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To be cited as: 10.1002/ange.202116515

Link to VoR: https://doi.org/10.1002/ange.202116515

Ultrasound-Responsive Aqueous Two-Phase Microcapsules for On-Demand Drug Release

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Keywords: aqueous two-phase systems, focused ultrasound, localized delivery, microfluidics, tunable delivery, polymers

ABSTRACT

Traditional implanted drug delivery systems cannot easily change their release profile in real time to respond to physiological changes. Here we present a microfluidic aqueous two-phase system to generate microcapsules that can release drugs on demand as triggered by focused ultrasound (FUS). The biphasic microcapsules are made of hydrogels with an outer phase of mixed molecular weight (MW) poly(ethylene glycol) diacrylate that mitigates premature payload release and an inner phase of high MW dextran with payload that breaks down in response to FUS. Compound release from microcapsules could be triggered as desired; 0.4 μg of payload was released across 16 on-demand steps over days. We detected broadband acoustic signals amidst low heating, suggesting inertial cavitation as a key mechanism for payload release. Overall, FUS-responsive microcapsules are a biocompatible and wirelessly triggerable structure for on-demand drug delivery over days to weeks.

Main text

Localized drug delivery can target intended sites in the body while reducing adverse off-target effects.[1] Many implanted drug-delivery systems, such as nanoparticles, hydrogels, and microdevices, work via passive release or exhibit a pre-programmed drug-release profile,[2] in contrast to localized drug-delivery systems that are externally triggerable for on-demand release.[2a, 3] These remotely-activated systems come in a variety of forms, including DNA-capped nanoparticles, microchips, and microcapsules, and can be responsive to visible and near-infrared (NIR) light, magnetic fields, cell membrane receptors, and enzymes. Additionally, these systems can target delivery to an entire region of the body or within an individual cell.[4] Triggerable release offers potential advantages of efficacious and selective delivery which can be controlled on demand;[2k, 5] solutions have included reversible NIR disruption of microcapsule membranes and the controlled erosion of layers of a bulk device to release payload.[6] Ultrasound-triggerable microscale systems such as microbubbles (Table S1) typically require destruction of the drug-delivery vehicle, limiting the triggered effect to a one-time bolus release. Towards personalized medicine, real-time tunable drug release could be important; examples include chronotherapy, which optimizes therapies to account for the body's circadian rhythm, and closed-loop drug delivery that releases a payload in response to defined physiological conditions.[7] Pulsatile delivery systems are largely developed at the macroscale[8] rather than microscale which can be implanted or injected, and stimuli-responsive microdevices for localized and repeated drug delivery remain challenging (Table S2).[9]

There is a growing interest in using ultrasound in various applications including increasing cell membrane permeability for the direct delivery of payloads[10]; probing the

response of cells to shear stresses[11]; and sorting and transporting cells.[12] Ultrasound is a promising mechanism for actuating release, as it is safe and noninvasive, can penetrate deep into the body, and can be focused to sub-millimeter dimensions.[5, 13] Ultrasound can cause both heating and cavitation in a material, with either of these effects capable of triggering drug release from an encapsulating material. For heating, disruption of gel structure can release drugs, but demonstrations have been mainly performed with bulk gels, and careful attention must be paid to avoid adverse effects from excessive localized heating.[2k, 14] By comparison, cavitation is the formation, growth, oscillation, and collapse of gas bubbles and begins with the formation or presence of gas pockets or dissolved gasses.[15] Oscillations and collapse of bubbles caused by inertial cavitation can release payload by eroding surfaces, disrupting membranes, and rupturing stiff microparticles. Cavitation has been used in conjunction with microbubbles that are loaded with drugs or injected alongside drug depots to achieve drug delivery, but microbubbles were originally designed for short-term diagnostic applications; as such, they have limited lifetimes (less than an hour), are fragile, and can only be triggered only once to achieve a burst release before they are destroyed (Tables S1-2).[4d, 16]

In this work, we present a method to fabricate hydrogel microcapsules that can be triggered by focused ultrasound (FUS) for on-demand release. We fabricate microcapsules using aqueous two-phase system (ATPS) on a microfluidic chip,[2d, 2e, 17] in which the two phases spontaneously separate above minimum polymer concentrations, when the decrease in enthalpy of demixing is greater than the gain in entropy of mixing.[18] These ATPS microcapsules can be formed with biocompatible polymers and without intermediate oil-based phases, which can adversely affect biocompatibility. For example, the van Esch group has created crescent-shaped

ATPS on a microfluidic chip using PEGDA and dextran, where a low molecular weight (MW) dextran (e.g. 20k Da) formed off-centered spheres that separated from low MW PEGDA (e.g. 700 Da) to yield a microcarrier bucket made only of PEGDA.[2e] Building on this work, we examined different MW of PEGDA and dextran to form stably biphasic microcapsules (Fig. 1a). In this type of envisioned microcapsule (Fig. 1b), the outer phase (PEGDA) blocks passive release of payload stored in the inner dextran phase which remains intact until triggered by FUS. A focused ultrasound transducer, with a cross-sectional focal area smaller than 2 mm^2 and focal depth of 10 mm, selectively actuates the microcapsules (Fig. 1c). The inner phase of dextran remains intact until triggered by FUS, at which point it breaks down, releasing the payload (Fig. 1d). The application of FUS primarily causes breakdown of the soft inner dextran phase while also causing some breakdown of the stiff outer PEGDA phase. Compared to microbubbles (which are typically several microns in diameter[2i, 5, 19]), these biphasic microcapsules can carry large payloads and be used for pulsatile payload release over days. An overview of our process can be seen in Movie S1.

