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## Electric charge-mediated coalescence of water droplets for biochemical microreactors

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This work proposes the use of charged droplets driven by the Coulombic force as solution-phase reaction chambers for biological microreactions. A droplet can be charged near an electrode under dc voltage by direct contact to the electrode. This process is called electrical charging of droplet (ECOD). This charged droplet can then be transported rapidly between electrodes following the arc of an electric field line by exploiting electrostatic force. As on-demand electrocoalescence, both alkalization of phenolphthalein and bioluminescence reaction of luciferase in the presence of adenosine triphosphate are studied to test the feasibility of the biochemical microreactors using ECOD. Two oppositely charged droplets are merged to have a color change immediately after microchemical reaction. The applicability of an ECOD-driven droplet to measurement of glucose concentration is also tested. The glucose concentration is measured using a colorimetric enzyme-kinetic method based on Trinder's reaction [J. Clin. Pathol. **22**, 158 (1969)]. The color change in the merged droplet is detected with an absorbance measurement system consisting of a photodiode and a light emitting diode. © 2010 American Institute of Physics. [doi:10.1063/1.3427356]

### I. INTRODUCTION

Droplet motion is electrically induced in various processes, such as storm cloud formation, ink-jet printing, petroleum dehydration, electrospray ionization, electrowetting, and laboratory-on-a-chip manipulations.<sup>1,2</sup> Droplets, as the common carriers and reactors for biochemical agents, have found growing importance in laboratory-on-a-chip design and biomicroelectromechanical system.<sup>3,4</sup> Moreover, it would help to explain the long-standing problem of cloud electrification in thunderstorms.<sup>5,6</sup> Additionally, it could further improve our understanding of electrocoalescence-based de-emulsification processes that are widely used to separate residual water from crude oil.

Droplet-based microfluidics is a relatively new microscale handling technique for independent control of droplets.<sup>7</sup> Referred to as digital microfluidics, this approach was pioneered in the early 2000s. Digital microfluidics is an alternative technology for laboratory-on-a-chip systems based on the micromanipulation of discrete droplets. Currently, most of the droplet-based microfluidics is based on the electrowetting on dielectric (EWOD). Recently Jung and Kang<sup>3</sup> proposed an alternative method to EWOD-based digital microfluidics. The droplets are charged at the electrode surfaces and transported to desired positions by the Coulombic force along the electric field lines. They called it the method based on *electrical charging of droplet* (in short ECOD as a contrasting word to EWOD).

When droplets are used as microreactors, the coalescence process must be controlled easily. The overall current understanding of electrocoalescence of water droplets in oil under the influence of an applied electric field was reviewed by Eow *et al.*<sup>8</sup> However, they did not fully explain

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the actual mechanisms underlying droplet-droplet coalescence. Electric control of electrically charged droplets inside microchannels was accomplished by Link *et al.*<sup>4</sup> They presented a generic and robust platform technology for manipulating and controlling individual droplets in microfluidic devices. An important issue about droplet coalescence is that oppositely charged drops have long been assumed to experience an attractive force that favors their coalescence. Recent observation, however, is different. Contrary to our conventional understanding, two oppositely charged droplets fail to merge when the electric field is stronger than a critical level.<sup>1-3</sup>

Based on the EWOD with switching of ac voltage, the transport of droplets, deflection of a droplet in either of two bifurcating paths, and the mixing of two droplets by coalescence were experimentally demonstrated by Washizu.<sup>9</sup> Similar works were accomplished by Taniguchi *et al.*<sup>10</sup> They successfully studied chemical reactions that included alkalization of phenolphthalein and the luciferin-luciferase reaction based on EWOD with beam guidance film.

As mentioned earlier, Jung and Kang<sup>3</sup> carefully studied the feasibility of a novel actuation method for manipulating conductive droplets based on ECOD. They noted that one potential use of an ECOD-based droplet microfluidic device is to encapsulate a varied population or library of molecules, cells, or particles into individual microreactors.<sup>3,4</sup> The present work is the continuation of previous work as ECOD-mediated microfluidics.

This work tests the proof-of-concept of ECOD-driven droplet microreactors for the biochemical reactions by studying on-demand electrocoalescence of two oppositely charged droplets. For the test, we have chosen three reactions that have been considered for EWOD systems by the previous researchers. The first two are the alkalization of phenolphthalein and the bioluminescence reaction. Taniguchi *et al.*<sup>10</sup> carried out the two reactions on a EWOD chip. The third test is chosen for the use of ECOD-driven droplets as biochemical reactors. We try the method of glucose detection based on the absorbance measurement system, which was originally proposed by Tinder<sup>11</sup> and Srinivasan *et al.*<sup>12</sup>

The precise manipulation of droplets in an immiscible fluid under an electric field is revolutionizing various droplet-based technologies in fields such as biochemical and biomedical engineering. The electrical micromanipulation of droplets could allow programmable operations. Thus, the electrically charged droplet could be an alternative platform technology to enable high-throughput droplet-based microreactors.

## II. EXPERIMENTAL SETUP

Chemical reactions can be induced by coalescence of two droplets containing the sample and reagent, respectively. ECOD-driven chip processes are developed for the alkalization of phenolphthalein, the luciferin-luciferase enzyme bioluminescence reaction, and the detection of glucose based on absorbance measurement.

Droplets are charged directly from an electrode (Fig. 1). The droplets are then transported by switching the electric field lines and they are merged by the attractive electrostatic force.<sup>3</sup> The forces exerted on a droplet are shown for the ECOD-driven actuation system (negatively charged case) in Fig. 2. Since the densities of water and silicone oil are slightly different, water droplets in silicone oil move along the arcs formed by the electric field.

