Operando Investigation of the Locally Enhanced Electric Field Treatment (LEEFT) Harnessing Lightning-Rod Effect for Rapid Bacteria Inactivation

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ABSTRACT. The growth of undesired bacteria causes numerous problems. Here, we show that locally enhanced electric field treatment (LEEFT) can cause rapid bacteria inactivation by electroporation without any side reactions. The bacteria inactivation is studied *in situ* at the single-cell level on a lab-on-a-chip that has nanowedge-decorated electrodes. Rapid bacteria inactivation occurs specifically at nanowedge tips where the electric field is enhanced due to the lightning-rod effect. The mechanism study shows that the bacteria inactivation is caused by electroporation induced by the locally enhanced electric field. The bacteria inactivation performance depends on the strength of the enhanced electric field instead of the applied voltage, and no ROS generation is detected when >90% bacteria inactivation is achieved. Quick membrane pore closure under moderate LEEFT indicates that electroporation is the predominant mechanism. LEEFT only requires facile treatment to achieve bacteria inactivation, which is safe for treating delicate samples and energy-efficient for large scale applications. The findings in this work can provide strong supports for the future applications of LEEFT.

KEYWORDS. Locally enhanced electric field treatment, Lightning-rod effect, Electroporation, Antimicrobial, Nanostructures, Bacteria, Operando investigation

Bacteria are indispensable for both ecological systems and human bodies, but the growth of undesired bacteria can also cause serious problems. Seeking approaches for bacteria inactivation is an everlasting effort. Most of our current practices for bacteria inactivation highly rely on the uses of chemicals, such as antibiotics for infection treatment, chlorine for water disinfection, antiseptics for food preservation, and chemical anti-fouling agents. They have been effectively inactivating bacteria, but caused new problems: overuse of antibiotics has already raised the concern of antibiotic resistance,¹ chlorination generates disinfection by-products (DBPs) that can be carcinogenic;² food antiseptics and anti-fouling agents themselves may be harmful to human health or the environment.

Effective physical processes, such as thermo/ultraviolet radiation,^{3, 4} acoustic vibration,^{5, 6} microwave,⁷ and electric field treatment (EFT),⁸ can be superior alternatives to chemical approaches for bacteria inactivation, although many of them suffer from high capital cost or energy consumption. Among these processes, the EFT is increasingly finding applications in food preservation and water disinfection.⁹⁻¹² The EFT aims to inactivate bacteria by electroporation: when a cell is exposed to a strong electric field, an induced transmembrane voltage (TMV) will cause pore formation on the lipid bilayer membrane,¹³⁻¹⁵ and when this external electric field is strong enough, the membrane damage, *i.e.*, the pores, will become lethal to the bacterial cells.¹⁰ The lethal electroporation threshold was found to be between 10 ~ 35 kV/cm.¹⁶ Typically, in order to achieve the strong enough electric field, the EFT processes will require high applied voltages (e.g., ~ 23 kV to achieve 35 kV/cm on the electrodes with 0.65 cm distance),¹⁷ which leads to safety issues, side reactions, and high energy consumption.

A strategy to realize the high electric field strength with lower voltages is to decorate the electrodes with sharp objects, such as nanowires or nanowedges. Attributed to the lightning-rod effect, the electric field near the tips could be largely enhanced depending on the aspect ratio of the electrode decorations.¹⁸ As a result, even with relatively low applied voltages, the locally enhanced electric field can still build up the transmembrane voltage that is sufficient to cause irreversible electroporation and bacteria inactivation. Although this concept has been claimed as the predominant mechanism for bench-scale EFT water disinfection devices equipped with nanowire-modified electrodes,¹⁹⁻²⁸ direct demonstration of lightning-rod effect for bacteria inactivation, especially at the single-cell level, is not yet done. Here, we conduct locally enhanced EFT (LEEFT) on a lab-on-a-chip device that has nanowedge-modified electrodes and investigate the microbial inactivation process *in situ*. Results show that the bacteria located at the tips of nanowedges on both positive and negative electrodes are rapidly inactivated with the voltages that are not sufficient to kill bacteria in bulk. Electroporation induced by the locally enhanced electric field attributed to the lightning-rod effect is demonstrated to be the predominant mechanism for this bacteria inactivation.

