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Eccentric Magnetic Microcapsules for Orientation-specific and Dual Stimuli-responsive Drug Release

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Abstract:

In this paper, we fabricated uniform polydimethylsiloxane (PDMS) magnetic microcapsule with eccentric internal structure, and employed it as a novel delivery system for orientation-specific and dual stimuli-responsive controlled drug release. These eccentric microcapsules contained Fe_3O_4 nanoparticles in the inner cores. Because of the paramagnetic Fe_3O_4 nanoparticles, the eccentric microcapsules could be accurately moved by a magnetic field, leading to a precise control of the location of microcapsules. Also, due to the eccentric structure of magnetic microcapsules, the capsules exhibited non-uniform magnetic property; the capsules could be aligned by magnetic fields with the thin wall facing towards the magnet, resulting in a precisely orientation-specific control of microcapsules. More interestingly, eccentric magnetic microcapsules demonstrated a dual stimuli-responsive controlled release of inclusions, where it was a sustained release under ultrasound and an intense release under laser stimulation, respectively. Furthermore, we studied the efficacy of doxorubicin (DOX) from the microcapsules regulated by laser stimulation by performing the *in vitro* cell test with and without a magnetic field applied. The cell test showed that under an orientation-specific control of microcapsules by magnetic field (when the thin wall of eccentric microcapsule was toward the cell), the efficacy of the drug released from microcapsules was improved. The results suggested that our eccentric magnetic microcapsules held all properties needed for a site-specific, orientation-specific and dual stimuli-responsive delivery system, demonstrating a great potential application for multifunctional controlled drug release.

Keywords: magnetic microcapsules; eccentric; orientation-specific; dual stimuli-responsive; controlled drug release

1. Introduction

Microcarrier systems are attracting increasing attentions in the field of drug delivery because they enable site-specific and controlled release of drugs.[1-5] An ideal microcarrier system for drug delivery is capable of improving the efficacy of the drugs at the site of interest while minimizing systemic side effects comparing to conventional techniques based on the administration of free drugs.[1-5] Such localized delivery of drugs is beneficial for patients, where only pathological cells are killed by cytotoxic drugs released from the microcarriers while the healthy tissues escape the toxic side effects.[6-10] Among many categories, polymeric microcapsules with well-defined structures are promising candidates for site-specific controlled drug release and targeted therapy.[11-15] To date, various types of polymer microcapsules have been developed for targeted drug delivery, achieved by means of, for example, covalent bonding with molecules that can enhance cell specific recognition,[7, 8] strong affinity for specific tissues,[9,10] or external guidance (*i.e.*, by magnetism).[16-18] In particular, targeted delivery by magnetic field has received much attention.[19-24] By loading the capsules with magnetic nanoparticles, polymeric microcapsules can be attracted and transported by externally applied magnetic field, thus achieving targeted localization.[17, 19-22] However, when the carriers containing cytotoxic drugs reach the target (*i.e.*, pathological tissues), the drugs are released and will typically diffuse in all directions.[6-10, 17, 18] In other words, although the drug carriers possess the capability to distinguish pathological tissues from normal ones, the isotropic release and diffusion of drug is still likely to elicit the damage of normal cells surrounding the region.[7-10] Therefore, it is highly necessary to devise an approach to focus the released drug molecules only onto the target and suppress the non-directional diffusion effect.

In order to tackle this issue, it has been attempted to prepare orientation-specific microcarriers, which show great potential in directing the drug molecules to the target for improved efficacy. For example, anisotropic polymer microtubes functionalized to present distinct properties for various segments achieved orientation-specific attachment on the target.[25, 26] Janus colloidal particles

are non-centrosymmetric particles with two distinct units, whose orientation can be controlled by external stimuli depending on the property of each unit.[27-29] Anisotropic magnetic polymer microcapsules can be easily manipulated under a magnetic field to change their orientations.[30-32] Although these microcarriers can all achieve orientation-specific controlled release of drugs, the manipulation of their rotation for more precise site-specific drug delivery is not straightforward. In addition, it is difficult for most anisotropic polymer microcapsules to achieve well-controlled sustained release of drug. Typically, the encapsulated drugs are released from the microcapsules either by chemical destruction of microcapsules or by exposing them to single external stimuli,[33-37] resulting in limited regulation of the release profiles of drug.[2, 12, 35, 38] To the best of our knowledge, a drug delivery system combining site and orientation specificity with multimodal controlled release for efficient delivery of drugs has not been reported.

Herein, we report the fabrication and evaluation of uniform polydimethylsiloxane (PDMS) eccentric microcapsules for site-/orientation-specific and dual stimuli-responsive controlled drug release. Using a three-phase microfluidic device,[39-41] we obtained uniform eccentric microcapsules containing magnetic nanoparticles in inner cavities after the solidification of PDMS. Because of the paramagnetism of nanoparticles in microcapsules, we could easily control the transport and orientation of microcapsules using a magnetic field, and therefore achieving a site/orientation-specific controlled release of encapsulated drugs. We demonstrated the orientation-specific and dual stimuli-responsive controlled drug release under ultrasonic and light regulation using rhodamine 6G as a model molecule. Furthermore, doxorubicin (DOX) was encapsulated into the eccentric magnetic microcapsules to prove the release behavior and drug efficiency on cells *in vitro*. The results suggested that the uniform eccentric magnetic microcapsules could be precisely manipulated under a magnetic field, which showed controllable, sustained release (under ultrasound) and intense release (under light) drug release profiles, respectively. Due to the large size and the slow degradability of PDMS, this kind of microcapsule is demonstrated here as a potential controlled release model

for local injection therapy and oral administration.

