Potassium Iodide Potentiates Bacterial Killing by Helium Atmospheric Pressure Plasma Jet

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ABSTRACT: Cold atmospheric plasma (CAP) is an effective new antimicrobial approach that is gaining increasing attention and has a wide range of potential applications in biomedical fields. Among all of the bactericidal factors generated by CAP, the synergy of reactive nitrogen species (RNS) and reactive oxygen species is generally considered as the main reason for its high bactericidal efficiency. However, the produced RNS (such as nitrite) may also pose potential risks to human health. Therefore, it is of significance to keep the high disinfection efficiency of CAP but with producing no or little harmful RNS. In this study, we investigated whether it is possible to improve the disinfection efficiency of CAP without producing the harmful RNS by adding a certain amount of inert halogen salt such as potassium iodide (KI). We found that the inactivation of both Gram-negative and Gram-positive bacteria by helium atmospheric pressure plasma jet (He-APPJ), one form of CAP, is enhanced consistently in the presence of a certain amount of KI. The mechanism of action is due to the fact that the He-APPJ-generated hydrogen peroxide (H₂O₂) oxidizes the iodide anion to triiodide (I₃⁻), which contributes to the major bactericidal activity. We believe that the results in this work can be highly relevant to the practical application of plasma for disinfection in the biomedical field.

1. INTRODUCTION

The rapid development of bacterial resistance against traditional antibiotics has led to the urgency to search for novel and efficient antimicrobial methodologies with which microbes are unable to develop resistance. In a review paper written by a group of 28 scientists from both academic and industrial backgrounds, the authors encouraged the “investigation of nonantibiotic approaches for the prevention of and protection against infectious diseases”. In this regard, cold atmospheric plasma (CAP) has come into the spotlight as a new alternative approach for nonsystemic infection as it shows effectiveness in inactivating a range of microorganisms, including antibiotic-resistant biofilm-forming strains and spores, and in reduction of bacterial load in chronic wounds. CAP refers to a partially ionized gas generated by electrical discharges, whose temperature is close to room temperature. During discharge, various types of plasma-chemical reactions are initiated and a number of physical and chemical bactericidal factors are produced, including electrons, ions, neutrals (fundamental and excited states), reactive oxygen/nitrogen species (ROS/RNS), UV light, electrical field, and so on. Among all of the active factors, the produced ROS/RNS have gained increasing attention because they can be dissolved into the liquid and initiate various chemical and biological effects. Increasingly more pieces of evidence have proved that the generated ROS and RNS are major factors for the antimicrobial effects of CAP.

Among various reactive species produced by CAP, hydroxyl radical, singlet oxygen, superoxide anion, hydrogen peroxide (H₂O₂), etc. are the main ROS generally considered to play the dominant role in the bacterial lethal effects in the CAP systems. RNS such as nitric oxide and its derivatives, including nitrite, nitrate, and peroxynitrite, can contribute crucially to the CAP-induced inactivation processes. Indeed, increasingly more works have suggested that the bactericidal effects of CAP are not simply ascribed to the ROS or RNS alone but to the complex synergy of both. For example, our laboratory and other groups have proved that during nitrogen–oxygen mixture plasma treatment, the generated nitrite can react with the generated H₂O₂ to form peroxynitrite/peroxynitrous acids, which are strong oxidizing RNS and can inactivate planktonic bacteria, and the production of nitrite and other RNS during plasma treatment results in the drop of pH in the plasma-treated solution, which is also critical for the reaction of H₂O₂ with nitrite. Ikawa et al. also found that the CAP applied to the surface of an aqueous solution only showed bactericidal effects to the

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Supporting Information

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bacteria suspended in the solution when the solution was sufficiently acidic (pH < 4.7), and if the pH was above 4.7, the bacteria were hardly affected by the CAP application. They thought the reason for this phenomenon is that in acidic medium, the generated superoxide anion radicals are converted to hydroperoxyl radicals, which can penetrate the cell membrane and damage the intercellular components. Therefore, the RNS with pH decrease may play a significant, perhaps even central, role in CAP-induced bacterial inactivation processes.

