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Letter

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Conducting Nanosponge Electroporation for Affordable and High-Efficiency Disinfection of Bacteria and Viruses in Water

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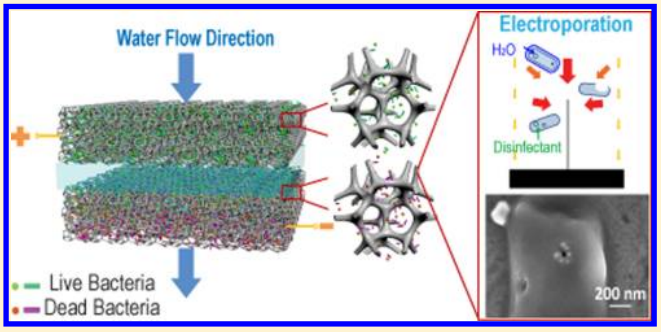
S *Supporting Information*

ABSTRACT: High-efficiency, affordable, and low energy water disinfection methods are in great need to prevent diarrheal illness, which is one of the top five leading causes of death over the world. Traditional water disinfection methods have drawbacks including carcinogenic disinfection byproducts formation, energy and time intensiveness, and pathogen recovery. Here, we report an innovative method that achieves high-efficiency water disinfection by introducing nanomaterial-assisted electroporation implemented by a conducting nanosponge filtration device. The use of one-dimensional (1D) nanomaterials allows electroporation to occur at only several volts, which is 2 to 3 orders of magnitude lower than that in traditional electroporation applications. The disinfection mechanism of electroporation prevents harmful byproduct formation and ensures a fast treatment speed of 15 000 L/(h·m^2), which is equal to a contact time of 1 s. The conducting nanosponge made from low-cost polyurethane sponge coated with carbon nanotubes and silver nanowires ensures the device's affordability. This method achieves more than 6 log (99.9999%) removal of four model bacteria, including *Escherichia coli*, *Salmonella enterica* Typhimirium, *Enterococcus faecalis*, and *Bacillus subtilis*, and more than 2 log (99%) removal of one model virus, bacteriophage MS2, with a low energy consumption of only 100 J/L.



KEYWORDS: *Electroporation, water disinfection, high-efficiency, one-dimensional nanomaterial, electric field*

Diarrheal illness is responsible for up to 1.3 million deaths of children under five worldwide every year.[1,2] One of the main transmission pathways is contaminated water. To increase the availability of clean water, efficient, affordable, and low energy water disinfection devices are needed,[3] especially in regions without access to improved water or recovering from natural disasters. The most common low-cost treatment method, chlorine disinfection, can cause the formation of carcinogenic disinfection byproducts.[4,5] The use of membrane filtration and UV disinfection are limited due to their high-cost, maintenance, and energy consumption.[6−8] Solar disinfection is inexpensive but time intensive.[9]

Electroporation, which is commonly used in the field of molecular biology to deliver polar substances (such as protein, DNA, drugs, etc.) into cells,[10−13] shows great potential for water disinfection. Under a strong electric field, cell membranes are damaged causing a large change in cell permeability and conductivity.[14,15] If membrane damage is too severe, death occurs immediately. Electroporation-based disinfection works for bacteria[16] and protozoa[17] as well as viruses.[18] No harmful chemical byproducts are known to form during electroporation. A few studies have investigated the feasibility of using electroporation to disinfect liquid for different purposes.[19−23] These studies concluded that although the process can achieve more than 3 logs (99.9%) of microorganism removal, voltages as high as 10^3 to 10^5 volts are required to maintain a high electric field. The use of high external voltage not only results in tremendous energy consumption and cost, but also raises operational safety concerns.

