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# Inactivation of Foodborne Microorganisms Using Engineered Water Nanostructures (EWNS)

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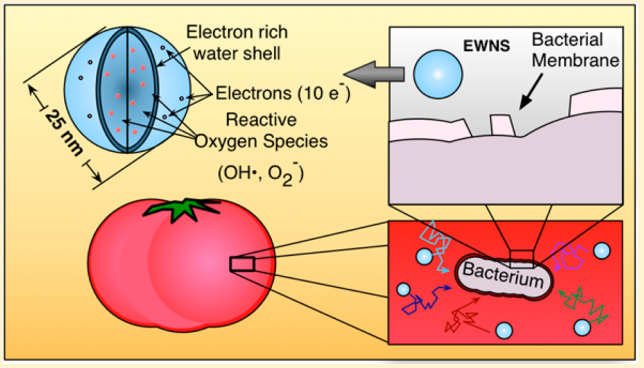
Supporting Information

**ABSTRACT:** Foodborne diseases caused by the consumption of food contaminated with pathogenic microorganisms or their toxins have very serious economic and public health consequences. Here, we explored the effectiveness of a recently developed intervention method for inactivation of microorganisms on fresh produce, and food production surfaces. This method utilizes Engineered Water Nanostructures (EWNS) produced by electrospraying of water vapor. EWNS possess unique properties; they are 25 nm in diameter, remain airborne in indoor conditions for hours, contain Reactive Oxygen Species (ROS) and have very strong surface charge (on average 10e/structure). Here, their efficacy in inactivating representative foodborne bacteria such as *Escherichia coli*, *Salmonella enterica*, and *Listeria innocua*, on stainless steel surfaces and on organic tomatoes, was assessed. The inactivation was facilitated using two different exposure approaches in order to optimize the delivery of EWNS to bacteria: (1) EWNS were delivered on the surfaces by diffusion and (2) a "draw through" Electrostatic Precipitator Exposure System (EPES) was developed and characterized for EWNS delivery to surfaces. Using the diffusion approach and an EWNS concentration of 24 000 #/cm$^3$, the bacterial concentrations on the surfaces were reduced, depending on the bacterium and the surface type, by values ranging between 0.7 to 1.8 logs. Using the EPES approach and for an aerosol concentration of 50 000 #/cm$^3$ at 90 min of exposure, results show a 1.4 log reduction for *E. coli* on organic tomato surfaces, as compared to the control (same conditions in regards to temperature and Relative Humidity). Furthermore, for *L. innocua*, the dose−response relationship was demonstrated and found to be a 0.7 and 1.2 logs removal at 12 000 and 23 000 #/cm$^3$, respectively. The results presented here indicate that this novel, chemical-free, and environmentally friendly intervention method holds potential for development and application in the food industry, as a "green" alternative to existing disinfection methods.




## INTRODUCTION

Raw or minimally processed fruit and vegetables can be a source of pathogenic microorganisms, such as bacteria, viruses, and parasites and are being implicated in foodborne disease outbreaks with increasing frequency.[1] Every year in the U.S.A., approximately 48 million people get sick, 128 000 are hospitalized, and 3000 die of foodborne diseases. Between 1990 and 2005, contaminated fresh produce accounted for 13% of all reported food borne outbreaks and for 21% of reported illnesses.[1−3] In Europe, according to the European Food Safety Authority (EFSA), foods of nonanimal origin, such as fresh produce, accounted for 10% of the outbreaks, 26% of the cases, 35% of the hospitalizations, and 46% of deaths between years 2007−2011.[4] Furthermore, a large number of foodborne outbreaks have also been traced back to cross contamination of surfaces where food is processed and prepared.[5]

Fruits and vegetables can become contaminated with pathogenic microorganisms anywhere in their production chain, starting from the field or orchard, during harvest,

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transport, processing, distribution, and marketing, or during preparation in food-service establishments and in the home (from farm to fork route). Possible pathogen sources include contaminated irrigation water, animals, birds, insects, soil, manure, and infected workers/food handlers. In addition, improperly cleaned and disinfected food preparation/contact surfaces can also be important reservoirs of pathogens.[6−8] In the food industry, the cleanliness of surfaces where produce is peeled/cut/processed, before sale to the consumer, is especially crucial. Stainless steel is one of the most common surfaces in commercial food production settings. The use of stainless steel in the food processing industry has gained popularity because of its mechanical strength, corrosion resistance, longevity, and ease of fabrication.[9] However, even on these surfaces, the potential for contamination, subsequent inadequate cleaning/disinfection, and pathogen survival is high.[10,11]

Cleaning and disinfection of the food preparation surface as well as the outer surface of the food itself (where applicable), is crucial in preventing foodborne disease. A number of different treatments are currently available for disinfecting the surfaces of whole and cut raw fruit and vegetables or their contact surfaces during production.[12] These mainly include the following: (1) chlorine-elemental or as a hypochlorite; (2) chlorine dioxide;[13,14] (3) peracetic acid;[15,16] (4) hydrogen peroxide;[17] (5) Quaternary Ammonium Compounds (QACs)—for wash water;[17] (6) ozone—gaseous and aqueous;[18] and (7) irradiation.[19]

Some of these methods leave behind chemical residues. Some others can induce visible damage to the treated goods if they are improperly used, while the ones using chlorine compounds cannot be used with organic food as there are restrictions for certain chemical use imposed by law.[20,21] Furthermore, most of the available methods cannot be used continuously as a means of reducing microbial risks during the food's journey from "farm to fork". The food industry is in need of new approaches to assist in reducing emerging public health hazards and in line with the new "green" environmental approaches and consumer preferences.