2. Experimental section

2.1 Chemicals and materials

Polydimethylsiloxane (PDMS, Dow Corning, USA) and dichloromethane (DCM, Damao Reagent, CHN) were used as the main components for microcapsule preparation (PDMS:DCM = 3:1). Polyvinyl alcohol (PVA, Sigma-Aldrich, USA) at a concentration of 2.0 wt.% served as the inner and outer water phases. Magnetic nanoparticle (~10 nm, modified by sephadex) aqueous solution at a concentration of 1.2 wt.% (mixed with rhodamine 6G or doxorubicin) served as the inclusion in inner phase. Rhodamine 6G (Aladdin, CHN) and doxorubicin (DOX, Beijing Huafeng United Technology CO., CHN) were used as examples of drugs. The two different glass capillary tubes (with inner/outer diameters of 0.70/1.23 and 0.45/0.65 mm respectively) were obtained from Ace Glass (USA). Deionized (DI) water used in all experiments was obtained by filtering through a set of Millipore cartridges (Epure, Dubuque, IA, USA).

2.2 Preparation and characterization of eccentric magnetic microcapsules

The microfluidic device was fabricated according to our published work with minor modification.[42] The schematic diagram is shown in Figure S1. Briefly, the fluidic device was consisted of two PVC tubes (0.60/1.50 and 1.45/2.30 mm in inner/outer diameters respectively), two glass capillary tubes and a 30G needle. The device was assembled by inserting the needle and glass capillary tubes into PVC tubes, which was fixed with epoxy adhesive. The inner, middle and outer phases were introduced using three syringe pumps (KD100, KD Scientific, USA) at independently adjustable flow rates. In a typical demonstration, the flow rates for the inner, middle and outer phases were kept at 0.004, 0.03 and 1.5 mL/min respectively to fabricate the eccentric microcapsules. The inner water droplets formed at the tip of the needle and flowed along the PVC tube into the small glass capillary tube, and then the water-in-oil droplets formed at the exit of the small glass

capillary tube and finally flowed along the big glass capillary tube into a 100 mL beaker containing the outer water phase. The PVA solution (2 wt.%) containing rhodamine 6G (or doxorubicin) and sephadex-modified Fe_3O_4 magnetic nanoparticles were served as the inner water phase. The mixture of PDMS and DCM at a mass ratio of about 3:1 was used as the middle oil phase. We used PVA solution (2 wt.%) as the outer water phase and collection solutions. The microcapsules suspension were kept at 60 °C for 30 min to get rid of DCM, and then at 90 °C for 30 min to solidify the microcapsules. For static solidification, microcapsules were kept static and inner water phase sank to the bottom of microdroplets due to the density contrast and gravity, thus we obtained uniform eccentric microcapsules which contained magnetic nanoparticles in their eccentric inner cavities. An optical microscope (Inverted fluorescence microscopy, NIKON, JPN) with a CCD camera (Digital Sight DS-Fi2, NIKON, JPN), a stereoscope (SZ760-DM601, OPTEC, CHN) and scanning electron microscopy (SEM, JSM-6010LA, JEOL, JPN) were used to observe the morphology and structures of the microcapsules. The average diameter and standard deviation were calculated by measuring the diameters of over 50 microcapsules randomly selected from the optical micrographs of each sample.

2.3 Manipulation of eccentric magnetic microcapsules

For the demonstration of the site-specific control of microcapsules, hydrogel (Sigma-Aldrich, USA) micropieces which contained phenolphthalein (Damao Reagent, CHN) was used as the targets to evaluate the locating capability of eccentric magnetic microcapsules. We also demonstrated the orientation capability of eccentric magnetic microcapsules by setting a series of attracting angles of magnetic field to control the orientation of microcapsules with a magnet (the magnetic intensity of the magnet is ~1600 G).

2.4 UV-*vis* spectra measurement

A spectrophotometer (UD730, Beckman, USA) was used to measure the release profiles of the drug molecules from the microcapsules. The drug molecules were

loaded into the microcapsules by directly adding them (at a final concentration of 0.6 mg/mL for rhodamine 6G, and 4.0 mg/mL for doxorubicin respectively) into the PVA solution which also contained magnetic nanoparticles as inner water phase. After solidification, the microcapsules (six capsules for each sample) were dispersed in glass containers (4.5 mm in diameter and 30.0 mm in height) containing 150 μL DI water. We applied an ultrasound (DL-360B, ZHI SUN INSTRUMENT, CHN) and a laser (F-20, Guangzhou Riton Laser CO., CHN) to regulate the drug release process (40 kHz, 360 W, 37 °C for ultrasound, and 1064 nm, 20 kHz, 2.0 W for laser). Every 15 min under ultrasound (or 5 seconds under laser), we measured the UV-*vis* spectra of the solutions of one sample. The absorption maximum was recorded as a function of exposure time.

2.5 *In vitro* cell test

2.5.1 Cell line and co-culture with microcapsules

HeLa cells were cultured in Dulbecco's modified eagle medium (DMEM, high glucose, modified with 10% fetal bovine serum and 1% streptomycin/penicillin), and incubated at 37°C with 5% CO_2. Human adipose-derived stem cells (ADSCs, PCS-500-011™, ATCC, USA) were cultured in 1:1 mixture of DMEM and Ham's F-12 (F-12, contained L-Glutamine), and incubated at 37°C with 5% CO_2. The medium was replaced every two days. At 80-90% confluence, the cells were washed twice with phosphate buffer solution (PBS), detached with 0.25% trypsin-EDTA, and then replanted (24 well for FDA staining and 96 well for MTT assay) at approximately 2×10^4 cells/cm^2. The cells were incubated until about 80–90% confluence and were used for subsequent experiments.

