JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

High Spatiotemporal Control of Spontaneous Reactions Using Ultrasound-Triggered Composite Droplets

Marine Bezagu,[†] Claudia Errico,[‡] Victor Chaulot-Talmon,[§] Fabrice Monti,[§] Mickael Tanter,[‡] Patrick Tabeling,*[,§] Janine Cossy,*[,†] Stellios Arseniyadis,*[,†] and Olivier Couture[‡]

[†]Laboratoire de Chimie Organique, Institute of Chemistry, Biology and Innovation (CBI), − UMR 8231 − ESPCI ParisTech/CNRS/PSL* Research University, 10 rue Vauquelin, 75231 Paris Cedex 05, France

[‡]Institut Langevin, ESPCI ParisTech, CNRS (UMR 7587), INSERM (U979), Paris, France

[§]Laboratoire de Microfluidique, MEMS et Nanostructures, ESPCI ParisTech, CNRS (UMR Gulliver 7083), Paris, France

Supporting Information

ABSTRACT: Achieving high spatial and temporal control over a spontaneous reaction is a particularly challenging task with potential breakthroughs in various fields of research including surface patterning and drug delivery. We report here an exceptionally effective method that allows attaining such control. This method relies on a remotely triggered ultrasound-induced release of a reactant encapsulated in a composite microdroplet of liquid perfluorohexane. More specifically, the demonstration was achieved by locally applying a focused 2.25 MHz transducer onto a microfluidic channel in which were injected composite microdroplets containing a solution of an azidocoumarin and an external flow containing a reactive alkyne.

Recent years have witnessed a considerable increase of interest in the development of devices or methods capable of improving both spatial and temporal control in spontaneous chemical reactions. These two criteria are all the more important as they are necessary for a number of synthetic applications including surface patterning,[1] polymer synthesis, and polymer modification[2] or in any situation which requires a controlled sequence of events. This is particularly true for drug delivery, which could greatly benefit from the possibility of generating drugs *in situ via* a controlled chemical reaction.

One way to achieve high spatial and temporal control over a spontaneous reaction is to isolate and remotely trigger the release of the different reactive partners. Gracias and co-workers, for instance, recently reported a chemical encapsulation in metallic containers with a remotely guided chemical release using magnetic fields.[3] However, although they were able to achieve good spatial and temporal control of the release through shape, size, porosity, and magnetic characteristics of their containers, the low penetration depth of radio frequency energy and the need to introduce an electrode to promote the reaction impedes any clinical application.

Bowman and co-workers[4] recently contributed to the field of controlled chemical reactions by attaining high spatial and temporal control in the copper-catalyzed alkyne−azide click cycloaddition (CuAAC)[5] by photochemically reducing *in situ* a Cu(II) catalyst to the corresponding Cu(I) species using

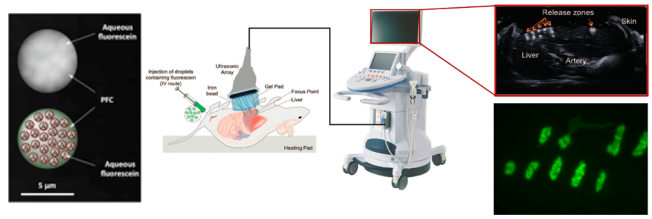

Figure 1. *In vivo* ultrasound-triggered release of composite microdroplets for tissue tattooing (previous work).[7]

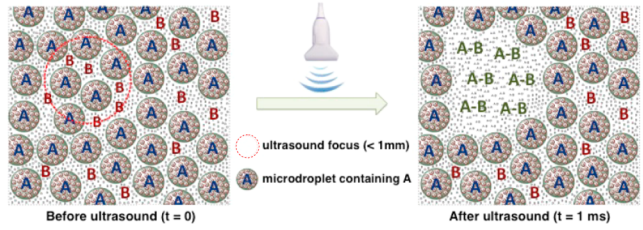

Figure 2. *In situ* ultrasound-triggered release of composite microdroplets for improved spatial and temporal control over a spontaneous reaction (this work).

