Electrohydrodynamic Micro-droplet Generation on Both Conducting and Non-conducting Surfaces by Electric Induction

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ABSTRACT

We report a brand-new printing method, so called, Electric Charge Concentration(ECC) method, to generate a single droplet in the range of pL to μL with a single size capillary nozzle by electric induction phenomenon. Using the method, a droplet is generated electrohydrodynamically from the capillary nozzle and the induced charges at the target substrate surface. Differently from the common EHD methods, the present method doesn't need a counter electrode, and can generate a tiny small droplet (as small as 2.6 pL) on any type of target substrates, such as a conductor or an insulator, and a solid or even liquid. Furthermore, in our method, the electric stress acting on the interface of droplet and target surface is 22 times less than in the common EHD methods. It is also proven possible to generate a single droplet containing live cells by controlling the drop size with no influence of high electric voltage on the cells. Cell viability is confirmed through the cell culture after the live cell dispensing on a cell chip. This new dispensing method may find applications such as generation of biological microarrays for high-throughput screening.

Keywords: Electric charge concentration, picoliter, live cell printing

1 INTRODUCTION

There is a rapid growing need of the drop-based delivery of small quantities of functional materials with specific biological functionalities to the well-defined locations on substrates. Thus, formation of drops with small volumes of nanoliter to picoliter is of great interest for many applications in science and technology, such as biological microarrays[1-7].

Numerous techniques have been developed and applied for drop formation. They are categorized as the contact and non-contact delivery methods. As the contact delivery method, there is a pin spotting which is primitive and robust, but drop-volume is limited up to 1 *nL*. As the non-contact delivery methods, there exist many ones, such as inkjet spotting[8-11], and electro-hydrodynamic (EHD) dispensing method[12-17]. One of them, the last EHD dispensing method is attractive because, unlike the other methods, it can generate drops of the sizes from micrometer to millimeter without changing the size of a capillary nozzle. Even with a millimeter-size capillary nozzle, the EHD dispensing method can generate micrometer-size drops, so that it is easy to manufacture a nozzle not prone to clogging and damage especially for cell printing. However, the printing by the EHD method is limited to the conducting target surface when the nozzle and the target surface are very close. That still has been left one of the major drawbacks even though the EHD printing has been developed up to sub-micrometer resolution[17].

We have developed a substrate surface-independent microdrop generation method for the drop size in the range of picoliter to microliter with a single size capillary nozzle by using the previously mentioned ECC method. The ECC method is completely different from the conventional EHD method, because it has fundamentally different drop formation mechanism. In the EHD method, a drop is formed by the electro-hydrodynamic force when an electric potential is applied to both the conducting nozzle (as an upper electrode) and the conducting target surface (as a lower electrode). On the other hand, in the ECC method, the drop is formed by the electro-hydrodynamic force when the electric potential is applied only to the conducting nozzle, while the target surface is just maintained to be close enough to make a liquid bridge between the nozzle and the target surface. Therefore, the ECC method is not limited by the physical properties of target surfaces, such as the electrical conductivity, the electric permittivity, and the phase, due to the mechanism of electric induction by nature. So, the target substrate surface can be a conductor or an insulator, and a solid phase or liquid phase. With this method, it has been shown that ECC method can generate a single drop that contains live cells by controlling the drop size. Through the cell culture after the live cell dispensing on a cell chip, it has also been shown that there is no influence of high electric voltage on the cell viability.

2 MATERIALS AND METHODS

2.1 Experimental Setup

The schematic experimental setup of the ECC method is shown in Fig. 1. Key components of the system are an upper electrode and a lower target substrate. The upper

