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Hybrid Iron Oxide-Graphene Oxide-Polysaccharides Microcapsule: A Micro-Matryoshka for On-demand Drug Release and Antitumor Therapy In Vivo

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KEYWORDS. microcapsules, layer-by-layer, magnetic hyperthermia, photothermal therapy, on-demand release

ABSTRACT. Premature drug release is a common drawback in stimuli drug delivery systems (DDS) especially if it depends on internal triggers, that are hard to control, or a single external stimulus, that can only have one function. Thus, many DDS systems were reported combining different triggers, however limited success has been established in fine-tuning the release process mainly due to the poor bioavailability and complexity of the reported designs. This paper reports the design of a hybrid microcapsule (h-MC) by a simple *layer-by-layer* technique comprising polysaccharides (Alg, Chi, HA), iron oxide, and graphene oxide. Electrostatic assembly of the oppositely charged polysaccharides and graphene sheets provided a robust structure to load drugs through pH control. The polysaccharides component ensured high biocompatibility, bioavailability, and tumor cells targeting. Alternative magnetic field and near infrared laser triggerable Fe₃O₄@GO component provided dual high energy and high penetration hyperthermia therapy. On-demand drug release from h-MC can be achieved by synchronizing these external triggers, making it highly controllable. The synergistic effect of hyperthermia and chemotherapy was successfully confirmed *in vitro* and *in vivo*.

INTRODUCTION

Stimuli responsive drug delivery systems (DDS) have emerged as an extremely functional platform in targeted tumor therapies.¹⁻⁴ However, the major mechanism of action for most of

these reported systems involves a “burst release effect”, where drug molecules are prematurely released in one shot before reaching the target site.⁵⁻⁷ Therefore, different stimuli were employed to further control and tune drug release including intracellular physiological signals such as, temperature,⁸ pH,^{9, 10} and redox.¹¹ However, systems operating under these triggers can only deliver drug passively. Moreover, these signals only show small variations between cancer cells and healthy cells, which reduce the sensitivity of DDS response.¹²⁻¹⁴ On the other hand, external stimuli such as electrical field,¹⁵ alternative magnetic field (AMF),¹⁶⁻²⁰ ultrasound,²¹ light²² or near infrared (NIR) laser irradiation have been vastly used in DDS.^{11, 23-27} Among these triggers, AMF and NIR irradiation attracted much attention because their synergic therapy can be used to overcome drug resistance and the unfavorable side effects of chemotherapy. DDS coupled with AMF or NIR irradiation as external triggers can release drug on demand, and also be used as agent for hyperthermia therapy provided a high uptake of the designed DDS is guaranteed to ensure spreading the heat and delivering the drug to the entire tumor issue. Comparing these two external triggers, AMF can penetrate deeper tissue but falls short from eliminating cancer cells at once owing to the heterogeneous heat distribution and low energy conversion.²⁸⁻³⁰ On the other hand, NIR irradiation has shown higher efficiency in eradicating cancer cells, due to its relatively high energy conversion,^{11, 31-35} but the narrow penetration range greatly limits its applicability.^{36, 37} Thus, combining both triggers in one highly bioavailable DDS can provide much of the needed control over the drug release process and consequently antitumor therapy.^{38, 39} Herein, we report the “micro-Matryoshka”, which is a hybrid microcapsule comprising polysaccharides, graphene oxide and iron oxide, as a multifunctional therapeutic platform. This biocompatible, easily scalable, and smart design innovatively improves DDS targeting, bioavailability, and eliminates the traditional drawbacks of conventional hyperthermia.

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3 Natural polysaccharides, such as sodium alginate (Alg) and chitosan (Chi), are usually
4 employed in DDS due to their biocompatibility and stimuli responsiveness (pH- and
5 temperature-responsive).^{40, 41} Moreover, polysaccharides have high adhesion capability so they
6 readily interact with cell membranes followed by internalization and chitosan have relatively
7 higher glass transition temperature (more than 203 °C) and rigid structure,⁴² which make them
8 more tolerable to the high temperature during hyperthermia. Hyaluronic acid (HA) is also one of
9 the most used natural polysaccharides specifically because of its higher bio-distribution in tumor
10 tissue. It is well known to degrade faster in cancer cells as hyaluronidase, the enzyme responsible
11 for breaking down HA, is overexpressed in most cancer cells.⁴³⁻⁴⁵

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13 Graphene oxide (GO) is one of the promising nano-carbon materials for use in drug delivery
14 systems since it can be triggered by NIR irradiation and has better colloidal stability and
15 dispersibility in water compared to carbon nanotubes.⁴⁶⁻⁴⁸ Graphene hollow capsules showed
16 extremely high drug (doxorubicin, DOX) loading compared to other GO composites with
17 triggered NIR release.⁴⁹ This is attributed to the strong sheet-like structure of GO, stabilizing the
18 capsule multilayers to avoid drug leakage.⁵⁰⁻⁵⁴ GO structure also plays a vital role in anchoring
19 magnetic nanoparticles such as Fe₃O₄ and FeCo. Fe₃O₄ decorated GO was also well studied in
20 synergistic magnetic hyperthermia and photothermal therapy.⁵⁵⁻⁵⁸

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22 This work presents an easily assembled hybrid DDS platform comprising iron oxide-
23 decorated GO nanosheet incorporated within Alg/Chi microcapsule (via LbL technique)⁵⁹⁻⁶² with
24 HA shell for the first time (Scheme 1). The iron oxide decorated GO nanosheet is envisioned to
25 be the functional layer between the alternating oppositely charged polysaccharides layers, so the
26 word “Matryoshka” can be used to illustrate our hybrid microcapsules. Matryoshka is a Russian
27 nesting doll. It refers to a set of wooden shells of decreasing size placed one inside another. Each
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layer during layer-by-layer assembly could be interpreted as “wooden shell” of Matryoshka. Most importantly, it can be employed as a dual-responsive trigger induced by both NIR and magnetic hyperthermia while the polysaccharides ensure high bioavailability and tumor accumulation (due to HA shell). Combining photothermal therapy (higher energy), magnetic hyperthermia (deeper penetration), and targeted chemotherapy (higher accumulation) in a biocompatible carrier, generated a synergistic effect to efficiently annihilate cancer cells.

