Bacterial inactivation by engineered water nanostructures generated by electrospraying

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ABSTRACT

Herein we present a novel method for microbial disinfection that utilizes the formation of unique Engineered Water Nanostructures (EWNS) generated via electrospraying. The physico-chemical properties of the EWNS such as their size distribution, surface charge, and reactive oxygen species were assessed. The potential of the generated EWNS to inactivate bacteria in air and on surfaces was assessed both quantitatively and qualitatively. *Serratia Marcescens* (gram-negative) bacteria were used for both the surface and for the air inactivation experiments. Our results show that the EWNS have a size of approximately 20 nm, which is very stable, and carry an average charge of 10 electrons per particle. The results from the surface inactivation of *Serratia Marcescens* showed that there is more than a 2-log10 reduction. Similarly the results from the air inactivation showed 50% bacterial removal at steady state.

Keywords: Engineered Water Nanostructures, nanoparticles, electrospray, bacterial inactivation, nanotechnology

1 INTRODUCTION

The burden of infectious disease worldwide, related to contamination via contact with non-contaminated surfaces (fomites) and airborne infection, is a growing issue. Globally, these infections are linked to an estimated 1.7 million deaths a year from diarrheal disease and 1.5 million deaths form respiratory infections [1]. The problem of surface and airborne infections can also be traced, in smaller scale, to households where common areas can accumulate pathogens that can potentially become a threat, especially to more sensitive population groups [2].

In this work we present a novel method to inactivate bacteria from surface that utilizes the formation of unique Engineered Water Nanostructures (EWNS) generated via the electrospraying of water. Electrospray is a widely used method for the aerosolization of liquid suspensions containing engineered nanomaterials (ENMs) [3], [4]. During the process high voltage is applied between a capillary that contains the targeted liquid and a collection plate. The technique relies on the balance between surface tension and electrostatic repulsion to break up large liquid droplets to fine particles [5]. Additionally, in this process by controlling the number of supplied charges (via the applied voltage) the aerosol size can also be controlled [3]. It is also known that during the electrospray process, various radicals including superoxides and hydroxyl radicals are also generated [6], [7].

2 MATERIALS AND METHOD

Here, an electrospray method is explored which generates water nanostructures via the condensation of atmospheric humidity. Initially, water vapor from the air condenses on a rounded electrode cooled by a Peltier element at 6 °C. At a distance of 5 mm from the condensing electrode, a ring counter electrode is concentrically arranged. High voltage is applied between the two electrodes by setting the condensing electrode to negative potential, -5 kV, while the counter electrode is grounded. Charges start to accumulate on the surface of the condensed water droplets and the applied voltage pulls the water towards the grounded electrode. Countering this force is the surface tension that holds the water on the Peltier-cooled electrode. Eventually, enough negative charges accumulate on the surface of the forming a water cone (Taylor cone) resulting, eventually, in the breaking of water droplets [8]. The highly charged droplets continue to break, due to the Rayleigh effect, until they go down to the nano regime.

Figure 1: The experimental setup for the surface inactivation. During the experiment the temperature, Relative humidity and ozone were measured.
2.1 Particle physical characterization

The particle number and size distribution as well as the surface charge were measured using a Scanning Mobility Particle Sizer (SMPS, Model 3936, TSI, MN) and a Faraday Aerosol electrometer (TSI, Model 3068B, MN). The Scanning Mobility Particle Sizer (SMPS) was operated at 0.5 l/min and a sheath flow of 5 l/min. The particle number and current were measured concurrently. The average charge was calculated by dividing the total charge by the respective number of particles.

\[
\bar{Q} = \frac{I \cdot \Delta t}{N_{\text{tot}}}
\]  

(1)

2.2 Surface inactivation

The bacteria inactivation experiments took place in a 45 l chamber (figure 1). The humidity in the chamber was maintained to approximately 50% by supplying an adjustable flow of humidified air. 200 µl of bacteria solution was placed on autoclaved stainless steel cups and let to dry for 30 mins in a biosafety hood. Three coupons were placed in a fixed distance of 5 cm below the electrospray device. Every 30 mins a coupon was removed and repetely washed with 2 ml of PBS to recover the bacteria. The agar rinsate was diluted and plated on tryptan agar plates. The plates were left at room temperature for 48 hours to grow. During the experiment environmental parameters such temperature, relative humidity and ozone in the chamber were also monitored in real time using a 205 Dual Beam Ozone Monitor™ (2B Technologies, Boulder, Co). A second identical chamber was also used without an electrospray device to expose bacteria to EWNS free, room air condition. (control experiments).

