Optimizing the distance for bacterial treatment using surface micro-discharge plasma

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Abstract. Reactive plasma species generated by a surface micro-discharge (SMD) electrode are delivered to the target by diffusion and/or convection. In humid air conditions, the diffusion process is coupled with complicated plasma chemical reactions, which affect the density profiles of bactericidal agents. One may expect that the production of reactive plasma species can be optimized at a certain distance. Our experimental results found the optimum distance for achieving the highest bactericidal efficiency with plasma treatment using an SMD electrode. The optimum distance is about 2–4 mm from the SMD electrode to the target and depends on the geometry of the experiment. The bactericidal efficiency in the plasma-treated area can be improved by a factor of 30 if the bacterial samples are placed at the optimum distance. The results show the predominant role of the long-lived reactive plasma species. It is seen that the diffusion model of multi-plasma species with coupled plasma chemical reactions would be highly important for understanding the bactericidal property of cold atmospheric plasmas and therefore for optimizing cold atmospheric plasma sources for medical and biological applications.

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Cold atmospheric plasmas (CAPs) have been extensively investigated in recent years with a focus on medical and biological applications [1–7]. It has been shown that CAPs can kill/inactivate different micro-organisms including bacteria, fungi, viruses and spores, destroy biofilms [8, 9] and remove proteinous contamination from surfaces [10, 11]. In vitro experiments show that CAPs can even break amyloid fibrils into smaller units [12]. Much effort has been devoted to developing different CAP sources, mainly by using dielectric materials [13, 14] such as the plasma jet/plume/needle [15–21] and the surface micro-discharge (SMD) [1, 22, 23], and the microwave plasma torch [24, 25]. The discharge dynamics of pin-to-plate dielectric barrier discharge [26] and the glow discharge without dielectric barrier [27, 28] have also been studied at atmospheric pressure recently. Furthermore, the bactericidal property of the CAPs has been investigated for different micro-organisms, different plasma conditions (frequency and waveform of the driven signals) and different environmental (humidity, temperature and gas compositions) and sample (substrate for bacteria deposition) conditions. The aim is to develop/optimize the CAP sources for different applications. Well-designed CAP sources can be applied to heat-sensitive and radiation-sensitive targets including human and animal tissues [29–32].

However, the killing/inactivation mechanism of the plasma treatment is not yet well understood. It has been attributed to several synergic effects including ultraviolet (UV) radiation, electric field, charged particles and reactive plasma species. The predominant components for the bactericidal efficacy can be different depending on different CAP sources and application methodologies. Normally a temperature gradient exists from the plasma source to the target, which helps to increase the diffusion speed of the reactive species and certainly improve the efficacy of the plasma treatment.

For the SMD source, plasma is ignited on the surface of a dielectric material that is sandwiched by a solid metal plate and a metal mesh. Ignition of the plasma results in energetic electrons adjacent to the mesh electrode. In ambient air conditions, oxygen molecules are dissociated by the electron impacting initially. The production of a complex plasma chemistry with reactive neutral species dominating is then triggered [33–35]. The bacterial sample is
Figure 1. Sketch of the SMD electrode. The mesh electrode is made of S/S, the glass epoxy board serves as the dielectric layer and the copper foil works as the large-area electrode. Plasma is ignited on the side of the mesh electrode by applying an HV signal (see figure 2) between the S/S mesh and the copper foil.

located normally several millimeters away from the SMD electrode and the reactive plasma species reach the bacterial sample by diffusion and/or convection. The diffusion properties have been well studied in low-temperature plasmas [36–38], but in most cases only electrons, ions and impurity particles are considered. The plasma chemistry generated in humid air by the SMD electrode consists of more than hundreds of plasma species (~20 of them are regarded as the main bactericidal agents). The long-lived reactive species may play important roles by changing the spatial and temporal density profiles of bactericidal agents during diffusion processes. In order to understand the bactericidal mechanism of the CAPs, coupling between diffusion (mass transport) and plasma chemical reaction concerning especially reactive plasma species would be highly necessary [39].

