Supplementary Information

Remotely Nano-Rupturable Yolk/Shell Capsules for Magnetically-Triggered Drug Release

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Experimental Section

Synthesis of yolk/shell capsules: To prepare the self-assembled magnetic nanoparticles and functional polymers, 5 mg of iron oxide was centrifuged at 6000 rpm for 10 min and then re-dispersed in 4 mL of a 1% ibuprofen (model drug) chloroform solution. Different ratios of PVA/F68 as shown in **Table 1** were heated in 10 mL of DI water at 70 °C until the solids had completely dissolved, and this clear solution was then cooled to room temperature. Next, the chloroform solution was added to the polymer solution and the resulting mixture was emulsified for 5 minutes with a 100 W ultrasonicator. The mixture was then stirred magnetically at room temperature to evaporate the organic solvent. After the evaporation of the organic solvent, the products were washed 3 times with DI water and centrifuged at 6000 rpm to collect the desired products. An ultra-thin silica shell was then added to the nanospheres using the modified Stöber method. In brief, 5 mg of nanospheres were dispersed in 4 mL of 99.5% ethanol and 0.1 mL of 33% NH_4OH for 30 min. Following this, 50 μm of tetraethylorthosilicate (TEOS) was slowly added to the mixture and the mixture was stirred for 12 h. After hydrolysis and condensation, the silica shell was coated onto the nanocomposites (NCs) to form the yolk/shell capsules.

Characterization: The morphologies of the yolk/shell capsules were examined using field emission scanning electron microscopy (FE-SEM, JEOL-6500, Japan) and transmission electron microscopy (TEM, JEM-2100, Japan). For SEM analysis, the capsules were dried on 0.5×0.5 cm silicon waters. After drying, the capsules were coated with an ultrathin metal layer using platinum sputtering to enhance the image quality. The magnetization of the yolk/shell capsules was measured using a superconducting quantum interference device (SQUID, MPMS-XL7) at 298 K and ±8000 G applied magnetic field. Before SQUID analysis, the iron oxide nanoparticles and yolk/shell capsules were dried in a vacuum oven at 60 °C for 2 days. The relative amount of magnetic nanoparticles that were associated with the gelatin was determined using thermo-gravimetric analysis (TGA, Perkin Elmer). Samples were dried under vacuum for approximately 48 hours and analyzed in the platinum plate at a heating rate of 10 °C/min under an atmosphere of nitrogen. The high-frequency magnetic field (HFMF) was produced by a power supply with a functional generator, amplifier, and cooling water. The strength of the magnetic field in these systems depends on the coils used; in this study, the coil had 8 loops with a frequency of 50 kHz and a magnetic field strength (H) of 2.5 kA/m. The temperature of the HFMF generator was controlled by cycling cooling water at 25 °C.

Drug Loading Efficiency and Release: This study used ibuprofen (IBU) as a model drug to examine the drug loading efficiency and release behaviors of the yolk/shell capsules. After the drug loading process, the drug-containing capsules were separated from the aqueous solution by centrifugation at 10,000 rpm for 15 min. The drug concentration in the supernatant was analyzed using a UV-vis spectrophotometer (Agilent, 8453 UV-Visible spectrophotometer) at a wavelength of 264 nm, where there is a strong absorption band from the IBU. There are three replicates at each time point for the release profiles. The amount of drug in the yolk/shell capsules was calculated by subtracting the residual IBU in the supernatant from the total amount of IBU measured. The encapsulation efficiency (EE) was obtained as follows: $EE=(A-B)/A\times 100$, where A is the total amount of IBU, B is the amount of IBU remaining in the supernatant. For the drug release tests, the capsules were washed with phosphate buffered saline (pH 7.4) and then DI water. Ibuprofen-containing capsules were

placed in PBS buffer solution for all drug-release experiments. A quantitative estimate of the IBU loading was obtained using UV-vis spectrophotometry, and the drug-release behavior of the capsules was measured using 20 mL of phosphate buffered saline solution (pH 7.4). To measure the concentration of released drug, 1.5 mL of the capsule-containing PBS medium was centrifuged at 10,000 rpm to remove the particles.