standard photolithography techniques. Unfortunately, the presence of the copper catalyst and the photoinitiator as well as the low penetration of coherent light through tissues (few hundred micrometers) hampers potential *in vivo* applications. In order to overcome the toxicity of both the copper catalyst and the photoinitiator and with the same goal of attaining high spatial and temporal control, Anseth and co-workers reported a particularly clever strategy involving sequential metal-free click reactions to build biologically functionalized gels and spatially tune their properties in the presence of cells.[6] Nonetheless, while they were able to overcome the presence of undesirable reactants, their photopatterning method implied the use of UV light which, as stated previously, impedes potential *in vivo* applications due to its low penetration.

In contrast to light-based techniques, ultrasound is non-invasive and can penetrate up to 10 cm deep into tissues while preserving a submillimetric resolution using standard scanners

Received: February 24, 2014
Published: May 2, 2014

   dx.doi.org/10.1021/ja5019354 | *J. Am. Chem. Soc.* 2014, 136, 7205−7208

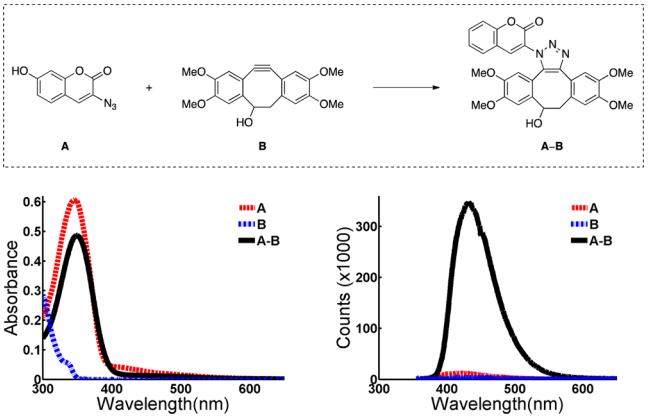

Figure 3. Copper-free click reaction between 3-azido-7-hydroxycoumarin **A** and TMDIBO **B**, chosen as a model reaction. Absorption (left) and emission (right) spectra of compounds **A** (red), **B** (blue), and **A**–**B** (black). Both compounds show an absorption maximum at 350 nm and an emission maximum at 450 nm, but the reaction product **A**–**B** shows a higher emission intensity than the starting materials **A** and **B**.

which are ubiquitous in hospitals worldwide. Considering these elements, the spatiotemporal control of a spontaneous reaction using acoustic waves could be particularly appropriate for *in vivo* applications.

We previously established that perfluorocarbon (PFC) composite droplets loaded with a desired molecule (in our case fluorescein) could release their content locally in a known process of acoustic vaporization to achieve internal tattooing of tissues for surgical guidance in rats (Figure 1).[7] Various advantages arose from this technology. First, as mentioned previously, ultrasound benefit from their high spatial (millimeter) and temporal (microsecond) resolution as well as their penetration depth (up to 10 cm into tissues). Second, the low ultrasound release threshold of the droplets enables the use of standard clinical scanners with the same resolution as that for imaging (millimetric zone within a microsecond time scale) and with low acoustic pressures, which are both compatible with potential *in vivo* applications. Finally, the liquid perfluorocarbon matrix allows the inner phase to be efficiently isolated from the outer medium prior to the release.

The potential use of composite droplets as ultrasound-induced carriers of chemotherapeutic agents would be particularly appealing, as large doses of cytotoxic drugs could be delivered specifically at the focus of the ultrasound scanner. Unfortunately, even if this approach increases the spatial specificity of the release, the question of unspecific release remains as for all drug-delivery methods reported so far. One way to circumvent this issue, and thus limit any undesired side effects which could occur downstream, would be to generate or annihilate the drug specifically in a zone of interest. In order to attain this goal, we first needed to prove that a biologically relevant reaction could be promoted with a high level of spatial and temporal control after ultrasound release of the composite microdroplet carriers (Figure 2). We present here the results of our endeavor.

