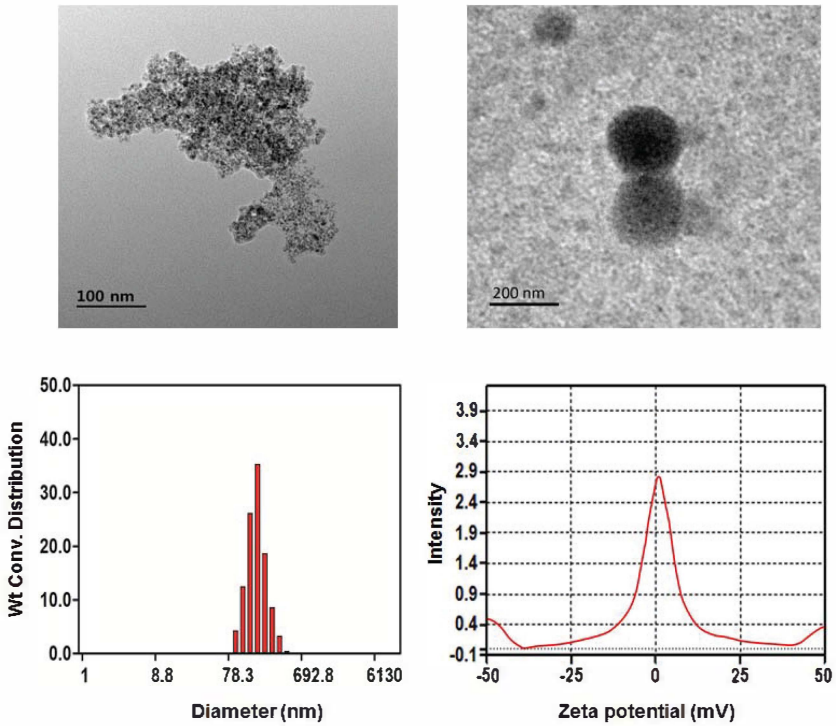

Fig. 1. Physical and chemical characterization of SPIO and MNL. TEM image of (a) SPIO and (b) MNL. (c) Size distribution of MNL. (d) Zeta potential of MNL.

was magnetically separated, washed three times using deionized water and then dispersed in 20ml of deionized water. The solution was then placed in an ultrasonic bath for 10 min and centrifuged (2000rpm, 10min) to remove the undispersed residue.

2.2 Preparation of Magnetic Nanoliposomes (MNL)

Liposome colloidal suspension was prepared by dissolving the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) in chloroform and transferred into round bottom flask. The organic solvent for the lipid solution was evaporated in a fume hood and completely removed in a vacuum for an additional 1h to obtain a dry film. The dried lipid film was then hydrated with 0.5wt% glycerol dissolved phosphate buffered saline (PBS, pH 7.4) to obtain a clear solution with final lipid concentration of 15mg/ml. Aliquots of this lipid solution were transferred into 5ml chromatography vials and mixed with DOX (1mg/ml) and SPIO (10mg/ml). MNL was synthesized by sonication for 5min (VC750, Sonics & Materials, Newtown, CT). The emulsion was centrifuged for 3min at 3000 rpm. The underlying liquid phase was removed from the top foam with a syringe and re-dispersed with fresh PBS buffer. This washing step was performed three times.

2.3 Characterization of SPIO and MNL

The size and size polydispersity of MVL were assessed using a dynamic light scattering method. The zeta potential of the MNL was measured in KCl 1mM with a Malvern Nano ZS at room temperature. The morphologies of the SPIO and MNL were determined using transmission electron microscopy (TEM). The SPIO loading efficiency of MNL was analyzed using inductively coupled plasma-optical emission spectrometer (ICP-OES) (PE-3300DV; Perkin Elmer, Norwalk, Connecticut). The DOX loading efficiency was analyzed using high performance liquid chromatography (HPLC) system.

2.4 Photothermal Study of MNL

An important feature of MNL is NIR light-induced thermal effect which could be used for thermal triggered drug release and hyperthermia treatment of solid tumors. To investigate temperature increase, we adopt NIR light irradiation in the presence of MNL. We used a continuous wave fiber coupled diode light (center wave length; 808nm) with an external adjustable power (CNI, Changchun New Industries Optoelectronics Tech. Co. Ltd). The light power and intensity were measured with an optical power meter (PM200, Thorlabs, NK, USA). 1ml of MNL sample solution upon to various concentration of SPIO were placed in cuvette tubes and irradiated by the light. The distance between the sample and the light was set to be 5cm and the light power was adjusted to 2W/cm^2. The photothermal transduction photographs were obtained through the thermal camera (America, FLIR E60; thermal sensitivity is 0.05°C).