In this paper, an SMD plasma device, which sustains in atmospheric air and operates without any gas flow, has been used to study the bactericidal mechanisms of CAPs. Bacterial samples were treated at different distances from the SMD electrode. The experimental results identified an optimum distance of approximately 2–4 mm where the highest bactericidal efficacy was achieved. This optimum distance can be affected by geometrical changes of the bacterial experiments. The log reduction in the plasma-treated area could be enhanced by as much as a factor of 30 when the optimum distance was chosen.

2. Device and experiments

2.1. The surface micro-discharge electrode

The SMD electrode was used for the production of an air plasma at atmospheric pressure. No gas flow or gas mixture was applied. Figure 1 shows a sketch of the SMD electrode used for our experiments. The SMD electrode consists of a stainless steel (S/S) mesh electrode, a glass epoxy board with a thickness of 1 mm and a layer of copper foil with a thickness of approximately 0.2 mm. The S/S mesh has a square pattern with 30 grids per inch. The wire of the S/S mesh has a diameter of approximately 0.2 mm. The copper foil was encapsulated by Kapton tape to avoid any discharge on the copper electrode side.

2.2. Electrical characterizations of the discharge

A high-voltage (HV) signal was provided by a self-made power supply unit. It consists of mainly rechargeable batteries, a precise timer, a MOSFET driver and a transformer. All the electronic elements are integrated into a printed circuit board. The HV signal was applied between the

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Figure 2. (a) The voltage and current waveforms for the plasma discharge are plotted in gray and black, respectively. The inset shows a single-current spike. (b) Schematic diagram for measuring the electrical properties of the discharge. HV: high voltage; SMD: surface micro-discharge electrode; $V_o$: the output voltage of the HV power supply with respect to the ground when the discharge is switched on; $V_R$: the voltage applied to the sampling pure resistor $R$.

mesh and the copper electrodes to produce plasma on the side of the mesh electrode. The height of the visible plasma glow was less than 1 mm from the epoxy surface. Figure 2(a) shows the HV signal applied for plasma generation, as well as the current waveform measured through the discharge circuit. As shown in figure 2(b), a 10Ω sampling pure resistor $R$ is connected in series with the discharge circuit to measure the current waveform. Both the voltage and the current waveforms were recorded by an oscilloscope (LeCroy, WavePro 725Zi) with a sampling rate of 20 G s$^{-1}$. The voltage signal on the sampling resistor $V_R$ was detected by an active voltage probe (LeCroy, HFP2500), which has a bandwidth of 2.5 GHz. The output voltage of the HV power supply $V_o$ (with discharge switched on) was measured by using a normal co-axial cable so that high-frequency information was not detected for $V_o$. Both $V_R$ and $V_o$ were measured with respect to the ground. The peak-to-peak value of the HV signal is about 7 kV with a repetition rate of approximately 6.75 kHz. The current waveform consists of abundant fast spikes. The instantaneous amplitude of a single current spike can be as high as 0.4 A. Each spike lasts for typically less than 1 μs. The shape of a single current spike is similar to a damping sine curve. An example of a single current spike is also presented in figure 2(a) as the inset for better understanding. In figure 3, the power spectral density was calculated from the fast Fourier transform of the current waveform. It shows that the current spikes have a typical frequency range between 10 and 200 MHz. The power density absorbed by the plasma discharge in the discharge area of 28 mm diameter, which was calculated based on the measured voltage and current waveforms, was approximately 0.5 W cm$^{-2}$.