Lab-on-a-chip has been intensively used for operando investigation of microbiology related processes.^{13, 29, 30} We developed a lab-on-chip device with gold nanowedges fabricated on both positive and negative electrodes (**Fig. 1a & Fig. S1**). The gap between the two electrodes is 50 μm . The length and thickness of the nanowedge are 8 μm and 200 nm, respectively. The width of the nanowedge tip is 200 nm, and it gradually increases to 1 μm to allow a steadier connection to the bulk electrode. This is the default chip design for our experiments unless otherwise stated. When an 18 V voltage is applied to the two

electrodes, the electric field near the nanowedge tips will be enhanced due to the lightning-rod effect, which is simulated using COMSOL Multiphysics (**Fig. 1b**).

The chip was pre-coated with poly-L-lysine, and the model bacteria *Staphylococcus epidermidis* (*S. epidermidis*) cells were uniformly attached on the chip (**Fig. 1c**). Since the bacteria cells are negatively charged, they are firmly immobilized by the positively charged poly-L-lysine and will remain at the original place even after electrical pulses. Live-and-dead cell distinguishing stain propidium iodide (PI) was added in the deionized water (DI water) medium before treatment (See experimental setup in **Fig. S2a**). After 500,000 electrical pulses at 18 V with 2 μ s pulse width and 100 μ s period are applied (denoted as 18 V/2 μ s/100 μ s/500,000 pulses, see the waveform in **Fig. S3**), the bacteria at the tips of nanowedges on both positive and negative electrodes show red fluorescence of the PI stain, indicating cell membrane damage, while cells anywhere else are intact (**Fig. 1d**). The zoom-in image clearly shows that only the cells located very close to the nanowedge tips are damaged, which is consistent with the electric field enhancement pattern (**Fig. 1b**). By comparison, for the electrodes that have no nanowedge modification but a smaller gap of 34 μ m, hardly any cells are affected (**Fig. S4**), suggesting that this electrical treatment is not sufficient to kill bacteria in bulk or on electrode edge. Therefore, LEEFT can cause bacteria inactivation with lower applied voltages than in bulk EFT.

The bacteria damage process was observed in real-time. The onset position of PI fluorescence indicates that the cell membrane damage takes place at the position adjacent to the nanowedge tip, where the nano-enhanced electric field has the highest strength. The circled bacteria cells at the nanowedge tips on the negative electrode (**Fig. 1e**) and positive electrode (**Fig. 1f**) do not show fluorescence before the treatment (0 s). The arrows indicate

the location where the cell membrane is adjacent to the nanowedge tip. After the treatment starts, the red fluorescence of PI stain first originates from the adjacent point indicated by the arrows (shown in 0.1 s, 0.2 s, and 0.4 s), suggesting that the part of the cell membrane subjected to the strongest electric field will be perforated first.

Figure 1. Bacteria damage in LEEFT. (a) Microscopy image of the lab-on-a-chip device. (b) The nano-enhanced electric field at the nanowedge tips at 18 V applied voltage. (c & d) Microscopy images of the immobilized bacteria cells before (c) and after (d) LEEFT treatment. Scale bars are 10 μm in normal images and 5 μm in the zoom-in images. (e & f) PI fluorescence onset indicating pore formation position of a cell at nanowedge tip on negative electrode (e) and positive electrode (f). The arrows indicate the position of the cell membrane adjacent to the nanowedge tip, which is also the onset position of PI fluorescence. Scale bars are 5 μm .

The bacteria damage occurs rapidly in LEEFT, which can be seen from the video **Movie S1**. To figure out how fast it is, different effective treatment time (i.e., the total time

