A microfluidic chip for controlled release of drugs from microcapsules

Wen-Chuan Cheng, Yuan He, An-Yi Chang, and Long Que^{a)}

Institute for Micromanufacturing, Louisiana Tech University, Ruston, Louisiana 71272, USA

(Received 5 September 2013; accepted 25 October 2013; published online 6 November 2013)

A new microfluidic device with liquid-droplet merging and droplet storage functions for the controlled release of drugs from microcapsules is reported. A switching channel is designed and integrated within the microfluidic device, facilitating the generation and capturing of uniform droplets by the storage chambers. The drug model is the MnCO_3 microparticle, which is encapsulated by a microcapsule and fabricated using a simple layer-by-layer nanoassembly process. The merging function is used for dynamically adding the control solution into the droplets, which contain drugs within the microcapsules ($\text{DW}\mu\text{Cs}$) and water. The storage chambers are used for collecting $\text{DW}\mu\text{Cs}$ -laden droplets so that the controlled-drug release in specific droplets can be monitored for an extended period of time, which has been experimentally implemented successfully. This technology could offer a promising technical platform for the long-term observation and studies of drug effects on specific cells in a controlled manner, which is especially useful for single cell analysis. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4829776>]

I. INTRODUCTION

Controlled drug release is very important for achieving the optimum therapeutic benefit, drug screen, and discovery.¹ To this end, a variety of technologies have been developed for the past decades.² One of the main controlled drug release techniques is achieved by the triggered release from polymer capsules. The triggering mechanisms include chemical, biological, light, thermal, magnetic, and electrical stimuli, which have been summarized and reviewed.^{2,3} This type of controlled release belongs to the *actively* controlled release. The polymer capsules can be fabricated using a layer by layer (LbL) nanoassembly process. In this case, the metal oxide microparticles are typically used as a core, and negatively and positively charged polyelectrolytes are then deposited on the core in a stepwise fashion, enabled by the electrostatic interaction between the layers of the polymers. After the coating of the multi-layers of polymers has been done, the core is dissolved. As a result, hollow, semi-permeable capsules are formed. Thereafter, the drugs are loaded into the empty capsules, and the capsules serve as a barrier for the drug diffusion/release. The actively controlled-drug release from the polymer capsules can then be realized by using a variety of triggering techniques as aforementioned.² Other controlled release techniques utilize some microdevices with micropores or nanopores,⁴ which are usually enabled by the diffusion process, and hence is a *passively* controlled release. For instance, one type of device is the nanopore thin film device. The nanopore thin film can be fabricated from silicon or aluminum. Specifically, the porous silicon is fabricated from p-type silicon using the electrochemical etching process.⁵ Additionally, the aluminum oxide (AAO) nanopores are fabricated from Al foil thin film (typically 1.0 mm thick, purity 99.998%) using a two-step anodization process.^{6,7} Once the nanopore thin film has been fabricated, the drugs can be loaded into the nanopores, followed by depositing a layer of polymer. As a result, the

^{a)} Author to whom correspondence should be addressed. Electronic mail: lque@latech.edu. Tel.: 1-318-257-5121, Fax: 1-318-257-5104 911.

polymer and nanopores serve as the diffusion barrier for the controlled drug release without any external stimuli.

While these technologies have been demonstrated to be successful, they are not necessarily suitable for large-scale studies for the drug effects and treatment. Due to the emergence of the droplet microfluidics technology,^{8,9} the large-scale studies have become possible. Using this microfluidics technology, hundreds or thousands of droplets with nano-liter volume can be generated very rapidly. Each droplet can serve as a reactor for studying the drug effects. By taking advantage of the polymer capsule technology and droplet microfluidics, a new technology for controlled drug effect analysis with high throughput could be developed.

