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Antimicrobial Particle-Based Novel Sanitizer for Enhanced Decontamination of Fresh Produce

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ABSTRACT Microbial food safety of raw or minimally processed fresh produce is a significant challenge. The current sanitation processes are effective for inactivation of bacteria in wash water but have limited efficacy (<2 log/g reduction) for inactivation of microbes on the surfaces of fresh produce. This study demonstrates a novel concept to enhance effectiveness of chlorine using a particle-based sanitizer to improve decontamination of fresh produce. In this concept, enhanced effectiveness is achieved by localized high concentration of chlorine bound to the surfaces of silica particles and improved surface contact of microparticles with the produce surface using mechanical shear during a washing process. The results of this study demonstrate that 500 ppm active chlorine can be bound to the surfaces of modified silica particles. These modified particles maintain over 90% of their initial chlorine content during extended storage in aqueous solution and provide improved inactivation of both *Escherichia coli* O157:H7, *Listeria innocua*, and *Pseudomonas fluorescens* in the presence of organic content in contrast to conventional chlorine sanitizer. The modified particles exhibit effective sanitation of fresh produce (>5-log reduction) in the presence of relatively high organic content (chemical oxygen demand of 500 mg/liter), demonstrating a potential to address a significant unmet need to improve fresh produce sanitation. The particle-based sanitizer had no significant effect on the quality of fresh lettuce.

IMPORTANCE The limitation of current sanitation processes for inactivation of microbes on the surfaces of fresh produce is due to nonspecific consumption of sanitizers by reactions with the food matrix and complexity of surface chemistries and structural features of produce surfaces. This study demonstrates a novel approach to enhance sanitation effectiveness of fresh produce using a particle-based sanitizer. The particle-based sanitizer concept provides localized high concentration delivery of chlorine to the surfaces of fresh produce and enables more than 5 logs of inactivation of inoculated bacteria on fresh produce surfaces without significant changes in produce quality. The results of this study illustrate the potential of this approach to address the unmet need for improving sanitation of fresh produce. Further validation of this approach using a scaled-up produce washing system will enable commercialization of this novel concept.

KEYWORDS foodborne pathogen, *N*-halamine, particle-based sanitizer, poly-L-lysine, sanitation of fresh produce, silica particles

Microbial food safety of raw or minimally processed fresh produce is a significant challenge. Globally, fresh produce consumption has increased significantly over the last two decades due to growing consumer awareness of the health benefits of fresh produce (1, 2). Considering that most fresh produce is consumed raw or after minimal processing, it is important to reduce the risk of its contamination with pathogenic microorganisms throughout the production chain (3, 4). It is reported that

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more than 46% of the foodborne illnesses in the United States from 1998 to 2008 are attributed to the consumption of raw or minimal processed fresh produce (5). Similarly, contamination of fresh produce with spoilage bacteria can also lead to significant reduction in shelf life of fresh produce. Despite significant advances in postharvest packaging and refrigeration technologies, a significant fraction of fresh produce (20 to 40%) is spoiled due to microbial contamination and overgrowth (6). Limitations in reducing pathogenic and spoilage microbes on minimally processed fresh produce are among the key constraints in maintaining safety and quality of fresh produce (7). Therefore, sanitation and washing procedures are vital steps for improving safety and shelf-life of fresh produce products by inactivating pathogenic and spoilage bacteria on the surfaces of food products (8, 9).

To address safety and spoilage issues of fresh produce, a number of decontamination methodologies for fresh produce have been reported (10, 11). Current chemical treatments such as chlorine, peroxyacetic acid, hydrogen peroxide, quaternary ammonium, and ozone are effective in reducing the microbial load in wash water but have limited efficacy for inactivation of bacteria on fresh produce surfaces (1- to 2-log reduction) (12–15). This limitation is due to nonspecific consumption of sanitizers upon reactions with the food matrix (16). Furthermore, complexity of surface chemistries (such as waxy cuticle) and structural features of surfaces of the produce (e.g., crevices and cracks) may shield bacteria from conventional sanitizers and thus limit the efficacy of these sanitizers (8, 17, 18). In addition, pathogenic bacteria may form complex biofilms in combination with other microbes on the surfaces of fresh produce. The complexity of the biofilm matrix on produce surfaces makes it challenging to inactivate bacteria in biofilms compared to planktonic bacteria in a wash water (17, 19, 20). In summary, there is a significant unmet need to develop a novel sanitizer for effectively inactivating pathogenic and spoilage bacteria on the surfaces of contaminated fresh produce during postharvest sanitation and washing process.

Particle-based drug delivery carriers have been developed for pharmaceutical and medical applications to enhance localized delivery of high concentration of active agents (21, 22). To extend this concept to sanitizers, the sanitizer should be localized at the surface of a particle so that it can inactivate microbes upon contact or particle can release adequate concentration of sanitizer molecules into the surrounding environment for inactivation of microbes.

With this overall goal, this study evaluated the concept of particle-based sanitizers for inactivation of bacteria in simulated produce wash water and on the surfaces of a model fresh produce. It is expected that localized delivery of high concentration of sanitizer to leaf surfaces using particle-based sanitizers will induce rapid inactivation of bacteria. In this study, the antimicrobial chlorine-based agent was immobilized on the surfaces of micron-scale silica particles using halamine chemistry. Silica microparticles were selected due to their biocompatibility, chemical inertia, and water dispersibility (23, 24). Furthermore, food-grade silica particles can be considered a processing aid if their mass concentration is below 1,250 mg/kg (25). In addition, coated micron-scale particles provide a large surface area in contrast to conventional applications of *N*-halamine chemistry on polymeric materials. This enhanced surface area may enable effective inactivation of bacteria in wash water and on the surfaces of fresh produce. To the best of our knowledge, none of the previous studies have evaluated the potential of antimicrobial microparticles to improve sanitation of fresh produce and wash water with suspended organic content. Development of a particle-based sanitizer for food products and food contact surfaces may improve safety of minimally processed products such as fresh produce, reduce spoilage, and reduce cross-contamination from food contact materials.

RESULTS

Characterization of synthesized SiO_2-PLL particles. The physicochemical properties of particles (SiO_2–poly-L-lysine [SiO_2-PLL]) were characterized based on size, surface charge, surface chemistry, and morphology. The results (Fig. 1a) of dynamic light

