Supplementary Information for

Synthesis of Iron Oxide Nanocubes Patched Janus Magnetic Nanocarriers for Cancer Therapeutic Applications

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Experimental Section

Material:

Iron(III) chloride hexahydrate (≥98%), *n* -docosane (99%), 1-octadecene (90%), *n* -hexane, ethanol, tetraethyl orthosilicate (98%) (TEOS), (3-Aminopropyl)triethoxysilane(99%) (APTES), Sodium Hydroxide (NaOH), ethyl acetate (EtOAc) were purchased from Sigma–Aldrich Co. (Milwaukee, WI). Sodium oleate (>97%, TCI America, Portland, OR). Cetyltrimethylammonium bromide (CTAB) (MP Biomedicals, LLC).

IONCs-P-JMNC synthesis:

5.40 g iron chloride hexahydrate was mixed with 18.25 g sodium oleate in a 250 ml round-bottom flask. 30 ml Milli-Q water, 70 ml *n* -hexane and 40 ml ethanol were sequentially added into the flask. The mixture was maintained under nitrogen atmosphere at $T = 70$ °C with vigorous stirring for 4 hours. The resulting solution was washed 3 times by Milli-Q water and collected organic phase by a separatory funnel. The dark red organic phase further underwent evaporation at 50-60 °C in a vacuum bin to remove excessive hexane. 1.57 g as-prepared iron oleate, 0.53 g sodium oleate, 6.0 g *n*-docosane and 11 ml of 1-octadecene were mixed in a 100 ml round-bottom flask. The flask was heated up to 120 °C under high vacuum and maintained for at least 30 minutes. Then the vacuum was replaced with nitrogen atmosphere and the mixture was constantly heated up to 325 °C at a rate of 3 °C/min with continuous stirring for 30 minutes. The final solution was cooled down to 80 °C and precipitated by a mixture composed of *n*-hexane and ethanol (volume ratio=2:3). The precipitation was collected and re-dispersed by a small amount of *n*-hexane. Ethanol washing steps are carried out at least 3 times. The final precipitation was dried under vacuum and re-dispersed in n-hexane at a concentration of 6 mg/ml, noted as INOCs. As-synthesized IONCs hexane solution (1 ml) was mixed with 0.01 M CTAB aqueous solution (10 ml) in a 20 ml vial and emulsified by a standard ½" diameter probe with 50% amplitude for 30 s (about 200 J). The mixture underwent vigorous magnetic stirring (1000 rpm) in 65 ℃ water bath for at least 1 h to evaporate all hexane. The resulting solution changed to transparent yellow. To synthesize IONCs-P-JMNC, aforementioned 10 ml CTAB stabilized IONC solution was diluted to 20 ml with Milli-Q water and adjusted pH to 11 by adding NaOH solution. 400 μl EtOAc and 100 μl TEOS were sequentially added to the system while mechanical stirring. The reaction carried out for 4 h in room temperature to result in IONCs-P-JMNC (noted as Si1 in **Fig. 5b**), which were washed with ethanol and collected by centrifugation (9,000 rpm,10 min). To generate a thicker silica coating, 150 μl, 225 μl and 300 μl was added into the system, noted as Si2, Si3 and Si4 **(Fig. 5a).** Excessive CTAB was extracted by ethanolic NH_4NO_3 solution (6 mg/ml) and kept stirring for 12h. Additional washing steps can be repeated for several times. To make particle water soluble, APTES was further applied to modify IONCs-P-JMNC surface. As-synthesized IONCs-P-JMNC was re-dispersed in 30 ml ethanol with 100 ml 30% ammonia and 300 μl APTES. The solution was mechanical stirring for 12 h before washed with ethanol. The final product was dispersed in water and prepared for further experiments.

Nanoparticles characterization:

The morphologies of the synthesized nanoparticles were observed by transmission electron microscopy (TEM; FEI Tecnai Spirit G2, Hillsboro, OR) operating at 80 kV. Dynamic Light Scattering (DLS) was performed by Zetasizer Nano ZSP in the Analytical bioNanoTechnology Core Facility of the Simpson Querrey Institute at Northwestern University.