Recently, our group developed a novel method for inactivating bacteria in the air and on surfaces/fomites. The method relies on generating engineered water nanostructures (EWNS) through electrospraying of water. The EWNS possess a unique set of physical and biological properties, which we have fully characterized in previous publications.[22−24] Our studies revealed that EWNS have an average of 10 electrons per structure and an average nanoscale size of 25 nm.[22,23] In addition, electron paramagnetic resonance (EPR) showed that the EWNS contain a large number of reactive oxygen species (ROS), primarily $OH^{\bullet}$ and superoxides (Figure 1). The EWNS remain airborne for a long time, potentially colliding with microorganisms suspended in air and present on surfaces, delivering their ROS payload, and resulting in microbial inactivation. Our earlier studies have shown the potential of EWNS to interact and inactivate both Gram-negative and Gram-positive bacteria including mycobacteria, on surfaces and in air.[22,23] The destruction of the outer membrane has been demonstrated in our recently published studies, both through TEM imaging and with a Lipid Peroxidation assay. It was shown that ROS plays a role in the microbial inactivation and results to oxidation and destruction of the outer cell membrane.[24] Although the direct measurement of the lifetime of the ROS within the EWNS remains a technical challenge, the presence of ROS in EWNS after their synthesis was indirectly confirmed, since the inactivation of pathogens occurred up to 45 min after their production.[22] It is worth noting that for the current inactivation study using the "draw through" EPES system, EWNS are delivered to fresh produce in a few seconds after their production. In this time scale, as indicated by the data presented in this study, EWNS can interact and inactivate bacteria on fresh produce. We have demonstrated that the ROS are one of the primary mechanisms of microbial inactivation due to the damage of the outer cell membrane.[24] Furthermore, preliminary acute animal inhalation studies showed no signs of adverse health effects even at doses much higher than those used to inactivate the bacteria on surfaces or in the air.[22]

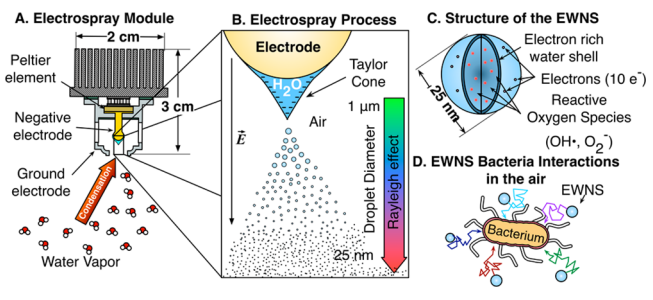


**Figure 1.** Synthesis and properties of the EWNS.

The aim of this study was to investigate the potential application of this novel, nanotechnology method in the battle against foodborne diseases and more specifically, to assess the ability of EWNS to inactivate classes of foodborne-related bacteria on fresh produce and on stainless steel surfaces. In addition, an electrostatic based exposure system was developed and characterized in order to optimize the delivery of EWNS on surfaces. Finally, potential sensory effects on the quality and appearance of the fruits were also evaluated using a panel study.

## MATERIALS AND METHODS

**Generation of the Engineered Water Nanostructures.** EWNS are synthesized via electrospray, a method widely used to aerosolize particles[25] and fibers for environmental/industrial applications,[26] and for delivering of DNA to bacteria cells.[27] Figure 1a,b illustrates the electro-spray module used in this study and the process for the synthesis of EWNS, respectively. Synthesis and physicochemical characterization of EWNS have been described in great detail in previously published work.[22−24] In brief, a gold-plated electrode is cooled down to 6 °C via a Peltier element. The atmospheric water vapor, condensed on the electrode, becomes the source of water for the electrospray. A high voltage of approximately 5 kV is applied between the Peltier electrode and a grounded counter electrode, causing the water to break into small droplets. The energy consumption for the electrospray module used in this study is 5 W and operates at 12 V DC. For this particular experimental setup, the operational environmental conditions were maintained at 21 °C and 50% Relative Humidity (RH).

In addition to the utilization of the electrospray modules, a EWNS generation system was developed to provide a volume of EWNS aerosol at variable concentrations and airflow levels. It consisted of an array of modules used to generate the EWNS. The humidity was maintained between 45 and 50% by mixing HEPA filtered air through a bubbler with dried air (HEPA filtered air drawn trough DryRite). Organic free, deionized water (18.1 MΩ-cm, purified with Barnstead Nanopure, Thermo Scientific, Rockford, IL) was used in the synthesis of


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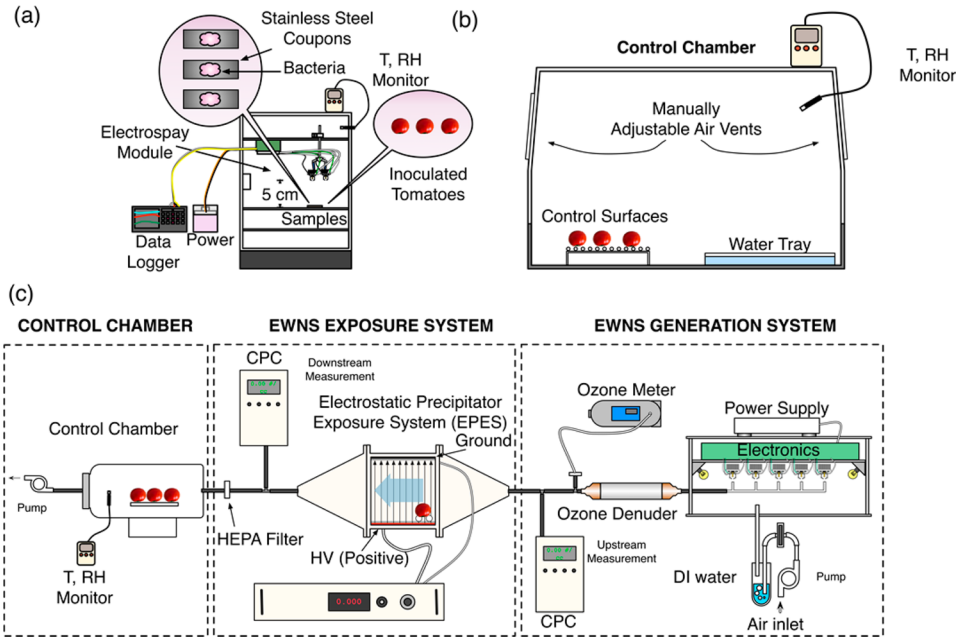


