

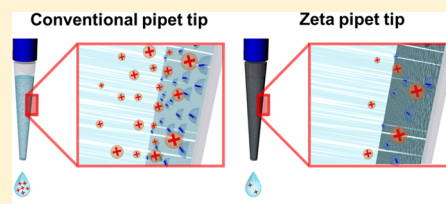
# A Zeta ( $\zeta$ )-Pipet Tip to Reduce the Spontaneously Induced Electrical Charge of a Dispensed Aqueous Droplet

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## Supporting Information

**ABSTRACT:** We report that the zeta potential of a pipet tip's inner surface is one of the crucial parameters for controlling the electrical charge of the dispensed droplet. Since the charge is unexpected and undesirable for most experiments in various fields of science and, thereby, they can cause unsuspected problems, reducing the charge on a dispensed droplet is important for the results of pipetting-based experiments. We fabricate a graphene-based nanocomposite-coated pipet tip, which we called a zeta-pipet tip, as a proof-of-concept example to reduce the zeta potential of the pipet tip's inner surface. The fabricated zeta-pipet tip can successfully mitigate the undesired droplet separation in the droplet merging experiments in an oil bath, which is one of the unexpected effects caused by the electrification. The findings of this study provide helpful guidelines for researchers in many fields of science and technology, who utilize a pipet tip in their respective experiments.



## INTRODUCTION

A pipet is a widely used laboratory tool for transporting an aliquot volume of liquid in experiments conducted in the fields of chemistry, biology, and pharmacy.<sup>1–3</sup> The pipet generally requires a disposable tip, which comes in contact with the liquid, to prevent the cross-contamination of analytical results and maintain efficiency. Recently, our group has reported that pipetting spontaneously induces a considerable amount of electrical charge in an aqueous droplet dispensed using conventional pipet tips.<sup>4</sup> We found that this droplet electrification was due to the interfacial charge resulting from the ionization of the surface chemical groups on a polymeric pipet tip. The amount of spontaneously generated charge in a 7  $\mu$ L deionized (DI) water droplet was measured to be about 0.2 nC. This value is one-quarter of the critical charge at which natural droplet fission occurs due to electrical instability, based on Rayleigh's instability criterion for a conducting droplet.<sup>5</sup> This relatively large amount might lead to experimental errors, such as decreasing surface tension, Coulombic fission of droplets, chemical instability of emulsions, as well as the combination and localization of charged biomolecules.<sup>4,6–12</sup> These charge effects are undesirable for most experiments in various fields of science and engineering; further, they can cause unsuspected problems, such as dispensing of tiny droplets as well as the merging, evaporation, and surface oscillation of droplets.<sup>4,13–15</sup> In particular, one of the strange phenomena observed in our previous experiments demonstrates that the droplets dispensed into an oil bath using a conventional pipet tip could not merge easily with others due to the electrical repulsion among them.<sup>4</sup> The micropipetting that dispenses a submicrometer sized droplet or emulsion could be used to fabricate the extremely small particles.<sup>16–18</sup> In this case, the charge effect on the experimental result might become more significant owing to the small size of

dispensed droplet or emulsion. Since the unexpected effects caused by the electrification on the results of pipetting-based experiments are considerable and undesirable, reducing the charge of a droplet is crucial in the experiments.

In this paper, using theoretical and experimental investigations, we demonstrate that the zeta potential,  $\zeta$ , of the pipet tip's inner surface material is one of the crucial parameters for controlling the electrical charge of the aqueous droplet. We also fabricate a graphene-based nanocomposite coated pipet tip, which we call a zeta-pipet tip, as a proof-of-concept example to reduce the zeta potential of the inner surface of the pipet tip, thereby diminishing the spontaneously induced electrical charge in a dispensed aqueous droplet. By utilizing the fabricated zeta pipet tip, we successfully mitigate the undesired droplet separation in the droplet merging experiments in an oil bath, which is one of the unexpected effects caused by the spontaneous electrification.

