Microfabrication

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Controllable Monodisperse Multiple Emulsions**

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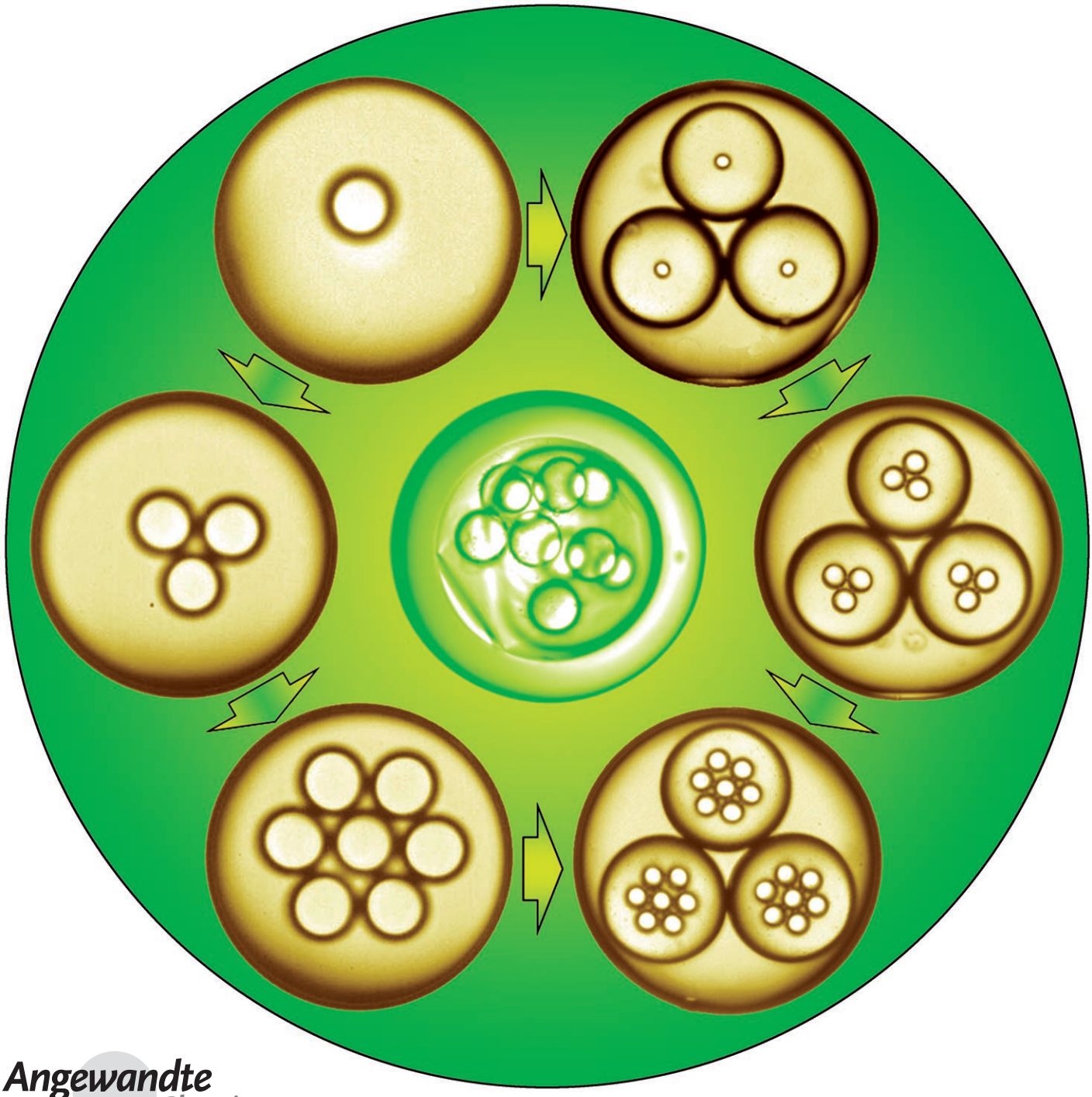