### A. Microchip system

A platform is designed to handle conductive droplets on a chip substrate (Fig. 3). Single de-ionized water droplets and silicone oil (KF-96 series produced from Shin-Etsu Silicone, Japan) are used as the conductive droplet and the dielectric fluid medium, respectively. Microdroplets are transported with sequentially switched dc voltage on the Y-type arrayed electrode device. The electrode array is connected to a switching system consisting of a dc voltage power supply (Trek, Inc., Medina, NY), a relay (mechanical switch), and a control software program (LABVIEW<sup>TM</sup>). Droplet actuation is achieved by applying voltages sequentially. The detailed procedures are explained in our previous work<sup>3</sup> (see Sec. II A). The circular copper electrode has a diameter of  $\sim 0.8$  mm. The pitch between the centers of the electrodes is  $\sim 10$  mm. Teflon plate is used as the

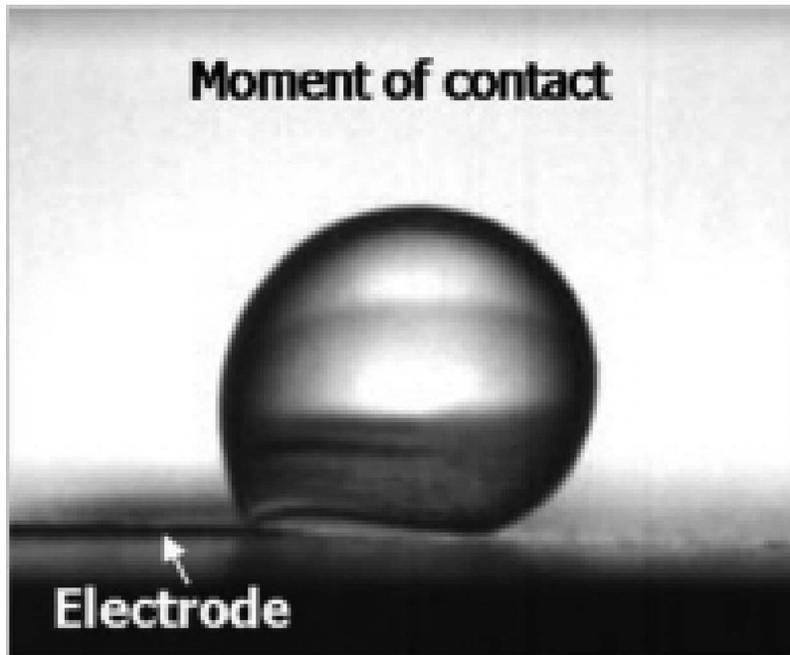


FIG. 1. Moment of contact of a droplet to the bottom electrode (Ref. 3).

chip substrate because of its hydrophobicity. This keeps the microdroplets spherical while it is at stationary state as well as during translation. The chip is divided into the initial transporting zone, the merging zone, and the zone of transporting after merging. To observe the behaviors of droplets, a high speed charge coupled device (CCD) camera (Photron Fastcam 1024 PCI model 100 K, San Diego, CA) at 6000 frames/s was mounted on the microscope. dc of 1–1.5 kV is applied to the electrode. A micropipette (Effendorf, Hamburg, Germany) is used to inject water microdroplets. The two droplets must be controlled individually since they should be oppositely charged. The time interval between switching of signals is related to the ECOD-droplet translational velocity so it can be set empirically.

### B. Materials for biochemical reactions

The ECOD process is tested for the feasibility of the chemical reactions with on-demand electrocoalescence. Both alkalization of phenolphthalein and bioluminescence reaction of

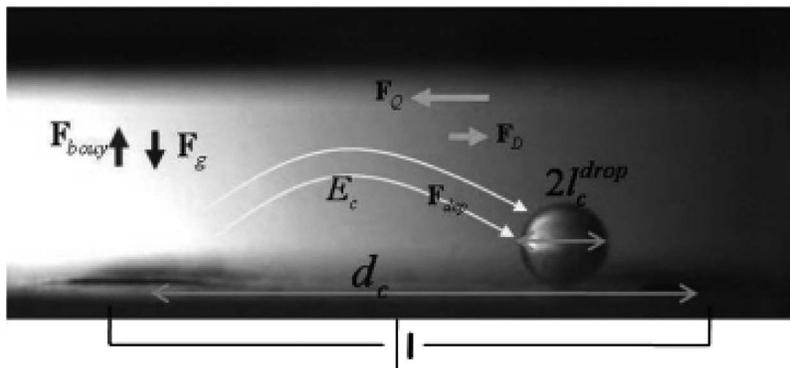


FIG. 2. ECOD actuation system and the forces exerted on the droplet.

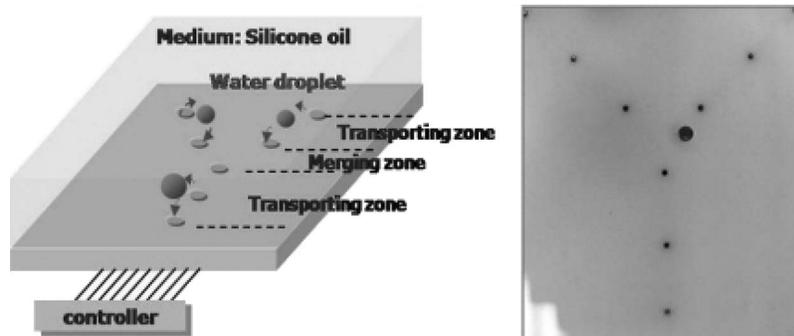


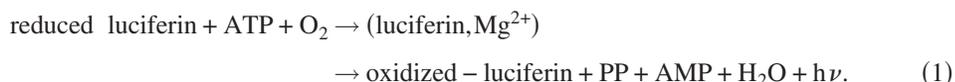
FIG. 3. The schematic view of the electrode dot array.

luciferase-luciferin enzyme in the presence of adenosine triphosphate (ATP) are performed through a chip process. As mentioned earlier, these reactions have been carried out on a EWOD-based chip by Taniguchi *et al.*<sup>10</sup>

Alkalization of phenolphthalein is a simple visible chemical reaction. Phenolphthalein is itself a weak acid, which can lose H<sup>+</sup> ions in solution. The phenolphthalein molecule is colorless. However, the phenolphthalein ion is pink. When a base is added to the phenolphthalein, the molecule ⇌ ions equilibrium shifts to the ion. This was predicted by Le Chatelier's principle, one of the central concepts of chemical equilibria.