We fabricated double-emulsion microcapsules using a microfluidic chip with two flow-focusing junctions in series, followed by a region for photopolymerization (Fig. 2a, Fig. S1, Supp. Methods).[2e, 20] These polymerized microcapsules have a soft inner dextran phase and an outer PEGDA shell that has low permeability to the model drug, although there is a diffuse phase boundary. We tested the effect of different materials on swelling and phase separation to inform the final selection of materials (Supp. Methods, Fig. S2). These are biphasic with an asymmetric inner phase (Fig. 2b) with a small exposed surface of the inner phase to bulk solution. The microcapsules were morphologically stable after 112 days of storage in deionized water (Fig.

2c), with much of the fluorescent compound in the outer PEGDA phase having passively diffused out.

We chose a set of fluid and flow conditions for payload-delivery microcapsules, and assessed the consistency of sizes of microcapsules generated. The innermost phase was high MW dextran loaded with fluorescein isothiocyanate (FITC)-dextran 20k Da as a model drug, and the second phase was a mixed MW PEGDA. These coflowing phases were broken up into droplets by an outer oil phase.[21] The flow rates were tuned so that the collected, washed microcapsules had an average diameter of 548.7 ± 34.0 μm, similar to prior demonstrations on a percentage basis (Fig. 2d, Supp. Methods).[2e]

To assess the ultrasound-responsiveness of the microcapsules, we first transferred the microcapsules into polydimethylsiloxane (PDMS) wells, with each well loaded with 24 microcapsules (corresponding to a cumulative delivery of ~15 μg of compounds, suitable for clinical applications[22]) in 25 μL of degassed deionized water. PDMS exhibits an acoustic impedance similar to that of water, minimizing the amount of wave reflected at the water-PDMS boundary and formation of standing waves.[23] We sealed the wells with a thin plastic film and mounted each well onto the coupling cone of a 1.1 MHz focused ultrasound transducer (Fig. 3a). The transducer was powered by a variable drive box and data from the hydrophone was high-pass filtered and amplified (Supp. Methods). An acoustic power of 150 W was used; this power, coupled with low duty cycle (5%), maximized cavitation effects while minimizing heating, and no adverse effects were detected when these parameters were used in a murine model (data not shown). We monitored thermal effects from ultrasound application by placing a thermistor next

to the microcapsule sample during select runs (Fig. S5). At high duty cycle (20%), we detected more than 4˚C of temperature rise, compared to less than 2˚C at a low duty cycle (5%), with less than 1˚C of heating occurring during the first 10 s of the pulsed FUS at low duty cycle, indicating that minimizing the total application time further minimizes thermal effects (Fig. 3b). According to the model for calculating thermal dose, the effective thermal dose for all applied FUS conditions is below the threshold for cell death.[24] For *in vivo* applications, we anticipate the observed heating at low duty cycle to be unlikely to damage tissue, particularly for short FUS applications (30 s or less).

We next used signal processing methods to analyze the cavitation occurring in and around our microcapsules during FUS applications (Fig. S6, Supp. Methods). In the frequency domain, spectral energy occurring at the harmonics of the f_0 frequency, 1.1 MHz, indicates emissions from stable cavitation. Additionally, the occurrence of spectral energy in the water-only sample is likely, in part, caused by the impedance change at the water/air interface on the top of the water bath. The spectral energy outside of those harmonic frequencies demonstrates broadband emissions, which were detected only when microcapsules were present (Fig. 3c). The detected broadband acoustic emissions during the application of FUS to microcapsules indicate inertial cavitation, as nucleation sites within the microcapsules collapse and cause shock waves, microjets, and microstreaming.[25] These broadband noise emissions over the course of a single FUS pulse are apparent in spectrogram data, which exhibits increased intensities across a range of frequencies and over the duration of the pulse within the microcapsules sample, compared to the water-only sample (Fig. 3d). The spectrogram collected from microcapsules in the sample shows both greater harmonics and broadband emissions.

Finally, we measured the effect of applied FUS on the structure and release of model drug from the microcapsule. As discussed previously in microfluidic fabrication (Fig. 2), some amount of fluorescent dextran was mixed into the PEGDA phase before phase separation. Upon ten FUS applications, the dextran phase broke down (Fig. 4a), depending on the FUS intensity and duration of FUS application (Fig. S7). Further applications of FUS, beyond when the dextran phase was disrupted, resulted in the PEGDA outer phase showing cracks and surface damage (Fig. 4b). Next, we measured the release of a fluorescent compound. By applying 16 periods of 30 s pulsed FUS applications, we released 0.552 ± 0.069 μg of model drug from a microcapsule; presumably, the compound was released through an area of inner phase exposed directly to solution. Over the same multi-day time period, a microcapsule passively released significantly less, 0.158 ± 0.013 μg of model drug (Fig. 4c, left). After a period of equilibration in the first two hours in solution, during which most measured passive release occurred (Fig. S8), FUS applications triggered the release of at least an additional 0.4 μg (Fig. 4c, right), which can take place over multiple doses and several days (Fig. 4d). We further studied the effect of FUS parameters, including power, frequency, and pulse length, on the amount of payload released and the overall stepwise profile (Fig. S9, Supp. Methods).