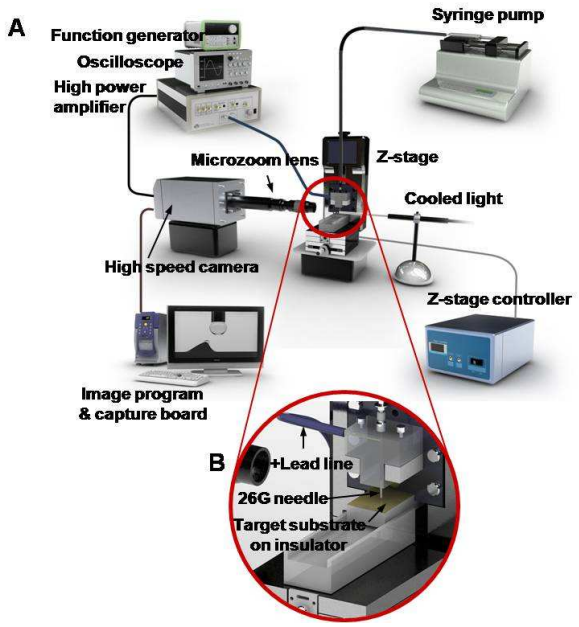

Figure 1. A schematic diagram of experimental setup. **(A)** Whole picture of the experimental setup. **(B)** Close-up image of the main body including a capillary nozzle and a target substrate.

electrode is a capillary nozzle of 26G (OD 460 μm, ID 230 μm, SUS, Hamilton) as a conductor. The lower target substrate is not confined to a conductor. Because of the nature of the electric induction, any surface can be used as the target substrate, which corresponds to the lower electrode of EHD method. Here, several materials were tested, for example, a SUS plate (100 μm-thick stainless steel), a PMMA-coated SUS plate (5 μm-thick PMMA), a cover glass (100 μm-thick), and oil surface (silicone oil, KF56). The upper electrode is installed on a high-precision motorized Z-stage which can control the distance between the capillary nozzle and the target substrate with the accuracy of 10 μm (Sigma Koki, Japan) and the target substrate is placed on the thick insulator (20 mm-thick Polycarbonate).

In our study, an AC electric potential is used as $\psi = \psi_{off} + \psi_{AC} \cos(2\pi ft)$, where ψ_{off} is a offset potential, ψ_{AC} an AC potential, f an AC frequency, and t time, for example, ψ_{off} = 1 kV, ψ_{AC} = 1 kV, and f = 10 Hz or 100 Hz. It is applied by a high voltage power amplifier (Trek, Model 10/10B) and a function generator (Agilent, Model 33120A). They can impose the AC potential (up to 10 kV) with the frequency range of 100 μHz ~ 15 MHz.

For formation and control of initial pendant drops, a syringe pump (KDS210, KD Scientific Inc.) is connected to the capillary nozzle by fluidic channels and adapters. Before application of electric field, an initial pendant drop is made by adjusting the position of the syringe pump very carefully. Once the initial drop becomes stable, the adjustment of the syringe pump position is stopped. That is to say, the flow rate is set by 0. However, during the process of drop elongation and breakup under electric field, slight movement of the syringe pump is allowed.

To study the dynamics of a falling drop in the applied electric field, the drop motion is captured by using a high speed camera (Motion Extra) and a microzoom lens (Navita, max. resolution 1.6 μm/px). It can take images right at the instance of forming and breaking of a liquid bridge occurring less than 1 ms.

2.2 Materials

Two kinds of working solutions are used in this study: one is 99.9 % purified water (HPLC grade, Aldrich), the other is cell-contained medium (Lung epithelial cell, 2×10^6 cells/mL).

For live cell printing, the A549 (Lung epithelial) cells are used and incubated in the presence of CellTracker™ Red CMTPX probes (Invitrogen™) in the cell culture medium (RPMI-1640 + FBS). These probes freely diffuse into live cells and traces for a long term (at least 72 hours at 37 °C). Once inside the cell, these probes react with intracellular components to produce cells that are both fluorescent and viable. Trypsinized cells were suspended again in the medium containing 10 % glycerol. After a drop is dispensed by the ECC method, the drop image containing the live cells is obtained by a inverted microscope (Leica, DMIRB).

3 RESULTS AND DISCUSSION

To investigate the mechanism of the ECC method, proof-of-principle experiments were conducted. The basic experimental setup for the proof-of-principle experiment about the ECC method is sketched in Fig. 2A. An initial pendant drop of water as a conducting liquid is prepared at the tip of a conducting nozzle (OD 460 μm, ID 230 μm, SUS) by a syringe pump. Only the nozzle is connected to the high voltage power supply with an electrical potential of a few kilovolts (2 kV), whereas the target surface is a thin paraffin film with a bump mounted on a perfect insulator which is *not* connected to the high voltage power supply. The corresponding electric circuit is an open state as shown in Fig. 2B. It mimics the situation of the electric induction like lightning onto a tall building.