Cell Culture: Three cell lines were used as normal cell models: retinal pigment epithelium (RPE) cells, ARPE-19 cells, and a monolayer of hexagonal cells that separate the neural retina from the underlying choroidal vascular bed. The cells were cultured on 75 cm^2 flasks at a density of 10,000 cells/cm^2 and were maintained in culture until the plates reached >95% confluence. Cultures were fed Dulbecco's modified Eagle's medium-nutrient mixture F-12 (DMEM-F-12; GIBCO, Invitrogen Corporation, Grand Island, NY) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 g/mL of streptomycin. Cells were cultured in complete medium at 37 ℃ in a humidified atmosphere of 5% CO_2 in air. In all experiments, cells were harvested from subconfluent cultures using trypsin and were resuspended in fresh complete medium before plating. The in vitro cytotoxicity of the capsules to the AREP-19 cells was tested with an external magnetic field treatment using an in vitro proliferation method with MTT. In brief, 1×10^4 cells were plated in 96-well plates to allow the cells to attach. The cells were then exposed to 10 μL/cc of capsules at 37 °C. At the end of the incubation period, 20 μL of MTT solution was added and the cells were incubated for an additional 4 h. The medium was then replaced with 200 μL of DMSO and the absorbance was monitored using a Sunrise absorbance microplate reader at dual wavelengths of 570 and 650 nm.

To estimate the cellular uptake of capsules, green emitting fluorescein dye was attached to the yolk/shell capsules (FITC-labeled yolk/shell capsules). First, fluorescein isothiocyanate (FITC) was mixed with an ethanolic 3-aminopropyltrimethoxysilane (APTMS) solution for 24 h at room temperature to form N-1-(3-trimethoxysilylpropyl)-N-fluoresceyl thiourea (FITC-APTMS). In a separate flask, 5 mg of yolk/shell capsules were dispersed in 4 mL of 99.5% ethanol and 0.1 mL of 33% NaOH solution for 30 min. Following this, 40 μm of

tetraethylorthosilicate (TEOS) and 10 μm of FITC-APTMS were slowly added to the mixture, which was then stirred for 12 h. After hydrolysis and condensation, the FITC-labeled silica was coated onto the capsules. The unreacted chemicals were removed by rinsing with DI water three times. The capsules were incubated with the cells for different times and then the cells were observed by confocal microscopy (Nikon, C1).

Table S1. Reagents used for the synthesis of thermosensitive yolk/shell capsules. The saturation magnetization (Ms) of yolk/shell capsules, and their encapsulation efficiency (EE) where the hydrophobic drug, ibuprofen, was used as a model drug.

sample	Weight Ratio			Ms (emu/g)	Fe_3O_4 (%)	EE (%)[a]	Drug content[b] (mg IBU/ mg capsules)
	Fe_3O_4	F68 /PVA	TEOS				
Yolk/shell-1	1	1/5	2.5	7.9	12	73±5	0.27
Yolk/shell-2	1	2.5/5	2.5	6.7	10	70±4	0.26
Yolk/shell-3	1	5/5	2.5	4.9	8	65±4	0.24
Yolk/shell-4	1	10/5	2.5	3.1	5	58±5	0.22

[a]**EE (%):** The encapsulation efficiency (EE) was obtained as follows: *EE=(A-B)/A×100,* where *A* is the total amount of IBU, *B* is the amount of IBU remaining in the supernatant.

[b]**Drug content:** mg IBU/mg Capsules. The drug-loaded capsules were dried and weighted. Then, theses capsules were dispersed in the PBS, and subjected to high-frequency magnetic field for 30 minutes for releasing all of the drug in the capsules. To measure the concentration of released drug, 1.5 mL of the capsule-containing PBS medium was centrifuged at 10,000 rpm to remove the particles. Finally, a quantitative estimate of the IBU loading was obtained using UV-vis spectrophotometry at a wavelength of 264 nm, where there is a strong absorption band from the IBU.