In summary, the study demonstrates two-phase aqueous microcapsules that can be repeatedly triggered to release a model drug, likely due to inertial cavitation within the microcapsule. This payload-delivery vehicle contributes a new capability to localized drug delivery for minimally-invasive repeated dosing, exhibiting stability over a multiweek period and

externally triggerable repeatable delivery using FUS. The amount released by the microcapsule is tunable depending on the applied FUS parameters, which allows for real-time dosage control.

Towards clinical use, for cancer treatment, timed delivery of growth factors and cytokines has been of interest,[26] including recent work on carcinomas,[27] consistent with observations that the body's sensitivity to cancer therapy changes throughout the day.[28] Similarly, certain drugs, such as pain relievers and insulin, have been shown to exhibit increased efficacy when the delivery is provided at different times.[29] Currently, this work demonstrated delivery of large molecules (i.e. above 20 kDa), which includes many growth factors and cytokines, and hydrophilic molecules, which preferentially partition into the dextran phase.[30] Future work could broaden the types of payloads, by incorporating small MW or hydrophobic molecules into carriers (such as nanoparticles),[31] incubating the microcapsules with UV-sensitive payloads after UV-polymerization,[32] or further coating the microcapsules to reduce passive release of small MW compounds,[33] to further expand the potential uses of this system

Acknowledgements

This research is sponsored by the Defense Advanced Research Projects Agency (DARPA) through Cooperative Agreement D20AC00004 awarded by the U.S. Department of the Interior (DOI), Interior Business Center. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred. R.D.F. was supported by the Irving Institute for Clinical and Translational Research's TL1 Training Program (TL1TR001875) at Columbia University.

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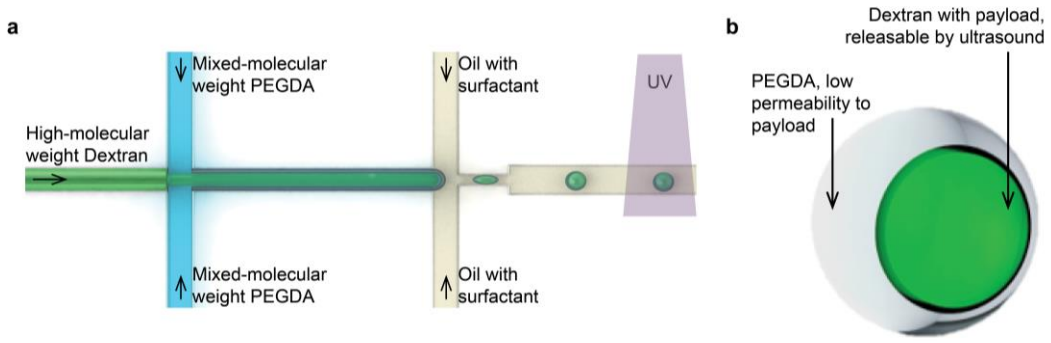

Microfluidic fabrication (left) of a two aqueous phase microcapsule, which releases a model drug payload in response to focused ultrasound actuation.

