Supplementary Information

Spontaneous electrical charging of droplets by conventional pipetting

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Supplementary Tables

Table S1

Droplet	Charge (x 10^{-11} C)
Protein suspension	-0.98±0.10
Cell suspension	-1.53±0.16
DNA solution	7.01±0.72
Phosphate buffered saline 1x	8.04±0.66
NaCl 154mM	9.65±0.67
Orange juice	-0.72±0.21
Milk	-1.95±0.66
Coffee	-1.79±0.69

Table S1: Charges of various solutions. We have measured the charges of solutions of bio-macromolecules (Protein suspension: Bovine serum albumin 2% with DI water, Cell suspension: fibroblast 5.6 million/mL in Dulbecco's modified eagle medium (DMEM)), DNA solution (DNA 200 μg/mL in diethylpyrocarbonate (DEPC) solution) and others. Droplet volume was 7-μL. Each charge value was the average of 8 measurements. Relative humidity (RH) was 25%.

Table S2

	Forward pipetting	Reverse pipetting
Charge (x 10^{-11} C)	18.0±1.38	4.5±0.64

Table S2: Comparison of the charge amount between forward pipetting (normal) and reverse pipetting. For the comparison, we have measured the charges of the 7-μL deionized water with two different pipetting modes; 1) forward 2) reverse. The charge amount of the droplet dispensed by reverse pipetting mode was 25% of that by forward pipetting mode.

Table S3

Droplet	Applied voltage (V)	Charge (x 10^{-10} C)
7-μL DI water	500	2.15
	600	2.32
	700	2.2
	800	2.12
	900	2.32
	1000	2.41

Table S3: Charge measurement varying the applied voltages. To test the effect of applied voltages on charge measurement, experiments were carried out under varying applied voltage (500 ~ 1000 V). We could get consistent results regardless of the applied voltage.

Table S4

Solution	Volume (μL)	Charge (x 10^{-11} C)	Applied voltage (V)
DI water	3	12.9	300
	5	14.9	500
	7	20.9	700
	9	26	800
PBS	3	3.85	800
	5	6	1000
	7	8	1100

Table S4: Applied voltages adjusted according to the size and the charge of the droplets. The falling velocity of a droplet in oil depended on the size and density of the droplet. For extracting the accurate velocity information of the droplet, it was needed to apply appropriate electric field which causes the deflection of the droplet movement. We applied higher electric field to droplets which have faster falling velocity or smaller amount of the charge.

