Supporting information for:

Controlled drug release from ultrasound-visualized elastic eccentric microcapsules using different resonant modes

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Table S1. Carriers for ultrasound-stimuli controlled drug release

Ultrasound controlled drug release carriers	Materials	Structure	Size	Active drug	Ref.
PLA microcapsules	PLA	Microcapsule	3.01±1.98 μm	IO@PLA/GO	[1]
Bubble liposomes	Cationic lipids	Liposome	600-700 nm	Plasmid DNA	[2]
SFNs	Silk fibroin	Nanoparticle	210-220 nm	FITC-BSA	[3]
eLipoDox	DPPC	Liposome	100-500 nm	Doxorubicin	[4]
Polymeric microcapsule	PAH, PSS	Multilayer microcapsule	3-5 μm	RBITC-labelled BSA	[5]
Polymer ultrasound contrast agents	Polylactic acid	Microbubble	1.2–1.8 μm	Doxorubicin	[6]
MB-Lipo	Lipids	Microbubble	~ 1.5 μm	Doxorubicin	[7]
PLGA-PEG-NP	PLGA, PEG	Nanoparticle	115.3±18 nm	miR-122	[8]
uPA-MB	Lipids	Microbubble	~ 3.13 μm	Urokinase	[9]

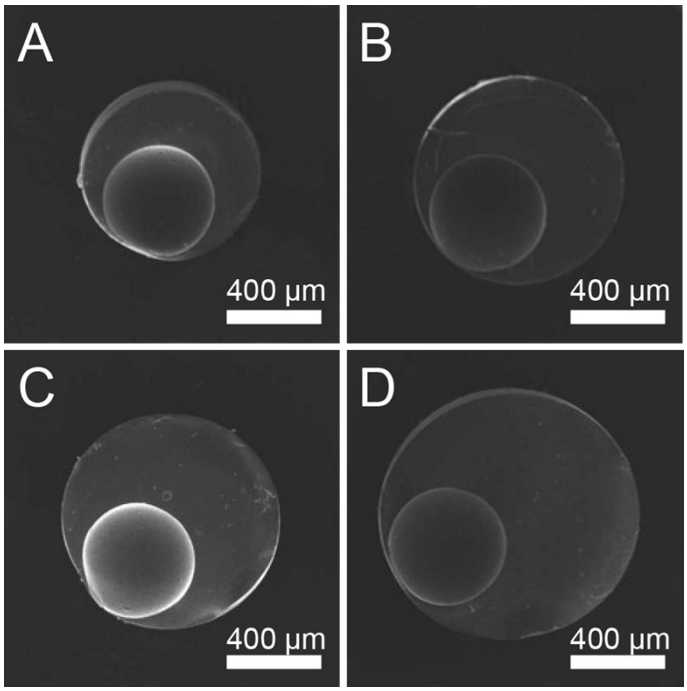

Fig. S1. Cross-sectional SEM images of EEMs at different external diameters (*i.e.* the

diameter of the overall EEM) at a constant diameter of inner spherical cavity.

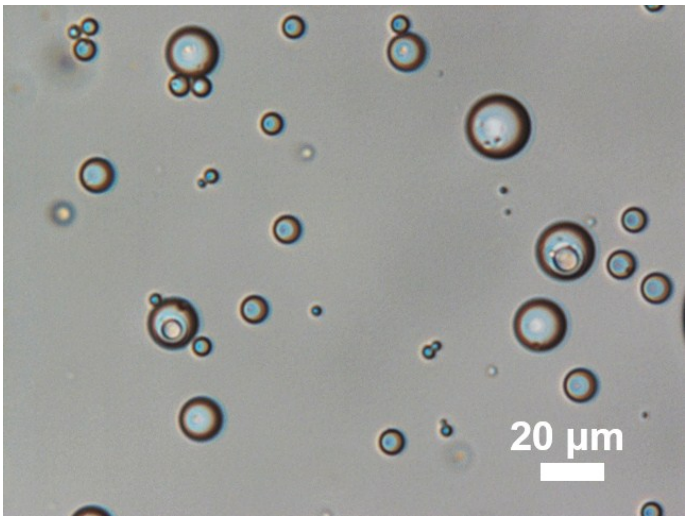

Fig. S2. Optical images of elastic eccentric microcapsules (EEMs) fabricated using the double emulsion method. The image was captured by a NIKON microscope. The scale bar is 20 μm.