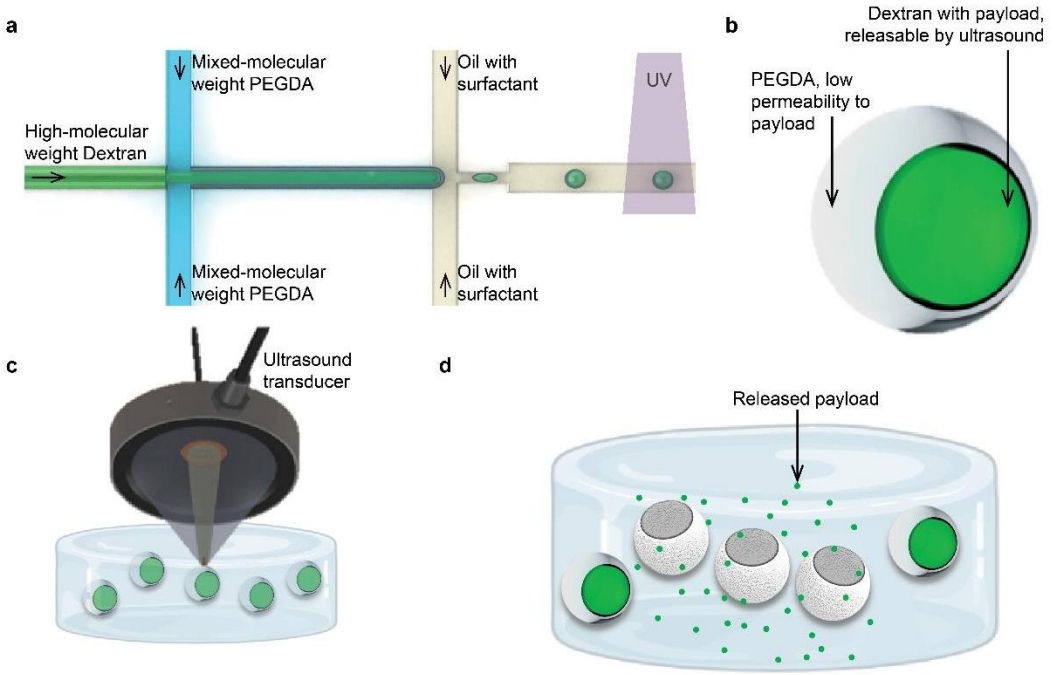

Figure 1. Fabrication and triggered release of model drug from biphasic microcapsules. a) Schematic diagram of the fabrication and UV polymerization of biphasic microcapsules in a microfluidic device. A mixed high and low MW PEGDA and a high MW dextran are used as the two phases. b) Schematic diagram of the biphasic microcapsule with a dextran inner phase loaded with FITC-dextran as a model drug (green) and a UV-polymerized mixed-MW PEGDA outer phase that is impermeable to payload (light grey). c) Schematic diagram of focused ultrasound transducer being used to selectively trigger a microcapsule. The focal area of the transducer is small enough to selectively actuate individual microcapsules. d) After focused ultrasound is applied to the microcapsule, the payload is released into the surrounding environment.

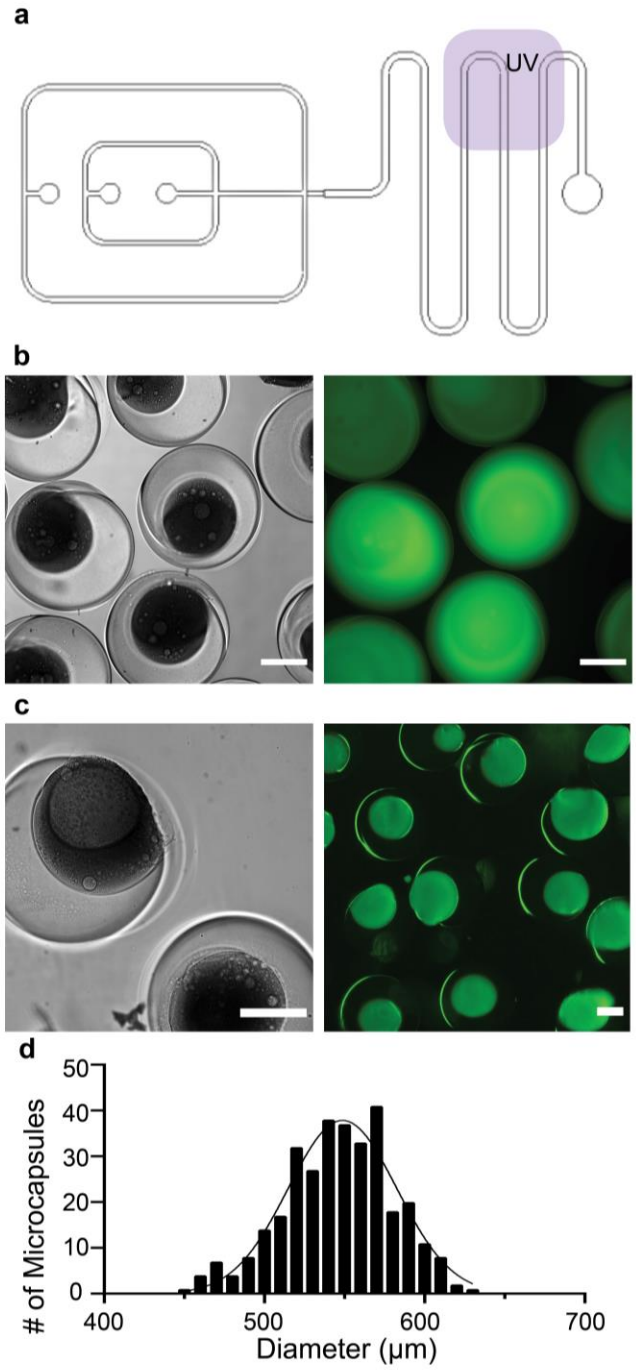

Figure 2. Microfluidic fabrication of microcapsules. a) Design of microfluidic chip, featuring two flow-focusing junctions followed by a serpentine area that allows for sufficient time to complete phase separation and for ultraviolet exposure (purple) to polymerize the PEGDA phase. b) Brightfield (left) and fluorescent (right) images of microcapsules after UV polymerization and washing. c) Brightfield (left) and fluorescent (right) images of microcapsules taken at 112 days

after fabrication. d) Frequency distribution of the microcapsule diameters, post-polymerization. Scale bars = 400 μm.

