The bactericidal effect of surface micro-discharge plasma under different ambient conditions

T Shimizu¹, J L Zimmermann and G E Morfill
Max-Planck Institute for Extraterrestrial Physics, Giessenbachstr.,
85748 Garching, Germany
E-mail: tshimizu@mpe.mpg.de

New Journal of Physics 13 (2011) 023026 (7pp)
Received 18 September 2010
Published 11 February 2011
Online at http://www.njp.org/
doi:10.1088/1367-2630/13/2/023026

Abstract. A series of experiments on the bactericidal properties of plasmas using a surface micro-discharge (SMD) device in an atmosphere under different ambient temperatures and humidities was carried out. This plasma dispenser was developed for use as a disinfection system in private and public places (hospitals, medical practices, etc). The bactericidal effect is due to the interplay of the plasma and the chemical products produced via interactions with O₂/N₂ and H₂O vapour in air. To use this device in different countries and therefore under various ambient conditions, it is important to understand its behaviour and efficiency, especially with respect to air temperature and humidity. The experimental results obtained in this study show that the bactericidal properties of the SMD plasma dispenser are not sensitive to the different temperatures and humidities.

Contents

1. Introduction 2
2. Experimental setup 2
3. Experimental result 4
4. Summary 6
Acknowledgments 7
References 7

¹ Author to whom any correspondence should be addressed.
1. Introduction

Various medical applications for atmospheric pressure plasma sources have been investigated and there has been growing interest in them [1]–[5]. One reason is that such plasma sources can combine many advantages, e.g. low cost, simple design and easy handling [6]. Another reason is that they have bactericidal and fungicidal properties due to reactive species, UV radiation, charged particles, etc produced by the plasma [3]. Since the plasma has gaseous form, it is possible to treat not only surfaces but also narrow cavities and fissures where fluid disinfection is not easily accessible. In addition, contact-free treatment can be achieved without any heating. This has opened the possibility for in vivo treatments without any painful or uncomfortable sensation. This suggests that plasmas have many advantages and correspondingly there are many research topics emerging in ‘plasma health care’, for example hygiene, dental care, treatment of skin diseases and chronic wounds, and cosmetics [4].

From the viewpoint of hygiene, disinfection and sterilization are a very important concern [7]. It is crucial to have a fast and efficient sterilization technique also for resistant bacteria, e.g. Methicillin-resistant Staphylococcus aureus (MRSA), because of the growth of nosocomial infections and the increase in the number of bacteria resistant to antibiotics. In our group, a large–area, scalable plasma dispenser has been developed and tested based on surface micro-discharge (SMD) technology (which incorporates both barrier and corona discharges (BCD)) with high bactericidal and fungicidal efficiency [8]. This device can be operated significantly below the WHO safety levels of UV radiation, toxicity and electromagnetics and is therefore destined for use in disinfection in private and public places (e.g. hospitals, medical practices, nursing homes, etc).

Since this dispenser uses the surrounding air for plasma production, it is very important to investigate the bactericidal properties of plasmas under various ambient conditions, especially focusing on air temperature and humidity. This is useful information if the plasma device is to be used in different countries. In addition, such measurements under different temperatures and humidities could lead to insights into the detailed mechanism for the killing of bacteria and fungi by cold atmospheric plasmas, as different ambient conditions can change the plasma chemistry totally.

In this work, we performed a series of plasma treatments of both Gram-negative Escherichia coli and Gram-positive Enterococcus mundtii under different ambient conditions.

2. Experimental setup

The plasma was produced by a $10 \times 10 \, \text{cm}^2$ SMD electrode. It consisted of an insulator Teflon plate of 1 mm thickness sandwiched between a copper plate and a wire mesh, as shown in figure 1(a). The wire mesh was made of stainless steel wires (0.5 mm diameter) with 6 meshes inch$^{-1}$. The plasma was driven by a sinusoidal ac voltage of $15 \, \text{kV}_{pp}$ with a frequency of 1 kHz between the metal plate and the electrically grounded wire mesh using a high-voltage power amplifier (model 10/10B, Trek, Inc.). A homogeneous plasma was formed on the surface of the wire mesh. This SMD plasma consisted of many micro-discharges of duration $\sim 100 \, \text{ns}$ and length a few mm. The power consumption was $0.4 \, \text{W cm}^{-2}$, estimated by the Lissajous figure method using a 1 $\mu\text{F}$ capacitor.

Gram-negative (Escherichia coli, DSM 1116) and Gram-positive (Enterococcus mundtii, DSM 3269) bacteria were used for our measurements. Bacteria suspensions with a density
of $\sim 10^8$ cells ml$^{-1}$ were prepared in phosphate-buffered saline using the Vitrek Densicheck System (BioMerieux). Subsequently, 100 µl of this suspension was smeared out on Mueller Hinton agar plates with a diameter of 8.6 cm. These agar plates inoculated with bacteria were kept in the ambient condition ($\sim 22^\circ$C, $\sim 40\%$ relative humidity, 955–960 hPa) for 30 min to dry the surfaces. After the treatment with plasma, the agar plates were incubated at 35 $^\circ$C for 18 h, so that the surviving bacteria could reproduce and form colonies. The colony-forming units (CFUs) were counted afterwards. For comparison, a $10^5$ diluted suspension for each bacteria type was smeared out on agar, dried for 30 min and incubated at 35 $^\circ$C for 18 h as a control.