Bioluminescence is a form of chemiluminescence or cold-light emission. It should not be confused with fluorescence or refraction of light. Chemiluminescence has a better signal-to-noise ratio and better sensitivity. Firefly and Renilla luciferases are widely used as reporter genes to study gene regulation and function, and for pharmaceutical screening.<sup>13</sup>

Bioluminescence of luciferase in the presence of ATP is shown as follows:



The droplet of luciferase solution and the droplet of luciferin (in the presence of ATP) are moved toward each other by exploiting Coulombic force. Mixing of the two droplets causes a chemical reaction; the fused droplet is luminescent with a yellow-green color. Bioluminescence of luciferase is often used to measure changes in ATP level for cell viability in microbiological reactions, such as phagocytosis, etc.<sup>13</sup>

### C. Absorbance measurement system

The microfluidic chip for ECOD-driven droplet microreactors has been improved [Fig. 4(a)]. Channel design is improved to reduce the distance between detection systems. The system also has an advantage in that the droplet can be rapidly mixed through repetitive W-type movement of the droplet caused by switching the electric field [Fig. 4(b)]. The optical system consists of a photodiode and a light emitting diode (LED) to detect absorption change (Fig. 5). The detection system and on-chip optical beam guidance are as in Fig. 5(a). The incident probe beam is deflected by 90° into the chip plane by an equilateral triangle-shaped V groove.<sup>14</sup> From the refractive index of acrylic ( $n=1.35-1.37$ ) and air ( $n=1$ ), the incident angle should exceed the critical angle, 41.8°,

$$\alpha_c = \sin^{-1}\left(\frac{n_{\text{air}}}{n_{\text{polymer}}}\right) > 41.8^\circ. \quad (2)$$

A green LED is used as the incident probe beam and a photodiode (TSL-257) is used to convert light to a voltage signal [Fig. 5(a)]. Dark conditions are required because the photodiode is extremely sensitive to ambient light. The voltage output captured by a 16 bit analog data

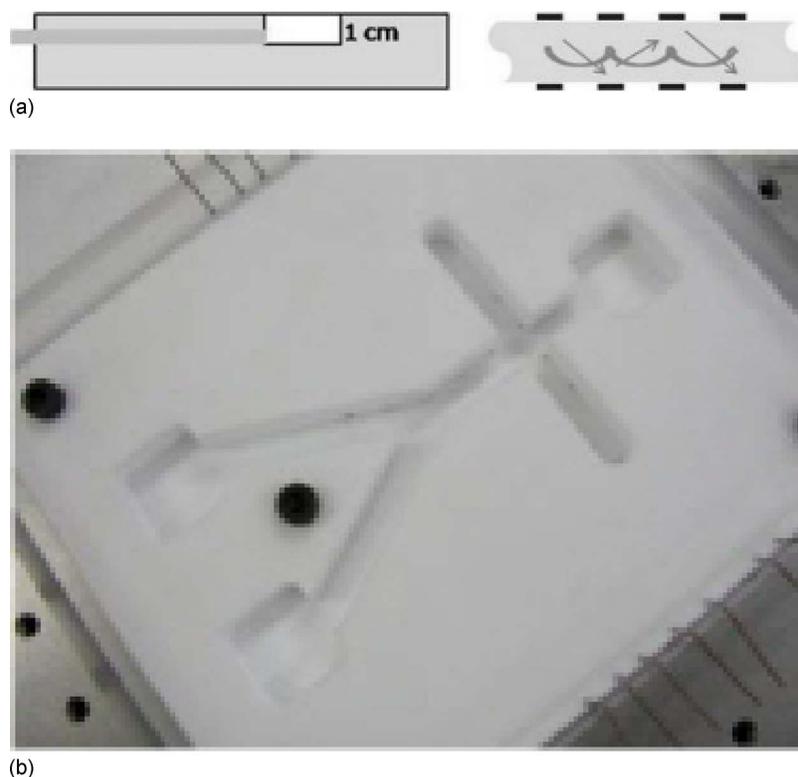


FIG. 4. The schematic view of the improved test channel: (a) Molded electrodes and (b) channel on chip.

acquisition board (NI-PCI-6251) and recorded using LABVIEW<sup>TM</sup>. The voltage is proportional to the light intensity incident on the photodiode and is related to the absorbance,

$$A(t) = \ln\left(\frac{V_0 - V_{\text{dark}}}{V(t) - V_{\text{dark}}}\right), \quad (3)$$

where  $V_0$  corresponds to zero absorbance (or 100% transmittance) and  $V_{\text{dark}}$  corresponds to the voltage output of the photodiode under dark conditions. Real time photographs are acquired by a real-time CCD.

The absorbance, as detected by voltage change, is measured for about 20–30 s, about 20 s after the droplets are merged. The merged droplet is stationary during the detection. All experiments are conducted at room temperature and ambient condition.

#### D. Glucose assay

The *in vitro* measurement of glucose in human physiological fluids is of great importance in clinical diagnosis of metabolic disorders, such as diabetes.<sup>12</sup> In diabetes, the body either fails to properly respond to its own insulin, does not make enough insulin, or both. This causes glucose to accumulate in the blood, often leading to various complications. Detection of glucose level in the blood is typically done using electrochemical methods or optical methods.

In this experiment, we propose an alternative glucose detection method using ECOD-driven droplet microreactors. This paradigm has some advantage in that very small sample/reagent volumes can be used and the contamination of sample on the chip substrate can be greatly reduced. Glucose concentration is measured using a colorimetric-kinetic method based on Trinder's reaction.<sup>11</sup> Glucose is enzymatically oxidized to gluconic acid and hydrogen peroxide in the