RESULTS AND DISCUSSION

Preparation and Characterization of Hybrid Microcapsule

Monodisperse spherical MF (HCl-soluble melamine formaldehyde resin) particles with a diameter of $\sim 2 \mu\text{m}$ were employed as a sacrificial template. Fe_3O_4 -decorated graphene oxide ($\text{Fe}_3\text{O}_4@\text{GO}$) was synthesized by a co-precipitation method with size of $191.2 \pm 7.8 \text{ nm}$ (measured by dynamic light scattering (DLS) method) and Zeta potential of $-14.2 \pm 6.48 \text{ mV}$. Alginate acid (Alg), chitosan (Chi) and hyaluronic acid (HA) were then used to prepare microcapsules applying layer-by-layer (LBL) method (refer to experimental section). MF particles were etched by HCl to yield the hollow center for DOX loading (Scheme 1). The LBL assembly progress of different polysaccharide layers and $\text{Fe}_3\text{O}_4@\text{GO}$ was monitored by Zeta potential measurements (Figure S1). The hybrid microcapsules with $\text{Fe}_3\text{O}_4@\text{GO}$ are denoted as Alg/Chi/ $\text{Fe}_3\text{O}_4@\text{GO}$ /Chi/HA (h-MC); and the microcapsules with only polysaccharides are denoted as Alg/Chi/Alg/Chi/HA (MC) and used as a control.

The morphology of microcapsules at the air-dried state can be seen in TEM images. The distribution of $\text{Fe}_3\text{O}_4@\text{GO}$ can be easily confirmed comparing the TEM images of h-MC and MC (Figure 1). TEM images of h-MC and MC before etching the MF templates are included in Figure S2. From the inductively coupled plasma test (ICP-OES), the iron ion concentration in

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3 1×10^9 h-MC was found to be 0.32 μg . The characteristic Raman peaks of GO (G band and D
4 band) and Fe_3O_4 can be seen in Figure S3, which further supports the successful consecutive
5 coatings. Owing to the presence of Fe_3O_4 in the wall of microcapsules, h-MC also showed
6 magnetism, as revealed by the field-dependent magnetization measurement (Figure S4). The
7 obtained magnetization value for h-MC was 1.1 emu/g. This can be explained by the presence of
8 polysaccharide layers and the GO that can disorder the spins of Fe_3O_4 causing a reduction in the
9 overall magnetization value. Moreover, the magnetic character allowed these microcapsules to
10 be heated by magnetic hyperthermia system (MHS). h-MC ($5 \times 10^9/\text{mL}$, 1 mL) was heated by
11 MHS with fixed parameters (7 kW, 80% output, 375 kHz) (Figure 2A). After 10 min, the
12 temperature was increased by 9.4 $^\circ\text{C}$ while water and MC solution could not be heated ($< 2^\circ\text{C}$).
13 The photothermal effect of incorporating GO (NIR into heat) was then verified as h-MC
14 irradiated by NIR laser ($0.5 \text{ W}\cdot\text{cm}^{-2}$, 808 nm) showed a temperature increase of 18 $^\circ\text{C}$ compared
15 to control solutions (Figure 2B).

Biocompatibility and Enhanced Accumulation of h-MC

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37 After investigating the magnetic properties and NIR conversion ability of the h-MC, it was
38 then tested as a cancer therapy platform. First, the cytotoxicity of h-MC was evaluated by
39 incubating HeLa cells with eight different ratios of microcapsules (MC and h-MC) for 24 h
40 followed by standard CCK-8 assay. In different number ratio (microcapsules to HeLa cells, 50:1,
41 10:1, 5:1, 1:1, 0.5:1, 0.1:1, 0.05:1, 0.01:1), h-MC did not show obvious toxicity since all the
42 polysaccharides employed in the microcapsules are biocompatible (Figure S5).

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50 Then, the cellular internalization of h-MC was investigated by incubating FITC-labelled h-
51 MC with HeLa cells for 12 h at 37 $^\circ\text{C}$. The nuclei were stained by Hoechst 33342. The co-
52 localization of h-MC (Figure S6) was then determined using confocal laser scanning microscopy

(CLSM). Z-stack was performed to identify the h-MC location, showing the orthogonal view of the three planes (x/y/z) of the internalized h-MC and tight binding to cell membrane. This verified cancer cell targeting and bioadhesion abilities of HA (Figure S6). Moreover, magnet (static magnetic field) and relatively low power NIR laser ($0.25 \text{ W}\cdot\text{cm}^{-2}$, 808 nm) with short time irradiation (5 min) were used to improve the cell internalization and bioadhesion. Magnetic field could be actively used to improve the cell internalization and bioadhesion of h-MC (Figure 3B') compared to the control (Figure 3A'). Low power NIR-induced heat can further increase the lipid fluidity of cell membrane without any serious injuries to the cells, inducing higher cell internalization (Figure 3C'), compared to the control (Figure 3A'). Thus, enhanced bioadhesion of h-MC to cell membrane was confirmed while MC samples (Figure 3A, 3B and 3C) did not show significant difference during magnet (Figure 3B) and NIR laser treatments (Figure 3C). These results were further confirmed by flow cytometry (Figure 4). The fluorescence of FITC-labelled h-MC in the cells or attached to the cells can be enhanced around 17-20% with the treatments with magnet or NIR laser (Figure 4B), however, the fluorescence of FITC-labelled MC is unchanged with different treatments (Figure 4A).

On-Demand Release of Doxorubicin in Solution

Doxorubicin (DOX) was employed as a model drug and mixed with h-MCs in ABS buffer (pH 5.5) at 37°C for 2 days. DOX can diffuse into the microcapsules and reach the osmotic equilibration. Then, h-MCs were washed by PBS buffer (pH 7.2) and the excess DOX was removed. According to the calibration curve of DOX, the average loading efficiency of DOX was calculated to be 13.2%, and the loading amount was $129.4 \mu\text{g}$ in 1×10^9 microcapsules. The cumulative release amount of DOX from MC (Figure 5A and 5C) and h-MC (Figure 5B and 5D) with or without magnetic hyperthermia (MHS, 7 kW, 80% output, 375 kHz, coil dimensions are

D=50 mm, H=50 mm, and number of turns is N=5) or NIR laser ($0.5 \text{ W}\cdot\text{cm}^{-2}$, 808 nm) was then investigated by testing the time dependent UV-vis spectra of supernant after dispersing the DOX-loaded microcapsules in buffer (pH 5.5 or 7.4).

Some interesting facts were shown during the DOX release process of MC and h-MC. First, it was obvious that the release rate of both MC and h-MC at pH 5.5 was much larger than that at pH 7.2, which confirms that they are pH-sensitive systems. This is due to the nature of the microcapsules that are comprised of weak polyelectrolytes. The intermolecular interactions between COOH and NH_2 groups in different polyelectrolyte chains could be weakened in an acidic environment, inducing a higher permeable structure. This is beneficial in cancer therapy, as the tumor is known for its mildly acidic environment (pH ~ 6.8). In addition, the endosome or lysosome of cancer cells can provide more acidic environment (pH 4.5~5.5) after the microcapsules are internalized and thus increasing the bioavailability of the drug. h-MC displayed relatively slower drug release than that of MC because the existence of GO layer restricted DOX diffusion. This can effectively reduce the unwanted leakage during delivery.