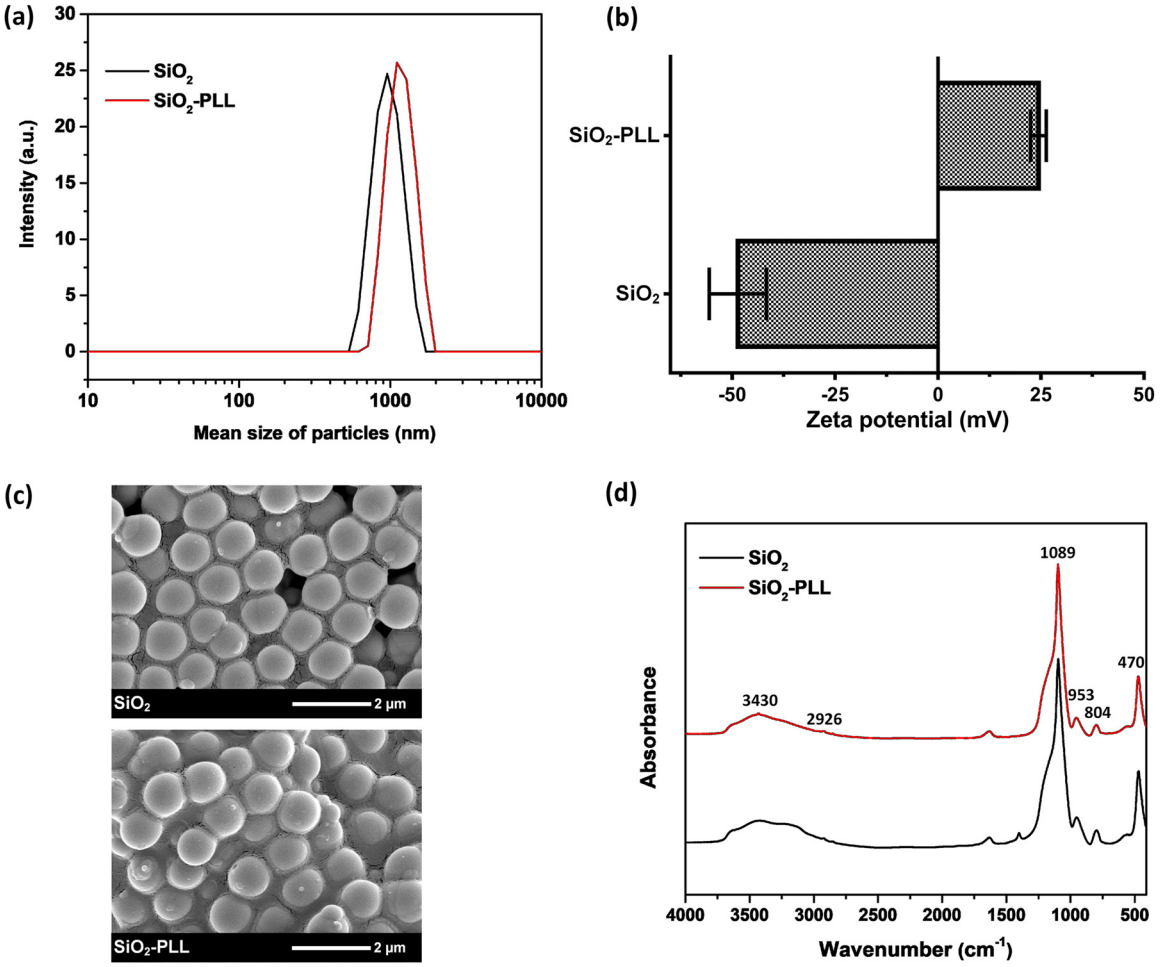

FIG 1 Characterization of synthesized SiO_2 and SiO_2-PLL particles. (a) Particle size measurements using dynamic light scattering; (b) zeta potential measurements before and after coating; (c) SEM images; (d) ATR-FTIR spectrum. Error bars represent the standard deviations of $n = 3$ values.

scattering measurements show that, as prepared, SiO_2 had a monomodal particle size distribution with a mean diameter of 955.4 nm and a polydispersity index of 0.251. These results indicate a relatively uniform particle size of silica particles (Fig. 1a). Coating silica particles with PLL based on electrostatic interactions only marginally increased the mean particle diameter to (1,085.7 nm) but reversed the negative zeta potential of silica particles from -48.6 ± 6.9 mV to 24.4 ± 1.9 mV (Fig. 1b). Scanning electron microscopy (SEM) imaging results (Fig. 1c) further confirmed that SiO_2 and SiO_2-PLL have a relatively similar particle size and morphology.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) measurements were performed on dried particles to characterize the surface chemistry of the coated particles as synthesized (Fig. 1d). In the case of as-synthesized SiO_2 particles, the characteristic peaks at 953, 804, and 1,099 cm^{-1} were ascribed to Si-OH stretching and to symmetric and antisymmetric stretching vibrations of the Si-O-Si bond, respectively (26, 27). In the FTIR spectrum of SiO_2-PLL particles, absorption bands at approximately 3,430 and 2,926 cm^{-1}, respectively, were attributed to N-H stretching and C-H bond stretching (28, 29). The presence of these bands further confirms surface modification of silica particles with poly-L-lysine (PLL) coating.

Chlorination efficiency. It is widely acknowledged that the biocidal mechanism of *N*-halamine modified polymers is based on the transfer of halogen atoms from the modified polymers to microbes upon contact. Therefore, the total halogen loading in a *N*-halamine polymer can significantly influence both the rate and extent of microbial

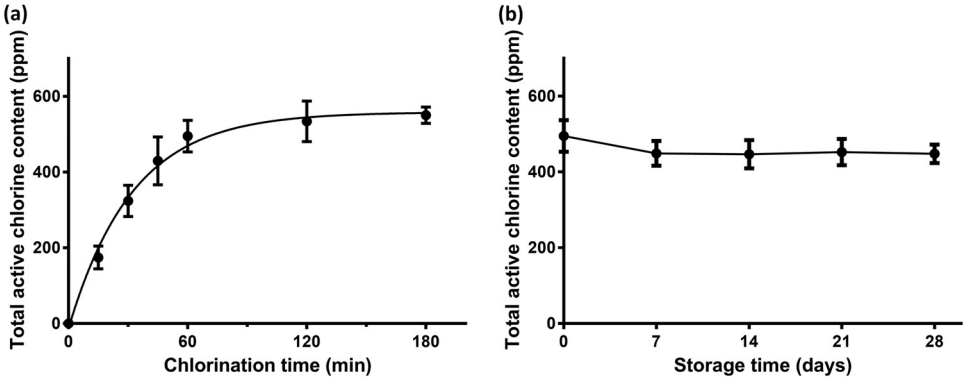

FIG 2 (a) Total active chlorine content of SiO_2-halamine as a function of chlorination time; (b) total active chlorine content of SiO2-halamine particles during storage in an aqueous suspension at 4°C for 28 days.

inactivation ($P < 0.05$). The N-H structure in amino rich polymers can be transformed to N-Cl group upon incubation of SiO_2-PLL particles with sodium hypochlorite to obtain the *N*-halamine functionality on the surfaces of coated silica particles (30, 31). In this study, the PLL polymer coated on silica particles was chlorinated by incubating these particles in a diluted household bleach (10% [vol/vol] of 8.25% commercial sodium hypochlorite solution) at pH 5 and room temperature for a range of time periods. The results in Fig. 2a illustrate an increase in chlorine content of PLL-coated silica particles as a function of incubation time. The results show a rapid increase [$C = 559.4 \times (1 - e^{-0.03t})$] in the chlorine content on silica-coated PLL particles reaching approximately 500 ppm, which means 0.5 mg of chlorine bound to the surfaces of 1 g of silica particles after 1 h of incubation with bleach. With extended incubation to 3 h, there was no significant increase in the total mass of chlorine per unit mass of silica particles ($P > 0.05$). This trend suggests saturation of the PLL polymer with chlorine. After charging of chlorine on PLL-coated silica particles, the stability of chlorine bound to the surfaces of PLL-coated silica particles was evaluated. The results in Fig. 2b illustrate no significant changes in the total chlorine content upon storage of chlorine charged PLL-coated silica particles for 28 days under refrigerated conditions ($P > 0.05$).

Antimicrobial activity of charged silica particles in water. To assess the antimicrobial activity of charged silica particles, water was selected as a model system. The water samples were inoculated with *E. coli* O157:H7, *L. innocua*, and *P. fluorescens*, respectively. The reduction in bacterial count upon treatment was characterized using a standard plate colony-forming assay. The results in Fig. 3 show the survival of *E. coli*, *L. innocua*, and *P. fluorescens* in wash water after 20 min of exposure to the control, SiO_2, SiO_2-PLL, conventional chlorine-based sanitizer, and SiO_2-halamine particles, respectively. SiO_2 and SiO_2-PLL did not exhibit antimicrobial properties, as expected, whereas

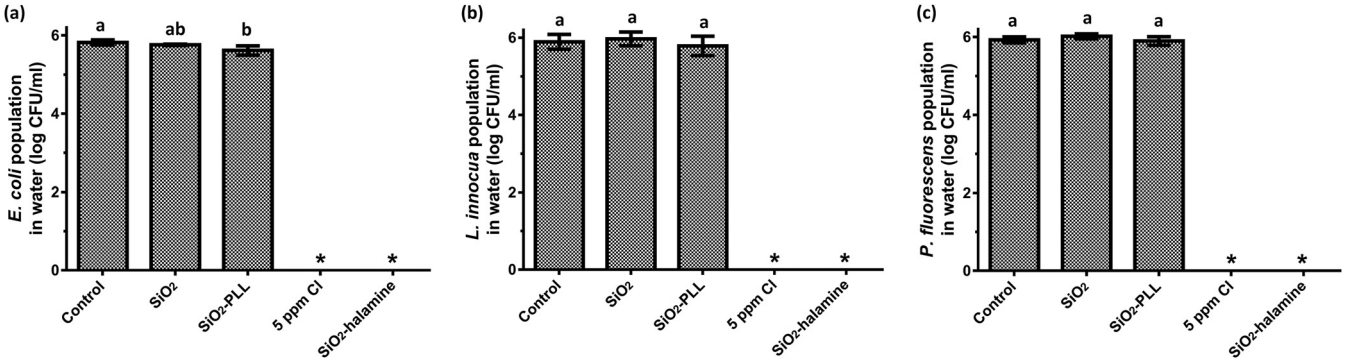

FIG 3 Sanitation of water by SiO_2-halamine particles. (a) *E. coli* O157:H7; (b) *L. innocua*; (c) *P. fluorescens*. The asterisk indicates that the viable/culturable counts for that experiment were below the detection limit (1 log CFU/ml). Treatments with different letters are significantly different ($P < 0.05$).