that the applied voltage is not zero, equals to pulse width $\times$ pulse number) was tested by applying different pulse numbers of 2 μ s/100 μ s pulses. Under 30 V and 18 V applied voltage, 0.1 ms and 1 ms of effective treatment times are long enough to achieve >80% bacteria damage (represented by the percentage of nanowedges inducing bacteria damage at tips), respectively, indicating that bacteria damage in LEEFT is very rapid (**Fig. 2a**). Under relatively lower applied voltages (14 V and 10 V), bacteria damage efficiency stays at low percentages up to 1 s of effective treatment time, suggesting that the limiting factor of the lower bacteria damage is the applied voltage rather than treatment time (**Fig. 2a**). Therefore, the bacteria damage efficiency at different applied voltages with 1 s effective treatment time were tested. In case the cell damage was reversible, we conducted parallel experiments but stained the cells with PI 2 hours after treatment. Since reversible membrane damages should already recover after 2 hours, the cells stained with PI are considered inactivated.¹⁴ There is no significant difference between the efficiency of cell damage and inactivation, except that cell inactivation is even higher especially at low voltages (Fig. 2b), which is due to random cell inactivation in all cells after 2 hours. The efficiency shows a positive correlation with the applied voltage (**Fig. 2b**). It starts at a low voltage of 10 V, and 20 V is already high enough to achieve bacteria inactivation for almost all nanowedges, and there is no significant difference between the positive and negative electrodes. This result indicates that with 1 s effective treatment time, most cell damage is irreversible, leading to cell inactivation. The phenomenon that damaged bacteria lost cell integrity and decayed after stored in nutrient broth at 35 °C for 6 hours further confirms cell inactivation (Fig. S5). Therefore, when 1 s effective time is used, we consider the cell inactivation efficiency is approximately the same with cell damage.

The high aspect ratio of the nanowedges is important to LEEFT, indicated by the control experiments with different chip designs (**Figs. 2c - f**). After treatment of 18 V/2 μ s/100 μ s/500,000 pulses, the nanowedges with 200 nm width at the tip (**Figs. 2c & e**) and 8 μ m length (**Figs. 2d & f**) show a much higher efficiency of bacteria inactivation than other wider or shorter electrode modifications. LEEFT also works for free-moving bacteria cells suspended in the medium. Syto 9 and PI-stained *S. epidermidis* are suspended in DI water before the treatment (**Fig. 2g upper**. See the experimental setup in **Fig. S2b**). During the LEEFT (18 V/2 μ s/100 μ s/500,000 pulses), bacteria cells are attracted toward the nanowedges on both positive and negative electrodes, especially to the tips. Subsequently, those near the tips get inactivation, indicated by switching from green fluorescence of Syto 9 to red fluorescence of PI (**Movie S2, Fig. 2g lower**). As the bacteria cells are negatively charged in DI water, most of them accumulated at the positive electrode because of the electrophoretic force. Some cells are attracted to the nanowedge tips on the negative electrode, which is probably due to the strong dielectrophoretic force induced by the electric field enhancement near the tips.^{27, 31} Two other kinds of bacteria, *Bacillus subtilis* (*B. subtilis*, Gram +) and *Escherichia coli* (*E. coli*, Gram -), are also tested, which show similar transport and inactivation phenomena with *S. epidermidis* (**Figs. 2h & i, Movies S3 & S4**), suggesting that LEEFT could be a wide spectrum bacteria inactivation method.

Figure 2. Bacteria inactivation characterization in LEEFT. (a) Bacteria damage efficiency (i.e., the percentage of nanowedges inducing bacteria damage) with different effective treatment time. Positive and negative electrodes are denoted as + and –, respectively. (b) Bacteria damage and inactivation efficiency versus the applied voltage with 1 s effective treatment time. (c - f) Bacteria inactivation with wedges of different width (c & e) and different length (d & f). (g – i) Microscopy images of different kinds of bacteria in suspension before (upper) and after (lower) the LEEFT. (g) *S. epidermidis*. (h) *E. coli*. (i) *B. subtilis*. The scale bars are 10 μm .

In LEEFT, only the bacteria located near nanowedge tips are inactivated, while bacteria in bulk are not affected. This pattern is consistent with the electric field enhancement of nanowedges due to the lightning-rod effect, suggesting that irreversible

electroporation induced by the enhanced electric field is the most possible mechanism for bacteria inactivation. Here, we investigated the mechanism, and the evidence collected is discussed below.