## EXPERIMENTAL SECTION

**Materials.** All KCl solutions were prepared by using deionized water (SAMCHUN Chemical, Korea). The graphene nanopowder (12 nm flake, grade: AO-3, Graphene Supermarket) was used to make graphene nanocomposite. HCl and KOH solutions were used to regulate pH of solutions. In droplet merging experiment, highly viscous silicone oil (Shin-Etsu, KF96 50 cSt) was used as the surrounding medium to make the aqueous droplets moving in the low Reynolds number regime.

**Zeta Potential Measurement.** The zeta potentials of the materials were measured by using the zeta potential analyzer (Zetasizer Nano ZS, Malvern, Britain). Especially, the zeta potential of the PE capillary and the Teflon® AF were measured by utilizing surface zeta potential cell

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(Malvern, Britain). The sample was flattened by using thermal flattening process and cut to appropriate size (5 mm × 4 mm) before the measurement. By using the surface zeta potential cell equipped to the Zetasizer Nano ZS, the magnitude of surface zeta potential could be easily measured. Since the zeta potentials of the same substrate in different physical forms are the same, PDMS and fabricated graphene-based nanocomposite were prepared by cutting and grinding the samples to grains to measure the zeta potentials.<sup>19</sup>

**Charge Measurement.** The Faraday cup method was used to measure the charge of a dispensed solutions. The charge was directly measured by using a Faraday cup connected to an electrometer (Keithley Model 6517A). When the charged solutions were dispensed into the Faraday cup, the counter charges were induced from the electrometer, and the electrometer measured the total induced charge.

**Fabrication of the Teflon-Coated Polyethylene Capillary.** The 6 wt % solution of Teflon AF 1600 (Du Pont, Wilmington DE) was diluted to 1 wt % with the FC-40 (Du Pont). To coat the desired fraction of polyethylene capillary with the Teflon solution, at first, the polyethylene capillary was equipped with the conventional pipet, and then aspirating and dispensing of the solution were mildly performed several times for achieving the dip coating. For perfect evaporation of solvent inside the capillary, the coated capillary was cured at 65 °C for 24 h.

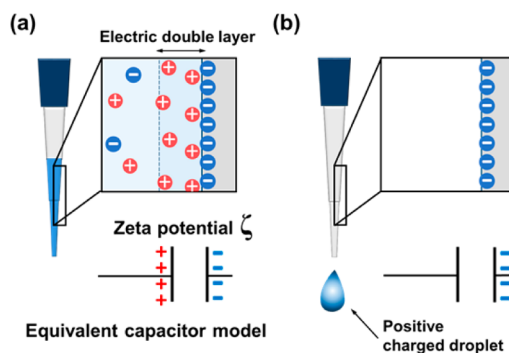
**Fabrication of the Zeta-Pipet Tip.** The PDMS oligomer and the graphene nanopowder were blended (100:6 ratio by weight) via a modified single-screw mixing method.<sup>20</sup> Since the viscosity of the blended nanocomposite was dependent on the nanopowder composition, the maximum blending ratio of the nanopowder was determined based on the nanocomposite viscosity. Then, the curing agent was added at a 10:1 ratio by weight. After equipping the conventional pipet tip with a pipet, aspirating and dispensing of the nanocomposite were performed several times for achieving the dip coating. The air was blown into the pipet tip to avoid blocking of the tip entrance, and then cured at 65 °C for 2 h.

