Supplementary Information

Nearly monodispersed core-shell structural Fe_3O_4@DFUR–LDH submicro particles for magnetically controlled drug delivery and release

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Experimental section

1) Preparation and Characterization of Fe_3O_4@DFUR-LDH submicro particles

Preparation of Fe_3O_4@C submicro particles. Fe_3O_4 submicro particles with diameter of 220 nm were first synthesized *via* a solvothermal reaction as previously described with proper modifications.[1] In the next step, 0.200 g Fe_3O_4 submicro particles were ultrasonicated for 10 min in 0.1 M HNO_3, followed by washing twice with deionized water. Then, the treated Fe_3O_4 ubmicro particles were redispersed in 0.5 M aqueous glucose solution. After ultrasonicating for 20 min, the suspension was transferred to an autoclave and kept at 180 ^oC for 8 h. After reaction, the autoclave was cooled naturally in air, and the suspensions was isolated with the help of a magnet and washed with deionized water and alcohol for three times, respectively, resulting in the product Fe_3O_4@C.

Preparation of Fe_3O_4@C@LDH and Fe_3O_4@LDO submicro particles. 0.100 g Fe_3O_4@C submicro particles were dispersed into a 60 ml methanol solution containing containing 0.420 g NaOH then ultrasonically agitated for 20 minutes to obtain a uniform suspension. Another 60 ml methanol solution containing 0.769 g $Mg(NO_3)_2{\cdot}6H_2O$ and 0.563 g $Al(NO_3)_3{\cdot}9H_2O$ was added dropwise into the above suspension with vigorous stirring until the final pH ca. 9.5. The resulting slurry was aged at 60^oC under N_2 atmosphere for 48 h to yield Fe_3O_4@C@LDH. In the next step, the Fe_3O_4@C@LDH was heated to 500 ^oC at a ramp rate of 0.5 ^oC min^{-1} under N_2 atmosphere and kept at this temperature for 2 h to remove NO_3^- and water. The obtained mixed oxides were denoted as Fe_3O_4@LDO and kept in a vacuum desiccator.

Preparation of Fe_3O_4@DFUR-LDH submicro particles. 0.150 g Fe_3O_4@LDO submicro particles were added into a 50 ml aqueous solution and then ultrasonicated under N_2 atmosphere for 30 min to form a uniform suspension. Another 50 ml aqueous solution containing 0.500 g doxifluridine (DFUR) was added into the above suspension with vigorous stirring. The resulting slurry was aged at 60^oC under N_2

atmosphere for 24 h, and then the obtained product was washed by decarbonated deionized water for three times and collected with the aid of the magnet. The final product was dried at 60°C in vacuum for 24 h and denoted as Fe_3O_4@DFUR-LDH.

Characterization. Powder XRD data were taken on a Shimadzu XRD-6000 diffractometer using Cu Kα radiation (λ = 1.5418 Å, 40 kV, 30 mA). The samples, as un-oriented powders, were step-scanned in steps of 0.02° (2θ) in the range of 2θ 3–70° using a count time of 4 s per step. The FT-IR spectra were obtained on a Bruker Vector-22 FT-IR spectrophotometer using KBr pellet technique (sample/KBr = 1/100). The SEM micrographs were recorded on Zeiss Supra 55 field emission scanning electron microscope. The TEM graphs were recorded on a HITACHI-800 transmission electron microscope. The HRTEM graphs were recorded on JEM 2010 transmission electron microscope. The actual metal incorporation contents were measured by inductively coupled plasma (ICP) emission spectroscopy on a Shimadzu ICPS-7500 instrument. The drug content in nanohybrid was determined based upon carbon, hydrogen and nitrogen (CHN) elemental analysis using an Elementar Vario Elemental Analyser. The magnetization of the magnetic submicro particles was tested on a JDM-13 vibrating-sample magnetometer at 298 K and ±15kOe applied magnetic field. The amount of drug released in PBS solution was determined by UV-Vis absorbance on a Shimadzu UV-2501 PC UV-Vis spectrophotometer.