Multiple emulsions, or "emulsions of emulsions," are complex systems in which dispersed droplets contain smaller droplets inside. They are widely used to encapsulate active ingredients in myriad applications, including drug delivery,[1–3] foods,[4,5] cosmetics,[6,7] chemical separations,[8,9] and syntheses of microspheres and microcapsules.[10–16] Monodispersity of size and accurate control of internal structure are critical for the versatility of such emulsions, because these attributes allow precise manipulation of the loading levels and the release and transport kinetics of the encapsulated substances.[10–21] Although there have been several reports on the preparation of monodisperse multiple emulsions,[10–13,17–23] concurrent and precise control over both the size and structure of the emulsions remains difficult to achieve, and current techniques are not scalable to higher-order multiple emulsions. Herein, we describe the fabrication of highly monodisperse multiple emulsions using capillary microfluidics to independently control both the size and the number of inner droplets. Importantly, our technique is easily scalable to higher-order multiple emulsions, which we demonstrate by making monodisperse, controllable triple emulsions. We illustrate the utility of the tight control afforded by this technique by fabricating a novel hydrogel microcapsule from a triple emulsion, and we demonstrate its use for simultaneous controlled release of two-phase (oil and aqueous phases) substances into the environment.

Multiple emulsions are typically formed through sequential bulk emulsification using shear, which usually results in polydisperse emulsions. Controlling the shear with constrained geometry,[24–28] porous membranes,[17,18] or microchannels[19] can produce emulsions that are nearly monodisperse; these can then themselves be emulsified to create multiple emulsions. However, although the volume fractions of both the initial and final emulsions can be controlled,[17–19,24,26–28] precise control over the number of inner droplets is difficult to attain with these techniques. There are, however, many cases where control of the number of inner droplets is more important than control of the volume fraction. For example, with accurate control of the number and size of inner droplets, transport kinetics of encapsulated substances can be precisely manipulated. Control of the number of inner droplets is also critical for structuring colloidal assemblies to produce nonspherical particles.[29] Furthermore, if the inner droplets are used as separate compartments for cell culture or evolution in the same large droplet, control of the number of inner droplets is essential.

Microfluidic devices offer an alternate route to produce monodisperse multiple emulsions. Cascading two T-junctions[20,22] can produce monodisperse double emulsions with some control over the number and size of the inner droplets; however, the standard two-dimensional microfluidic channels require precise, localized modification of the surface chemistry to control the wettability to enable the production of multiple emulsions, which ultimately limits the utility of such channels. By contrast, coaxial flow-focusing[10,12,13] geometries relax the wetting constraints but are still limited in the range of fluids that can be used and in the precise control afforded over the number and size of inner droplets. Moreover, current microfluidic methods cannot be scaled to make higher-order multiple emulsions, such as triple emulsions.

To overcome these limitations, we present a highly scalable microcapillary technique that simultaneously controls the droplet monodispersity as well as the number and size of the inner droplets (see the Supporting Information for experimental details). The first emulsification step is accomplished using a coaxial, co-flow geometry. It comprises the injection tube (a cylindrical capillary with a tapered end), which is inserted into the transition tube (a second cylindrical capillary with an inner diameter D_2), as shown in Figure 1a. Both cylindrical capillary tubes are centered within a larger square capillary; alignment is ensured by matching the outer diameters of the cylindrical tubes to the inner dimensions of the square ones. The other end of the transition tube is also tapered and is inserted into a third, coaxially aligned cylindrical capillary tube, the collection tube, of inner diameter D_3. We generate uniform monodisperse multiple emulsions in two emulsification steps. Droplets of the innermost fluid are emulsified in the first stage of the device by coaxial flow of the middle fluid (Figure 1b). The single emulsion is subsequently emulsified in the second stage through coaxial flow of the outermost fluid, which is injected in the outer stream through the square capillary (Figure 1a). In both emulsification steps, droplets form immediately at the exit of the tapered capillary (Figures 1b and c); this dripping mechanism[30] ensures formation of highly monodisperse drops. The separation of the two emulsification steps affords independent control over each; we achieve this control by adjusting the device dimensions as well as the inner, middle, and outer fluid flow rates (Q_1, Q_2, and Q_3, respectively). The