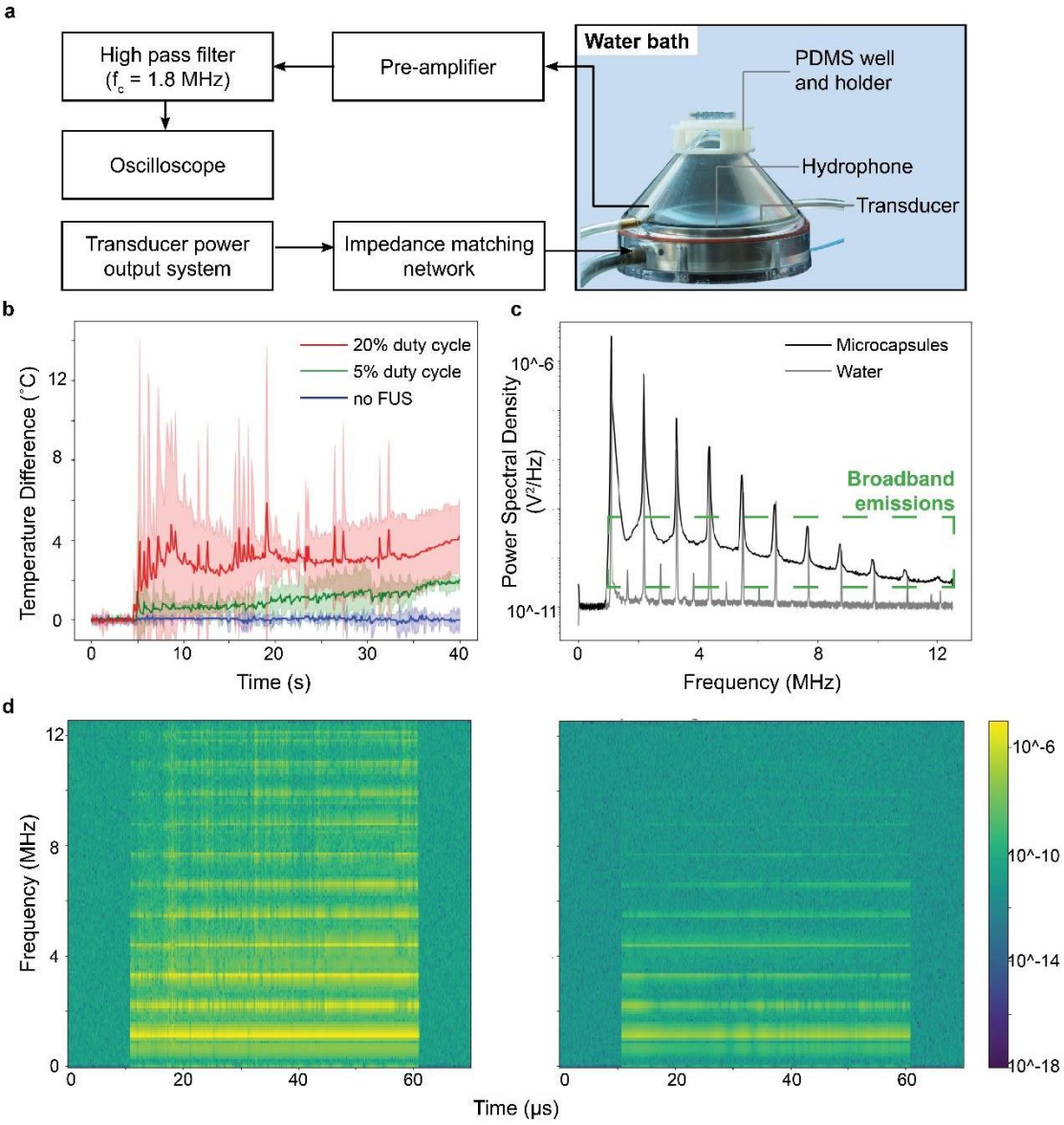

Figure 3. FUS setup and results, showing inertial cavitation and minimal heating in the microcapsules. a) Block diagram of the FUS experimental setup for FUS application and data processing. b) Measured thermal effects of 30 s pulsed FUS application, with first FUS application occurring at t = 5 s. Shaded areas represent 95% confidence intervals. c) Power spectral density of the hydrophone signal with microcapsules in the sample well compared to only degassed water in the well. Broadband noise (dotted green box) was observed when the capsules are present, indicative of inertial cavitation. d) Spectrogram of microcapsule sample (left) and degassed water (right) over a 50 ms pulse of FUS. Increased brightness (yellow) is indicative of increased cavitation.