## RESULTS AND DISCUSSION

**Hypothesis and Experimental Validation.** Most solid materials that come in contact with an aqueous medium acquire a surface electrical charge through various mechanisms, such as the ionization of surface groups, isomorphic substitution, or specific ion adsorption.<sup>21</sup> Regardless of the mechanism by which the charge is acquired, the presence of a charged interface influences the redistribution of the free ions in the aqueous medium that, in turn, generates an electrical double layer (EDL). The zeta potential is an electrical potential, and has been widely used for quantifying the magnitude of the electrical charges in the EDL. According to our previous report, the charging procedure of an aqueous droplet inside the pipet tip is the same as the procedure described above.<sup>4</sup> After aspirating the aqueous medium, the EDL is formed near the pipet tip's inner surface (Figure 1). The EDL accumulates electrical charge and acts as an electrical capacitor. In this regard, the capacitance of the EDL can be calculated as follows:

$$C_{\text{EDL}} = \epsilon \frac{A}{\lambda_D} \quad (1)$$

where  $\epsilon$  is the electrical permittivity of the solution,  $A$  is the contact area between the pipet tip's inner surface and the solution, and  $\lambda_D$  is the thickness of the EDL, which has no information regarding the properties of the solid surface. The accumulation of the charge is a response to the electrical potential difference,  $\zeta$ , between the pipet tip's inner wall and the bulk solution. Thus,  $\zeta$  can be applied across an electrical capacitor (Figure 1a). Therefore, the amount of surface charge developed inside the EDL can be calculated as follows:



**Figure 1.** EDL formation during pipetting and its equivalent circuit. (a) The electric double layer is formed when aspirating the solution, and the zeta potential,  $\zeta$ , is a double-layer potential. The EDL acts as an electrical capacitor with the electrical potential difference  $\zeta$  between the pipet tip's inner wall and the solution. (b) The positive charges inside the EDL that comprise the zeta potential are dispensed with the solution.

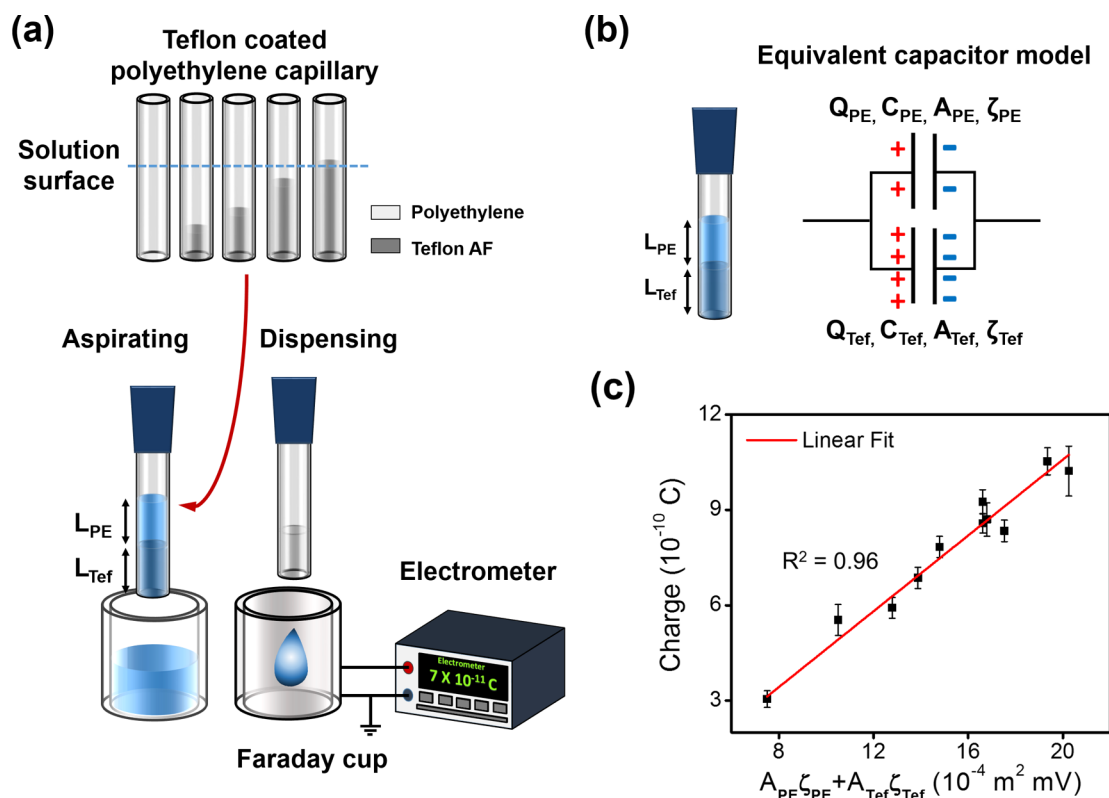
$$Q_{\text{EDL}} = C_{\text{EDL}} \times \zeta = \epsilon \frac{\zeta A}{\lambda_D} \quad (2)$$

