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The antibacterial activity of clove oil/chitosan nanoparticles embedded gelatin nanofibers against *Escherichia coli* O157:H7 biofilms on cucumber

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ABSTRACT

This study aims to evaluate the antibacterial activity of clove oil-loaded chitosan nanoparticles (CO@CNPs) and gelatin electrospun nanofibers against *Escherichia coli* O157:H7 (*E. coli* O157:H7) biofilms on cucumbers. The optimal CO@CNPs were prepared when the initial concentration of clove oil (CO) was 2.5 mg/mL according to the ionic crosslinking method. CO@CNPs showed high antibacterial activity against *E. coli* O157:H7 biofilms. After 8 h treatment, almost 99.98% reduction in *E. coli* O157:H7 population was achieved when CO@CNPs were applied at 30% (w/v). Subsequently, the prepared CO@CNPs were incorporated successfully within gelatin nanofibers by electrospinning. After 9 mg/mL gelatin/CO@CNPs treatment for 24 h, the population of *E. coli* O157:H7 biofilm reduced by about 99.99% *in vitro*. Further, the application of gelatin/CO@CNPs nanofibers on cucumber against *E. coli* O157:H7 biofilm was evaluated as well. After 6 mg/mL and 9 mg/mL gelatin/CO@CNPs nanofibers treatment at 12 °C for 4 days, 4.28 and 4.97 $\log_{10}$ reductions of *E. coli* O157:H7 biofilm in population were observed, respectively. Finally, the sensory evaluation results implied that the gelatin/CO@CNPs nanofibers treatment could maintain the color and flavor of cucumber well for > 4 days.

1. Introduction

Bacterial infections, due to their potential to be a public health hazard, have caused an increasing social concern (Ongeng et al., 2011). Recently, outbreak of foodborne illness caused by *E. coli* O157:H7 is often associated with consumption of infected solely fresh products such as cucumbers and cherry tomato (Olomon et al., 2002). The bacterium often survives on the food surface as a biofilm even at worst conditions, which has a more stable structure (Rayner et al., 2004). Biofilm is a complex matrix of microorganisms attached to an inert or living surface, usually consisting of polysaccharide, and organisms within are able to excrete various virulence factors and enzymes (Srey et al., 2013). The formation of *E. coli* O157:H7 biofilms has a high occurrence rate on cucumber (Almasoud et al., 2015; Cui et al., 2016). Therefore, the risk of human bacterial infections caused by *E. coli* O157:H7 biofilms is a serious challenge in the field of food safety due to their enhanced antimicrobial resistance (Sheen and Hwang, 2010).

For the purpose of minimizing the incidence of disease caused by *E. coli* O157:H7 biofilm, some thermal treatments are utilized in the food industry frequently. However, some nutrients in food may be denaturalized or destroyed during the heating process. As a consequence, the application of non-thermal sterilization technology has become the keystone of research (Li and Farid, 2016). Essential oil (EO) has gained increasing attraction among consumers as healthy preservatives because of its safety, high efficiency and hypotoxicity (Bajer et al., 2017).

Clove oil (CO) is one kind of natural essential oil that possesses high antimicrobial and antioxidant activities (Cui et al., 2015). Unfortunately, CO is difficult to inhibit bacteria for a long term due to its volatility and instability (Mulla et al., 2017). In the resent years, the antibacterial activity and stability of CO has been enhanced by using nanoencapsulation with the development of nanotechnology.

As a biopolymer material, chitosan has been widely applied to prepare drug-loaded nanoparticles due to its good biocompatibility and high cost-effectiveness (Feyzioglu and Tornuk, 2016; Liang et al., 2017). It has also been used for encapsulation of natural essential oils such as cinnamon, limonene, and oregano essential oil (Cui et al., 2017).

Further, electrospinning is an economic and versatile technique in the food packaging industry that allows the incorporation of different kinds of agents into a wide variety of polymers and blends in the form of nanofibers (Bhardwa and Kundu, 2010). Electrospinning technique is an easy method to fabricate micro and nanofibers with high surface area (Surucu and Sasmazel, 2016). Previous studies have shown that electrospun nanofibers achieved long-term efficiency when it was used for the encapsulation of antimicrobial agents (Rieger and Schiffman, 2014; Rieger et al., 2016). Therefore, the coating of vegetable surfaces