Ten microcapsules were added into each well, and medium was removed and replaced by fresh medium. Every well was then treated with laser (1064 nm, 20 kHz, 1.0 W; the laser wavelength is in the biologically "friendly" window which can minimize the absorption of laser light by cells and tissue) in time range from 0 to 50 seconds. After that, cells were incubated for another 24 h.

2.5.2 FDA staining for optical observation

Fluorescein diacetate (FDA) was dissolved in acetone to obtain 5 mg/mL stock solution and was stored at 4 °C and diluted to 5 μg/mL for final cell viability test. At the test point, the medium was removed and replaced by 200 μL of FDA solution (5 μg/mL). After incubation at room temperature for 10 min, the solution was removed and the cells were washed in PBS. An optical microscope (Inverted fluorescence microscopy, NIKON, JPN) with a CCD camera (Digital Sight DS-Fi2, NIKON, JPN) was used to observe the HeLa cells, and took the optical images.

2.5.3 MTT assay

MTT solution was prepared as 5 mg/mL stock in sterile PBS, 0.2-μm filtered and was stored as 2 mL aliquots at -20°C. Dimethylsulfoxide (DMSO) was used as the solvent. After the cells were incubated for another 24 h post drug release, the medium was removed and replaced by 120 μL of MTT solution (20 μL MTT stock solution diluted with 100 μL medium) for 4 h. The MTT solution was then replaced by 100 μL of DMSO to each well and left for 10 min at 37 °C. The solution of MTT-formazan in DMSO was transferred to a new plate. The absorbance at 490 nm was measured using a plate reader to determine the cell viability as a percentage of control group.

3. Results and discussions

3.1 Sizes and inner structures of eccentric magnetic microcapsules

To fabricate eccentric magnetic microcapsules, we used PDMS as the base material of microcapsules due to its biocompatible and widespread use as oral pharmaceutical excipients.[38, 43] Moreover, PDMS is a water-permeable and elastomeric material allows for adjustable pore size for drug release.[38, 43] Figure 1A and 1B show optical images of the eccentric magnetic microcapsules. The microcapsules were obtained at flow rates of 0.004, 0.03, and 1.5 mL/min for the inner water phase, middle oil phase, and outer water phase, respectively. Due to the high water-solubility of the magnetic nanoparticles, we could directly load these

nanoparticles into the inner water phase, evidenced by the brownish-red color typical of the nanoparticles. The uniform PDMS microcapsules contained magnetic nanoparticles in the cavities. Interestingly, we were able to obtain eccentric microcapsules instead of core-centric microcapsules.[42] In the fabrication process, the emulsion droplets were solidified under a static condition without any mechanical disturbance (i.e., stirring). Since the density of the inner water phase is higher than that of PDMS in the outer phase, the water phase would sink to the bottom of a microcapsule as dragged by the gravitational force. Nevertheless, these water droplets could not penetrate through PDMS and escape due to the low surface energy of PDMS,[44] leading to the eventual formation of eccentric microcapsules with a PDMS thin-wall on the side.

Using scanning electron microscopy (SEM), we further examined the internal structure and inclusions of the eccentric magnetic microcapsules. As shown in Figure 1, C and D, the microcapsules were eccentric with the papyraceous material in the hollow core, which was a mixture of Fe_3O_4 and polyvinyl alcohol (PVA, the surfactant) after drying. The transmission electron microscopy (TEM) image of Fe_3O_4 nanoparticles is shown in Figure S2. According to our measurement, the average diameters of the microcapsules and the internal spherical cores were 808.8 ± 3.5 μm and 482.2 ± 2.0 μm, respectively. The microcapsules possessed a wall of non-uniform thickness, and the thinnest wall in each microcapsule was about 8.1 ± 0.3 μm, which can be potentially used as an “exit” for the inclusions to escape. By adjusting the flow rate of each phase, we can control the size of the internal structures which has been demonstrated in our published work,[42] and also the size of the microcapsule. The results of the size controlling of microcapsules are shown in Figure S3. However, it’s worth mentioning that we could further reduce the diameters of eccentric microcapsules by fabricating them in double emulsion without a microfluidic chip, and the results are showed in Figure S4. The average diameters of these eccentric microcapsules were ~11.6 μm.