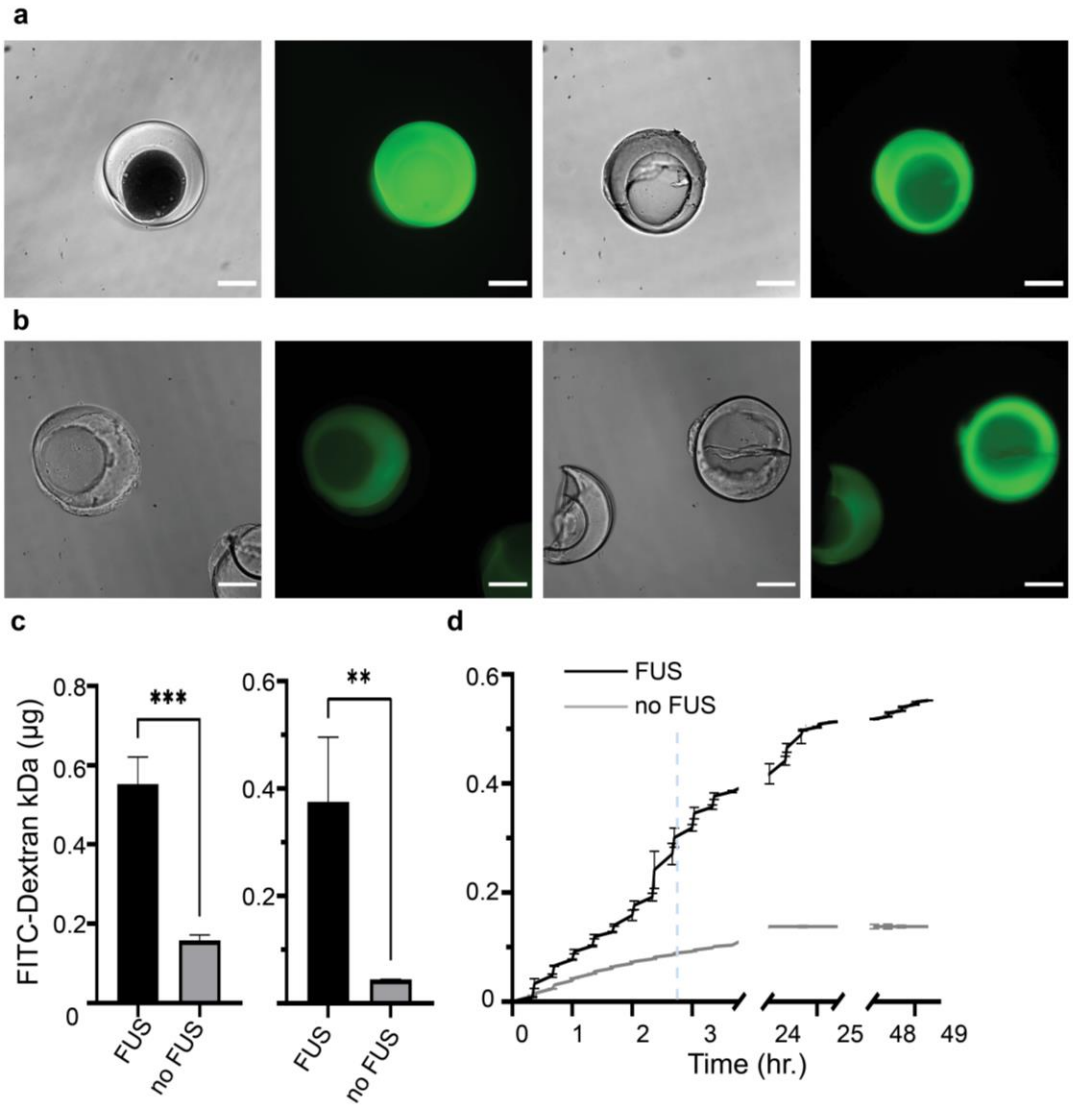

Figure 4. FUS-triggered release. a) Microcapsule shown prior to FUS in brightfield and fluorescence (left), and after 10 FUS applications at 150 W and 5% duty cycle in brightfield and fluorescence (right). b) Microcapsules after 16 pulsed FUS applications, with the dextran portion of the microcapsule fully released and pitting in the PEGDA phase (left) and cracking of the microcapsule structure (right). c) Total FITC-dextran released over three days (left) and total FITC-dextran released over three days when excluding the initial 2 hours equilibration period, during which surface leaching occurs (right). ***$P < 0.006$, **$P < 0.0089$. d) Release profile of FITC-dextran from a microcapsule undergoing repeated FUS applications over three days, compared to a control sample that does not undergo FUS. FUS was applied for 30 s every 20 minutes, for 10 cycles on day 0 and 3 cycles on days 1 and 2. The dashed line demarcates the end of the equilibration period for the passively releasing microcapsules.

Sources:

[1] a) S. A. Stewart, J. Domínguez-Robles, R. F. Donnelly, E. Larrañeta, *Polymers* **2018**, *10*, 1379; b) S. Talebian, J. Foroughi, S. J. Wade, K. L. Vine, A. Dolatshahi-Pirouz, M. Mehrali, J. Conde, G. G. Wallace, *Advanced Materials* **2018**, *30*, 1706665.

