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Disinfection of Dental Equipment—Inactivation of *Enterococcus mundtii* on Stainless Steel and Dental Handpieces using Surface Micro-Discharge Plasma

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ABSTRACT: Cold atmospheric plasma produced by a surface micro discharge (SMD) device was used to investigate the bactericidal efficacy for *Enterococcus mundtii* inoculated and dried on stainless steel and dental handpieces. The novelty of this approach can be explained by the used setup incorporating a circular plasma system. The circular plasma system produces reactive plasma species, which are transported through the narrow cavities of a dental handpiece into a separate treatment chamber and which are subsequently recycled and transported back to the SMD electrode. To investigate the efficacy of this circular plasma setup, stainless-steel plates inoculated with *E. mundtii* were placed in the treatment chamber and treated with plasma. The results show that a 5 log reduction in bacterial number can be achieved within only 15 min of plasma treatment time, although the plasma species had to pass the narrow channels of the dental handpiece before entering the treatment chamber. First results with *E. mundtii* inoculated directly on the outer side of the dental handpiece showed a 2 log reduction within 15 min of plasma application. Further investigations targeting this latter issue are nevertheless necessary to investigate the plasma treatment time to achieve full disinfection also on dental handpieces.

KEY WORDS: cold atmospheric plasma, bacteria inactivation, disinfection, dentistry, dental equipment

I. INTRODUCTION

Hygiene in hospitals and practices is of major concern due to increasing numbers of bacteria developing resistance against antibiotics and due to decreasing numbers of newly released antibiotics.[1] In the field of dentistry, prevention of contamination and cross-contamination is one of the primary issues.[2] Since handpieces, contra-angles or dental turbines must be reused, appropriate as well as effective and fast disinfection methods are required to avoid the transmission of microorganisms not only from one patient to the other but also from/to the user.

The autoclaving process is a well-established and proven method for disinfection

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and sterilization and used for disinfecting/sterilizing dental equipment. However, there are handpieces, contra-angles or dental turbines that do not withstand the autoclaving process without changes or alterations. It was reported that if the number of autoclaving processes is high, ball bearings in some handpieces are slightly modified.[3,4] In addition, the autoclave treatment is time consuming and therefore cannot be carried out within the pause of patient change. Therefore, alternative methods for disinfecting and/or sterilizing dental equipment are needed.

The use of cold atmospheric plasma (CAP) a partly ionized gas, could function as a possible option for the disinfection/sterilization of dental equipment.[5–8] CAPs consist of charged particles (electrons and ions), excited atoms and molecules, reactive species, and photons. In previous investigations, it was demonstrated that CAPs are bactericidal, virucidal, fungicidal and even sporicidal. The degree of efficacy depends strongly on the used plasma device and the produced plasma.[9–11] These effects demonstrate that many investigations are still needed to identify which of the produced species in the respective plasma are responsible for the reported inactivation mechanisms. Up to now it is believed that the produced reactive species are the main players for inactivating microorganisms.[12] It was furthermore shown that CAPs can be designed in such a way that no damage is induced to healthy tissue (for example see Isbary et al.[13]). This means that it is possible to minimize the damage to materials if appropriate parameters are used for the plasma production.

In this paper, we propose that CAPs are a suitable method for disinfection of dental handpieces, contra-angles, or dental turbines. We investigated the inactivation efficacy on Enterococcus mundtii inoculated on stainless steel in a newly developed circular plasma system incorporating a plasma box with two surface micro-discharge (SMD) electrodes, a treatment chamber, a humidifier, and a pump. The novelty of this circular plasma system is that the reactive species produced in the plasma box are transported into the treatment chamber by passing the narrow channels and cavities of a dental handpiece and are then transported back into the plasma box for recycling. This so-called plasma boosting system confines the plasma species and increases their concentration within the volume of the system.

For investigation of the efficacy of this circular plasma system, the bacterial samples of *Enterococcus mundtii* inoculated on stainless steel were placed in the treatment chamber. Furthermore, the direct inactivation effect for *E. mundtii* inoculated on the outer side of the dental handpiece was analyzed.