**Figure 2.** Experimental setups used in the various inactivation experiments: (a) The chamber used to deliver EWNS via diffusion on the surface of tomatoes and stainless steel coupons. (b) Diagram of control chamber. (c) The EPES inactivation setup.

the EWNS aerosol. Any generated ozone was removed by passing the aerosol through freshly coated, glass honeycomb denuders, as previously developed by the authors.[28]

**Test Surfaces.** Two different surfaces were selected for inoculation and exposure to EWNS due to their high relevance to the food industry—(a) stainless steel, which represents the most common food preparation surface, and (b) tomato, a popular vegetable, consumed raw, which has been implicated in foodborne illness, in the U.S. and other parts of the world.

*Stainless Steel Coupons.* Stainless steel coupons (Stainless Steel Supply, NC) with a mirror finish, cut to a size of 1 × 1 $cm^2$ were used. Prior to use, the coupons were wiped with 70% (v/v) ethanol and autoclaved. The coupons were then inoculated with selected bacterial suspensions as described in the section below.

*Tomatoes.* Tomato was chosen due to its popularity among consumers, having a central place in the daily diet; the fact it is eaten raw, thus being a high microbial risk item; its implication in many foodborne disease outbreaks; the fact that it is smooth, easily washable, and has no hidden sites of attachment to complicate inoculation experiments in this concept paper. Organic grape tomatoes between 1/2−3/4 in. were selected and stored at 4 °C until use, washed thoroughly in a dilute soap solution, and rinsed three times in deionized water prior to use. The tomatoes were washed thoroughly in a dilute soap solution and rinsed three times in deionized water prior to use. As with the stainless steel surfaces, tomatoes were surface-inoculated with the selected bacterial populations.

**Microbiological Procedures.** *Test Microorganisms.* Three bacterial species were chosen for inoculation on test surfaces: *Escherichia coli* (ATCC #27325), a known fecal indicator and a surrogate to enteropathogenic strains; *Salmonella enterica* (ATCC # 53647), a pathogen and surrogate to other pathogenic *Salmonella* strains, and *Listeria innocua* (ATCC # 33090), an environmental indicator and surrogate to pathogenic *Listeria monocytogenes*. All strains were obtained directly from ATCC (Manassas, VA).

*Bacterial Inoculum Preparation.* The contents of the bacterial vial were thawed at room temperature and cultured on tryptic soy agar plates (Becton Dickinson, Franklin Lakes, NJ). For each exposure experiment, the inoculum was prepared by transferring a single colony from the previously seeded plates to 5 mL of tryptic soy broth (Becton Dickinson, Franklin Lakes, NJ) and grown overnight for 18−24 h in a shaking water bath at 37 °C. *S. enterica* and *L. innocua* inoculants were then prepared by resuspending 1 mL of overnight culture in 1 mL of 1XPBS to yield a suspension with a final concentration of $10^8$ CFU/mL. In the stainless steel and tomato experiments, the *E. coli* test suspension was concentrated to a final concentration of ~$10^{10}$ CFU/mL since *E. coli* was particularly sensitive to air-drying.

*Inoculation of Surfaces with Bacterial Suspensions.* Inoculation took place in a CleanSpot PCR/UV Workstation (Coy Laboratory Products, Grass Lake, MI). Ten $\mu$L of the bacterial suspension, split in ten droplets, were added on each surface (stainless steel coupons or "upper" surface of tomato) and air-dried (~15 min) at room temperature (~21 °C). In the case of the tomatoes, the droplets were scattered in an area of 1 cm in diameter while during the exposure the inoculated section was placed facing upward. Once the surfaces were dry, a random subset was taken to assess bacteria loss due to air-drying (referred to as time zero $-t_0$). Nine coupons or tomatoes were then used for the EWNS inactivation experiments.

*Recovery of Bacteria from Test Surfaces.* To recover the bacteria from the stainless steel coupons each coupon was transferred into a 50 mL beaker containing 1 mL 1XPBS and rinsed well using an analog vortexer (VWR, Radnor, PA) at medium speed for 30 s. Similarly, each tomato was transferred to a 50 mL sterile conical polypropylene tube (VWR, Radnor, PA) containing 20 mL 1 X PBS and rinsed using an analog vortexer at the same speed. The recovery protocols were evaluated in depth, in terms of the recovery efficiency (Supporting Information, SI).


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The rinsate was then serially diluted and dilutions were plated on tryptic soy agar using the drop-plate method. Each agar plate was divided into quadrants. Five 10-$\mu$L drops of each dilution were dropped onto each quadrant, making a total of 20 drops on one plate. Plates were then incubated for 24−48 h at 37 °C prior to colony counting.

**EWNS Delivery/Exposure Approaches.** The various inoculated surfaces (stainless steel coupons and tomatoes) were exposed to EWNS under two distinct delivery/exposure approaches. In the first method, the inoculated surfaces were exposed in an atmosphere containing the EWNS aerosol, allowing them to reach and interact with the test surfaces via diffusion. This method has been used in the past for a number of bacteria, and is well understood, and has been described in detail previously.[23] The second delivery approach utilizes a newly developed, in-house built electrostatic precipitation exposure system (EPES), which was developed and used to take advantage of the EWNS high surface charge to efficiently deposit EWNS on the test surfaces as they flow through the system. The EPES provides for a more targeted, fast deposition of generated EWNS on the test surface.