More importantly, h-MC showed a physically triggered release compared to MC. Since alternating magnetic field with high frequency is known to heat the inner magnetic particles and NIR is also known to cause local heating through the photothermal conversion of GO, the induced heat would destroy the intermolecular configuration of the $\text{Fe}_3\text{O}_4@\text{GO}$ layer. When the h-MC was treated with magnetic hyperthermia system (MHS) or irradiated with NIR laser, the $\text{Fe}_3\text{O}_4@\text{GO}$ in the wall of microcapsules heated their surroundings and induced the relaxation of polymer chains and even disassembly of polyelectrolyte interactions. Moreover, external alternating magnetic fields in MHS induced mechanical vibration and motion of Fe_3O_4 , which can increase the stress in the wall of h-MC or even destroy it. All these physical factors

synergistically contribute to the increased permeability and on demand release from h-MC (Figure 5B and 5D). The accelerated release zones are highlighted in gray, showing the “open” stage. However, as a control experiment, this phenomenon could not be observed in MC (Figure 5A and 5C). The accelerated release is proved to be halted in h-MC when the MHS or NIR is removed. As a protective layer, chitosan has a relatively high glass transition temperature (T_g), which makes microcapsule robust and the assembled structure would not be severely destroyed during the MHS treatment or NIR irradiation. Moreover, the polysaccharides could form a “gel-like” structure through hydrogen bonding between chains, which are destroyed upon applying MHS or NIR. Removing the stimuli allows for the hydrogen bonding to form again and the h-MC returned to their original state, resulting in stopping the release. This further confirms that the release from h-MC is an on demand process and not a premature burst release.

Antitumor Therapy *in vitro*

To estimate the cancer therapy efficacy of h-MC, a series of cell viability studies were conducted on HeLa cells incubated with different samples under different conditions (Figure 6). The added samples were free DOX (5 $\mu\text{g/mL}$ DOX), MC (MC, $\sim 5 \times 10^7$), h-MC (h-MC, $\sim 5 \times 10^7$), DOX-loaded MC (DOX/MC, equiv. 5 $\mu\text{g/mL}$ DOX) and DOX-loaded h-MC (DOX/h-MC, equiv. 5 $\mu\text{g/mL}$ DOX), separately. HeLa cells with no sample (Cells, $\sim 1 \times 10^6$) were also evaluated as controls. All the HeLa cell groups were first incubated with different samples for 24 h. Then, one plate from each group was treated by MHS for 30 min; the second plate was irradiated by NIR laser for 30 min; the third plate was treated by both MHS and NIR. The mixture of HeLa cells and samples were later collected and placed in 96-well plates for another 4 h of incubation. Finally, the relative cell viability was measured by CCK-8 assay (Figure 6). As shown in Figure 6, magnetic hyperthermia and NIR laser irradiation did not show toxicity to

HeLa cells from the control groups (cells) and MC groups (MC) as there are no magnetic materials in their plates, and the cell culture media or polysaccharides used in MC cannot convert any NIR light into heat. Even for the DOX group (DOX) or DOX-loaded MC group (DOX/MC), the cell death is only caused by the chemotherapeutic effect of DOX. The cell viability of group (DOX/MC) is higher than (DOX) since DOX cannot be totally released in the experimental time slot, which also can be confirmed by the release curves. On the other hand, the cell viability of the group (DOX/h-MC) was highly affected by MHS or/and NIR treatments. It showed the lowest cell viability with both treatments.

The Live/Dead assay was also performed to display the difference in cell death visually (Figure 7). In the experiments, it is found that the microcapsules have very strong absorption to live/dead dye. Therefore, only a low portion of microcapsules were used and they could not kill a large number of cells, comparing to the cytotoxicity experiments (Figure 6). However, these effects of AMF or NIR still can be observed. Control (Cells), MC (MC, $\sim 1 \times 10^6$), h-MC (h-MC, $\sim 1 \times 10^6$) were exposed to MHS or/and NIR treatments. Control and MC groups did not show obvious difference in cell death. However, in h-MC group, MHS or/and NIR treatments induced higher cell death.

Antitumor Therapy *in vivo*

In-vivo efficacy of this synergistic therapy was verified in HeLa-bearing BALC/nude mice. First, BALC/nude mice were injected in the right front leg with HeLa cell line. All the mice were divided into five groups (3 mice in each group). DOX-loaded h-MC was injected into tumors (intratumoral) of groups 2-5 (Figure 8), the injection dose of DOX was 0.25 mg/kg. Saline was injected into tumors of group 1 (intratumoral) as control. After 1 h, the tumors of group 3 were treated with MHS (6 kW, 100% output, 425 kHz) for 30 min. The tumors of group 4 were

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3 irradiated with NIR laser ($2\text{ W}\cdot\text{cm}^{-2}$, continuous) for 3 min. The tumors of group 5 were treated
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5 with both MHS and NIR. The size of tumors was recorded during therapies (Figure 8). Finally,
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7 the mice were sacrificed, and then the tumors were fixed and sectioned for histology analysis
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9 (Figure 9 and S7). In a characteristic tissue section, nuclei are stained blue by haematoxylin and
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11 extracellular matrix is stained pink by eosin (H&E staining). Comparing the histology analysis,
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13 magnetic hyperthermia and NIR irradiation associated chemotherapy (group 5) showed higher
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15 efficiency than either of them only. After 5 rounds of treatment, group 5 showed highest cell
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17 death and tissue necrosis. Moreover, granulation tissue was also observed. Therefore, combined
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19 hyperthermia therapy (magnetic and photothermal) can achieve better synergy with
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27 **CONCLUSIONS**
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29 In summary, we have developed a new therapeutic hybrid platform (h-MC) by a simple and
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31 feasible layer-by-layer technique comprising polysaccharides (Alg, Chi, HA), iron oxide, and
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33 graphene oxide. Electrostatic assembly of the oppositely charged polysaccharides provided a
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35 robust structure to load drugs through pH control and the exterior HA shell/ coat enhanced
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37 internalization and bioadherence to cancer cells with magnetic field or mild NIR irradiation. A
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39 dual magnetic hyperthermia and NIR triggered $\text{Fe}_3\text{O}_4@\text{GO}$ layer provided better scope of
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41 hyperthermia effect. On-demand drug release from h-MC can be achieved by applying
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43 synchronizing these external triggers, making it highly controllable. This synergistic effect of
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45 hyperthermia and chemotherapy was confirmed in vitro and in vivo. Such biocompatible hybrid
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47 microcapsules are promising for further clinical testing and broader biomedical applications.
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MATERIALS AND METHODS