Although the plasma-generated ROS and RNS exhibited several potential beneficial applications, they could have damaging effects on the biological systems, especially the latter. As will be illustrated later, the concentration of hydrogen peroxide in 1 mL of plasma-treated water, the main long-lived ROS produced by plasma, is several to dozens of micromolars even after treatment for 2 min, significantly lower than the concentration used at home (3–9%). However, nitrate and nitrite, the main long-lived RNS species produced by plasma, are precursors of endogenously formed N-nitroso compounds, most of which are potent animal carcinogens. Food frequency questionnaire-based median nitrate intakes were 68.9 and 74.1 mg per day and the nitrite intakes were 1.3 and 1.0 mg per day in men and women, respectively. In 1 mL of water after air plasma treatment for 1 min, the produced nitrate and nitrite are about 80 and 120 μM (equal to 5 and 5.6 mg), respectively, exceeding the recommended intake value of nitrate as mentioned above. Using other gases (such as helium, argon, and oxygen), except nitrogen and air, as the plasma working gas can rule out or diminish RNS production, but this also weakens the disinfection capability of CAP. Therefore, a selective method for potentiation of the antimicrobial activity of CAP without or with diminishing production of harmful chemicals is required for practical applications of plasma. This is important not only for the treatment efficiency but also for the potential risks associated with the direct plasma action on human chronic wounds.

In one of our latest research works, we found that the chloride anion can selectively potentiate the bacterial killing induced by corona discharge plasma with oxygen as the working gas. However, a high concentration of chloride anion (as high as 100 mM) is required, and its working mechanisms are very complex. In this work, we tried another halogen anion, namely, iodide (I−), at low concentration (e.g., 10 μM) in the application of helium atmospheric pressure plasma jet (He-APPJ) for achieving the potentiation effect. He-APPJ is a typical CAP, which has only the high-voltage (HV) electrode. The discharge is generated between the HV electrode and the surrounding atmosphere, and the generated plasma can reach the treated sample with the flow of injected helium. Helium gas can provide a homogeneous plasma at room temperature, thus will not cause any thermal damage to the objects to which it works on. Therefore, it is the preference used to treat living objects, such as the chronic wounds in humans. A disadvantage of He-APPJ is that it only produces ROS but no or little RNS, so its disinfection capability is normally not high enough. Herein, with addition of 10 μM potassium iodide (KI), we found that the killing of pathogenic bacteria, including Pseudomonas aeruginosa (Gram-negative), Escherichia coli (Gram-negative), and Staphylococcus aureus (Gram-positive), was clearly potentiated under He-APPJ treatment. The underlying mechanisms were also investigated, and for this purpose, we compared the inactivation behaviors of bacteria induced by plasma-activated water (PAW) and plasma-activated KI (PAI), and by PAW in the absence or presence of KI. PAW is a product resulting from a cascade of chemical reactions between plasma-generated active particles and water molecules, which is with the presence of a rich diversity of long-lived ROS and RNS, such as hydrogen peroxide, nitrite, nitrate, and so on. From the comparisons, we confirmed that the long-lived ROS generated by He-APPJ oxidized I− to active iodine, which can inactivate the bacteria efficiently. Furthermore, we also tried to determine which long-lived ROS was mainly responsible for the I− oxidation and which types of active iodine were produced. We believe that this work may be helpful for practical applications of He-APPJ for disinfection in the biomedical field.