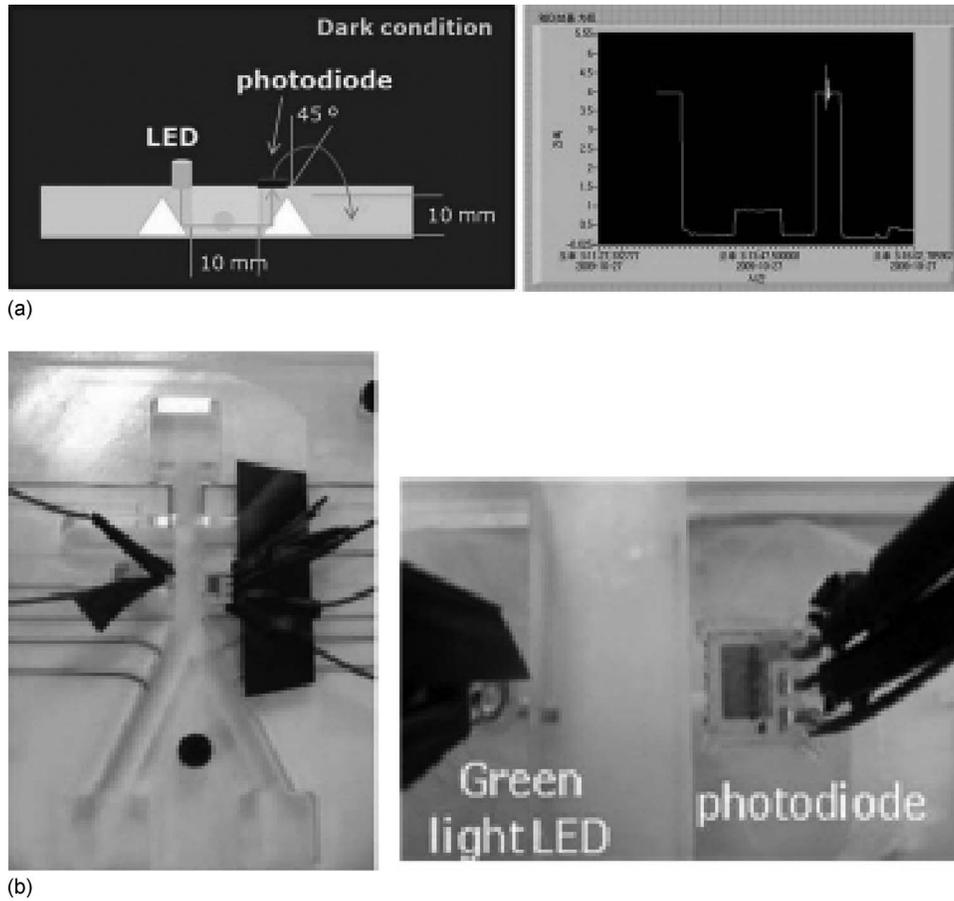
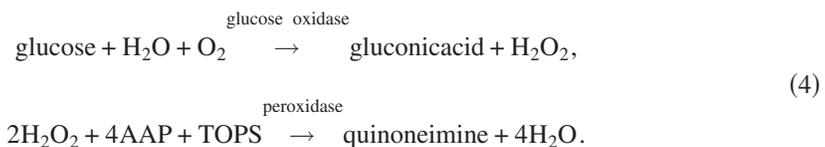


FIG. 5. On-chip optical detection system: The incident light from source LED is deflected into the cell chip and is detected by a molded focusing lens in front of photodiode. (a) The voltage is proportional to the light intensity incident on photodiode and is recorded by LABVIEW<sup>TM</sup> program. (b) The enlarged plan of the detecting section.

presence of glucose oxidase, and then hydrogen peroxide reacts with 4-amino antipyrine (4-AAP) and N-ethyl-N-sulfopropyl-m-toluidine (TOPS) in the presence of peroxidase to form violet colored quinoneimine, which has an absorbance peak at 545 nm,



The rate equations for the glucose concentration can be written as

$$V_i = V_{\max} \frac{[g]}{K_M \times \text{DF}} \quad \text{if} \quad \frac{[g]}{\text{DF}} \ll K_M, \quad (5)$$

where  $V_i$  is the initial rate of the reaction,  $[g]$  is the glucose concentration in the sample, DF is the dilution factor of the sample in the assay mixture,  $K_M$  is the Michaelis constant, and  $V_{\max}$  is the limiting maximum reaction rate, which is proportional to the enzyme activity.

The initial rate is measured as the rate of formation of the colored product quinoneimine,

$$\frac{dA(t)}{dt} = \varepsilon LV_i. \quad (6)$$

The relation between the glucose concentration and the initial rate of change in absorbance can be written as

$$\frac{dA(t)}{dt} = \frac{\varepsilon LV_{\max}}{K_M \times DF} [g] = S[g], \quad (7)$$

where  $A(t)$  is the absorbance at time  $t$ ,  $L$  is the optical path length, and  $\varepsilon$  is the extinction coefficient of quinoneimine under the chip conditions. So, in this stage, analytical sensitivity  $S$  is determined as follows:

$$S = \frac{\varepsilon LV_{\max}}{K_M \times DF}. \quad (8)$$

Note that the sensitivity  $S$  is equivalent to the initial rate of change in the absorbance divided by glucose concentration. Thus, it is the linear slope of the calibration curve in absorbance rate versus sample glucose concentration. The calibration curve can then be used to determine the glucose concentration when the absorbance rate is known.

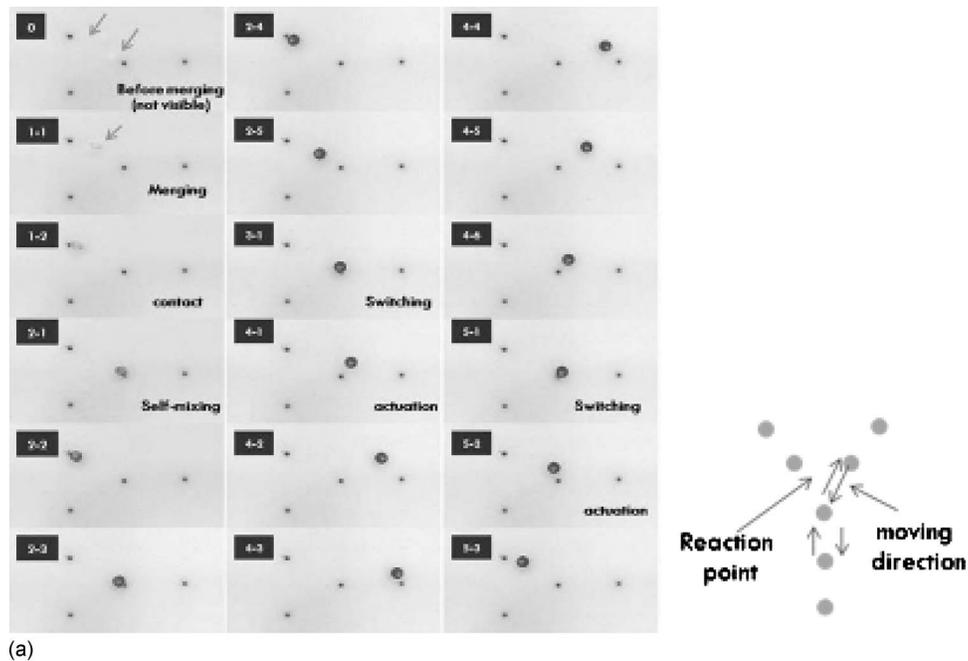
For comparison with reliable data, experimental conditions are chosen to be the same with that of Srinivasan *et al.*<sup>12</sup> The sample and reagent droplets are about 1  $\mu\text{l}$  individually and the dilution factor is about 2 for both liquids. Glucose oxidase (G-7141), peroxidase (P-8375), 4-aminoantipyrine (A-4382), and TOPS (E-8506) were purchased from Sigma, St. Louis, MO. The reagent was constituted such that the reagents mixture consists of 6 U/ml glucose oxidase, 6 U/ml peroxidase, 6 mM 4-aminoantipyrine, and 10 mM TOPS in 0.1M phosphate buffered saline (pH 7.0). Reference glucose solutions of different concentrations were prepared by diluting 50 and 100 mg/dl standards (G-7528) with de-ionized water.