Since the  $\epsilon$  and  $\lambda_D$  are the properties of the electrolyte solution, we could treat these properties as constants for a given electrolyte solution. Finally, we can obtain a directly proportional relationship from eq 2 between the charge developed inside the EDL and the zeta potential. This is expressed as follows:

$$Q_{\text{EDL}} \propto \zeta A \quad (3)$$

In the dispensing stage, the charged ions (positively charged ions in the case of Figure 1) inside the double layer, which comprise the zeta potential, are swept and dispensed with the aqueous medium. Thus, we hypothesize that there is a linearly proportional relationship between the charge amount of a liquid droplet dispensed from a pipet tip and the zeta potential of the pipet tip's inner surface material according to eq 3. In addition, the zeta potential of the pipet tip's inner surface material can be used as a quantification parameter of the magnitude of the induced electrical charge of the dispensed droplet.

We conduct the pipetting experiment to verify the above-mentioned hypothesis (Figure 2). The inner surfaces of the polyethylene (PE) capillary tubes are coated with Teflon AF in various coating fractions, resulting in different levels of Teflon AF surfaces inside the PE capillary tubes (Figure 2a). The PE and the Teflon AF are selected as the appropriate materials owing to their hydrophobicity, i.e., the ability to minimize the residual solutions inside the capillary tubes during the dispensing stage. The contact angles (CAs) of the PE and the Teflon AF are measured as 96° and 110°, respectively (SmartDrop, Femtofab, South Korea). The zeta potentials of the PE and the Teflon AF in the KCl 10<sup>−3</sup> M solution were measured as −40 and −108 mV, respectively. Using the zeta potential analyzer (Zetasizer Nano ZS, Malvern, Britain), we found that their magnitudes were similar to those reported in the literature.<sup>22,23</sup> Conventional pipetting procedures, such as aspirating and dispensing the solution, are performed after inserting the coated PE capillary tubes into the pipet (Figure 2a). The amount of induced charge of the dispensed droplet is precisely measured using the Faraday cup method, as plotted in Figure 2c. Considering the charge amount described in eq 3, we developed an equivalent circuit model for the coated capillary, which comprised two capacitors connected in parallel (Figure 2b). According to the electrical circuit theory, the total charges inside the EDL could be



**Figure 2.** Dispensing experiment with a series of Teflon AF-coated PE capillary tubes with various coating fractions and equivalent circuit. (a) The schematic of the pipetting experiment for measuring the charge of a dispensed aqueous medium by the Teflon AF-coated PE capillary tubes with the Faraday cup method. (b) Equivalent circuit of the Teflon AF-coated capillary tube with aqueous solution. The contact length, contact area, zeta potential, and developed charge are denoted by  $L$ ,  $Q$ ,  $A$ , and  $\zeta$ , respectively. (c) Mean measured amount of charge ( $n = 10$ , bars  $\pm$  s.d.) of 200  $\mu$ L KCl  $10^{-3}$  M aqueous solution versus the sum of the product of the contact area and the zeta potential ( $A_{PE}\zeta_{PE} + A_{Tef}\zeta_{Tef}$ ). For the linear plot, the correlation coefficient is  $R^2 = 0.96$ .