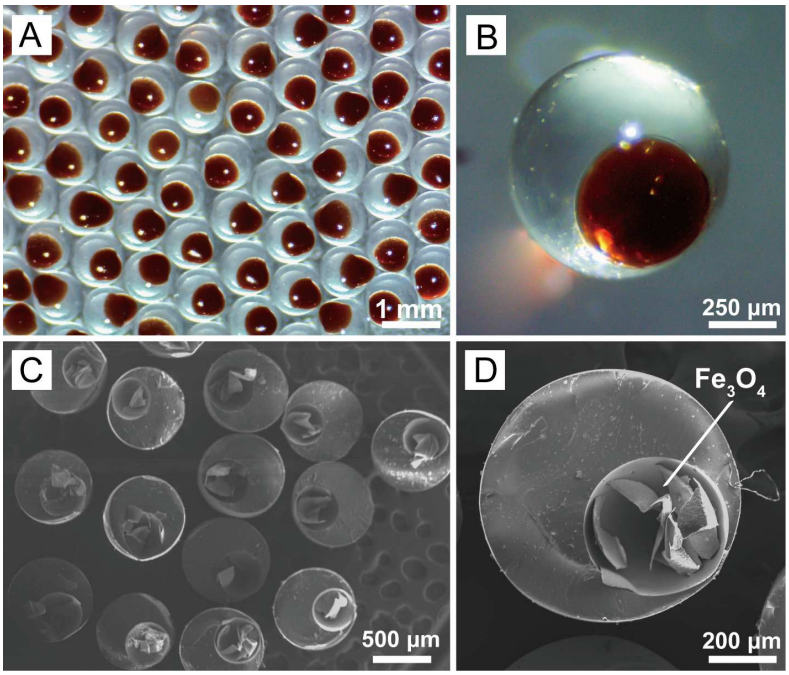

Figure 1. Observation of eccentric magnetic microcapsules. (A, B) Optical images of eccentric magnetic microcapsules. The inner aqueous phase contained magnetic nanoparticles. (C, D) Scanning electron microscope (SEM) images of eccentric magnetic microcapsules. The internal space contained magnetic nanoparticles which were dried after baking for imaging.

We then obtained hollow eccentric magnetic microcapsules by baking the microcapsules at an elevated temperature after the solidification process. The magnetic substances were aggregated onto the thin wall of the hollow eccentric microcapsules under a magnetic field during the baking process. Figure S5 shows the optical images of hollow eccentric magnetic microcapsules post drying. The dried microcapsules floated on the surface of water upon mixing (Figure S6A), indicating a lower density of hollow eccentric magnetic microcapsules than that of water. In contrast, the microcapsules would instead settle at the bottom of water (Figure S6A). We further loaded the dehydrated microcapsules into a Petri dish filled with water where the microcapsules were floating on the surface. When the microcapsules were exposed to a magnet placed above or beneath the Petri dish, the microcapsules could be rotated, with the thin wall facing the top or bottom of Petri dish, respectively, as shown in Figure S6, B and C. The non-centric distribution of magnetic

nanoparticles inside the microcapsules demonstrated the guiding effect regarding the orientation and rotation of these hollow microcapsules.

3.2 Site- and orientation-specific control of eccentric microcapsules

The location of the microcapsules, as well as the orientation of individual capsules, could be controlled by a magnetic field, because the Fe_3O_4 nanoparticles encapsulated are paramagnetic and responsive to magnet field. Interestingly, by adjusting the mass concentrations of magnetic nanoparticles in the core, we were able to manipulate the magnetic-responsive property of the microcapsules, leading to the production of a series of microcapsules with different mobility under a constant magnetic field. As shown in Figure S7, with increasing mass fraction of magnetic nanoparticles from 0.46 to 0.58, 0.77, 1.15, and 2.30%, the movement speeds of microcapsules was raised from 0.40 to 0.91, 1.67, 3.33, and 5.00 mm s^{-1}, indicating a positive correlation.

In order to demonstrate the site-specific control of microcapsules, we placed one microcapsule into a Petri dish filled with water, and manipulated the capsule by a magnet. We set three targets (*i.e.* hydrogel micropieces containing phenolphthalein, purple color) at different locations and sequentially routed the microcapsule (containing dilute HCl at 12 mM) using a magnet to the target sites one by one. Figure 2A shows the initial state, whereas Figure 2, B-D, show the attachment of the microcapsule to each hydrogel micropiece. As expected, the microcapsule was successfully located at all three target site on after another, and the released HCl from the microcapsule induced the fading of phenolphthalein in the hydrogels. This result suggests that the eccentric magnetic microcapsule is responsive to the magnetic field, and can be moved to the locations of interest as needed.

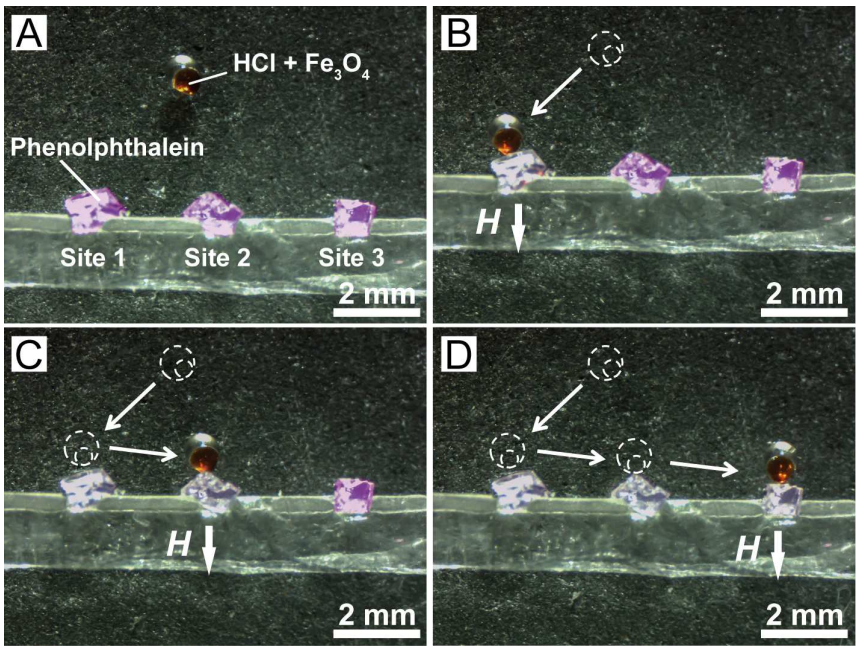

Figure 2. Site-specific control of one eccentric magnetic microcapsule under magnetic field. (A) Optical image of an eccentric magnetic microcapsule and a PDMS receptacle with three targets at different sites. (B, C, and D) Optical images showing the process of site-specific control of the microcapsule. Microcapsule was moved and located to the three target sites sequentially under the attraction of a small magnet.