2.3. Bacterial sample preparation

An Escherichia coli (E. coli) bacterial strain (DSM1116) was used for the experiments in this study. The bacterial samples were prepared by using a stock culture of E. coli. The stock culture was reserved in the refrigerator at a temperature of 4°C. The secondary culture was prepared several days before the experiments. On the day of the experiments, a phosphate
buffer saline (PBS; Biochrom, Germany; NaCl 8 g l$^{-1}$, KCl 0.2 g l$^{-1}$, Na$_2$HPO$_4$ 1.15 g l$^{-1}$, KH$_2$PO$_4$ 0.2 g l$^{-1}$ without Ca$^{2+}$ and Mg$^{2+}$) suspension of *E. coli* was made by using isolated colonies on the secondary culture plate. This ‘master’ suspension was then distributed evenly on each Müller–Hinton agar plate that has an inside diameter of around 85 mm. Before the plasma treatment, the agar plates were kept under ambient conditions for approximately 30 min in order to dry the bacterial samples. To control the number of bacteria on the plasma-treated samples, the ‘master’ suspension was diluted one million times and the same volume of the diluted solution was then distributed on the agar plate. The control plates were also kept under ambient conditions for a 30 min drying period. After the plasma treatment, the plasma treated and the control plates were incubated for approximately 14 h at a temperature of 35°C. After incubation, the colony-forming units (CFUs), which equalled the number of bacteria on the plates, were then counted to evaluate the efficiency of the plasma treatment.

2.4. Experimental arrangement

For the plasma treatments, the SMD electrode as well as the power supply unit (for plasma generation) was housed in a cylindrical tube made of polyoxymethylene-copolymer (POM-C). The POM-C tube was vertically mounted with the SMD electrode oriented horizontally. The bacterial samples were placed horizontally underneath the SMD electrode. The agar surface with bacteria-deposited faces upwards. The experimental arrangement is sketched in figure 4(a). The size of the plasma area ($A_p$) was 28 mm in diameter, i.e. 615 mm$^2$. After the plasma treatment, a certain area on the agar plates, where the bacterial growth was significantly inhibited, was induced. This area was referred to as the inhibited area ($A_i$). The CFUs in the inhibited area $A_i$ were countable after plasma treatments. The log reduction ($L_R$) of the bacterial load in the inhibited area was then obtained by making a comparison with the number of CFUs on the control plates.

Note that there is 1 mm distance from the mesh electrode to the front end of the POM-C tube. The distance from the mesh electrode to the agar plate, which will be referred to as $\Delta$, was changed by adjusting the height of the plasma device (the POM-C tube) using a
Figure 4. (a) Sketch of the experimental arrangement. The two oblique dashed lines mark the total inhibited area $A_i$ induced by the plasma treatment. The two vertical dashed lines restrict the central inhibited area $A_{iC}$, which has the same size as the surface area where the plasma is generated, i.e. $A_p$. (b) Photograph of a plasma-treated agar plate for the ‘open volume’ condition. $A_{iC}$ is marked with a black circle.

A micro-positioning stage. The bactericidal efficiency was then evaluated at different distances $\Delta$. These experiments were conducted for both ‘closed volume’ and ‘open volume’ configurations. For the ‘closed volume’ configuration, ring adapters made of POM-C (with an inside diameter of 28 mm) with different heights (for plasma treatments with different distances $\Delta$) were mounted between the plasma device and the agar samples to close the volume between the SMD electrode and the target surface. The ring adapters touched both the surface of the agar plate and the front surface of the POM-C tube; however, the joints were not air-tight.

Depending on the plasma treatment time $t$ and the distance $\Delta$ from the SMD electrode to the agar surface, the inhibited area $A_i$ can be larger or smaller than the surface plasma area $A_p$. For the ‘closed volume’ configuration, the inhibited area cannot be larger than the surface plasma area because the target area is limited by the ring adapters. For all experiments presented in this paper, we have $A_i \geq A_p$ for all plasma treatments. The central inhibited area $A_{iC}$, marked with two vertical dashed lines in figure 4(a), was used to evaluate the log reduction $L_R$ from the plasma treatment. The central inhibited area $A_{iC} = A_p$ was cropped from the center of the total plasma-inhibited area $A_i$. For the ‘open volume’ configuration, no ring adapter was used and the total area $A_i$ inhibited by plasma treatments was also obtained with respect to different distances $\Delta$. Figure 4(b) shows a photograph of the plasma-treated agar plate (after incubation). There is a clear boundary between the inhibited area and the unaffected region. The central inhibited area $A_{iC}$ is cropped by the black circle.