### III. RESULTS

#### A. Alkalinization of phenolphthalein

Microdroplets containing NaOH aqueous solution and phenolphthalein solution are set in the device for the ECOD-microfluidic chip (Fig. 6). By applying voltage (Fig. 6, 0), the NaOH solution droplet and the phenolphthalein solution droplet are charged individually and then the two oppositely charged droplets are moved toward each other by the electrostatic force. They are coalesced by exploiting the Coulombic force. The mixing of the two droplets causes a chemical reaction, and the merged droplet becomes pink in color due to alkalization of phenolphthalein [Fig. 6(a), 1-1–2-5]. The merged droplet can be transported to the nearest electrode [Fig. 6(a), 1-2]. The reason is that the charged droplets are injected using a micropipette. So, the two droplets do not have exactly the same volume. Different volumes induce the different amounts of charges,<sup>15</sup> causing the net charge of the merged droplet not to be neutral. In addition, electric conductivities of microdroplets containing the sample and the reagent are changed, compared to the pure water droplets. This would also change the amount of charging quantities of microdroplets, even if the volumes were the same. Because the merged droplet has a slight electric charge, it can be translated toward the electrode [Fig. 6(a), 1-2], where it is recharged. After several to-and-fro movements of the droplet [Fig. 6(a), 2-1–2-5], the chemical reaction makes the color more and more red. The movement direction of the droplet can be controlled by switching the electric field [Fig. 6(a), 3-1–5-3].

A detailed view of the electrocoalescence is shown in Fig. 7. The local electric field strength between two oppositely charged droplets becomes higher because the water droplet is an excellent conductive material. The surfaces of the microdroplets are pulled toward each other by their electrostatic force (see Fig. 11 in Ref. 3). The microscopic capillary bridge is formed between the two droplets.<sup>16</sup> Due to the capillary force, two oppositely charged droplets are merged by interplay between interfacial tension and the geometry of the capillary bridge. Immediately after the drop-



(a)



(b)

FIG. 6. Two charged droplets of phenolphthalein solution and NaOH solution are merged by the attractive electrostatic force: (a) Detailed view (the distance between the electrodes is 10mm), (b) movie (enhanced online) [URL: <http://dx.doi.org/10.1063/1.3427356.1>].

lets are merged, the shape of the resultant droplet is distorted due to their inertia but the shape is recovered by the interfacial tension. After the resultant droplet is recharged from the electrode, it can be moved further.

## B. Bioluminescence of luciferase

In the same manner with alkalization of phenolphthalein, the bioluminescence of luciferase in the presence of ATP is accomplished as on-a-chip process (Fig. 8). The luciferin droplet has a pale yellow color and the luciferase droplet is transparent (Fig. 8, 0). When the electric signal is applied, the two droplets are oppositely charged and merged by electrostatic force (Fig. 8, 1-1). The resultant droplet makes a yellow-green light through the microchemical reaction (Fig. 8, 1-2). After several to-and-fro movements (Fig. 8, 2-1–2-5), the resultant droplet produces more and more luminescence. Also, the luminescent droplet can be moved using ECOD (Fig. 8, 2-1–4-4),