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

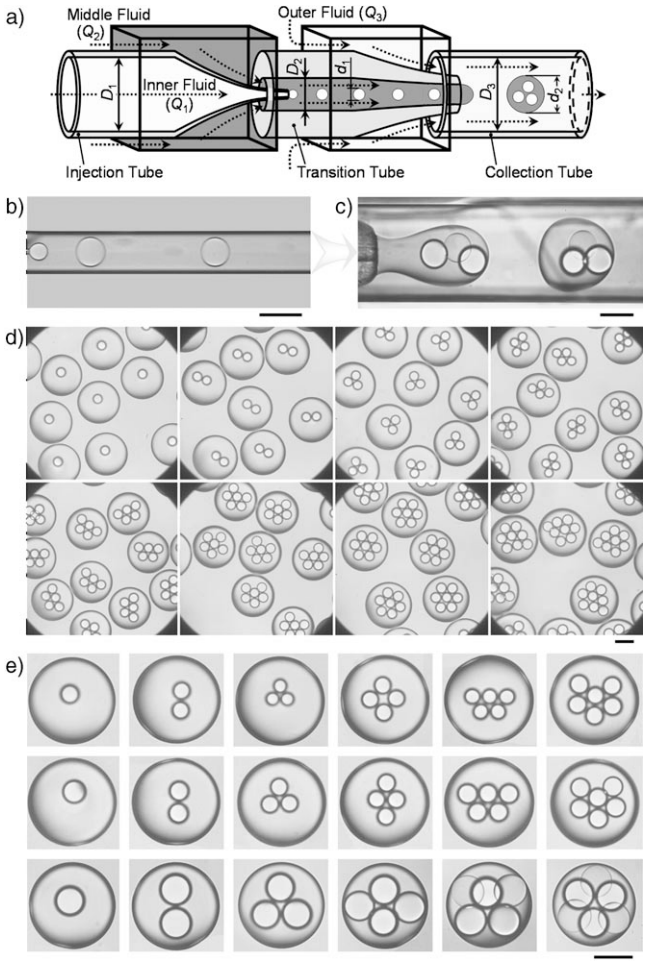

Figure 1. Capillary microfluidic device and the formation of precisely controlled monodisperse double emulsions. a) Schematic diagram of the device geometry. The outer fluid must be immiscible with the middle fluid and the middle fluid must be immiscible with the inner fluid. b) and c) High-speed optical micrographs of the first (b) and second (c) emulsification stages. d) Optical micrographs of monodisperse double emulsions containing a controlled number of monodisperse single emulsions. e) Optical micrographs of monodisperse double emulsions showing controlled increase of the diameter of the inner droplets while the number is constant. All double emulsions were made in the same device and with the same fluids. The flow rates of the inner, middle, and outer fluids in (b) and (c) are $Q_1 = 350$, $Q_2 = 2000$, and $Q_3 = 5000\ \mu L\,h^{-1}$, respectively. In (d), the flow rates of middle and outer fluids are fixed at $Q_2 = 2000$ and $Q_3 = 5000\ \mu L\,h^{-1}$, and those of the inner fluid are $Q_1 = 20$, 55, 70, 85, 150, 200, 225, and 240 $\mu L\,h^{-1}$, from the left top to the right bottom. In (e), the variation ranges for the flow rates of the inner, middle, and outer fluids are $Q_1 \approx 20$–600, $Q_2 \approx 1600$–5000, and $Q_3 \approx 2000$–8000 $\mu L\,h^{-1}$ (in each case, Q_3 is larger than Q_2). All scale bars are 200 μm.

number of innermost droplets can be precisely controlled, as illustrated by the double emulsions containing one to eight inner droplets (Figure 1d). Similarly, the size of the innermost droplets can be precisely controlled, as shown by the three sets of double emulsions with different inner droplet sizes in Figure 1e. In each case, all droplets are highly monodisperse. The coefficient of variation (CV, defined as the ratio of the standard deviation of the size distribution to its arithmetic mean) is used to characterize the size monodispersity of emulsion droplets. In all experiments, the CV values for diameters of internal droplets and for double emulsions are less than 2.3% and 1.6%, respectively. Furthermore, the coaxial structure of this capillary microfluidic device has the advantage that no surface modification of wettability is necessary, allowing the same device to be used to prepare either water-in-oil-in-water (W/O/W) or the inverse oil-in-water-in-oil (O/W/O) multiple emulsions.