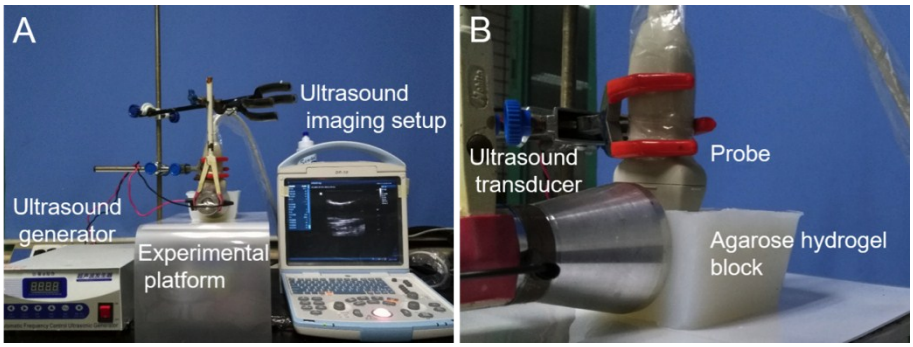

Fig. S3. An experimental setup for imaging and simultaneous application of external ultrasound stimuli for ultrasound-visualized controlled drug release from EEMs. The system consists of three parts: an artificial phantom containing a channel, an ultrasound imaging system, and an ultrasound generator at low frequency as the external ultrasonic stimuli. The sample stage was comprised of a hydrogel block containing a channel as an artificial lumen, an iron stand and three extension clamps. The distance between the side wall and the channel is 20.0 mm. The ultrasound probe used for visualization, was fixed

by an extension clamp perpendicular to the channel. The ultrasound generator (the frequency of which ranges from 20 kHz to 40 kHz) was attached to the side wall of the hydrogel block, and used as external ultrasonic stimuli.