We next investigated the orientation-specific control of eccentric magnetic microcapsules. We set a series of attracting angles of magnetic field, ranging from 0° to 45° and 90°, to demonstrate the control over the orientation of the microcapsules. As shown in Figure 3A, the microcapsules were initially randomly orientated towards all directions on a slide before exposure to a magnet. After a magnetic field was applied, the microcapsules were immediately aligned and the thin walls of the microcapsules turned to face the magnet (Figure 3B). As the attracting angle changed, the orientation of all the microcapsules followed, with the thin wall of microcapsules facing the magnet (Figure 3, C-F). These results indicate that we could control the orientation of individual microcapsule due to the non-uniform magnetic property of eccentric magnetic microcapsules under a magnetic field.

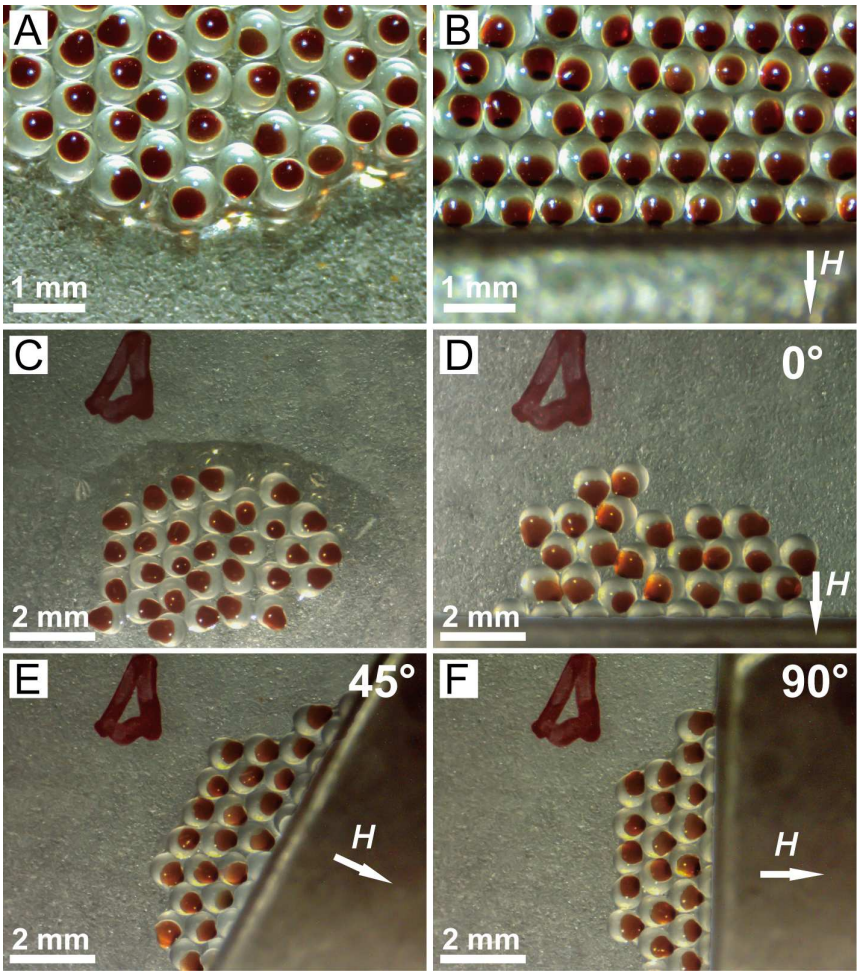

Figure 3. Orientation-specific control of eccentric magnetic microcapsules at different angles under magnetic field, showing that the capsules could be aligned and rotated by magnetic field. The thin wall sides of microcapsules turned to face the magnet. (A) Eccentric magnetic microcapsules before exposing to a magnet. (B) Eccentric magnetic microcapsules under attraction of a magnetic field. (C) Eccentric magnetic microcapsules before exposing to a magnet. (D-F) Eccentric magnetic microcapsules under magnetic fields at different angles (0°, 45°, 90°).

3.3 Dual stimuli-responsive release profiles of inclusions from the microcapsules

To study the release of drugs from the eccentric magnetic microcapsules *in vitro*, we used rhodamine 6G as a model drug and applied ultrasound or laser to regulate the drug release process. In all cases, the encapsulation efficiency of rhodamine 6G into the inner water phase approached 100%, suggesting that all drug molecules were encapsulated in the final microcapsules.