To see the bactericidal effect under different ambient conditions, the plasma dispenser was placed in the environmental chamber (test chamber of type 3636/15, Feutron GmbH, $77 \times 102 \times 74.5$ cm$^3$), as shown in figure 1(b). Through holes (7.5 cm diameter) in the chamber, all electrical cables were connected. First, for every environmental condition, agar plates

Figure 1. (a) Cross-sectional view of the experimental setup. Agar plates were placed upside down on the SMD electrode so that the distance between the agar surface and the electrode was always 6 mm. (b) Environmental chamber used in this study.
Figure 2. Number of surviving bacteria as a function of treatment time. *E. mundtii* and *E. coli* were used in this experiment. For comparison, the level of $10^5$ dilution is shown by solid lines.

inoculated with bacteria were placed inside the chamber next to the electrode. In the next step, the ambient parameters were set, and in approximately 10 min both the temperature and relative humidity reached the set value. To ensure stable ambient conditions, we waited another 20 min before treating the bacteria with plasma. Through the holes in the wall of the environmental chamber, we placed every agar plate upside down on the lower electrode of the plasma dispenser, so that the distance between the electrode and the surface of the agar was always 6 mm (figure 1(a)). *E. mundtii* was treated for 15 s and *E. coli* for 30 s.

After the treatment with plasma, the agar plates were removed from the chamber and new plates were placed inside the chamber for the next experiment. For every ambient condition (temperature–humidity pair), three agar plates were treated in total. In this study, we analysed the bactericidal properties of the plasma dispenser at 15, 25 and 35°C and 20, 40, 60 and 80% relative humidity.

For reactive gas, we measured the concentrations of O$_3$ and NO$_2$ at 6 mm above the electrode using a UV absorption ozone analyser (Teledyne Advanced Pollution Instrumentation, model 400E) and a gas detector (Dräger Multiwarn II), respectively. The gas was sampled through a tube. In the ambient condition outside the environmental chamber (22°C, 40% relative humidity, 955–960 hPa), the concentration of O$_3$ was about 3.2 ppm and that of NO$_2$ was 2.3 ppm.

The UV light power density was measured with a Hamamatsu UV-power meter C8026 at the sample position. The power density was 0.41 µW cm$^{-2}$. The optical spectrum can be found in [8].

3. Experimental result

Figure 2 shows the number of CFUs, i.e. the number of surviving bacteria, for different plasma-treatment times. This experiment was carried out in the normal ambient conditions (22°C, 40%
Figure 3. Number of CFUs, i.e. the number of bacteria, that survived the plasma treatment: (a) *E. mundtii* and (b) *E. coli*. For both bacteria types, no statistically significant difference in bactericidal property under various temperatures and relative humidities was detectable. The error bars show the spread in measurements from the three samples.

relative humidity). It was shown that there is a fast reduction initially for a few seconds, followed by a slower decay. About five orders of magnitude reduction in bacterial load was obtained in 40–50 s for both *E. mundtii* and *E. coli*.

Figure 3 shows the number of CFUs ((a) *E. mundtii* and (b) *E. coli*) for different temperatures and relative humidities. The solid lines in figures 3(a) and (b) refer to the 10$^5$-diluted control sample. For every condition (temperature–humidity pair) and bacteria type, three agar plates were treated with the plasma—15 s for the treatment of *E. mundtii* and 30 s for the treatment of *E. coli*. These treatment times were chosen because at them the bacteria number was decreasing relatively slowly. For *E. mundtii*, as shown in figure 3(a), a reduction of approximately five orders of magnitude was obtained for a plasma-treatment time of 15 s. In the current set of experiments, the reduction by a factor of 10$^5$ took longer than reported previously [8], because we applied a lower power and voltage to the SMD electrode, resulting in a correspondingly lower plasma density. A reduced plasma density was used in order to observe the bactericidal properties with respect to the treatment time more clearly.

For *E. coli*, a reduction of approximately four–five orders of magnitude was obtained for a plasma-treatment time of 30 s (figure 3(b)).

In both cases, namely for *E. mundtii* (figure 3(a)) and *E. coli* (figure 3(b)), statistically no significant difference in bactericidal property was detected for different temperatures and humidities. From these results we conclude that the bactericidal properties of our SMD plasma dispenser are not very sensitive to ambient conditions over a wide range of conditions for both Gram-positive and -negative bacteria and therefore can be used in different countries in different environmental conditions with similar and predictable performance.

It is well known that different humidities in the gas used for plasma production can entirely change the chemistry of plasmas. For instance, through the dissociation of water molecules by
electron impact and through the reaction of excited O with water vapour, hydroxyl radicals are produced [9], which are known to have bactericidal properties.