2) In vitro magnetically controlled drug release of Fe_3O_4@DFUR-LDH submicro particles

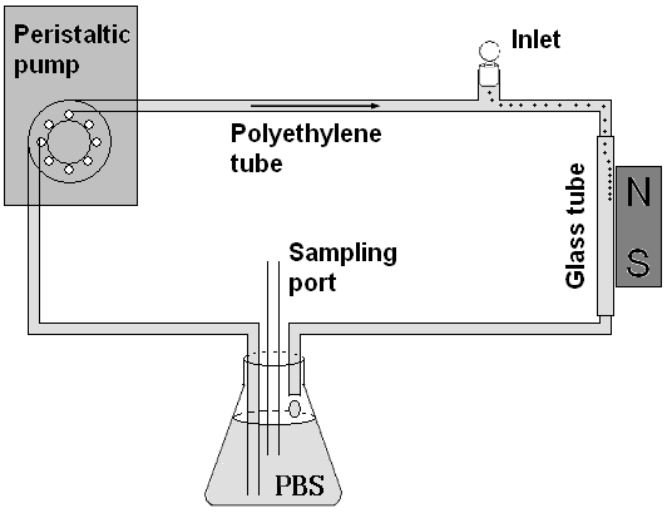
In vitro drug release without an external magnetic field (MF). A solution simulated gastrointestinal and intestinal fluid at pH 7.45 without pancreatine (phosphate buffered solution, Chinese pharmacopoeia 2005) was employed as release medium. The Fe_3O_4@DFUR-LDH submicro particles (0.05 g) was suspended in 250 ml release medium in a flask and incubated in a water bath at 37±0.5°C with paddle rotation speed of 50 rpm. Aliquots (3 ml) of the release medium was taken at prefixed time intervals, replaced by a same volume of fresh PBS, centrifuged at 12000 rpm and the supernatant DFUR content was determined by UV-Vis absorbance (λ_{max} =270nm).

In vitro drug release with an external MF of 0.15 T. In order to investigate the drug

release behavior of the Fe_3O_4@DFUR-LDH submicro particles under the magnetic drug targeting operation, a stable MF of 0.15 Tesla (surface magnetic flux density) was applied to emulate the magnetic location and magnetically controlled release process (i.e. "MF on" mode). The magnet was placed just beside the flask and the position of the magnet relative to the flask is unchangeable during the whole release process.

In vitro drug release with MF On-Off switch operation. The drug release behavior of the Fe_3O_4@DFUR-LDH submicro particles under quasi MF On-Off switch operation was also examined through applying and removing a MF of 0.15 T alternatively for several cycles to study the in-time drug release property of the magnetic nanohybrids. At the prefixed time of "MF On", the magnet was placed beside the flask, while at the prefixed time of "MF Off", the magnet was removed immediately and other conditions kept unchanged.

Simulated magnetic drug targeting and controlled release. To investigate their magnetic drug targeting and controlled release properties, the Fe_3O_4@DFUR–LDH submicro particles were submitted to a device designed for simulating the circulatory fluid system in human body as shown in Scheme S1.



Scheme S1. The device for simulating the circulatory fluid system in human body for studying the magnetic drug targeting and controlled release property

The device is composed of a peristaltic pump, a permanent magnet with surface magnetic flux density of 0.45 T, a water bath, a flask, a glass tube and polyethylene

tubes. The release medium (250 ml PBS) is pumped from the flask incubated in the water bath (37.5 ± 0.5°C) and flows along the polyethylene tubes with a fixed flow velocity to the targeted site, the glass tube beside the permanent magnet, and then flows back to the flask to form a circulatory system as that in human body. The Fe_3O_4@DFUR–LDH submicro particles (0.05 g, dispersed in 10 ml deionized water) are injected into the circulatory system in the inlet site, and flow along with the release medium. When the Fe_3O_4@DFUR–LDH submicro particles reach the glass tube, they were captured by the magnet beside the glass tube and retained there. Aliquots (3 ml) of the release medium was taken at prefixed time intervals, replaced by a same volume of fresh PBS, centrifuged at 12000 rpm and the supernatant DFUR content was determined by UV-Vis absorbance (λ_{max} =270nm).