Supplementary Figures

Figure S1

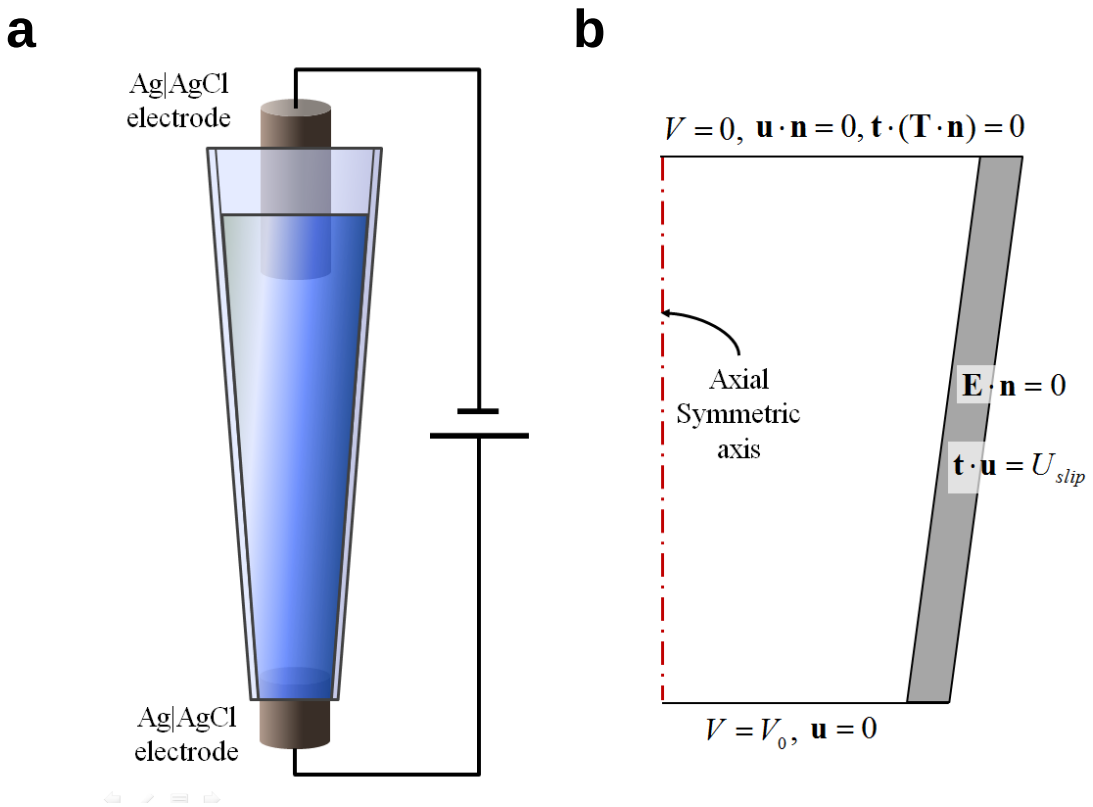

Figure S1: Schematic diagrams of the experiment and numerical analysis for electroosmosis in pipette tips. (a) Ag|AgCl electrodes were used to apply electric field for preventing bubble formation. The distance between two electrodes was about 23 mm. (b) Domain of numerical analysis and applied boundary conditions.

Figure S2

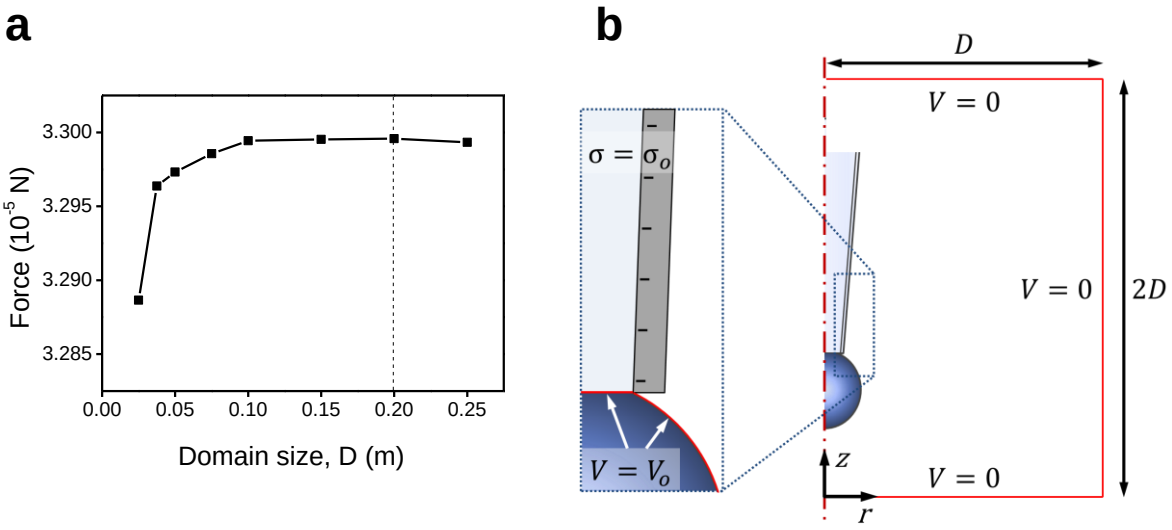

Figure S2: Numerical results of the forces on the charged droplet depending on the domain size and the schematic diagrams of the numerical analysis for evaluating the electrostatic force. (a) The magnitude of the force on the charged droplet is saturated with increasing domain size D. (b) Domain of numerical analysis and applied boundary conditions.