3. Experimental results

3.1. Survival curve

First of all, the survival curve of *E. coli* was obtained for the ‘open volume’ configuration by applying different plasma treatment times at a distance of $\Delta = 2$ mm. This configuration
Figure 5. The log reduction $L_R$ of the bacteria (E. coli) due to the plasma treatment is plotted with respect to different plasma treatment times $t$. For these experiments, an ‘open volume’ configuration was used. The distance from the mesh electrode to agar surface equalled 2 mm, resulting 1 mm gap between the front surface of the POM-C tube and the agar surface.

resulted in a 1 mm gap between the front surface of the POM-C tube and the agar surface. Figure 5 shows 5 log reduction of the bacterial load in the area $A_{iC}$ within 10 s of plasma exposure. For 20 and 30 s plasma treatments, more than 6 log reduction was achieved. The error bars represent the standard deviations calculated from three repetitions of each plasma treatment (the same for figures 6 and 7).

3.2. Distance dependence

To evaluate the bacterial reduction dependence on the distance between the electrode and the bacteria surface $\Delta$, experiments were then performed for both the ‘open’ and the ‘closed volume’ configurations for different distances $\Delta$. The results are presented in figure 6. For all experiments, the plasma treatment time was kept the same (20 s). The distance dependence of the log reduction $L_R$ for both configurations indicates the existence of an optimum distance $\Delta_{Opt}$. This optimum distance is approximately 3 mm for the ‘open volume’ configuration (figure 6(a)) and 4.5 mm for the ‘closed volume’ configuration (figure 6(b)), respectively.

In particular for the ‘closed volume’ configuration (figure 6(b)), a linear increase of the log reduction $L_R$ in the area of $A_{iC}$ when increasing the distance $\Delta$ from the nearest point ($\sim 2.5$ mm) to the optimum distance $\Delta_{Opt}$ ($\sim 4.5$ mm) can be seen. A linear extrapolation of the log reduction $L_R$ in the distance range of $\Delta \leq \Delta_{Opt}$ gives approximately a 4 log reduction ($L_R = 4$) directly on the surface of the electrode when it is applicable. If the distance is increased further beyond the optimum distance $\Delta_{Opt}$, the log reduction $L_R$ in the area $A_{iC}$ rapidly decreases. Note that as high as 5 log reduction was still achieved at a distance of approximately 10 mm for the ‘closed volume’ configuration. More experimental results show that more than 4 log reduction can be achieved even at a distance of 20 mm for the ‘closed volume’ configuration. This indicates that long-lived neutral reactive species produced by CAPs are important for the bactericidal efficacy. It also suggests that these long-lived species are not likely fully absorbed by the interaction with the POM-C wall (the ring adapter). They are at

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Figure 6. The distance dependence of the log reduction by plasma treatment is plotted for the ‘open volume’ (a) and ‘closed volume’ (b) configurations, respectively. The plasma treatment time is the same for all the treatments and equals 20 s.

Figure 7. Inhibited area by a 20 s plasma treatment for the ‘open volume’ configuration at different distances $\Delta$.

least partially reflected when they hit the inside wall of the ring adapter. This reflection rate may also depend on the wall material and should be considered to improve the boundary conditions for numerical simulations. Furthermore, it would also be interesting to investigate the effect of using different wall materials. This will be addressed in future experiments. Concluding, for the
‘closed volume’ configuration, it is clear that the plasma inactivation efficiency can be increased by as much as a factor of 30 when placing the bacterial samples at an appropriate distance, i.e. the optimum distance $\Delta_{\text{Opt}}$.