The retentions of the Fe_3O_4@DFUR–LDH submicro particles were also tested in this device. The release medium PBS was changed as deionized water to prevent the release of the DFUR from Fe_3O_4@DFUR–LDH submicro particles. *A* g of Fe_3O_4@DFUR–LDH submicro particles was injected in the inlet site, and then the retained particles in the glass tube were collected after 5 min and dried at 60 °C for weighing (*B* g). The retention of Fe_3O_4@DFUR–LDH submicro particles was calculated by the following equation:

$$Retention = \frac{B}{A} \times 100\%$$

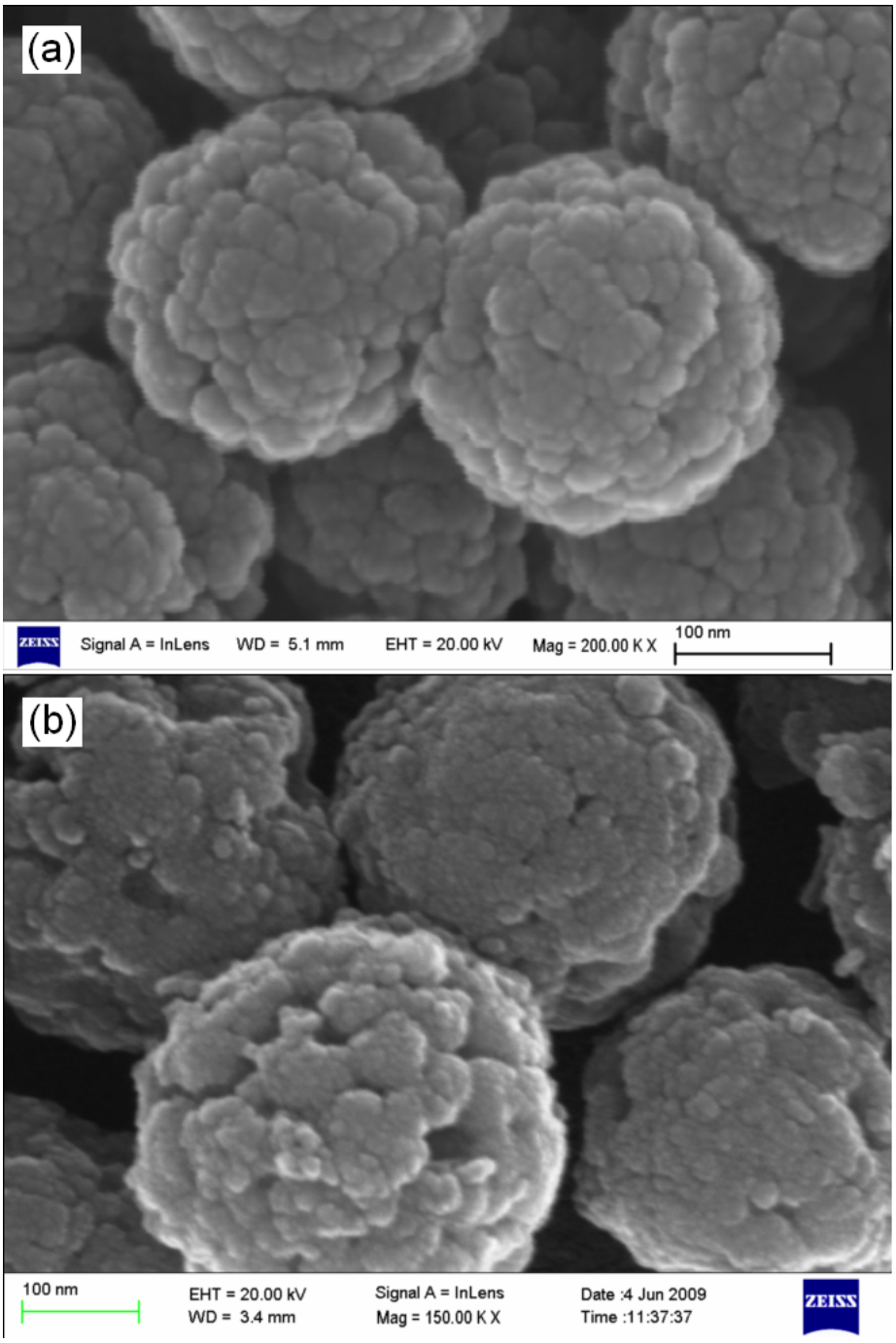


Fig. S1. SEM images of (a) Fe_3O_4 and (b) Fe_3O_4@DFUR-LDH.

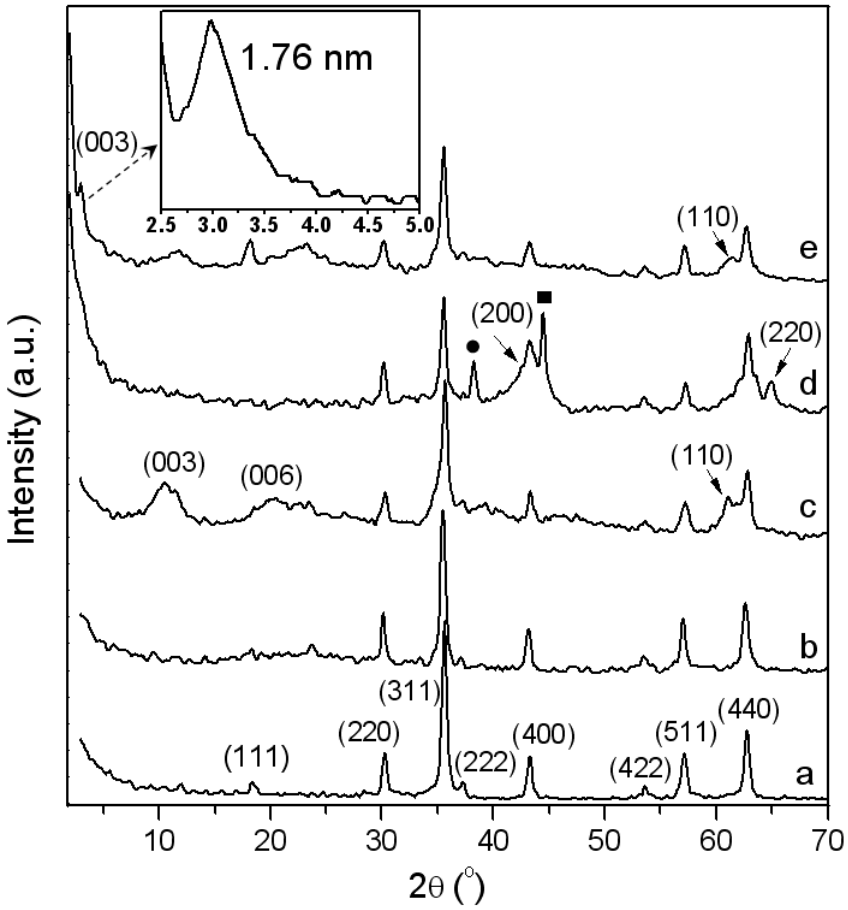


Fig. S2 XRD patterns of (a) Fe_3O_4, (b) Fe_3O_4@C, (c) Fe_3O_4@C@LDH, (d) Fe_3O_4@LDO (● goethite phase, ■ α–Fe phase) and (e) Fe_3O_4@DFUR–LDH.