The high degree of control and stability afforded by the dripping mechanism allows us to quantitatively determine the flow-rate dependence of the diameters of the inner and outer drops (d_1 and d_2, respectively) and to predict the number of inner droplets N_1. For fixed device dimensions and solution conditions, the droplet diameter in coaxial flow is inversely proportional to the velocity of the surrounding flow[30] in the dripping regime. We calibrate the dependence of d_1/D_2 on Q_1/Q_2 and find the linear dependence depicted in Figure 2a. We find a similar linear dependence of d_2/D_3 on $(Q_1+Q_2)/Q_3$ (Figure 2b). Using these empirical relations, we can quantitatively predict the number of inner droplets as $N_1 = f_1/f_2$, where f_1 and f_2 are the formation rates of the inner and outer droplets. From mass conservation for each stream [Eq. (1)],

$$N_1 = \frac{f_1}{f_2} = \frac{Q_1/(\pi d_1^3/6)}{(Q_1+Q_2)/(\pi d_2^3/6)} = \frac{Q_1}{Q_1+Q_2}\frac{d_2^3}{d_1^3} \qquad (1)$$

using the linear fits to the experimental data in Figures 2a and b, we can predict the number of encapsulated droplets as a function of the flow rates [Eq. (2)], where a_1 and a_2 are the

$$N_1 = \frac{Q_1}{Q_1+Q_2}\frac{D_3^3}{D_2^3}\left(\frac{a_2\,(Q_1+Q_2)/Q_3 + b_2}{a_2\,(Q_1/Q_2) + b_1}\right)^3 \qquad (2)$$

slopes, and b_1 and b_2 are the intercepts obtained from the fits in Figures 2a and b. The experimentally measured N_1 values are well-described by Equation (2), with no free parameters, as shown by the solid line in Figure 2c; this correlation allows easy calibration of our device. When the number predicted is an integer, N_1 can be precisely controlled; however, when the number predicted is between two integers, the operation is in a transition zone, and N_1 takes either integral value. However, precise control over N_1 can be achieved through correct adjustment of the flow rates.

This method for fabricating multiple emulsions has the significant advantage that it can be very easily extended, thus enabling us to generate additional hierarchical levels of multiple emulsions. We illustrate this concept by fabricating monodisperse triple emulsions, which consist of water-in-oil-in-water-in-oil (W/O/W/O) drops. This preparation is accomplished by adding a second transition tube at the outlet of the first and injecting the outermost fluid to flow coaxially around this tube to form the third level of emulsification at its outlet, as shown in Figure 3a. The individual steps of drop formation leading to the triple emulsions are shown in Figures 3b–d. Although there are large deformations of the droplets during their formation as they flow through the tapered regions of the capillaries, the emulsification process remains stable. Again, both the diameter and the number of the individual drops at every level can be precisely controlled, as illustrated