First, we found that the bacteria inactivation depends on the strength of the nano-enhanced electric field rather than the applied voltage. Chips of different positive/negative electrode gaps (25 μm , 50 μm , 100 μm) and with nanowedges of different intervals (0.8 μm , 4 μm , 40 μm) were tested for bacteria inactivation. The strength of the nano-enhanced electric field is reversely proportional to the gap between the two electrodes (**Figs. S6a, b & c**). Therefore, with the same applied voltage, chips with a smaller gap achieve a higher percentage of bacteria inactivation (**Fig. 3a & Fig. S6d**). Similarly, because of the stronger lightning-rod effect for electric-field enhancement (**Figs. S7a & b**), the nanowedges with a larger interval in between could achieve higher bacteria inactivation under the same applied voltage (**Fig. 3b & Fig. S7c**). When all the results are analyzed, the percentage of bacteria inactivation at the tips of nanowedges shows a positive correlation with the electric field strength (**Fig. 3c left**), but not with the applied voltages (**Fig. 3c right**). This result indicates that the bacteria inactivation is attributed to the nano-enhanced electric field.

The bacteria inactivation is not attributed to reactive oxygen species (ROS). Although inducing ROS is a commonly used antimicrobial method, it has some side effects, including generating by-products or causing sample damage when high sample quality needs to be preserved, such as blood sample or liquid food. Electric-field treatment systems could generate ROS, especially under high voltages or long treatment times. To test if the bacteria inactivation is attributed to ROS damage, DCFH-DA stain was used to detect ROS generation.^{32, 33} In the experiment group with 30 V/2 μs /100 μs /100,000 pulses treatment,

DCFH-DA stained cells show no fluorescence (**Fig. 3d upper**), suggesting no ROS generation. Meanwhile, >90% bacteria inactivation is achieved (**Fig. 3d lower & experiment group, no DMSO in Fig. 3h**), indicating that this bacteria inactivation is not due to ROS damage. To confirm this ROS detection method is valid, we intentionally induced ROS generation with a much longer pulse width in the positive control (20 V/200 μ s/10 ms/1000 pulses). The significant green fluorescence of DCFH-DA stained cells shows that ROS is generated near the positive electrode (**Fig. 3e upper**). The positive electrode shows more inactivated bacteria at each nanowedge tip than the experiment group and negative electrodes (**Fig. 3e lower & positive control, no DMSO in Fig. 3h**), which could be attributed to the ROS damage.

To further confirm that the bacteria inactivation at 30 V/2 μ s/100 μ s is not due to ROS, a ROS scavenger, DMSO, was added to the medium at 15% (w/w) to quench ROS and protect bacteria from its damage.³⁴ In the positive control group, the bacteria at the positive electrodes are largely protected by DMSO (**Fig. 3g & positive control in Fig. 3h**), proving that 15% DMSO is able to protect bacteria from ROS damage. In the experiment group, even with the ROS scavenger, the bacteria inactivation percentage and inactivated cell number are not affected (**Fig. 3f & experiment group in Fig. 3h**), which further confirms that the bacteria inactivation is not due to ROS damage.

Figure 3. Antibacterial mechanism investigations. (a) Bacteria inactivation on chips of different gaps between positive and negative electrodes. (b) Bacteria inactivation on chips of different intervals between nanowedges. (c) Relationship between the bacteria inactivation and the electric field strength (EF) at the tip of the nanowedge (0.1 μm away from the nanowedge tip) (left), and the applied voltage (right). (d) Fluorescence of DCFH-DA-stained cells (upper), and bacteria inactivation with no DMSO lower in experiment group (lower). (e) Fluorescence of DCFH-DA-stained cells (upper), and bacteria inactivation in positive control group (lower). Green fluorescence indicates ROS

generation. (f & g) Bacteria inactivation in (f) experiment group and (g) positive control with 15% DMSO. (h) Bacteria inactivation percentage and average inactivated cell number at each nanowedge tip. Scale bars are 10 μm .