2. RESULTS AND DISCUSSION

2.1. KI Potentiates the He-APPJ-Induced Killing of Planktonic Bacteria. KI is a nontoxic inorganic salt and has been used in medicine for over a century. It is still a good candidate for the therapy of several dermatoses as a drug of first or second choice. Even applying KI with dose at 2.8–3.5 g per day to the area infected with sporotrichosis in adult never causes serious adverse events. Herein, our initial experiments involved the comparison of the killing of both Gram-negative and Gram-positive bacteria in the absence or presence of KI. We added different concentrations of KI into P. aeruginosa, E. coli, and S. aureus suspensions and then the suspensions were

Figure 1. (a) Effect of KI at different concentrations on He-APPJ-induced inactivation of P. aeruginosa, E. coli, and S. aureus. The treatment time was 20 s. (b) Dependence of the inactivation of P. aeruginosa, E. coli, and S. aureus in the absence or presence of 100 μM KI by He-APPJ on the treatment time. The initial bacterial density for plasma treatment was at about 107 CFU mL−1. Each experiment was repeated three times. Error bars indicate ±SD obtained from the average calculation of three experimental data.
exposed to He-APPJ treatment for 20 s. The initial densities of all of the three bacteria were approximately 10⁷ CFU mL⁻¹. After treatment, the samples were diluted 10⁴ times with water and then examined with colony-forming unit (CFU) measurements. Figure 1a shows the results of the survival fraction of the three bacteria with additions of increasing concentrations of KI. Regardless of the bacterial strains, we observed a pronounced increase in bacterial killing with addition of certain amount of KI. For example, in the absence of KI, the survival ratio of P. aeruginosa after He-APPJ treatment for 20 s was 94.8%, while with addition of 1 μM KI, it was 77.9%. With further increasing the KI concentration to 100 μM and 10 mM, the survival ratio was 60.7% and 0, respectively. A similar potentiation effect was also observed for E. coli and S. aureus. Addition of 10 μM KI into the bacterial suspension increased the inactivation ratio from 1.1 to 21.3% for E. coli and from 33.9 to 73.8% for S. aureus. When the added KI reached 1 mM, both E. coli and S. aureus were completely killed after treatment for the same time. To illustrate the potentiation effect of KI, the photographs of CFU measurements of E. coli without or with different concentrations of KI after He-APPJ treatment are shown in Figure S1. Considering the possible toxicity of KI, we added different concentrations of KI in bacterial suspension and found that even 100 mM KI could not induce any bacterial death at all (see Figure S2). This result is consistent with other researchers’ report, in which the authors showed that even 100 mM KI had no toxic effects on bacterial viability.  

Moreover, to further verify the potentiation effect of KI, 100 μM KI (final concentration) was added to P. aeruginosa, E. coli, and S. aureus suspensions, and then the bacterial samples were exposed to He-APPJ treatment for increasing time (from 20 to 60 s). After treatment, the suspensions were also diluted and CFU measurements were performed as described above. The survival ratio of the three bacteria in the presence of 100 μM KI after treatment for different times were compared to that in the absence of KI (Figure 1b). For both P. aeruginosa and E. coli, the potentiation effect of KI on the plasma-induced bacterial killing was increasingly pronounced with increasing treatment time. For example, the survival ratios for P. aeruginosa and E. coli without KI after He-APPJ treatment for 20 s were 85.1 and 82.2%, which decreased to 43.8 and 77.1% after treatment for 60 s, respectively. However, in the presence of 100 μM KI, the survival ratios of P. aeruginosa and E. coli after He-APPJ treatment for 20 s were 78.5 and 53.8%, which decreased to 3.9 and 0% after treatment for 60 s, respectively. For S. aureus, which was more susceptible to the He-APPJ than P. aeruginosa and E. coli, the potentiation effect of KI on its killing was more pronounced with 20 s plasma exposure. Its survival ratios in the absence or presence of 100 μM KI after plasma treatment for 20, 40, and 60 s were 37.8, 12.7, and 2.9% and 21.3 0.1, and 0%, respectively. All of these results suggest that KI can greatly potentiate the He-APPJ killing of both Gram-negative and Gram-positive pathogenic bacteria with density at 10⁷ CFU mL⁻¹ even at 10 μM. The slight increase of survival of E. coli with KI at 1 μM in Figure 1a is probably due to that the salt (KI) provides normal conditions for bacterial survival, such as ionic strength or osmotic pressure. The nonmonotonous decrease of survival of E. coli in Figure 1b is most probably due to that the bacterial suspension without salt caused large errors of the experimental data.