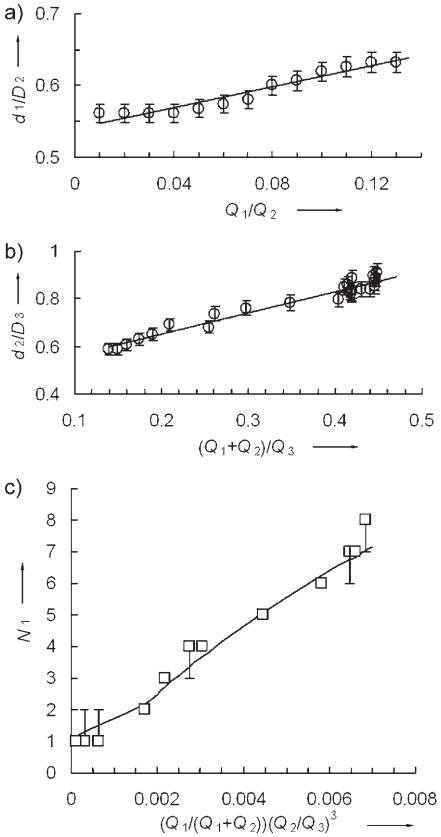

Figure 2. Effect of the relative flow rates on drop diameter for both the inner and outer drops and on the number of encapsulated droplets in the double emulsions. a) Scaled drop diameter d_1/D_2 as a function of Q_1/Q_2. The solid line is a linear fit with slope $a_1 = 0.72$ and intercept $b_1 = 0.54$. b) Scaled double emulsion drop diameter d_2/D_3 as a function of $(Q_1+Q_2)/Q_3$. The solid line is a linear fit with slope $a_2 = 0.88$ and intercept $b_2 = 0.48$. c) Experimentally measured number of encapsulated droplets in each double emulsion drop (□) as a function of the flow rates. We note that d_1 is inversely proportional to Q_2 and d_2 is inversely proportional to Q_3; thus, rather than using the expression in Equation (1), we plot the data as a function solely of flow rates. Equation (2) is used to predict the number of encapsulated drops (——). We use the slopes and intercepts from (a) and (b) in Equation (2) for this calculation. The variation ranges for the flow rates of the inner, middle, and outer fluids are 20–260, 2000, and 5000–15000 μL h^{-1}, respectively.

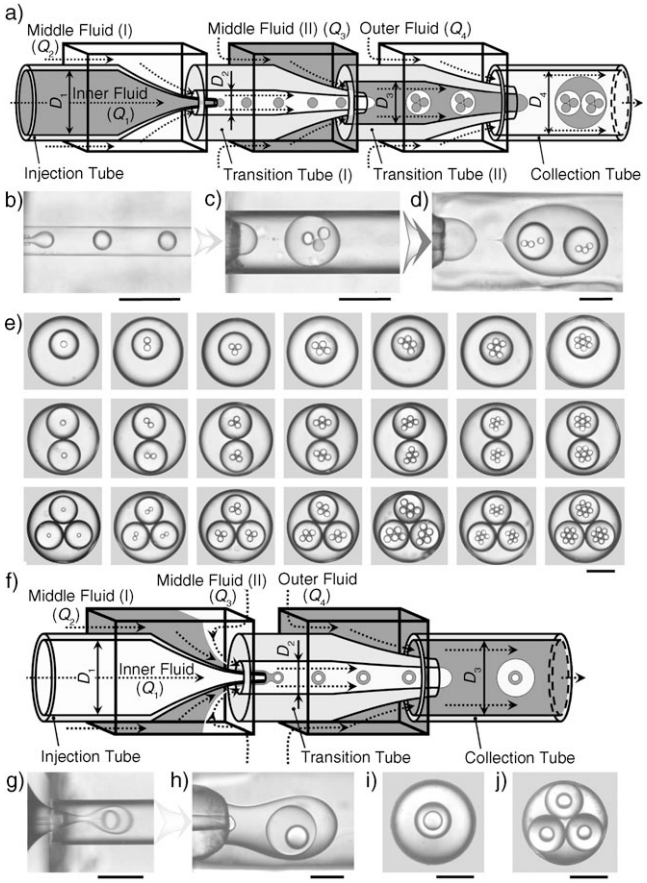

Figure 3. Generation of highly controlled monodisperse triple emulsions. a) Schematic diagram of the extended capillary microfluidic device for generating triple emulsions. b)–d) High-speed optical micrographs displaying the first (b), second (c), and third (d) emulsification stages. The flow rates of the inner, middle (I), middle (II), and outer fluids in (b)–(d) are $Q_1 = 50$, $Q_2 = 500$, $Q_3 = 2000$, and $Q_4 = 5000$ μL h^{-1}. e) Optical micrographs of triple emulsions that contain a controlled number of inner and middle droplets. The variation ranges for the flow rates are $Q_1 \approx 5$–100, $Q_2 \approx 200$–1000, $Q_3 \approx 2000$–3500, and $Q_4 = 5000$ μL h^{-1}. f) Schematic diagram detailing an alternate method for generating triple emulsions where the middle fluid (II) is injected from the entry side of the first square tube, leading to flow-focusing of the first middle fluid into the transition capillary. g) and h) High-speed optical micrographs showing the formation of double emulsions in a one-step process in the transition capillary (g) and the subsequent formation of triple emulsions in the collection capillary (h). i) and j) Optical micrographs of triple emulsions that contain a different number of double emulsions. The variation ranges for the flow rates in (g)–(j) are $Q_1 = 50$–200, $Q_2 = 1600$–2500, $Q_3 = 4000$–8000, and $Q_4 = 5000$–12000 μL h^{-1}. The scale bar in all images is 200 μm.

by the series of triple emulsions with one to seven innermost drops and one to three middle drops in each outer drop (Figure 3e). In all experiments, the CV of the diameters of triple emulsions is less than 1.5%. Furthermore, the technique can clearly be sequentially scaled to even higher levels of emulsification if desired; for example, quadruple emulsions could be made by adding an additional stage.