In all experiments, the relative humidity (RH) and temperature ($T$) of the exposure and experiments are monitored in real time and kept at same levels (RH = 50 ± 5%, $T$ = 21 ± 2 °C) in order to ensure high viability levels of the bacteria and the sensitivity of the microbial experiments. The temperature and relative humidity was monitored with an H32B-C2, a data logger by Omega (Omega, Lake Success, New York). In our preliminary control viability experiments, relative humidity lower than 40% resulted in very low bacterial viability (data not shown). Furthermore, as shown in our previous published work,[22] for the 30−60% RH range, the EWNS's lifetime is on the order of hours, significantly greater than the time scale of the EPES exposure approach (order of seconds) used in this study.

*Diffusion Approach.* A 40 L plastic chamber was used to house 4 EWNS electrospray modules. The modules were fixed from a shelf with downward orientation at a distance of 5 cm from the inoculated surfaces (Figure 2a). The humidity in the chamber was maintained at 50% by the influx of humidified air generated by passing environmental air through a water bubbler. A real time aerosol monitoring system (P-Trak, TSI, Shoreview, MN) was used to measure the EWNS number concentration within the environmental chamber during the experiment.

*Control Experiments.* As a control (not exposed to EWNS), a plant growth chamber with a clear plastic hood was used to maintain the control treatment at similar conditions of RH and $T$ as those of the EWNS chamber (Figure 2b). The humidity inside the chamber was regulated through an adjustable vent on the top and a tray of DI water placed at the bottom to match same EWNS exposure conditions. The vents were adjusted manually to match the RH to that of the exposure chamber.

*Electrostatic Precipitator Exposure System (EPES) Approach.* Figure 2c illustrates the newly developed "draw through" Electrostatic Precipitation Exposure System (EPES). It consists of a PVC chamber, which houses two parallel metal plates placed at 15.24 cm apart. The plates were connected to an external high voltage source (Bertran 205B-10R, Spellman, Hauppauge, NY). The bottom plate is always set to positive voltage, and the top plate is always set at ground (floating ground). The voltage difference can be modulated to be between 0 and +10 kV, resulting in an electric field up to 6.66 kV/m. The chamber has a front-loading door that allows the test surfaces to be placed on a plastic rack that keeps them elevated from the lower plate in order to avoid interference from the high voltage. The walls of the chamber were coated with aluminum foil that was grounded to prevent particle losses. The chamber was repeatedly pressure tested to assess potential leakage. Although the ozone was monitored in real time and found to be at low levels (ppb range), it was scrubbed using the ozone denuders[28] to limit any inactivation effects to only the EWNS.

The EPES was connected to the EWNS generation system (Figure 2c) and was utilized for the exposure studies of the inoculated organic tomatoes. Ideally, the electric field will drive the negatively charged EWNS onto the inoculated tomato placed on the plastic rack at the edge of the bottom plate as they flow through the system. On the basis of the specific geometry of the EPES as well as the EWNS charge, theoretical calculations showed that a voltage difference of +3 kV would be sufficient to deposit all the particles on the lower plate. The calculations are based on the theory developed by Han et al.[29]

*Control Experiments.* A second cylindrical chamber was connected in series to the EPES system, utilizing an HEPA filter in between them, to remove the EWNS. This chamber was used as the control chamber and had identical atmosphere ($T$, RH, Ozone levels) as the EPES, but without the EWNS (Figure 2c). The effective EWNS removal from the control chamber was confirmed with the P-Track (data not shown).

**Characterization of the EWNS Deposition Efficiency of the EPES System.** *Deposition Efficiency.* The EWNS deposition efficiency in the EPES system was evaluated prior to the inactivation experiments to assess the number of particles deposited. The EWNS concentration was measured upstream and downstream (after the EPES) of the electrostatic precipitation chamber ($C_B$ upstream, $C_A$, downstream) using a Condensational Particle Counter (CPC) (TSI, Shoreview, MN). At a given air flow and voltage setting, the deposition efficiency ($\alpha$) of the aerosol due to the Electric Field was calculated as follows:

$$\alpha = \frac{C_B - C_A}{C_B} \tag{1}$$

The deposition efficiency was calculated for a variety of flow rates (0.5, 1, 2 L/min) and voltages (+0.5, +1, and +3 kV).

Further, the calculation of the total number of EWNS deposited in the EPES system, $N_{Dep}$, can be estimated as follows:

$$N_{Dep} = \alpha \times N \times \phi \times t \tag{2}$$

where $N$ is the EWNS number concentration at the inlet of chamber, $\alpha$ is the deposition efficiency, $\phi$ is the flow through the chamber, and $t$ is the exposure time. It is worth noting, however, that $N_{Dep}$ is not the number of EWNS deposited on the test surface, but the total number of EWNS deposited in the EPES system during the exposure experiment. $N_{Dep}$ is a good exposure dose metric for the EPES experiments and was used here as such.

*EWNS Losses in the EPES System.* The same protocols as before were used to estimate the EWNS losses through the EPES system. For these characterization experiments, the high voltage was turned off and all the metal parts were grounded. The difference between the upstream and downstream EWNS