Materials

Doxorubicin (DOX), Phosphate buffered solutions (PBS, pH 7.4) and sodium acetate buffer solution (ABS, pH 5.5) were purchased from Sigma-Aldrich and diluted to 10 mM before use. The other organic solvents were purchased from Sigma-Aldrich and used without any further purification. The human cervical tumor cell line (HeLa) was purchased from ATCC (USA). EMEM medium supplemented with 10 % fetal bovine serum was purchased from Invitrogen (USA). 4, 6-diamino-2-phenylindole (DAPI) was obtained from Sigma-Aldrich. Graphene Oxide (GO) was purchased from Nanjing XFNANO Materials Tech Co., Ltd (China). HCl-soluble melamine resin (MF) particles: 5 %w/v (50 mg/mL), diameter: 2.07 μm , particle concentration: 7.1×10^9 /mL. (Microparticles GmbH, Germany)

Preparation of nano Graphene Oxide (nanoGO)

The suspension of nanosize Graphene Oxide sheet (nanoGO) was prepared by ultrasonication of the GO solution. Briefly, NaOH (200 mg) and Chloroacetic acid (150 mg) were added to 10 mL of GO suspension (1 mg/mL) and the mixture was stirred at 45 °C for 4 h in a circulating water bath. Then, the solution was sonicated by a Sonics VCX 500 (500 W; Sonics & Materials Inc., USA) system with probe (Sonics cv334) for 1 h. The parameters were set as: Output=70 %, On=2 seconds, Off=4 seconds. After sonication the suspension was neutralized by dialysis (3,500 Da) against DI water for 2 days with frequent change of water. The neutralized suspension was further sonicated for 1 h and then centrifuged to remove the large aggregates. The nanoGO solution was stored at 4 °C for characterization and further use.

Preparation of iron oxide-loaded graphene oxide ($\text{Fe}_3\text{O}_4@\text{GO}$)

NanoGO was mixed and stirred in NaOH aqueous solution (pH 12) for several hours to transform the carboxylic acid groups to carboxylate anions, followed by dialysis against water until the dialysate became neutral. The resulting product was then purged with N₂ for 30 min. A solution of FeCl₃·6H₂O and FeCl₂·4H₂O (Fe³⁺:Fe²⁺=1:2) in water (5 mL) was added to the flask. The mixture was stirred overnight under N₂ for ion exchange. After removing excess iron salts, the solid product was redispersed in water and then NaOH aqueous solution (2 mL, 5 M) was added dropwise under N₂. The mixture was kept stirring at 65 °C for extra 2 h. Then the mixture was washed with water.

Preparation and Characterization of Microcapsules

Two types of microcapsules without or with Fe₃O₄@GO were prepared according to the followed steps:

1. The MF particles (1% w/v in water) were incubated in alginate solution (0.5 mg/mL in 0.5 M NaCl) for 30 min under continuous shaking. The coated microparticles were collected through centrifugation and washed 3 times with water.
2. Then they were incubated in chitosan solution (0.5 mg/mL in 1% acetic acid aqueous solution containing 0.5 M NaCl) using the same procedure.
3. The coated microparticles were incubated in Fe₃O₄@GO aqueous solution (equiv. 0.01 mg/mL of GO) for 60 min under continuous shaking, then washing and being collected.
4. Same as 2.
5. The microparticles were incubated in hyaluronic acid solution (0.5 mg/mL aqueous solution containing 0.5 M NaCl) using the same procedure.

The multilayer coatings with different configurations were formed by alternate deposition of corresponding materials. Fe₃O₄@GO in the 3rd step could be replaced by alginate solution as 1st

step for the preparation of capsules without $\text{Fe}_3\text{O}_4@\text{GO}$ as a control. Finally, the hollow microcapsules were obtained after removing the MF templates by using HCl solution (pH ~ 1.1). The microcapsule without $\text{Fe}_3\text{O}_4@\text{GO}$ was denoted as Alg/Chi/Alg/Chi/HA (MC); and the microcapsule with $\text{Fe}_3\text{O}_4@\text{GO}$ was denoted as Alg/Chi/ $\text{Fe}_3\text{O}_4@\text{GO}$ /Chi/HA (h-MC).

The FITC labelling of microcapsules involves the following steps. First, chitosan solution (0.5 mg/mL in 1% acetic acid aqueous solution) was mixed with FITC ethanol solution. The primary amine of chitosan to FITC ratio was about 5:1. Then, the mixture was purged with nitrogen for 10 min and stirred for 6 hours in dark. Second, the FITC-labelled chitosan was precipitated by adding 0.1 M NaOH to the mixture. It was then centrifuged and washed with ethanol (70% in water). Third, the FITC-labelled chitosan was re-dissolved in 1% acetic acid and dialyzed against water for 2 days. Finally, the FITC-labelled chitosan was lyophilized for further use. During layer-by-layer assemble; chitosan solution (above-mentioned step 4) was replaced to FITC-labelled chitosan solution. The final microcapsules will be denoted as FITC-labelled MC and FITC-labelled h-MC.

The LBL products were studied by a Malvern NanoZS Zetasizer. The morphology of microcapsules was observed by TEM. The Fe content in h-MC was measured by inductively coupled plasma optical emission spectrometry (ICP-OES). The h-MC suspension was centrifuged and the solvent was removed, then nitric acid was added to make ferric ion in the solution. The magnetic properties of h-MC (dried state) were measured by a commercial Quantum Design superconducting quantum interference device (SQUID) magnetometer.

Characterization

Zeta potential (ζ) was measured by Malvern NanoZS Zetasizer at 20 °C. Raman spectrometer LabRAM Aramis (Horiba Jobin Yvon) was employed for Raman spectra and the range of 100–

3500 cm^{-1} was explored. Transmission electron microscopy (TEM) images were obtained with a FEI Tecnai T12 microscope. TEM samples were prepared by drop casting aqueous dilute solutions onto carbon-coated Cu grids, and drying them in air for 12 hour before the measurement. UV-vis-NIR spectra of Doxorubicin (DOX) during release test were recorded with Varian 5000 UV-Vis-NIR spectrophotometer.

Magnetic hyperthermia effect of h-MC

Magnetic hyperthermia system (MHS, 7 kW, output power of 80 %, 375 kHz) was obtained from MSI Automation (USA). h-MC dispersion (5×10^9 /mL, 1 mL) was put in NMR tube and treated with MHS. The real-time temperature was recorded by Neoptix Reflex Fiber Optic Signal Conditioner (Neoptix inc.).