Fig. 2C shows the results of the proof-of-principle experiments. When a positive high voltage (single sine pulse, offset 1 kV) is applied to the nozzle like in Fig. 2A, a fat water pendant drop (403 nL) is elongated toward the bump with an angle of 52° from the center of the nozzle. Then it touches and forms a liquid bridge between the nozzle and the bump. After the liquid bridge breaks up, a small drop (~ 400 pL) is left on the top of the bump.

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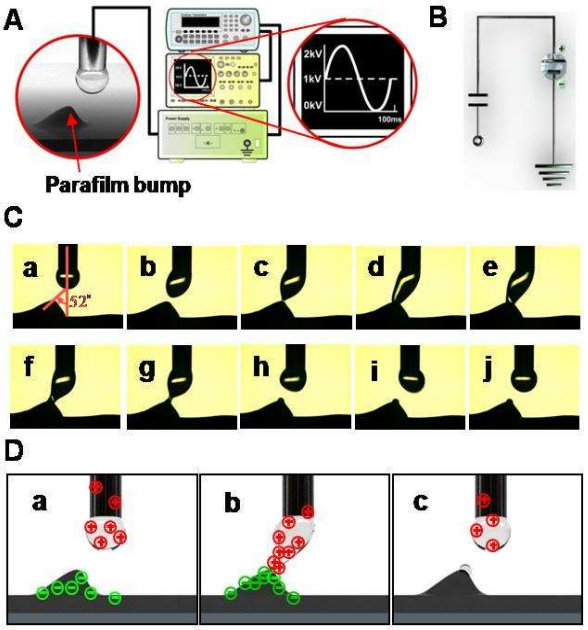

Figure 2. **(A)** A typical experimental setup for proof-of-principle experiments and an electric signal. The positive electric potential is applied to the capillary nozzle (OD 460 μm, ID 230 μm, SUS) and the target substrate is parafilm with a bump as an insulator which is not connected by the power supply. **(B)** The open electric circuit corresponding to (A). **(C)** Successive falling behaviors are captured by high speed camera with 2500 fps and the disintegrated drop volume is about 400 *pL*. **(D)** A schematic drawing for charge distributions of the ECC mechanism.

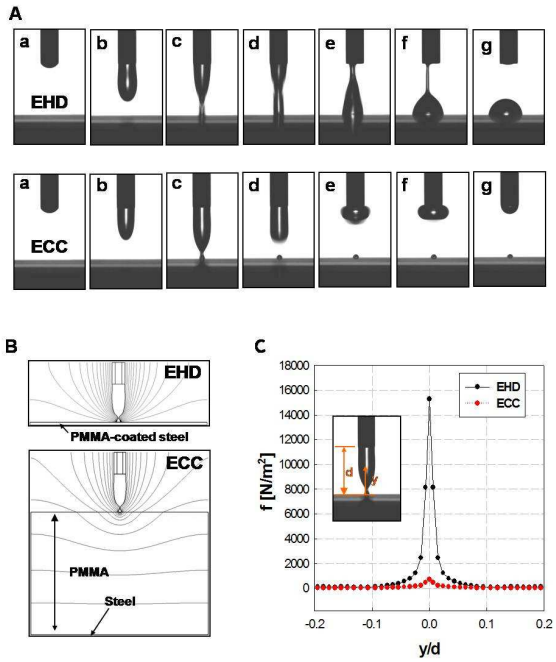

Figure 3. Comparision between EHD and ECC method: **(A)** Successive falling behaviors captured by a high speed camera with 2000 fps. The nozzle size and the electric potential applied to the nozzle are the same between them. Time interval (EHD). **(B)** Electric potential distributions for each EHD and ECC method. EHD: 5 μm-thick PMMA-coated steel plate, ECC: 4 mm-thick PMMA on the steel plate. **(C)** Normal electric stress along the drop surface.