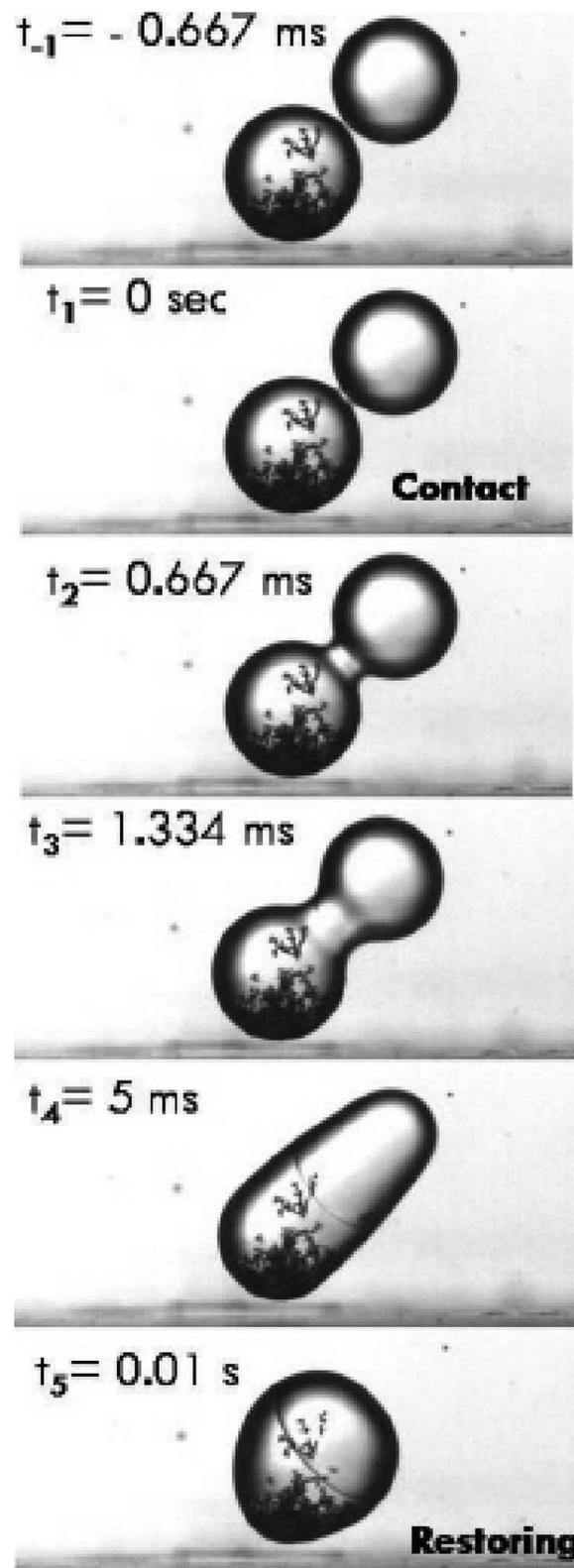


FIG. 7. High speed photographs on the moment of merging of two droplets containing phenolphthalein solution and NaOH solution separately.

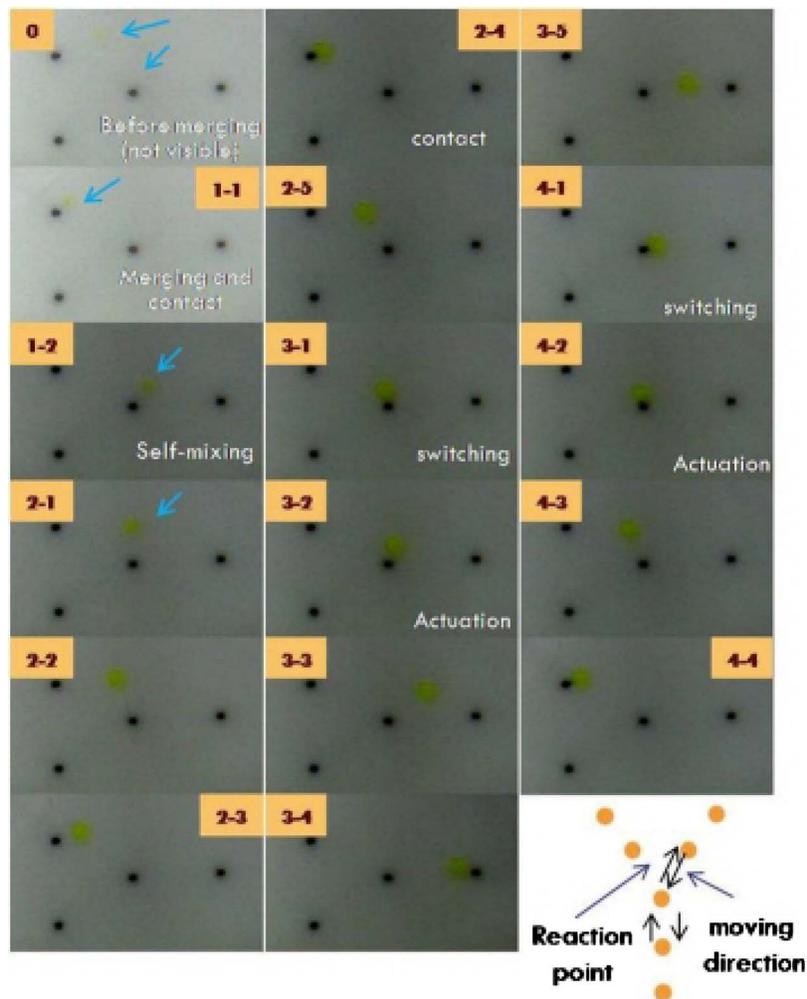


FIG. 8. Bioluminescence reaction of luciferase by using ECOD is accomplished on a chip process. The merged droplet makes the yellow-green light after the self-microchemical reaction.

Fig. 9 shows the droplet with and without ambient light to illustrate that this is a luminescence reaction by biomaterial and enzyme, not a fluorescence reaction nor a chemical reaction with yellow color.

When the reacted droplet is stationary when the electric signal is off, the luminescent level of droplet can be detected by the pertinent absorbance measurement system. From these data, change in ATP levels can be measured for cell viability in microbiological reaction.

### C. Glucose detection reaction and absorbance measure for glucose concentration

Photographs of the *in situ* glucose reaction are shown in Fig. 10. Two microdroplets containing sample and reagent are charged oppositely and then merged. The merged droplet makes a violet color immediately. Then, the absorbance of the stationary droplet is measured. After detection, it can be moved by using ECOD.

The absorbance as a function of time  $A(t)$  is shown for concentrations of 50 and 100 mg/dl in Fig. 11 to acquire the absorbance rate. In each plot of Fig. 11, y-axis is the absorbance converted from the voltage output of the photodiode and the x-axis is the time after merging. At the concentration of 50 mg/dl, the absorbance rate is about 0.005 83. At 100 mg/dl, the absorbance rate is about 0.009 62. These absorbance rate values are used to plot the calibration curve of the absor-

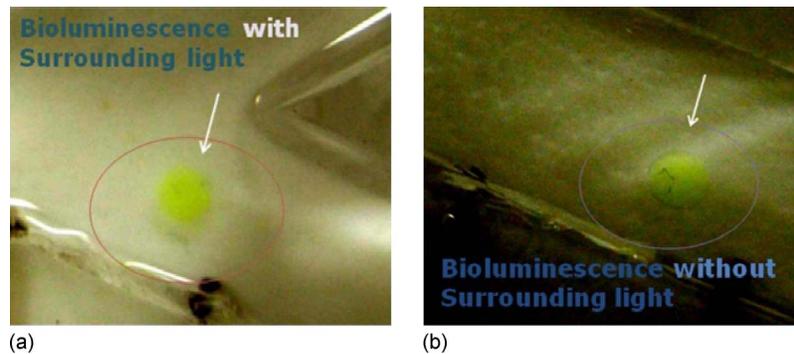


FIG. 9. Bioluminescence is a form of cold-light emission. It should not be confused with fluorescence or chemical reaction with yellow color.

bance rate as a function of the sample glucose concentration. The slope of the calibration plot is  $75.8 \times 10^{-06}$  (Fig. 12). From the rate kinetic equations [Eqs. (3)–(8)], the slope value is equivalent to the sensitivity  $S$  defined in Eq. (8).