2.3 Air inactivation

A high volume EWNS aerosol generator was constructed with an array of 20 electrospray modules which enables the generation of high flow (up to 15 l/min), high concentration aerosol. High efficiency honey comb denuders were used to remove Ozone downstream of the generator. For the bioaerosol generation, a colison nebulizer was used to aerosolize a *Serratia* solution. The generated aerosol of EWNS and bioaerosol were mixed in a 1000 l environmental chamber (figure 2). The residence time in the chamber was 19.5 mins representing approximately 3 air exchanges an hour. After three air exchanges the system is considered to be at steady state. In order to monitor the bacteria concentration while reaching steady state, a sample was taken every 20 minutes (one air exchange) with the Collison biosampler. The Collison biosampler was operated at 28.3 lpm with the Stage #6 (>650 nm) loaded with a trypsin agar plate. Once at steady state the concentration of the bacteria in the chamber was measured in triplicate. Immediately after, all the flows to and from the chamber were shutted and the bacteria concentration was monitored for one hour by sampling every 20 mins (decay). The plates were left for 48 hrs for the culture to grow and subsequently counted. As control the same protocol was used without the EWNS aerosol.

3 RESULTS

3.1 Physical Characterisation of EWNS

The ENWS exhibit a narrow size distribution with a mode of 18 nm and average size approximately 20 nm (Fig 3). The integration of the current as function of time, showed that the average total charge measured resulted in 67.8±8.7 pCu, which represents approximately \((42.31±5.4) \times 10^7\) electrons. At the same time the particle air inactivation. (control experiments).
counter measured approximately \((4.4\pm0.5)\times10^7\) total particles, which results in approximately \(10\pm2\) electrons per particle.

### 3.2 Surface inactivation

Figure 4 shows the results of the surface inactivation. *Serratia Marcescens* exhibits more than a \(2\log_{10}\) reduction in 90 mins of exposure at \(9000\ #/\text{cm}^3\). The control experiments reveal that there is a small inactivation due to the drying of the bacteria, which, however, is negligible compared to the effect of the EWNS. Ozone control experiments also showed that the levels of the generated ozone (150 ppbv) are not enough to inactivate the *serratia*.

### 3.3 Air inactivation

In the environmental chamber the average EWNS particle number concentration and bacteria concentration in the chamber was found to be \(40,000\ #/\text{cm}^3\) and \(5\ \text{cfu/l}\) respectively. Figure 5 summarizes the results of the air inactivation experiments. The EWNS stop the growth of the bacteria aerosol by \(50\%\) when reaching steady state. During the decay experiment the EWNS expedite the inactivation of the bacteria achieving complete elimination at 45 mins.

## 4 DISCUSSION

It was clearly demonstrated in this study that Engineered Water Nanostructures have the potential to be effective and efficient in inactivating from surfaces representative gram-negative bacteria. Moreover, inactivation was found to be dose dependent. Similarly, the air inactivation experiment showed that the EWNS can also inactivate bioaerosol in an indoor environment and reduce the bacterial population build up by \(50\%\). More importantly, EWNS were found to be effective in accelerating significantly the bioaerosol from the indoor air environment.

The charge of the EWNS is a very important physical property of the EWNS. These results are in agreement, earlier published research by Yamauchi et al. [9] and for other studies related to electrospaying [4], [10], [11] For an average of 10 electrons the Rayleigh critical diameter, is \(12.5\ \text{nm}\), which is less than the measured EWNS average size (20 nm). This means that the average charge and diameter of the EWNS satisfies the Rayleigh condition, explaining why the EWNS size is not further reduced and remains stable. Furthermore, it was recently shown that for charged water nanodroplets the charges accumulated on the surface effectively increases the surface tension of the water reducing the evaporation rate [12].

This is in agreement with our experiments and explains why the generated EWNS do not instantly evaporate [11] and remain airborne enough to reach the target bacteria. (Typically for particles of this size the RMS Brownian motion is \(1.5\times10^7\ \text{cm/s}\). Assuming that Brownian motion is the major mode of the particle delivery, it means that the EWNS require an average 5.5 mins to cover the 5 cm distance to the targeted bacteria on surfaces).

## 5 CONCLUSIONS

The electrospayed generated Engineered Water Nanostructures seem to be effective and efficient in disinfecting surfaces and air from vegetative bacteria. Their unique properties such as surface charge, nanoscale size, make them ideal ROS delivery vehicles for bacteria inactivation. Furthermore, there is no chemical trail related to this disinfection method since the EWNS become water vapour after a short period of time (minutes).

This novel nanotechnology has the potential to be used in a large number of applications related to surface and air...
dissinfection ranging from clinical nosocomial environment, to food safety.

REFERENCES