### 2.2. Bacterial Inactivation Induced by PAW and PAI

In one of our latest reports, we found that chlorine could potentiate the bacterial killing induced by corona discharge plasma in oxygen, and the reason for the potentiation effect was due to that the chloride is oxidized to active chlorine by plasma-produced hydroxyl radicals. We speculate that the enhancement effect of KI on bacterial killing was due to the oxidation of I⁻ by He-APPJ to form active iodine species. To confirm this hypothesis, disinfection capabilities of PAW and PAI with different initial KI concentrations were compared, and the result is shown in Figure 2. If the oxidation of I⁻ by He-APPJ is responsible for the potentiation effect of KI on bacterial killing shown in Figure 1, PAI should have stronger disinfection capability than PAW. For all of the three bacterial strains, PAI showed really stronger disinfection capability than PAW, which represented a KI-concentration-dependent manner. As the initial concentration of KI increased, higher bacterial reduction was achieved by PAI exposure. For both P. aeruginosa and E. coli, the disinfection efficiency of PAW was nearly negligible, which was only 5.6% for both strains. While when the initial KI concentration reached 100 μM, the PAI showed clearly stronger disinfection capability than PAW, which inactivated 51.2% P. aeruginosa and 63.2% E. coli, respectively. With further increasing the initial KI concentration to 1 mM, the PAI could inactivate almost 100% P. aeruginosa and E. coli. For S. aureus, PAW showed modest sterilization capability, which inactivated 49.2% bacteria. PAI-1 mM and PAI-10 mM showed stronger sterilization capability, which inactivated 77.7 and 99.9% bacteria, respectively. The slight increase of survival of E. coli after PAI-1 μM and PAI-10 μM treatment is probably due to that the salt (KI) in it provides certain ionic strength or osmotic pressure, which are beneficial to the bacteria survival. For excluding the possible role of increase of conductivity of solution with addition of KI, we compared the bacterial inactivation by He-APPJ in the presence of KCl, KBr, and KI at different concentrations. Although all of the three salts have potentiation effects at certain concentration, their capabilities are different, especially at 1 or 10 mM. KI has the strongest potentiation effect, followed by KBr and then KCl (Figure S3). If the potentiation effect is due to the increase of conductivity of solution, the potentiation would be the same for the three salts at the same concentration, and also the PAI should not have stronger disinfection capability than PAW.
Indeed, several previous studies have shown that PAW has a notable broad-spectrum biocidal activity against bacteria, 
fungus, and biofilms. The ROS and RNS in PAW, or their synergy, play a crucial role in its biocidal activity, which results in a high oxidation-reduction potential and low pH value. Some research works have shown that the synergy of nitrite and H2O2 in acidified medium in PAW form peroxynitrite, which is a strong oxidant and plays a crucial role in the disinfection ability of PAW. In this work, the nozzle of the plasma device was inserted into the well of the 24-well plate, and so the plasma was generated in an airtight environment. This way prevented the incorporation of air into the helium and also inhibited the nitrite production and pH decrease in the solution. In addition, the plasma treatment time for PAW and PAI was really short (20 s). Therefore, the PAW exhibited extremely weak disinfection capability.