Fig. S2 shows the XRD patterns of the samples at various stages of the fabrication of Fe_3O_4@DFUR–LDH composites. For Fe_3O_4@C (Fig. S2b), the broad band at $2\theta = 25^o$ can be assigned to the Carbon shell.[1] The other diffraction peaks can be readily indexed to a cubic structure of magnetite according to JCPDS card No. 19–0629. In the case of Fe_3O_4@C@LDH, besides of the characteristic peaks of Fe_3O_4 and Carbon, the obvious diffraction peaks at $2\theta = 11^o$, 22^o and 62^o can be indexed to the (003), (006) and (110) plane of the LDH material, suggesting the successful crystallization of NO_3–LDH on the surface of Fe_3O_4@C as the interlayer spacing d_{003} of 0.86 nm.[2] After calcined at 500 ^oC for 2 h, the LDH phase disappears, instead of mixed oxides characterized by two broad peaks indexed to (200) and (220) plane of the MgO,[2] small amount of goethite phase

(marked with ●) and α–Fe phase (marked with ■) can also be detected. [3] As shown in Fig. S2e, when this mixed oxides was dispersed into the DFUR solution for 24 h, the LDH phase characterized by (003) and (110) plane emerges, indicating that the DFUR–LDH was successfully coated on the surface of the Fe_3O_4 by the calcination-reconstruction process. The d_{003} of the coated DFUR–LDH shell crystallite is 1.76 nm, may be ascribed to the bilayer arrangement of the DFUR between the LDH layers, considering the size of 0.81 nm for DFUR molecule along its long axes. The particle size along the (110) plane of the coated DFUR–LDH shell crystallite is also calculated by the Scherrer equation ($D = 0.89\lambda / (\beta\cos\theta)$, where λ is the X-ray wavelength (0.1542 nm), θ the Bragg diffraction angle, and β the FWHM of the XRD lines). It should be mentioned that the exact evaluation can be carried out through the deconvolution of overlapped (110) and (113) peaks by Original Pro 7.0. [4] As calculated, the D_{110} value of the coated DFUR–LDH crystallites is ca. 12 nm, conformed to the SEM result.

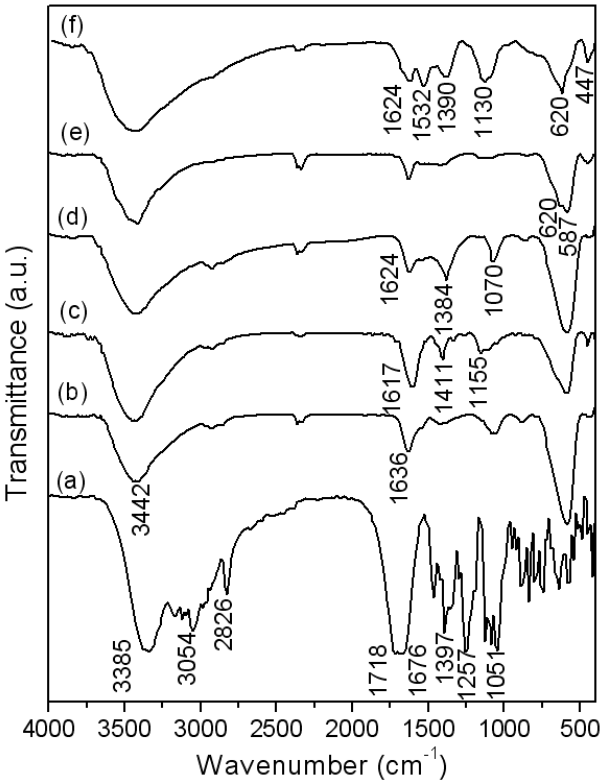
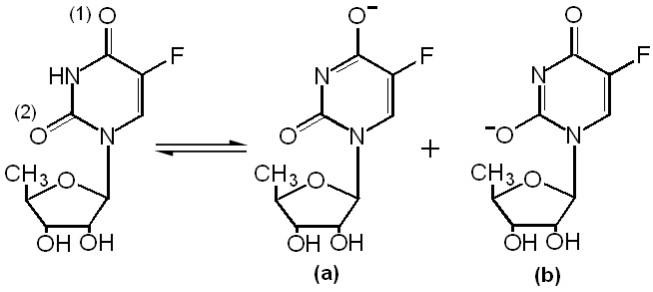


