

Timing controllable electrofusion device for aqueous droplet-based microreactors†

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This paper describes an electrofusion device for controlling the precise moment of fusion between droplets by applying an electric field. This device allows (i) accurate determination of the start of chemical/biological reactions, (ii) minimum contact of reactants with channel walls—eliminating surface absorption problems, (iii) easy fabrication and (iv) continuous observation of initiated reaction. We demonstrated the fusion of β -galactosidase and fluorescein di- β -D-galactopyranoside (FDG) droplets, and observed the enzymatic reaction using fluorescence microscopy. In addition, sequential fusion of pico-litre droplets was also accomplished.

1. Introduction

Small vessels such as liposomes,^{1,2} microchambers,^{3,4} and oil-dispersed droplets^{5,6} have been used as microreactors for detection and observation of chemical/biological reactions where only a small number of molecules is concerned. In these vessels, there are no problems of dilution. This is an attractive attribute for reactions where product molecules are formed, as the concentration of these molecules can change dramatically, facilitating observation and detection.

Among the methods proposed, the method employing oil-dispersed droplets remains very promising and represents an active area of research.^{7,8} Monodisperse droplets ranging from hundreds to several micrometres can be easily generated in microchannels,^{9–12} allowing us to work with nano, pico and even femto litres. One of the advantages of oil-dispersed droplets is that the encapsulation of biomolecules such as DNA and proteins in the droplets isolates these molecules from channel walls and substrate, avoiding the problem of non-specific binding.¹³

The work described here is motivated by the need for simple devices for the formation, manipulation and fusion of droplets for down-scaled analytical techniques. One problem when handling small droplets is the difficulty of adding reactants and determining the exact start of reactions. In the preparation of aqueous droplets in microchannels, surfactants are frequently added to stabilize the emulsion. Surfactants are compounds that are amphipathic; they contain both hydrophobic and hydrophilic groups. These surfactant molecules tend to absorb to the interfaces of the droplets, allowing metastable colloids to be formed. However, this

treatment makes it difficult to mix different types of reactants in droplets.

Here, we extend the use of electrofusion to add reactants to droplets. Electrofusion was discovered during the study of electrophysiology of plant protoplasts,¹⁴ and has been used to fuse cells^{15–18} and liposomes.¹⁶ Briefly, when an electric field is applied, the membrane (cell membrane, liposome, *etc.*) destabilizes and pores are formed (electroporation). When two destabilized membranes come into contact, they fuse. We found that this technique for fusion is not limited only to membranes, but application of an electric field can also cause otherwise stable droplets in the presence of surfactants to fuse. The electric field has to be applied parallel to the axis of contacting droplets in order to initiate fusion. This phenomenon only occurs for aqueous droplets in oil and not oil droplets in water. Using this technique, we have designed an electrofusion device for aqueous droplets in microchannels. Two types of experiment, namely, fusion of dynamic and static droplets were performed with this device. We believe that this approach using electrofusion allows the exact start of chemical/biological reactions to be accurately determined. The process is achieved under minimal wall contact as water droplets in an oil medium are almost spherical. This simple device is realized without complicated fabrication and multiple-electrodes integration. Size of droplets, and hence the volume of reactants to be added, are determined by channel geometry and flow conditions.

2. Concept, design and operation

Expanding channel and fusion chamber

In order to fuse the droplets, they have to be in contact with each other when the electric field is applied. Tan *et al.*¹⁹ demonstrated droplet fusion in a passive device using flow rectifying design to coalesce droplets. Their device used an expanding channel to bring droplets together. As surfactant was not used, droplets fused instantaneously upon contact with each other. Here, we present a simple analysis of the contact criterion for droplets in an expanding channel junction. In addition, we will show how this design is modified

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† Electronic supplementary information (ESI) available: Three video clips showing: device operating under continuous flow without and with application of voltage (playback speed for operation without and with application of voltage is 0.3 \times and 0.5 \times of the original clip, respectively); high speed camera video of fusion of water droplets and droplets containing fluorescent beads. See DOI: 10.1039/b517178d

to bring moving droplets into contact and simultaneously align them in parallel to the applied electric field.

Fig. 1(a) shows the schematic of aqueous droplets in organic phase in an expanding channel. Droplets in the narrow and wide channels are assumed to be transported at mean velocities $u_{m,n}$ and $u_{m,w}$, respectively.

$$u_{m,n} = \frac{Q}{w_n d} \text{ and } u_{m,w} = \frac{Q}{w_w d}, \quad (1)$$

where Q , w_n , w_w and d are respectively the total rate of flow of organic and aqueous phases, the width of the narrow channel, the width of the wide channel and the depth of the channel.