In this study, electroporation for water disinfection is induced by applying an extremely low external voltage to a pair of nanosponge filters, which are made from commercial polyurethane sponges modified by 1D nanomaterial carbon nanotubes (CNTs) and silver nanowires (AgNWs). These 1D nanomaterials help to functionalize the polyurethane sponges as conductive electrodes constructed with sharp nanoscale tips

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in order to build up a strong electric field for electroporation. The material choice of CNTs and AgNWs is based on their promising performances in other water filtration applications.[24−28] In the nanosponge filter, the coating of CNTs applied to the sponge makes it conductive and the coating of AgNWs produces of a large number of nanoscale sharp tips distributed on the sponge's surface. Because of AgNW's high conductivity and the nanowire's tip structure,[28] the electric field in AgNW's vicinity is increased several orders of magnitude over the electrical field generated only by flat surfaces without nanowires (Figure 1a,b). Bacteria and viruses in the strong electric field would be electroporated and die from consequences such as sudden intercellular environment disruption due to large amount of water inflow, inability to repair irreversible pores, or being poisoned by disinfectants that entered from generated pores (Figure 1c,d).

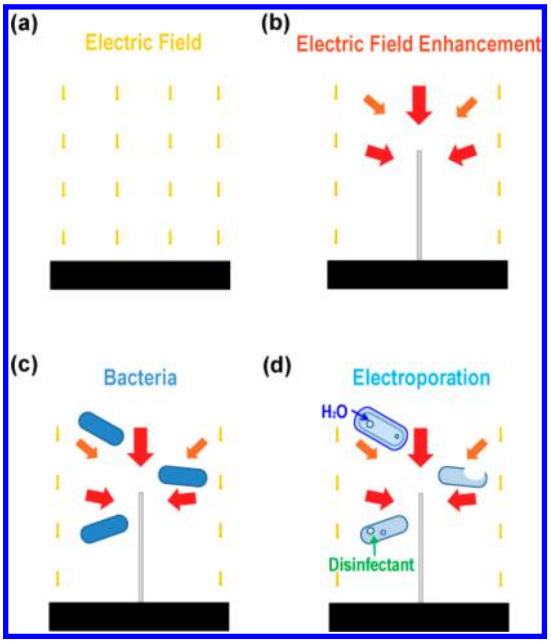

Figure 1. Schematics showing conducting nanosponge disinfection mechanism of 1D AgNW-assisted electroporation. (a) Conductive electrode without nanowire structure. Yellow arrows show the electric field. (b) Conductive electrode with AgNW. Red arrows show the electric field strength enhanced by AgNW (not quantified). (c) Bacteria in water flow to the vicinity of AgNW. (d) Bacteria are electroporated and inactivated due to the strong electric field. (Bacteria shown in the figure represent the possible inactivation means including sudden intercellular environment disruption due to large amount of water inflow, inability to repair irreversible pores and being poisoned by disinfectants entered from generated pores.)

The configuration of the nanosponge filtration device is shown in Figure 2a. The device is operated by applying an external voltage to the two parallel nanosponge electrodes contained inside an in-line filter holder. The fabrication process of the nanosponge is shown in Figure 2b−d. Commercial polyurethane sponges were coated with CNTs followed by AgNWs. After coating, CNTs and AgNWs were evenly distributed on the sponge surface, and the framework of the sponge did not change as shown in the scanning electron microscopy (SEM) images in Figure 2e−g. The resistivity of the nanosponge electrode was very low, ∼1 Ω·cm. The use of sponge material as the backbone provides several advantages. First, its continuous framework without internal loops provides