The third piece of evidence is quick cell membrane recovery after LEEFT, which also supports that electroporation is the main bacteria inactivation mechanism. Reversible electroporation is a phenomenon that pores formed on the lipid bilayer membrane reseal automatically after the electric field is removed. It occurs when the cell is exposed to a relatively weaker electric field or shorter treatment time than irreversible electroporation.¹⁴ The PI fluorescence intensity of four cells under 14 V/2 μs /100 μs intermittent treatment shows that when the treatment is on (red shadow, 10,000 pulses for 1 s total time), the fluorescence increases, which means pore formation and PI dye inflow (**Fig. 4a**). When the treatment is removed (gray shadow, 5 s), the fluorescence stops rising immediately, suggesting that the pores close and the membrane regains its integrity after the treatment stops (**Fig. 4a**). This kind of quick cell membrane recovery is a common phenomenon in reversible electroporation,^{13, 14} but is hard to find in other kinds of membrane damages, such as direct oxidation. Therefore, quick pore reseal is strong evidence for reversible electroporation.

Reversible electroporation was also tested using a double staining method with SYTOX Green and PI, which can only enter cells with compromised membrane.³⁵ SYTOX Green is first added to the medium (Time point 1, **Fig. 4b**). After the LEEFT is applied, perforated cells are stained with SYTOX Green and show green fluorescence (Time point 2, **Fig. 4b**). After 10 minutes, PI is added, which could only stain the cells that still have compromised membrane. Thus, the cells that are not stained with PI are considered as

having reversible pores (Time point 3, **Fig. 4b**). With a relatively low applied voltage at 14 V (2 μ s/100 μ s/20,000 pulses), some already perforated cells could not then be stained with PI, indicating the pores formed on the cell membrane are reversible (**Fig. 4c**). While under a high applied voltage at 80 V (1 μ s/1 ms/10 pulses), almost all cell perforation is irreversible (**Fig. S8**). This phenomenon conforms to the feature of electroporation, indicating that electroporation is the predominant mechanism for bacteria inactivation in LEEFT. Note that when 1 s effective treatment time (500,000 pulses) is used, there is no significant reversible damage as shown in Fig. 2b. It is mainly due to the longer treatment time, which greatly increases the cell death possibility. It is also possible that although reversible pores close after LEEFT, they damage cell function, which leads to cell inactivation ultimately.

Figure 4. Detection of reversible electroporation. (a) Increase of PI stain fluorescence of four cells at nanowedge tips on positive and negative electrodes, respectively, with intermittent LEEFT. The red shadow indicates that the LEEFT is on, which are 14 V/2 μ s/100 μ s/10,000 pulses for 1 s total time. The gray shadow indicates that the LEEFT is off, which is 0 V for 5 s. The inserted images show Cell No. 1 at the positive electrode (orange spheres). (b) Schematic of double staining method

with SYTOX Green and PI for reversible electroporation detection. (c) Microscopy images showing reversible electroporation under 14 V. The cells inside the yellow frames had reversible pores on membrane since they are stained with SYTOX Green at Time point 2 but are not stained with PI at Time point 3. The cells inside the red frames have irreversible pores since they are first stained with SYTOX Green and then stained with PI. Scale bars are 5 μm .

Due to the lightning-rod effect, the electric field at tips of metal rods with a high aspect ratio will be greatly enhanced compared to that in bulk. Therefore, this strong electric field could be sufficient to charge cell membrane, cause irreversible electroporation, and kill bacteria even under lower applied voltages. Although bench-scale LEEFT for water disinfection was developed based on this concept, the mechanism was only supported by control experiments done with electrodes with/without nanowire modifications.^{19, 20} There was no direct evidence confirming that the bacteria were inactivated due to the locally enhanced electric field and electroporation. The results achieved in this study provide important evidence on the mechanism. Firstly, only the bacteria in the area of the locally enhanced electric field are inactivated while others in bulk are intact (**Fig. 1b & d**). The inactivation percentage shows a positive correlation with the strength of the locally enhanced electric field instead of the applied voltage (**Fig. 3c**). Furthermore, when >90% bacteria inactivation is achieved with LEEFT at 30 V/2 μs /100 μs , there is no significant ROS generation (**Fig. 3d**), indicating this bacteria inactivation is not due to ROS damage. Reversible electroporation is detected under relatively low applied voltage (**Fig. 4**), suggesting that LEEFT could induce electroporation, and irreversible electroporation causing bacteria inactivation could be dominant at higher voltages.