CTAB and oleic acid interaction:

0.02 M CTAB and 0.02M sodium oleate solution were prepared as stock solutions. Both solutions were heated up to 50 ℃ to fully dissolve surfactants before use. For CTAB: sodium oleate mole ratio 10:5, 5 ml 0.02 M CTAB was mix with 5 ml 0.01 M sodium oleate to achieve a 10 ml system in a 20 ml vial. 1 ml *n* -hexane was sonicated with above mixture under standard ½" diameter probe with 50% amplitude for 30 s (about 200 J) and underwent evaporation by vigorous stirring at 65 ℃. Different mole ratio combinations were achieved by mixing different volumes of CTAB and sodium oleate solution and added up Milli-Q water to maintain 10 ml system.

MRI T2* Relaxivity Properties:

T2*-weighted MRI was performed by a 7 T MRI scanner (BioSpec, Bruker, Billerica, MA, USA). Phantoms Image samples were prepared by using 1% agar in 1 ml Eppendorf tubes at various concentrations of IONC and IONCs-P-JMNC (in equivalent to Fe concentration ranging from 0.1–1 mM). A multiecho gradient recalled echo (GRE) T_2*-weighted

imaging sequence was utilized (TR (repetition time) = 0.2 s, 4 TE (echo time) ranging from 2.3 to 10.8 ms). R_2* time constants were determined by fitting signal decay curves to the exponential function $S(TE) = M_0 e^{-TE/T2^*}$. The corresponding T_2* were extracted and fitted into linear equation with respect to Fe concentration. The slope of the resulting linear fit line provided the relaxivity rate(r_2^*) in the unit of $s^{-1} \cdot mM^{-1}$.

Magnetic field triggered DOX drug release and cellular response:

DOX-loaded IONCs-P-JMNC were prepared by co-incubation of 5 mg nanoparticles and 0.3 mg/ml DOX PBS solution (15 ml) under light-sealed conditions for 24 h. DOX-loaded nanoparticles were collected by centrifugation (9,000 rpm,10 min) and re-washed with PBS three times. The supernatant was collected and measured the absorption at 480 nm by a Synergy™ HT Multi-Detection Reader (BioTek Instruments, Winooski, VT). DOX loading amount was decided by free DOX amount, which could be calculated based on the standard curve. Resulting DOX-loaded IONCs-P-JMNC were re-dispersed in PBS (pH=7.4) and stored at 4 ℃. To study magnetic field triggered magneto-mechanical drug release, 1 ml of DOX-loaded IONCs-P-JMNC (3 mg/ml) were sealed into a dialysis device (Spectrum™ Spectra/Por™ Float-A-Lyzer™ G2 Dialysis Devices), which was then submerged in 20 ml PBS at 37 ℃. The AC magnetic field (0.5 Hz, 35 Oe) was generated by a AFG-2225 Instek Arbitrary Waveform Generator (Valuetronics International, Inc. IL) and applied at each time point for 10 min (1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h). The generator probe was constantly placed at 0.7 cm away from the dialysis membrane. Before each time of applying magnetic field, 1 ml of release media was extracted from the system and another 1 ml PBS solution was replenished (1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48 h). Drug release amount was calculated by UV-vis at the wavelength of 480 nm. To test release rate at different duration, same samples were prepared and the AC magnetic field was applied at 2 h, 6 h, 8 h for 1 min, 4 min, or 10 min. UV-vis data were collected at 2 h, 3 h, 6 h, 7 h, 8 h, and 9 h. The DOX release rate was calculated by subtraction in the unit of %/h. Cell toxicity tests were conducted by Clone 9 (ATCC® CRL-1439™) hepatocyte cells and Hep G2 (ATCC® HB-8065™) cancer cells, which were seeded at a density of 6×10^3 cells in 96-well plate and allowed for 12 hours to attach to the wall. IONCs-P-JMNC (for Clone9 and HepG2) and DOX-loaded IONCs-P-JMNC (for HepG2) were added at varies concentrations (6.25-200 μg/ml) and incubated for 16 h. The AC magnetic field with the same parameters was applied to DOX-loaded IONCs-P-JMNC at 1 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h with generator probe moved regularly and evenly under 96-well plate for 10min. Then Cell Counting Kit-8 (CCK-8) was used to determine cell viability. For cellular uptake imaging, Hep G2 cells were seeded at a density of 1×10^5 cells on a 6-well plate and allowed for 12 hours to attach to the wall. 10 μg DOX-loaded IONCs-P-JMNC (100μg /ml) was added and waited for one hour before fluorescent images were taken under red channel or bright field with different magnification. (CKX53 Cell Culture Microscope, Olympus Co.)