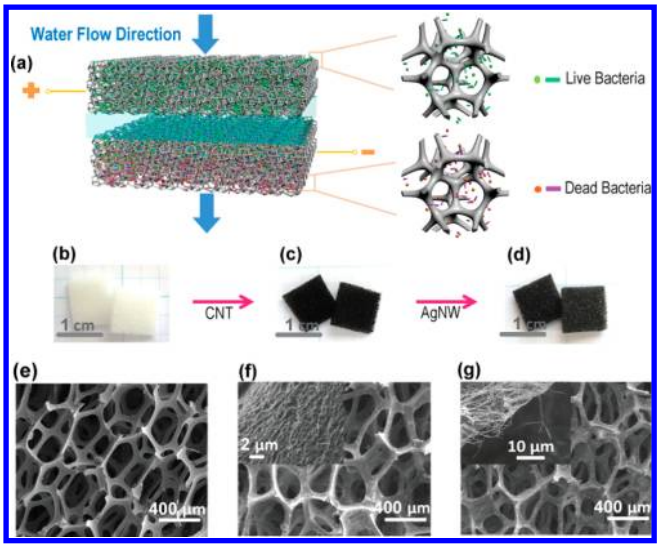

Figure 2. Conducting nanosponge configuration, fabrication, and characterization. (a) Schematics of nanosponge electrode in filtration device during operation. (b) Polyurethane sponge substrate before coating. (c) Polyurethane sponge after CNTs coating. (d) Polyurethane sponge after CNTs coating and AgNWs coating. (e) SEM image showing polyurethane sponge porous framework before coating. (f) SEM image showing polyurethane sponge uniformly coated with CNTs. The inset is a high-magnification SEM image showing the polyurethane sponge's surface after CNTs coating. (g) SEM image showing polyurethane sponge uniformly coated with AgNWs. Inset is high-magnification SEM image showing AgNWs on sponge surface.

a natural percolation pathway for the CNTs and AgNWs coating to easily make the whole filter conductive. The pore size (∼500 μm) of the sponge, characterized by SEM, is 2 to 4 orders of magnitude larger than bacterial, protozoan, or viral pathogens, so the water can be kept at a high flow rate without sieving the microorganisms out of water. The possibility of biofouling is also reduced because of the large pore size of sponge combined with the antimicrobial coating of AgNWs.[29,30] The filtration setup and filtration device photos are shown in Supporting Information, Figure S1.

Disinfection performance was evaluated using four different bacteria: *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Enterococcus faecalis*, and *Bacillus subtilis*. These bacteria represent Gram-negative and Gram-positive species (Supporting Information, Figure S2). Gram-positive bacteria have a thicker layer of peptidoglycan than Gram-negative bacteria,[31] while Gram-negative bacteria have an outer membrane, which Gram-positive bacteria do not. Electroporation of Gram-positive bacteria usually require a higher electric field than Gram-positive bacteria since the peptidoglycan layer performs most of the resistance to physical stress.[32] In addition to being gram-positive, *B. subtilis* is also a spore forming bacterium and hence may serve as a model for protozoa in disinfection experiments.

During the disinfection experiments, different external voltages ranging from 0 to 20 V were applied to the two nanosponge electrodes. The device flow rate was maintained at 1.5 L/h or 15 000 L/(h·m^2) using a peristaltic pump. The energy consumption of this device was very low. For example, at a voltage of 10 V, the energy consumption was ∼100 J/L compared to above 500 J/L needed for membrane filtration.[33,34] The energy consumption is likely to be further reduced significantly when going to a larger scale filtration

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system. The influent to the device contained ~10^7 colony forming units (CFU)/mL of bacteria; the effluent was considered treated water. Concentrations in the influent and effluent were determined using standard spread plate techniques (Supporting Information, Figure S2). Log removal efficiencies for the four bacterial strains at different voltages are shown in Figure 3a–d. Bacterial inactivation was enhanced with increasing external voltage for all four bacteria, indicating that a stronger electric field results in more efficient disinfection. For voltages above 10 V, all four bacteria achieved over 6 log (99.9999%) removal, corresponding to no culturable bacteria detected in the effluent, with retention time of only 1 s. *E. coli* and *S.* Typhimurium were inactivated immediately after filtration, while *Enterococcus* required a resting period of two hours without any additional treatment before it was inactivated (Supporting Information, Figure S3).