It is worth noticing that electric field enhancement by nanowedges is the same for both positive and negative electrodes (**Fig. S6 & S7**). Consistently, all the bacteria inactivation phenomena discussed above do not show a significant difference between positive and negative electrodes. An electrochemical disinfection study reported that anode induced significantly higher bacteria inactivation than cathode, suggesting that electrical reduction should not cause the same level of cell damage as electrical oxidation.³⁶ Our positive control group for ROS detection also confirms that (**Fig. 3e**). Therefore, the same phenomenon on both electrodes found in this work indicates that electrical oxidation/reduction should not be the mechanism causing bacteria inactivation. Besides that, metal ions should not play a role in bacteria inactivation, neither. Since gold is electrochemically stable, under the very short 2 μ s pulses at voltages lower than 30 V, there should be no significant gold ion release. Therefore, electroporation is demonstrated as the predominant mechanism causing bacteria inactivation in LEEFT.

Since electroporation is the predominant mechanism for bacteria inactivation, the induced transmembrane voltage (TMV), which is the increased potential difference across the cell membrane resulting from exposure to an external electric field, was analyzed theoretically using finite element simulation to compare LEEFT and bulk EFT. Both the on-chip system like the one used in this work (**Fig. 5**) and a 3D system with standing nanowire (**Fig. S9**) were simulated. Two cells in LEEFT and bulk EFT respectively are compared, which is Cell No. 1 located at the nanowedge tip (**Fig. 5a left**), and Cell No. 2 located between two electrodes without nanowedge (**Fig. 5a right**). The simulation results show that the voltage drop across the membrane, i.e., the electric field, is greatly enhanced at Cell No. 1 near the nanowedge tip (**Fig. 5b left**) compared to Cell No. 2 (**Fig. 5b right**).

The maximum TMVs of the two cells show that with the same applied voltage, the cell No. 1 in LEEFT located at the nanowire tip can achieve around 7.5 times higher TMV than cell No. 2 in bulk EFT (**Fig. 5c**), indicating that much lower voltage could be applied to achieve the same level of TMV on cells in LEEFT than bulk EFT.

Figure 5. Theoretical analysis of cell TMV in LEEFT and bulk EFT. (a) Simulation set up for LEEFT (left) and bulk EFT (right). (b) Left view of the middle cutting plane showing the electric field across the cell membrane of cell No. 1 in LEEFT (left) and cell No. 2 in bulk EFT under 20 V applied voltage (right). The arrows indicate the direction of the electric field. The scale bars are 0.5 μm. (c) Maximum TMV on cell No. 1 and cell No. 2 under different applied voltages.

This work is a single-cell level proof-of-concept study of LEEFT, which directly proves that combining lighting rod effect and electroporation can be used for antimicrobial purpose. We are optimistic that LEEFT technique can be developed to have plenty of

specific applications. LEEFT effectively kills bacteria with mild treatment conditions without causing side effects, making it suitable for high-quality sample processing, such as liquid food or blood sample, and also safe for medical applications, such as for wound healing. Lower voltages and short pulses make it is an energy-efficient approach that is applicable for large-scale treatment processes, such as water treatment. Since the bacteria inactivation is highly localized, it is perfect for surface treatment, such as biofilm and biofouling prevention, and it can also find applications in continuous flow system, since bacteria could be attracted to the nanowedge tips and get inactivated. The as-shown rapid cell damage and the effectiveness of both electrodes further improve its efficiency. Since electroporation targets the phospholipid membrane, LEEFT should work on a broad range of cell types with more applications, including intracellular molecule delivery and cell lysing.

In real applications, the efficiency of LEEFT could be further improved through several ways. Electrodes all covered with nanowires could be developed to increase the effective zones. The treatment system and operation process could also be improved depending on different applications. For instance, fluid mixing could be introduced in continuous flow systems to improve the possibility of transporting bacteria to the nanowire tips. The parameters of electrical pulses, such as pulse width, directions, and voltages, could be altered to effectively manipulate cells in the flow and to attract bacteria cells to the nanowire tips. For large scale applications, the cost could be reduced by using cheaper electrode materials, such as Cu electrodes with CuO or Cu₃P nanowires synthesized on the surface. The synergistic effect of electroporation with metal ions,^{37, 38} ozone,²⁵ and other antimicrobial reagents could also be applied to further improve the efficiency of LEEFT.

