Magnetically Vectored Delivery of Cancer Drug Using Remotely On–Off Switchable NanoCapsules

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Hollow-sphere-structured magnetic nanocapsules containing intentionally trapped iron oxide nanoparticles and anticancer drugs have been prepared to provide a powerful magnetic vector under moderate gradient magnetic fields. It is shown that these nanocapsules can penetrate into the interior of tumors and allow a controlled ON–OFF switchable release of the anticancer drug cargo via remote 100 KHz RF field. This smart drug delivery system is nanoscale compact, with the drug molecules and magnetic nanoparticles contained within the hollow capsules having $\sim 80 \sim 150$ nm diameter. *In vitro* results using a mouse model indicate that such a nanocapsule-mediated, on-demand drug release is effective in reducing tumor cell growth.

***Index Terms*—Anticancer, drug release, hollow nanocapsule, magnetic nanoparticles, RF field.**

I. Introduction

FOR efficient use of many types of therapeutic drugs, a desired drug concentration range has to be maintained. The drugs tend to be somewhat ineffective at low levels, but are toxic beyond certain concentration ranges. Therefore, the controlled drug release approaches from various drug carriers by external stimuli such as temperature, electric or magnetic fields, light radiation, and pH changes [1]–[4] have received much attention for their potential in regulating and maintaining drug delivery. The controlled drug release improves the efficacy of the delivered drugs and minimizes toxic side effects [5]. However, most of the existing techniques are not capable of carefully controlled, on-demand drug release.

For therapeutic drugs to be most efficient, the placement of the drug-containing vehicles close to the disease region is essential [6]. In the case of anti-cancer therapies, there is a major challenge in the delivery of drugs because of the size and thickness of the multiple cell layers that need to be penetrated, and certain abnormal properties of the tumor itself, even with small molecule drugs [7], resulting in drug molecule penetration of only a few cell layers. Currently, no method overcomes these limitations. Therefore, a new delivery technique to allow deeper penetration of drug molecules into tumor aggregates is desirable.

Spherical carriers with hollow inside have become a subject of intense investigations in recent years for various applications including controlled drug release [8]–[10]. Some nanospheres ($\sim 50 - 200$ nm diameters) having pores in their outer shell allows for the chemical dissolution removal of the interior materials for synthesis of a completely hollow containers. The pores in the nanosphere shell can allow for drug loading and releasing based on a passive drug delivery system and slowed-down kinetics. Currently, there is no convenient and established way of a triggered, on-demand drug release from such hollow nanospheres.

Manuscript received July 09, 2012; revised September 26, 2012; accepted September 28, 2012. Date of current version December 19, 2012. Corresponding author: S. Jin (e-mail: jin@ucsd.edu).

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Digital Object Identifier 10.1109/TMAG.2012.2223202

Here, we describe novel and versatile drug-delivery hollow capsules containing a desired drug together with magnetic nanoparticles, which can provide a powerful magnetic vector for penetration into a tumor and on-demand drug release on external radio frequency (RF) stimulus.

II. Experimental Procedures

All chemical reagents utilized were procured from Alfa Aesar and Sigma-Aldrich, Inc. The nano-screenMAG/G-Chitosan particles (core: magnetite, 100 nm hydrodynamic diameter, ~40% in magnetic volume) were purchased from Chemicell GmbH (Berlin, Germany). The structure and properties of synthesized nanoparticles were characterized and the experimental measurements were carried out using a transmission electron microscope (FEI Tecnai G2 Sphera with 200 kV accelerated voltage), FT-IR (Nicolet 6700 analytical MCT FT-IR Spectrometer), SQUID (Quantum Design MPMS2) and UV/VIS spectrophotometer (Thermo BioMate3). For the generation of RF magnetic field, Lepel LSS-2.5 RF power supply equipped with a water cooled solenoid was used.

We have synthesized silica coated magnetic nanocapsules (SiMNCs) according to the previous paper [11]. A mixture of 24 g $FeCl_3 \cdot 6H_2O$ and 9.82 g $FeCl_2 \cdot 4\,H_2O$ was reacted with 50 ml of ammonium hydroxide at 80 °C for 1.5 hours after the addition of 3.76 g of oleic acid. The magnetite nanoparticles were washed and then were transferred *in situ* into octane. The magnetite-miniemulsion and styrene miniemulsion were prepared using ultrasound and microporous glass membrane (SPG membrane, SPG Technology Co., Ltd). With these emulsions, the mono disperse Fe_3O_4/polystyrene nanospheres were synthesized at 80 °C for 20 h in nitrogen atmosphere. The synthesized Fe_3O_4/polystyrene nanospheres were redispersed into 10 ml 0.5 wt.% polyoxyethylene sorbitan monolaurate aqueous solution. The suspension was added into 20 ml 2-propanol, and then 0.5 ml ammonium hydroxide and 20 μl TEOS were consecutively added into the above reaction solution. The silica encapsulation reaction was performed at room temperature for 48 h. The resultant silica magnetic nanospheres were collected by centrifugation and washed. For fabrication of hollow SiMNCs, the polystyrene (PS) within the silica shell was burnt out at 400 °C–500 °C for 3–6 h.