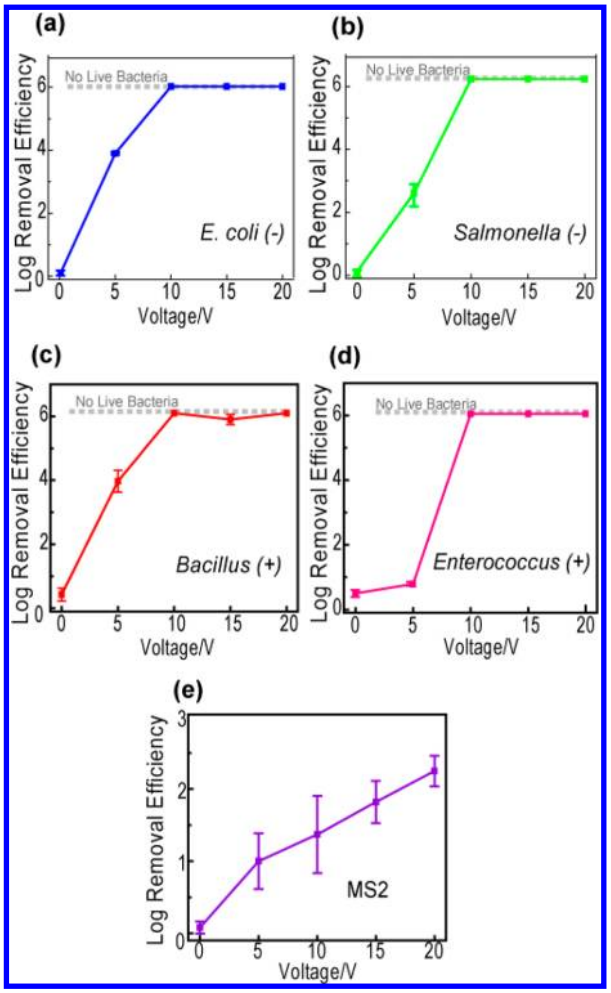

Figure 3. Bacteria and virus disinfection performance. (a–d) Disinfection efficiency of both Gram negative and Gram positive bacteria: *Escherichia coli*, *Salmonella enterica serovar* Typhimurium, *Bacillus subtilis*, and *Enterococcus faecalis* under five different voltages. (e) Disinfection efficiency of bacteriophage MS2 at five different voltages. Error bar represents the standard deviation of three replicate measurements.

Nanosponge effectiveness was also evaluated using a model virus, MS2, an F+ bacteriophage of *E. coli* that is often used as a surrogate for human enteric viruses. Water containing ~10^7 plaque forming unit (PFU)/mL was tested under different applied voltages at the same flow rate as used for the bacterial experiments. Viruses were tittered using a double agar layer plaque assay with *E. coli* Famp as a host (Supporting Information, Figure S2). Inactivation increased with increasing external voltage as shown in Figure 3e. With a voltage of 20 V, 99.4% of influent viruses were inactivated.

The bacterial disinfection mechanism was confirmed to be electroporation based on the studies of model bacteria *E.coli* and *Enterococcus*. First, electroporated pores were observed on bacteria membranes using SEM. *E. coli*, both untreated and treated by the nanosponge filtration device, were fixed for SEM characterization. The SEM images (Figure 4a–c) clearly show bacterial membrane damage after filtration. Small pore structures are present on the bacteria surfaces; some bacteria have more than one pore on their surface. More SEM images of bacteria with electroporation pores are shown in Supporting Information, Figure S4.

Moreover, dye staining experiments further identified that electroporation led to compromised cellular membranes and likely cell death. Propidium iodide (PI), a DNA stain, enters cells when their membranes are compromised and appears red under an epi-fluorescent microscope. PI dye was used to stain *Enterococcus* immediately after filtration, and bacteria were washed using phosphate buffer solution (PBS) to remove excess free PI dye molecules in solution. Figure 4d shows both the bright filed image and fluorescent image of *Enterococcus* samples treated under different applied voltages. The statistic results of bacteria staining percentage under each applied voltage show that the percentage of stained cells increased as the applied voltage increased, and at 20 V, 95.2 ± 4.2% of the bacteria was stained. This again indicates that electroporation is occurring at low voltages with the help of nanowire structures. The 0 V result of 7.6 ± 2.5% staining could be from bacteria's natural death or from the contact of bacteria with CNTs or AgNWs.[35] A very interesting phenomenon was observed with *Enterococcus*, which strongly supports the electroporation mechanism. Immediately after filtration, the bacteria were stained and the membrane damage was observed. However, when plated at this time, some bacteria were still able to reproduce and form colonies on the agar. For example, the PI staining results showed that 52.8 ± 4.9% of the cells had compromised membranes at 15 V and that 95.2 ± 4.2% of the cells had compromised membranes at 20 V (shown in Figure 4e). The cell culture result at the time of staining gives a removal efficiency $[-\log(c/c_0)]$ of 0.13 and 0.77 for 15 and 20 V, respectively, which is equal to a death percentage $[1 - c/c_0]$ of 26% and 83%, respectively. The percentage of dead bacteria measured by culture-based methods is smaller than the percentage of bacteria with compromised membranes measured by staining methods, which means that some bacteria with membrane damage could repair themselves after being plated on agar plates. This implies that some electroporation-generated pores are repairable, and cells with repairable pores might be able to reproduce in a nutrient rich environment. However, if bacteria with reversible electroporation pores were kept in water for 2 h prior to plating without any additional treatment or nutrients, they were not able to reproduce. Thus, it appears that during the rest period the cells die potentially due to osmotic pressure differences or chemical imbalance between the intercellular and extracellular environment.