339 ASSOCIATED CONTENT

340 Supporting information

341 Movie S1: Bacteria inactivation process in LEEFT (MP4)

342 Movie S2: Transport and inactivation of free-moving *S. epidermidis* cells in LEEFT

343 (MP4)

344 Movie S3: Transport and inactivation of free-moving *E. coli* cells in LEEFT (MP4)

345 Movie S4: Transport and inactivation of free-moving *B. subtilis* cells in LEEFT (MP4)

346 Materials and methods; figures S1-S9 showing the digital photo of the lab-on-a-chip, the

347 experimental setup, the waveform, the electrodes have no nanowedges as a control, the cell

348 decay after LEEFT, the chips of different electrode gap, the chips with nanowedges of

349 different interval, the irreversible electroporation at 80 V, the simulation of TMV; screen

350 shots of Movie S1-S4 and the captions (PDF).

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364 **Author Contributions**

365 X.X. and T.W. designed research. T.W. performed research. D.K.B. contributed new
366 reagents/analytic tools. T.W. and X.X. analyzed data and wrote the paper.

367 **Competing Interest Statement**

368 The authors declare no competing interests.

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REFERENCES

1. Neu, H. C. *Science* **1992**, 257, (5073), 1064-1073.
2. Sedlak, D. L.; von Gunten, U. *Science* **2011**, 331, (6013), 42-43.
3. McKinney, C. W.; Pruden, A. *Environmental science & technology* **2012**, 46, (24), 13393-13400.
4. Hijnen, W. A. M.; Beerendonk, E. F.; Medema, G. J. *Water Research* **2006**, 40, (1), 3-22.
5. Gao, S.; Hemar, Y.; Ashokkumar, M.; Paturel, S.; Lewis, G. D. *Water research* **2014**, 60, 93-104.
6. Lu, H.; Mutafooulos, K.; Heyman, J. A.; Spink, P.; Shen, L.; Wang, C.; Franke, T.; Weitz, D. A. *Lab on a Chip* **2019**, 19, (24), 4064-4070.
7. Plazas-Tuttle, J.; Das, D.; Sabaraya, I. V.; Saleh, N. B. *Environmental Science: Nano* **2018**, 5, (1), 72-82.
8. Grahl, T.; Markl, H. *Applied Microbiology and Biotechnology* **1996**, 45, (1-2), 148-157.
9. Barba, F. J.; Parniakov, O.; Pereira, S. A.; Wiktor, A.; Grimi, N.; Boussetta, N.; Saraiva, J. A.; Raso, J.; Martin-Belloso, O.; Witrowa-Rajchert, D. *Food Research International* **2015**, 77, 773-798.
10. Kotnik, T.; Frey, W.; Sack, M.; Meglič, S. H.; Peterka, M.; Miklavčič, D. *Trends in biotechnology* **2015**, 33, (8), 480-488.
11. Bendicho, S. I.; Barbosa-Cánovas, G. V.; Martín, O. *Trends in Food Science & Technology* **2002**, 13, (6-7), 195-204.
12. Gusbeth, C.; Frey, W.; Volkmann, H.; Schwartz, T.; Bluhm, H. *Chemosphere* **2009**, 75, (2), 228-233.
13. Sengel, J. T.; Wallace, M. I. *Proceedings of the National Academy of Sciences* **2016**, 113, (19), 5281-5286.
14. Kotnik, T.; Rems, L.; Tarek, M.; Miklavčič, D. *Annual review of biophysics* **2019**, 48, 63-91.
15. Tieleman, D. P.; Leontiadou, H.; Mark, A. E.; Marrink, S.-J. *Journal of the American Chemical Society* **2003**, 125, (21), 6382-6383.
16. Wang, T.; Chen, H.; Yu, C.; Xie, X. *Environment international* **2019**, 132, 105040.
17. Yildiz, S.; Pokhrel, P. R.; Unluturk, S.; Barbosa-Canovas, G. V. *Food Research International* **2021**, 140.
18. Rojas-Chapana, J. A.; Correa-Duarte, M. A.; Ren, Z.; Kempa, K.; Giersig, M. *Nano Letters* **2004**, 4, (5), 985-988.
19. Liu, C.; Xie, X.; Zhao, W.; Liu, N.; Maraccini, P. A.; Sassoubre, L. M.; Boehm, A. B.; Cui, Y. *Nano letters* **2013**, 13, (9), 4288-4293.
20. Huo, Z.-Y.; Xie, X.; Yu, T.; Lu, Y.; Feng, C.; Hu, H.-Y. *Environmental science & technology* **2016**, 50, (14), 7641-7649.
21. Huo, Z.-Y.; Zhou, J.-F.; Wu, Y.; Wu, Y.-H.; Liu, H.; Liu, N.; Hu, H.-Y.; Xie, X. *Journal of Materials Chemistry A* **2018**, 6, (39), 18813-18820.
22. Huo, Z.-Y.; Liu, H.; Wang, W.-L.; Wang, Y.-H.; Wu, Y.-H.; Xie, X.; Hu, H.-Y. *Journal of materials chemistry A* **2019**, 7, (13), 7347-7354.
23. Huo, Z.-Y.; Kim, Y.-J.; Suh, I.-Y.; Lee, D.-M.; Lee, J. H.; Du, Y.; Wang, S.; Yoon, H.-J.; Kim, S.-W. *Nature Communications* **2021**, 12, (1), 1-11.
24. Huo, Z.-Y.; Liu, H.; Yu, C.; Wu, Y.-H.; Hu, H.-Y.; Xie, X. *Chemical Engineering Journal* **2019**, 369, 1005-1013.
25. Zhou, J.; Wang, T.; Xie, X. *Environmental Science & Technology* **2020**, 54, (21), 14017-14025.
26. Zhou, J.; Wang, T.; Chen, W.; Lin, B.; Xie, X. *Environmental Science: Nano* **2020**, 7, (2), 397-403.