For the ‘open volume’ configuration, the optimum distance $\Delta_{\text{Opt}}$ is shorter than that for the ‘closed volume’ configuration. In addition, the enhancement of the bactericidal efficiency by placing the bacterial samples at $\Delta_{\text{Opt}}$ is not that significant in comparison to the ‘closed volume’ configuration. Both cases can be explained by looking at the geometrical change of the bacterial experiment. For the ‘open volume’ configuration, the size of the target area is much larger than the size of the surface plasma $A_p$. Furthermore, there is no side wall to confine/direct the diffusion of the plasma species to the target. The loss of reactive plasma species (RNS/ROS) from side diffusion must be taken into account. Due to the open air configuration, a higher exchange rate between the reactive plasma species and the fresh air is expected, so that the reactive plasma species in the central volume can be diluted. (The diffusion time for the air molecules and the ROS/RNS is much shorter than the plasma treatment time.) The results show that an increase in the distance $\Delta$ results in a much faster decrease of the log reduction $L_R$ for the ‘open volume’ configuration, compared with the results for the ‘closed volume’ configuration. In addition, the side diffusion of the reactive plasma species in the ‘open volume’ configuration results in a larger inhibited area $A_i$ ($> A_p$) for short distances (when $\Delta$ is less than $\sim 6$ mm). Therefore the inhibited area $A_i$ can also be used to evaluate the bactericidal efficacy for the ‘open volume’ configuration. Figure 7 shows the distance dependence of $A_i$. For the ‘open volume’ configuration, the experimental results indicate that the inhibited area $A_i$ is smaller than the surface plasma area $A_p$ for $\Delta > 6$ mm.

4. Discussion and summary

SMD technology—a robust and simple design of an electrode for the generation of CAPs—can be easily scaled up and machined into complex structures. It is particularly suitable for large-area disinfection, for example, hand disinfection [22]. It can be fully encapsulated by insulating the materials so that this technology can be integrated into different household and industrial products for the regular disinfection of different surfaces.

In addition, the SMD plasma is also a novel source for studying the bactericidal mechanisms of CAPs. It does not need any gas flow so that no physical force due to the vortex of plasma-assisted gas flow is applied to the bacterial sample. The treatment is dominated by diffusion of plasma species from the core or by reactive species which are generated along the diffusion channel. In principle, SMD technology uses a remote/indirect plasma treatment. The charged particles are found to play a negligible role; therefore, the surface tension on the bacteria membrane due to the charged particles can also be neglected. The SMD electrode uses a self-standing plasma generation principle, so that there is no need for the bacterial sample to serve as a counterelectrode. This leads to stable and reproducible plasma generation for all treatments. Furthermore, it was found that the plasma production is not affected even when the sample was placed less than 1 mm away from the SMD electrode, which was confirmed by visual inspection of the discharge glow and also the discharge current.

The bactericidal property of the SMD plasma can be attributed to neutral reactive species, including radicals, atoms and molecules. The high electric field (due to the applied HV) adjacent to the mesh electrode produces high-energy electrons which initiate the air plasma chemistry by impacting and dissociating oxygen molecules. By including 630 chemical reactions, a humid air
plasma chemistry model developed by Sakiyama and Graves [33–35] for the SMD configuration predicts 21 neutral reactive species; among them, O$_3$, O$_2^*$ (singlet oxygen), O (oxygen atom), N$_x$O$_y$ (including NO, NO$_2$, N$_2$O, NO$_3$, N$_2$O$_5$), N (atomic nitrogen), HNO$_2$, HNO$_3$, H$_2$O$_2$, HO$_2$ and OH are the most abundant ones. Their model also predicts the obvious change of the plasma chemistry by including the air humidity. It shows that the charged particle densities are about two orders of magnitude lower than those of the reactive neutral species. The charged particles are found mainly in the core plasma region and are quickly recombined in the diffusion process.

SMD technology uses the diffusive plasma/gas species as bactericidal agents. In these experiments the target sample is often placed several millimeters away from the electrode. A monotonic decrease of the bactericidal efficacy is normally expected when increasing the distance from the electrode to the bacterial sample. Our experiments found an optimum distance where the highest bactericidal efficacy can be obtained. This result is crucial for understanding the bactericidal mechanisms of CAPs, especially for those using the SMD electrode. Our results indicate that long-lived neutral species, including meta-stable atoms and molecules, are dominant for the bactericidal property of the SMD electrode. These bactericidal agents are not fully absorbed by insulating wall material so that they can be transported for remote treatments.

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