In the experiment, the major time-consuming step is the elongation step before making a liquid bridge (10.4 ms) and the retraction and oscillation step. The former elongation step is governed by the accumulation of electric charges at the apex of the drop. In the meantime, the counter-electric charges are induced on the top of the bump maximally. So, the strong electric field is induced between the drop surface and the bump due to two different electric charges. The drop elongation is facilitated by the electric stress acting on the drop surface as the drop moves toward the bump more and more. After the liquid bridge is formed, the electric charges are instantaneously neutralized between them and the electric force disappears. Subsequently, the liquid bridge breaks up. This is the basic mechanism of the ECC method drawn in Fig. 2D schematically.

The EHD and the ECC methods are similar in the viewpoint of the electric circuit except the connection of the lower electrode. However, the resulting phenomena are totally different between them as shown in Fig. 3A. For the same initial pendant drop of water, the same electric potential is applied to the nozzle. The target substrate is a PMMA-coated steel plate (5 μm-thick PMMA, 100 μm-thick steel) mounted on the perfect insulator, and the distance between the nozzle and the target substrate is 2.0 mm. The final volumes of the formed drops are quite different, such as 133 nL for the EHD method and 791 pL for the ECC method. The difference in the resulting volumes is due to the different drop formation mechanisms. For the EHD method, the mechanism is divided into two stages; drop elongation by an electric force and breakup of a liquid bridge with the assistance of electrowetting tension[14]. Because of the electrowetting tension, it takes long time to sustain the liquid bridge between the nozzle and the substrate (61.5 ms, Fig. 3A-EHD c ~ f). On the other hand, the time to maintain the liquid bridge for ECC method is very short (1 ms, Fig. 3A-ECC c) even though the ECC method has a similar two-stage mechanism. It is due to the latter stage of the mechanism, which is breakup of liquid bridge with the assistance of charge neutralization as shown in Fig. 2D.

As shown in Fig. 3B, the distributions of electric potential for both methods are surely different at the interface of the drop and the substrate. The electric stress normal to the drop surface is calculated along the drop surface and the results are shown in Fig. 3C. The electric stress for the EHD method increases sharply toward the surface of the target substrate and its magnitude of the EHD method is 22 times larger than that of the ECC method. From the results, it is surely expected that the drop touched on the target substrate spreads more in the EHD case than in the ECC case. Consequently a larger drop is formed in the EHD case.

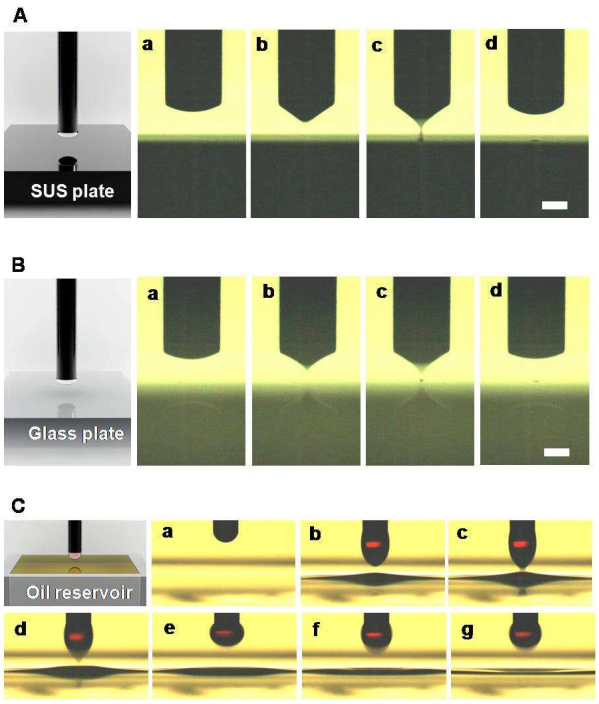

Figure 4. ECC method's independency of target substrates: Electric potential is a single sine pulse (2kV, offset 1 kV, 10 ms). **(A)** Target substrate is SUS plate and the disintegrated drop volume is 17 *pL*. **(B)** Target substrate is glass plate and the disintegrated drop volume is 5 *pL*. **(C)** Target substrate is silicone oil (KF56). White scale bars are 200 μm.