2.5 NIR Triggered DOX Release

Drug release behavior from MNL was evaluated using dialysis method. DOX loaded MNL was put in dialysis

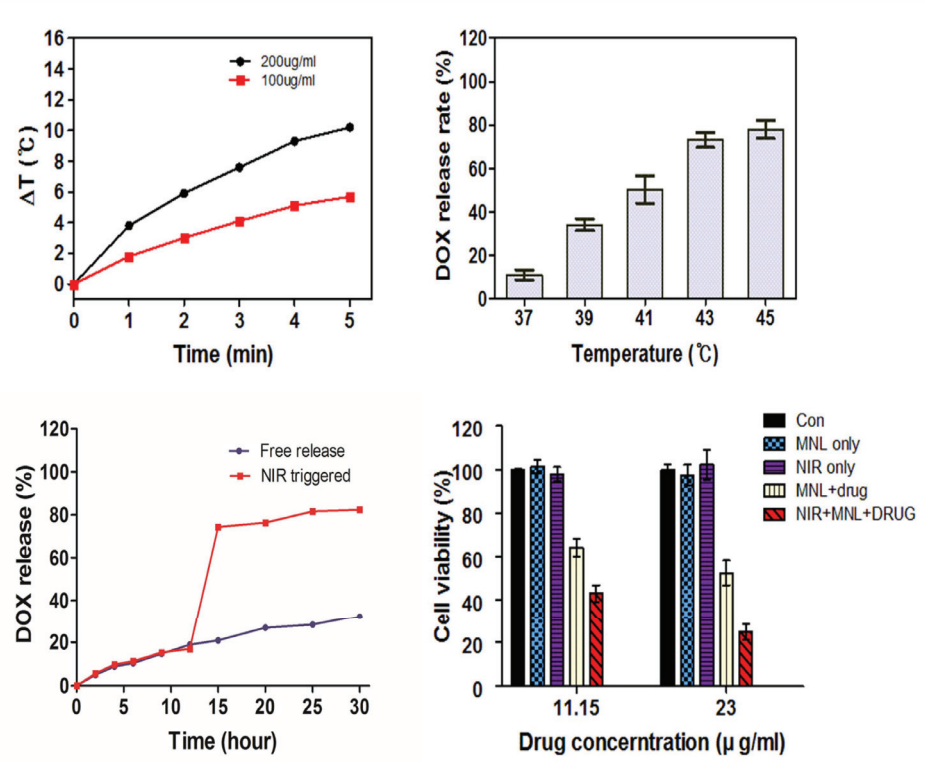

Fig. 2. a) Temperature increase induced by NIR light irradiation when the Fe concentration are 100μg/ml and 200μg/ml. b) DOX release behavior from MNL upon to various temperature. c) In-vitro DOX reelase profiles. d) Tumor cell viability test of MNL combined with NIR irradiation

bag (MW: 3500), immersed in PBS buffer solution (pH 7.4) and then the solution was placed in a shaking incubator (37°C, 150rpm). Then, at determined time period, 1 ml release medium was sampled and the equal volume of fresh medium was added to maintain the sink conditions. After the certain period of 12h, the sample with PBS solution was placed in heating mantle to keep the surround temperature at 37°C, and then irradiated with NIR light for 3 min. 1ml of release medium was collected and replaced with fresh medium before NIR irradiation. The negative control group was tested with the same process without NIR irradiation. The released DOX was measured by HPLC system.

2.6 In vitro Cytotoxicity Test

The cytotoxicity of MNL was determined by a MTT cell proliferation assay. Briefly, 4T1 breast cancer cells were seeded at a density of 10^4 cell per well in 96-well plates. Next day, the used medium was replaced with different formulation solutions: fresh medium, MNL and DOX loaded MNL with different DOX concentrations (11.5μg/ml and 23μg/ml), where the DOX concentration was based on the corresponding SPIO concentration when DOX and SPIO are co-loaded (Fe concentrations: 100μg/ml and 200μg/ml). After 12h, the cell wells were placed on sample holder at 37°C and the certain cell line group was irradiated with NIR light for 3min. After following incubation for 12h, supernatants were removed. Then, the wells were washed twice with PBS and incubated with DMEM containing MTT (5mg/ml) for an additional 2h. The MTT solution was removed and dimethylsulphoxide (DMSO) was added to dissolve the formazan crystals. The absorbance at 570 nm was measured by a microplate reader and the untreated cells were taken as a negative control groups.

