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Microfluidic Fabrication of Perfluorohexane-Shelled Double Emulsions for Controlled Loading and Acoustic-Triggered Release of Hydrophilic Agents

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ABSTRACT

The ability of low boiling point liquid perfluorocarbons (PFCs) to undergo a phase change from a liquid to a gas upon ultrasound irradiation makes PFC-based emulsions promising vehicles for triggered delivery of payloads. However, loading hydrophilic agents into PFC-based emulsions is difficult due to their insolubility in PFC. Here, we address this challenge by taking advantage of microfluidic technologies to fabricate double emulsions consisting of large aqueous cores and a perfluorohexane (PFH) shell, thus yielding high loading capacities for hydrophilic agents. Using this technology, we efficiently encapsulate a model hydrophilic agent within the emulsions and study its response to ultrasound irradiation. Using a combination of optical and acoustic imaging methods, we observe payload release upon acoustic vaporization of PFH. Our work demonstrates the utility of microfluidic techniques for controllably loading hydrophilic agents into PFH-based emulsions, which have great potential for acoustically triggered release.

Introduction

An important goal of advanced drug delivery is to controllably supply drugs to specific sites in the body, which often requires the use of carrier vehicles that efficiently encapsulate payloads and release them in response to an external trigger; examples of such triggers include light, magnetic fields and ultrasound.[1,2,3,4] Ultrasound is, in fact, an ideal trigger because it provides both spatial and temporal control over the transmission of thermal and mechanical energy;[5,6] this enables highly-localized heating or mechanical disruption of carrier vehicles,1[,2,4] and hence rapid release of entrapped payloads. Carrier vehicles for ultrasound drug delivery often contain small gas bubbles; these serve as cavitating bodies that concentrate acoustic pressure waves to facilitate disruption of the carrier vehicles. Unfortunately, such vehicles have limited shelf-lives due to the inherent instability of gas bubbles. A promising alternative is to utilize emulsion drops composed of low boiling point liquid perfluorocarbons (PFCs); these undergo a liquid-to-gas phase transition when insonified.[7,8,9,10,11,12,13,14,15,16] These drops have a longer shelf-life than bubbles, can circulate in blood for hours rather than minutes,[17] and can extravasate through leaky tumor vasculature.[17,18] In addition to imaging applications, these PFC drops can be combined with ultrasound for highly localized delivery of payloads.7[,11] Conventionally, PFC emulsion drops are coated using polymer or lipids; these not only provide stability to the emulsion drops but also allow for drug loading.[19] Payloads can either be dissolved in the emulsion drop or embedded in its coatings. However, due to the poor solubility of hydrophilic agents in both amphiphilic coatings and PFCs, the utility of these emulsion drops as carrier vehicles is restricted to hydrophobic or amphiphilic payloads. To address this limitation, hydrophilic agents are pre-dissolved in water and subsequently emulsified with PFCs through high shear mixing;[20,21,22,23,24] unfortunately, this strategy leads to wide distributions in both loading capacities and drop sizes.

Microfluidic technologies enable the encapsulation of these mixtures into micron-sized droplets with narrow size distributions;[25] however, this strategy still results in uncontrolled loading capacities. These issues severely limit the utility of PFC-based emulsions as acoustically-activated vehicles for controlled delivery of hydrophilic payloads. It is therefore essential to develop an approach for the production of PFC-based emulsions with uniform sizes and controlled loading capacity for hydrophilic agents.

In this work, we report a microfluidic technique for the production of perfluorohexane (PFH)-shelled double emulsion drops with uniform sizes and controlled loading capacities for hydrophilic agents. Using this technique, we efficiently encapsulate a model hydrophilic agent, a colored compound, in the cores of the double emulsions. Moreover, we study the response of these hydrophilic loaded double emulsions to ultrasound irradiation using a combination of optical and acoustic imaging methods; we observe the controlled release of the core resulting from the vaporization of PFH. Our work demonstrates a straightforward and versatile approach for the production of PFH-shelled double emulsion drops with uniform sizes and controlled loading capacity for hydrophilic agents; this further enhances the potential of PFH-based emulsions for acoustically triggered release of payloads.