2.3. Bacterial Inactivation Induced by PAW in the Presence of KI. When a plasma jet directly interacts with the surface of the liquid medium, both long-lived species (H2O2, ozone, nitrite, nitrate, etc.) and short-lived radicals (hydroxyl radical, superoxide anion, hydrated electron, etc.) are produced. For direct plasma treatment, both short-lived reactive species (such as hydroxyl radicals, superoxide anions, etc.) and long-lived species (H2O2) can oxidize I-. However, for PAW, only the long-lived species can oxidize I- because the lifetime of short-lived radicals is very short (ns to μs). To elucidate which reactive species (short-lived or long-lived) are responsible for the generation of bactericidal iodine species during He-APPJ treatment, PAW was mixed with certain amount of KI solution, followed by addition with bacterial suspension. If the presence of KI can potentiate the PAW-induced bacterial inactivation, then the long-lived species generated by He-APPJ oxidize KI to active iodine species, which further inactivate the bacteria. If not, then the short-lived radicals are responsible for the KI oxidation. The inactivation behavior of the three bacterial strains by PAW in the absence or presence of KI was accounted, and the result is shown in Figure 3. For both P. aeruginosa and E. coli, there is no discrepancy in the inactivation rate when the KI is in the range of 0–100 μM. When the KI concentration reached 1 mM, 99% P. aeruginosa and 63.1% E. coli were inactivated. When the KI concentration reached 10 mM, the PAW inactivated almost all E. coli. For S. aureus, KI at 10 mM could effectively potentiate the PAW-induced bacterial inactivation. The difference of the data between 0 μM and PAW in Figure 2 may be due to the difference of initial bacterial density in different experiments, and it is known that inactivation rate is dependent on the initial bacterial density. These results imply that the long-lived reactive species in PAW, which itself cannot inactivate the bacteria, can eventually oxidize the KI to active iodine, which induces bacterial inactivation.

2.4. Oxidation of Iodide by He-APPJ Treatment. Upon oxidation, iodide will be transformed to a series of iodine species, including HOI, I2, I3-, iodate, etc. Iodate is an inert and nontoxic form of iodine. Our first thought about the active iodine species resulting from the KI oxidation by He-APPJ are I2 and HOI, which are typical disinfectants. To confirm whether I2 and HOI were produced or not, the mixture of KI (10 mM) and phenol (0.9 mM) was exposed to He-APPJ treatment for different times and then analyzed by high-performance liquid chromatography (HPLC). Scavenging of I2 and HOI by phenol was expected to form iodophensols, which can be identified by HPLC. The HPLC images of the mixture after treatment for 60–360 s are shown in Figure 4.

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Figure 3. Inactivation of P. aeruginosa, E. coli, and S. aureus by PAW in the absence or presence of different concentrations of KI. The bacterial density for PAW and PAI treatment was at about 10⁵ CFU mL⁻¹. Each experiment was repeated three times. Error bars indicate ±SD obtained from the average calculation of three experimental data.

Figure 4. HPLC images of the mixture of KI (10 mM) and phenol (0.9 mM) after He-APPJ treatment for 60–360 s.
increasing treatment time from 20 to 60 s, the yield $I_3^-$ concentration increased from 3.9 to 15.4 $\mu$M, which increased to 35.5 $\mu$M on further increasing the treatment time to 120 s.

As mentioned above, the long-lived species are responsible for the generation of bactericidal active iodine species during He-APPJ treatment. To further verify this speculation, we compared the $I_3^-$ generation by direct plasma treatment to that by PAW. It can be seen from the comparison results (Figure 5b) that there was no significant difference for $I_3^-$ formation between direct He-APPJ treatment group and PAW group. Therefore, we conclude that the main reactive species responsible for $I^-$ oxidation are really long-lived species.