3. Results and Discussion

3.1 Characterization of SPIO and MNL

SPIO were characterized in terms of morphology and size using transmission electron microscopy (TEM) (Fig. 1a), TEM results showed a spherical morphology with uniform size ranges from 7 to 12 nm and an apparent aggregation was not detected.

MNL were prepared using the thin film hydration and sonication method. The TEM image showed that MNL exhibited a smooth spherical morphology and the presence of hydrophilic SPIO in MNL was demonstrated by the enhanced contrast manifested as dark domains (Fig. 1b). All the MNL presented neutral zeta potentials and the sizes were between 95 to 382 nm (Fig. 1c and d). It is well known that the enhanced permeability and retention (EPR) effect is exploited to allow nanocarriers the passive targeting of tumors. Therefore, the size of nanocarriers plays an important role. The payloads of SPIO and DOX were 2.4mg/ml and 274μg/ml, which were measured with ICP-OES and HPLC method, respectively.

3.2 Characterization of Drug-loaded MNL

We determined the temperature increase induced by NIR light irradiation by optical thermal camera. Fig. 2a shows the temperature profiles for 5 min NIR irradiation of MNL solutions with SPIO concentration of 100 and 200μg/ml (SPIO concentration was normalized by considering the ICP-OES results). At a SPIO concentration of 200μg/ml, the MNL solution shows significant temperature elevation about more than 7°C and 10°C, when the NIR irradiation time were 3min and 5min, respectively, which are much higher than transient temperature of liposome (41°C), sufficient to control drug release from DOX-loaded MNL.

To verify the thermal responsive drug release behavior, DOX release rate upon to various temperatures was performed. Fig. 2b shows that DOX can be released from MNL over than 68 and 72% in 3min at 41°C and 43°C, which shows obvious possibility of controlled drug release by NIR irradiation. Next, we performed controlled DOX release form MNL combined with NIR light irradiation (Fig. 2c). Two drug release samples were prepared, one sample was freely released in shaking incubator, and the other was triggered with NIR irradiation for 3min when the time interval was 12 h. The drug release behavior of MNL at 37°C was very slow and the drug release rate was only 32% even 30 hours later. In contrast, the drug release of NIR light triggered sample was similar slow release before NIR irradiation and the drug release rate was increase more than 56% after irradiate NIR light for 3min. And then, the drug release was retained slow release mode again without NIR irradiation. This result shows the drug release from MNL combined with NIR light triggering almost meets the requirement of controlled drug release that can stably retain drug in physiological conditions and release it with stimuli existence.

3.2 In vitro Cytotoxicity

In addition, the tumor cell killing effect of MNL combined with NIR irradiation was evaluated (Fig. 2d). The cell survival rates of control group and MNL only group showed no significant differences. However, the drug loaded MNL group showed obvious tumor cell killing effect. Furthermore, the drug loaded MNL combined with NIR irradiation group showed much higher tumor killing effect. It may be caused by the enhanced drug release, the increased cell permeability and the photothermal therapy effect by NIR light irradiation [4, 12].

4. Conclusion

In this study, we provided a novel NIR light sensitive MNL, which contains SPIO nanoparticles and DOX, and verified the photothermal effect and the controlled release of DOX by NIR light irradiation. The rapid DOX release from MNL could be achieved by NIR light irradiation, which could be mainly attributed to the phase transition and the increased permeability of liposome membranes due to the heat diffusion from SPIO particles in liposomes. The significantly enhanced cytotoxicity for DOX and SPIO loaded MNL combined with NIR irradiation was achieved, which could be attributed the PTT effect, the increased intracellular DOX concentration due to the triggered release of DOX from MNL and the synergistic interaction between the photothermal effect and the cytotoxic effect of DOX. Therefore, we expect that this study can be used for a promising application of drug-loaded MNL using NIR irradiation for photothermal-chemotherapy in cancer treatment.

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