In this section, we present the concept of a dual stimuli-responsive controlled

drug release. By applying ultrasound or light stimulation, we could achieve different release profiles of the same model drug by using eccentric magnetic microcapsules as carriers. The release profiles of rhodamine 6G from eccentric magnetic microcapsules in the absence/presence of ultrasonic stimulation are shown in Figure 4, A and B. We applied a magnetic field (at 1600 G) to fix the orientation of the microcapsules, and used ultrasound to stimulate the release of drug molecules from the microcapsules. Figure 4, a1 to a4, show fluorescence images of eccentric magnetic microcapsules at different ultrasonic stimulation time. There was a time-dependent decrease in fluorescence intensity of microcapsules, suggesting the release of rhodamine 6G from microcapsules into the surrounding medium. Figure 4B shows the release profile. Rhodamine 6G was barely released without ultrasonic stimulation, indicating a fine sealability of rhodamine 6G in the microcapsules. However, when the microcapsules were exposed to the ultrasound, the drug release started immediately, and continued until the ultrasound was stopped. The amount of rhodamine 6G molecules released to the surrounding medium increased with the time of ultrasonic stimulation. We assume that, ultrasound may change the porosity of the PDMS in a nondestructive manner or destroy the walls of the microcapsules, resulting in the increasing release of drugs from the PDMS microcapsules.[35, 42] After 2 h, the accumulated release percentage was about 50%. It took nearly 5 h until most of the drugs released from the microcapsules. This result demonstrated that eccentric magnetic microcapsules showed sustained release under ultrasonic stimulation.

The release of the same drug under light stimulation was also investigated and compared to the situation under ultrasonic stimulation.[45] The release profiles of rhodamine 6G from eccentric magnetic microcapsules in the absence/presence of laser stimulation are shown in Figure 4, C and D. Similarly, we applied a magnetic field to fix the orientation of the microcapsules and then used laser (at 2.0 W) to stimulate the release of drug. Figure 4, c1 to c4, show the fluorescence images of eccentric magnetic microcapsules at different laser stimulation time. After applying laser stimulation for 5 s, the fluorescence intensity of microcapsules decreased

drastically and the capsule surface became chapped, suggesting an intense and rapid release of rhodamine 6G. Figure 4D shows the time-dependent release profiles. The release of rhodamine 6G was negligible without laser stimulation, which is similar with the situation where no ultrasound was applied. After the microcapsules were exposed to the laser, the drug release from the microcapsules started immediately and intensely. The amount of rhodamine 6G molecules released increased with the time of laser stimulation. The release process lasted for only less than 1 min, indicating that eccentric magnetic microcapsules underwent burst release triggered by laser light. After about 30 seconds, the accumulated release percentage was nearly 95%. Comparing to the drug release under ultrasonic stimulation, the drug release rate was much faster under laser stimulation. As a result, we could easily regulate the drug release profiles from eccentric magnetic microcapsules by ultrasonic stimulation (for sustained release) or by laser stimulation (for burst release).

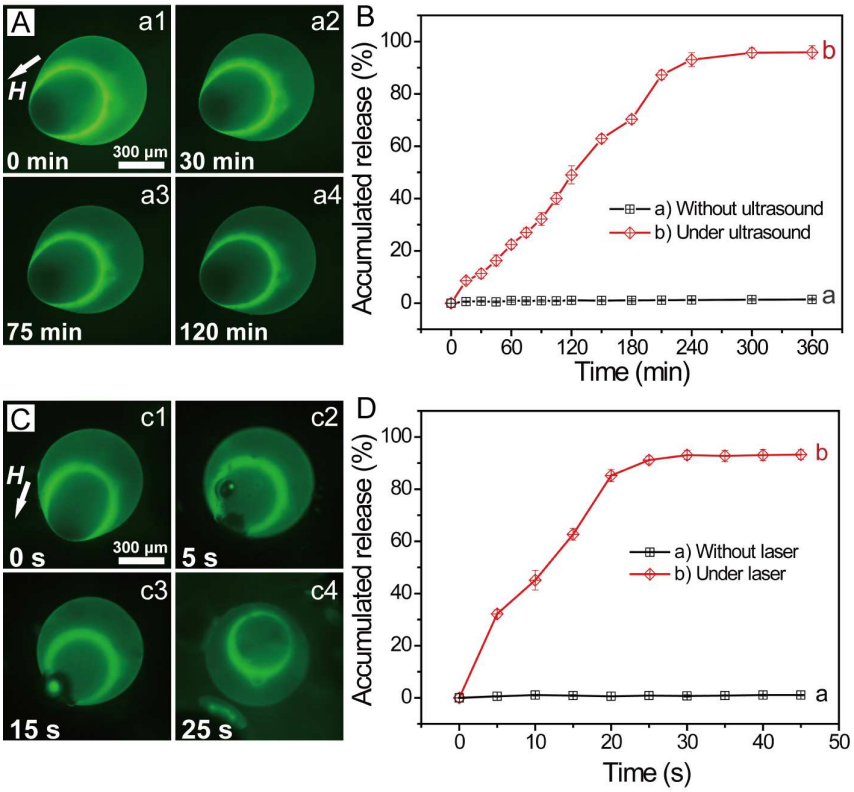

Figure 4. Dual stimuli-responsive controlled release of rhodamine 6G (under ultrasound or laser stimulation) from eccentric magnetic microcapsules. (A) The fluorescence images of eccentric magnetic microcapsules under different time of ultrasonic stimulation (a1 to a4). (B) Time-dependent release profile of encapsulated rhodamine 6G to the surrounding medium under ultrasonic stimulation. (C) The fluorescence images of eccentric magnetic microcapsules under different time of laser stimulation (c1 to c4). (D) Time-dependent release profile of encapsulated rhodamine 6G to the surrounding medium under laser stimulation.

We also used the microcapsules as vehicles to deliver doxorubicin (DOX), a type of anticarcinogen to further investigate the efficacy of released drug. We studied the release of DOX from the microcapsules under laser stimulation. Figure 5 shows the release profile of DOX from eccentric magnetic microcapsules. The release profile is similar to that of rhodamine 6G under laser stimulation, demonstrating that such regulation of drug release is also applicable to conventional drugs for *in vitro* cell test.