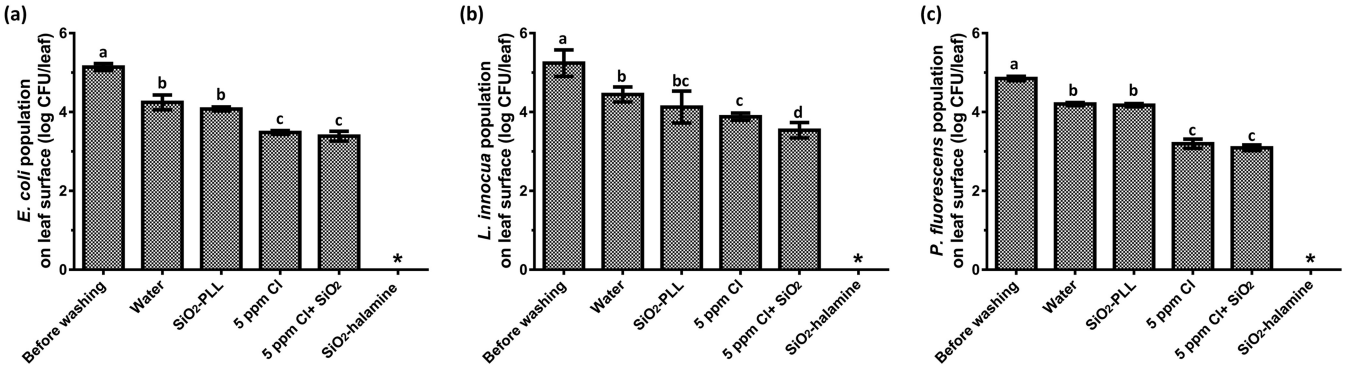

FIG 4 Antimicrobial efficacy of synthesized SiO_2-halamine particles and conventional chlorine-based sanitizer for the sanitation of the lettuce leaf surface in the absence of organic load in wash water. (a) *E. coli* O157:H7; (b) *L. innocua*; (c) *P. fluorescens*. The asterisk indicates that the viable/culturable counts for these measurements were below the detection limit (1 log CFU/leaf). Treatments with different letters are significantly different ($P < 0.05$).

conventional chlorine-based sanitizer (containing 5 ppm free chlorine, which was equivalent to the chlorine content on 2 mg/ml charged SiO_2-halamine particles) and charged (SiO_2-halamine) particles inactivated 6 logs of *E. coli* O157:H7, *L. innocua*, and *P. fluorescens*. These results suggest significant antimicrobial potential of SiO_2-halamine particles against both Gram-positive and Gram-negative bacteria.

Sanitation of fresh produce surfaces. Current sanitizers, including chlorine, cannot achieve adequate (>2-log) inactivation of bacteria on the surfaces of fresh produce due to nonspecific interactions of the sanitizer with organic content on the surfaces of fresh produce. In this next set of experiments, we assessed the efficacy of particle-based sanitizer for the decontamination of fresh produce. In this study, lettuce leaf samples were inoculated with *E. coli* O157:H7, *L. innocua*, and *P. fluorescens*, respectively, using a novel spin-coating approach to generate a relatively uniform distribution of bacteria on produce surfaces, as described in our previous work (32). The contaminated leaf samples were treated with sterile water, modified particles before charging (SiO_2-PLL), conventional sanitizer containing 5 ppm free chlorine, SiO_2 particles with 5 ppm free chlorine, and charged modified particles (SiO_2-halamine), respectively. Based on the standard plate count assay (Fig. 4a and b), a <1-log reduction in *E. coli* and *L. innocua* concentrations from inoculated leaf samples was obtained by washing with sterile water for 20 min, respectively. In the case of *P. fluorescens*, only a 0.65 $\pm$ 0.03 log CFU/leaf decrease in bacterial concentration on lettuce leaf samples was obtained upon washing leaf samples with sterile water for 20 min (Fig. 4c). The presence of uncharged SiO_2-PLL did not significantly improve the inactivation efficacy for both Gram-positive and Gram-negative bacteria on leaf surfaces ($P > 0.05$). After exposure to 2 mg/ml of SiO_2-halamine particles for 20 min, the bacterial population on inoculated lettuce leaves was reduced by approximately 5 log CFU/leaf. Meanwhile, to simulate the typical washing procedure used in fresh produce industry, the addition of conventional chlorine-based sanitizer (5 ppm free chlorine) in wash water resulted in approximately 1.66-, 1.37-, and 1.36-log reductions, for *E. coli*, *L. innocua*, and *P. fluorescens*, respectively. In addition, increasing the free chlorine content in wash water to 10 ppm did not significantly enhance the microbial reduction on lettuce leaf surfaces (data not shown). To further validate the enhanced efficacy of SiO_2-halamine particles, the inoculated lettuce sample was treated with control silica particles in combination with 5 ppm chlorine. The results show no significant enhancement in antimicrobial activity of chlorine in this case compared to the conventional chlorine treatment ($P > 0.05$) for *E. coli* and *P. fluorescens*. A small but statistically significant enhancement in antimicrobial activity of chlorine was observed for *L. innocua* with a combination of chlorine and silica particles ($P < 0.05$).

To further simulate a realistic produce washing situation, the sanitation efficacy of SiO_2-halamine particles against *E. coli* O157:H7 inoculated on lettuce leaf surfaces was

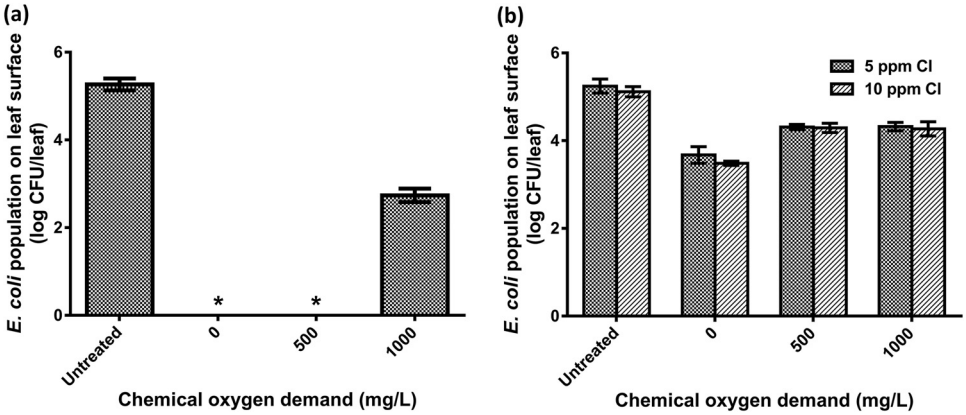

FIG 5 (a and b) Efficacy of synthesized SiO_2-halamine (a) and conventional chlorine-based sanitizer (b) on the sanitation of *E. coli* O157:H7 on lettuce leaves in the presence of organic load in wash water. An asterisk indicates that the viable/culturable counts for these measurements were below the detection limit (1 log CFU/leaf).

assessed in a simulated wash water with dissolved organic load. A diluted solution of appropriate Luria-Bertani (LB) medium was used for simulating the chemical oxygen demand (COD) levels encountered conditions during the washing of fresh produce. In the case of wash water with a COD ranging between 0 and 500 mg/liter, the SiO_2-halamine particles caused a >5-log CFU/ml reduction in inoculated *E. coli* from fresh produce surfaces (Fig. 5a). In the case of COD value of 1,000 mg/liter in a simulated wash water, the SiO_2-halamine particles achieved a reduction of 2.5-log units of inoculated bacteria on fresh produce surfaces. The decrease in antimicrobial activity in the presence of high COD conditions could be attributed to partially quenching of active chlorine on the particle surface upon reaction with high organic content in wash water. To compare these results with conventional sanitation using chlorine, two levels of conventional sanitizers with 5 and 10 ppm for free chlorine were evaluated (Fig. 5b). Under the same set of experimental conditions as in the case of SiO_2-PLL, conventional chlorine-based sanitizer achieved only a 0.5-log reduction in the inactivation of bacteria from leaf surfaces for COD contents ranging between 500 and 1,000 mg/liter. In addition, increasing the free chlorine concentration from 5 to 10 ppm did not affect the sanitation efficacy in the presence of organic content. It is important to note that to achieve a free chlorine concentration of 5 and 10 ppm in a high-COD environment, the total chlorine added to the wash water ranged between 70 and 150 ppm. Overall, these results demonstrate the superior antimicrobial potential of SiO_2-halamine particles for reducing an inoculated bacterial load on the surfaces of fresh produce.