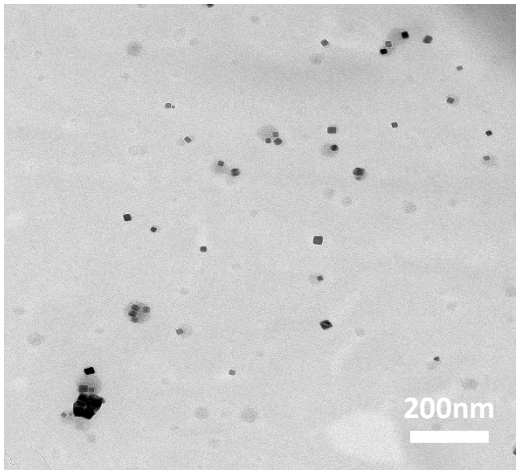

Figure S1. TEM of monodisperse IONCs after ultrasonication at standard ½" diameter probe with 90% amplitude for 120 s, about 1900 J. Spherical complexes are still visible, in agreement with sonication-induced rupture effect that could generate smaller complex. Scale bar is 200 nm.

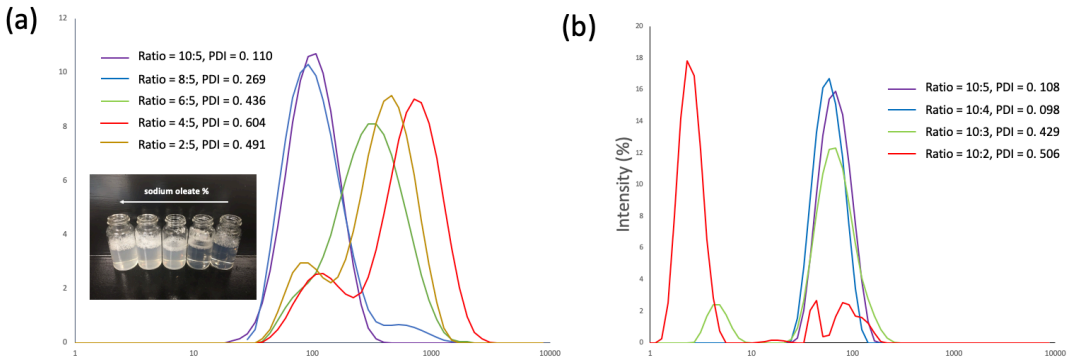

Figure S2. DLS measurement of different components ratio: (a) CTAB concentration and (b) oleic acid concentration in emulsion systems. Inset photo shows creaming process in demulsification when increasing sodium oleate amount, while solution remain clear when increasing CTAB. Ratio: ratio of mole concentration of CTAB/sodium oleate.

The generation of stable nanostructure in the emulsion process involved interplay between CTAB, IONCs and oleic acid (whose concertation is positively correlated to IONCs concentration). The regular nanostructure templates are more likely to depend on oleic acid and CTAB than IONCs themselves. The effect of CTAB and oleic acid interaction on defining templates are investigated by different ratios of CTAB and sodium oleate without addition of IONCs. Polydispersity index (PDI) in DLS measurement is used to describe the degree of "non-uniformity" of nanoparticles distribution. PDI near or below 0.1 is considered monodisperse, while bigger than 0.4 is considered broad polydisperse. When maintaining sodium oleate mole concentration (denominator noted as 5) and decreasing CTAB concentration (numerator noted from 10 to 2), PDI increases from 0.110 up to 0.604 and size distribution right shift, meaning comparatively high sodium oleate concentration will deform uniform spherical micelles and demulsify into an unstable creaming system. The demulsification system can be visualized by inset picture, as increasing sodium oleate concentration, emulsion solution becomes turbid. When maintaining CTAB concentration (numerator noted as 10) and decreasing sodium oleate concentration (denominator noted from 5 to 2), PDI increases from 0.108 up to 0.506 and size distribution left shift, indicating uniform smaller micelles generated in the system. These trends are confirmed by TEM in **Fig. 3**: As the optimum ratio of IONC(also indicating the oleic acid inside) and CTAB is 6 mg: 0.01 M, 6 mg: 0.002M (top in **Fig. 3a**)and 18mg: 0.01M (bottom in **Fig. 3b**) are equivalent to comparatively higher oleic acid amount than the optimum, then IONCs patches structure are not well-guided by spherical curvature of nanostructure, probably due to polydispersity or non-uniform shape. 6 mg: 0.2 M (bottom in **Fig. 3a**) and 0.5 mg: 0.01 M (top in **Fig. 3b**) are equivalent to comparatively higher CTAB amount than the optimum, then spherical templates show more contrast than 6 mg: 0.01 M (middle in **Fig. 3a and 3b**), probably due to small CTAB micelles deposit onto the spherical template. However, the optimum ratio with and without IONCs are different, probably because IONCs have distinguish effects on micelles structure formation and oleic acid distribution.