In order to demonstrate that we were able to remotely trigger a specific reaction with high spatial and temporal control, we first needed to select a specific spontaneous reaction. The bioorthogonal copper-free click between an azide and a strained alkyne such as cyclooctyne derivatives to form the corresponding triazole appeared to be ideal for this study. Indeed, it has a

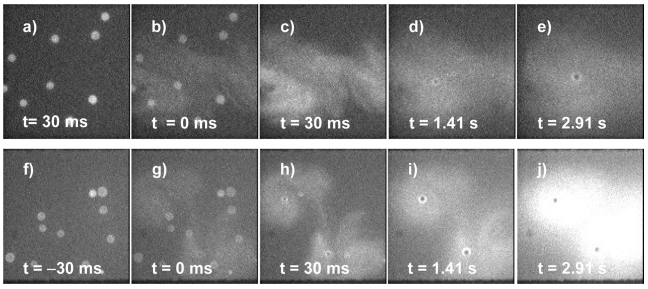

Figure 4. Droplets loaded with 3-azido-7-hydroxycoumarin **A** in a flow of DMSO (a to e) or DMSO + TMDIBO **B** (f to j). Sequences of images taken at 34 Hz, with ultrasonic pulse occurring at images b and g, respectively. The width of each image is 100 μm.

relatively high reaction rate at low reagent concentration, it is compatible with biological environments (physiological pH, temperature and pressure), and it is inert to abundant biological nucleophiles, electrophiles and redox-active metabolites. As a matter of fact, this strain-promoted azide–alkyne cycloaddition has been extensively applied in many fields including drug discovery,[8] material science,[9] and bioconjugation[10] to probe biomolecules in living systems or to label cells.[11]

We decided to initiate our study using coumarin derivative **A** and the strained-alkyne tetramethoxydibenzocyclooctyne (TMDIBO, **B**) recently developed by Leeper et al. (Figure 3).[12] Indeed, 3-azidocoumarin **A** had previously been proven to be a good fluorogenic compound and could therefore be used to monitor the reaction,[13] while TMDIBO (**B**) appeared to be a good candidate as a strained alkyne due to its relatively direct synthetic access and its inherent stability. Most importantly, the product (**A**–**B**) resulting from the cycloaddition demonstrates a stronger fluorescence than either starting materials (λ_{ex} = 350 nm, λ_{em} = 430 nm). Spatiotemporal control of the reaction was achieved by encapsulating a DMSO solution of **A** (3.0% w/v) into composite microdroplets of perfluorohexane following the procedure previously reported.[7] TMDIBO **B** on the other hand was dissolved in the external flow of DMSO (0.3% w/v). They were both mixed together prior to the injection in a microfluidic channel with a width of 100 μm and a depth of 40 μm.[14] A 2.25 MHz transducer was focused (focus = 38 mm, f/*d* = 1) within the channel (Figure 4). Single bursts of 30 cycles were generated at 2.25 MHz by an arbitrary waveform generator and amplified to 12.3 MPa peak-negative pressure by a radio frequency amplifier.

Prior to and after the ultrasonic pulse transmission, a camera (34 fps) mounted on a fluorescent microscope (Leica, 20× or 10×, DAPI filter) recorded the fluorescence induced during the reaction. Within the microfluidic channel, this single acoustic pulse was able to vaporize several droplets (8 μm in diameter), leading to the release of their content in the surrounding medium. As shown in Figures 4 and 5, a significant difference of fluorescence between the control experiment (only one reactant encapsulated in a flow of pure solvent) and the reaction experiment (one reactant encapsulated in a flow of the other) demonstrated the subsequent formation of the product upon ultrasound release. Indeed, the average intensity in the observation window increased drastically as the released cloud spread by diffusion and the click reaction occurred. At 34 fps, the ultrasound disruption of the droplets appeared instantaneous since the pulse duration was 13 μs. After 5 s, the fluorescence intensity reached a plateau whereas the fluorescence intensity of the control experiment remained