Let t be the time taken for droplet 1 to travel a distance, i , i.e. ,

$$t = \frac{i}{u_{m,n}}, \quad (2)$$

where i is the interval between droplet 1 and droplet 2.

In the same time period, t , droplet 2 will travel a distance L , where,

$$L = u_{m,w} t = \frac{u_{m,w} i}{u_{m,n}}. \quad (3)$$

In order for contact between droplet 1 and droplet 2,

$$L \leq \frac{\phi_1 + \phi_2}{2}, \quad (4)$$

or

$$i \leq \frac{(\phi_1 + \phi_2)}{2} \frac{u_{m,n}}{u_{m,w}}, \quad (5)$$

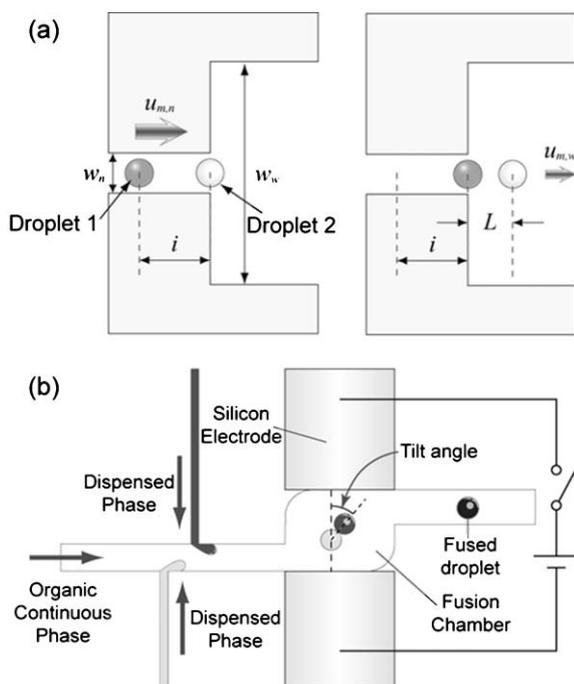


Fig. 1 Schematic diagrams of (a) droplets in an expanding channel, and (b) electrofusion device. Widening of the chamber brings droplets into contact. Unique shape of the chamber aligns the droplets in parallel to the electrodes.

where ϕ_1 and ϕ_2 are the diameters of droplet 1 and droplet 2, respectively.

Using eqn (1), we arrive at the final expression,

$$i \leq \frac{(\phi_1 + \phi_2)}{2} \frac{w_w}{w_n}. \quad (6)$$

Two droplets separated by an interval that satisfies eqn (6) will come into contact when they enter the wider channel in an expanding channel junction. In a device, the geometry and hence the width ratio, w_w/w_n , is fixed. Diameters of the droplets can be changed within a certain range by varying flow conditions. However, the most direct way to control contact of the droplets is by adjusting the interval between droplets. This can be realized by careful manipulation of syringe pumps during experiments.

In our early experiments, we observed that the electric field for fusion was lowest if the axis of the contacting droplets was parallel to the field (Fig. 1(b)). We postulate that this is because the droplets' contact point coincided with local maximum of the electric field due to the polarization of droplets. While fusion occurred when the axis was tilted ($<90^\circ$) with respect to the applied field, a stronger field was required to obtain the same local electric field to achieve fusion. This is analogous to the electrofusion of cells.^{17,18} Tilting of the axis of contacting droplets was achieved by modifying the shape of the fusion chamber. The resulting "stomach-shaped" fusion chamber serves two purposes: (i) to bring droplets that are formed within a certain distance into contact, and (ii) to align droplets to the electric field for fusion. The tilt angle is a function of the width ratio, w_w/w_n . Larger w_w/w_n results in smaller tilt angle and hence fusion can be initiated with a weaker electric field. Increasing w_w/w_n has two other consequences: (i) higher voltage is required to produce the same local electric field, and (ii) the interval, i , within which droplets will come into contact in the fusion chamber increases (eqn (6)). These two consequences set a practical limit to how much the tilt angle can be varied. As a result, our final design is a compromise between tilt angle, applied voltage and interval between droplets.

Electrodes

Tresset and Takeuchi¹⁶ reported that liposomes were aligned using two dimensional gold-evaporated electrodes, but electrofusion was not successful as the induced electric field was not uniform. Theoretically, flat electrodes should be able to induce electrofusion. However, due to non-uniformity of the electric field, the field will be substantially weakened at the plane at which the droplets contact one another. In order to produce an electric field that is strong enough to initiate fusion, it will require high operating voltages. Here, we adopted the same approach as Tresset and Takeuchi¹⁶ to create a uniform electric field by means of high aspect-ratio electrodes formed from silicon.