Figure S3

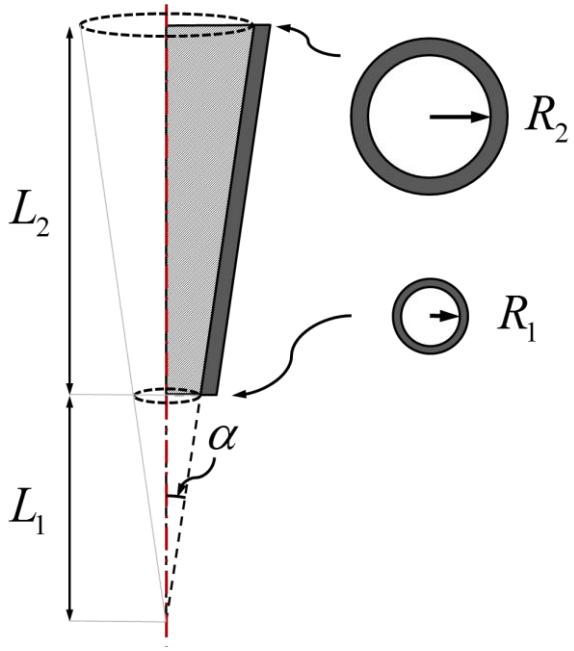

Figure S3: Schematic diagram of the pipette geometry. To measure the geometry of pipette tips, we cut the pipette at the middle. The distance between the narrow end of the pipette tip and the cross section, L_2, and the radii of them, R_1 and R_2, were measured.

Supplementary note

Calculation of the contact area between liquid and a pipette tip

To calculate the contact area between the liquid and the pipette tip according to the aspirated volume, we got the geometry information of the pipette tip from the cut piece of the pipette tip as shown in Supplementary Figure S3. Since the inner radius of the pipette tip is linearly narrowed, we need only obtain the distance between the narrow end of the pipette tip and the cross section, L_2, and the radii of them, R_1 and R_2. Using the measured values, the inclined angle of the inner surface of the pipette tip,

$$\alpha = \mathrm{atan}\left(\frac{R_2 - R_1}{L_2}\right),$$

can be calculated. Here, we introduce an imaginary cone which is drawn by extending the narrow end of the pipette as shown in Supplementary Fig. S3. The height and volume of the cone, L_1 and V_1, are expressed by

$$L_1 = R_1 \cot(\alpha) \quad \text{and} \quad V_1 = \frac{1}{3}\pi R_1^3 \cot(\alpha),$$

respectively. Finally, we obtain the contact area as

$$A = 2\pi \sec(\alpha)\left[R_1 L + \frac{1}{2}\tan(\alpha) L^2\right],$$

where the height and volume of the aspirated liquid are $L = \left(\sqrt[3]{1 + V/V_1} - 1\right) L_1$ and V, respectively.

Additional information on technical interests in pipetting

Pipette tip brand

There are many manufacturers of pipette tips. They may have different coating material and geometry of the tips. Therefore, pipette tip brands can be a parameters affecting the amount of charge. Figure S4 shows the effect of pipette tip brands on the charge of a droplet. Pipette tip brands affected both the magnitude of the charge and its rate of increase with respect to droplet volume.

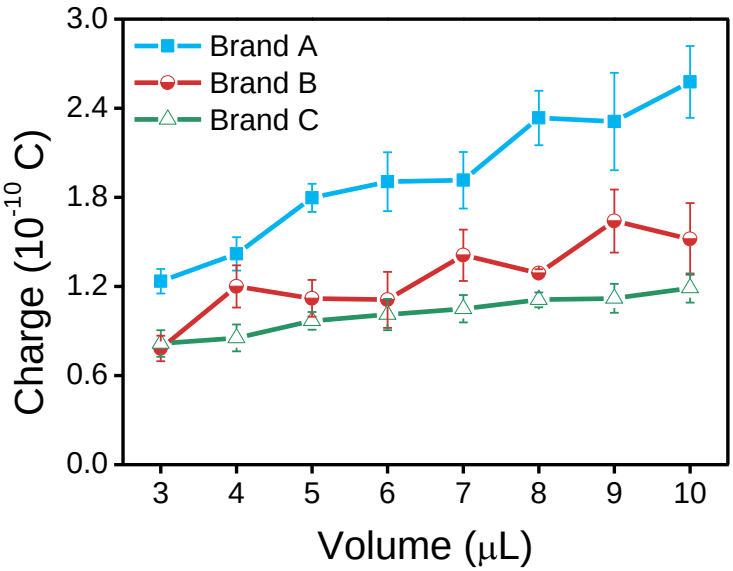

Figure S4: Effect of pipette tip brands on the charge of a droplet. Mean measured charge of a deionized water droplet at relative humidity (RH) 50 % and pH = 7.4.

The reasons for these differences could be the differences in the geometry of the tips, the mold surface texture, the coating material used in them. The difference in the geometry of the tips leads to that in the contact areas corresponding to the volume of each droplet. However, the difference in the contact area was not as great as that in the charge amount, as shown in Supplementary Table S5. The mold surface texture can affect to the effective contact area.