The mechanism of disinfection was also determined to be electroporation by differentiating the effect of electroporation from electrochemically generated species (e.g., Ag^+), which are

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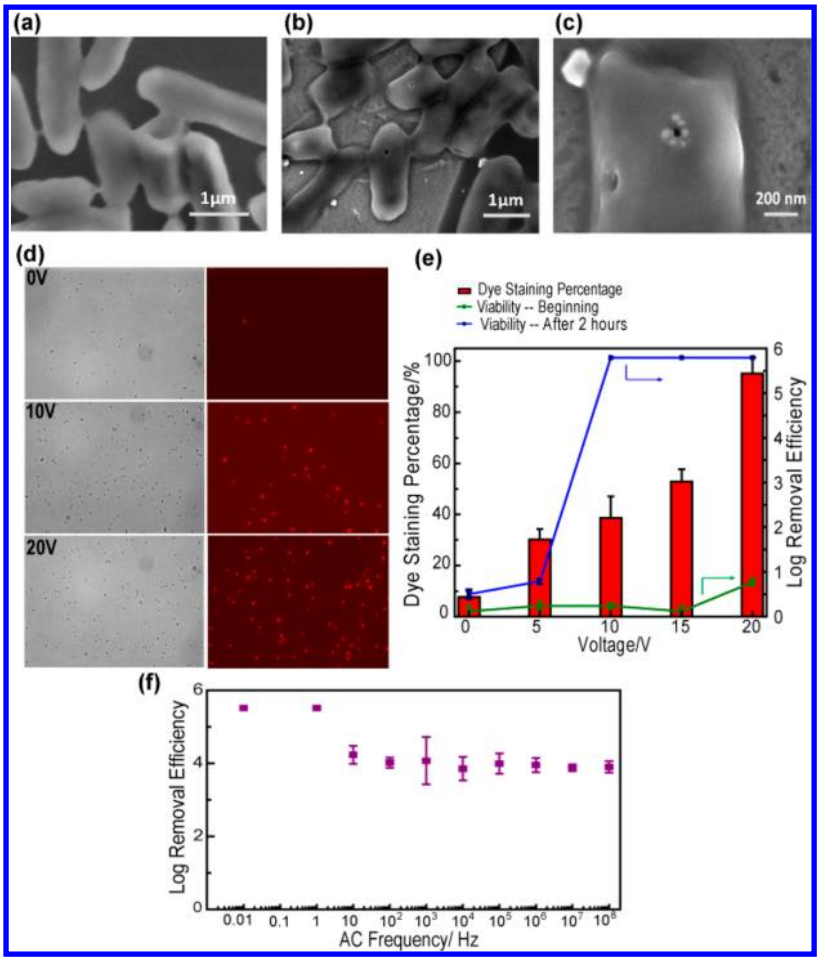