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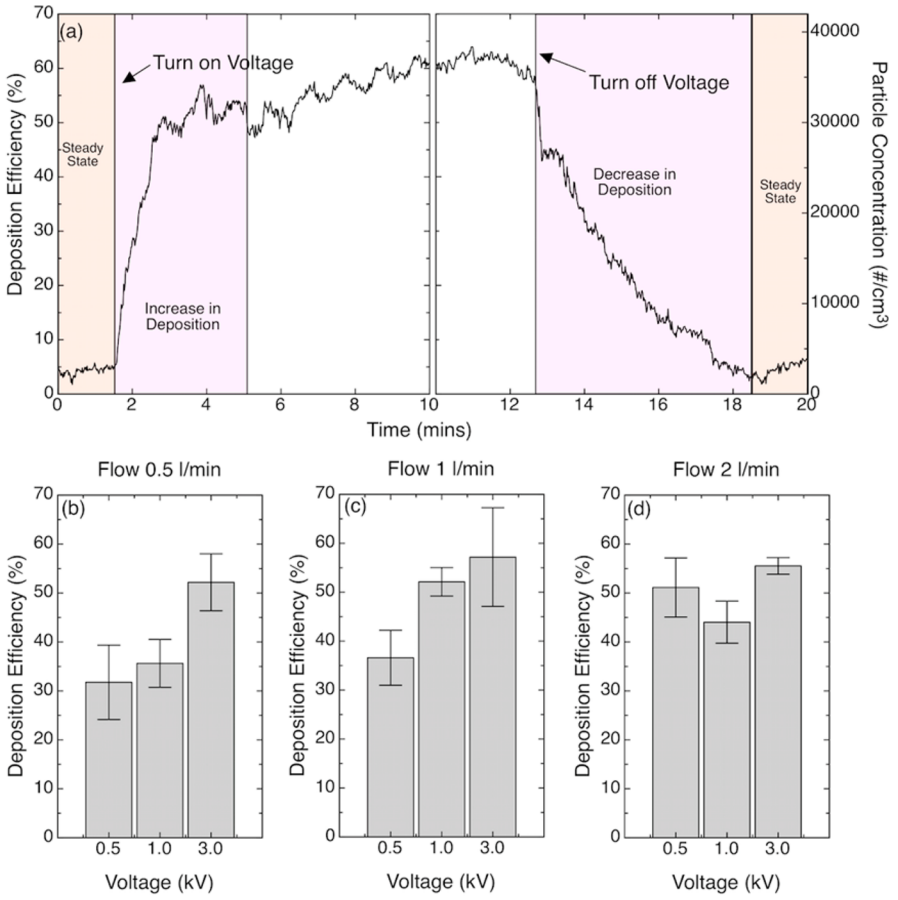


**Figure 3.** Characterization of the EPES (a) The deposition efficiency of the EWNS when the electric field is activated. (The flow through the chamber was set at 2 L/min and the voltage at +3 kV). (b)−(d) The deposition efficiency for three different flows and three different voltages.

concentration was used to calculate the EWNS particle losses through the EPES platform.

**Bacteria Inactivation Experiments.** *Experimental Protocol Used in the Diffusion Delivery Approach.* When the humidity inside the EWNS exposure chambers reached and stabilized at 45% to 50%, the EWNS electrospray modules were turned on. In addition to the relative humidity (RH), the ozone levels, and the temperature ($T$) were also monitored in real time during the experiment. Once the particle concentration was stabilized, the inoculated surfaces were inserted and exposed to the EWNS over time. For each different bacterial evaluation experiment, 21 surfaces were inoculated, 9 of which were used to test for inactivation efficacy, 9 for the control treatment (no EWNS, RH 50%, and $T$ 21 °C) and three to assess bacterial levels at time zero ($t_0$). The exposure was allowed for 30, 60, and 90 min for all three bacterial species and for both tomatoes and stainless steel coupons. The only exception was the *E. coli* inactivation assessment on the stainless steel coupons where the exposure was for 15, 30, and 45 min, as *E. coli* was found to be very sensitive to drying.

*Experimental Protocol Using the EPES Delivery Approach.* The experiments were performed at optimum conditions in terms of deposition efficiency (see the Results section below) with a flow through the chamber of 2 L/min and the voltage set at +3 kV. The EWNS generator was turned on, and the concentration of the EWNS was measured using the P-Trak (TSI, Shoreview, MN) downstream in real time. In addition to the ozone levels, RH and $T$ were also monitored, in real time, during the experiments. Before the exposure experiments, all surfaces within the chamber were scrubbed with ethanol soaked wipes. Similarly, the tomatoes were handled with sterile equipment (gloves, forceps, and Petri-dishes). During the placement step, tomatoes were transferred from container to container by holding them with sterile gloves from their noninoculated sides. As it was explained above, the tomatoes were inoculated only on the top so the bottom and the sides were available for handling.

Once the particle concentration at the EWNS generation chamber was stabilized, the inoculated surfaces were inserted in the EPES chamber and exposed to EWNS aerosol over time. Six more tomatoes were placed in the control chamber (Figure 2C). The opening and closing of the EPES chamber takes only a few seconds and even though it momentarily affects the concentration inside the EPES chamber, the EWNS concentration returns to the steady state levels in a matter of minutes (data not shown). Such perturbation of EWNS concentration is negligible compared to 45 and 90 min of exposure time. Three more were used for the $t_0$ time point. The system was allowed to reach equilibrium once again (stabilization of the number concentration measured downstream of the electrostatic chamber), and the high voltage was turned on. The reduction in the EWNS concentration was recorded and used to estimate the amount of those deposited in the chamber EWNS during the various exposure time points. At $t$ = 45 min, three tomatoes were removed and processed. At $t$ = 90 min, three more tomatoes were removed.

As an additional control, six tomatoes were placed in the EPES chamber without the electric field. Thereafter, the same