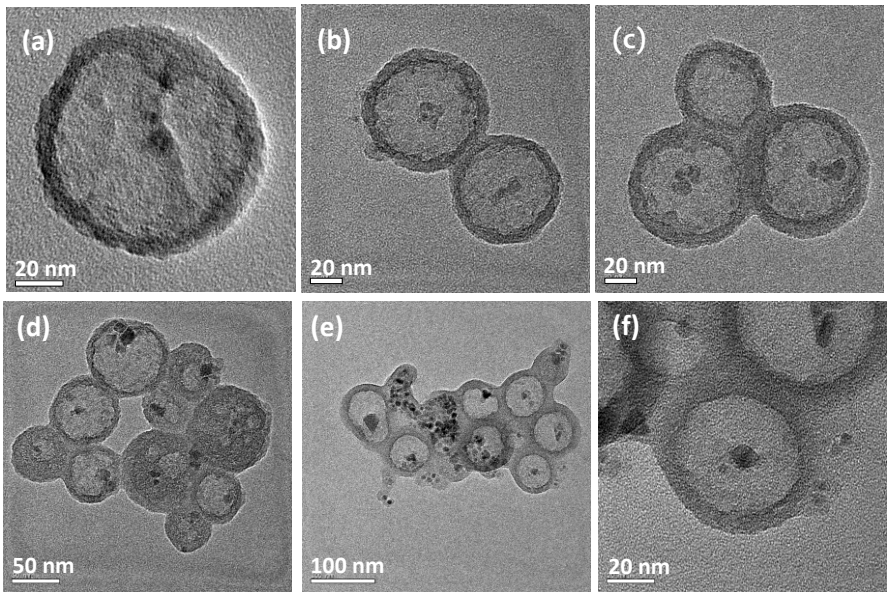

Figure S1. TEM images of (a) yolk/shell-2, (b) yolk/shell-3, and (c) yolk/shell-4. (d-e) TEM images of yolk/shell capsules without PVA. Without PVA as a stabilizer, the nanocapsules displayed a un-uniform morphologies. Some magnetic nanoparticles were not encapsulated in the nanocapsules. Removing PVA from this system resulted in yolk/shell capsules with non-uniform morphologies This finding suggests that PVA plays an important role in stabilizing the core-shell structure and further in promoting the regular adsorption of the silica precursor on the surface. This could result from the high density of the hydroxide functional group in PVA, which forms strong hydrogen-bonding interactions with the -OH group on TEOS.

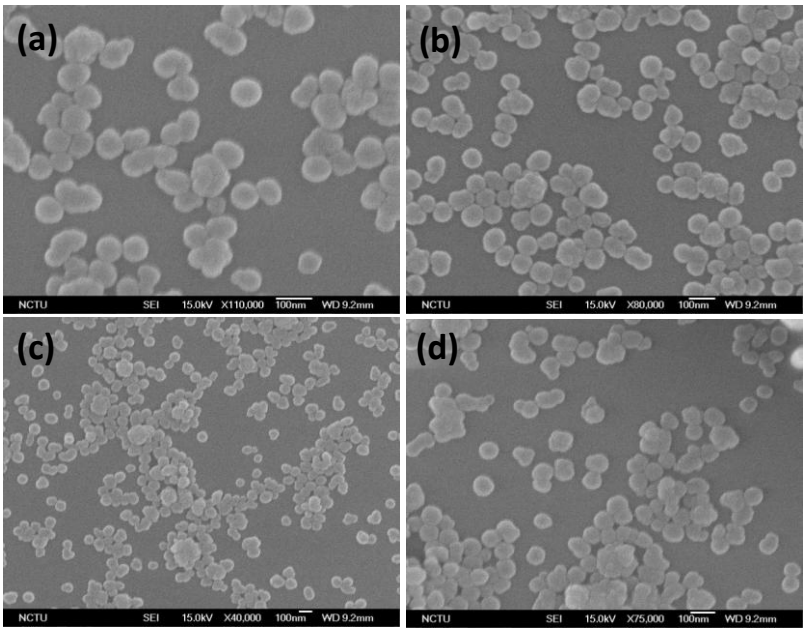

Figure S2. SEM images of (a) yolk/shell-1, (b) yolk/shell-2, (c) yolk/shell-3, and (d) yolk/shell-4. SEM images of yolk/shells-2, 3 and 4 displayed morphologies similar to those of yolk/shell-1, which had a spherical geometry. The ultra-thin silica shell was designed to be a physical barrier that could eliminate undesired drug release and regulate desired release in a controllable manner.

The magnetic properties of the yolk/shell capsules were investigated using a superconducting quantum interference device (SQUID) at 298 K with a magnetic field sweeping from -10000 to +10000 G. **Figure S3** shows the correlation of the magnetization with the magnetic field from yolk/shells-1 to -4, where the curves are similar for all yolk/shells and display negligible hysteresis. The increased polymer content from yolk/shell-1 to yolk/shell-4 dilutes the concentration of iron oxide particles, resulting in a decreasing saturation magnetization (Ms). The weight % (wt%) of iron oxide nanoparticles in the capsules shown in **Table S1** were determined by the relative intensities of saturation magnetization (Ms) between the Fe_3O_4 and the yolk/shell capsules. The wt% of iron oxide nanoparticles in the yolk/shell capsules ranged from 5-12%, suggesting a relatively low concentration of these nanoparticles.