Figure 4. Electroporation evidence: SEM images, dye staining results, and AC disinfection. (a) SEM image showing morphologies of *E. coli* without any treatment. (b) SEM image showing pores formed on different *E. coli* surfaces after filtration under 20 V. (c) High-magnification SEM showing more than one pore formed on *E. coli* surface after filtration under 20 V. (d) Both bright field and fluorescent microscope images of *E. faecalis* samples immediately after filtration under voltages of 0, 10, and 20 V. (e) The percentage of *E. faecalis* stained immediately after filtration accompanied with viability results of both immediately after filtration (beginning) and 2 h after filtration. (f) Disinfection efficiency of *E. coli* with 10 V of applied AC voltage at different frequencies.

the top two possible contributors to bacteria and viruses inactivation in a nanosponge filtration device. When involving electricity during filtration, diverse processes occur at the same time such as electron transport in the electrodes, electroporation, electrochemical reaction, ion diffusion, etc. These physical processes have different response times to the applied external voltage. The process of electroporation, which is at the time scale of ns, is fast enough to be differentiated from electrochemical reactions by changing the frequency of applied voltage.[36,37] When the frequency of alternating current (AC) increases from 0 to 10^8 Hz at 10 V of applied voltage, electrochemical reactions decrease gradually. The inactivation efficiency of *E. coli* using AC in Figure 4f shows that disinfection is almost constant at high frequencies from 100 Hz to 100 MHz of around 4 log removal, which points to electroporation as the mechanism of cell death. The disinfection efficiency difference between low frequencies (<100 Hz) and high frequencies (>100 Hz) reveals the occurrence of reversible electroporation at 10 V. Though, adding the electrochemical factor like silver ions could further improve pathogen inactivation since silver ions could enter bacteria cell easily after cell membrane damage and react with inner cellular components to disruption cell functions. Silver ions alone in the absence of the electric field showed little disinfection effect (Supporting Information, discussion and Figure S5).

The concentration of silver in the effluent was examined to determine whether it poses a health risk. Total silver (ion and particle) concentrations in the filter effluent were measured using inductively coupled plasma mass spectrometry (ICP–MS). Between applied voltages of 5 to 15 V, the effluent has a silver concentration around 70 ppb. The highest silver release occurred at 20 V with the total silver concentration in the effluent of 94 ppb, which is still below the national drinking water standard of 100 ppb[38] (Supporting Information, Figure S5).

In conclusion, the nanosponge water filtration device presented in this letter takes advantage of 1D nanomaterials to achieve high-efficiency disinfection by electroporation for both bacteria and viruses. Electroporation shows a promising future as a disinfection mechanism for bacteria, viruses, and protozoa. The low cost, low energy consumption, and fast treatment speed makes this disinfection technology likely to be adopted as a point-of-use device and also be scaled up to combine with water treatment systems as a pretreatment or posttreatment step.

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Methods. *Sponge Filter Synthesis.* CNT ink was prepared by dispersing 0.1% single-walled CNTs (Carbon Solution, Inc., CA) in water with 1% sodium dode-cylbenzene sulfonate (SDBS) as a surfactant. Then CNTs were dispersed in water by 5 min bath sonication and 30 min probe sonication.[39] AgNWs were synthesized by polyol method. Twenty-five milligrams of AgCl was reduced in 30 mL of ethylene glycol (EG) with 330 mg of poly(vinylpyridine) at 170 °C, and then 110 mg of $AgNO_3$ in 10 mL of EG was added slowly. After synthesis was done, AgNWs were transferred into methanol by centrifuging at 6000 rpm (FISHER accuSpin 400) for 3 times.[40] A polyurethane sponge (McMaster-Carr, CA) was dipped into CNT ink, dried at ∼90 °C, and washed with DI water.[41] This dipping process was repeated three times. After the sponge was treated with CNT, the sponge was dipped in AgNWs ink and dried at ∼90 °C washed with DI water. After being coated in CNT and AgNWs, sponges were cut into desired size (1 cm × 1 cm × 0.2 cm). Finally, coated sponges were put into the filtration device and connected to a peristaltic pump for operation.