In our experiment, the airtight environment where plasma generated prevented the incorporation of air into the helium and also inhibited the generation of nitrite, nitrate, and ozone. Therefore, the most possible long-lived species responsible for $I^-$ oxidation is $H_2O_2$, which can react with $I^-$ to form $I_3^-$. The presence of catalase completely inhibited the formation of $I_3^-$, implying that the plasma-generated $H_2O_2$ was really the principle species which oxidized $I^-$ to $I_3^-$ during the He-APPJ treatment. Furthermore, to confirm that the oxidation of $I^-$ by plasma-generated $H_2O_2$ is the reason for the observed potentiation effect of $I^-$, we mixed $H_2O_2$ at 7 $\mu$M (equal to the concentration in PAW with 20 s treatment) with different concentrations of KI (1 $\mu$M to 10 $\mu$M) and then evaluated the disinfection ability of the mixture. Consistent with the results in Figure 3, when the final KI concentration was at 1 $\mu$M, the mixture exhibited obvious disinfection ability (Figure S5). This result further confirms that the oxidation of $I^-$ to $I_3^-$ by plasma-generated $H_2O_2$ is responsible for the observed potentiation effect.

Compared with iodine-containing disinfectants, the advantage of the combination of He-APPJ and KI provides an “in situ” and “on-demand” route to production of iodine species together with ROS, whose synergy exhibits stronger sterilization ability than the two alone. For example, several studies have proved that the antimicrobial activity of $I_3^-$ is enhanced by $H_2O_2$. Indeed, $I_3^-$ is the polyiodide ion of $I_2$ which may release $I_2$. Additionally, compared to commercial iodine-containing disinfectants, the concentrations of utilized KI and produced $I_3^-$ are really low in our case; therefore, it is mild to the human and may not cause damage to the mammalian cells. Therefore, we believe that this method may have practical applications in disinfection of local infection.

3. CONCLUSIONS

In conclusion, KI can enhance the bactericidal efficiency of He-APPJ against both Gram-negative and Gram-positive bacteria dramatically. By comparing the inactivation behaviors induced by PAW and PAI, and by PAW in the absence or presence of KI, we confirmed that the reason for the potentiation effect is due to active iodine species oxidized from $I^-$. The reactive substance responsible for $I^-$ oxidation is plasma-generated $H_2O_2$, which can oxidize $I^-$ to $I_3^-$ efficiently. The produced $I_3^-$ is a moderate oxidant which possesses a certain extent of bactericidal activity. We think that the combination of KI and He-APPJ has the potential for clinical disinfection applications in the biomedical field.

4. EXPERIMENTAL SECTION

4.1. Bacterial Suspension Preparation, He-APPJ Treatment, and Colony-Forming Units (CFU) Measurements. 

$P. aeruginosa$, $E. coli$, and $S. aureus$ stock (20 $\mu$L) at $20^\circ C$ was inoculated into 100 mL of liquid nutrient media ($P. aeruginosa$) or LB media ($E. coli$ and $S. aureus$) and then incubated at 37 $^\circ C$ overnight with shaking (180 rpm). After incubation, the bacterial pellet was obtained by centrifugation (2188g, 5 min) and then suspended in $H_2O$ with density at $10^7$ CFU mL$^{-1}$. The working bacterial suspension was obtained by mixing 1/10 volume of $H_2O$ or KI stock solution at certain concentration with 9/10 volume of bacterial suspension. The final KI concentration in the working bacterial suspension was from 1 $\mu$M to 10 $\mu$M. The working suspension (1 $\mu$L) was deposited into the well of 24-well plates and then exposed to He-APPJ treatment. The plasma jet device was constructed by us, which had been described previously with minor changes. The HV stainless steel electrode with a diameter of 2 mm is inserted into a ceramic tube with one end closed. The outer and inner diameters of the ceramic tube are 5 and 3 mm, respectively. The ceramic tube along with the HV electrode is inserted into a hollow barrel pipet made of Teflon, which has the diameter of the hollow barrel about 8 mm, and the...
diameter of the nozzle is about 3 mm. When helium with a flow rate of 1 L min$^{-1}$ is injected into the hollow barrel and the HV voltage is applied to the HV electrode, a plasma jet can be generated in front of the end of ceramic tube reaching the sample surface. The distance between the end of the plasma jet and the suspension surface was about 1 cm. The output voltage was about 9.6 kV, and the frequency was 9.5 kHz. After plasma treatment for certain period, the bacterial suspension was diluted with H$_2$O and then transferred and spread on solid agar plates. After incubating at 37°C overnight, CFU were counted and the survival ratio was calculated.