The drug effect analysis on single cells in droplets has been demonstrated in a recent effort.^{10,11} However, in this technology, the drug dose applied on the cells is rapid, immediate, and fixed; the studies of the controlled drug dose effect on cells cannot be achieved. In another recent work, the controlled-drug release in a droplet has been demonstrated,^{12,13} showing a great promise for realizing the drug effect analysis on single cells in a controlled manner on a microdevice. In this demonstration, drugs (i.e., Cucurmin) with nanostructured capsules are encapsulated in droplets by a microfluidic device. Experiments find that the drug release in a droplet can be controlled by *dynamically* changing the pH of the control solution (water + NaOH). By observing the fluorescent images of the droplets, the drug release inside a droplet environment can span from several minutes to several weeks by the control solutions of a different pH.

However, in order to monitor the drug effect on a specific cell in a droplet, it is desirable that the position of a droplet can be fixed inside a microdevice; and thus, the drug effects on a cell can be monitored for an extended period of time. Hence, the arrangement and storage of the droplets in specific locations inside a microdevice can become very useful and important, especially for high throughput applications.

Herein, a microfluidic device with liquid-droplet merging and droplet storage functions for monitoring the controlled drug release has been developed and is reported. A schematic of a device is given in Fig. 1(a). Filtered mustard oil is used as the continuous flow phase and the carrier fluid. Along the flowing direction of the fluids as illustrated in Fig. 1(a), this device consists of a T-shape droplet generator, a liquid-droplet merger, a serpentine switching-channel (s-channel), and the droplet storage-chambers (chambers). The droplet generator forms drug-laden droplets. The liquid-droplet merger is used to add drug-release control-solution (c-solution) to each drug-laden droplet. The s-channel is designed to prevent any air bubbles or non-uniform droplets from entering and occupying the chambers at the beginning of the operation of the device. Specifically, at the beginning of the operation, the outlet connecting to the chambers is closed, while the outlet of the s-channel is open. As a result, the fluids inside the device will flow toward and through the s-channel. Once the uniform droplet generation is established, the s-channel is closed, and the outlet of the chambers is open. As a result, the droplets will flow toward the chambers, thereby entering and occupying them one by one. A photo of a fabricated device is shown in Fig. 1(b). The close-up photos of the droplet generation and the merging function are given in Figs. 1(c) and 1(d), respectively. A photo of the chambers is given in Fig. 1(e), which consists of a series of cascaded circular-shape structures with inputs for the droplet and outputs for the flowing liquid. The width of the input is much larger than that of the output for each chamber to facilitate the capture and storage of the droplet. Another zigzag channel running along all the chambers is used to facilitate the capture of the droplets one by one by each chamber.

II. METHODS AND MATERIALS

A. Design and fabrication of the microfluidic device

It has been routinely found that if no s-channel is integrated in the device as shown in Fig. 1(a), some chambers will be occupied by air bubbles or non-uniform droplets; since at the beginning operation of the device, uniform droplets usually cannot be generated immediately.^{13,14} To this end, the use of the s-channel is necessary, and care should be taken to design the dimensions of the device. Specifically, in order to facilitate the switching of the flow of the fluids between the s-channel and the chambers, the fluidic resistance of the device should be

FIG. 1. (a) Sketch of a microfluidic device showing the droplet generating function, the merging function, the s-channel, and the chambers; (b) photo of a fabricated device; (c) close-up of the T-shape droplet generator; (d) close-up of the merging function; (e) close-up of some chambers; (f) the equivalent fluidic circuit of the device; (g) close-up of a chamber: input size is $D1$, chamber diameter is $D2$, and output size is $D3$.