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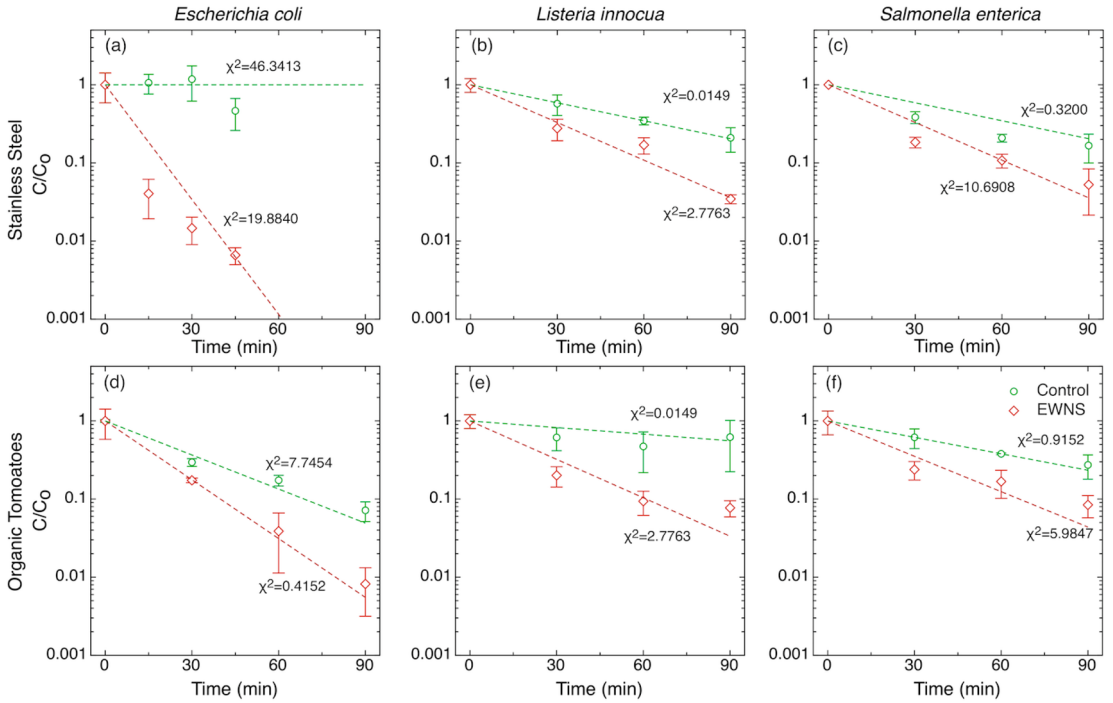


**Figure 4.** Bacterial inactivation on the surface of the stainless steel coupons and tomatoes with the EWNS delivered by diffusion. The inactivation on the Stainless Steel Coupons for (a) *E. coli*, (b) *L. innocua*, and (c) *S. enterica*. The inactivation on the tomato surface for (d) *E. coli* on tomatoes, (e) *L. innocua* on tomatoes, and (f) *S. enterica* on tomatoes. The average concentration of the EWNS was 24 000 #/#/cm$^3$.

protocols were followed in order to assess the effect of the electric field and targeted EWNS deposition in the inactivation process.

**Statistical Analysis.** All the experiments were performed in triplicate, and the standard deviation was used as the measurement error. Log-reductions of bacterial concentrations were computed for a given condition according to the following

$$\log \text{red.} = \log_{10}\left(\frac{C_n}{C_0}\right) \tag{4}$$

where "log red." is the log reduction, $C_0$ is the bacteria concentration of the control coupon at time 0 (i.e., after surface was dried, but before placed into a chamber), and $C_n$ is the bacteria concentration of the surface after $n$ minutes of exposure. The data are presented in terms of log reduction compared to the control (no-EWNS exposure).

To calculate the removal rate, the data were fit with an exponential decay equation:

$$\frac{C_{(t)}}{C_0} = e^{-t/\tau} \tag{5}$$

where the $C_0$ and $C_{(t)}$ are the bacterial number concentration at time 0 min and time $t$, respectively, and $\tau$ is a constant that is the time required to remove 63.21% of the bacteria. This was further converted to Log Reduction Rate (LRR, removal rate in terms of Logs/min):

$$\text{LRR} = [\text{Logs/min}] = 0.43/\tau \tag{6}$$

To represent the Log Reduction as a function of the deposited EWNS the data had to be normalized with the respective control to account for the natural log reduction, which is different for each exposure time. In that case, the Log Reduction was calculated as follows:

$$\log \text{red.} = \log_{10}\left(\frac{C_n}{C_{n\text{-control}}}\right) \tag{4}$$

where "log red." is the log reduction, $C_n$ is the bacteria concentration of the control coupon at time $n$, while $C_{n\text{-control}}$ is the concentration of the control bacteria at time $n$.

The curve fit was calculated with ProFit (QuantumSoft, Uetikon am See, Switzerland). The parameter $\chi^2$ was used to evaluate the quality of the fit. The LRR between control and exposed surfaces were analyzed with $t$-test (SI).

**Sensory Evaluation.** In the case of tomatoes, the effect of the EWNS on their sensory qualities of was evaluated in a separate experiment. The parameters examined after exposure to EWNS were as follows: the overall quality and appeal of the tomatoes, their color, their texture, their skin integrity, and their aroma. The protocol and the results are described in detail in the SI.

## RESULTS AND DISCUSSION

**Deposition Efficiency of the EPES System.** Figure 3a shows the EWNS deposition efficiency of EPES as a function of time, for the 2 L/min flow rate and +3 kV voltage settings. At the time prior to the voltage activation, the EWNS deposition efficiency represents the particle losses inside the chamber, which were found to be minimum and in the order of 3−5%. When the high voltage is applied, the EWNS deposition gradually increases due to the presence of the electric field. The time required for the deposition to reach equilibrium depends on the flow through the chamber. After approximately 3 min, the deposition efficiency reaches a maximum and steady value of approximately 58%. The assessment of the deposition efficiency for other flow and electric field conditions is shown collectively in Figure 3b−d. The deposition efficiency for various flow/voltage combinations in the chamber varies from


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40% to 60%. The maximum achieved deposition of 58% was obtained at +3 kV voltage and 2 L/min flow rate operational conditions.

It is evident that for a flow of 2 L/min (maximum EWNS levels for current EWNS generation system used here), the voltage does not seem to have any significant effect on the deposition efficiency. Similarly for the voltage of 3 kV, the flow rates have minimum effect on the EWNS deposition efficiency in the EPES system. It is also worth noting that higher voltage (in the order of +5 kV) was tried but sporadic "sparks" were evident between the positive plate and the grounded walls of the chamber with no significant increase of the deposition efficiency (data not shown). Therefore, the 2 L/min flow and +3 kV voltage conditions are considered optimum for the current EPES configuration. A scale up, high throughput system for commercial applications, which will enable generation of high flow (10 L/min) and concentration of EWNS (up to 500 000 #/cm$^3$) is currently under development by our group. For such different operational conditions, EPES design will need to be optimized following the same approach described here in order to optimize the EWNS delivery on fresh produce for commercial applications.