Experimental

Materials: Perfluorohexane (PFH) is purchased from Strem Chemicals and used without further purification. Krytox-PEG-Krytox is synthesized as described elsewhere,[26] suspended in PFH at 1 wt.% and used as the middle oil phase. Zonyl-FSO 100 from Du Pont is dissolved in de-ionized water (milli-Q) or in a 10 wt.% PVA aqueous solution at a final concentration of 0.1 wt.%; these solutions are used as the inner and outer water phases, respectively. Sulforhodamine

B from Sigma is added to the inner water phase at 1 mg/mL, thereby serving as a model hydrophilic agent.

Acrylamide hydrogel phantoms: To prepare acrylamide hydrogels we use a 40% acrylamide/bis-acrylamide 19:1 solution purchased from Bio-Rad. We mix 2.1 mL of this solution with 1.2 mL of Tris buffer at 1 M, 4.1 mL of de-ionized water, 0.1 mL of ammonium persulfate solution (APS, Sigma) and 12 μL of N,N,N',N'-tetramethylethylenediamine (TEMED, Sigma) in the specific order mentioned. We stir the mixture and pour it into a mold, which is approximately 2 × 2 cm wide and 0.5 cm tall. Immediately after pouring the mixture into the mold, we add an aliquot of the double emulsions into the mixture; these rapidly sink and are immobilized upon gelation of acrylamide at the hydrogel bottom region. We carefully remove the hydrogel from the mold after approximately 1 hour.

Optical imaging: We record the production of double emulsion drops within the microfluidic device using a 4× objective on an inverted microscope (Leica) equipped with a high speed camera (Phantom V9). We monitor the ejection of the colored hydrophilic agent, resulting from the acoustic vaporization of PFH with a color camera (Unibrain) and we visualize the alignment of microparticles and penetration of an external bubble into the emulsion drops using a fast camera (Phantom V7). All our experiments are performed at room temperature. For the images, we place the double emulsion drops between a microscope slide and a micro cover glass (VWR) separated with a thin spacer and sealed using 5 minute® Epoxy (Devcon). The microscope slide is fixed with Epoxy within a small glass container that we use as a water bath to immerse the tip sonicator (Fisher Scientific, Model 120 Sonic Dismembrator) used as the ultrasound source.

Acoustic imaging: We use a Terason 2000 diagnostic ultrasound for acoustic visualization of PFH vaporization as described previously.[17] Briefly, a 2-MHz single-element spherically focused

transducer (aperture of 64 mm, radius of curvature of 63 mm) is affixed to a water tank with dimensions 35 cm length, 28 cm width and 15 cm height. The transducer is utilize as ultrasound source (SonicConcepts, Woodinville, WA, USA), and is driven with sinusoidal waves (Function Generator 33250A, Agilent, Santa Clara, CA, USA in series with a 150W RF amplifier, ENI A150, Rochester, NY). The amplifier output impedance is matched to the transducer impedance through a matching network provided by the transducer manufacturer. We monitor the transducer input signal with an oscilloscope (Waverunner 6050A, Lecroy,Chestnut Ridge, NY). In our experiments, the acrylamide hydrogels are placed in a rectangular acrylic frame with openings on all sides and positioned at the transducer focus using a manually controlled three-axis translation stage.

Results and Discussion

We use a flow-focusing glass capillary device to fabricate water-in-perfluorohexane-in-water (W/PFH/W) double emulsion drops. The device consists of two tapered cylindrical capillaries inserted into opposite ends of a square capillary; this configuration aligns the axes of the cylindrical capillaries. The injection capillary, on the left, is inserted into the collection capillary, on the right, as illustrated schematically in Figure 1(a). To direct the flow of the fluids and controllably emulsify them, it is essential to appropriately modify the capillary surfaces. To accomplish this, the injection capillary is treated with 0.01 vol.% Heptadecafluoro-1,1,2,2-tetrahydrodecyl trichlorosilane (Gelest, Inc.) dissolved in HFE (Novec, 7500); this favors the contact of the PFH with the outer wall of the injection capillary and focuses the PFH flow toward the collection capillary. In addition, the collection capillary is treated with 2-C methoxy(polyethileneeoxy)-propyltrimethoxysilane (Gelest, Inc.) to prevent PFH from wetting