After the fabrication of the SiMNCs, externally triggered drug release measurements were carried out. The Camptothecin (Cpt) loading into the hollow SiMNCs was carried out using vacuum insertion approach The SiMNCs were placed in a small vacuum chamber ($\sim 10^{-4}$ torr) at room temperature for approximately 30 min. to rid nanopores of any trapped air. The chamber valve was then open and the liquid drug was allowed to get sucked into the chamber and the hollow capsules. ~1 mg of Cpt in 1 ml of THF (tetrahydrofuran) was loaded into 1 mg of SiMNCs. The amount of externally triggered drug release was measured by UV/vis spectrophotometer. Drug-loaded SiMNCs in a liquid medium were exposed to a 100 KHz RF field for 10 s. Then the SiMNCs were temporarily settled down to the container bottom by a magnet pull for a duration of 4 min. After the Cpt supernatants were cleared of floating particles, the drug content in the clear part of the solution was measured using UV/vis absorption. This step is denoted as the switch "ON" measurement. Then the SiMNCs were suspended again by stirring, and the solution was left for 5 min without RF to check on the leakage. Next, SiMNCs were settled down by magnet for 4 min, and the amount of the drug released was measured again by UV/vis spectrophotometer. This process represents the switch "OFF" state. The "ON–OFF" measurements were taken alternately and the drug release profile as RF field cycle was graphed.

We also performed cell viability test and deep tumor penetration experiments as described below.

a) **Cell viability:** The cells cultivated for *in vitro* experiments were MT2 mouse mammary tumor cells derived from a tumor of MMTV-c-Neu Tg mice and B16/BL6 mouse melanoma cells from Dr. Michael Karin's lab (University of California, San Diego, USA). MT2 cells were maintained in F12 medium (invitrogen) supplemented with 10% fetal bovine serum, 10 ng/ml EGF, 5 μg/ml of insulin, 1 μg/ml hydrocortisone, 100 U/ml penicillin, and 100 μg/ml of streptomycin and B16/BL6 cells were cultured on in 5% FBS in RPMI-1640 and supplemented with 2 mM L-Glutamine with 100 units/ml penicillin and 100 μg/ml streptomycin. All cells were cultured at 37 °C in a 5% CO_2 and 95% air humidified atmosphere. Initially counted MT2 and B16-BL6 cells were seeded into 4 plates (35 × 10 mm style tissue culture plates) in 2 ml of complete media and incubated overnight. After that, four different nanoparticle preparations were added to the cells two plates, 2 ml media with emptied capsules; another two plates, 2 ml media with including drug-loaded capsules- drug release was performed with vs. without RF for the two sets of two. After 2 h incubation, fresh 2 ml media was replaced. The B16/BL6 cells were incubated for 12 h and the MT2 cells were incubated for 48 h after either no exposure or exposure to RF. After incubation, MTT assay was performed to determine the viability of the cells and normalized to cells incubated with empty capsules without RF field.

b) **Deep tumor penetration:** subconfluent MT2 cells were trypsinized and evenly resuspended at a density of 2×10^5 cells/3 ml of F12 medium containing 0.4% agar, antibiotics, and 7.5% fetal bovine serum. Cell suspensions were layered in 6-well dishes over a 0.7% agar base. Cells were grown at 37 °C with an addition of 0.5 ml of normal medium every 3 days. One week later, colonies were collected and seeded onto poly-L-Lysine-coated cover slips and incubated at 37 °C for overnight. The magnetic nanocapsules were applied to the 1 ml medium and incubated for two hours with or without magnetic field of ~1,000 Oe (or an average gradient of ~2,000 Oe/cm). The magnet was placed at the bottom of the dish with ~0.5 cm distance from the colony.