calculated by summing up the amount of charges of the two capacitors. This is expressed as follows:

$$Q_{\text{Total}} \propto A_{PE}\zeta_{PE} + A_{Tef}\zeta_{Tef} \quad (4)$$

Equation 4 implies that the total charges of a droplet dispensed from the coated capillary tube has a linearly proportional relationship with the sum of the product of  $\zeta$  and  $A$ . Figure 2c indicates that the experimental results are in very good agreement with the theoretical model shown in eq 4, thus verifying our hypothesis. This result states that (i) the induced charge amount of a droplet dispensed from a pipet tip has a nearly linearly proportional to the zeta potential of its inner surface material, and (ii) the zeta potential could be a quantification measure of the magnitude of the induced electrical charge of a dispensed aqueous droplet.

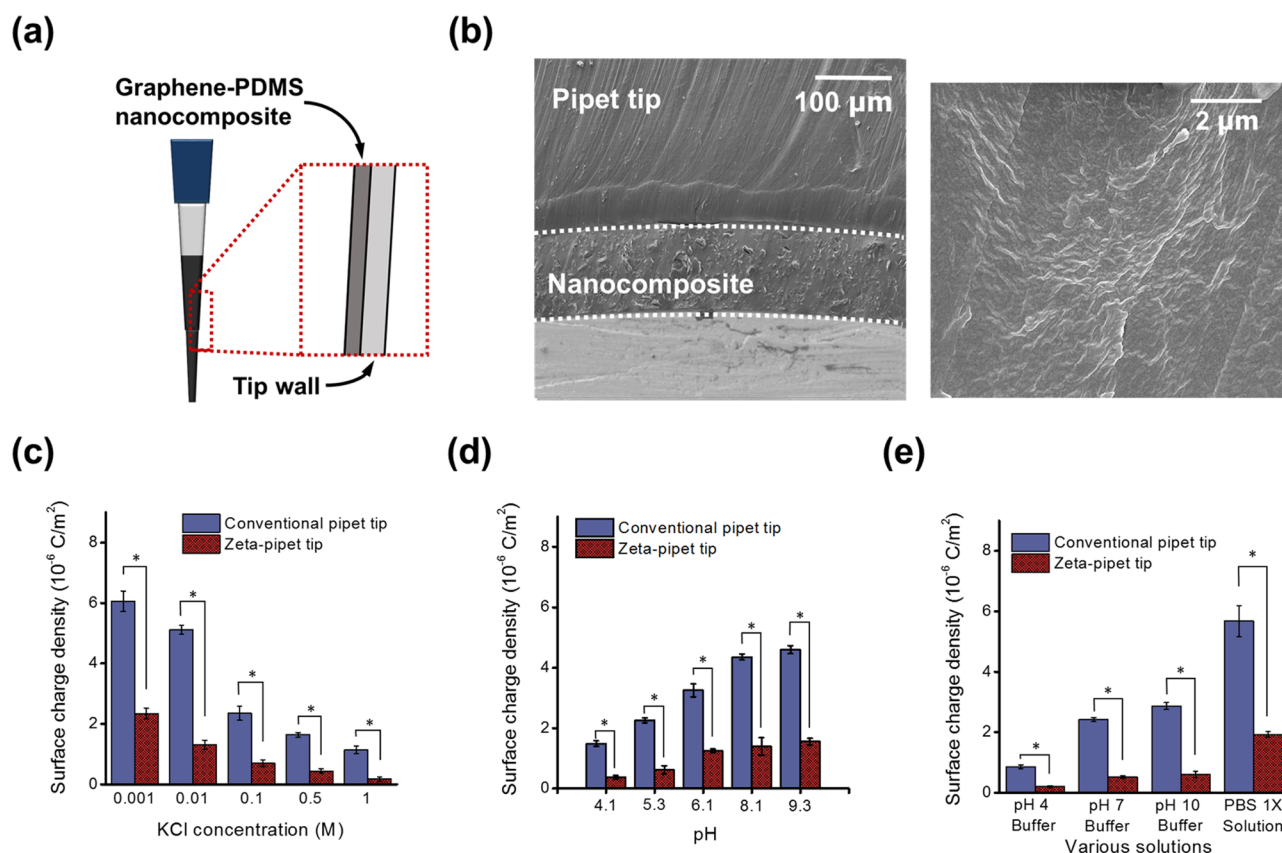
**Zeta ( $\zeta$ )-Pipet Tip.** According to the validated hypothesis in this study, we can reduce the induced charge amount of the dispensed droplet by reducing the zeta potential of the pipet tip's inner surface material. The simplest way to reduce the zeta potential is to coat the inner surface of the pipet tip with an appropriate material that has a low zeta potential and the proper level of hydrophobicity to enable aqueous droplets to easily detach from it.