NIR triggered Photothermal effect of h-MC

NIR laser was obtained from Changchun New Industries Optoelectronics Technology Co., Ltd (cnilaser, China). h-MC dispersion (5×10^9 /mL, 1 mL) was put in NMR tube and irradiated with NIR laser ($0.5 \text{ W} \cdot \text{cm}^{-2}$, 808 nm). The real-time temperature was recorded by Neoptix Reflex Fiber Optic Signal Conditioner (Neoptix inc.).

Doxorubicin Loading and Release

Microcapsules (5.3×10^9) were washed with ABS buffer (sodium acetate buffer solution, pH 5.5) and centrifuged. Then, they were mixed with 2 mL of DOX solution (5 mg/mL) and incubated at 37 °C overnight. The DOX-loaded microcapsules were washed with PBS (pH 7.4), and the supernatant was collected to calculate the loading efficiency after centrifugation.

The release experiment was performed in two different buffers (pH 7.4, pH 5.5) at 37 °C. The release behavior with the intermittent treatment of magnetic hyperthermia system or NIR laser was monitored. The release process was carried out as follows: DOX-loaded microcapsules were

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3 mixed with 1 mL of PBS, 600 μ L of supernatant was taken at different time slots for absorbance
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5 measurement ($\lambda=490$ nm), and then the supernatant was poured back into the tube. Finally, the
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7 cumulative release was obtained.
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10 **Cytotoxicity of MC and h-MC**

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12 The viabilities of HeLa cells incubated with MC and h-MC were evaluated using CCK-8
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14 assay. Cells were seeded at a density of 5×10^3 cells per well in 96-well flat bottom plate and
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16 incubated with EMEM medium containing 10% FBS and 0.1% penicillin-streptomycin at 37 $^{\circ}$ C
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18 in a humidified 5% CO_2 atmosphere for 12 h. After cell attachment, they were washed with
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20 DPBS and incubated with different proportional concentrations (50:1, 10:1, 5:1, 1:1, 0.5:1, 0.1:1,
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22 0.05:1, 0.01:1) of microcapsules solutions in EMEM media for 24 h. Cell viability was evaluated
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24 by the CCK-8 colorimetric procedure.
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29 **Intracellular localization and internalization of MC and h-MC**

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31 HeLa cells were seeded in CLSM dish and cultured in EMEM medium containing 10% FBS
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33 and 0.1% penicillin-streptomycin at 37 $^{\circ}$ C in a humidified 5% CO_2 atmosphere. After cell
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35 attachment, FITC-labelled microcapsules were added and incubated for 12 h. Nuclei were
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37 stained with Hoechst 33342 for 30 min and then washed 3 times with DPBS. Finally, cells were
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39 fixed by 4% paraformaldehyde for confocal laser scanning microscopy (CLSM, Zeiss LSM 710
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41 upright confocal microscope).
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46 In the enhanced internalization and bioadherence experiments, after adding the microcapsules,
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48 the cells were further incubated with a magnet (Neodymium-iron-boron alloy 30/150) under the
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50 petri dish for 24 h; or the cells were irradiated with NIR laser 5 times in the first 10 h (interval 2
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52 h), followed by another 14 h. The cells were further analyzed by flow cytometry (BD
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54 Biosciences BD Influx Cell), the data was processed by flowjo software.
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Synergistic therapy

The viability of HeLa cells incubated with MC, h-MC, DOX-loaded MC, and DOX-loaded h-MC, followed by different therapeutic treatments were evaluated using CCK-8 assay. Cells were seeded in 35 mm petri dish and incubated with EMEM medium containing 10% FBS and 0.1% penicillin-streptomycin at 37 °C in a humidified 5% CO₂ atmosphere for 12 h. After cell attachment, they were washed with DPBS and incubated with different microcapsules in EMEM media for 24 h. Herein, the ratio of microcapsules to cells in all different dishes is around 50. Then the extra microcapsules were removed by three times of DPBS washing. The cells were treated with magnetic hyperthermia (30 min, 7 kW, output power of 80%, 375 kHz) or/and NIR laser (10 min, 0.5 W·cm⁻², 808 nm). After that, the cells were collected and incubated in 96-well plate for another 4 hrs. At last, cell viability was evaluated by the CCK-8 colorimetric procedure.

Live/dead assay of HeLa cells

HeLa cells were seeded on glass cover slides, and cultured in EMEM medium containing 10% FBS and 0.1% penicillin-streptomycin at 37 °C in a humidified 5% CO₂ atmosphere. After cell attachment, they were washed with DPBS and incubated with different microcapsules in EMEM media for 24 h. Herein, the ratio of microcapsules to cells in all different dishes is around 1, because the excess microcapsules could absorb live/dead dye and affect the final images. Then the glass slides with cells were put into 50 mL tubes and treated with magnetic hyperthermia (30 min, 7 kW, output power of 80%, 375 kHz) or/and NIR laser (10 min, 0.5 W·cm⁻², 808 nm). Then, the cells were stained with live/dead assay for 30 min. Finally, cells were observed with confocal laser scanning microscopy (CLSM, Zeiss LSM 710 upright confocal microscope).

In vivo study

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3 *in vivo* experiments were performed with female BALB/C mice (Vital River Laboratories,
4 China). Mice were housed in rooms under standard and sterile conditions (20 °C, 50% relative
5 humidity, 12 hrs dark-light cycles), with water, food *ad libitum*. All *in vivo* studies were
6 approved by the Ethical Committee of the Institute of Biomedical Engineering, Chinese
7 Academy of Medical Sciences & Peking Union Medical College (No. 2014-012).
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11 *In vivo* efficacy of synergistic therapy was conducted in HeLa-bearing BALC/nude mice.
12 First, BALC/nude mice were injected in the right front leg with HeLa cell line. All the mice were
13 divided into five groups (3 mice in each group). DOX-loaded h-MC were injected into tumors
14 (intratumoral) of group 2~5, the injection dose of DOX was 0.25 mg/kg. Saline was injected into
15 tumors of group 1 (intratumoral) as control. After 1 h, the tumors of group 3 were treated with
16 MHS (6 kW, 100% output, 425 kHz) for 30 min. The tumors of group 4 were irradiated with
17 NIR laser (2 W·cm⁻², continuous) for 3 min. The tumors of group 5 were treated with both of
18 physical therapies. The size of tumors was recorded during therapies. Finally, the mice were
19 sacrificed, and then the tumors were fixed and sectioned for histology analysis.
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Scheme 1. Schematic illustration of hybrid microcapsules (h-MC) via layer-by-layer assembly.

Figure 1. TEM images of h-MC (A, B) and MC (C, D).

Figure 2. Temperature increase of H_2O , MC, h-MC treated by magnetic hyperthermia system (MHS) (A), and near infrared laser irradiation (NIR) (B).