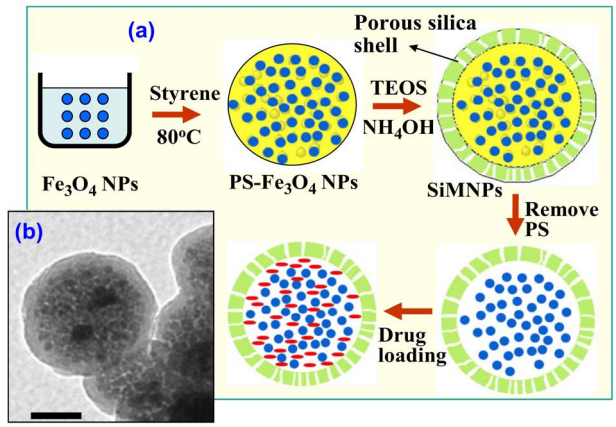

Fig. 1. Hollow SiMNCs as a drug carrier. (a) Schematic illustration of the process for drug-trapped hollow SiMNCs. (b) TEM micrograph showing trapped magnetic nanoparticles in SiMNPs. Scale Bar = 50 nm.

III. Results and Discussion

A multiple-step synthesis technique was employed to produce silica coated magnetic nanoparticles (SiMNPs). The magnetic particles were placed within the interior of SiMNPs as the trapped iron oxide nanoparticles (~10 nm diameter). Such a structure was obtained by using the polystyrene (PS) emulsion approach. The PS nanoparticles (~100 nm average diameter) were then coated with silica shell which is nanoporous, and the PS removed by either solvent dissolution or by burning. Shown in Fig. 1(a) is a schematic illustration of such hollow silica capsule, with trapped magnetic nanoparticles, into which the drug molecules were inserted. The PS inside the particle can be dissolved or burnt out to create the hollow core space [12] for subsequent drug insertion [Fig. 1(a)]. The TEM micrograph [Fig. 1(b)] represents the resultant silica coated core/shell spheres with a high density of trapped magnetic particles. The diameter of core with high dense magnetic particles is ~100 nm and the thickness of silica shell is ~25 nm. The amount of Fe_3O_4 nanoparticles is also estimated to be ~80 wt% (~45 volume %) [13].

Switchable ON–OFF release of hydrophobic drug, Camptothecin (Cpt), from our hollow SiMNCs was demonstrated by applying RF field. Fig. 2 shows the drug release data based on a hydrophobic drug carrier system using our SiMNC and Cpt, presenting the measured amount of released drug during ON–OFF switch cycle of applying the RF magnetic field (at 100 kHz).

It is apparent from Fig. 2 that a dramatic change in the amount of released drug occurs when the remote magnetic field is switched "ON" versus "OFF". Such drug release ON–OFF cycles are repeated at least several cycles in the experiment. Uniquely, we have designed our system so that the empty

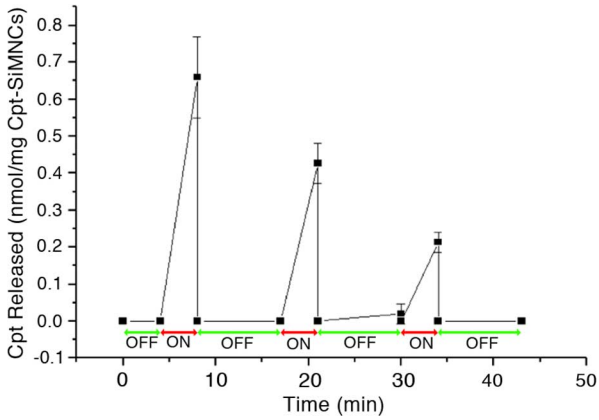

Fig. 2. ON–OFF controlled drug release from Cpt-containing SiMNCs by RF magnetic field.

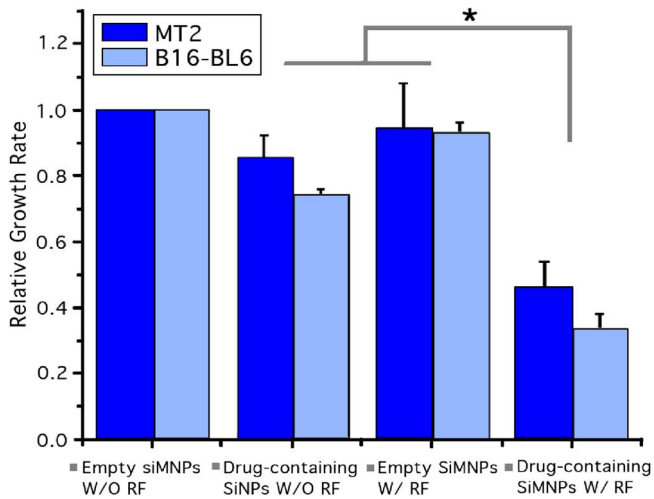

Fig. 3. Comparative growth rates (MTT assay) of B16/BL6 and MT2 in the presence as absence of activated SiMNCs. P values after performing ANOVA (Student-Newman-Keuls Method) reaching statistical significance $P < 0.05$ are marked on the graphs: * denotes significance between bracketed groups.