II. MATERIALS AND METHODS

A. Circular Plasma System

In Fig. 1, the used experimental setup is shown. The circular plasma system consists of a membrane pump (5 slm), two SMD electrodes, a treatment chamber, and a humidifier. All components are connected by plastic tubes 6 mm in diameter. In this experimental system, the gas is cycled and no gas exchange between the plasma gas and the ambient

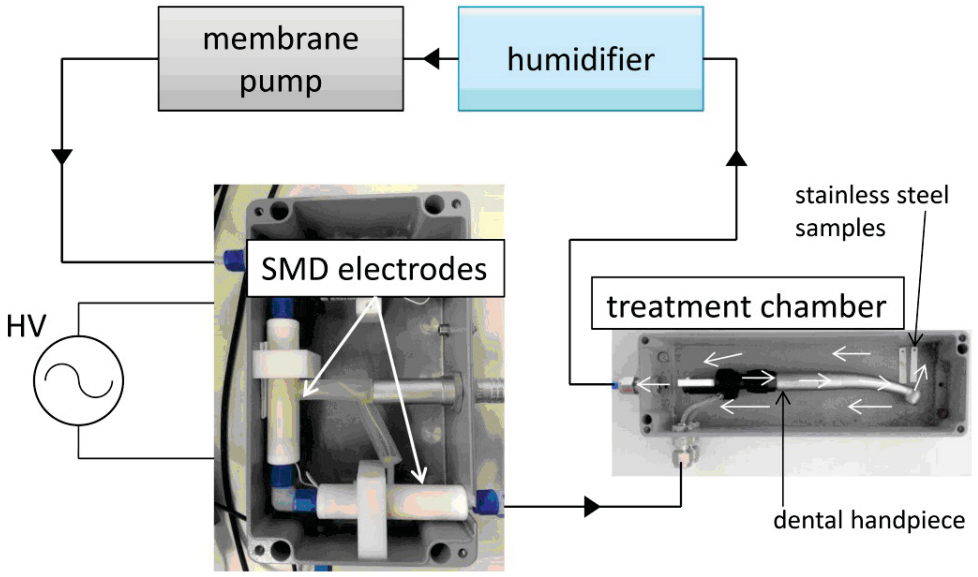

FIG. 1: Sketch of the circular plasma system. The plasma gas is cycled and confined within the system composed of SMD electrodes, treatment chamber, humidifier and pump. Black arrows represent the direction of the gas flow. In the treatment chamber (lower right), the dental handpiece and two stainless steel samples inoculated with bacteria are shown. The white arrows show how the plasma gas flows within the treatment chamber.

air takes place. For the CAP source, two SMD electrodes with a coaxial structure were used. A stainless-steel mesh electrode with a spring shape is covered by a quartz tube 1-mm in thickness. The quartz tube is surrounded by a copper tube. The length and inner diameter of one electrode are 10 cm and 1 cm, respectively. Between the mesh electrode and the copper tube, high voltage of 6.4 kV *pp* at 10 kHz is applied using a high voltage amplifier (model 10/40A, Trek, Inc., Lockport, New York). The SMD plasma is then produced on the side of the mesh electrode. The typical power consumption for two electrodes is ~3.7 W.

As already stated, the plasma gas is transported from the SMD electrodes to the treatment chamber with a size of ~340 cm^3 by a pump. In the treatment chamber, the dental handpiece GENTLEsilence 8000C (Kavo Dental GmbH, Germany) is placed so that the plasma gas has to pass through the channels of the dental handpiece. As shown in Fig. 1 the plasma gas flows through the handpiece and is released at the head of the handpiece. To evaluate the bactericidal efficacy of this newly developed CAP treatment, two stainless-steel (V4A) samples (5 mm–30 mm) inoculated with *E. mundtii* were placed in front of the gas exit of the handpiece. Please note that the plasma gas flows through the small channels of the dental handpiece before it is able to spread in the treatment chamber.

From the treatment chamber, the plasma gas is transported to a humidifier (option-

ally), a gas washing bottle of 500 ml in volume. Using 200 ml of highly purified water (Ampuwa, Fresenius Kabi AG, Germany), the relative humidity in the circular plasma system reached ~90% whereas the ambient atmosphere was ~40%–50%. After passing through the humidifier the plasma gas flows back to the pump. The whole circular plasma system has a volume of ~1300 cm^3.

Due to the size of the setup, the plasma species are not instantly distributed within the whole system. As ozone is one of species produced by SMD plasma, the ozone concentration was quantified as a measure for how fast the plasma species are distributed within the system.[14] For the ozone measurement, a small chamber of 60 cm^3 equipped with quartz windows was inserted in between the SMD electrodes, i.e., the plasma box and the treatment chamber. The ozone concentration was measured by absorption spectroscopy using UV light at 254 nm. The humidifier was also used for the inactivation experiments. As shown in Fig. 2, it takes approximately 5 min to reach a steady state concentration of 800–900 ppm. Because the ozone is not quenched and therefore is present throughout the measurements, the main plasma products produced by these two SMD electrodes are reactive oxygen species.[15]

B. Microbiological Samples

As mentioned, stainless steel was chosen as a carrier material for the bacteria because it is one of the main materials used for dental handpieces or turbines.