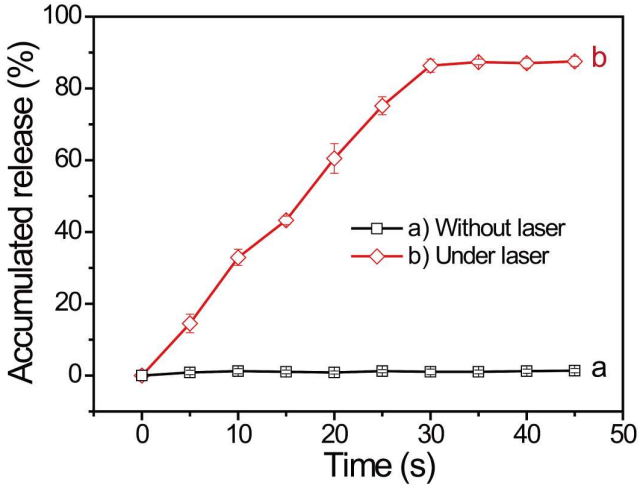

Figure 5. The release profiles of doxorubicin from eccentric magnetic microcapsules under laser stimulation.

3.4 *In vitro* cell test of eccentric magnetic microcapsules

In order to evaluate the efficacy of DOX under orientation-specific controlled release, we carried out the *in vitro* cell test. First, we evaluated the *in vitro* cytotoxicity of the eccentric magnetic microcapsules alone (*i.e.* without any drugs) based on the viability of HeLa cells and ADSCs. In these cases, the cells were co-cultured with eccentric magnetic microcapsules at different mass concentrations. Cell survival percentage was characterized by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results are shown in Figure 6. Figure 6A shows the cell viability of HeLa cells based on MTT assay. As the concentration of microcapsules increased the cell viability decreased but not significant decreased. We also study the cell viability of ADSCs, and the results are similar to HeLa's (Figure 6B). Even under a concentration of as high as 9 mg/mL and co-culture for up to 5 days, both HeLa cells and ADSCs could still maintain a reasonable viability of approximately 90%. These results indicate that the eccentric magnetic microcapsules alone had minimum cytotoxicity towards HeLa cells and ADSCs.

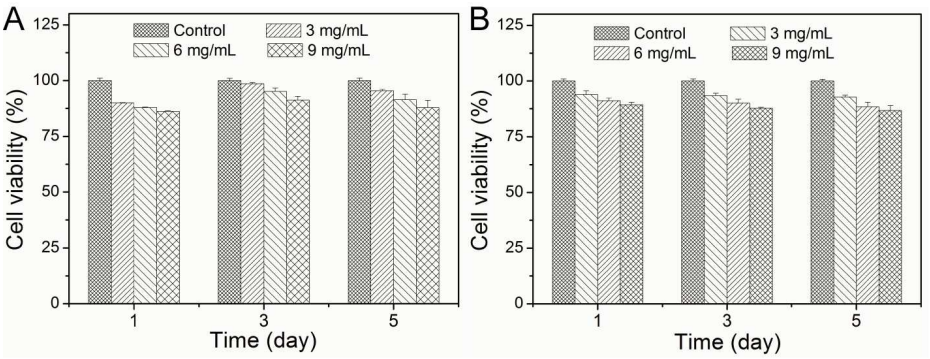

Figure 6. Cell viability MTT assay of (A) HeLa cells and (B) ADSCs treated with different mass concentrations of microcapsules without doxorubicin for different days.

Secondly, we carried out the *in vitro* experiment to study the laser induced damage of cells based on the viability assay of HeLa cells. In this case, HeLa cells were illuminated with laser beam operated at 1064 nm with a power of 2.0 W for different time period. Cell survival percentage was measured and shown in Figure S8. There was no significant difference in cell viability between the cells before and after laser illumination for up to 30 seconds.

Finally, we studied the cytotoxic efficacy of the drugs released from the capsules under orientation-specific control by co-culturing HeLa cells and microcapsules with magnetic field manipulation. The microcapsules were randomly distributed without magnetic field applied, while the microcapsules turned to a fixed orientation to face the HeLa cells under magnetic field. The capsules were exposed to the laser stimulation for different periods ranging from 0 to 50 seconds and then the cells were returned back to culture for another 24 h. We then performed the fluorescein diacetate (FDA) staining for optical observation of living cells and MTT assay for evaluating the cell viability. Figure 7 shows the fluorescence images of HeLa cells after laser exposure for different periods. More DOX was released from the capsules after longer exposure time (Figure 5). The experimental group (Figure 7, α, β, and γ) in which the drug was released under a magnetic field showed less living cells than the control group (Figure 7, A, B and, C) without magnetic field. Meanwhile, the dead

cells in the experimental group appeared to be in small aggregated islands, while the dead cells in control group seemed to be evenly distributed. Figure 8 shows the cell viability based on MTT assay. There was an exposure time-dependent decrease on cell viability and the experimental group where the drug was released under a magnetic field showed lower cell viability than the control group without magnetic field. The result of MTT assay was consistent with the optical observation. The above results indicated that, under an orientation-specific control, the efficacy of the drugs released from the eccentric magnetic microcapsules could be improved.