designed properly. The resistance-equivalent circuit of the device is shown in Fig. 1(f).¹⁴ The fluidic resistance $R6$ of the s-channel should be equal to the fluidic resistance $R7$ of the chambers. A close-up photo of one chamber is given in Fig. 1(g), the width of the input is $D1 = 50 \mu\text{m}$, the diameter of the chamber is $D2 = 120 \mu\text{m}$, and the width of the output is $D3 = 10 \mu\text{m}$. Once a droplet enters the chamber through the input, it flows toward the output due to the flowing carrier fluid oil. As a result, the output of the chamber is blocked, and no droplets will enter the chamber any more. Instead, the subsequent droplets will flow through the zigzag channel, parallel to the chambers, and will enter the chambers one by one in sequence. One typical design of this device and its dimensions are summarized in the supplementary material.¹⁵ Devices of these dimensions have been fabricated and tested for the technical demonstrations. As experimentally confirmed in Sec. III, this simple fluidic-circuit model gives a very viable design guide.

The device is fabricated using a soft-lithography process.¹⁶ Briefly, a $50 \mu\text{m}$ thick SU-8 mold of the device is formed on a silicon substrate using conventional optical lithography. Polydimethylsiloxane (PDMS) is then casted on the mold, followed by 1.5 h of curing at the temperature of 65°C . Finally, the PDMS microfluidic layer is peeled off from the mold, and then is bonded with a glass substrate after oxygen plasma treatment for 10 s. The input and output holes are made in the PDMS layer for the delivery of the samples to the chip, followed by assembling input and output tubing (Upchurch Scientific, Inc.), and being connected with syringes controlled by several syringe pumps (KD Scientific, Inc.).

B. Materials and fabrication of the drugs within the microcapsules ($DW_{\mu}\text{Cs}$)

MnCO_3 microparticles (MnCO_3 μPs) with diameters of $\sim 5 \mu\text{m}$ purchased from PlasmaChem GmbH, Inc. are used as the drug model. Poly(allylamine hydrochloride) (PAH),

Poly(sodium styrene sulfonate) (PSS), and Fluorescein isothiocyanate (FITC) are purchased from Sigma, Inc. All of them have been used without any further purification.

The fabrication process of the DW μ Cs is illustrated in Fig. 2(a). The DW μ Cs have been fabricated by a LbL nanoassembly process.¹² Briefly, the solutions of PAH (2 mg/ml), PSS (2 mg/ml), FITC, and drug (2 mg/ml) are prepared. The process starts with the drug model (MnCO_3 μ Ps), and they are coated by a PAH layer and then by a PSS layer. Thereafter, this coating process is repeated 3 times. Note that after each layer has been coated, the drug solution must be rinsed and centrifuged before the next layer is coated. Similarly, the outer two layers of PAH and FITC are coated in sequence, resulting in DW μ Cs. Thereafter, the DW μ Cs are suspended in water for the following controlled-release experiments. Note that a layer of FITC, as shown in Fig. 2(a), is assembled in order to facilitate the confirmation of the success of the formation of microcapsules by observing their fluorescent images. Optical and fluorescent images of the fabricated DW μ Cs are shown in Figs. 2(c) and 2(d). Since the FITC is the out-most layer assembled on the microcapsules, the almost identical profiles/features of the two types of images indicate the drug model has been indeed encapsulated in the FITC-labeled microcapsules. The DW μ Cs appear *optically* dark as shown in Fig. 2(d).

FIG. 2. (a) Procedure to fabricate a DW μ C using a LbL nanoassembly process; (b) a sketch showing the controlled drug release: the MnCO_3 μ P is dissolved by the c-solution and diffuses through the microcapsule; (c) fluorescence images of the DW μ Cs on a cover slide; (d) the corresponding optical images of the DW μ Cs (e) optical images of the DW μ Cs on a cover slide 5 min after adding c-solution; (f) optical images of the DW μ Cs on a cover slide 10 min after adding c-solution. The size of the MnCO_3 μ Ps is ~ 5 μm .