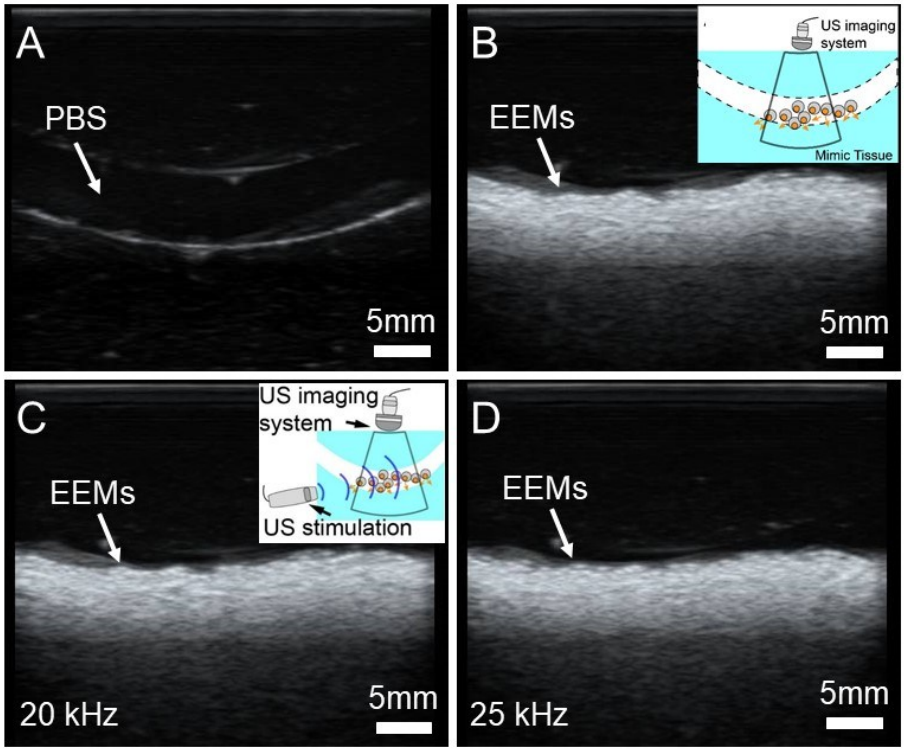

Fig. S4. Ultrasound images of the channel within a tissue mimic tissue filled with EEMs in PBS solution. (A) The ultrasound image of the channel filled with PBS in an agarose hydrogel block with no EEMs. (B) The ultrasound image of the channel completely filled with EEMs without application of external ultrasonic stimulation. The inset graph is the schematic diagram of the experiment setup for visualizing the location of the EEMs. (C) The ultrasound image of the channel filled with EEMs upon the application of external ultrasonic stimulus at the frequency of 20 kHz. The inset graph is the schematic diagram of the experiment setup for visualizing the location of the EEMs and the application of external ultrasonic stimuli. (D) The ultrasound image of the channel filled with EEMs after exposure to the external ultrasonic stimuli at the frequency of 25 kHz for 30 minutes.

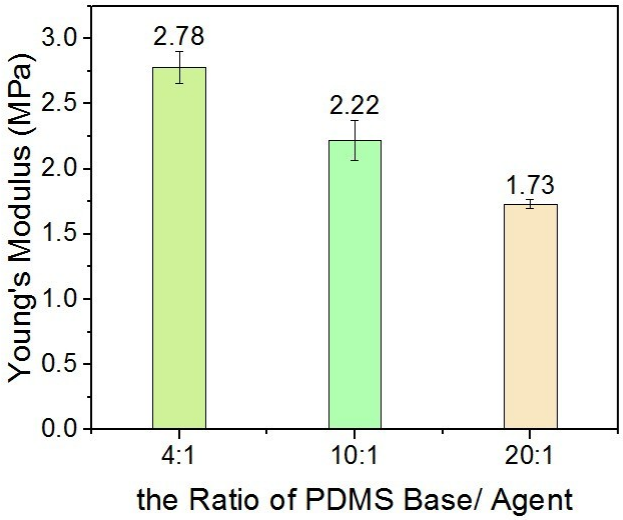

Fig. S5. The Young's modulus of PDMS at different ratios of base/agent under the compression tests by a universal testing machine. The Young's modulus of PDMS at the ratio of 10:1 (base : cross-linker) is 2.22 MPa. A universal testing machine (UTM, LR10K Plus, LLOYD, UK) with a 100 N sensor was employed to gauge the Young's modulus of PDMS in the compression tests. We prepared three types of PDMS with different mass ratios of base : cross-linker at 4:1, 10:1, and 20:1, respectively. Dichloromethane (DCM) was added into the as-prepared PDMS mixture, and the mass ratio of the mixture and DCM was about 3 to 1 (PDMS : DCM). After stirring, the mixture was placed in a vacuum tank for de-gassing. The mixture was then poured into cylindrical glass molds to make test-pieces. The same solidification process for the preparation of EEMs was also applied to solidify these test-pieces.

