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# A nano-carrier platform for the targeted delivery of nature-inspired antimicrobials using Engineered Water Nanostructures for food safety applications

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## ABSTRACT

Despite the progress in the area of food safety, foodborne diseases still represent a massive challenge to the public health systems worldwide, mainly due to the substantial inefficiencies across the farm-to-fork continuum. Here, we report the development of a nano-carrier platform, for the targeted and precise delivery of antimicrobials for the inactivation of microorganisms on surfaces using Engineered Water Nanostructures (EWNS). An aqueous suspension of an active ingredient (AI) was used to synthesize iEWNS, with the 'i' denoting the AI used in their synthesis, using a combined electrospray and ionization process. The iEWNS possess unique, active-ingredient-dependent physicochemical properties: i) they are engineered to have a tunable size in the nanoscale; ii) they have excessive electric surface charge, and iii) they contain both the reactive oxygen species (ROS) formed due to the ionization of deionized (DI) water, and the AI used in their synthesis. Their charge can be used in combination with an electric field to target them onto a surface of interest. In this approach, a number of nature-inspired antimicrobials, such as $H_2O_2$, lysozyme, citric acid, and their combination, were used to synthesize a variety of iEWNS-based nano-sanitizers. It was demonstrated through foodborne-pathogen-inactivation experiments that due to the targeted and precise delivery, and synergistic effects of AI and ROS incorporated in the iEWNS structure, a pico-to nanogram-level dose of the AI delivered to the surface using this nano-carrier platform is capable of achieving 5-log reductions in minutes of exposure time. This aerosol-based, yet 'dry' intervention approach using iEWNS nano-carrier platform offers advantages over current 'wet' techniques that are prevalent commercially, which require grams of the AI to achieve similar inactivation, leading to increased chemical risks and chemical waste byproducts. Such a targeted nano-carrier approach has the potential to revolutionize the delivery of antimicrobials for sterilization in the food industry.

## 1. Introduction

Safe and nutritious food is an important aspect of public health assurance worldwide (Newell et al., 2010); however, microbiologically contaminated food, across the farm-to-fork continuum, is a constant threat to the agricultural and food processing sector. The annual toll of foodborne disease resulting from the consumption of microbiologically contaminated food is on the rise and has reached an alarming 600 million cases and 420,000 deaths worldwide (World Health Organization, 2015). In the United States, the Center for Disease Control and Prevention (CDC) estimates that 52% of all foodborne diseases are from fresh produce, 22% from meat and poultry, 20% from eggs, and 6% from seafood (Interagency Food Safety Analytics, 2015). Further, it is estimated that, on average, there are approximately 65

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outbreaks annually due to fresh produce, more than any other food commodity (Interagency Food Safety Analytics, 2015).

Fruits and vegetables can become contaminated with pathogenic microorganisms anywhere in their production chain starting from the field or orchard, during harvest, transport, processing, distribution, and marketing, or during preparation in food-service establishments and at the home. Possible pathogen sources include contaminated irrigation water (Steele & Odumeru, 2004), animals, birds, insects, soil, manure, and infected workers/food handlers (Berger et al., 2010). In addition, improperly cleaned and disinfected food preparation/contact and storage surfaces can also be important reservoirs of pathogens (Buchholz, Davidson, Marks, Todd, & Ryser, 2012; Kaminski, Davidson, & Ryser, 2014; van Asselt, de Jong, de Jonge, & Nauta, 2008). In recent years, these emerging food safety issues can also be attributed to newly established factors and trends, such as the globalization of the food supply chain (Trienekens & Zuurbier, 2008); growing consumer demand for minimally processed foods, especially fresh produce; and a surge in organic produce consumption (Brackett, 1999; Harvey, Zakhour, & Gould, 2016; Mercanoglu Taban & Halkman, 2011). Modern supermarkets are full of products that are out of season, transported halfway around world to reach the consumer (Schaadt, 2013), usually stacked in containers for weeks (Athukorala & Jayasuriya, 2003), increasing the risk of cross contaminations.

The antimicrobial strategies currently used by the food industry for decontamination mainly include the use of chemicals, such as chlorine-based products, especially sodium hypochlorite (Parish et al., 2003); ozone (Horvitz & Cantalejo, 2014); ultraviolet radiation (Chang et al., 1985; Pearson, Hunt, & Mitchell, 1997); and high-pressure processing (Balasubramaniam & Farkas, 2008; Shearer, Dunne, Sikes, & Hoover, 2000). Other alternative disinfection methods being investigated for fresh produce disinfection include alternative chemical methods, such as vaporized hydrogen peroxide (Ukuku, Bari, Kawamoto, & Isshiki, 2005); electrolyzed water (Izumi, 1999; Koseki, Yoshida, Isobe, & Itoh, 2004); biological methods (bacteriocins, bacteriophages, enzymes and phytochemicals); (Meireles, Giaouris, & Simões, 2016); physical technologies, such as non-thermal electrical plasma discharges, ionizing radiation, ultrasounds, etc.; and combination methodologies (Vaze, Park, Brooks, Fridman, & Joshi, 2017; Wan, Coventry, Swiergon, Sanguansri, & Versteeg, 2009).