the inner wall of the collection capillary. The inner water phase consists of an aqueous solution of 0.1 wt.%Zonyl-FSO containing 1 mg/mL of sulforhodamine to enable visualization and is flowed through the injection capillary. The middle PFH phase, which contains 1 wt.% of the surfactant, Krytox-PEG-Krytox, is flowed in the same direction through the interstices between the injection and square capillaries. The outer water phase, which consists of a 0.1 wt.% Zonyl-FSO in 20 wt.% poly(vinyl alcohol) (PVA, MW = 13-23 kDa) aqueous solution, is injected through the interstices between the collection and square capillaries, and consequently flows in the opposing direction of the inner and middle phases; this focuses the inner, middle and outer phases at the orifice of the injection capillary, which is slightly inserted into the collection capillary. Using this arrangement, we are able to produce monodisperse W/PFH/W double emulsion drops, as illustrated schematically in Figure 1(a) and shown in the optical microscope images of Figures 1(b-c) and Movie S1 of the Supporting Information. Upon collection in a volumetric excess of 10 - 20 mL deionized water, the double emulsions rapidly sink because of the high density of PFH (1.76 g/mL). These double emulsion drops can be stored in water for several weeks without evidence of rupture or coalescence, due to the high stability provided by the surfactants present in the inner, middle and outer phases.

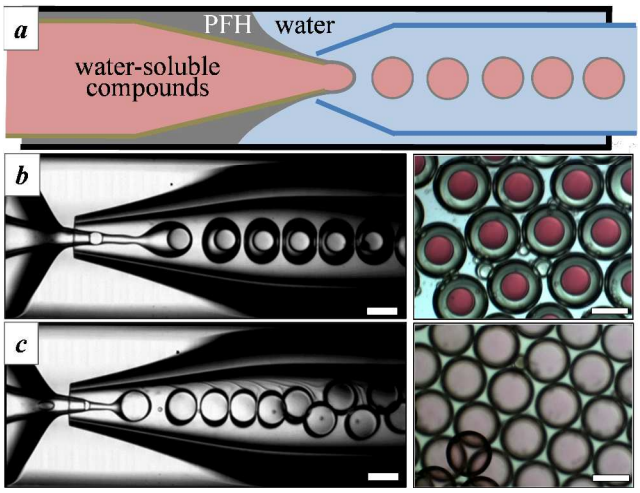

Figure 1. (a) Schematic illustration of the microfluidic device used to fabricate water-in-perfluorohexane-in-water double emulsion drops. (b) Optical microscope images showing the fabrication for typical flow rates of the inner, middle and outer phases of 500, 1000 and 2000 μL/h or (c) 750, 750 and 2000 μL/h, respectively, and the monodispersity of the resultant drops upon collection. Scale bars are 200 μm.

Precise control of the flow rates of the inner, middle and outer phases within the microfluidic device enables us to finely tune the thickness of the PFH shell; this allows us to carefully vary the loading capacity of these double emulsion drops, herein defined as the volume of the inner water core relative to the total volume of the double emulsion drop. For example, using flow rates for the inner, middle and outer phases of 500, 1000 and 2000 μL/h, respectively, we produce thick-shelled double emulsion drops with a loading capacity of approximately 34% as shown in Figure 1(b). Increasing the flow rate of the inner water phase and decreasing the flow rate of the middle phase yields thinner-shelled double emulsion drops, as exemplified in Figure 1(c), for typical flow rates of the inner, middle and outer phases of 750, 750 and 2000 μL/h, respectively. These thin-shelled double emulsion drops have a loading capacity of approximately 84%; this value is over 10% higher than capacities that have been reported previously.[11]

Using this technology, we fabricate double emulsion drops with diameters on the order of several tens of microns; this allows us to easily monitor their response to ultrasound irradiation using optical microscopy. To conduct these studies, we isolate individual double emulsion drops in sealed chambers that contain de-ionized water, and place the chambers in a water bath. We expose these individual double emulsion drops to continuous ultrasound irradiation using a tip sonicator that is immersed in the water bath, while monitoring their response using an inverted microscope, as illustrated schematically in Figure 2(a). The microscope is equipped with a color camera to visualize the color of the sulforhodamine molecules encapsulated within the aqueous cores of the double emulsions; these molecules serve as model hydrophilic agents. Prior to ultrasound excitation, the core of the double emulsion is uniformly colored and localized approximately in the center of the double emulsion drop, as shown in Figure 3(a). Approximately 35s after ultrasound exposure, the red-colored core begins to darken, still remaining approximately in the center of the double emulsion drop as shown in Figure 3(b). The darkening continues after further ultrasound exposure, as shown in Figure 3(c). Immediately after these color changes, we observe the sudden ejection of the red-colored hydrophilic agent from the double emulsion drop into the outer aqueous phase; this is shown in Figure 3(d). The complete process is shown in Movie S2 of the Supporting Information.