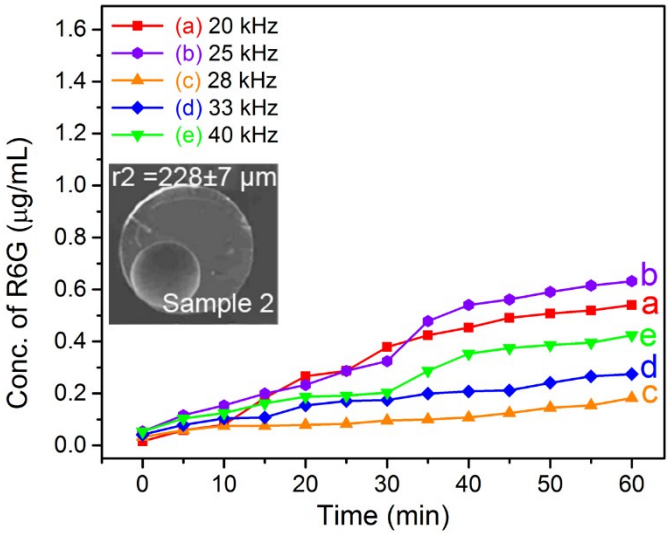

Fig. S6. The experimental release profiles of R6G from another type of EEMs (*r*= 228±7 μm, *R* = 440 μm, described as Sample 2) under external ultrasonic stimuli at frequencies of (a) 20, (b) 25, (c) 28, (d) 33 and (e) 40 kHz. R6G was loaded into the inner spherical cavity of EEMs as the model drug.

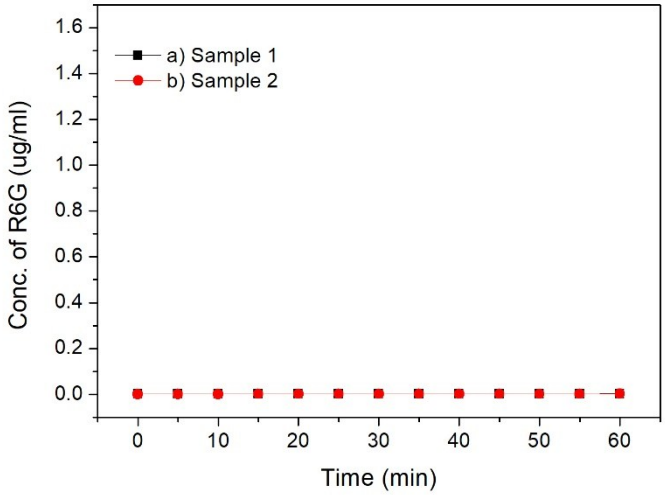

Fig. S7. The experimental release profiles of R6G from two types of EEMs (*r*= 285±9 μm, *R* =400 μm, described as Sample 1; *r*= 228±7 μm, *R* = 440 μm, described as Sample 2) without external ultrasonic stimuli. R6G was loaded into the inner spherical cavity of EEMs as the model drug.

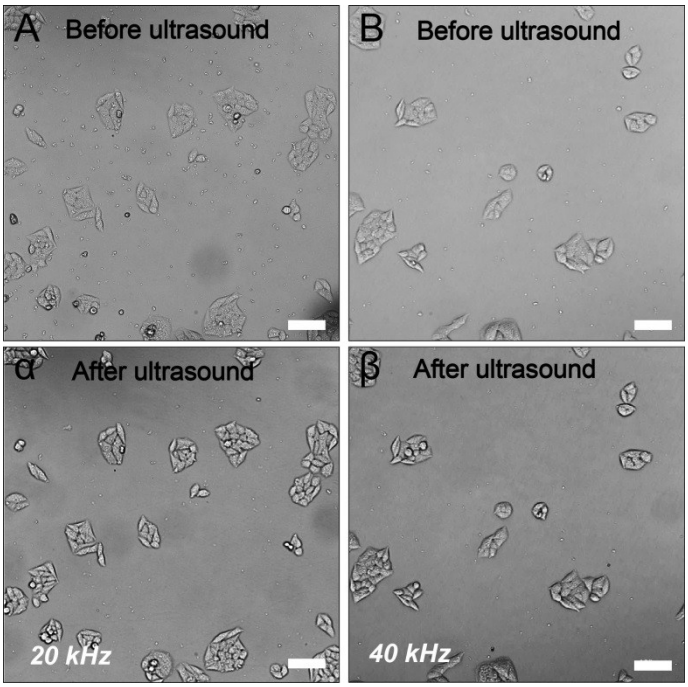

Fig. S8. Bright-field microscope images of HeLa cancer cells. HeLa cells were exposed to ultrasonic stimuli at the frequencies of 20 kHz and 40 kHz for 30 min at room temperature. The scale bar is 100 μm.