space in the hollow core can contain hydrophobic drugs, while the porous interior of the silica shell and the outer silica shell surface has hydrophilic characteristics, which will naturally resist the leakage of hydrophobic drug once loaded based on opposing surface properties. Therefore, in principle, the trapped hydrophobic drug in the interior of SiMNCs does not easily diffuse out of the capsule without additional stimuli to overcome the hydrophilic shell surface repulsion.

It is hypothesized that the remotely applied magnetic field primarily induces magnetic particle heating and causes the localized liquid temperature inside the nanosphere to rise. This mechanism is in agreement with the well-established magnetic nanoparticle heating in the case of magnetic hyperthermia treatment of cancer cells [14]. We have demonstrated that the temperature of an aqueous solution containing iron-oxide nanoparticles can be raised substantially (as high as $\sim 60\,^{\circ}\mathrm{C}$) using 100 kHz RF magnetic field stimulation [11]. The rate of drug diffusion during the "on-demand" cycle of drug release is controllable by the degree and duration of magnetic heating as well as the predetermined nanoporosity of the silica shell material and the amount of magnetic materials and nanocapsules involved. The resultant temperature gradient between inside as. outside of the sphere is a key element in inducing the diffusional delivery of the stored drug on-demand when the field is applied for a specific duration.

To evaluate potential applicability of the cancer cell treatment for *in vivo* drug delivery, the growth rates of B16-BL6 and MT2 cells were evaluated in the presence of SiMNCs. Fig. 3 shows the viability of cells in a media with empty SiMNCs (e-SiMNCs) and Cpt-loaded SiMNCs (Cpt-SiMNCs) with (+RF) vs without (−RF) the exposure to RF. First, it should be noted that the cell viability was tested by exposing the cells to RF field in the absence of particles in order to ensure that the cell viability was never compromised upon control exposure of RF (data not shown). Secondly, no noticeable change in MTT viability (Fig. 3) was observed in the cells incubated with e-SiMNCs for both cases of with and without RF exposure. This result implies that the RF and SiMNC exposures by themselves do not give any negative influence on the growth properties of cells. There was slightly less viability of cells incubated with Cpt-SiMNCs vs e-SiMNCs without RF field exposure (control), possibly due to slow diffusional leakage through the nanopores of the shell (estimated to be a few nm size). However, a dramatic change in viability is seen in cells incubated with Cpt-SiMNCs and exposed to RF field. The on-demand RF exposure caused increased diffusional drug release rates, which caused a much decreased viability in both cancer cell lines (Fig. 3). The novelty of our system is that in the switch "OFF" state (without RF exposure), the diffusional drug release was minimized, but in the switch "on" state (with RF exposure), the drug release is maximized for therapeutic efficacy by delivering a desired drug concentration to the *in vitro* cancer cells causing a decrease in viability. The slow leakage of drugs from unactivated capsules can be substantially reduced by a temporary coating of a biodegradable polymer or a lipid monolayer, which will be reported in future publications.

For further characterization of the SiMNPs, the M-H magnetization loop of the capsules was measured as shown in Fig. 4(a). Compared with the same amount of loose magnetic nanoparticles with similar individual particle size (∼10 nm), and commercially available nano-screenMAG particles, which have the same overall particle size as our SiMNP (∼150 nm) and contain comparable amount of magnetic Fe_3O_4 particles and fluorophores for visualization in the core, our SiMNPs provide substantially improved magnetization ($\sim \times 13$ times and $\sim \times 1.5$ times higher at 500 Oe, respectively) over these other types of magnetic particles. This can be attributed to the proximity and interaction of magnetic particles trapped in the capsule-confined geometry. The higher magnetization property of our SiMNPs is important for magnetic vector enhancement for the purpose of guiding the capsule through cell boundaries and cell cytoplasm toward the interior of the cells or deep into the midst of tumor cell aggregates. Fig. 4(b) shows that SiMNP movements in an aqueous solution, in the presence of ∼500 Oe applied field at ∼1.5 cm distance from a Sm-Co permanent magnet, exhibit superior magnetic vector at a movement speed of ∼0.24 cm/sec. By contrast, the speed of dispersed nano-screenMAG in the same solution was ∼4 times slower under identical magnetic field. These high-density trapped magnetic nanoparticles inside the silica shell can exclusively provide our drug-containing SiMNCs a strong penetrating