Operation

In this paper, we performed two types of experiments, namely fusion of dynamic and static droplets. Fig. 1(b) shows the schematic of the electrofusion device. Except for the

differences in channel sizes, devices used for fusion of both dynamic and static droplets were similar in design. Surfactant was added to the organic phase to prevent undesired coalescence of droplets upon contact. Fusion initiated by application of electric field allows the exact start of reactions to be accurately controlled with this device. Unlike microdroplets in open air, there is no problem with evaporation and contamination.

Dynamic droplets. In a typical run, aqueous droplets containing different types of reagent were formed at separate T-junctions and transported downstream into the fusion chamber by the continuous phase. The brief pausing of one of the pumps controlling the dispensed phase resulted in the delay of droplet formation for that reagent, changing the interval between droplets of one reagent with respect to another. This was repeated until the droplets came into contact in the fusion chamber. For fusion of dynamic droplets, *i.e.*, moving droplets, 5 pulses of DC voltage signal (50–200 V, 10 μ s) were applied at an interval of 0.2 s.

Static droplets. For continuous monitoring of the reaction brought about by droplet fusion, we performed electrofusion of static droplets in the range of nano and pico-litres. For electrofusion of static droplets in the nano-litre range (ϕ 100–250 μ m), the operation is similar to that of dynamic droplets except that the flow was stopped after the droplets came into contact in the fusion chamber. A single pulse of DC voltage (50 V, 10 μ s) was then applied. To form pico-litre droplets (ϕ 10–30 μ m), devices with smaller channels were used. The pico-litre droplets were formed by slowly introducing the dispensed phase into the main flow. Once the droplets were formed at the T-junction, the pump for the dispensed phase was immediately stopped and put into withdrawal mode for a short period of time to eliminate the residual pressure. After the formed droplets were carried into the fusion chamber by the continuous flow, the pump controlling the continuous flow was stopped. The droplets were then manipulated, brought into contact, and aligned using laser tweezers. A single pulse of DC voltage (200 V, 10 μ s) was then applied. As laser tweezers were too weak to effectively manipulate micro-litre droplets, it was not used for the nano-litre droplet experiments.

3. Experimental

Device fabrication

A summary of the fabrication process is presented in Fig. 2(a). Silicon electrodes were formed by anodic-bonding silicon to Pyrex glass and then etched by DRIE with aluminium layer as mask. A 200 μ m thick silicon wafer measuring about 15 mm by 30 mm (single side mirror) and a 500 μ m thick Pyrex glass measuring 26 mm by 56 mm were cleaned by Piranha solution prior to bonding. Anodic bonding was carried out at 500 $^{\circ}$ C at 1000 V. Subsequently, a thin film of aluminium was deposited on the silicon using SANYU Evaporation system (SVC-700TURBO-TM). 3 μ m thick S1818 (Shipley) photoresist was spin-coated onto the silicon wafer and patterned using standard lithography methods (Double-side mask aligner, PEM-800, Union Optical Co. Ltd.). Photoresist S1818 was

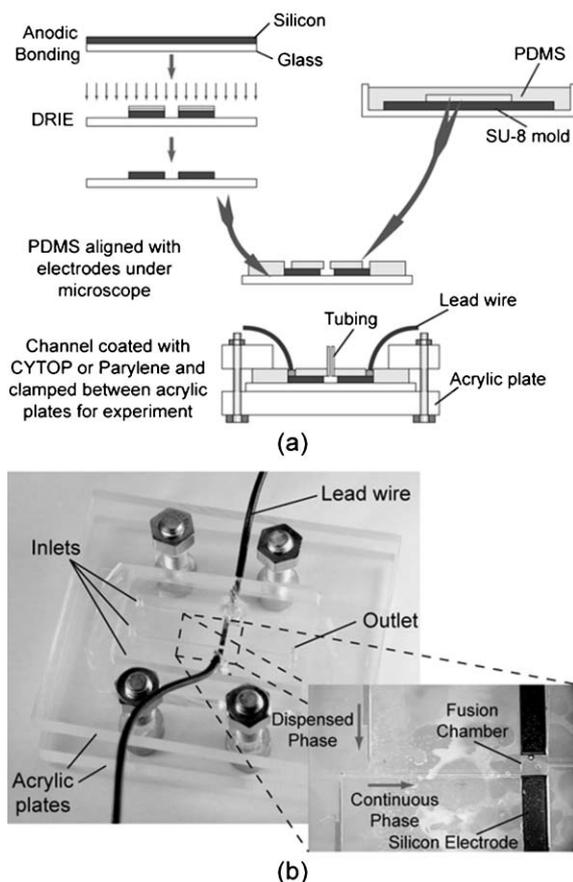


Fig. 2 (a) Process flow, and (b) electrofusion device.

developed using TMAH2 38% (NMD-3, Tokyo-Ohka). Aluminium was then etched using aluminum etchant (Wako Pure Chemical Industries, Ltd.) with S1818 photoresist as mask. This was followed by DRIE of silicon wafer to form the electrodes. The process described above allowed high aspect-ratio electrodes to be fabricated without resorting to cumbersome procedures like electroplating.