To assess the sanitation efficacy of particle-based sanitizer on the natural flora of fresh produce, the total aerobic mesophilic plate count and yeast and mold count were measured before and after a simulated washing process. The aerobic plate counts of leaf surfaces before washing were 6.1 $\pm$ 0.2 log CFU/g (Fig. 6a). The counts of total aerobic mesophilic bacteria were reduced by 3 log CFU/g after a washing step with 2 mg/ml of SiO_2-halamine. Washing with sterile water, uncharged SiO_2-PLL, and conventional sanitizer (i.e., 5 ppm free chlorine) only led to a limited reduction of total aerobic mesophilic bacteria, i.e., a <1-log CFU/g reduction compared to unwashed leaves. For yeast and mold on leaf surfaces, the initial count was 5.1 $\pm$ 0.1 log CFU/g (Fig. 6b). Washing with charged modified particles (SiO_2-halamine) for 20 min resulted in an approximately 1.6-log CFU/g reduction in yeast and mold counts. In comparison, yeast and mold counts were not significantly reduced after washing with sterile water, modified particles before charging (SiO_2-PLL), conventional sanitizer containing 5 ppm free chlorine, or SiO_2 particles with 5 ppm free chlorine, respectively.

Evaluation of the quality of fresh produce. The effect of particle-based sanitizer on the quality of fresh produce during storage was evaluated based on measuring changes in the total color and texture after treatment with selected sanitizers. Changes

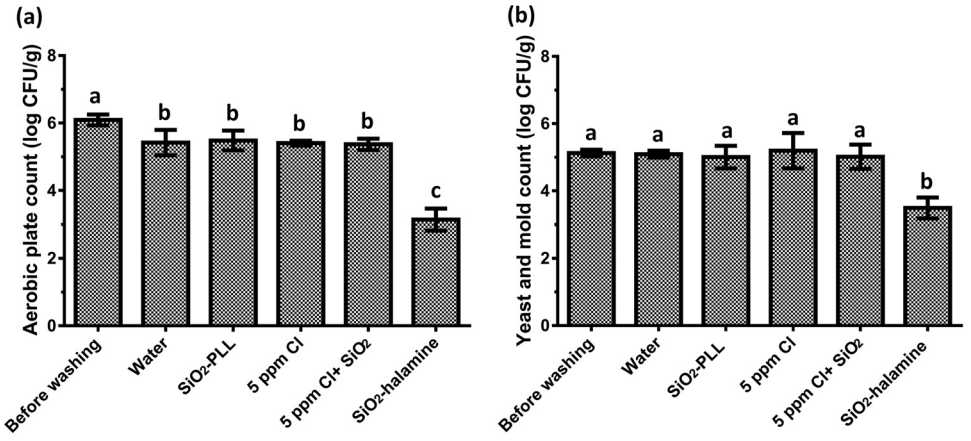

FIG 6 (a and b) Aerobic mesophilic counts (a) and yeast and mold counts (b) in examined samples of leaf surfaces before and after washing (log CFU/g). Different letters indicate statistically significant differences ($P < 0.05$).

in the total color index of lettuce leaves upon treatment were compared to the unwashed leaves in Fig. 7a. Based on the observed color difference measured after treatment (day 0), the washing process by water, SiO_2 particles, and SiO_2-halamine particles had a negligible impact on the net color change. However, the addition of conventional chlorine-based sanitizer resulted in a slight change in color. In addition, changes in the color and texture of treated and control leaves were evaluated during extended storage for 6 days. During storage, no significant changes in the color of the treated leaves compared to unwashed leaf samples ($P > 0.05$) were observed. Mea-

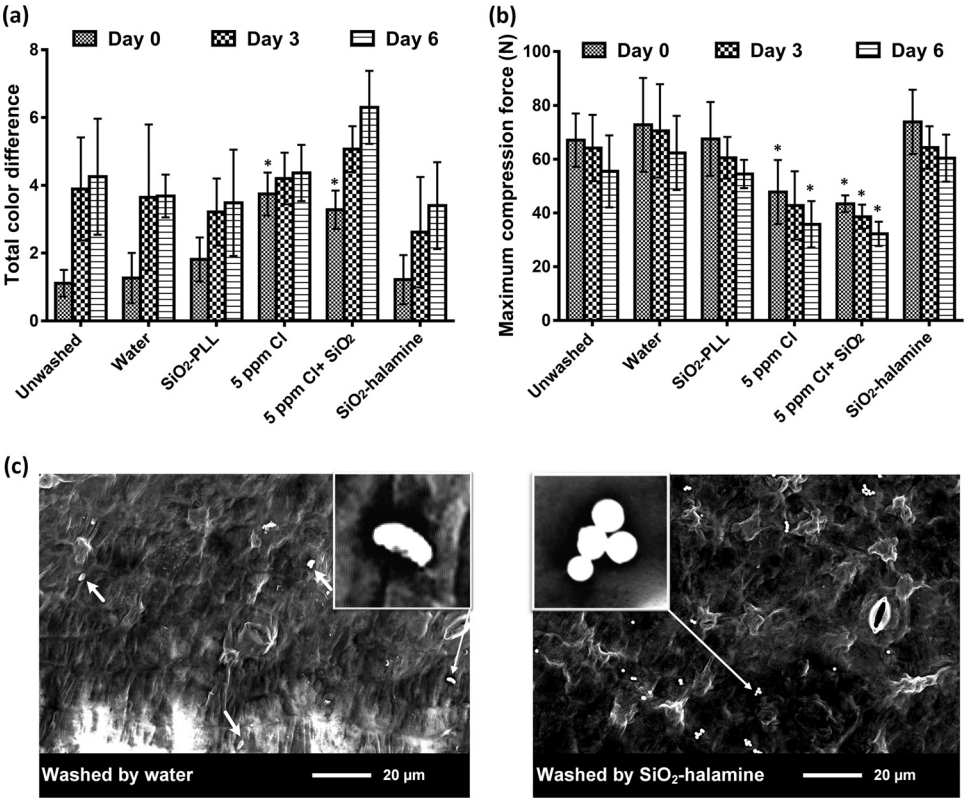

FIG 7 Effect of particle-based sanitizer on the quality parameters of lettuce leaves during storage. (a) Total color difference measured using the Hunter scale; (b) maximum compression force (N); (c) representative SEM images of leaf surfaces washed by water and SiO_2-halamine particles. An asterisk (*) indicates statistical significance between the test group and the unwashed leaf samples ($P < 0.05$).

surement of changes in the maximum compression force of lettuce samples upon treatment with selected sanitizer (Fig. 7b) showed that the addition of conventional sanitizer resulted in a significant decrease in the maximum compression force of leaves ($P < 0.05$), whereas no significant changes in the texture of leaves treated with SiO_2-halamine were observed ($P > 0.05$). This negative influence of conventional sanitizer on the texture of leaves was also observed during extended storage, while no significant changes in texture were observed for leaves treated with SiO_2-halamine during extended storage ($P > 0.05$). Overall, these results illustrate no significant changes in the quality of fresh produce upon treatment with SiO_2-halamine particles.

Electron microscopy of leaf surfaces shows that inoculated bacterial cells can be detected on a leaf surface after the leaves are washed with wash water, but in the presence of SiO_2-halamine there was significant reduction in the presence of bacteria on leaf surfaces (Fig. 7c). This suggests that inactivation of bacteria with SiO_2-halamine may also result in removal of bacteria from leaf surfaces. In addition, SEM images also suggest the possible retention of a few silica particles on the lettuce leaf surface.