By increasing the ambient temperature and relative humidities, the concentration of water vapour can be significantly increased and the production of hydroxyl radicals may be expected to be correspondingly higher. Haehnel et al [10] reported that, using their dielectric barrier discharge plasma device, the reduction rate of Bacillus spores was strongly dependent on the concentration of water vapour in the surrounding gas—the reduction rate of Bacillus spores was greatly increased at high relative humidities. From these results, Haehnel et al [10] concluded that hydroxyl radicals were a major candidate responsible for killing the Bacillus spores. However, our experiments under different ambient conditions did not show a significant change in bactericidal property, both for Gram-positive and -negative bacteria. This result in itself is interesting and, in connection with Haehnel et al [10], even more so: it implies either that hydroxyl radicals alone are not the major candidate responsible for killing or inactivating the bacteria, or that the mechanism for killing the spores is different from that for killing the bacteria.

The bactericidal effect of plasmas is believed to be a ‘compound’ one [11]: (i) excited atoms may make the cell membrane permeable to nano-sized ‘agents’ (up to 5 nm [12, 13]); (ii) different reactive species may thus penetrate the cell and interact with e.g. the metalloproteins in the cell wall, with the cytoplasm or with the DNA directly (if this is not specifically protected inside a nucleus as in the case of mammalian cells); (iii) the reaction chemistry may be quite complicated, involving reactive oxygen species (ROS), reactive nitrogen species (RNS), hydroxyl radicals, etc; and (iv) the role of ions, both positive and negative, may also be important, but this is currently not well understood.

The general picture that has emerged is that the plasma bactericidal efficiency is due to the ‘cocktail’ of products generated in the non-equilibrium chemistry—a cocktail that contains long- and short-lived components not otherwise available and whose individual properties may complement or enhance the overall effect. For instance, increased humidity leads to an increase in hydroxyl radicals. It also leads to an increase in NO production [9, 14]. If both types of reactive species are important for inactivating bacteria, then they will complement one another: RNS being important at low humidities and hydroxyl radicals at high humidities, with ROS presumably following the same trend as RNS. We could not measure nitrogen dioxide in our system under different ambient conditions; however, Laroussi and Leipold’s measurements [9, 14] support the ‘cocktail hypothesis’ in the sense that they showed that nitrogen dioxide alone is not responsible for the bactericidal effect.

4. Summary

In this paper, we investigated the bactericidal properties of our SMD plasma dispenser under different ambient conditions. A wide range of different temperatures and relative humidities did not lead to a significant difference in bactericidal properties for either Gram-positive E. mundtii or Gram-negative E. coli. Such measurements can shed light on the dominant processes responsible for plasma disinfection. Our measurements support the hypothesis that plasmas act through a number of reactive agents that are produced in non-equilibrium chemical reactions—the ‘cocktail hypothesis’. Due to the demonstrated insensitivity of disinfection efficiency to ambient conditions, this SMD plasma dispenser is suitable for use in disinfection in hospitals and other public places, and in private places, to reduce the spread of bacterial diseases.
The present results are applicable only to our setup. For the other plasma devices, e.g. the plasma jet, plasma torch, etc, another study is required.

Acknowledgments

We thank Professor W Stolz and Dr G Isbary (Clinic of Dermatology, Allergology and Environmental Medicine, Munich-Schwabing, Germany) for fruitful discussions. We also thank Dr H-U Schmidt (Institute for Medical Microbiology, Hospital Munich Schwabing, Germany) and Mr B Steffes from our institute for technical support. This study was supported by the Max-Planck Society under grant nos 01.M.TT.A and 02.M.TT.A.

References

[1] Stoffels E 2007 Contrib. Plasma Phys. 47 40
[2] Fridman G, Shekhter A B, Vasilets V N, Friedman G, Gutsol A and Fridman A 2008 Plasma Process Polym. 5 503
[3] Laroussi M 2002 IEEE Trans. Plasma Sci. 30 1409
[4] Kong M G, Kroesen G, Morfill G E, Nosenko T, Shimizu T, van Dijk J and Zimmermann J L 2009 New J. Phys. 11 115012
[5] Weltmann K D, Kindel E, von Woedtke T, Haehnel M, Stiebe M and Brandenburg R 2010 Pure Appl. Chem. 82 1223
[6] Park J, Henins I, Hermann H W, Selwyn G S and Hicks R F 2001 J. Appl. Phys. 89 20
[7] Jernigan J A, Clemence M A, Stott G A, Titus M G, Alexander C H, Palumbo C M and Farr B M 1995 Infect. Control Hosp. Epidemiol. 16 686
[8] Morfill G E, Shimizu T, Steffes B and Schmidt H-U 2009 New J. Phys. 11 115019
[9] Laroussi M and Leipold F 2004 Int. J. Mass Spectrom. 233 81
[10] Haehnel M, von Woedtke T and Weltman K-D 2010 Plasma Process. Polym. 7 244
[11] Nosenko T, Shimizu T and Morfill G 2009 New J. Phys. 11 115013
[12] Leduc M, Guay D, Leask R L and Coulombe S 2009 New J. Phys. 11 115021
[13] Yonson S, Coulombe S and Leask R L 2006 J. Phys. D: Appl. Phys. 39 3508
[14] Laroussi M 2009 IEEE Trans. Plasma Sci. 37 714