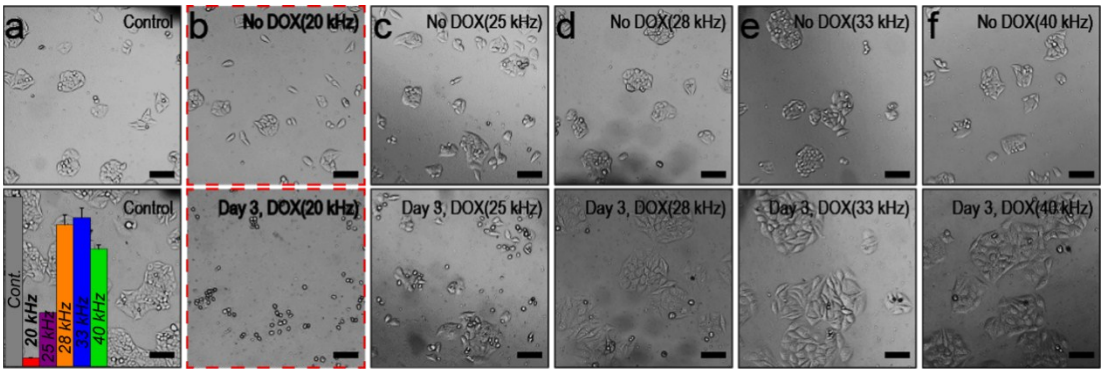

Fig. S9. Optical images of HeLa cells before and after exposing to the doxorubicin hydrochloride (DOX) released from EEMs (r = 285±9 μm, R =400 μm, Sample 1) under external ultrasonic stimuli at frequencies of 20, 25, 28, 33 and 40 kHz. (a)➌(f): the cellular morphologies before DOX was added and 3 days later after DOX was added (Day 3). The scale bar is 100 μm. The confluences of HeLa cells were observed before the released DOX was added. At Day 3 (3 days later after DOX was added), the control group (Fig. S9a) presented a significant cell proliferation, while the others showed cells lysis or suppressed

proliferation at varying degrees (Fig. S9a❸S9f). Especially, the cells in Fig. S9b, which shows the highest value of cell, exhibited lysis, when the cells were treated with the DOX released under external ultrasonic stimuli at a frequency of 20 kHz.

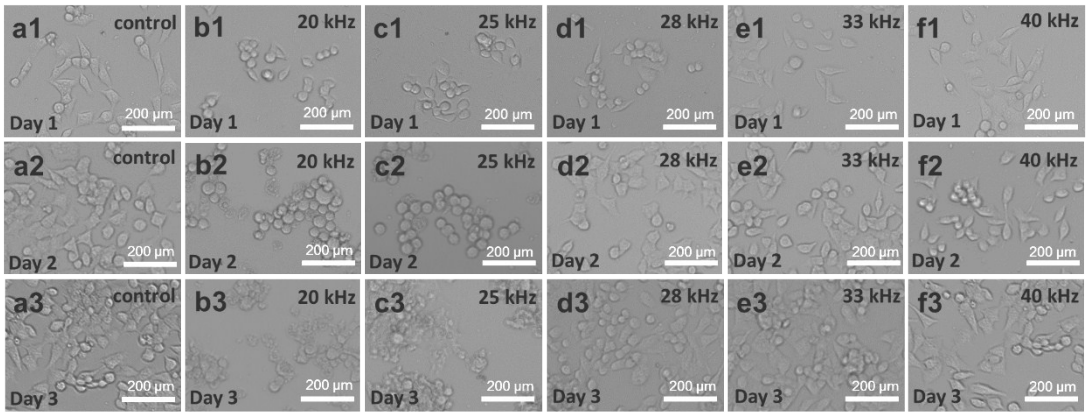

Fig. S10. Optical images of HeLa cells co-cultured with EEMs before and after exposing to the external ultrasonic stimuli at frequencies of 20, 25, 28, 33 and 40 kHz.