DISCUSSION

Enhanced antimicrobial activity of chlorine on silica particles. This study demonstrates that chlorine bound to the surfaces of silica particles can inactivate 5 logs of model pathogenic and spoilage bacteria on fresh produce in the presence of a relative high organic content (500 mg/liter COD) within 20 min of incubation. In contrast, 70 to 150 ppm chlorine added to wash water did not inactivate more than 2 logs of bacteria on the surfaces of fresh produce in the presence of 500 mg/liter COD wash water. Furthermore, antimicrobial efficiency of added chlorine to wash water was not adequate to inactivate 2 log or more of bacteria on the surfaces of fresh produce even without the presence of organic content in wash water. The decontamination assay of multiple leaves demonstrates that particle-based sanitizer was able to achieve 3- and 1.6-log CFU/g reductions in naturally occurring bacteria and yeast and mold counts, respectively, on leaf surfaces, while the sanitation efficacy of conventional chlorine-based sanitizer was very limited. As illustrated in Fig. 8, we attribute this enhanced antimicrobial activity of chlorine bound to the surfaces of silica particles to two factors: (i) the localized high concentration of chlorine bound to the surfaces of silica particles and (ii) the enhanced surface contact of silica particles with leaf surfaces and the resulting high surface shear exerted by the circulation of these microparticles in wash water. The approach developed in this study is distinct from prior efforts to enhance chlorine activity. Previous efforts mainly focused on maintaining the pH conditions and improving stability of chlorine in the presence of organic content by reducing its reactivity (33, 34). In these studies, a chlorine stabilizer T-128 (New Leaf Food safety Solutions, LLC, Salinas, CA) was added to chlorinated wash water (pH at 5.0) that reduced the depletion of free chlorine in wash water containing a high organic content. These improved formulations show enhanced inactivation of inoculated bacteria in wash water (5-log reduction). Despite these promising results, it is still not feasible to consistently achieve more than 2 logs of bacterial inactivation on fresh produce surfaces during postharvest processing, as indicated by the results of this and other studies (35, 36). We hypothesize that this limitation results from the lack of a localized high concentration of chlorine at the leaf surface since chlorine suspended in wash water can be rapidly depleted at the leaf surface upon reaction with organic matter at the leaf surface (35, 37). Furthermore, the hydrophobicity of the leaf surface and structural properties may limit uniform wetting of the leaf surface, thus limiting the antimicrobial activity of chlorine in wash water. Previous studies have also suggested that waxy cuticle on produce surfaces, as well as microscale features of the leaf surface, may provide a physical barrier to the antimicrobial activity of chlorine and other antimicrobial agents (38).

In contrast, the ability of silica particles to bind 0.5 mg of chlorine per g of silica particles on their surfaces enhances the localized delivery of a relatively high concentration of chlorine to the leaf surface. Furthermore, the enhanced shear force generated

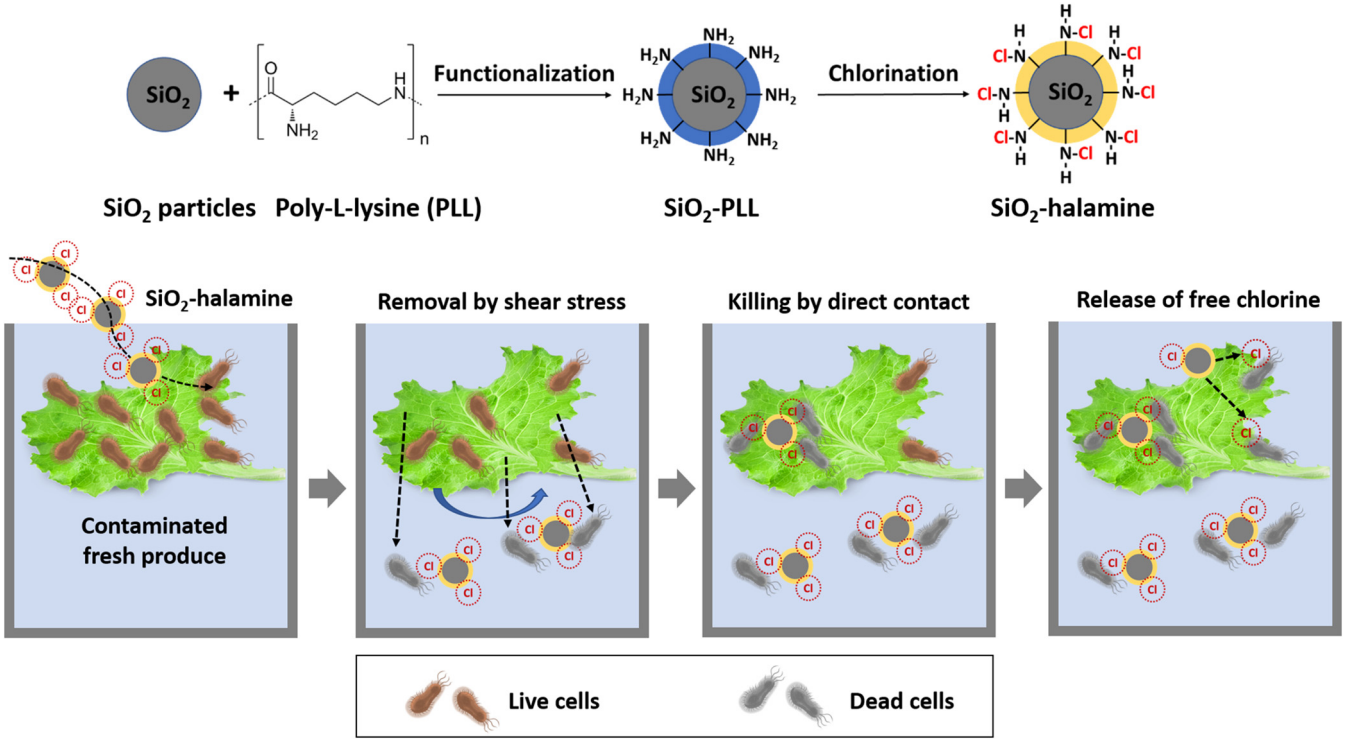

FIG 8 Illustration of enhanced antimicrobial activity by particle-based sanitizer.

by movement of microparticles near the leaf surface also improves contact between chlorine modified particles and bacteria attached on the surfaces of fresh produce. In addition, retention of small fractions of silica particles on the surfaces of fresh produce may also contribute to enhanced antimicrobial activity since these particles can provide localized release of chlorine on the surface. Overall, the results of this study highlight a novel approach to address both the chemical and the physical barriers to improve the sanitation of fresh produce and to achieve 4 to 5 logs of bacterial inactivation on fresh produce surfaces in a relatively short incubation time.

Translation of particle-based sanitizers to industrial applications. Extended storage stability of sanitizer formulations is critical for successful industrial applications. The results of this study demonstrate that the formulation developed in this study retained >90% of the active chlorine after 4 weeks of refrigerated storage, a common environmental condition for fresh produce processing. Unlike prior studies that have focused on the stability of chlorine charged polymers in dry conditions (30, 39–41), this study evaluated the stability of an aqueous suspension of SiO_2-halamine. Since most of the sanitizing agents in the food industry are stored and applied in aqueous suspension, maintaining the stability of chlorine on the surface of as-synthesized SiO_2-halamine is critical for industrial applications of these materials. Furthermore, an aqueous formulation can significantly reduce the cost of drying these formulations prior to industrial applications.

Maintaining produce quality after sanitation is essential for successful translation of sanitizers to industrial practice. The results demonstrate no significant influence on texture and color of fresh produce upon treatment with particle-based sanitizers compared to the controls. The results based on SEM imaging suggest the retention of a few silica particles on the surfaces of leaf samples. It is important to note that SEM is a high-resolution imaging method that has the sensitivity to detect trace amounts of particles on the surfaces of fresh produce (0.01% [wt/wt]). In addition, it is possible that washing processes, including the use of ultrasound treatment during washing, could be used to remove the residual particles from fresh produce surfaces after sanitation.

Further studies may also be designed to evaluate the influence of particle-based sanitizers on internalized bacteria in fresh produce. It is also anticipated that use of particle-based sanitizer may reduce the amount of total chlorine used during the sanitation of fresh produce and its associated environmental impact. Further studies are needed to validate this potential benefit of these novel formulations.

Conclusions. In summary, we demonstrate here a novel approach to enhance the sanitation effectiveness of fresh produce using a particle-based sanitizer. The particle-based sanitizer concept provides localized high-concentration delivery of chlorine to the surfaces of fresh produce and causes >5 logs of inactivation of inoculated bacteria on fresh produce surfaces in the presence of 500 ppm COD without significant changes in produce quality. Our results illustrate the potential of this approach to address the unmet need for improving the sanitation of fresh produce. Further validation of this approach using a scaled-up produce washing system will enable commercialization of this novel concept.