In conclusion, the use of the electric field had a positive effect on the deposition of the EWNS in the EPES, which resulted in a deposition reaching up to 58%. It is worth noting that based on theoretical calculations, for a flow of 2 L/min and an EWNS average electric charge of 10 electrons, it is estimated that 3 kV should be enough to deposit all of the EWNS on the plate, which, however, was not the case in the experimental data presented here. This is expected since it is known that the EWNS aerosol has a distribution of charges ranging from 1 to 40 electrons per structure with an average of 10 electrons.[30]

**Inactivation of Bacteria on Stainless Steel and Tomato Surfaces.** *Diffusion Exposure Approach. Stainless Steel Surfaces.* Figure 4a−c summarizes the results of the inactivation experiments on the surface of the stainless steel coupons for the three bacterial species under evaluation. The temperature and relative humidity for control experiments were in close proximity to the actual exposure levels (RH = 50 ± 5%, $T$ = 21 ± 2 °C) (data not shown). All three bacteria showed between 0.6 to 1.8 log reductions, as compared to the control, for a 45 to 90 min EWNS exposure of 24 000 #/ cm$^3$ delivered by diffusion. More specifically, *E. coli* (Figure 4a) was susceptible to air-drying on the stainless steel coupons, and therefore the inactivation was documented for only 45 min, at 10 min intervals. After 45 min of EWNS exposure, the results show an approximate 1.8 log reduction, which represents an average removal rate of 0.0726 ± 0.0064 logs/min while the control showed 0.0054 ± 0.0031 logs/min. This represents a rate of inactivation 30 times as fast as the control. *L. innocua* exposed to EWNS (Figure 4b) was reduced by an average 0.8 logs in 90 min, as compared to the control, which represents a removal rate of 0.0163 ± 0.0014 logs/min, while the control showed a removal rate of 0.0051 ± 0.0024 logs/min. Finally, similar results were obtained for *S. enterica* exposed to EWNS (Figure 4c) which was reduced by 0.6 logs in 90 min, as compared to the control, representing a removal rate of 0.0162 ± 0.0014 logs/min, while the control was reduced by 0.0076 ± 0.0007 logs/min. The *S. enterica* inoculated stainless steel coupons exposed to EWNS had a 90% higher removal compared to the control. It is worth noting that the inactivation potential presented here for the three foodborne bacteria is based on a specific low EWNS aerosol concentration of 24 000 #/ cm$^3$. As will be demonstrated below, higher levels of EWNS will increase the inactivation potential. On the basis of the published literature under our experimental conditions, the formation of biofilms is not likely. For the formation of biofilm, a rich nutritional environment and a minimum of 2 h is required (the entire length of the inactivation experiment used here is 90 min, and the PBS used to disperse the bacteria does not contain any nutrients).[31]

Current effective treatments for disinfection of stainless steel surfaces include the use of chlorine-based compounds and quaternary ammonium compounds (QACs). Chlorine-based compounds are traditionally used because of the short contact time required for efficient disinfection. However, these compounds can also damage the exposed surfaces and are potentially hazardous to workers and the environment.[32] QACs, on the other hand, are deficient against Gram-negative bacteria, exhibit reduced activity in hard water, produce foam in clean-in-place (CIP) equipment, and leave residual film on surfaces which may be undesirable.[33] The nanotechnology approach described in the current study might be a promising green alternative to surface disinfection in the food industry, not utilizing toxic or corrosive chemicals, just pure water vapor.

*Tomatoes.* Figure 4d−f summarizes the inactivation results for the tomatoes. *E. coli* (Figure 4d) was able to survive air drying much better when inoculated on tomato surfaces. After 90 min of EWNS exposure at 24 000 #/ cm$^3$, the results show an approximate 0.9 logs reduction, as compared to the control, representing an average removal rate of 0.0249 ± 0.0009 logs/min, while the control showed a removal of 0.0144 ± 0.0008 logs/min. Similarly, *L. innocua* (Figure 4e) showed an average of 0.7 log reduction in 90 min. This represents a removal rate of 0.0162 ± 0.0014 logs/min, while the control showed a removal rate of 0.0051 ± 0.0024 logs/min. Finally, similar results were observed with *S. enterica* (Figure 4f). An average 0.5 logs reduction in 90 min was obtained, as compared to the control, which represents a removal rate of 0.0150 ± 0.0017 logs/min, while the control showed 0.0069 ± 0.0001 logs/min.

Currently available treatments, such as chlorine dioxide, provide good microbial control at low concentrations and minimal exposure times. However, the control is most effective in water or on wet surfaces.[14] When bacteria were dried on to tomato surfaces, the recommended dose of 5−10 ppm and exposure time of 1 min did not produce an observable reduction in bacteria concentration.[14] In a study on cucumbers, the researchers reported that a high concentration of $ClO_2$ (105 ppm) was unable to significantly reduce the microbial populations.[13] Taking into account these results, the potential ill effects of the treatment on the produce, as well as potential toxicity effects on the handlers, renders the use of Chlorine dioxide problematic.