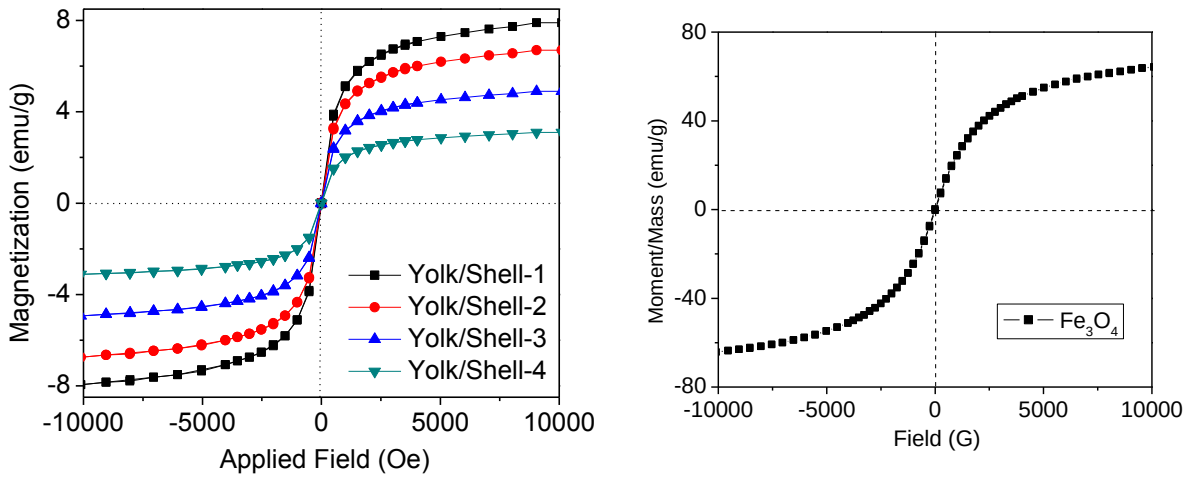

Figure S3. Field-dependent magnetization curves of yolk/shell-1 to yolk/shell-4, showing the capsules are superparamagnetic. Field-dependent magnetization curves of magnetic nanoparticles. The saturation magnetization (Ms) of iron oxide nanoparticles is 67 emu/g.

To investigate the drug-loading capacity of the yolk/shell capsules, the hydrophobic model drug ibuprofen (IBU) was loaded into the capsules. The encapsulation efficiency (EE) of IBU with different PVA/F68 ratios is shown in **Table S1.** The capsules encapsulate the drug molecules through a microemulsion process. This process enhances the drug loading efficiency because it gathers the hydrophobic substances like iron oxide nanoparticles and

drug molecules into the oil-phase of the droplets. During evaporation of the organic solvent, the droplets form stable nanospheres that contain the hydrophobic molecules and nanoparticles. Furthermore, the hydrophobic chains of the amphiphilic polymer that are in the center of the nanospheres efficiently bind the hydrophobic drug through hydrophobic interaction. Because the hydrophobic drug has a low solubility in aqueous solutions, the drug molecules were preferentially encapsulated in the nanospheres rather than remaining in water. With an increase in the F68 ratio, the IBU encapsulation efficiency decreased from 0.73 to 0.58. The high encapsulation efficiency of IBU in the capsules resulted from three factors: first, the vast space of the polymer matrix in the yolk/shell capsules allows for a high payload. Second, PVA forms strong intrinsic hydrogen bonds, tightening the nanocomposites and making drug encapsulation more stable. Finally, silica shells are effective shielding layers to seal the drug molecules into the yolk/shell capsules and prevent leakage.