In this regard, we choose a graphene that has a low zeta potential (about  $-30$  mV in KCl  $10^{-3}$  M solution) and appropriate hydrophobic property as a coating material for the pipet tip's inner surface.<sup>24,25</sup> The graphene nanoflakes (12 nm flake, grade: AO-3, Graphene Supermarket) themselves are unsuitable for coating, so we prepare a graphene-based

nanocomposite solution, which we blend via a batch-type screw mixer.<sup>20</sup> Polydimethylsiloxane (PDMS) oligomer has the following properties: easy handling, good adhesion to the pipet tip, and ideal hydrophobicity. Owing to these properties, the PDMS oligomer is chosen as a solvent for the preparation of the graphene-based nanocomposite. The zeta potential of the nanocomposite with respect to KCl  $10^{-3}$  M solution is measured to be about  $-60$  mV. This value is between the zeta potentials of the pristine graphene nanoflakes (about  $-30$  mV) and the PDMS (about  $-80$  mV) in the KCl  $10^{-3}$  M solution.<sup>24,26</sup> After preparing the graphene-based nanocomposite, the conventional pipet tip's inner surface is coated with a relatively thin, uniform nanocomposite film ( $\sim 100$   $\mu$ m) through the dip-coating method (Figure 3b).

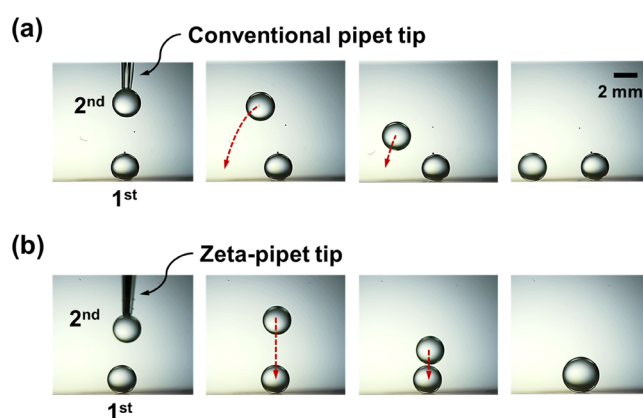
Utilizing the graphene-based nanocomposite-coated pipet tip (zeta-pipet tip), we performed the pipetting experiments to compare the charge amounts of droplets produced by the conventional pipet tip and our zeta-pipet tip. A series of solutions are used by changing KCl concentrations (Figure 3c), pH (Figure 3d) and the types of solutions (Figure 3e), in order to show the independency of the charge reduction behavior on the solution constituents. The results reveal that the induced charge of the droplet from the zeta-pipet tip is always lower than that from the conventional pipet tip, regardless of the concentration, pH level, and type of solution. With this result, we could guess the zeta potential of the conventional pipet tip that we used in this experiments (detailed information in the Supporting Information).