The effective contact area increases as the roughness of the inner surface of pipette tips increases. But the surface of pipette tips was very smooth (Supplementary Figure S5), so the roughness effect might be negligible. Therefore, the major reason for the difference between the tip brands would be the coating material used in them, as the results shown in Figure 6b in the paper. The materials of the pipette tips cannot be reported because of a trade secret, although it may enhance fundamental understanding and provide more information on charging mechanism.

Volume (μL)	Contact area (mm^2)		
	Brand A	Brand B	Brand C
3	16.5	17.2	16.9
5	24.3	25.3	24.9
7	31.3	32.5	32.0
9	37.6	39.1	38.5

Table S5: Contact area between liquid and a pipette tip corresponding to the volume of droplets.

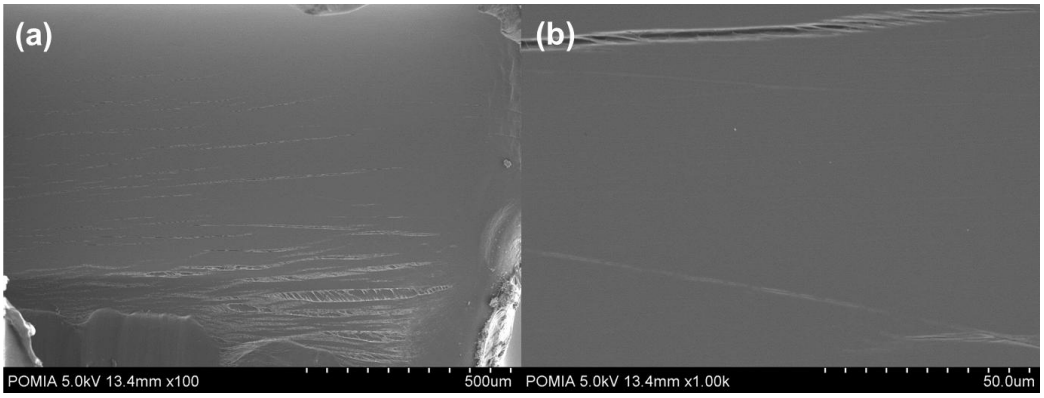

Figure S5: Scanning electron microscopy (SEM) images of inner surface of pipette tips. (a) Low-magnification SEM image. The cracks were caused in cutting to open the inner

surface of the pipette tip. (b) High-magnification SEM image. The undamaged surface of the pipette tip was very smooth.

Effect of pipetting rate

Pipetting rate was also one of the interesting parameters. To investigate the effect of the pipetting rate, we controlled the pipetting rate by using a syringe pump and syringe which was connected to a pipette tip. Aspirating and dispensing were performed by withdrawing and infusing a syringe, respectively. The pipetting rate was from 30 to 600 μL/min. No significant effect of pipetting rate was observed as shown in Figure S6.

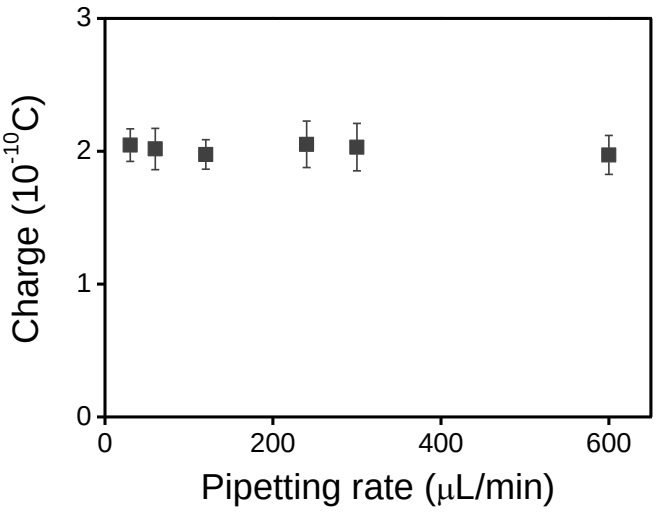

Figure S6: The relationship between the droplet charge and the pipetting rate. 7-μL deionized water droplets were dispensed with different pipetting rates. No significant effect of pipetting rate was observed.