C. Monitoring of the controlled release of drugs from microcapsules

The controlled release procedure is described in Fig. 2(b). The drug release can be dynamically controlled by the addition of the c-solution (water + HCl) to DW μ Cs. In order to evaluate the drug model (MnCO₃ μ P) diffusion process under the c-solution, some DW μ Cs are deposited on a microscope slide, then the c-solution is added. In Figs. 2(d)–2(f), the optical images show the dissolving process of the drug model inside the microcapsules on a microscope slide. Before the c-solution is added, the DW μ Cs appear dark. Five minutes after adding c-solution, majority of the drugs (MnCO₃ μ Ps) inside the microcapsules have been dissolved and diffuses through the microcapsules, resulting in transparent, empty microcapsules. Ten minutes later, all the drugs inside the microcapsules have been dissolved and diffused through the microcapsules. In this case, the c-solution serves as a dissolving solution of the drugs inside the microcapsules, and thus controls the drug diffusion actively and dynamically. The microcapsule, on the other hand, serves as a passive diffusion barrier for the drug. This procedure can also be used to observe/monitor the qualitative drug release process in the droplets.

On the other hand, the quantitative release of the drugs (MnCO₃ μ Ps) from the microcapsules into the water can be determined by measuring the changed optical absorption by the water surrounding the DW μ Cs.^{17,18} However, given the small size (~ 50 – 80 μ m in diameter) of the droplet generated by the microfluidic chip, it is difficult to directly measure the release of the drugs into the water inside the droplet. In order to address this issue and facilitate the measurements, the drug release in a droplet can be mimicked as the following. The DW μ Cs suspension (DW μ Cs in water) of the same concentration as used in the microfluidic chip is added to a transparent plastic tube. The control-solution (i.e., water + HCl) is then added to it, followed by adding drops of oil to encapsulate it to simulate the drug release in a droplet. The optical absorption by the water is then measured in real-time continuously until the absorbance is saturated using an optical fiber-based spectrometer (Ocean Optics, Inc.). In these experiments, the concentration of DW μ Cs suspension is 2 mg/ml and the volume of DW μ Cs suspension is 1.5 ml. Three different concentrations (0.025 M, 0.05 M, and 0.1 M) of the c-solution are used to evaluate the relationship between the release rate of the drugs from the microcapsules and the c-solution concentration.

D. Instrumentation

In order to observe the storage of the droplets in chambers and the controlled drug release in droplets, the microfluidic device is fixed on a fluorescence microscope (Olympus, Inc.) for the experiments. Both the fluorescent images and bright field optical images are obtained using the same microscope. The oil, c-solution, and DW μ Cs suspension are transported to the device through the assembled plastic tubing (Upchurch Scientific, Inc.) by syringes controlled by syringe pumps (KD Scientific, Inc.).

E. Experimental data analysis

Both the bright field optical images and fluorescent images of the DW μ Cs are used to monitor and analyze the controlled drug release. In addition, the control experiment has been performed to form droplets containing DW μ Cs and water, which is used as a reference for droplets containing DW μ Cs and c-solution. The drug release in a droplet is monitored qualitatively by observing the bright field optical images of the DW μ Cs. The DW μ Cs appear dark before the drug model is dissolved and diffused from the microcapsules, and become transparent after the drug has been completely released from the microcapsules.

III. RESULTS AND DISCUSSION

A. Merging, switching, and storage of drug-laden droplets

As aforementioned, the merging function is to add the drug-release c-solution to the drug-laden droplets, thereby realizing a dynamical control of the drug release from the

microcapsules. Optical micrographs showing the liquid-droplet merging function are given from Figs. 3(a) to 3(c). When a drug-laden droplet arrives at the merging region, it will merge with some amounts of c-solution. As a result, a larger droplet is formed. The amount of the c-solution to be merged can be dynamically tuned by adjusting the flowing rate of the c-solution, which is another parameter for the controlled drug release. The function of s-channel is demonstrated from Figs. 3(d) to 3(f). In Fig. 3(d), the outlet connecting to the chambers is closed while the outlet of the s-channel is open, hence the droplets are flowed toward the s-channel. Once the outlet connecting to the chambers is open and the outlet of the s-channel is closed, the droplets start to flow toward the chambers as shown clearly in Figs. 3(e) and 3(f).