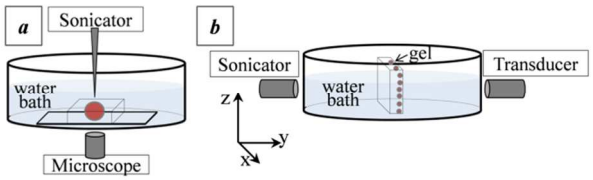

Figure 2. Schematic illustration of (a) the optical-acoustic setup equipped with a tip sonicator and a color or fast camera to optically-observe the response of PFH-shelled double emulsions in

aqueous medium to ultrasound irradiation, and (b) the acoustic setup equipped with a focused ultrasound transducer to insonate and acoustically-observe the vaporization of PFH-shelled double emulsions that are immobilized in a polyacrylamide gel.

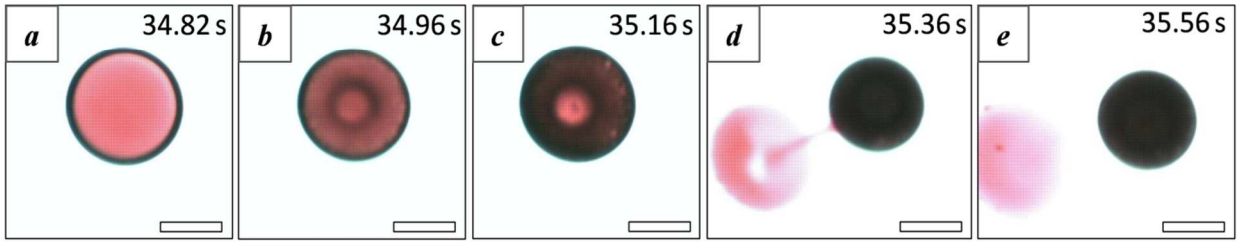

Figure 3. Time series of a single W/PFH/W droplet (r~191μm; t~17μm) isolated in a transparent sample chamber undergoing continuous ultrasound irradiation. The sonicator tip is fixed at a position ~ 1 cm above the sample chamber for the duration of insonation. Scale bars are 200 μm.

We hypothesize that the release of encapsulated material is triggered by the acoustic vaporization of the PFH shell. To evaluate this hypothesis, we measure the radius, r, and shell thickness, t, of the double emulsion drop showed in Figure 3(a), which are approximately 191 and 17 μm, respectively, and use these values to calculate the volume of liquid perfluorohexane in the double emulsion drop. If vaporization does not occur, the liquid perfluorocarbon volume must be preserved in the single emulsion drop following the release of the innermost aqueous core. Therefore, one would expect the resultant drop to have a radius of 119 μm; however, the radius that we measure from Figure 3(e), is approximately 162 μm. This corresponds to a volume expansion of 2.5 times that of the original volume. According to the ideal gas law, one would expect a volume increase of approximately fifteen times that of the original volume, rather than our measured 2.5 increase. A possible explanation for this discrepancy is that vaporization of

PFH is incomplete because part of it is ejected along with the aqueous core. Indeed, we frequently observe additional small drops surrounding the resultant perfluorohexane bubble as shown for example in Figure 4e. In addition, the thermal expansivity of the perfluorohexane may be decreased by the presence of the polymer, PEG-Krytox-PEG, dissolved in the perfluorohexane. Therefore, our results indicate the vaporization of the perfluorocarbon.