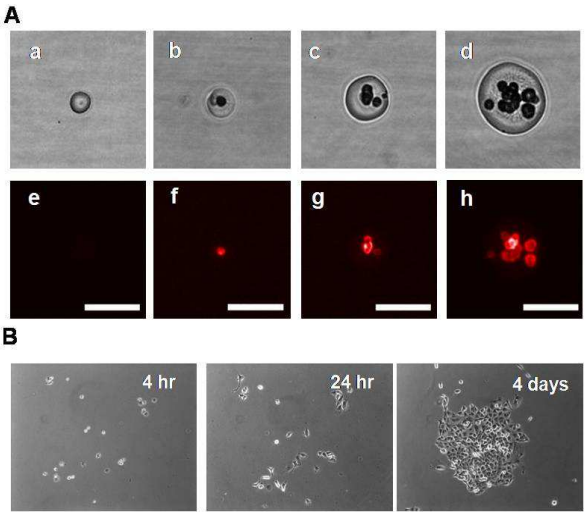

Figure 5. **(A)** Live cell dispensing on a glass plate. **a** ~ **d**: phase-contrast images, **e** ~ **h**: fluorescent images. **a**. 0 cell (spot size 35 μm), **b**. 1 cell (53 μm), **c**. 4 cells (80 μm), **d**. 11 cells (124 μm). Electric potential is the same as in Figure. 4, Scale bar is 100 μm, **(B)** Cell division test in the culture medium after a cell-containing drop is dispensed into the medium.

One of the unique characteristics of the ECC method is the independency of the target surface compared to the EHD method. Most EHD methods require a "conducting electrode" as the target substrate (or a conducting electrode coated with thin insulating material). However, ECC method works on any surface, such as a conductor and an insulator, or a solid and liquid. Figure 4 shows three examples. In Fig. 4A, the target surface is a SUS plate as a conductor, and, in Fig. 4B, a glass plate as an insulator. Two examples show the same successive falling behaviors with the tiny small disintegrated water drops on the target substrate (17 pL for the SUS plate, 5 pL for the glass plate). In addition, Fig. 4C shows an interesting phenomenon, in which the pendant drop is water and the target surface is oil (silicone oil). The deformation of liquid surface is the most obvious example to show the direction of force by nature. When the electric potential is applied to the upper nozzle, the pendant drop is elongated toward the oil surface. Meanwhile, the oil surface rises toward the pendant drop at the same time. After they touch, a small drop is disintegrated into the oil reservoir quickly and the oil surface moves up and down in accordance with the up-and-down motion of the pendant drop.

The cell dispensing is one of several key requirements for the effective use of cell-based assays in high-throughput screening. Traditional and flow-through liquid handlers can be destructive to live cells, and have problems with contamination and clogging of flow paths. As a potential application, the live cell dispensing was conducted by the ECC method without contamination and clogging of flow paths (see Fig. 5A). Lung epithelial cells (A549, 2×10^6 cells/mL) are prepared in the presence of CellTracker™ Red CMTPX probes (Invitrogen™) that freely diffuse into live cells. Once inside the cell, these probes react with intracellular components to produce cells that are both fluorescent and viable. The upper images (Fig. 5A-a ~ d) are obtained by phase contrast microscopy, and the corresponding fluorescent images are Fig. 5A-e ~ h. They show that the cells are viable after being dispensed by the ECC method and a single cell dispensing is possible. The number of cells in the disintegrated drops can be controlled by the drop size.

The test of cell division is a common method to check if cells have their own function. Fig. 5B shows the results of cell division after a cell-containing drop (Lung epithelial cell, A549) is dispensed into the cell culture medium by the ECC method. The results show that the cells in the dispensed drop still have their own function and they are not affected functionally by the ECC method.

4 CONCLUSION

In this paper, we report that a novel method has been developed for drop dispensing that is substrate surface-independent. A single drop of the size in the range of picoliter to microliter can be generated with a single size

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capillary nozzle by using the "electric charge concentration (ECC)" method. Besides providing us with new insights about the electric induction phenomenon, this method is potentially useful for making biological microarrays for various targets, such as viable cells and tissues.

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