MATERIALS AND METHODS

Chemicals and reagents. Pure ethyl alcohol (>99.5%), tetraethyl orthosilicate (TEOS; 99.999%), poly-L-lysine (PLL; molecular weight, 30,000 to 70,000), and sodium hypochlorite (10%) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Ammonium hydroxide (29.7%), sodium carbonate, sodium thiosulfate, and hydrochloric acid (HCl) were purchased from Fisher Scientific (Waltham, MA). DPD (*N*,*N*-diethyl-1,4-phenylenediamine sulfate)-free chlorine reagent powder was purchased from Hach (Loveland, CO). LB broth, tryptic soy broth (TSB), nutrient broth, LB agar, tryptic soy agar, and nutrient agar were from Fisher Scientific (Waltham, MA).

Bacterial strains, media, and growth conditions. *Escherichia coli* O157:H7 (ATCC 700728) and *Listeria innocua* (ATCC 33090) were selected as a model Gram-negative foodborne pathogen and a surrogate for a Gram-positive foodborne pathogen, respectively. *Pseudomonas fluorescens* (ATCC 13525) was chosen as a model spoilage bacteria that is associated with spoilage of ready to eat salads. A liquid nitrogen stock of each bacteria was streaked onto to agar plates and grown overnight. LB agar, tryptic soy agar, and nutrient agar plates were used for plating of *E. coli*, *L. innocua* and *P. fluorescens*, respectively. Before each experiment, one colony was picked from an agar plate, cultured in a liquid medium, and incubated to achieve the stationary-phase cultures. *E. coli* O157:H7 strain was grown in LB broth supplemented with 25 μg/ml tetracycline with constant shaking (250 rpm) at 37°C. *L. innocua* strain was grown in TSB supplemented with 50 μg/ml rifampicin with constant shaking (250 rpm) at 30°C. *P. fluorescens* strain was grown in nutrient broth with constant shaking (250 rpm) at 30°C. After liquid culture, cells were centrifuged, washed twice in sterile phosphate-buffered saline (PBS) and resuspended in sterile PBS at a concentration of approximately 1.0×10^9 CFU/ml.

Lettuce leaf preparation. Prebagged romaine lettuce leaves were purchased from a local supermarket and stored at 4°C 1 day before the experiments. Exterior leaves of the lettuce were removed and discarded. The leaves without any visual evidence of tissue damage and similar surface properties to that of the majority of the leaves were selected and washed by deionized water. Then the chosen leaves were used for following assays.

Synthesis of SiO_2-PLL particles. Silica particles were prepared according to the modified Stöber method (42–44). The reaction mixture (solution I) was prepared with water (6.75 ml), ethanol (65 ml), ammonium hydroxide (9 ml), and KCl electrolyte (0.017 g). An ethanolic solution of TEOS (solution II) was prepared with TEOS (3.95 g), and ethanol (33.3 ml). The synthesis of silica particles was conducted in a 250-ml flask where the solution II was continuously supplied with a syringe pump (0.2 mL/min) to the solution I. After further reaction (300 rpm, 40°C) for 15 h, the particles obtained were purified by centrifugation and washed three times with ethanol.

The synthesis process of the PLL-functionalized particles was accomplished using a previously published method (45). Portions (50 mg) of silica particles were suspended in 25 ml of 0.6 M sodium carbonate solution (active buffer) for 15 min by bath sonication. The suspension was centrifuged to remove the supernatant. Activated silica particles were resuspended in 10 ml of PBS buffer (pH 7.4) for 20 min by sonication. PLL (80 nmol) was added dropwise to the particle suspension with continuous stirring for 21 h at 4°C. PLL-functionalized silica particles were washed four times with deionized (DI) water and then resuspended in 10 ml of DI water and stored at 4°C before use.

Characterization of SiO_2-PLL particles. The size and surface charge (ζ potential) of the synthesized particles were measured using dynamic light scattering (Nano-ZS; Malvern Instruments, Worcestershire, UK) after dilution in DI water. The refractive index of silica used in the light scattering calculation was 1.459 (46). Surface chemistry of synthesized particles was further characterized by ATR-FTIR spectroscopy. The morphology and structure of the particles were characterized by SEM using a FEL XL30 scanning electron microscope.

Total chlorine content and release of free chlorine in wash water. Next, 0.1 g of SiO_2-PLL particles were suspended in 10 ml of dilute household bleach (10% [vol/vol] of 8.25% commercial sodium hypochlorite solution at pH 5) at room temperature and incubated under constant shaking at 250 rpm for a predetermined period of time (15 min to 3 h). After chlorination, the particles were washed three times with DI water and then resuspended in 10 ml of sterile DI water.

The total active chlorine content was measured by titration method. Portions (0.1 g) of charged particles were added to 15 ml of 0.001 N sodium thiosulfate solution with shaking for 30 min. The residual sodium thiosulfate was subsequently titrated with a 0.001 N iodine standard solution. The active chlorine content (ppm) of the particles was calculated according to the following equation:

$$\text{active chlorine content} = \frac{35.45 \times N(V_0 - V_S)}{m_S} \times 10^6 \quad (1)$$

where N is the normality (equivalents/liter) of the iodine solution; V_0 and V_S are the volumes (in milliliters) of the iodine solution consumed in titration without or with charged SiO_2-PLL particles, respectively; and m_S is the weight (in grams) of the charged SiO_2-PLL particles.

Chlorination kinetics were determined by fitting data based on an exponential model as described in equation 2 using GraphPad Prism software V.5.04 (GraphPad Software, Inc., La Jolla, CA):

$$C = C_{\text{asympt}} \times (1 - e^{-kt}) \quad (2)$$

where C is the total chlorine content in ppm, C_{asympt} is the asymptotic chlorine content in ppm, which indicates a theoretical amount of chlorine at saturation; k is the exponential rate constant (per minute); and t is the time (in minutes).

Antimicrobial activity of modified particles in wash water. The antimicrobial activities of each treatment were evaluated (SiO_2, SiO_2-PLL, and charged modified particles [SiO_2-halamine]). Conventional chlorine-based sanitizer containing 5 ppm free chlorine was used as an additional control group, which was equivalent to the free chlorine content released from SiO_2-halamine in 20 min. The bacterial suspension of approximately 6 log CFU/ml was prepared by dilution. A volume of 1 ml of bacterial suspension was added to a test tube with 2 mg/ml of particles and incubated for 20 min with a rotating speed of 250 rpm at room temperature. A bacterial suspension without any particles was used as a negative control. After 20 min of incubation, the bacterial suspension of each treatment was serially diluted using PBS. A volume of 100 μl of each dilution was inoculated onto agar plates and incubated at 37°C for 48 h.

Sanitation of fresh produce surfaces. Before inoculation, the bacteria were centrifuged at 3,100 $\times$ *g* for 10 min at room temperature, washed twice with PBS buffer, and then resuspended in the same buffer to obtain a final bacterial cell concentration of 1×10^6 CFU/ml. A novel spin-coating approach as described in our previous study (32) was performed to obtain a uniform distribution of bacterial coating on the leaf surface. Briefly, cut leaf pieces (3 $\times$ 3 cm) were placed in petri dishes, and 0.5 ml of the bacterial suspension was dropped by pipette onto each leaf surface. The sealed petri dish was then fixed on the disk of a spin coater. A thin coating of bacterial cells on leaf surfaces was generated using a spin coater device at 500 rpm for 30 s. After inoculation, the leaf samples were stored at 4°C for 24 h. All of the leaf samples after inoculation were cut into 1-cm-diameter disks with a sterile cork borer before any of the selected treatments for the inoculated leaves.