To further investigate the mechanism of release through acoustic vaporization, we expose our individual double emulsions to pulsed ultrasound irradiation. We observe the appearance of a bubble within the double emulsion, which forces the innermost aqueous core to move towards one side of the double emulsion; this is accompanied by a change in shape in the double emulsion from circular to elliptical, as shown in Figure 4(a-b). After further ultrasound exposure we observe that the bubble increases its size, as shown in Figure 4(c-d); this likely produces stretching of the surfactant layer adsorbed at the water/perfluorocarbon interface, and thus an increase in interfacial tension, which ultimately results in the coalescence of the innermost aqueous core of the double emulsion with the outer aqueous phase. We also estimate the volume expansion for the example shown in Figure 4, which yields a factor of 3 that is comparable to the estimation performed from the example shown in Figure 3.

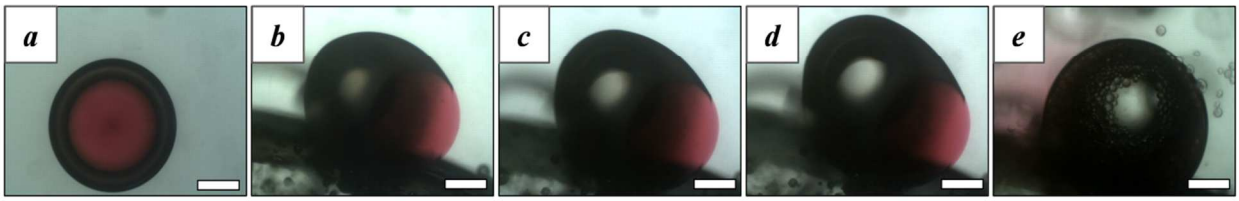

Figure 4. Evolution of a W/PFC/W emulsion droplet (r~300μm, t~20μm) isolated in a transparent sample chamber undergoing pulsed ultrasonic radiation. The sonicator tip is fixed at

a position ~ 1 cm above the sample chamber for the duration of insonation. Scale bars are 200 μm.

To visualize the fluid flow generated in the system upon ultrasound exposure, we add 2 μm polystyrene microparticles to the solution surrounding an isolated double emulsion and equip the optic-acoustic setup, illustrated in Figure 2a, with a high-speed camera to improve the time resolution for image acquisition. Upon sonication, the sound waves cause the outer solution to flow in a process known as acoustic streaming;[27] this makes the microparticles surrounding the double emulsion align around it, as shown in Figure 5(a-b). Surprisingly, about half a second after the start of sonication, a small bubble most likely formed by cavitation, enters the field of view from the top right, while the microparticles continue to align around the double emulsion, as shown by the white region in Figure 5(b). The small bubble accelerates toward the double emulsion, leaving a trail in the microparticles, as shown in Figure 5(c). The small bubble penetrates the double emulsion, as shown in Figure 5(d), likely acting as a nucleation site for the vaporization of the perfluorohexane in the shell of the double emulsion droplet. Bubble incorporation is followed by the sudden growth of the bubble within the double emulsion as shown in Figure 5(e). The complete process is shown in Movie S3 of the Supporting Information.

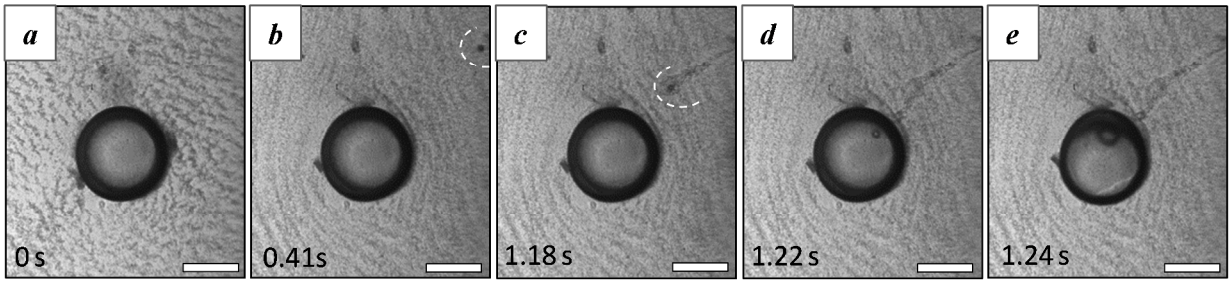

Figure 5. Time series of optical microscope images of (a) an isolated W/PFC/W droplet surrounded by an aqueous dispersion of 1μm polystyrene nanoparticles; these enable visualization of the fluid streams created upon continuous ultrasound irradiation from the sonicator tip positioned ~1cm above the droplet contained within the sample holder. (b-c) Acceleration of an external bubble toward the double emulsion droplet, as highlighted by the white region, (d) incorporation of the bubble into the double emulsion drop and (e) rapid growth of the bubble. Scale bars are 200 μm.