As aforementioned, at the beginning, the outlet of the s-channel remains open, while the outlet connecting the chambers is kept closed before the stable generation of the uniform droplets is achieved. Otherwise, the chambers are *usually* occupied by some air bubbles or by multiple droplets of different sizes. One experimental example is given in Fig. 4(a). In contrast, use of the s-channel to flow the droplets at the beginning of the device operation, each chamber can be routinely occupied by only one droplet with uniform size. One representative experimental result is given in Fig. 4(b). As shown, the droplet inside a chamber flows toward and then blocks the output of the chamber, no additional droplets will enter the chamber thereafter. As a result, the droplet will not be coalesced with or replaced by the subsequent droplets. Note that the size of the droplet in each chamber can be readily changed by the droplet generator and the merging function.

B. Controlled drug release in droplets

Optical micrographs of the droplets containing DW μ Cs are given in Fig. 5. In Fig. 5(a), the DW μ Cs are in water. Under this condition, the drug release from the microcapsule is very slow. The optical image of the DW μ Cs appears dark as shown in the close-up of the DW μ Cs inside the droplet. After several days, the optical image of the DW μ Cs essentially remains unchanged. In contrast, in Figs. 5(b)–5(d), the DW μ Cs are in the c-solution (HCl + water). Under this condition, as expected, the drugs have been dissolved, and thus diffused through the microcapsules into the water inside the droplet. Specifically, immediately after the DW μ Cs and c-solution are encapsulated in a droplet, the DW μ Cs appear dark. The DW μ Cs gradually become optically transparent with time (i.e., 4 min for this demonstration), since only the microcapsules remain. Hence, the release procedure of the drugs in a droplet can be monitored in real-time qualitatively using an optical microscope. It should be noted that the DW μ Cs

FIG. 3. ((a)–(c)) Photos showing a drug-laden droplet merged with the c-solution; ((d)–(f)) photos showing the generated droplets switched from the s-channel to the channel leading to the chambers.

FIG. 4. (a) Photo showing the chambers occupied by air bubbles or non-uniform droplets obtained using a device without an integrated s-channel; (b) a photo showing each chamber occupied by one droplet using a device with an integrated s-channel. Each droplet blocks the output of the chamber, no other droplet can enter the same chamber. The diameter of all chambers is $120\ \mu\text{m}$.

randomly move around in the droplet due to the local turbulence flow or advection of the liquid inside the droplet during the process of the droplet formation and being captured by a chamber at the beginning.¹⁹ After a certain period of time, the DW μ Cs are eventually become stationary inside the droplet when the turbulence flow or advection of the solution inside the droplet becomes negligible, which has been observed as shown from Figs. 5(b) to 5(d).

Using the scheme described in the Sec. II, the release rates of the drugs from the microcapsules inside the “simulated droplets” under different concentrations of the c-solution have been measured and are shown in Fig. 6. As expected, the release rate of the drugs can be dynamically changed by the concentration of the c-solution. The higher the concentration of the c-solution, the larger release rate of the drugs from the microcapsules, since the MnCO_3 μ Ps are dissolved faster and thus diffused more rapidly through the microcapsules into the surrounding water. The release kinetics can be summarized as the following. After the c-solution is added, the MnCO_3 μ Ps are dissolved and start diffusing through the microcapsules. As shown in Fig. 6, for the c-solution of different concentrations, within first several minutes to 10 min, the release from the microcapsules is fast. Thereafter, the release through the microcapsules slows down and eventually approaches negligible, when the drugs have been totally released. Specifically, all the drugs have been released in ~ 6 min by adding the c-solution at a concentration of 0.1 M, while it takes ~ 12 min for the c-solution at a concentration of 0.025 M.