The simplicity of this method also enables us to incorporate alternate emulsification schemes. For example, we can inject a second fluid at the inlet of the transition tube to create double emulsions by flow-focusing of a coaxial stream[10] with a subsequent emulsification step at the outlet of the transition tube to create triple emulsions, as shown in Figure 3f. While only two drop-formation steps are required (Figures 3g and h), the ability to independently control the number and size of innermost drops is reduced. Nevertheless, highly monodisperse triple emulsions can be fabricated, albeit with only a single innermost droplet (Figures 3i and j).

The ability to precisely control the formation of multiple emulsions offers new opportunities to engineer novel materials. To illustrate this potential, we use the triple emulsion structure to fabricate a microcapsule made with a thermosensitive, poly(*N*-isopropylacrylamide) hydrogel that can be used for controlled release of active substances. Since each fluid layer is sandwiched between two immiscible fluids, we are able to perform a polymerization reaction in a specific

layer. In this case, we form a W/O/W/O triple emulsion and add monomer, crosslinker, and initiator in the outer aqueous layer. We also add an accelerator to the inner oil phase, where it diffuses into the outer aqueous shell and speeds polymerization of the hydrogel. The result is a microcapsule consisting of a shell of thermosensitive hydrogel that encapsulates an oil drop containing several water droplets, all in a continuous oil phase, as shown in Figure 4a. Upon heating from 25° to 50 °C, the thermosensitive hydrogel rapidly shrinks by expelling water; however, because of the incompressibility of the inner oil, the hydrogel shell breaks, providing spontaneous, pulsed release of the innermost water droplets into the continuous oil phase, as shown in Figures 4b–e. This structure has a Trojan-horse-like behavior, protecting the innermost water droplets in the hydrogel shell until their temperature-induced release. This experiment demonstrates the utility of our technique to generate highly controlled capsules with multiple internal volumes that remain separate from each other; it also highlights the potential of this microfluidic device to create highly engineered structures for controlled release of active substances. Further refinements could adjust the thickness of the layers and the number of droplets, thus enabling fine control over diffusion of compounds contained within the innermost droplets, which would facilitate their highly controlled release.

The high degree of control and scalability afforded by this method makes it a flexible and promising route for engineering highly controlled multiple emulsions and microcapsules. The approach presented herein circumvents the difficulties in controllably forming multiphase structures. It can be used to optimize multiple emulsion systems for a broad range of applications in pharmaceutics, foods, cosmetics, and separations. Moreover, its generality will enable fabrication of novel materials containing complex internal structures. Future work must focus on refining methods proposed herein to allow the full potential of the technique to be realized. Moreover, applications of these devices to technological applications would require operating a large number of devices in parallel; this feat might best be accomplished through other fabrication techniques. Nevertheless, these results provide an essential guide to assess the utility of the structures that can be produced.

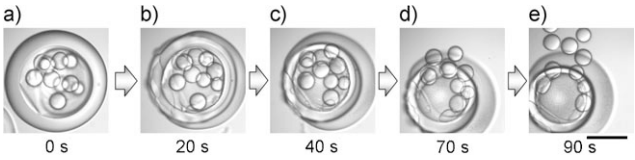

Figure 4. Temperature-sensitive hydrogel microcapsule for pulsed release. a) Optical micrograph of a microcapsule with a shell comprised of a thermosensitive hydrogel containing aqueous droplets dispersed in oil. Upon increase of the temperature, the hydrogel shell shrinks by expelling water. This capsule was generated from a triple emulsion, where the continuous liquid is oil, the hydrogel shell is aqueous, the inner middle fluid is also oil, and the innermost droplets are aqueous. b)–e) Optical micrograph time series showing the forced expulsion of the oil and water droplets contained within the microcapsule when the temperature is rapidly increased from 25 to 50 °C. The time series begins once the temperature reaches 50 °C. The extra layer surrounding the microcapsule in (b)–(e) is water that is squeezed out from the hydrogel shell as it shrinks. The coalescence of the expelled inner oil with the outer oil can not be resolved, since both liquids have the same index of refraction. The scale bar is 200 μm.

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