dx.doi.org/10.1021/ja5019354 | *J. Am. Chem. Soc.* 2014, 136, 7205–7208

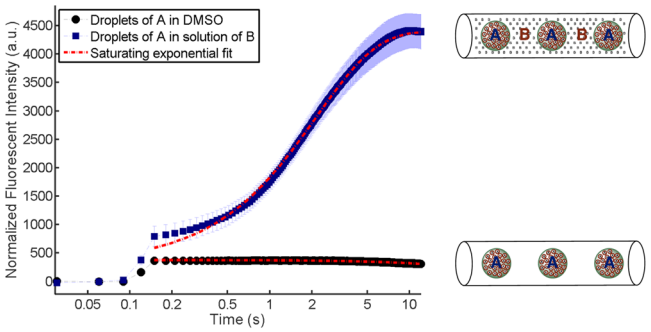

Figure 5. Fluorescence increase after the ultrasound release at t = 100 ms. Comparison between control experiment (droplets of 3-azido-7-hydroxycoumarin **A** in DMSO, black curve) and reaction (droplets of 3-azido-7-hydroxycoumarin **A** in a solution of TMDIBO **B** in DMSO, 0.3% w/v, blue curve). The reaction rate is well described by a saturating exponential fit with a time constant of 2.2 s (R^2 = 0.99).

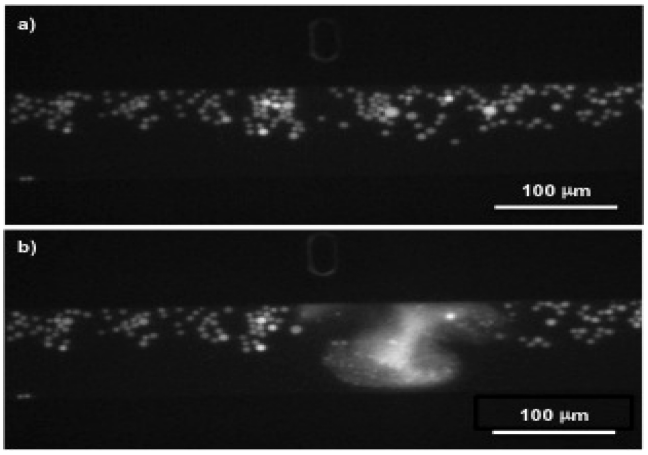

Figure 6. Microfluidic channel containing droplets of 3-azido-7-hydroxycoumarin **A** in a flow of TMDIBO **B** in DMSO before (a) and after (b) ultrasonic pulse.

stable after a slight increase upon release due to a decrease in the autoquenching phenomenon. Moreover, the intensity of the control experiment remained 10 times lower than the value of the plateau reached by the reaction's fluorescence.

As shown in Figure 6, the reaction remained spatially localized. The average diameter of the releasing cloud is in the same order of magnitude as the size of the focal spot, which is 600 μm (full-width at half-maximum). All droplets within that zone were disrupted, while those outside the focal spot remained undisturbed.

In contrast to the previous setup using a single-focus transducer, an ultrasonic scanner equipped with a multielement transducer can focus over several spots within a plane by using electronic delays. To further demonstrate the high level of spatial control as well as the reproducibility, droplets were injected within a cell-culture plate placed under a 4 MHz, 196-element probe (Vermon, France) connected to a clinical ultrasound scanner (Aixplorer, Supersonic Imagine, France).[15] By scanning remotely the focus zone of the ultrasound clinical system, several spots of release could be generated over each line, initiating the chemical reaction in specific zones of the plate. Thus, a 3 cm high representation of a landmark of Paris could be created point-by-point within 6 s (Figure 7).