*Targeted Inactivation of Bacteria via the EPES Exposure Approach.* Figure 5 collectively shows the inactivation results for the *E. coli* and *L. innocua* on the surface of the tomatoes at various EWNS exposure levels and times using the EPES system. Figure 5a shows the log reduction of *E. coli* on the surface of the tomatoes as a function of the exposure time. As shown in Figure 5a, in the case of *E. coli*, the tomatoes exposed to an EWNS aerosol of 50 000 #/cm$^3$ at +3 kV and 2 L/min settings, a 2.3 logs reduction was observed (1.4 log reduction compared to the control). This is translated to a removal rate of 0.028 logs/min that is approximately three times that of the control. The unexposed inoculated tomatoes in the control treatment showed a reduction of approximately 0.1 logs after 90


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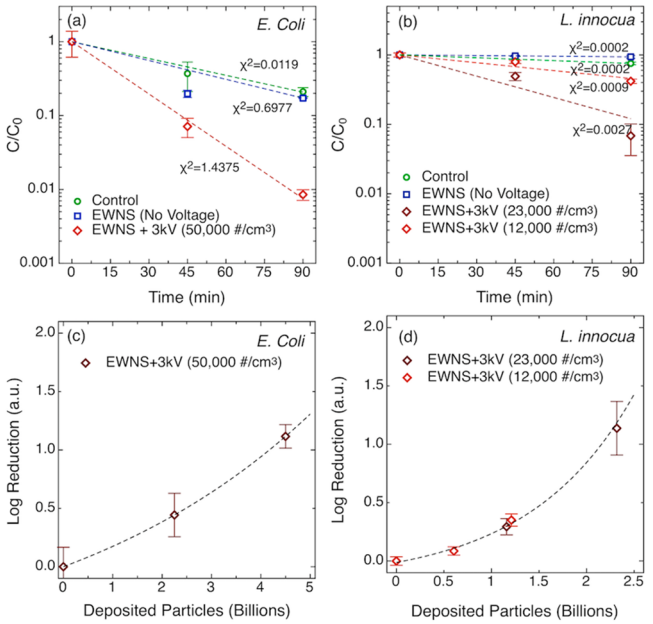


**Figure 5.** Inactivation of (a) *E. coli* and (b) *L. innocua* on the surface of tomatoes using the EPES. The log reduction compared to the control, as a function of the exposure time and the number of deposited EWNS for (a) *E. coli* and (b) *L. innocua*.

min, which represents a removal rate of 0.008 logs/min. Without the electric field, the removal rate was 0.009 logs/min, which is at the same level as the control (no exposure to the EWNS). This is indicative that the electric field enhances the EWNS deposition and increases the inactivation potential.

Similarly, Figure 5b shows the removal of *L. innocua* on the tomato surface for two different concentrations of EWNS: 12 000 and 23 000 #/cm$^3$. In the case of the 12 000 #/cm$^3$, a 0.5 log reduction was observed compared to the control representing a removal rate of 0.005 logs/min, while at 23 000 #/cm$^3$ exposure a 1.2 log reduction was observed representing a removal rate of 0.011 logs/min. The bacteria that were not exposed to the EWNS (control) showed a log reduction of 0.07 logs, representing a removal rate of 0.0003 logs/min. For the control case of EWNS exposure with no voltage, the removal was at the same level as the no EWNS exposure control as above, with a removal rate of 0.0006 logs/min, irrespective of the EWNS concentration (Figure 5b). The ozone levels were kept below the 150 ppb levels, which is significantly less (20 times) than the required levels for bacteria removal as reported in the literature.[23] Our EWNS generation system is equipped with ozone denuders to keep the ozone levels in the ppb range (Figure 2). More importantly, the "ozone only" control bacteria experiments performed here show no bacteria inactivation at this ppb ozone levels (Figure 5a,b, 0 kV).

Figure 5c,d shows log reduction, as compared to the control, as a function of the number of the deposited EWNS, calculated with eq 1, for the *E. coli* and *L. innocua*, respectively. More particularly, for *L. innocua*, all the data-points for the two different exposure doses fall on the same dose response curve. This showcases that the inactivation is a function of the EWNS dose. This dose–response relationship between the removal of bacteria and the EWNS concentration shows that by further increasing the dose, greater inactivation levels can possibly be obtained.

In conclusion, the targeted delivery of EWNS using the EPES exposure approach, as expected, showed an increase in the inactivation potential in the presence of the strong electric field. The absence of the electric field resulted in no inactivation, as expected, because very few EWNS particles were deposited in such a flow-through system. More importantly, it is evident from the presented dose–response data for the case of *L. innocua* that higher inactivation potentials can be obtained with higher exposure levels (doubling the dose almost doubled the inactivation potential—0.6 log reduction for the 12 000 #/cm$^3$ 1.2 log reduction for the 23 000 #/cm$^3$).

**Sensory Evaluation.** As the visual appearance of the treated fruit is important, a preliminary sensory evaluation was done to assess any potential damage on the fruits. The EWNS treatment did not bring about any noticeable alterations to the produce used in this study (see Sensory Evaluation section in the SI). The EWNS exposure dose used here was 100% higher than the one used in the inactivation experiments, and the exposure time was prolonged up to a period of 2 h. Therefore, these results confer an advantage to the EWNs nanotechnology method compared to other currently used fresh produce disinfection methods, such as heat and chemicals, that have the potential to damage/alter the exterior or the interior of fresh produce sensory characteristics. It should be noted that this is a preliminary sensory evaluation to evaluate certain organoleptic parameters, and the intent here was not to directly measure possible changes on properties (i.e., color).

Overall, the nanotechnology-based method for the inactivation of food-borne bacteria on food production and fresh produce surfaces, demonstrated promising results. The EWNS were effective in inactivating three distinct classes of microorganisms particularly important to the food industry: *E. coli*, *S. enterica*, and *L. innocua*. The inactivation potential was further increased when the delivery of the EWNS on surfaces was better targeted using the developed electrostatic precipitator based exposure system. More importantly, it was clearly shown that increased EWNS exposure doses result in higher inactivation potential. Last but not least, the very promising microbial inactivation results as well as the absence of any sensory alterations on the exposed products render this method a promising intervention technology, which can be used in the battle against foodborne disease. In future studies, our efforts will be focused on further understanding the EWNS–pathogen interactions using natural flora and also assess the EWNS effect on shelf life expectancy.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Details of the sensory evaluation (methods, results, discussion, and the questionnaire that was used), the statistical analysis of the inactivation rates (*t* test) and the discussion of the bacteria recovery and viability. 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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