Flow channels were formed with PDMS (Sylgard 184 silicone elastomer, Dow Corning) using SU-8 (MicroChem) masters. Access holes were punched and the PDMS slab was subsequently aligned with the silicon electrodes under a microscope. Lead wires were connected to the silicon electrodes using Dotite[®] conductive epoxy adhesive (Fujikura Kasei Co., Ltd.). Next, the device was baked at 65 $^{\circ}$ C for 2 h in an oven to harden the epoxy. Channels were coated with CYTOP[®] (Asahi Glass Co., Ltd.) by introducing the solution into the channels using a syringe. Excess CYTOP[®] solution was removed from the channels by gently blowing with an air gun through the access holes. The device was then baked for another 2 h at 65 $^{\circ}$ C in an oven.‡

‡ The device was not baked at higher temperatures as we observed cracking of Pyrex glass substrate at higher temperatures (>95 $^{\circ}$ C). This is due to a mismatch in thermal expansion coefficients between Pyrex glass and PDMS, leading to bending and cracking of the Pyrex glass. We found that this failure mode can be avoided by simply attaching another PDMS slab on the other side of the glass substrate during baking to counter the bending stress.

Alternatively, channels can be coated with Parylene N with SCS Parylene Deposition System. Coating of channels was to prevent swelling of PDMS by organic solvents such as hexadecane and mineral oil. CYTOP[®]-coated channels could be used for the duration of the experiments (~1 h) without significant swelling. On the other hand, Parylene-coated channels could be used for more than 24 h without any observable swelling. As the PDMS slab was not permanently bonded to the glass substrate, the device was sandwiched between two acrylic plates (Fig. 2(b)) to prevent leakage. Unless otherwise specified, dispensed phase and continuous phase channels were 100 μm and 250 μm wide, respectively. The fusion chamber measured 1000 μm by 750 μm and channels had a depth of 200 μm .

Chemicals

n-Hexadecane were purchased from Wako Pure Chemical Industries, Ltd.. Span 80 was purchased from Kanto Chemical Co., Inc. β -Galactosidase and di- β -D-galactopyranoside (FDG) was obtained from Sigma-Aldrich, Inc. Blue ink was purchased from Pilot Corporation. Ultra pure water having a specific resistance of 18 M Ω cm was used for all experiments.

Equipment

Syringe pumps (KDS 200, KD Scientific and Micro 4, World Precision Instruments) were used to control the flow rate of all phases. Both organic and aqueous phases were infused into the device using 100 or 250 μL Hamilton Gastight syringes (1700 series, TLL). The continuous phase was n-hexadecane with Span 80 (4 wt%). Blue ink diluted with ultra pure water, fluorescent beads suspended in water, β -galactosidase or FDG were used as the dispensed phase. Video images were taken with Keyence digital microscope (VH-5000C). High speed images were captured with Photron or Phantom high speed camera mounted to an inverted microscope (Eclipse, TE300, Nikon). Electric pulses were applied with an Electro Cell Fusion unit (LF101, Nepa Gene). Laser tweezers (Micro Manipulation System, Sigma Koki) were also used to manipulate droplets.

4. Results and discussion

Electrofusion and device operation

When an electric field is applied parallel to the axis of contacting aqueous droplets in an organic phase, the droplets fuse. Organic phase has a lower dielectric constant than that of aqueous phase. The dielectric constant of n-hexadecane and water is around 2 and 80, respectively. Consequently, electric flux density in aqueous droplets is higher than that in organic phase. We postulate that this concentration of electric field disrupts surfactant molecules at droplet interfaces, causing them to coalesce. This explanation is consistent with the observation that electrofusion only occurs for aqueous droplets in oil and not oil droplets in water. For the case of oil droplets in water, the electric flux density is lower in the oil droplets compared to the external aqueous phase. As a result, the electric field in the oil droplets is not able to initiate fusion. In our test experiments, electrical discharge occurred between