4.2. Bacterial Inactivation by PAW or PAI Solution. H$_2$O or KI solution (1 mL) at different concentrations (1 μM to 10 mM) was deposited into the well of 24-well plates and then exposed to He-APPJ treatment for 20 s. The plasma-activated water and plasma-activated KI with different initial KI concentrations were defined as PAW, PAI-1 μM, PAI-10 μM, ..., PAI-10 mM, respectively. After treatment, 100 μL bacterial suspension with density at about 10$^8$ CFU mL$^{-1}$ was added into the PAW/PAI solutions immediately. The survival ratio of the bacteria after PAW/PAI treatment for 30 min was calculated from CFU measurements.

4.3. Bacterial Inactivation by PAW in the Absence or Presence of KI. H$_2$O (1 mL) was exposed to He-APPJ treatment for 20 s and then 10 μL of KI solution was added into the PAW immediately. The final KI concentration was from 1 μM to 10 mM. A bacterial suspension (100 μL) with density at about 10$^6$ CFU mL$^{-1}$ was added into the mixture immediately and then incubated at room temperature for 30 min. The survival ratio of the bacteria was calculated from CFU measurements.

4.4. Analytical Methods. Active iodine species, including I$_2$ and HOI, are powerful disinfectants, which may be responsible for the potentiation effect of KI on He-APPJ-induced bacterial killing. For confirming whether I$_2$ and HOI were generated or not, KI solution in the presence of phenol was exposed to He-APPJ treatment and then analyzed by high-performance liquid chromatography (HPLC). KI (100 μL, 100 mM) was added to 900 μL of phenol (1 mM), and then the mixture was exposed to He-APPJ treatment for certain time. The treated samples were analyzed by HPLC, which was done on a C18 column with an eluent consisting of 60% water and 40% acetonitrile. The flow rate was 1.0 mL min$^{-1}$ and the detection wavelength was at 280 nm. Both I$_2$ and HOI reacted quickly with phenol to form iodophenols, which was analyzed quantitatively by HPLC and compared to standard 2-iodophenol, 3-iodophenol, and 4-iodophenol.\(^{45}\)

Triiodide (I$_3^-$), which is a moderate oxidant and shows inferior antibacterial activity compared to I$_2$, may also be responsible for the potentiation effect of KI. For detecting the possible generated I$_3^-$, KI solution (1 mL, 10 mM) was deposited into 24-well plates and then exposed to He-APPJ treatment for certain time (20–120 s). After treatment, the UV absorbance spectra of the mixture were measured for evaluating the generated I$_3^-$ concentration.

For evaluating the role of He-APPJ-generated H$_2$O$_2$ in KI oxidation, 4S μL of catalase (3 mg mL$^{-1}$) was mixed with 5 μL of KI at 1 M and then added with 450 μL of PAW. The mixture was centrifuged at 12 000 rpm for 5 min and the UV–vis absorbance spectra of the supernatant were recorded.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00160.

Photographs of CFU measurements of *E. coli* without or with different concentrations of KI after He-APPJ treatment for 20 s; photographs of CFU measurements of *E. coli* without or with KI exposure (10 and 100 mM); effect of KCl, KBr, and KI at different concentrations on He-APPJ-induced inactivation of *P. aeruginosa*; concentration of H$_2$O$_2$ in 1 mL of water after He-APPJ treatment for different times; and killing of *P. aeruginosa* upon exposure to the mixture of H$_2$O$_2$ at 7 μM with KI at different concentrations (PDF)

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### Notes

The authors declare no competing financial interest.

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