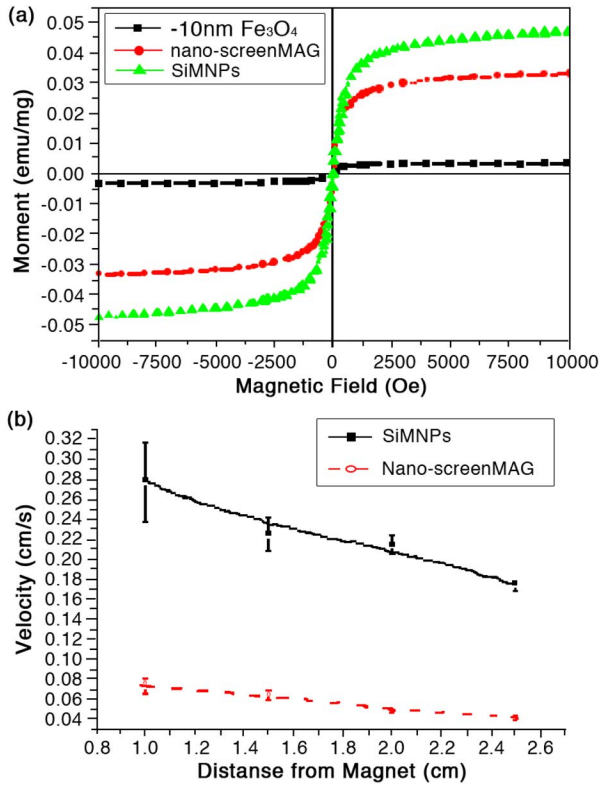

Fig. 4. (a) M-H loops showing a significant increase in magnetic strength in SiMNPs configuration. (b) Velocity of magnetic nanocapsule movement in a fluid as distance from the magnet (1 cm and 2.5 cm distance represent ∼900 Oe and ∼200 Oe, respectively).

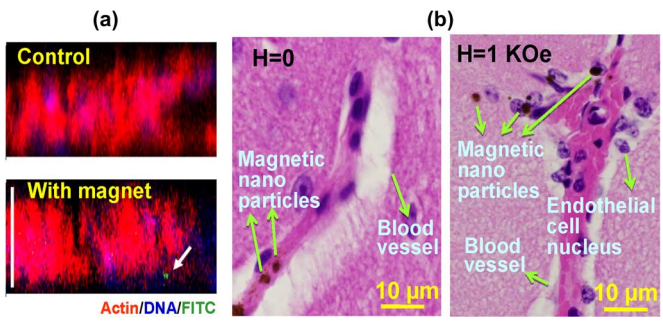

Fig. 5. (a) Confocal microscopy images of the nanoparticle (green dot) penetration of MT2 colony using magnetic force (scale bar $= 50\ \mu$m). (b) H&E image showing BBB crossing of magnetic nanocapsules by applied field.

power into mouse cancer colony, for instance, under a moderate applied magnetic field of several hundred to thousand oersteds. In our observations, the SiMNPs' incredible driving force penetrated through ∼10 cell layers, which is very encouraging for efficient cancer treatment to deal with thick layers of tumor tissue. Fig. 5(a) shows the efficient penetration of the capsules into the *in vitro* cultured cancer colony. The green-fluorescent nanoparticles (see the arrow) were magnetically driven by the applying a magnetic field of ∼1000 Oe to penetrate the cancer colony all the way through the tissue, which afterwards appear at the bottom of the colony. Shown in Fig. 5(b) is a 10-week old mouse brain model H&E (hematoxylin and eosin stain) section imaging. This preliminary data shows that the tail-vein injected magnetic nanocapsules in the blood can penetrate the blood brain barrier (BBB) in mouse brain at ∼1000 Oe level applied magnetic field at ∼1 cm distance from the mouse brain. Such BBB crossing can be useful for brain cancer treatment as well as for therapeutics of central nervous system diseases such as Alzheimer's Disease.

IV. Conclusion

Triggerable nanocapsules of 80–150 nm size (containing magnetic nanoparticles together with a loaded drug inside the hollow spheres) have been successfully created. The capsules can provide a powerful magnetic vector for tumor penetration, and are responsive to remote RF field for ON–OFF switchable drug release. *In vitro* results indicate that the drug-containing capsules are effective in reducing tumor growth.

Acknowledgment

This work was supported in part by Iwama Fund at UC San Diego and NSF under Grant DMR-1006081.

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