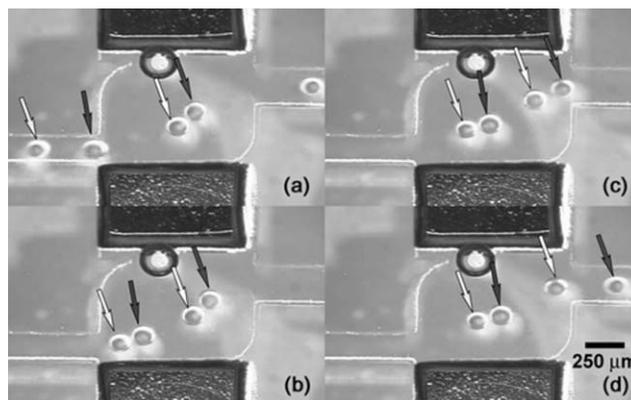


Fig. 3 Droplets brought into contact in the fusion chamber do not fuse without the application of electric field. (a) Due to the widening of the chamber, the droplets slow down and make contact when it enters the fusion chamber. (b) The presence of surfactant prevents the droplets from coalescing when in contact. (c) Droplets near the exit of the chamber speed up. (d) Droplets that were previously in contact exit the chamber separately. The round particle near the top electrode was an air bubble.

electrodes before fusion could be observed in the case of oil droplets in water.

Fig. 3 shows the device operating under continuous flow without application of voltage. Flow rates for the continuous and dispensed phase were 100 nl s^{-1} and 2 nl s^{-1} , respectively. Droplets within a certain distance contacted each other, while those separated by a large interval did not come into contact in the fusion chamber. Surfactant in the continuous phase prevented fusion during contact, and contacting droplets separated again near the exit of the fusion chamber. When an electric field of 67 kV m^{-1} was applied 5 times for 10 μs at an interval of 0.2 s, contacting droplets fused (Fig. 4). In this experiment, flow rates for continuous and dispensed phase were 40 nl s^{-1} and 0.6 nl s^{-1} , respectively. Even though we did succeed in initiating fusion for moving droplets with a single or

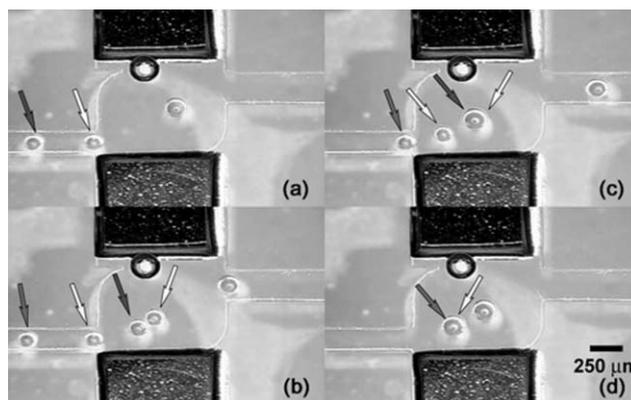


Fig. 4 Electrofusion of droplets in the fusion chamber. (a) Droplets formed upstream enter the fusion chamber. (b) Due to the widening of the chamber, the droplets slow down and make contact when it enters the fusion chamber. (c) Upon the application of an electric field (67 kV m^{-1}), the contacting droplets coalesce. (d) Photo showing two coalesced droplets. The round particle near the top electrode was an air bubble.

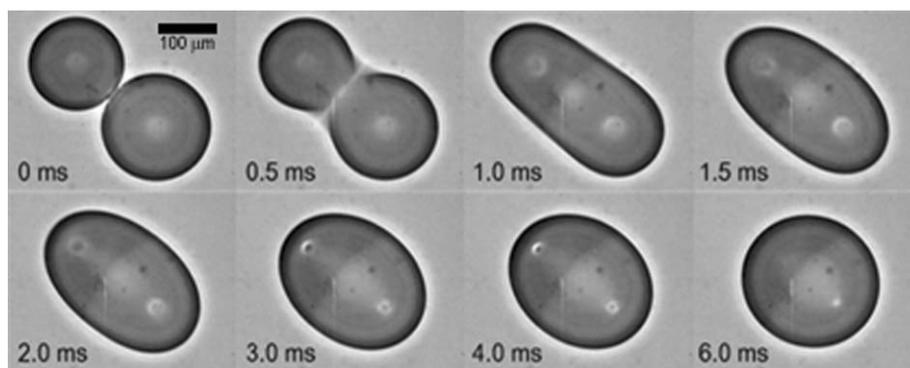


Fig. 5 High speed camera images of the fusion process. Throughout the fusion process, the darker colored blue ink droplet (top-left) was distinctly separated from the lighter colored water droplet (bottom-right)

double pulse, it was difficult to time the pulses with the contact of the droplets. In addition, stationary droplets could fuse with lower voltages compared with moving droplets. For stationary droplets (ϕ 100–250 μm), fusion could be initiated with electric field as low as 26.7 kV m^{-1} applied for 10 μs in a single try. Stationary droplets that could not be fused in a single try were due to poor contacts between the droplets.