The result of the present work is compared to the result of Srinivasan *et al.*<sup>12</sup> in Fig. 13. As we can see, they obtained the sensitivity  $S=27.2 \times 10^{-06}$  for their system. Since the optical path length of our system  $L$  is 3.02 times longer than that of Srinivasan *et al.*, we expect the sensitivity value of  $82.3 \times 10^{-06}$ . [As shown in Eq. (8),  $S$  is proportional to  $L$ . Thus we expect  $27.2 \times 3.02=82.3$ .] As mentioned above, the result of the present work shows a smaller value of  $75.8 \times 10^{-06}$  (Fig. 13). This value shows some difference from that of Srinivasan *et al.* However, we can still think that it shows qualitatively good agreement, which may be enough for the proof of concept.

The quantitative difference in two results is probably due to following facts. Since we wanted to test the feasibility rather than developing a fully established method, only two sample concentrations were used in the experiments. In addition, the experimental system was under less-stable and less-controllable condition. As we can see in Fig. 10, the detection region is too wide for a small droplet. These negative effects might be avoided if a more confined geometry is used for the

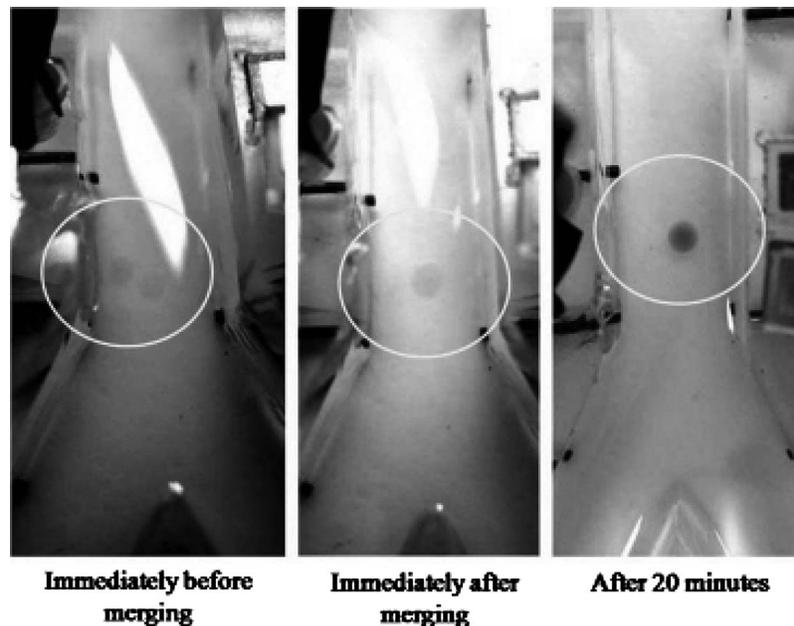


FIG. 10. Photographs of the *in situ* glucose detection reaction.

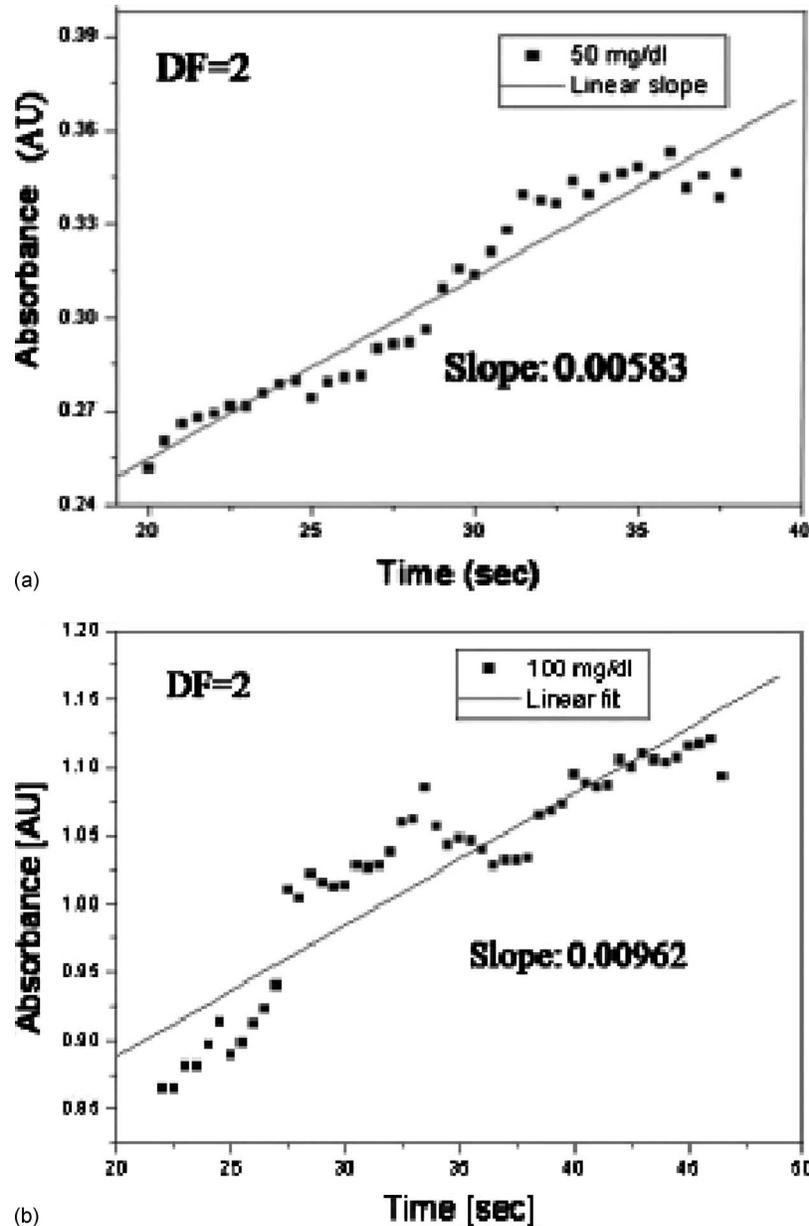


FIG. 11. Absorbance vs time: Dilution factor=2, glucose concentration=50 mg/dl [case (a)] and 100mg/dl [case (b)].

detection region. If the above mentioned improvements are made in the future works, we may expect more precise measurement of glucose concentration based on the ECOD-driven droplet microreactors.