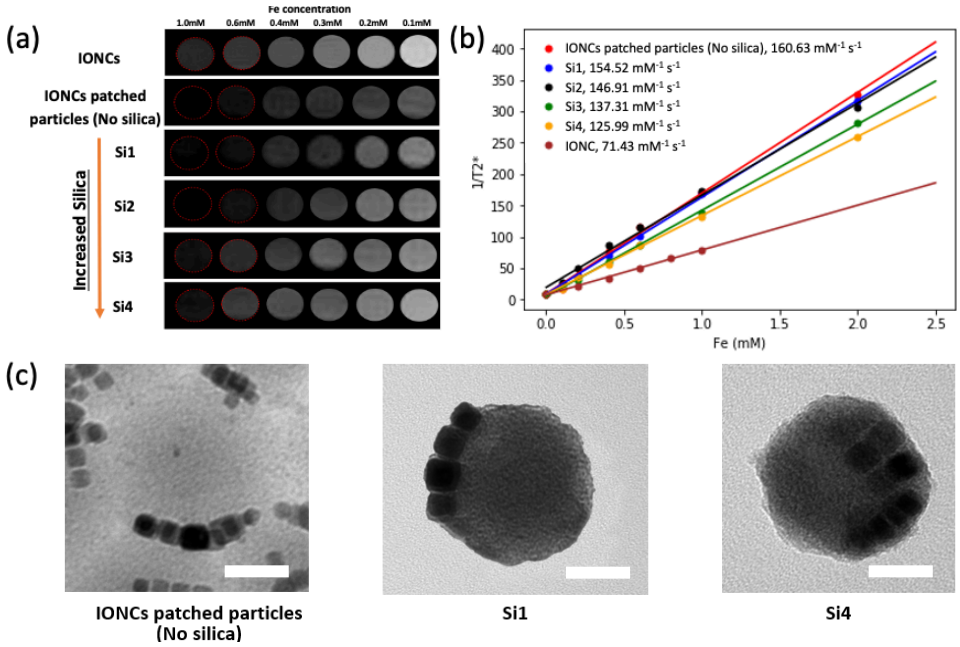

Figure S3. (a) T2*-weighted MR images and (b) R2* relaxation time of various concentrations of IONCs-P-JMNC with different amount of silica (TEOS); (c) TEM images of IONCs patched nanoparticles without silica, IONCs patched nanoparticles with 100µl TEOS (Si1) and IONCs patched nanoparticles with 300µl TEOS (Si4). Scale bars are 50 nm.