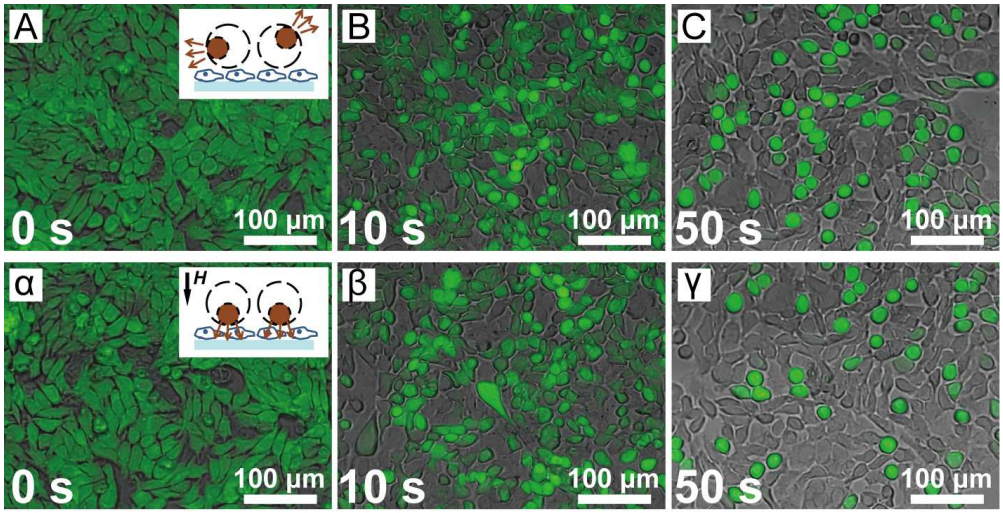

Figure 7. Fluorescence images of HeLa cells exposed to doxorubicin (DOX) released from the eccentric magnetic microcapsules under length of laser exposure. In (A), (B) and (C), DOX was released without a magnetic field applied. In (α), (β) and (γ), DOX was released under a magnetic field. A magnet was placed at the bottom of the Petri dish. The insets are schematic graphs showing the different situation between experimental group and the control group.

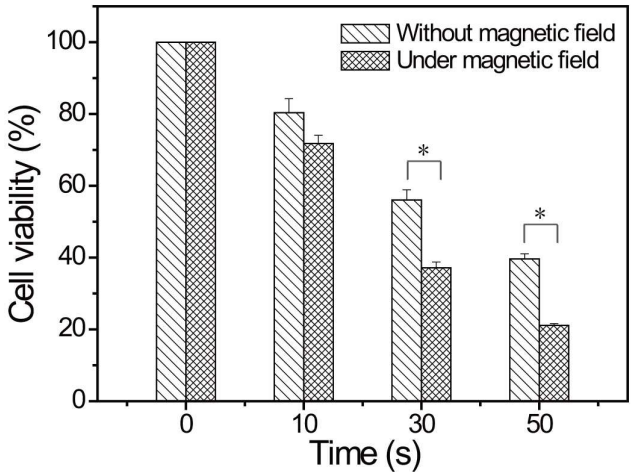

Figure 8. Cell viability based on MTT assay of HeLa cells co-cultured with the eccentric magnetic microcapsules (containing DOX) under different period of laser stimulation time without or under a magnetic field applied.

4. Conclusions

In summary, we have demonstrated a new class of eccentric magnetic microcapsules, for site-specific and orientation-specific controlled drug release. The *in vitro* cell evaluation indicated that, under an orientation-specific controlled release, the efficacy of the drugs could be improved. Comparing with the microcapsules reported in literatures, our drug delivery system based on eccentric magnetic microcapsules offer a range of advantages: *i*) we could achieve the reproducible preparation of uniform eccentric magnetic microcapsules with non-uniform magnetic property, whereas magnetic nanoparticles together with the drug molecules could be directly loaded inside the microcapsules during the fabrication process; *ii*) we could manipulate the location of individual microcapsules precisely as needed under magnetic field, realizing more accurate site-specific control of eccentric magnetic microcapsules; *iii*) due to the non-uniform magnetic property of eccentric magnetic microcapsules, the thin wall of microcapsules could be tuned to face toward the targets (*e.g.* cancer cells), achieving the orientation-specific control of drug release from the microcapsules; and *iv*) the eccentric magnetic microcapsules showed dual stimuli-responsive controlled drug release under ultrasound or laser, resulting in an optional release profile as needed. We believe that these novel microcapsules, as

well as the drug release strategy, hold great application potential for controlled drug release, separation, immunoassays, and as contrast agent for diagnostics and therapeutics. It is worth mentioning that, when the microcapsules are intended for *in vivo* study (*e.g.* injecting into the blood circulation system), the PDMS should be changed into biodegradable materials, and the size of the microcapsules should be reduced as well.

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Supplementary data

Supplementary data associated with this article is available online.

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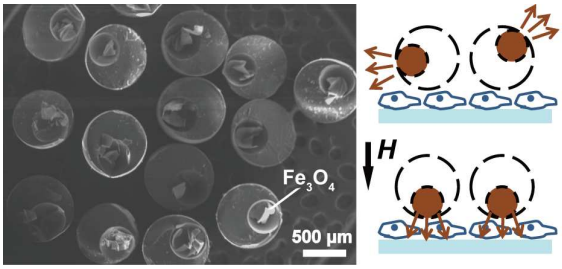
Table of contents entry for

Eccentric Magnetic Microcapsules for Orientation-specific and Dual Stimuli-responsive Drug Release



Uniform eccentric magnetic microcapsules show controlled-release behavior for orientation-specific and dual stimuli-responsive drug delivery under ultrasound and laser regulation.