In the assay of particle-based sanitation of lettuce leaves, a 15-ml test tube was filled with 2 ml of sterile DI water containing 2 mg/ml of charged SiO_2-PLL particles. An individual inoculated leaf sample was added to each test tube, and the samples were shaken at 250 rpm for a predetermined period (0, 5, 10, and 20 min). The total chlorine content in 2 mg/ml of charged SiO_2-PLL particles was equivalent to 5 ppm. The control groups were inoculated leaf samples treated with wash water, water with 2 mg/ml of uncharged SiO_2-PLL particles, and water with 5 ppm free chlorine, as well as wash water containing a combination of 5 ppm free chlorine in solution and suspended SiO_2 particles. All samples were treated in triplicate for each treatment. At the end of each treatment, the lettuce sample was collected with a sterile forceps from each tube and rinsed twice by sterile DI water to remove loosely adhered bacterial cells. The leaf samples were then transferred to a sterile test tube containing 1% (wt/vol) sodium thiosulfate in 1 ml of maximum recovery diluent (MRD; Sigma, St. Louis, MO) and allowed to stand for 2 min (47). After vortexing at full speed for 1 min in the tube by a vortex mixer (Fisher Scientific, Waltham, MA), the bacterial numbers were quantified by serial dilution and duplicate spread plating on agar. Bacterial counts were determined after incubation at 37°C for 48 h and are expressed as the log CFU/leaf.

A complementary assay was performed to evaluate the influence of the organic load on the sanitation efficacy of inoculated leaf samples. In this assay, charged SiO_2-PLL particles were suspended in the wash water with chemical oxygen demand (COD) of 500 and 1,000 mg/liter, respectively. LB medium was diluted with DI water by 40 and 20 times to simulate the COD levels of 500 and 1,000 mg/liter, respectively. After a washing step at 250 rpm for 20 min, the lettuce leaves were sampled for microbial analysis using the approach described above. The control groups were wash water with free chlorine concentrations at 5 and 10 ppm, respectively.

To compare the sanitation efficacy of particle-based sanitizer and conventional chlorine-based treatment on natural microflora on leafy greens, a 300-ml beaker was filled with 200 ml of sterile wash water and placed on a magnetic mixer. Then, 5 g of leaves was added to the sterile wash water. Leaves were treated with 2 mg/ml of SiO_2-halamine or equivalent free chlorine, i.e., 5 ppm for 20 min at room temperature. The other control treatments included washing of leaves with sterile water, uncharged SiO_2-PLL, and SiO_2 particles with 5 ppm chlorine, respectively. At the end of each treatment, the leaves were collected, rinsed twice with sterile DI water, and placed in a stomacher bag. It was diluted in 45 ml of MRD buffer and homogenized for 2 min at 260 rpm, using a stomacher (model 400 circulator; Seward, Norfolk, England). The suspension was serially diluted and analyzed for aerobic plate count, yeast, and molds. For the determination of aerobic mesophilic bacteria, 0.1 ml of each decimal dilution was added to the nutrient agar plates, and the agar plates were incubated for 48 h at 37°C. For the determination of yeast and mold counts, 0.1 ml of each decimal dilution was spread on the dichloran rose bengal

chloramphenicol agar plates. Colonies were counted after incubation at 25°C for 5 days and are expressed as the log CFU/g. All the treatments were performed in triplicate.

Evaluation of quality of fresh lettuce leaves. The effect of each treatment on the quality attributes of lettuce leaves were evaluated during six days of storage at 4°C in a petri dish. The treatments included leaf samples treated by wash water, water with 2 mg/ml of uncharged SiO_2-PLL particles, water with 2 mg/ml of charged SiO_2-PLL particles, and water with 5 ppm free chlorine, as well as wash water containing 5 ppm free chlorine and SiO_2 particles. The multiple comparisons for two-way analysis of variance (ANOVA) were performed to determine the significant difference between the particle treated washed leaves and the control unwashed leaves ($P < 0.05$). For color measurement, one lot of lettuce leaves was tested and analyzed using a ColorFlex EZ spectrophotometer (Hunter Lab, Reston, VA). Hunter's color values (L, a, and b) were analyzed by using EasymatchQC software (Hunter Lab). Triplicate measurements were performed for each of the four slices of lettuce leaves, and the averages and standard deviations based on 12 readings for each treatment are reported. The total color difference (ΔE) was determined according to the following equation:

$$\Delta E^*_{ab} = \sqrt{(L^*_1 - L^*_0)^2 + (a^*_1 - a^*_0)^2 + (b^*_1 - b^*_0)^2} \quad (3)$$

where L^*_0, a^*_0, and b^*_0 are Hunter's color values from a reference, and L^*_1, a^*_1, and b^*_1 are Hunter's color values from treated samples.

The compressibility of fresh lettuce leaves was measured using a TA-TXPlus texture analyzer (Texture Technologies Corp., Scarsdale, NY). A slice of leaf sample was positioned in the press holder, and the plunger was moved down at a velocity of 1 mm/s from its initial position to a final position at 2 mm below the bottom of the leaf holder. The maximum compression force was recorded using Texture Expert software (version 2.64; Texture Technology Corp.). Three slices of lettuce leaves were measured for each treatment set.

Statistical analysis. Statistical analysis was performed using Prism software (v.5.04; GraphPad Software, Inc., La Jolla, CA). All experiments were performed in triplicate. Unless otherwise stated in the corresponding section, the significant differences between treatments were determined through one-way ANOVA, followed by Tukey's pairwise comparisons with a 95% confidence interval.

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REFERENCES

1. Wiseman M. 2008. The Second World Cancer Research Fund/American Institute for Cancer Research Expert Report: food, nutrition, physical activity, and the prevention of cancer—a global perspective. Proc Nutr Soc 67:253–256. https://doi.org/10.1017/S002966510800712X.
2. Olaimat AN, Holley RA. 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol 32:1–19. https://doi.org/10.1016/j.fm.2012.04.016.
3. Predmore A, Li J. 2011. Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. Appl Environ Microbiol 77:4829–4838. https://doi.org/10.1128/AEM.00174-11.
4. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States' major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.091101p1.
5. Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg Infect Dis 19:407–415. https://doi.org/10.3201/eid1903.111866.
6. Phillips CA. 1996. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. Int J Food Sci Tech 31:463–479. https://doi.org/10.1046/j.1365-2621.1996.00369.x.
7. Huang K, Wrenn S, Tikekar R, Nitin N. 2018. Efficacy of decontamination and a reduced risk of cross-contamination during ultrasound-assisted washing of fresh produce. J Food Eng 224:95–104. https://doi.org/10.1016/j.jfoodeng.2017.11.043.
8. Gil MI, Selma MV, López-Gálvez F, Allende A. 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. Int J Food Microbiol 134:37–45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021.
9. Capozzi V, Fiocco D, Amodio ML, Gallone A, Spano G. 2009. Bacterial stressors in minimally processed food. Int J Mol Sci 10:3076–3105. https://doi.org/10.3390/ijms10073076.
10. Allende A, Tomás-Barberán FA, Gil MI. 2006. Minimal processing for healthy traditional foods. Trends Food Sci Technol 17:513–519. https://doi.org/10.1016/j.tifs.2006.04.005.
11. López-Gálvez F, Allende A, Selma MV, Gil MI. 2009. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. Int J Food Microbiol 133:167–171. https://doi.org/10.1016/j.ijfoodmicro.2009.05.017.
12. Rosenblum J, Ge C, Bohrerova Z, Yousef A, Lee J. 2012. Ozonation as a clean technology for fresh produce industry and environment: sanitizer efficiency and wastewater quality. J Appl Microbiol 113:837–845. https://doi.org/10.1111/j.1365-2672.2012.05393.x.
13. Wani S, Maker KJ, Thompson RJ, Barnes J, Singleton I. 2015. Effect of ozone treatment on inactivation of *Escherichia coli* and *Listeria* sp. on spinach. Agriculture 5:155–169. https://doi.org/10.3390/agriculture5020155.
14. De Giusti M, Aurigemma C, Marinelli L, Tufi D, De Medici D, Di Pasquale S, De Vito C, Boccia A. 2010. The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy. J Appl Microbiol 109:996–1006. https://doi.org/10.1111/j.1365-2672.2010.04727.x.
15. Chaidez C, Lopez J, Castro-del Campo N. 2007. Quaternary ammonium compounds: an alternative disinfection method for fresh produce wash water. J Water Health 5:329–333. https://doi.org/10.2166/wh.2007.009b.
16. Ölmez H. 2010. Effect of different sanitizing methods and incubation time and temperature on inactivation of *Escherichia coli* on lettuce. J Food Saf 30:288–299. https://doi.org/10.1111/j.1745-4565.2009.00206.x.
17. Cossu A, Ercan D, Wang Q, Peer WA, Nitin N, Tikekar RV. 2016. Antimicrobial effect of synergistic interaction between UV-A light and gallic acid against *Escherichia coli* O157:H7 in fresh produce wash water and biofilm. Innov Food Sci Emerg Technol 37:44–52. https://doi.org/10.1016/j.ifset.2016.07.020.
18. Yaron S, Römling U. 2014. Biofilm formation by enteric pathogens and its role in plant colonization and persistence. Microb Biotechnol 7:496–516. https://doi.org/10.1111/1751-7915.12186.
19. Simões M, Simões LC, Vieira MJ. 2010. A review of current and emergent