In conclusion, a copper-free click reaction was remotely induced with ultrasound by releasing an encapsulated reactant

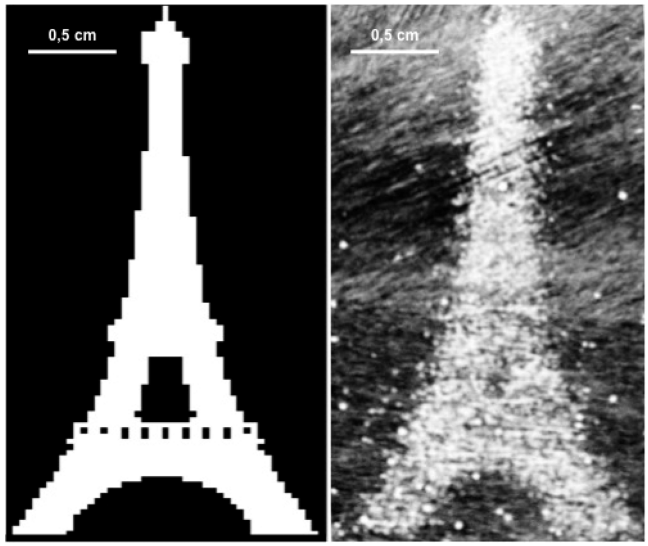

Figure 7. Drawing generated by focusing pulses according to the pattern with an ultrasound scanner within a plate filled with droplets of 3-azido-7-hydroxycoumarin **A** in a medium of TMDIBO **B** (0.15% w/v) in DMSO. Photography made under UV light (histogram equalized) a few seconds after the end of the release.

locally into a flow containing a reactive partner. This reaction was triggered by a single ultrasound pulse specifically within the focus of the transducer (0.6 mm in width) and within a time of less than 3 ms. Since these acoustic pulses could be generated by a clinical ultrasonic scanner, the generation of a chemical reaction deep into the tissue can be envisioned. Considering that a wide range of compounds can be encapsulated using this technology, we expect that such targeted chemistry will lead to the localized release of prodrugs or the localized production of drugs *in vivo* that are either too toxic or too unstable to be injected directly in patients.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

patrick.tabeling@espci.fr
janine.cossy@espci.fr
stellios.arseniyadis@espci.fr

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by LABEX WIFI (Laboratory of Excellence ANR-10-LABX-24) within the French Program "Investments for the Future" under reference ANR-10- IDEX-0001-02 PSL*. M.B. and C.E. would like to thank the Institut Pierre-Gilles de Gennes pour la microfluidique and the Fondation Pierre-Gilles de Gennes respectively for financial support.

■ REFERENCES

(1) (a) Arnold, R. M.; Locklin, J. *Langmuir* **2013**, *29*, 5920−5926. (b) Pauloehrl, T.; Delaittre, G.; Winkler, V.; Welle, A.; Bruns, M.;