All these methods have their inherent inadequacies and limitations. For example, chlorine-based products used as a “wet” approach in washes and sprays leave behind chemical residues that can be toxic, can have an effect on the sensory quality of food (i.e. color, taste), and generate large chemical waste, which in turn can create environmental problems (Karaca & Velioglu, 2007; Y.; Luo et al., 2018; Ventola, 2015). These methods have also been implicated in the loss of nutrients in the treated food items (Z. Chen, Zhu, & Han, 2011). Further, the majority of these chemical-mediated approaches require the tumbling of the produce items in pools of a sanitizer solution or spraying of the produce items with powerful jets of the sanitizer, which is a violent process that can damage delicate produce items, such as berries (Yaguang Luo et al., 2011). Some of these processes can also impart physical damage on the produce, reducing their visual appeal and quality (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). In addition, many of these chemicals cannot be used with produce that carry the organic label, due to FDA and USDA restrictions.

These aforementioned inadequacies, especially the lack of precision in the delivery of active ingredients (AIs) stress the need for developing new antimicrobial control strategies to supplement or replace existing ones and effectively combat foodborne pathogens at critical control points. Nanotechnology has emerged as an enabling technology to assist our society in combating infections (Eleftheriadou, Pyrgiotakis, & Demokritou, 2017). For example, engineered nanomaterials (ENMs) with antimicrobial properties, such as nanosilver, photocatalytic $TiO_2$ and ZnO nanoparticles, are being used to produce antimicrobial surfaces (Chaloupka, Malam, & Seifalian, 2010; Espitia et al., 2012); however, these ENMs cannot be implemented for fresh produce disinfection or as an aerosol treatment for airborne disinfection due to associated risks from ingestion and inhalation (H. Chen et al., 2017; Chia, Tay, Setyawati, & Leong, 2015; G. DeLoid et al., 2016; G. M. DeLoid et al., 2017; Giovanni et al., 2015; Lu et al., 2016; McClements et al., 2016; Pirela et al., 2016; Servin & White, 2016; Setyawati et al., 2018; Sohal, O'Fallon, Gaines, Demokritou, & Bello, 2018).

Here, we report the development and evaluation of a nano-carrier platform for the precise and targeted delivery of minute quantities of antimicrobials using the Engineered Water Nanostructures (EWNS) recently developed by the authors (Pyrgiotakis et al., 2016; Pyrgiotakis, McDevitt, Bordini, et al., 2014). In this study, this EWNS platform was expanded and used to incorporate other AIs into the EWNS structure in order to further enhance their antimicrobial potency. These new structures are referred to as iEWNS, where ‘i’ denotes the AI used. As shown, these iEWNS possess unique, active-ingredient-dependent physicochemical properties: i) they are engineered to have a tunable size in the nanoscale; ii) they have excessive electric surface charge, and iii) they contain both the reactive oxygen species (ROS) formed due to the ionization of deionized (DI) water, and the AI used in their synthesis. One of the novel features of these structures is the electrical charge that can be used in combination with an electric field to target them onto the surface of interest. As a result, the iEWNS deliver the AI with high precision, minimizing the amount required without compromising their effectiveness.

In this study, the focus was on using nature-inspired antimicrobials, such as hydrogen peroxide, citric acid, their combination, and lysozyme. These iEWNS were investigated for their physicochemical and antimicrobial properties and their efficacy was evaluated against foodborne pathogens.

## 2. Materials and methods

### 2.1. Synthesis and targeted delivery of iEWNS

**Synthesis:** Detailed description of the synthesis of the iEWNS is illustrated in Fig. 1 and has been explained in great detail in previous publications (Pyrgiotakis et al., 2016; Pyrgiotakis, McDevitt, Gao, et al., 2014).

In brief, an aqueous solution of the utilized antimicrobial is prepared (Fig. 1a) by adding the appropriate amount of the AI in DI water. The solution is held in an airtight bottle. High air pressure in the bottle created by an air compressor pushes the aqueous solution through a 0.3-mm ID Teflon tubing to an emitter-capillary (30G stainless steel needle). The emitter is held across an aluminum counter electrode that is grounded. The counter electrode has a 0.64-cm diameter aperture in the center and sits on top of a grounded aluminum funnel that is connected via brass tubing to the various sampling instruments for characterization.

A high voltage source (Spraybase, Dublin, Ireland) is used to apply voltage between the emitter and the counter electrode to form a strong electric field (Fig. 1b). As shown in a previous publication (Pyrgiotakis et al., 2016), during this process two distinct phenomena take place: i) electrospray and ii) ionization of the water. The charges accumulated at the water-air interface at the tip of the emitter in combination with the strong electric field causes the formation of the Taylor cone (Taylor, 1964). A strong electrical force, created by the interfacial charge and the strong electric field, results in the emission of highly charged particles (Fig. 1c - inset). At the same time, the electric field causes the water molecules to split and ionize, resulting in the generation of ROS, which end up along with the AI in the iEWNS particles (Fig. 1c). A digital camera (Point Grey Cameleon, FLIR Integrated Imaging Solutions Inc., Richmond BC, Canada) is used to visually monitor the formation and stability of this Taylor cone. In our current design, three emitters/generation modules are operating in parallel.

It is worth noting that the operational parameters that can be