27. Zhou, J.; Wang, T.; Yu, C.; Xie, X. *Frontiers of Environmental Science & Engineering* **2020**, 14, 1-12.
28. Zhou, J.; Yu, C.; Wang, T.; Xie, X. *Journal of Materials Chemistry A* **2020**, 8, (25), 12262-12277.
29. Jiang, X.; Hu, J.; Petersen, E. R.; Fitzgerald, L. A.; Jackan, C. S.; Lieber, A. M.; Ringeisen, B. R.; Lieber, C. M.; Biffinger, J. C. *Nature communications* **2013**, 4, (1), 1-6.
30. Wang, T.; Yu, C.; Xie, X., *Microfluidics for Environmental Applications*. Springer Berlin Heidelberg: Berlin, Heidelberg, **2020**; pp 1-24.
31. Lapidco-Encinas, B. H.; Simmons, B. A.; Cummings, E. B.; Fintschenko, Y. *Analytical chemistry* **2004**, 76, (6), 1571-1579.
32. Zhang, T.; Wang, T.; Mejia-Tickner, B.; Kissel, J.; Xie, X.; Huang, C.-H. *Environmental Science & Technology* **2020**, 54, (15), 9652-9661.
33. Huang, X.; Chen, X.; Chen, Q.; Yu, Q.; Sun, D.; Liu, J. *Acta biomaterialia* **2016**, 30, 397-407.
34. Guo, Y.; Cheng, C.; Wang, J.; Wang, Z.; Jin, X.; Li, K.; Kang, P.; Gao, J. *Journal of hazardous materials* **2011**, 192, (2), 786-793.
35. Vaessen, E.; Timmermans, R.; Tempelaars, M.; Schutyser, M.; den Besten, H. *Scientific reports* **2019**, 9, (1), 1-11.
36. Liu, H.; Ni, X.-Y.; Huo, Z.-Y.; Peng, L.; Li, G.-Q.; Wang, C.; Wu, Y.-H.; Hu, H.-Y. *Environmental science & technology* **2019**, 53, (6), 3238-3249.
37. Zhou, J.; Wang, T.; Xie, X. *Environment international* **2019**, 128, 30-36.
38. Chen, W.; Jiang, J.; Zhang, W.; Wang, T.; Zhou, J.; Huang, C.-H.; Xie, X. *Environmental science & technology* **2019**, 53, (13), 7504-7512.

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