Börner, H. G.; Greiner, A. M.; Bastmeyer, M.; Berner-Kowollik, C. *Angew. Chem., Int. Ed.* **2012**, *51*, 1071−1074.
(2) Chatani, S.; Sheridan, R. J.; Podgorski, M.; Nair, D. P.; Bowman, C. N. *Chem. Mater.* **2013**, *25*, 3897−3901.
(3) (a) Leong, T.; Gu, Z.; Koh, T.; Gracias, D. H. *J. Am. Chem. Soc.* **2006**, *128*, 11336−11337. (b) Ye, H.; Randall, C. L.; Leong, T. G.; Slanac, D. A.; Call, E. K.; Gracias, D. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 4991−4994.
(4) Adzima, B. J.; Tao, Y.; Kloxin, C. J.; DeForest, C. A.; Anseth, K. S.; Bowman, C. N. *Nat. Chem.* **2011**, *3*, 256−259.
(5) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.
(6) DeForest, C. A.; Polizzotti, B. D.; Anseth, K. S. *Nat. Mater.* **2009**, *8*, 659−664.
(7) Fluorescein was encapsulated and locally released to achieve internal tattooing of tissues for surgical guidance in rats; see: (a) Couture, O.; Pannacci, N.; Babataheri, A.; Servois, V.; Tabeling, P.; Tanter, M. *Med. Phys.* **2011**, *38*, 1116−1123. (b) Couture, O.; Dransart, E.; Dehay, S.; Nemati, F.; Decaudin, D.; Johannes, L.; Tanter, M. *Mol. Imaging* **2011**, *10*, 135−143. For other examples of localized delivery, see: (c) Fabiilli, M. L.; Haworth, K. J.; Sebastian, I. E.; Kripfgans, O. D.; Carson, P. L.; Fowlkes, J. B. *Ultrasound in Med. & Biol.* **2010**, *36*, 1364−1375. (d) Fabiilli, M. L.; Lee, J. A.; Kripfgans, O. D.; Carson, P. L.; Fowlkes, J. B. *Pharm. Res.* **2010**, *27*, 2753−2765.
(8) (a) Srinivasan, R.; Li, J.; Ng, S. L.; Kalesh, K. A.; Yao, S. Q. *Nat. Protoc.* **2007**, *2*, 2655−2664. (b) Evans, M. J.; Saghatelian, A.; Sorensen, E. J.; Cravatt, B. F. *Nat. Biotechnol.* **2005**, *23*, 1303−1307.
(9) (a) Franc, G.; Kakkar, A. *Chem. Commun.* **2008**, 5267−5276. (b) Malkoch, M.; Vestberg, R.; Gupta, N.; Mespouille, L.; Dubois, P.; Mason, A. F.; Hedrick, J. L.; Liao, Q.; Frank, C. W.; Kingsbury, K.; Hawker, C. J. *Chem. Commun.* **2006**, 2774−2776. (c) Polizzotti, B. D.; Fairbanks, B. D.; Anseth, K. S. *Biomacromolecules* **2008**, *9*, 1084−1087.
(10) (a) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249−1262. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064. (c) Franke, R.; Doll, C.; Eichler, J. *Tetrahedron Lett.* **2005**, *46*, 4479−4482.
(11) (a) Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *9*, 666−676. (b) Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 3688−3690. (c) Kuzmin, A.; Poloukhtine, A.; Wolfert, M. A.; Popik, V. V. *Bioconjugate Chem.* **2010**, *21*, 2076−2085. (d) Sletten, E. M.; Nakamura, H.; Jewett, J. C.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 11799−11805. (e) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422−9425. (f) Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769−15776. (g) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253−2255. (h) Codelli, J. A.; Baskin, J. M.; Agard, N. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2008**, *130*, 11486−11493. (i) Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793−16797. (j) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. A. *J. Am. Chem. Soc.* **2004**, *126*, 15046−15047.
(12) Stockmann, H.; Neves, A. A.; Stairs, S.; Ireland-Zecchini, H.; Brindle, K. M.; Leeper, F. J. *Chem. Sci.* **2011**, *2*, 932−936.
(13) (a) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. A. *Org. Lett.* **2004**, *24*, 4603−4606. (b) Zhou, Z.; Fahrni, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 8862−8863.
(14) **Ultrasound-triggered release of composite droplets in a microfluidic channel.** Composite droplets of **A** were suspended by magnetic stirring in a DMSO solution of **B** (0.3% w/v) and injected in the microfluidic channel using a pressure controller (Fluigent, MFCS-100). The microfluidic channel was 100 μm in width and 40 μm in depth. It was placed at the focus of a 2.25 MHz single-element transducer, which was immersed in a water bath. A single pulse of 30 cycles was emitted, which triggered a camera (Andor iXon, 34 Hz) mounted on a fluorescent microscope (Leica, 10× or 20×, DAPI) that recorded the fluorescence induced during the reaction.
(15) **Ultrasound-triggered release of composite droplets in a cell-culture plate.** The composite droplets of **A** were diluted in a DMSO solution of **B** (0.15% w/v, degassed prior to injection) and injected in an OptiCell plate made of two plastic membranes, transparent to both ultrasound and light, separated by 2 mm. The bottom membrane was placed at the focus of a 5 MHz ultrasound probe which was immersed in a water bath maintained at 12 °C. The ultrasonic probe was driven by a clinical ultrasound scanner (Aixplorer, Supersonic Imagine, France), emitting a single pulse of 2 cycles on each spot of the release, where the distance between the several spots of the release is equal to the pitch number of the transducer (0.3 mm). Release spots could be selected anywhere on the plate using a software interface. The chemical cloud was excited with a UV light, while the observation of the Eiffel tower was performed through a UV filter.