It should be noted that the MnCO_3 μ Ps as the drug model and the HCl as the c-solution are only used for the demonstration. This microfluidic device in principal can be applied to any other *real* drugs for the on-chip controlled release. Evidently, the c-solution will be different

FIG. 5. (a) Photo showing the DW μ Cs in a droplet with DI water: no drug release after several days. Photos of the DW μ Cs in a droplet with the c-solution: (b) 0 min, (c) 2 min, (d) 4 min, clearly showing the dynamic release of the drug from the microcapsules. The diameter of all chambers is 120 μ m.

depending on the mechanisms for inducing the controlled-drug release from microcapsules. For instance, the wall permeability of microcapsules fabricated by LbL nanoassembly can be reversely switched from the open state to the closed state through the variation in pH or through the variation in the solvent polarity.^{20,21} When the microcapsules are inside a solution with low pH or inside a solution with ethanol, the polyelectrolyte network of their walls swells and thus becomes permeable. As a result, the microcapsules are in an *open state*. Upon increasing the pH value or dispersing the suspension in the original medium without ethanol, the polyelectrolyte network shrinks, the walls are no longer permeable. The microcapsules hence are in a *closed state*. Thus, the microcapsules can be switched back and forth from an open state to a closed state, which can be used for the controlled-drug release. Obviously, this controllable procedure can also be easily implemented using this droplet microfluidic platform.

Due to the integrated multiple functions on a single microfluidic device, this technology could provide a technical platform for facilitating the drug effects analysis on single cells. As

FIG. 6. The release rates of the drugs from the microcapsules at different concentrations of the c-solution inside the "simulated" droplets. The concentration of the DW μ Cs used in these experiments is 2 mg/ml.

we know, the widely used microtiter-plate-based high throughput analysis is basically approaching its physical limit, and also has the following limitations:^{22–24} (i) it requires robots to operate; (ii) it consumes a large volume of agents and drugs, hence the whole process is expensive; (iii) it is still a relatively time-consuming process with relatively low throughput; (iv) the reagents are easily evaporated, especially for the 1 μ l-volumes of 1536-well plates; (v) it is not convenient to monitor the effects of the combination of different potential drugs; and (vi) it is not easy to monitor the *dynamic* effects on cells by changing the drug dose, which requires the capability to control the drug release. In contrast, this droplet microfluidic platform essentially could address all the aforementioned issues, thereby paving a way for the controlled drug effect analysis with high throughput. For instance, each droplet can serve as a reactor (equivalent to a micro titter well). High throughput analysis can be readily achieved by simply generating hundreds of thousands of droplets in a very short period of time. Hence, high throughput screen of the drug effects on single cells under a variety of different conditions might be possible in an efficient manner. In addition, compared to the previously reported work,^{11,12} this platform also provides droplet chambers to ensure droplets to become stationary, thereby enabling the observation of the drug effects for an extended period of time, which will greatly benefit the drug screen and discovery research.

IV. CONCLUSIONS

A microfluidic device with liquid-droplet merging and droplet storage functions has been developed for the controlled release of DW μ Cs. The merging function is used to add the c-solution to the drug-laden droplets, thereby dynamically modifying the drug release. The storage function is to capture drug-laden droplets, thereby facilitating the observation of the drug release in a specific droplet for an extended period of time. The s-channel is designed and integrated with the device at the upper stream of the chambers to prevent the air bubbles or non-uniform droplets from entering and occupying the chambers. These functions have been demonstrated successfully using MnCO₃ μ Ps as the drug model and the HCl as the c-solution. Using this type of microfluidic device, the drug release procedure can be monitored in real-time. Because of the feasibility of generating hundreds or thousands of droplets and storing them in the chambers rapidly, and combined with the DW μ Cs, this type of device could provide a very useful technical platform for analyzing the controlled drug effects on single cells with high throughput.