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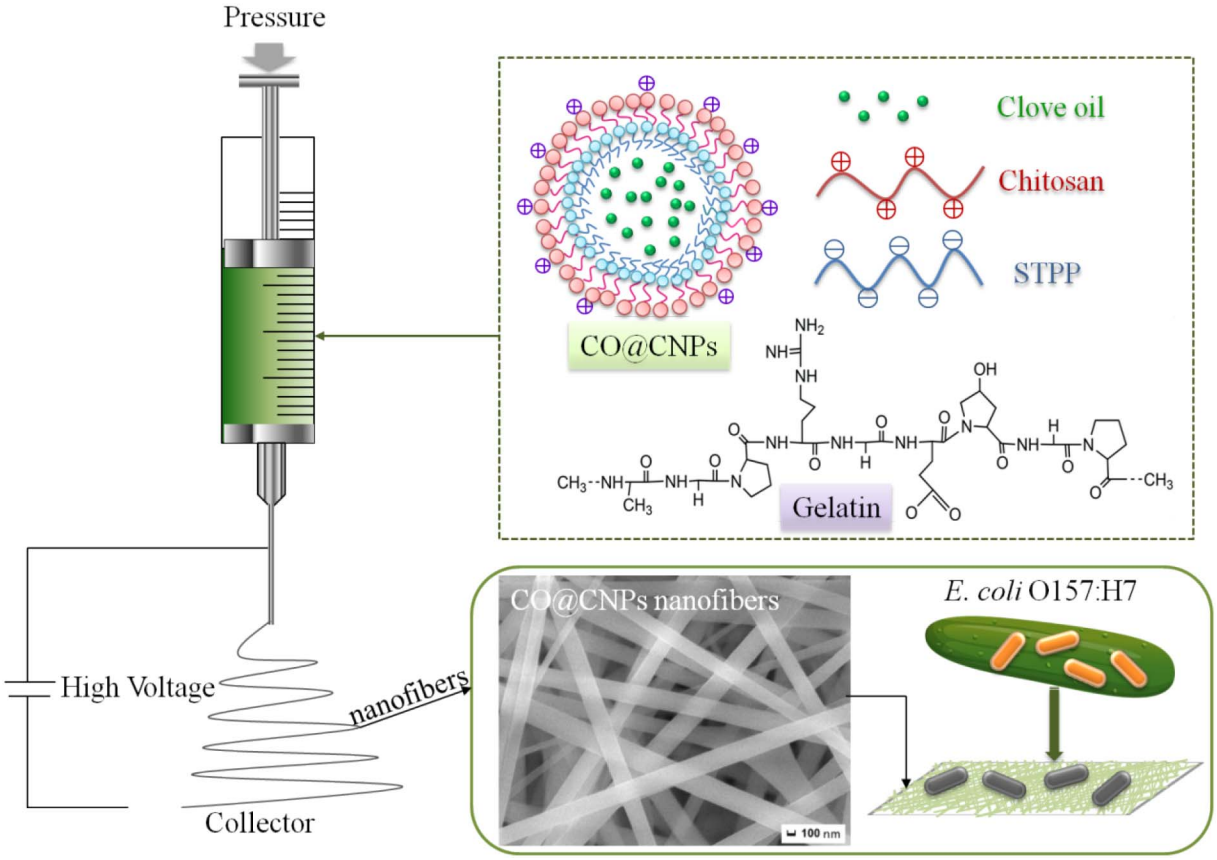

Fig. 1. The experimental design of this study.

with CO@CNPs loaded gelatin electrospun would be a novel strategy to inhibit microbial growth in vegetables.

As a proof-of-concept, this study attempted to enhance the stability and long-term antibacterial activity of CO by loading the CO in chitosan nanoparticles. Furthermore, electrospinning technique was employed to realize the application of chitosan nanoparticles in food packaging. The CO@CNPs loaded gelatin nanofibers were prepared successfully in the present work (Fig. 1). The antibacterial activity of CO@CNPs loaded gelatin nanofibers against *E. coli* O157:H7 biofilms on cucumber was evaluated as well.

2. Materials and methods

2.1. Materials and culture

CO was bought from J.E. International (Caussols plateau, France). Chitosan (85% deacylated) and sodium tripolyphosphate (STPP, technical grade) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). Gelatin (type B from porcine skin), Tween 80 and acetic acid (> 99.7% purity) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The tested strain of *Escherichia coli* O157:H7 CICC 21530 was purchased from China Center of Industrial Culture Collection (Beijing, China). The strain was cultured in nutrient broth (NB, AOBOX, Beijing, China) at 37 °C with shaking for 24 h. The viable count of *E. coli* O157:H7 was observed after culturing in nutrient agar (NA, AOBOX, Beijing, China) for 24 h at 37 °C. Fresh Holland cucumber was purchased from the local supermarket.

2.2. Chemical compositions of CO and antibacterial activity of three main compounds

The chemical compositions of CO were determined by GC–MS (Agilent 6890GC/5973NMSD, NYSE: A, USA). A fused silica capillary Agilent Technology HP-5ms (5% phenyl methyl siloxane) column (30 m × 0.25 mm × 0.25 μm, film thickness 0.1 μm) was used for separation. The temperatures in injector and detector were fixed at 250 and 280 °C, respectively. The initial temperature of the column was 60 °C for 2 min, and it was raised up to 250 °C at 2 °C/min and maintained at 250 °C for 5 min. Component identification was made on the basis of mass spectral fragmentation, retention time comparison with authentic constituents' mass spectral and retention time (matching with commercial and in-house libraries).

The bactericidal activities of CO and three main components were detected by time-kill curve assay. The extracted fractions were added into tubes containing *E. coli* O157:H7 (10^5–10^6 CFU/mL) suspension in sterile phosphate buffer solution (PBS, pH 7.2). As a control, bacterial suspension in PBS without extracted fractions was prepared as well. Subsequently, 100 μL solution were sampled from different tubes and respectively cultured in NA under the same condition as mentioned above (section 2.1). Finally, the total viable count of *E. coli* O157:H7 was observed after 24 h.

2.3. Preparation of CO@CNPs

CO@CNPs were performed in two steps, namely the preparation of oil-in-water (o/w) emulsion and ionic-gelation of chitosan using STPP based on the method reported by Woranuch and Yoksan (2013). Briefly, chitosan (CS) (0.4% w/v) was dissolved in acetic acid solution (1% v:v, 20 mL) and mixed well under magnetically stirring for 30 min. Then, the prepared solution of CS was diluted with double distilled water to