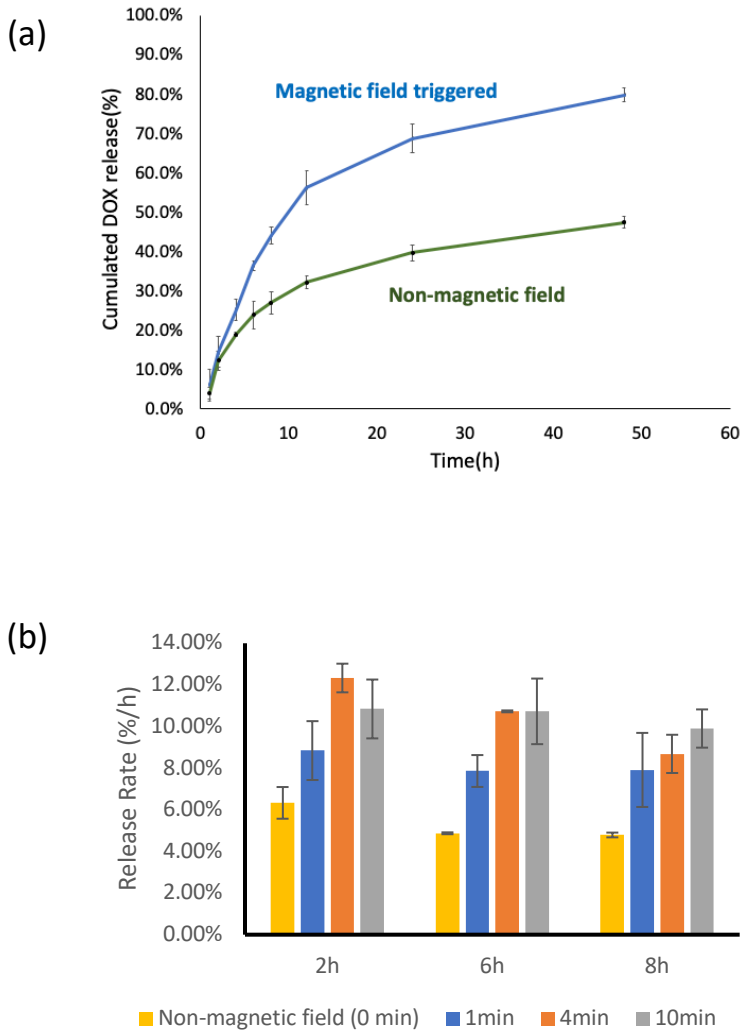

Figure S4. *In vitro* DOX drug release from (a) DOX loaded IONCs-P-JMNC under no magnetic field or AC magnetic field application. (b) DOX loaded IONCs-P-JMNC dependent on the duration (0, 1, 4, and 10 min) of AC magnetic field application. AC magnetic field was applied at each incubation time point (2, 6 and 8 hours).

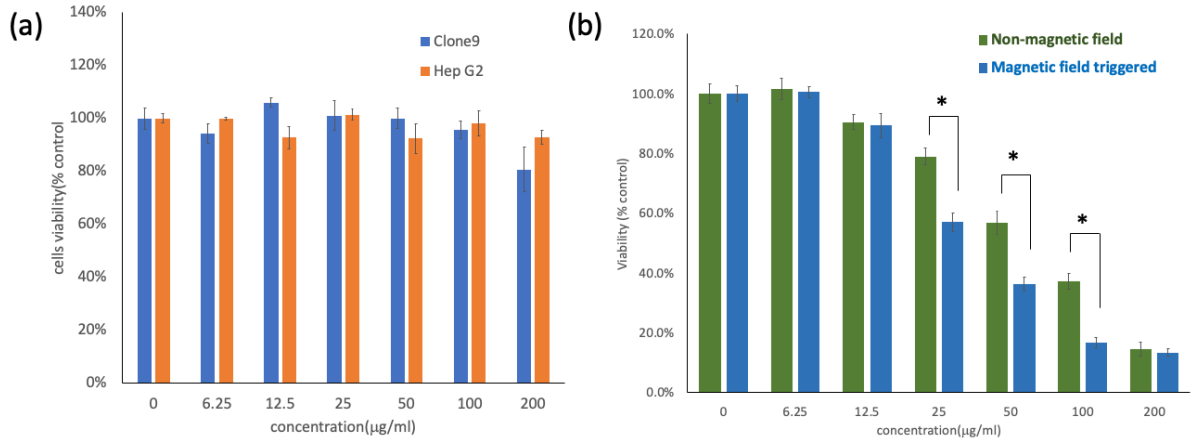

Figure S5. (a) Cell viability of Clone9 hepatocyte cells and HepG2 cancer cells with different concentrations of IONCs-P-JMNC (6.25 μg/ml – 200 μg/ml). (b) Cell viability of DOX loaded IONCs-P-JMNC treatment groups with non-magnetic field or AC magnetic field appliction (DOX loaded IONCs-P-JMNC concentration: 6.25 μg/ml–200 μg/ml, *P<0.05)