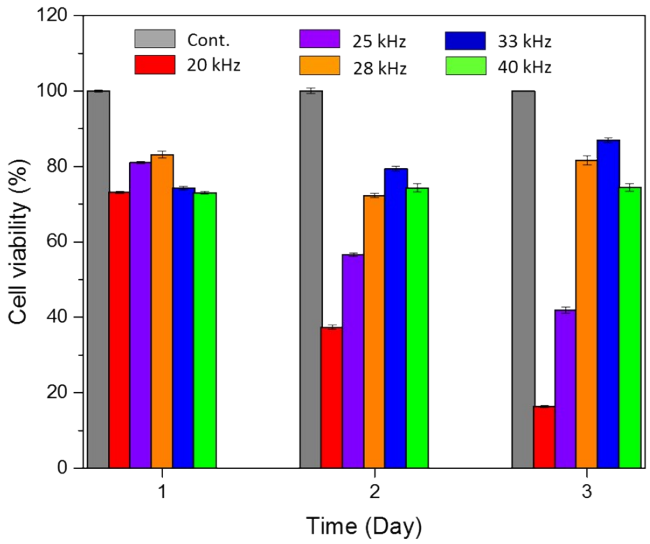

Fig. S11. Cell viability MTT assay of HeLa cells co-cultured with EEMs before and after exposing to the external ultrasonic stimuli at frequencies of 20, 25, 28, 33 and 40 kHz.

References

1. X. D. Li, X. L. Liang, X. L. Yue, J. R. Wang, C. H. Li, Z. J. Deng, L. J. Jing, L. Lin, E. Z. Qu, S. M. Wang, C. L. Wu, H. X. Wu, Z. F. Dai, *J. Mater. Chem. B*,

2014, **2**, 217.
2. Y. Endo-Takahashi, Y. Negishi, A. Nakamura, D. Suzuki, S. Ukai, K. Sugimoto, F. Moriyasu, N. Takagi, R. Suzuki, K. Maruyama, Y. Aramaki, *Biomaterials*, 2013, **34**, 2807.
3. D. Huang, L. L. Wang, Y. X. Dong, X. Pan, G. Li, C. B. Wu, *Eur. J. Pharm. Biopharm.*, 2014, **88**, 104.
4. C. Y. Lin, M. Javadi, D. M. Belnap, J. R. Barrow, W. G. Pitt, *Nanomedicine*, 2014, **10**, 67.
5. A. M. Pavlov, V. Saez, A. Cobley, J. Graves, G. B. Sukhorukov, T. J. Mason, *Soft Matter*, 2011, **7**, 4341.
6. J. R. Eisenbrey, M. C. Soulen, M. A. Wheatley, *IEEE Trans. Biomed. Eng.*, 2010, **57**, 24.
7. Y. I. Yoon, Y. S. Kwon, H. S. Cho, S. H. Heo, K. S. Park, S. G. Park, S. H. Lee, S. I. Hwang, Y. I. Kim, H. J. Jae, G. J. Ahn, Y. S. Cho, H. Lee, H. J. Lee, T. J. Yoon, *Theranostics*, 2014, **4**, 1133.
8. T. Y. Wang, J. W. Choe, K. Pu, R. Devulapally, S. Bachawal, S. Machtaler, S. M. Chowdhury, R. Luong, L. Tian, B. Khuri-Yakub, J. Rao, R. Paulmurugan, J. K. Willmann, *J. Control. Release*, 2015, **203**, 99.
9. S. T. Ren, X. N. Kang, Y. R. Liao, W. Wang, H. Ai, L. N. Chen, H. T. Luo, R. G. Fu, L. F. Tan, X. L. Shen, B. Wang, *J. Thromb. Thrombolysis*, 2014, **37**, 303.