Finally we would like to give a comment on the effect of charge exchange between the droplet and an electrode. The charge exchange could imply that electrochemical reactions take place at the metal-liquid interface. However, the effect seems to be negligibly small. In our previous work,<sup>15</sup> we have estimated the amount of charge acquired by a droplet from the electrode. In the case of the droplet of  $0.2 \mu\text{l}$  (radius= $363 \mu\text{m}$ ), the acquired charge is  $1.03 \times 10^{-11}$  C. This is equivalent to  $1.07 \times 10^{-16}$  moles of ions with the valence number 1. If hydrogen gas is generated at standard condition, this would be about  $1.2 \times 10^{-15}$  l (about  $6 \times 10^{-9}$  times of the droplet volume). This

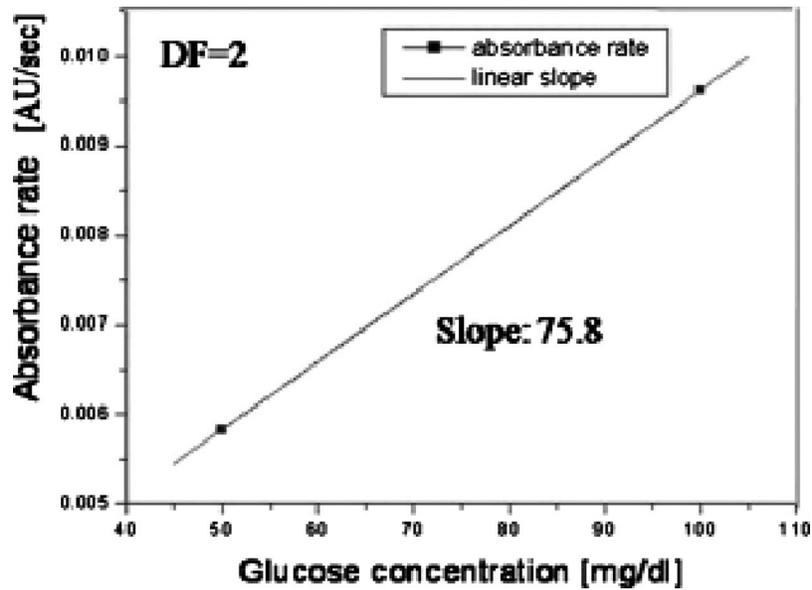


FIG. 12. The calibration plot of the absorbance rate vs sample glucose concentration.

shows that the amount of gas generated due to surface reaction is negligibly small. Indeed, we have not been able to observe any gas bubbles formed inside droplets during our experiments.

#### IV. CONCLUSIONS

We have demonstrated the feasibility of on-demand electrocoalescence of conductive droplets using ECOD for biochemical microreactions. Bioluminescence and glucose detection by electrocoalescence provide the proof of concept for ECOD-driven droplet-based biomicroreactors. Consequently, it is thought that the electrical charging phenomenon can be used as a tool to transport a single micro- or nanoliter droplet in a microchannel without moving the medium fluid. Thus, the

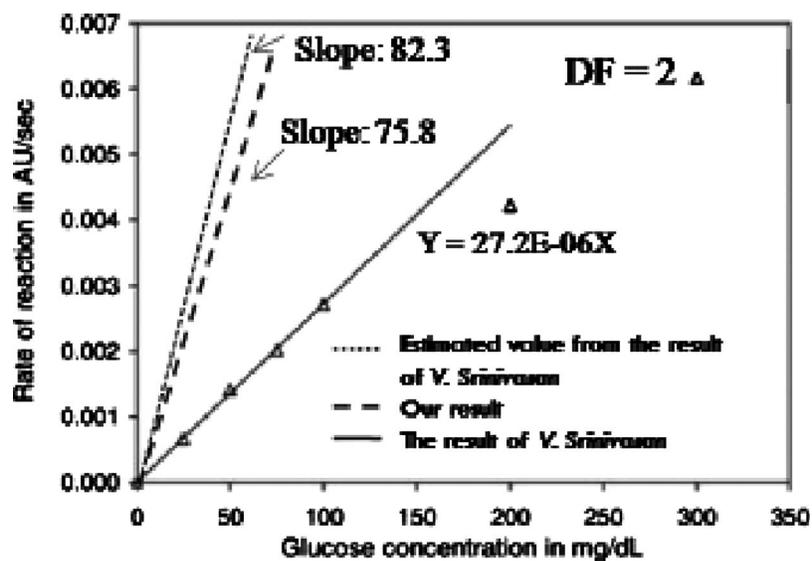


FIG. 13. The comparison with the results of Srinivasan *et al.* (Ref. 12). In the figure, the slopes 82.3 and 75.8 mean  $82.3 \times 10^{-6}$  and  $75.8 \times 10^{-6}$ , respectively.

droplets can be used as solution-phase reaction chambers. Glucose concentration has been successfully detected on ECOD-driven droplet microchip with the absorbance measurement system. This measurement chip would be directly useful to test latex agglutination, for example, prostate-specific antigen, in real biomedical application. The latex agglutination test is a laboratory method to check for certain antibodies or antigens in a variety of bodily fluids including saliva, urine, cerebrospinal fluid, or blood. Since our method provides both on-demand electrocoalescence and a stationary droplet, it can be applied in real biomedical applications that have relatively long reaction times. Therefore, ECOD-driven droplet microchip can be a promising method for droplet-based microchemical assay.

### A. Future work

Complete integration of ECOD-based actuation method for droplet-based microfluidics requires some enabling technologies for high-throughput droplet microfluidic reactors: Droplet generation system, system of performing serial reactions, precise microcontroller system, and so on. The realization of on-a-chip system for the droplet containing various biological fluids still requires the development of integrated microfluidic systems for subnanoliter levels of samples and reagents.

### ACKNOWLEDGMENTS

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