Fig. S3. FT–IR spectra of (a) DFUR, (b) Fe_3O_4, (c) Fe_3O_4@C, (d) Fe_3O_4@C@LDH, (e) Fe_3O_4@LDO and (f) Fe_3O_4@DFUR–LDH.

The FTIR spectrum of Fe_3O_4@DFUR–LDH (Fig. S3f) shows a new band at 1532 cm^{-1} due to the appearance of conjugated ionized C–O^- in tautomer (a) of the DFUR molecules, while that at 1676 cm^{-1} of C=O(2) keeps unchanged and presents as a shoulder band. The band at 1397 cm^{-1} downshifts to 1384 cm^{-1} is due to the cleavage of the CN–H.[5] These phenomena indicate that the DFUR species between the LDH layers can only be recognized as a negatively charged resonance tautomer (a) in the coated DFUR–LDH.



Resonance structure of DFUR.

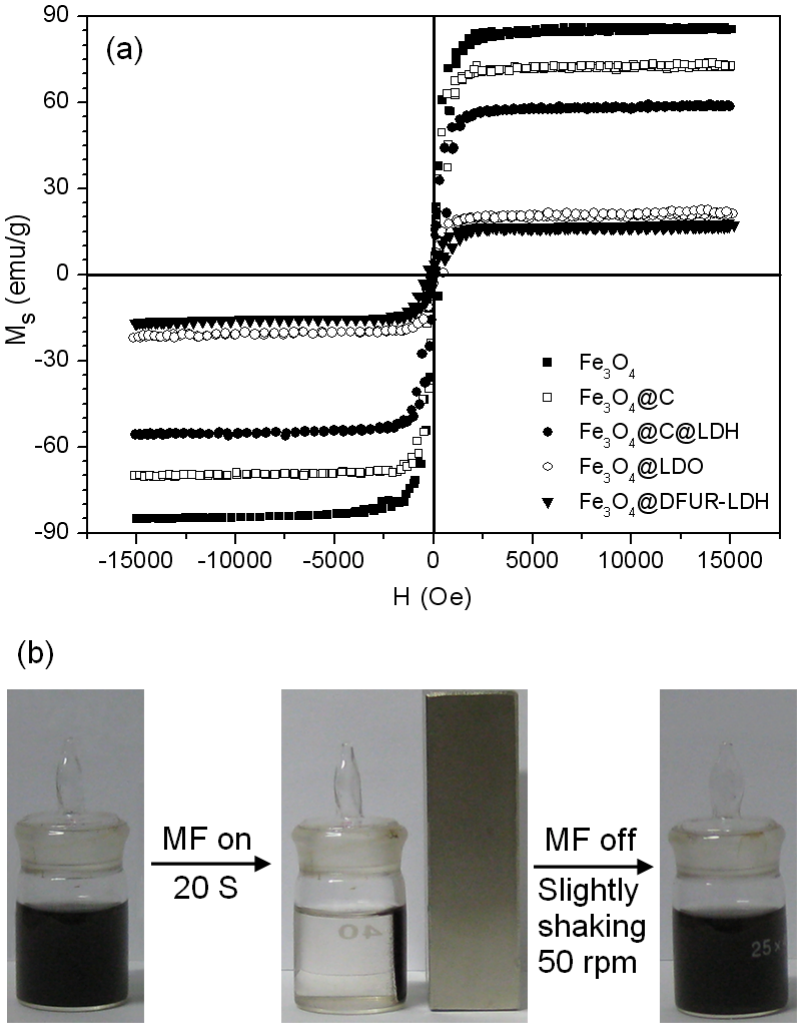


Fig. S4. (a) Magnetization curves of the samples at various stages of the fabrication of Fe_3O_4@DFUR–LDH submicro particles, (b) magnetic separation and redispersion of Fe_3O_4@DFUR–LDH submicro particles.