While this series of images provides a mechanism by which the PFH may be vaporized upon sonication, it does not imply that an external bubble is required for vaporization. In fact, several studies have shown that ultrasound-triggered vaporization can be nucleated can be nucleated within a perfluorocarbon droplet.[28,29,30] These studies were conducted with megahertz-frequency pulses, which is more clinically relevant than the frequency transmitted continuously by the tip sonicator. Thus, we investigate vaporization of PFH in the double emulsions with 2-MHz pulses, and monitor vaporization using a diagnostic ultrasound scanner; this setup is illustrated schematically in Figure 2(b). To avoid the formation of a bubble external to the double emulsion, we embed the PFH-shelled double emulsion drops into a degassed, acoustically-transparent hydrogel. Studies have shown that bubbles do not form in the degassed hydrogel at the acoustic pressures tested without the addition of PFC emulsion drops.[31] Vapor PFH is more compressible and more echogenic than liquid PFH and thus appears brighter in a diagnostic ultrasound image. We focus high-amplitude ultrasound pulses into the droplet-filled hydrogels, and we increase the pressure until bright spots with an ellipsoidal shape appear in the diagnostic ultrasound images, as shown in Figure 6(a); this corresponds to the geometry of the transducer focus. In addition, we observe eventual movement of these bright spots as indicated by the arrows in Figure 6(b) and

Movie S4 of the Supporting Information; these results compared to the empty-hydrogels indicate that these observations are due to PFH vaporization. Thus, diagnostic ultrasound imaging confirms that PFH vaporization can occur without the penetration of an external bubble. Furthermore, these observations highlight the utility of using focused ultrasound to precisely control the vaporization of PFH to induce payload release; this enhances the potential of our approach for acoustically trigger and monitor drug release.

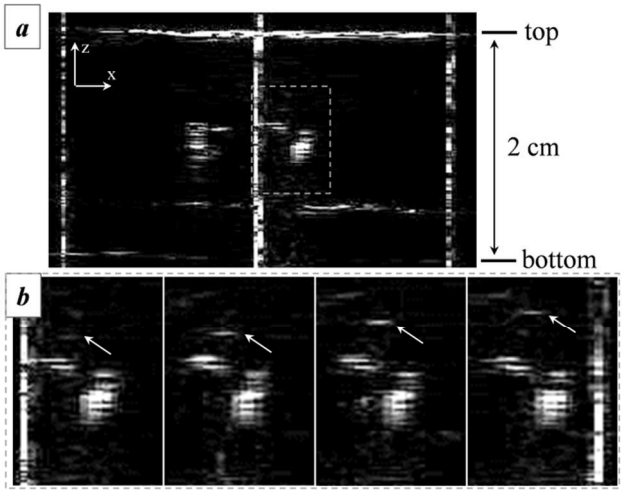

Figure 6 (a) B-mode ultrasound image showing the presence of PFH-shelled double emulsions as bright spots. The dash line encloses the region of interest enlarged below. (b) Sequence of B-mode ultrasound images with increasing amplitude pressure, showing the region of interest during movement of a vaporized drop, as pointed by the white arrows.

Conclusion

We report a microfluidic technique for the production of PFH-shelled double emulsion drops with controlled sizes and loading capacities for hydrophilic agents. Careful tuning of the flow rates of the fluids within the microfluidic device results in the formation of double emulsion drops with very large aqueous cores and thus high loading capacities for hydrophilic agents. We show that hydrophilic agents can be released from the double emulsion drops using ultrasound as

an external trigger; this controlled release results from vaporization of encapsulated perfluorohexane. These results demonstrate the potential of PFC-based double emulsions as a powerful tool for image-guided acoustically-triggered drug delivery.

ASSOCIATED CONTENT

Supporting Information. Microfluidic fabrication of perfluorohexane-shelled double emulsion drops, release of hydrophilic agents from an isolated drop, penetration of an external bubble into an isolated drop and proof of perfluorohexane vaporization is shown in Movies S1, S2, S3 and S4, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.‡These authors contributed equally.

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