Fusion dynamics and mixing

It has been reported that the start of an enzyme reaction can be defined at the moment of droplet formation out of a coflow of enzyme and substrate separated by a buffer solution.²⁰ In our approach, the exact start of the reaction is defined at the instant of fusion. In order to utilize droplets as microreactors, there is a need to understand the mixing process during and after the fusion process. To this end, electrofusion of droplets were initiated, and the instant was captured using a high speed camera. Fig. 5 shows high speed camera images of the fusion process between a droplet containing blue ink and a water droplet. This fusion process is almost instantaneous. The two droplets combined into one single “peanut-shaped” droplet within about 1 ms. It took about another 5 ms for the droplet to adopt a spherical shape under the effect of surface tension. Throughout the fusion process, the darker colored blue ink droplet (top-left) was distinctly separated from the lighter colored water droplet (bottom-right). In another experiment, fluorescent particles were included in the droplets to aid visualization. From both experiments, we could observe that no vortices were formed during the fusion process; there was no mixing between the contents of the droplets during and immediately after fusion. This indicates that mixing is dictated by diffusion after fusion.

Time scale for diffusion can be approximated by:

$$t = \frac{x^2}{2D}, \quad (7)$$

where t , x and D are the time for diffusion, diffusion distance and diffusion coefficient, respectively. For a small molecule in water at room temperature, D is in the order of $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, and it will take about 10 s to diffuse a distance of 100 μm .²¹ For large molecules like proteins and DNA, D is in the order of 10^{-7} to $10^{-8} \text{ cm}^2 \text{ s}^{-1}$ and the time for mixing

based on diffusion may be unacceptably long.²¹ In this case, diffusion time can be shortened by generating smaller droplets (decreasing diffusion distance) or by promoting mixing using chaotic mixing²⁰ after fusion.

Timing controllability

In the study of biological reactions and chemical kinetics, in particular single molecule detection, it is important to determine the exact start of reactions. Introduction of reagents to droplet microreactors by electrofusion not only allows chemicals to be added, it also enables the start of the reaction to be determined without any ambiguity. We used this device as a tool in the study of enzymatic activity to demonstrate timing controllability of the fusion process. With the electrofusion device, we prepared β -galactosidase and FDG droplets at T-junctions and stopped the flow when the droplets came into contact in the fusion chamber. Application of electric field fused the droplets. The instant at which droplets fused corresponded to $t = 0$ s, and this fusion process was completed in the order of milliseconds. The time for complete mixing computed based on diffusion time (eqn (7)) was around 10 s for this experiment. Mixing of the droplets caused hydrolysis of FDG substrate by β -galactosidase, producing a fluorescent compound, fluorescein, and two galactose residues. The enzymatic activity of β -galactosidase can subsequently be measured by monitoring the development of fluorescent intensities with time. Fig. 6 shows the increase in intensity *versus* time when we observed the reaction at about 1 min intervals. Mixing alone is not the main factor influencing the increase in fluorescent intensity. Its role is to ensure a homogeneous distribution of molecules. After mixing (~ 10 s), time is needed for reaction amongst the now homogeneously mixed liquid. The rate of enzymatic reaction is dependent on factors such as substrate and enzyme concentrations, pH, and temperature. This explains why the maximum intensity was not reached at the time at which mixing would have been completed.

Handling and sequential fusion of pico-litre droplets

Biological reactions and chemical synthesis often involve more complicated reactions that require sequential addition of reagents at predetermined intervals. By using smaller

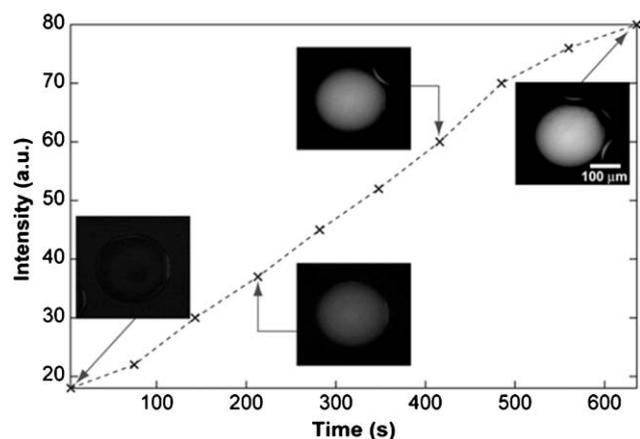


Fig. 6 Intensity vs. time graph for the reaction when β -galactosidase and FDG droplets were fused. The exact start of the reaction could be determined with electrofusion. Fluorescence microscopy was used to observe the reaction at intervals of about 1 min.

channels to form smaller pico-litre droplets and laser tweezers to manipulate and move the droplets, sequential fusion of 4 droplets was successfully accomplished (Fig. 7). The dispensed phase and the continuous phase channels were 7 μm and 100 μm wide, respectively. The fusion chamber measured 610 μm by 700 μm and channels had a depth of 63 μm .