The magnetic separability of such magnetic submicro particles was tested in ethanol by placing a magnet near the flask. The black particles in solution were attracted towards the magnet within 10 s, directly demonstrating that the Fe_3O_4@DFUR–LDH particles can be separated readily from a sol or a suspension system to carry drugs to targeted locations with the aid of an external magnetic field (MF). After shaking at 50 rpm for 30 s, the Fe_3O_4@DFUR–LDH particles can be redispersed again to form a homogenous suspension.

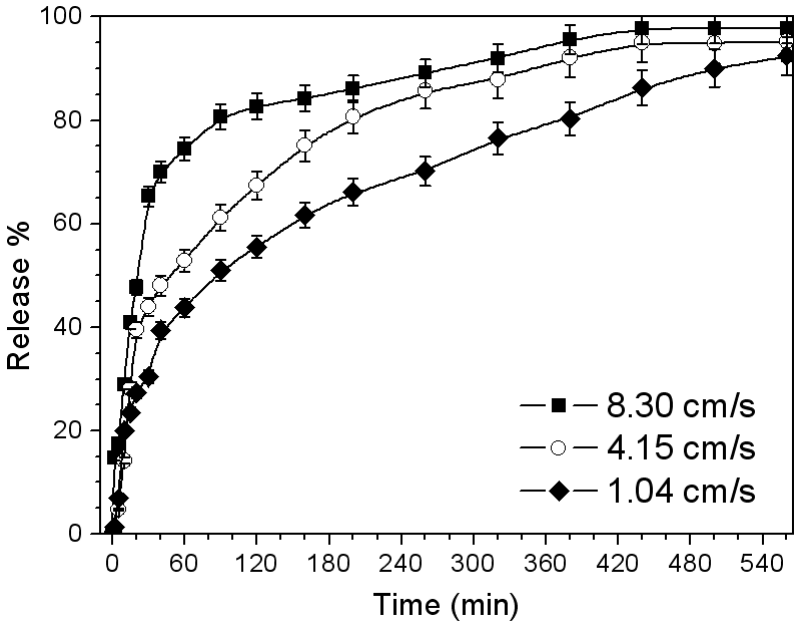


Fig. S5 Release profiles of Fe_3O_4@DFUR–LDH under a MF of 0.45 T in the circulatory device with the release medium flow velocity of 1.04, 4.15 and 8.30 cm/s.

The drug release of Fe_3O_4@DFUR–LDH particles in the circulatory device was also studied by setting the *d* of 0 cm and the *V* of 1.04, 4.15 and 8.30 cm/s (as linear flow rate of blood is appoximately 30 cm/s in large arteries while 0.5 cm/s in capillaries[6]), respectively. As shown in Fig. S5, when *V* is 1.04 cm/s, the $t_{0.5}$ (the time for release fraction of 50%) for Fe_3O_4@DFUR–LDH is ~90 min with t_{eq} (the time for release reaches equilibrium) of ~650 min. when *V* is 4.15 cm/s, the $t_{0.5}$ for Fe_3O_4@DFUR–LDH is ~50 min with t_{eq} of ~440 min. Further increase the *V* to 8.30 cm/s, the $t_{0.5}$ for Fe_3O_4@DFUR–LDH decreases to ~20 min. These findings strongly suggest that the drug release rate increases as increasing the *V* of the release medium, probably due to the decreasing retentions of the Fe_3O_4@DFUR–LDH particles and the increasing diffusion rate of DFUR in the circulatory system.

Reference

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