[2] a) Y. Zhang, J. Yu, H. N. Bomba, Y. Zhu, Z. Gu, *Chemical reviews* **2016**, *116*, 12536-12563; b) W. Zhang, L. Qu, H. Pei, Z. Qin, J. Didier, Z. Wu, F. Bobe, D. E. Ingber, D. A. Weitz, *Small* **2019**, *15*, 1903087; c) S. Guo, G. Kang, D. Phan, M. Hsu, Y. Por, C. Chen, *Scientific reports* **2018**, *8*, 1-10; d) T. Watanabe, I. Motohiro, T. Ono, *Langmuir* **2019**, *35*, 2358-2367; e) Q. Liu, M. Zhao, S. Mytnyk, B. Klemm, K. Zhang, Y. Wang, D. Yan, E. Mendes, J. H. van Esch, *Angewandte Chemie International Edition* **2019**, *58*, 547-551; f) A. G. Skirtach, B. G. De Geest, A. Mamedov, A. A. Antipov, N. A. Kotov, G. B. Sukhorukov, *Journal of Materials Chemistry* **2007**, *17*, 1050-1054; g) A. M. Pavlov, V. Saez, A. Cobley, J. Graves, G. B. Sukhorukov, T. J. Mason, *Soft Matter* **2011**, *7*, 4341-4347; h) J. Chen, S. Ratnayaka, A. Alford, V. Kozlovskaya, F. Liu, B. Xue, K. Hoyt, E. Kharlampieva, *ACS Nano* **2017**, *11*, 3135-3146; i) S. Tsirkin, R. Goldbart, T. Traitel, J. Kost, *ACS Applied Materials & Interfaces* **2021**, *13*, 25748-25758; j) M. C. Cochran, J. Eisenbrey, R. O. Ouma, M. Soulen, M. A. Wheatley, *Int J Pharm* **2011**, *414*, 161-170; k) N. Huebsch, C. J. Kearney, X. Zhao, J. Kim, C. A. Cezar, Z. Suo, D. J. Mooney, *Proc Natl Acad Sci U S A* **2014**, *111*, 9762-9767.

[3] a) Y. Wang, M. S. Shim, N. S. Levinson, H. W. Sung, Y. Xia, *Advanced functional materials* **2014**, *24*, 4206-4220; b) C. S. Linsley, B. M. Wu, *Therapeutic delivery* **2017**, *8*, 89-107; c) A. M. Goodman, O. Neumann, K. Nørregaard, L. Henderson, M.-R. Choi, S. E. Clare, N. J. Halas, *Proceedings of the National Academy of Sciences* **2017**, *114*, 12419-12424.

[4] a) C. H. Lu, I. Willner, *Angew Chem Int Ed Engl* **2015**, *54*, 12212-12235; b) B. P. Timko, T. Dvir, D. S. Kohane, *Adv Mater* **2010**, *22*, 4925-4943; c) A. G. Skirtach, A. Muñoz Javier, O. Kreft, K. Köhler, A. Piera Alberola, H. Möhwald, W. J. Parak, G. B. Sukhorukov, *Angew Chem Int Ed Engl* **2006**, *45*, 4612-4617; d) Y. Wang, D. S. Kohane, *Nature Reviews Materials* **2017**, *2*, 17020.

[5] T. Boissenot, A. Bordat, E. Fattal, N. Tsapis, *J Control Release* **2016**, *241*, 144-163.

[6] a) A. G. Skirtach, P. Karageorgiev, M. F. Bédard, G. B. Sukhorukov, H. Möhwald, *Journal of the American Chemical Society* **2008**, *130*, 11572-11573; b) M. Dang, A. J. Koh, T. Danciu, L. K. McCauley, P. X. Ma, *Adv Healthc Mater* **2017**, *6*.

[7] K. Scholten, E. Meng, *International journal of pharmaceutics* **2018**, *544*, 319-334.

[8] a) R. Vadlapatla, E. Y. Wong, S. G. Gayakwad, *Journal of Drug Delivery Science and Technology* **2017**, *41*, 359-366; b) T. Albuquerque, A. R. Neves, T. Quintela, D. Costa, *Journal of Molecular Medicine* **2021**, 1-23; c) A. Potruch, S. T. Khoury, Y. Ilan, *Seizure* **2020**; d) D. W. Kim, E. Zavala, J. K. Kim, *Current Opinion in Systems Biology* **2020**.

[9] R. D. Field, P. N. Anandakumaran, S. K. Sia, *Applied Physics Reviews* **2019**, *6*, 041305.

[10] a) J. N. Belling, L. K. Heidenreich, Z. Tian, A. M. Mendoza, T.-T. Chiou, Y. Gong, N. Y. Chen, T. D. Young, N. Wattanatorn, J. H. Park, L. Scarabelli, N. Chiang, J. Takahashi, S. G. Young, A. Z. Stieg, S. De Oliveira, T. J. Huang, P. S. Weiss, S. J. Jonas, *Proceedings of the National Academy of Sciences* **2020**, *117*, 10976; b) J. Rich, Z. Tian, T. J. Huang, *Advanced Materials Technologies* **2022**, *7*, 2100885.

[11] W. Wang, S. Li, L. Mair, S. Ahmed, T. J. Huang, T. E. Mallouk, *Angew Chem Int Ed Engl* **2014**, *53*, 3201-3204.

[12] P. Zhang, H. Bachman, A. Ozcelik, T. J. Huang, *Annual Review of Analytical Chemistry* **2020**, *13*, 17-43.

[13] A. Y. Rwei, B. Wang, T. Ji, D. S. Kohane, *Journal of controlled release* **2018**, *286*, 55-63.

[14] a) O. Ordeig, S. Y. Chin, S. Kim, P. V. Chitnis, S. K. Sia, *Scientific Reports* **2016**, *6*, 22803; b) G. T. Haar, C. Coussios, *Int J Hyperthermia* **2007**, *23*, 89-104.