Bacteria Inactivation. *Escherichia coli* (JM109, Promega), *Salmonella enterica* serovar Typhimurium LT2 (ATCC 700720), *Enterococcus faecalis* (ATCC 19433), and *Bacillus subtilis* (ATCC 6633) were cultured to log phase (4−6 h), harvested by centrifugation at 900 × *g*, and resuspended in DI water, after which they were centrifuged and resuspended in DI water 2 more times. Bacteria were then diluted using DI water to ∼10^7 CFU/mL. Twenty milliliters of the solution was stored as control at room temperature. Fifty milliliters of each bacterial solution was passed through the nanosponge filter at 1.5 L/h, while different voltages (0, 5, 10, 15, and 20 V) were applied. The effluent was collected in autoclaved containers. Bacterial concentrations in the effluent and control solutions were measured using standard spread plating techniques. Each sample was serially diluted, and each dilution was plated in triplicate and incubated at 37 °C for 18 h. Treated and control samples were compared to determine the extent of inactivation.

Virus Inactivation. Bacteriophage MS2 was grown with the *E. coli* Famp host on a shaker table set to 25 rpm at 37 °C for 24 h. MS2 was isolated and concentrated using the polyethylene glycol (PEG) precipitation method.[42] A solution of ∼10^7 PFU/mL was made. Some of this was stored at room temperature in the dark to serve as a control. The remaining solution was passed through the nanosponge in 50 mL aliquots while using different voltages (0, 5, 10, 15, and 20 V) at a flow rate of 1.5 L/h. MS2 was enumerated in the control and filter effluent using a double agar layer method. Treated and control samples were compared to determine the extent of inactivation.

Bacteria Sample Preparation for Scanning Electron Microscopy (SEM). Both the control and the sample treated using the nanosponge at 20 V for *E. coli* was pelleted by centrifuging at 900 × *g* for 10 min, and the supernatant was removed. Both samples were fixed using a solution of 0.1 M sodium cacodylate buffer (pH 7.3), 2% glutaraldehyde, and 4% paraformaldehyde and stored at 4 °C overnight. Then, the cells were washed with the same buffer for 5 min. Samples were dispersed on a metal grid and air-dried in preparation of SEM characterization.

ICP−MS Measurement. Each of the five treated bacterial solutions (0, 5, 10, 15, and 20 V) was divided into two parts. The first part of the five bacterial solutions and the control sample were treated by adding HNO_3 to 2%. The second part of bacterial solutions were treated with 70% HNO_3 and kept in a 50 °C water bath and then diluted to 2% HNO_3 solution. All samples were filtered with a 0.2 μm pore size filter. The first part of the bacterial solutions and control were used to measure the silver ion concentration, and the second part of bacterial solutions were used to measure the total silver concentration.

Growth Curve. An original ∼10^7 CFU/mL *E. coli* solution was divided into three aliquots. The first aliquot was used as the control sample. The second aliquot was filtered through the nanosponge device with a 10 V charge applied and with a flow rate of 1.5 L/h. The third aliquot was treated with 100 ppb silver ions. After treatment, 1 mL of each of the three aliquots was added to 30 mL of tryptic soy broth (TSB) medium and grown at 37 °C on a shaker table set to 25 rpm for 24 h. Optical density at 670 nm wavelength was measured using a spectrometer every 30 min to determine the cell concentration and generate a growth curve.

PI Dye Staining Experiment. After filtering bacterial solutions through the nanosponge filter with different voltages (0, 5, 10, 15, and 20 V), 10 μL of 1 mg/mL propidium iodide (PI) dye solution was added into 10 mL of each effluent for a final concentration of 1 μg/mL. This mixture was then centrifuged at 900 × *g* for 10 min. These samples were then washed using PBS for 3 times. Samples were examined using fluorescent microscopy.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and discussion on the examination of silver's bactericidal effect. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Author Contributions

C.L. and Y.C. conceived the concept. C.L. conducted most of experiments, and X.X., W.Z., N.L., P.A.M., and L.M.S. contributed parts of experiments. C.L., A.B.B., and Y.C. analyzed the data and cowrote the paper. All the authors discussed the whole paper.

Notes

The authors declare no competing financial interest.

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