biofilm control strategies. LWT Food Sci Technol 43:573–583. https://doi.org/10.1016/j.lwt.2009.12.008.
20. Srey S, Jahid IK, Ha S. 2013. Biofilm formation in food industries: a food safety concern. Food Control 31:572–585. https://doi.org/10.1016/j.foodcont.2012.12.001.
21. De Jong WH, Borm PJA. 2008. Drug delivery and nanoparticles: applications and hazards. Int J Nanomedicine 3:133–149.
22. Rizvi SAA, Saleh AM. 2018. Applications of nanoparticle systems in drug delivery technology. Saudi Pharm J 26:64–70. https://doi.org/10.1016/j.jsps.2017.10.012.
23. Tang F, Li L, Chen D. 2012. Mesoporous silica nanoparticles: synthesis, biocompatibility and drug delivery. Adv Mater 24:1504–1534. https://doi.org/10.1002/adma.201104763.
24. Asefa T, Tao Z. 2012. Biocompatibility of mesoporous silica nanoparticles. Chem Res Toxicol 25:2265–2284. https://doi.org/10.1021/tx300166u.
25. Maged Y, Peter A, Fernando A, Riccardo C, Birgit D, Filipič M, Frutos MJ, Pierre G, David G, Gundert-Remy U, Kuhnle GG, Leblanc JC, Lillegaard IT, Peter M, Alicja M, Agneta O, Ivan S, Waalkens-Berendsen I, Woutersen RA, Matthew W, Polly B, Dimitrios C, Gürtler R, Pasquale M, Parent-Massin D, Paul T, Natalia K, Rincon AM, Alexandra T, Lambré C. 2018. Re-evaluation of silicon dioxide (E 551) as a food additive. EFSA J 16:e05088.
26. Zhang K, Wu W, Meng H, Guo K, Chen J. 2009. Pickering emulsion polymerization: Preparation of polystyrene/nano-SiO_2 composite microspheres with core-shell structure. Powder Technol 190:393–400. https://doi.org/10.1016/j.powtec.2008.08.022.
27. Dong Q, Dong A. 2015. Evaluation of novel antibacterial *N*-halamine nanoparticles prodrugs towards susceptibility of *Escherichia coli* induced by DksA protein. Molecules 20:7292–7308. https://doi.org/10.3390/molecules20047292.
28. Li C, Xue L, Cai Q, Bao S, Zhao T, Xiao L, Gao G, Harnoode C, Dong A. 2014. Design, synthesis, and biocidal effect of novel amine *N*-halamine microspheres based on 2,2,6,6-tetramethyl-4-piperidinol as promising antibacterial agents. RSC Adv 4:47853–47864. https://doi.org/10.1039/C4RA08443H.
29. Rozenberg M, Shoham G. 2007. FTIR spectra of solid poly-L-lysine in the stretching NH mode range. Biophys Chem 125:166–171. https://doi.org/10.1016/j.bpc.2006.07.008.
30. Chen Z, Sun Y. 2006. *N*-Halamine-based antimicrobial additives for polymers: preparation, characterization, and antimicrobial activity. Ind Eng Chem Res 45:2634–2640. https://doi.org/10.1021/ie060088a.
31. Hui F, Debiemme-Chouvy C. 2013. Antimicrobial *N*-halamine polymers and coatings: a review of their synthesis, characterization, and applications. Biomacromolecules 14:585–601. https://doi.org/10.1021/bm301980q.
32. Huang K, Nitin N. 2017. Enhanced removal of *Escherichia coli* O157:H7 and *Listeria innocua* from fresh lettuce leaves using surfactants during simulated washing. Food Control 79:207–217. https://doi.org/10.1016/j.foodcont.2017.03.032.
33. Yang Y, Yaguang L, Patricia M, Daniel S, Xiangwu N. 2012. Enhanced chlorine efficacy against bacterial pathogens in wash solution with high organic loads. J Food Process Pres 36:560–566. https://doi.org/10.1111/jfpp.12000.
34. Nou X, Luo Y, Hollar L, Yang Y, Feng H, Millner P, Shelton D. 2011. Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *Escherichia coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing. J Food Sci 76:M218–M224. https://doi.org/10.1111/j.1750-3841.2011.02046.x.
35. Banach JL, van Bokhorst-van de Veen H, van Overbeek LS, van der Zouwen PS, van der Fels-Klerx HJ, Groot MNN. 2017. The efficacy of chemical sanitizers on the reduction of *Salmonella* Typhimurium and *Escherichia coli* affected by bacterial cell history and water quality. Food Control 81:137–146. https://doi.org/10.1016/j.foodcont.2017.05.044.
36. Niemira BA. 2008. Irradiation compared with chlorination for elimination of *Escherichia coli* O157:H7 internalized in lettuce leaves: Influence of lettuce variety. J Food Sci 73:M208–M213. https://doi.org/10.1111/j.1750-3841.2008.00746.x.
37. Behrsing J, Winkler S, Franz P, Premier R. 2000. Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. Postharvest Biol Tec 19:187–192. https://doi.org/10.1016/S0925-5214(00)00092-2.
38. Jahid IK, Ha S. 2012. A review of microbial biofilms of produce: Future challenge to food safety. Food Technol Biotechnol 21:299–316. https://doi.org/10.1007/s10068-012-0041-1.
39. Si Y, Cossu A, Nitin N, Ma Y, Zhao C, Chiou B, Cao T, Wang D, Sun G. 2017. Mechanically robust and transparent *N*-halamine grafted PVA-co-PE films with renewable antimicrobial activity. Macromol Biosci 17:1600304. https://doi.org/10.1002/mabi.201600304.
40. Sun X, Cao Z, Porteous N, Sun Y. 2012. An *N*-halamine-based rechargeable antimicrobial and biofilm controlling polyurethane. Acta Biomaterialia 8:1498–1506. https://doi.org/10.1016/j.actbio.2011.12.027.
41. Qiao M, Ren T, Huang T, Weese J, Liu Y, Ren X, Farag R. 2017. *N*-Halamine modified thermoplastic polyurethane with rechargeable antimicrobial function for food contact surface. RSC Adv 7:1233–1240. https://doi.org/10.1039/C6RA25502G.
42. Stöber W, Fink A, Bohn E. 1968. Controlled growth of monodisperse silica spheres in the micron size range. J Colloid Interface Sci 26:62–69. https://doi.org/10.1016/0021-9797(68)90272-5.
43. Yu B, Cong H, Xue L, Tian C, Xu X, Peng Q, Yang S. 2016. Synthesis and modification of monodisperse silica microspheres for UPLC separation of C60 and C70. Anal Methods 8:919–924. https://doi.org/10.1039/C5AY02655E.
44. Huang K, Chen J, Nugen SR, Goddard JM. 2016. Hybrid antifouling and antimicrobial coatings prepared by electroless codeposition of fluoropolymer and cationic silica nanoparticles on stainless steel: efficacy against *Listeria monocytogenes*. ACS Appl Mater Interfaces 8:15926–15936. https://doi.org/10.1021/acsami.6b04187.
45. Zhu S, Xiang J, Li X, Shen S, Lu H, Zhou J, Xiong W, Zhang B, Nie X, Zhou M, Tang K, Li G. 2004. Poly(L-lysine)-modified silica nanoparticles for the delivery of antisense oligonucleotides. Biotechnol Appl Biochem 39:179–187. https://doi.org/10.1042/BA20030077.
46. Xi JQ, Schubert MF, Kim JK, Schubert EF, Chen M, Lin S, Liu W, Smart JA. 2007. Optical thin-film materials with low refractive index for broadband elimination of Fresnel reflection. Nat Photon 1:176–179. https://doi.org/10.1038/nphoton.2007.26.
47. Villa-Rojas R, Zhu M, Paul NC, Gray P, Xu J, Shah DH, Tang J. 2017. Biofilm forming *Salmonella* strains exhibit enhanced thermal resistance in wheat flour. Food Control 73:689–695. https://doi.org/10.1016/j.foodcont.2016.09.021.