Figure 3. CLSM images of MC incubated HeLa cells (A-C) and h-MC incubated HeLa cells (A'-C'). A and A' has no treatments, B and B' were incubated with magnet (Neodymium-iron-boron alloy 30/150), C and C' were treated with NIR laser. MC and h-MC were labelled with FITC.

Figure 4. Flow cytometry curves of HeLa cells incubated with FITC-labelled MC (A) and FITC-labelled h-MC (B) with different treatments.

Figure 5. Magnetic hyperthermia (MHS)-triggered DOX release from MC (A) and h-MC (B). Near infrared laser (NIR)-triggered DOX release from MC(C) and h-MC (D). Each treatment is 10 min (gray column). The setup of MHS is: 7 kW, 80% output, 375 kHz, and the continuous NIR laser (808 nm) is set to $0.5 \text{ W} \cdot \text{cm}^{-2}$.

Figure 6. Cell viability after incubating HeLa cells with different samples: control (without samples), free doxorubicin (DOX), MC capsules (MC), h-MC capsules (h-MC), DOX loaded MC and h-MC (DOX/MC, DOX/h-MC). All the HeLa cells were further treated without or with magnetic hyperthermia treatment (MHS) and near infrared laser irradiation (NIR).

Figure 7. CLSM images of dead cells incubated with different samples: control (without samples), MC and h-MC. All the HeLa cells were further treated without or with magnetic hyperthermia treatment (MHS) and near infrared laser irradiation (NIR).

Figure 8. *In vivo* study of h-MC capsules. (A) HeLa-bearing mice after the injection of DOX loaded h-MC (DOX/h-MC) with/without magnetic hyperthermia treatment (MHS) and NIR laser irradiation (NIR). (B) Tumor sizes after 5 rounds of treatments. (C) Tumor therapy using DOX loaded h-MC with/without MHS and NIR.

Figure 9. Histology analysis of H&E stained slices (amplification 400 X) after different incubation and treatments, including control sample (Saline), DOX-loaded h-MC incubation (DOX/h-MC), DOX-loaded h-MC incubation with further magnetic hyperthermia treatment (DOX/h-MC+MHS), DOX-loaded h-MC incubation with further near infrared laser irradiation (DOX/h-MC+NIR), DOX-loaded h-MC incubation with both treatments (DOX/h-MC+MHS+NIR).

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>. Additional zeta potential data and TEM for *layer-by-layer* process, Raman spectra of MC and h-MC, SQUID curve of h-MC, cytotoxicity and cell internalization of microcapsules, more histology analysis.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. § L. Deng and Q. Li contributed equally.

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REFERENCES

(1) Mura, S.; Nicolas, J.; Couvreur, P. Stimuli-responsive Nanocarriers for Drug Delivery. *Nat Mater* **2013**, 12, 991-1003.

- (2) Allen, T. M.; Cullis, P. R. Drug Delivery Systems: Entering the Mainstream. *Science* **2004**, 303, 1818-1822.
- (3) Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an Emerging Platform for Cancer Therapy. *Nat Nano* **2007**, 2, 751-760.
- (4) Cheng, R.; Meng, F.; Deng, C.; Zhong, Z. Bioresponsive Polymeric Nanotherapeutics for Targeted Cancer Chemotherapy. *Nano Today* **2015**, 10, 656-670.
- (5) Hasan, A. S.; Socha, M.; Lamprecht, A.; Ghazouani, F. E.; Sapin, A.; Hoffman, M.; Maincent, P.; Ubrich, N. Effect of the Microencapsulation of Nanoparticles on the Reduction of Burst Release. *Int. J. Pharm.* **2007**, 344, 53-61.
- (6) Acharya, G.; Park, K. Mechanisms of Controlled Drug Release from Drug-eluting Stents. *Adv. Drug Delivery Rev.* **2006**, 58, 387-401.
- (7) Tan, J. P. K.; Wang, Q.; Tam, K. C. Control of Burst Release from Nanogels via Layer by Layer Assembly. *J. Controlled Release* **2008**, 128, 248-254.
- (8) Chen, K. J.; Liang, H. F.; Chen, H. L.; Wang, Y.; Cheng, P. Y.; Liu, H. L.; Xia, Y.; Sung, H. W. A Thermoresponsive Bubble-generating Liposomal System for Triggering Localized Extracellular Drug Delivery. *ACS Nano* **2013**, 7, 438-446.
- (9) Gillies, E. R.; Jonsson, T. B.; Fréchet, J. M. J. Stimuli-Responsive Supramolecular Assemblies of Linear-Dendritic Copolymers. *J. Am. Chem. Soc.* **2004**, 126, 11936-11943.
- (10) Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. A Polymersome Nanoreactor with Controllable Permeability Induced by Stimuli-Responsive Block Copolymers. *Adv. Mater.* **2009**, 21, 2787-2791.

(11) Kim, H.; Lee, D.; Kim, J.; Kim, T.-i.; Kim, W. J. Photothermally Triggered Cytosolic Drug Delivery via Endosome Disruption Using a Functionalized Reduced Graphene Oxide. *ACS Nano* **2013**, 7, 6735-6746.

(12) Yin, Q.; Shen, J.; Zhang, Z.; Yu, H.; Li, Y. Reversal of Multidrug Resistance by Stimuli-responsive Drug Delivery Systems for Therapy of Tumor. *Adv. Drug Delivery Rev.* **2013**, 65, 1699-1715.

(13) Ge, Z.; Liu, S. Functional Block Copolymer Assemblies Responsive to Tumor and Intracellular Microenvironments for Site-specific Drug Delivery and Enhanced Imaging Performance. *Chem. Soc. Rev.* **2013**, 42, 7289-7325.

(14) Qiu, Y.; Park, K. Environment-sensitive Hydrogels for Drug Delivery. *Adv. Drug Delivery Rev.* **2012**, 64, 49-60.

(15) Ge, J.; Neofytou, E.; Cahill, T. J.; Beygui, R. E.; Zare, R. N. Drug Release from Electric-Field-Responsive Nanoparticles. *Acs Nano* **2012**, 6, 227-233.

(16) Lee, J.-H.; Chen, K.-J.; Noh, S.-H.; Garcia, M. A.; Wang, H.; Lin, W.-Y.; Jeong, H.; Kong, B. J.; Stout, D. B.; Cheon, J.; Tseng, H.-R. On-Demand Drug Release System for In Vivo Cancer Treatment through Self-Assembled Magnetic Nanoparticles. *Angew. Chem. Int. Ed.* **2013**, 52, 4384-4388.

(17) Kong, S. D.; Zhang, W. Z.; Lee, J. H.; Brammer, K.; Lal, R.; Karin, M.; Jin, S. H. Magnetically Vectored Nanocapsules for Tumor Penetration and Remotely Switchable On-Demand Drug Release. *Nano Lett.* **2010**, 10, 5088-5092.