Laser tweezers trap a dielectric material if the refractive index is higher than the fluid medium that it is suspended in. In the case of aqueous droplets in oil, water has a lower refractive index than oil and the water droplets tend to move away from the laser focus. In this experiment, laser tweezers were used to push and dribble droplets into contact. Contacting droplets were then aligned in parallel to the electrodes before an electric field (286 kV m^{-1}) was applied.

Compared with previous electrofusion experiments, we notice that the minimum electric field to initiate electrofusion is a function of the droplet size. Droplets of diameter in the order of 100 to 200 μm fused with an electric field of around

67 kV m^{-1} . On the other hand, smaller droplets with diameter in the range 10 to 50 μm often required electric fields of up to 286 kV m^{-1} . A 5 fold decrease in diameter from about 150 μm to 30 μm (average values are used) resulted in a 4.2 fold increase in electric fields that is needed to initiate fusion. This result seems consistent with that reported for electrofusion of cells where the external field strength required for taking the membrane potential to its critical breakdown value is inversely proportional to the radius.^{17,18}

For *in vitro* protein expression studies, sequential addition of reagents after a long incubation time can be very important. Electrofusion assisted with optical tweezers may be a good method. However, protein molecules have small diffusion coefficients ($\sim 5 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$), which translates to a long mixing time that would restrict its applicability. To circumvent this problem, smaller droplets in the pico-litre range have to be used for such applications. The use of pico-litre droplets ($\sim 10 \mu\text{m}$ in diameter) will reduce the mixing time to around 1 s for typical proteins. Compared to configurations using plugs enhanced by chaotic mixing (where a mixing time of around 2 ms was reported at high flow rates), the mixing time of our approach using static droplets is long. However, our approach permits continuous monitoring of the reaction for long periods of time.

5. Conclusions

The electrofusion technique is not limited to membranes of cells or liposomes. Application of an electric field can also cause otherwise stable droplets in the presence of surfactants to fuse. Based on this phenomenon, we have designed an electrofusion device that can be easily fabricated with standard lithographic and MEMS processes. With this device, we have managed to form, handle and fuse nano-litre to pico-litre scale droplets without complicated control. Such devices allow reactants and reagents to be easily added to droplets. The exact start of reactions can also be clearly determined at the moment of fusion. The fusion process is instantaneous (in the order of

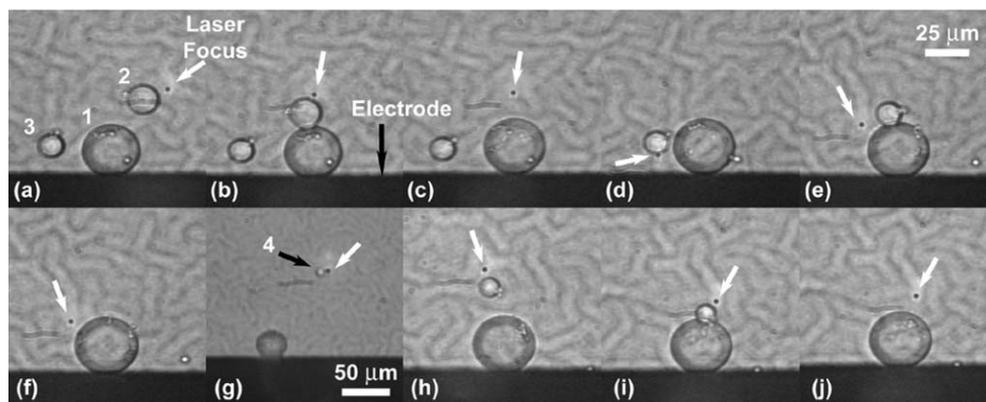


Fig. 7 Handling and sequential fusion of droplets. Droplets were in the fusion chamber between two electrodes (the top electrode is out of view). The white arrow indicates the position of the laser focus. (a) Position of 3 droplets that were going to be fused. (b) Droplet 1 and 2 were aligned in parallel to the electrodes by laser tweezers. (c) Droplets coalesced when an electric field of 286 kV m^{-1} was applied for 10 μs . (d) Manipulation of droplet 3. (e) Droplets were aligned in parallel to electrodes for fusion. (f) Droplets coalesced when electric field was applied. (g) Initial position of 4th droplet with respect to fused droplets observed at lower magnification. (h) Manipulation of droplet 4. (i) Droplets were aligned in parallel to electrodes for fusion. (j) Droplets coalesced when electric field was applied. Scale bar applies to all photos except (g).