ACKNOWLEDGMENTS

The research was funded in part by a NSF Grant and NSF-Pfund-Louisiana 2012.

- ¹J. Santini, A. Richards, R. Scheidt, M. Cima, and R. Langer, *Angew. Chem., Int. Ed.* **39**, 2396 (2000).
- ²A. Esser-Kahn, S. Odom, N. Scotto, S. White, and J. Moore, *Macromolecules* **44**, 5539 (2011).
- ³B. Radt, T. Smith, and F. Caruso, *Adv. Mater.* **16**, 2184 (2004).
- ⁴E. Gultepe, D. Nagesha, S. Sridhar, and M. Amiji, *Adv. Drug Delivery Rev.* **62**(3), 305 (2010).
- ⁵V. Doan and M. Sailor, *Science* **256**, 1791 (1992).
- ⁶T. Zhang, Z. Gong, R. Giorno, and L. Que, *Opt. Express* **18**(19), 20282 (2010).
- ⁷T. Zhang, P. Pathak, S. Karandikar, R. Giorno, and L. Que, *Biosens. Bioelectron.* **30**, 128 (2011).
- ⁸T. Lagus and J. Edd, *J. Phys. D: Appl. Phys.* **46**, 114005 (2013).
- ⁹T. Tran, F. Lan, C. Thompson, and A. Abate, *J. Phys. D: Appl. Phys.* **46**, 114004 (2013).
- ¹⁰Z. Gong, F. Nie, T. Zhang, P. Pathak, Z. Wang, H. Zhao, S. Wong, and L. Que, in *Proceedings of IEEE International Conference on MEMS* (2010), p. 1015.
- ¹¹Z. Gong, H. Zhao, T. Zhang, F. Nie, P. Pathak, K. Cui, Z. Wang, S. Wong, and L. Que, *Biomed. Microdevices* **13**(1), 215 (2011).
- ¹²Y. He, W. Cheng, Y. Zhao, Y. Lvov, and L. Que, in *Proceedings of IEEE Sensors* (IEEE, 2012), pp. 781–784.
- ¹³W. Cheng, Y. He, and L. Que, “Controlled drug release in a microfluidic device with droplet merging and storage functions,” *Proc. IEEE Sensors* (in press).
- ¹⁴S. Bithi and S. Vanapalli, *Biomicrofluidics* **4**, 044110 (2010).
- ¹⁵See supplementary material at <http://dx.doi.org/10.1063/1.4829776> for the detailed dimensions of the microfluidic device.
- ¹⁶Z. Gong, S. Penmesta, Z. Zheng, Y. Lvov, and L. Que, in *Proceedings of Solid-State Sensors, Actuators and Microsystems Conference* (IEEE, 2009), p. 1043.
- ¹⁷E. Anglin, M. Schwartz, V. Ng, L. Perelman, and M. Sailor, *Langmuir* **20**, 11264 (2004).

- ¹⁸S. Simovic, D. Losic, and K. Vasilev, [Chem. Commun.](#) **46**, 1317 (2010).
- ¹⁹M. Bringer, C. Gerdt, H. Song, J. Tice, and R. Ismagilov, [Philos. Trans. R. Soc. London, Ser. A](#) **362**, 1087 (2004).
- ²⁰Y. Lvov, A. Antipov, A. Mamedov, H. Mohwald, and G. Sukhorukov, [Nano Lett.](#) **1**, 125 (2001).
- ²¹P. Gil, L. del Mercato, P. del Pino, A. Munoz Javier, and W. Parak, [Nanotoday](#) **3**, 12 (2008).
- ²²S. Sundberg, [Curr. Opin. Biotechnol.](#) **11**, 47 (2000).
- ²³D. Dunn and I. Feygin, [Drug Discovery Today](#) **5**, S84 (2000).
- ²⁴J. Wolcke and D. Ullman, [Drug Discovery Today](#) **6**, 637 (2001).