(18) Kong, S. D.; Choi, C.; Khamwannah, J.; Jin, S. Magnetically Vectored Delivery of Cancer Drug Using Remotely On-Off Switchable NanoCapsules. *Ieee T Magn* **2013**, 49, 349-352.

(19) Zhang, W.; Deng, L.; Wang, G.; Guo, X.; Li, Q.; Zhang, J.; Khashab, N. M. Low-Magnetization Magnetic Microcapsules: A Synergistic Theranostic Platform for Remote Cancer Cells Therapy and Imaging. *Part. Part. Syst. Character.* **2014**, 31, 985-993.

(20) Sanson, C.; Diou, O.; Thévenot, J.; Ibarboure, E.; Soum, A.; Brûlet, A.; Miraux, S.; Thiaudière, E.; Tan, S.; Brisson, A.; Dupuis, V.; Sandre, O.; Lecommandoux, S. Doxorubicin Loaded Magnetic Polymersomes: Theranostic Nanocarriers for MR Imaging and Magneto-Chemotherapy. *ACS Nano* **2011**, 5, 1122-1140.

(21) Schroeder, A.; Honen, R.; Turjeman, K.; Gabizon, A.; Kost, J.; Barenholz, Y. Ultrasound triggered Release of Cisplatin from Liposomes in Murine Tumors. *J. Controlled Release* **2009**, 137, 63-68.

(22) Kostianinen, M. A.; Kasyutich, O.; Cornelissen, J. J. L. M.; Nolte, R. J. M. Self-assembly and Optically triggered Disassembly of Hierarchical Dendron-Virus Complexes. *Nat Chem* **2010**, 2, 394-399.

(23) Yavuz, M. S.; Cheng, Y.; Chen, J.; Cobley, C. M.; Zhang, Q.; Rycenga, M.; Xie, J.; Kim, C.; Song, K. H.; Schwartz, A. G.; Wang, L. V.; Xia, Y. Gold Nanocages covered by Smart Polymers for Controlled Release with Near-infrared Light. *Nat Mater* **2009**, 8, 935-939.

(24) Zhao, P. F.; Zheng, M. B.; Luo, Z. Y.; Gong, P.; Gao, G. H.; Sheng, Z. H.; Zheng, C. F.; Ma, Y. F.; Cai, L. T. NIR-driven Smart Theranostic Nanomedicine for On-demand Drug Release and Synergistic Antitumour Therapy. *Sci. Rep.* **2015**, 5, 10.1038/Srep14258.

(25) Timko, B. P.; Dvir, T.; Kohane, D. S. Remotely Triggerable Drug Delivery Systems. *Adv. Mater.* **2010**, 22, 4925-4943.

(26) Xiao, Z.; Ji, C.; Shi, J.; Pridgen, E. M.; Frieder, J.; Wu, J.; Farokhzad, O. C. DNA Self-Assembly of Targeted Near-Infrared-Responsive Gold Nanoparticles for Cancer Thermo-Chemotherapy. *Angew. Chem. Int. Ed.* **2012**, 51, 11853-11857.

(27) Zhong, Y.; Wang, C.; Cheng, R.; Cheng, L.; Meng, F.; Liu, Z.; Zhong, Z. cRGD-directed, NIR-responsive and Robust AuNR/PEG-PCL Hybrid Nanoparticles for Targeted Chemotherapy of Glioblastoma in vivo. *J. Controlled Release* **2014**, 195, 63-71.

(28) Kumar, C. S. S. R.; Mohammad, F. Magnetic Nanomaterials for Hyperthermia-based Therapy and Controlled Drug Delivery. *Adv. Drug Delivery Rev.* **2011**, 63, 789-808.

(29) Kozissnik, B.; Bohorquez, A. C.; Dobson, J.; Rinaldi, C. Magnetic Fluid Hyperthermia: Advances, Challenges, and Opportunity. *Int. J. Hyperthermia* **2013**, 29, 706-714.

(30) Deatsch, A. E.; Evans, B. A. Heating Efficiency in Magnetic Nanoparticle Hyperthermia. *J. Magn. Magn. Mater.* **2014**, 354, 163-172.

(31) Bagley, A. F.; Hill, S.; Rogers, G. S.; Bhatia, S. N. Plasmonic Photothermal Heating of Intraperitoneal Tumors through the Use of an Implanted Near-infrared Source. *ACS Nano* **2013**, 7, 8089-8097.

(32) Huang, C.-C.; Su, C.-H.; Li, W.-M.; Liu, T.-Y.; Chen, J.-H.; Yeh, C.-S. Bifunctional Gd₂O₃/C Nanoshells for MR Imaging and NIR Therapeutic Applications. *Adv. Funct. Mater.* **2009**, 19, 249-258.

(33) Yang, K.; Feng, L.; Shi, X.; Liu, Z. Nano-graphene in Biomedicine: Theranostic Applications. *Chem. Soc. Rev.* **2013**, 42, 530-547.

(34) Tian, B.; Wang, C.; Zhang, S.; Feng, L.; Liu, Z. Photothermally Enhanced Photodynamic Therapy Delivered by Nano-Graphene Oxide. *ACS Nano* **2011**, 5, 7000-7009.

(35) Yang, K.; Zhang, S.; Zhang, G.; Sun, X.; Lee, S.-T.; Liu, Z. Graphene in Mice: Ultrahigh In Vivo Tumor Uptake and Efficient Photothermal Therapy. *Nano Lett.* **2010**, 10, 3318-3323.

(36) Smith, A. M.; Mancini, M. C.; Nie, S. Bioimaging: Second Window for in vivo Imaging. *Nat Nano* **2009**, 4, 710-711.

(37) Padalkar, M. V.; Pleshko, N. Wavelength-dependent Penetration Depth of Near Infrared Radiation into Cartilage. *Analyst* **2015**, 140, 2093-2100.

(38) Di Corato, R.; Béalle, G.; Kolosnjaj-Tabi, J.; Espinosa, A.; Clément, O.; Silva, A. K. A.; Ménager, C.; Wilhelm, C. Combining Magnetic Hyperthermia and Photodynamic Therapy for Tumor Ablation with Photoresponsive Magnetic Liposomes. *ACS Nano* **2015**, 9, 2904-2916.

(39) Chu, M.; Shao, Y.; Peng, J.; Dai, X.; Li, H.; Wu, Q.; Shi, D. Near-infrared Laser Light Mediated Cancer Therapy by Photothermal Effect of Fe₃O₄ Magnetic Nanoparticles. *Biomaterials* **2013**, 34, 4078-4088.