[15] a) A. A. Atchley, A. Prosperetti, *The Journal of the Acoustical Society of America* **1989**, *86*, 1065-1084; b) B. M. Borkent, M. Arora, C.-D. Ohl, *The Journal of the Acoustical Society of America* **2007**, *121*, 1406-1412; c) B. M. Borkent, S. Gekle, A. Prosperetti, D. Lohse, *Physics of fluids* **2009**, *21*, 102003.

[16] a) I. Lentacker, B. Geers, J. Demeester, S. C. De Smedt, N. N. Sanders, *Mol Ther* **2010**, *18*, 101-108; b) F. Dong, J. Zhang, K. Wang, Z. Liu, J. Guo, J. Zhang, *Nanoscale* **2019**, *11*, 1123-1130; c) E. Stride, C. Coussios, *Nature Reviews Physics* **2019**, *1*, 495-509.

[17] L. Zhang, L. H. Cai, P. S. Lienemann, T. Rossow, I. Polenz, Q. Vallmajo-Martin, M. Ehrbar, H. Na, D. J. Mooney, D. A. Weitz, *Angewandte Chemie* **2016**, *128*, 13668-13672.

[18] a) B. Y. Zaslavsky, *Aqueous two-phase partitioning: physical chemistry and bioanalytical applications*, CRC press, **1994**; b) R. Hatti-Kaul, Springer, **2001**; c) A. G. Teixeira, R. Agarwal, K. R. Ko, J. Grant-Burt, B. M. Leung, J. P. Frampton, *Advanced healthcare materials* **2018**, *7*, 1701036.

[19] J. J. Kwan, R. Myers, C. M. Coviello, S. M. Graham, A. R. Shah, E. Stride, R. C. Carlisle, C. C. Coussios, *Small* **2015**, *11*, 5305-5314.

[20] Q. Liu, M. Zhao, S. Mytnyk, B. Klemm, K. Zhang, Y. Wang, D. Yan, E. Mendes, J. H. van Esch, *Angewandte Chemie* **2019**, *131*, 557-561.

[21] S. Bashir, M. Bashir, J. M. Rees, W. B. J. Zimmerman, *BioChip Journal* **2014**, *8*, 122-128.

[22] Q. Jin, P. X. Ma, W. V. Giannobile, *Adv Skin Wound Care* **2010**, *1*, 375-381.

[23] I. Leibacher, S. Schatzer, J. Dual, *Lab on a Chip* **2014**, *14*, 463-470.

[24] S. A. Sapareto, W. C. Dewey, *Int J Radiat Oncol Biol Phys* **1984**, *10*, 787-800.

[25] a) M. L. Fabiilli, K. J. Haworth, N. H. Fakhri, O. D. Kripfgans, P. L. Carson, J. B. Fowlkes, *IEEE transactions on ultrasonics, ferroelectrics, and frequency control* **2009**, *56*, 1006-1017; b) M. Lafond, J.-L. Mestas, F. Prieur, K. Chettab, S. Geraci, P. Clézardin, C. Lafon, *Ultrasound in medicine & biology* **2016**, *42*, 220-231; c) C. Coussios, C. Farny, G. Ter Haar, R. Roy, *International journal of hyperthermia* **2007**, *23*, 105-120.

[26] A. J. Croockewit, P. P. Koopmans, B. E. de Pauw, *Clinical Pharmacology & Therapeutics* **1996**, *59*, 1-6.

[27] G. L. Re, D. A. Santeufemia, F. L. Re, R. Bortolus, P. Doretto, W. Marus, L. Buttazzi, O. Lenardon, A. Falda, R. Piazza, S. Sulfaro, *Cytokine* **2020**, *128*, 154984.

[28] a) S. Chan, L. Rowbottom, R. McDonald, G. A. Bjarnason, M. Tsao, C. Danjoux, E. Barnes, M. Popovic, H. Lam, C. DeAngelis, E. Chow, *Clinical Oncology* **2017**, *29*, 231-238; b) R. C. Hermida, D. E. Ayala, J. R. Fernández, A. Mojón, M. H. Smolensky, *European Journal of Clinical Investigation* **2018**, *48*, e12909.

[29] a) A. Y. Rwei, J. L. Paris, B. Wang, W. Wang, C. D. Axon, M. Vallet-Regí, R. Langer, D. S. Kohane, *Nature Biomedical Engineering* **2017**, *1*, 644-653; b) S. H. Lee, Y. B. Lee, B. H. Kim, C. Lee, Y. M. Cho, S.-N. Kim, C. G. Park, Y.-C. Cho, Y. B. Choy, *Nature Communications* **2017**, *8*, 15032.

[30] Y. Song, A. Sauret, H. Cheung Shum, *Biomicrofluidics* **2013**, *7*, 061301.

[31] R. M. Ramanan, P. Chellamuthu, L. Tang, K. T. Nguyen, *Biotechnol Prog* **2006**, *22*, 118-125.

[32] G. Sun, C.-C. Chu, *Carbohydrate Polymers* **2006**, *65*, 273-287.

[33] a) K. Chojnacka-Górka, K. Wolski, S. Zapotoczny, *ACS Applied Materials & Interfaces* **2021**, *13*, 1562-1572; b) S. Ye, C. Wang, X. Liu, Z. Tong, B. Ren, F. Zeng, *Journal of Controlled Release* **2006**, *112*, 79-87.