ms) and mixing is dominated by diffusion after fusion. The time to complete mixing is determined by the diffusion coefficient and diffusion distance. This mixing time can be shortened by promoting chaotic mixing after fusion.²⁰ In our approach, there is minimum contact of reactants with channel walls, eliminating surface absorption problems when dealing with biomolecules such as DNA and proteins. We believe that besides using this device as a tool to study chemical kinetics, it can also be useful for biological analysis such as *in vitro* protein expression when substances such as DNA, RNA, ribosome, enzymes, *etc.* are included during droplet formation.²² Simple modifications such as smaller channels will make it possible to work with smaller volumes. In the future, we may be able to use such devices to trap single molecules such as DNA and proteins in pico or femto-litre droplets, add reagents by means of electrofusion and observe the reaction right from the start.

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References

- 1 D. T. Chiu, C. F. Wilson, F. Ryttsén, A. Strömberg, C. Farre, A. Karlsson, S. Nordholm, A. Gaggari, B. P. Modi, A. Moscho, R. A. Garza-López, O. Orwar and R. N. Zare, *Science*, 1999, **283**, 1892–1895.
- 2 D. Stamou, C. Duschl, E. Delamarche and H. Vogel, *Angew. Chem. Int. Ed.*, 2003, **42**, 5580–5583.
- 3 Y. Rondelez, G. Tresset, T. Nakashima, Y. K. Yamada, H. Fujita, S. Takeuchi and H. Noji, *Nature*, 2005, **433**, 773–777.
- 4 Y. Rondelez, G. Tresset, K. V. Tabata, H. Arata, H. Fujita, S. Takeuchi and H. Noji, *Nat. Biotechnol.*, 2005, **23**, 3, 361–365.
- 5 M. Gratzl, H. Lu, T. Matsumoto, C. Yi and G. R. Bright, *Anal. Chem.*, 1999, **71**, 2751–2756.
- 6 M. Nakano, J. Komatsu, S. Matsuura, K. Takashima, S. Katsura and A. Mizuno, *J. Biotechnol.*, 2003, **102**, 117–124.
- 7 D. L. Chen, C. J. Gerdtz and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2005, **127**, 9672–9673.
- 8 M. He, J. S. Edgar, G. D. M. Jeffries, R. M. Lorenz, J. P. Shelby and D. T. Chiu, *Anal. Chem.*, 2005, **77**, 1539–1544.
- 9 S. Xu, Z. Nie, M. Seo, P. Lewis, E. Kumacheva, H. A. Stone, P. Garstecki, D. B. Weibel, I. Gitlin and G. M. Whitesides, *Angew. Chem. Int. Ed.*, 2005, **44**, 724–728.
- 10 S. Takeuchi, P. Garstecki, D. B. Weibel and G. M. Whitesides, *Adv. Mater.*, 2005, **17**, 1067–1072.
- 11 B. G. D. Geest, J. P. Urbanski, T. Thorsen, J. Demeester and S. C. D. Smedt, *Langmuir*, 2005, **21**, 10275–10279.
- 12 T. Nisisako, T. Torii and T. Higuchi, *Lab Chip*, 2002, **2**, 24–26.
- 13 J. Y. Yoon and R. L. Garrell, *Anal. Chem.*, 2003, **75**, 5097–5102.
- 14 M. Senda, J. Takeda, S. Abe and T. Nakamura, *Plant Cell Physiol.*, 1979, **20**, 1441–1445.
- 15 D. T. Chiu, *Curr. Opin. Chem. Biol.*, 2001, **5**, 609–612.
- 16 G. Tresset and S. Takeuchi, *Biomed. Microdev.*, 2004, **6**, 213–218.
- 17 U. Zimmermann and J. Vienken, *J. Membr. Biol.*, 1982, **67**, 165–182.
- 18 U. Zimmermann and G. A. Neil, *Electromanipulation of Cells*, CRC Press, 1996, pp. 11–17.
- 19 Y. C. Tan, J. S. Fisher, A. I. Lee, V. Cristini and A. P. Lee, *Lab Chip*, 2004, **4**, 292–298.
- 20 H. Song, J. D. Tice and R. F. Ismagilov, *Angew. Chem. Int. Ed.*, 2003, **42**, 767–772.
- 21 D. Bray, *Cell Movements*, Garland Publishing, 2nd edn, 2001, p. 6.
- 22 P. S. Dittrich, M. Jahnz and P. Schuille, *Micro Total Analysis Systems 2004*, ed. T. Laurell, J. Nilsson, K. F. Jensen, D. J. Harrison and J. P. Kutter, RSC, Cambridge, 2004, vol. 1, pp. 52–54.