(40) Yang, Y.-J.; Tao, X.; Hou, Q.; Ma, Y.; Chen, X.-L.; Chen, J.-F. Mesoporous Silica Nanotubes Coated with Multilayered Polyelectrolytes for pH-controlled Drug Release. *Acta Biomater.* **2010**, 6, 3092-3100.

(41) Zhao, Q.; Han, B.; Wang, Z.; Gao, C.; Peng, C.; Shen, J. Hollow Chitosan-Alginate Multilayer Microcapsules as Drug Delivery Vehicle: Doxorubicin Loading and in vitro and in vivo Studies. *Nanomed. Nanotechnol. Biol. Med.* **2007**, 3, 63-74.

(42) Sakurai, K.; Maegawa, T.; Takahashi, T. Glass Transition Temperature of Chitosan and Miscibility of Chitosan/Poly(N-vinyl pyrrolidone) Blends. *Polymer* **2000**, 41, 7051-7056.

(43) Lapčák, L.; Lapčák, L.; De Smedt, S.; Demeester, J.; Chabreček, P. Hyaluronan: Preparation, Structure, Properties, and Applications. *Chem. Rev.* **1998**, 98, 2663-2684.

(44) Toole, B. P. Hyaluronan: from Extracellular Glue to Pericellular Cue. *Nat. Rev. Cancer* **2004**, 4, 528-539.

(45) Morra, M. Engineering of Biomaterials Surfaces by Hyaluronan. *Biomacromolecules* **2005**, 6, 1205-1223.

(46) Hong, B. J.; Compton, O. C.; An, Z.; Eryazici, I.; Nguyen, S. T. Successful Stabilization of Graphene Oxide in Electrolyte Solutions: Enhancement of Biofunctionalization and Cellular Uptake. *ACS Nano* **2012**, 6, 63-73.

(47) Huang, X.; Qi, X.; Boey, F.; Zhang, H. Graphene-based Composites. *Chem. Soc. Rev.* **2012**, 41, 666-686.

(48) Abdelsayed, V.; Moussa, S.; Hassan, H. M.; Aluri, H. S.; Collinson, M. M.; El-Shall, M. S. Photothermal Deoxygenation of Graphite Oxide with Laser Excitation in Solution and Graphene-Aided Increase in Water Temperature. *J. Phys. Chem. Lett.* **2010**, 1, 2804-2809.

(49) Ye, C. H.; Combs, Z. A.; Calabrese, R.; Dai, H. Q.; Kaplan, D. L.; Tsukruk, V. V. Robust Microcapsules with Controlled Permeability from Silk Fibroin Reinforced with Graphene Oxide. *Small* **2014**, 10, 5087-5097.

(50) Hu, S. H.; Fang, R. H.; Chen, Y. W.; Liao, B. J.; Chen, I. W.; Chen, S. Y. Photoresponsive Protein-Graphene-Protein Hybrid Capsules with Dual Targeted Heat-Triggered Drug Delivery Approach for Enhanced Tumor Therapy. *Adv. Funct. Mater.* **2014**, 24, 4144-4155.

(51) Jin, Y.; Wang, J.; Ke, H.; Wang, S.; Dai, Z. Graphene Oxide modified PLA Microcapsules Containing Gold Nanoparticles for Ultrasonic/CT Bimodal Imaging guided Photothermal Tumor Therapy. *Biomaterials* **2013**, 34, 4794-4802.

(52) Hong, J.; Char, K.; Kim, B.-S. Hollow Capsules of Reduced Graphene Oxide Nanosheets Assembled on a Sacrificial Colloidal Particle. *J. Phys. Chem. Lett.* **2010**, 1, 3442-3445.

(53) Kurapati, R.; Raichur, A. M. Graphene Oxide based Multilayer Capsules with Unique Permeability Properties: Facile Encapsulation of Multiple Drugs. *Chem. Commun.* **2012**, 48, 6013-6015.

(54) Kurapati, R.; Raichur, A. M. Near-infrared Light-responsive Graphene Oxide Composite Multilayer Capsules: a Novel Route for Remote Controlled Drug Delivery. *Chem. Commun.* **2013**, 49, 734-736.

(55) Chen, W.; Yi, P.; Zhang, Y.; Zhang, L.; Deng, Z.; Zhang, Z. Composites of Aminodextran-coated Fe₃O₄ Nanoparticles and Graphene Oxide for Cellular Magnetic Resonance Imaging. *ACS Appl Mater Interfaces* **2011**, 3, 4085-4091.

(56) Yang, K.; Hu, L.; Ma, X.; Ye, S.; Cheng, L.; Shi, X.; Li, C.; Li, Y.; Liu, Z. Multimodal Imaging Guided Photothermal Therapy using Functionalized Graphene Nanosheets Anchored with Magnetic Nanoparticles. *Adv. Mater.* **2012**, 24, 1868-1872.

(57) Ma, X.; Tao, H.; Yang, K.; Feng, L.; Cheng, L.; Shi, X.; Li, Y.; Guo, L.; Liu, Z. A functionalized Graphene Oxide-Iron Oxide Nanocomposite for Magnetically targeted Drug Delivery, Photothermal Therapy, and Magnetic Resonance Imaging. *Nano Res.* **2012**, 5, 199-212.

(58) Peng, E.; Ding, J.; Xue, J. M. Concentration-dependent Magnetic Hyperthermic Response of Manganese Ferrite-loaded Ultrasmall Graphene Oxide Nanocomposites. *New J. Chem.* **2014**, 38, 2312-2319.

(59) Tong, W.; Song, X.; Gao, C. Layer-by-layer Assembly of Microcapsules and Their Biomedical Applications. *Chem. Soc. Rev.* **2012**, 41, 6103-6124.

(60) Cui, J.; van Koeven, M. P.; Müllner, M.; Kempe, K.; Caruso, F. Emerging Methods for the Fabrication of Polymer Capsules. *Adv. Colloid Interface Sci.* **2014**, 207, 14-31.

(61) De Cock, L. J.; De Koker, S.; De Geest, B. G.; Grooten, J.; Vervaet, C.; Remon, J. P.; Sukhorukov, G. B.; Antipina, M. N. Polymeric Multilayer Capsules in Drug Delivery. *Angew. Chem. Int. Ed.* **2010**, 49, 6954-6973.

(62) Yan, Y.; Björnmalm, M.; Caruso, F. Assembly of Layer-by-Layer Particles and Their Interactions with Biological Systems. *Chem. Mater.* **2014**, 26, 452-460.

TOC

Therapeutic Micro-Matryoshka (h-MC) is developed as a multifunctional theranostic platform displaying targeted bioavailability, on-demand chemotherapy, and possible MRI-traceability. It also successfully achieved synergistic magnetic hyperthermia and photothermal therapy *in vitro* and *in vivo*.