**Figure 3.** Fabricated graphene-based nanocomposite-coated pipet tip (zeta-pipet tip) and the charge amount comparison results between the droplet dispensed by the conventional pipet tip and the zeta-pipet tip. The volume of the droplets is 7  $\mu\text{L}$ . (a) Schematics of the fabricated tip. (b) Scanning electron microscopy (SEM) images of the cross-section of the pipet tip and the graphene/PDMS nanocomposite. (c) Experimental results comparing the charge amount of the droplets dispensed by conventional pipet tip and the zeta-pipet tip with the solutions of a series of KCl concentrations. (d) Experimental results with various pH solutions. The electrolyte concentrations of the solutions are all  $10^{-2}$  M. (e) Charge comparison results with various kinds of solutions. The charge reduction behaviors are independent of the solution constituents. At the 5% significance level, all of the results show significant difference.

Utilizing the zeta-pipet tip, we performed an aqueous droplet merging experiment in an oil bath as a proof-of-concept application. Due to the electrical repulsion between two microliter-sized droplets consecutively dispensed from the conventional pipet tip, it was difficult for them to merge in an oil bath (detailed information in the Supporting Information).<sup>4</sup> The critical charge amount required for a droplet to merge with another is estimated using a force balance model (detailed calculation process description in the Supporting Information). The estimated critical charge can be considered as a maximum charge amount for the spontaneous merging of the charged droplets in the oil bath. When two 7  $\mu\text{L}$  droplets of KCl  $10^{-2}$  M solution are separated at a distance of 2 mm, similar to the diameter of the droplet, a critical charge amount can be calculated as  $3.2 \times 10^{-11}$  C. The charge amount of the 7  $\mu\text{L}$  droplet of KCl  $10^{-2}$  M solution dispensed from the conventional pipet tip is  $7.6 \times 10^{-11}$  C, which is beyond the calculated critical charge, thus preventing the spontaneous merging of the two droplets in oil (Figure 4a, Supporting Video 1). In the case of the zeta-pipet tip, the charge amount of the 7  $\mu\text{L}$  droplet of KCl  $10^{-2}$  M solution is about  $2.7 \times 10^{-11}$  C. This value is lower than the critical charge calculated above, thus resulting in the successful spontaneous merging of the droplets in oil (Figure 4b, Supporting Video 1).



**Figure 4.** Results of the droplet merging experiment in an oil bath. Two 7  $\mu\text{L}$  KCl  $10^{-2}$  M droplets are dispensed using (a) the conventional pipet tip and (b) the zeta-pipet tip. (Supporting Video 1).

## CONCLUSION

The spontaneously induced electrical charge of an aqueous droplet dispensed using a pipet tip is successfully reduced based on the reduction of the zeta potential of the tip's inner surface material. Using an equivalent electrical circuit model and pipetting experiments with Teflon AF-coated PE capillary tubes, we show that the charge amount of the dispensed aqueous

droplet is a nearly linearly proportional to the zeta potential of a pipet tip's inner surface material. To reduce the charge amount of the dispensed droplet, we fabricated a novel zeta-pipet tip by coating the pipet tip's inner surface with a graphene/PDMS nanocomposite, which has a low zeta potential. Using the zeta-pipet tip, we mitigate the undesirable droplet separation in the droplet merging experiments in an oil bath. Since the charge of a dispensed droplet is unexpected and undesirable for most experiments, reducing the charge on a dispensed droplet is crucial on the results of pipetting-based experiments. In this regard, this study first provides a helpful guideline for researchers in many fields of science and technology who utilize pipet tips that suffer from the unexpected electrification phenomena of the dispensed droplet from pipet tip.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Guessing about the zeta potential of a conventional pipet tip, detailed information about the droplet merging experiment and calculation procedure of critical charge. A movie clip showing the results of droplet merging experiment by using conventional pipet tip and zeta pipet tip. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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### Notes

The authors declare no competing financial interest.

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