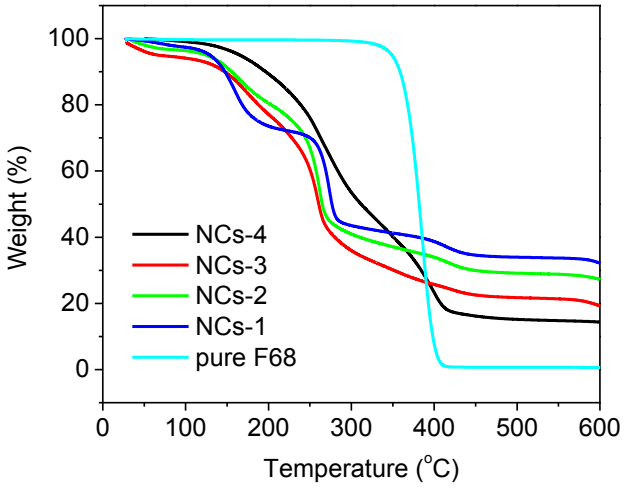

Figure S4. TGA analysis of yolk/shell capsules and pure F68 polymer. Furthermore the weight ratios of organic substances in the yolk/shell capsules ranged from 66-85% as estimated by TGA analysis. This could be advantageous for conjugating therapeutic molecules because a large proportion of organic material such as the polymer matrix can trap more therapeutic agents, thus increasing the payload in each capsule.

Table S2. Summary of fitting constants (n and k) for cumulative release under the magnetic field treatment. The F68/PVA nanocomposites (NCs) weight lost after 1 minute of magnetic stimulus, estimated by TGA.

Sample	Org./Ino. after MF (TGA)	F68/PVA Lost (%)	n	K X 10^3
Yolk/shell-1	31/69	35	0.40	42
Yolk/shell-2	26/74	47	0.39	30
Yolk/shell-3	23/77	57	0.39	24
Yolk/shell-4	19/81	66	0.39	14

Weight loss of F68/PVA after the magnetic field stimulus

We investigated the weight loss of F68/PVA after the magnetic field stimulus by thermogravimetric analysis (TGA) in **Figure S4**. After a 1 min period of magnetic field treatment, the amount of F68/PVA decreased 35-66% from yolk/shell-1 to yolk/shell-4 as shown in **Table S2,** indicating that substantial portions of the core phase did not become permanently incorporated into the cores. This was especially true for capsules with high F68 ratios. These findings suggest that the weight loss of the F68/PVA in the core results from outward diffusion caused by the rupture of the capsules and the formation of large cracks. This provides a reasonable explanation of why the drug release remained rapid after the burst-like release behavior. However, the release behavior in Figure 4a also demonstrates a positive relationship between F68 concentration and the release rate after the removal of the magnetic field. This further supports the conclusion that the thermosensitive polymer regulates the rupture effect of the shells. In other words, subjection to the magnetic field

perturbed the nanostructure of the inner core phase, considerably accelerating the movement of the IBU molecules due to the high stress of the core phase. Concurrently, thermally induced expansion and then shrinking of the core phase led to a significant release of IBU to the environment. This resulted in a burst-like release behavior for these yolk/shell capsules. However, once the stimulus was removed, the inner core phase could not be restored to its original state; thus, drug release continued following removal of the magnetic field. This increases the likelihood that the drug molecules will not be heated and harmed by magnetic heating, allowing them to reach high active concentrations in areas where release is triggered for local therapy.

Release kinetics

To estimate the mechanism of drug release from the yolk/shell capsules under magnetic field treatment, the release kinetics can be characterized using the equation,

$$Mt/M=kt^n,$$

where Mt is the mass of drug released at time t, M is the mass released at infinite time, k is a rate constant, and n is a characteristic exponent related to the mode of transport of the drug molecules.[1] This analysis was performed for $Mt/M<0.6$, and the fitting parameters are listed in Table S1. Under magnetic induction the exponent constants (n) are all approximately 0.4, which is beyond the regime of the Fickian diffusion mode. This indicates that the rapid release may result from a strong driving force such as the rupture of the capsules that allows for effective diffusion of drug from the core. Accordingly, the diffusion of drug from yolk/shell capsules occurs along two paths after rupture. One path is from the core of the yolk/shell capsules, which would be caused by the magnetic heating-induced volume/hydrophobicity transition that would pump the drug molecules from the polymer matrix toward the external environment. This path would have relatively fast release kinetics. The second path involves free diffusion towards the capsule outlet. Moreover, the rate

constant (k) in Table S1 clearly shows that there is a systematic growth of k from yolk/shell-4 to yolk/shell-1. These results indicate that the drug release from the yolk/shell capsules is a thermally activated process, and the thermosensitive F68 polymer in the capsules plays an important role in accelerating the magnetically triggered release.

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[1] P. L. Ritger, N. A. Peppas, *J. Control. Release* **1987,** *5*, 37– 42.