Preparation of the bacterial samples, plasma treatment and recovery of bacteria:

1. Preparation of a master suspension using *E. mundtii* (ATCC 43186, DSM 4838)

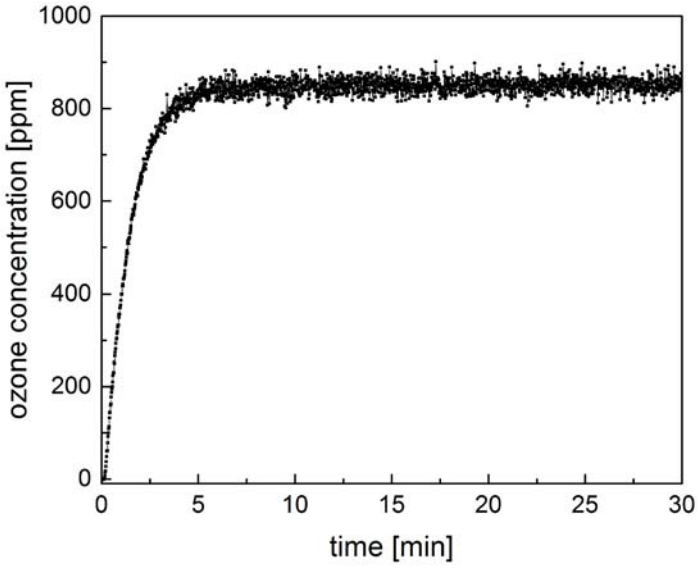

FIG. 2: Time evolution of the ozone concentration in the circular plasma system

in Ampuwa. The density of bacteria was set to ~10^8 bacteria/ml.

2. 10 μl of this master suspension was subsequently spread on a stainless-steel sample as shown in Fig. 3(a) and dried for 30 min in the ambient air under the safety workbench.
3. Immediately after the drying process, the samples were placed in the treatment chamber of the circular plasma system as shown in Fig. 1 for the plasma treatment.
4. The stainless-steel plates inoculated with dried bacteria were positioned in front of the head of the handpiece as shown in Fig. 1. Note that the dental handpiece is directly connected to the plasma gas flow so that the concentration of reactive species inside the handpiece is the same as that in the treatment chamber as soon as the steady state is reached after 5 min (see Fig. 2).
5. After the plasma treatment, the stainless-steel samples were added to 5 ml of Ampuwa and vortexed for 1 min. This was followed by a 30-min ultrasonic treatment and another vortexing period of 1 min.
6. After repetition of point 5, another 5 ml of Ampuwa was added.
7. To evaluate the number of survived bacteria, 100 μl of the recovered suspension was plated on agar plates. The rest of the suspension was filtered, and the filters were placed on agar plates.
8. The agar plates were then incubated for up to 16 hours at 37°C.
9. After incubation, the number of colony forming units (CFU) was counted to evaluate the log reduction.

A similar protocol was carried out for the inoculation of bacteria on the dental handpiece itself. In this case 10 µl of the bacterial master suspension was injected onto the head of the handpiece. As shown in Fig. 3(b), the surface of the handpiece is very hydrophobic. After the injection, the handpiece was placed in the ambient to dry for 30 min under the safety workbench.

According to our test results (Fig. 4), the detection limits in log reduction of the used bacteria were 5 and 4 for the stainless-steel samples and the dental handpiece, respectively.

III. RESULTS AND DISCUSSION

In this study, the inactivation of *E. mundtii* inoculated on stainless-steel plates and dental handpieces using a circular plasma system incorporating two SMD plasma electrodes, a humidifier, a pump and a treatment box was investigated in detail. Figure 5 shows the reduction of *E. mundtii* inoculated on stainless-steel plates and dental handpieces as a function of the plasma treatment time at 90% relative humidity.

For the stainless-steel plates, a 5 log inactivation was reached within 15 min of plasma treatment. Please note that a 5 log reduction represents the detection limit for this experiment. After 15 min of plasma treatment the experimental results showed that not a single bacterium survived the treatment. During the performed experiments, the plasma

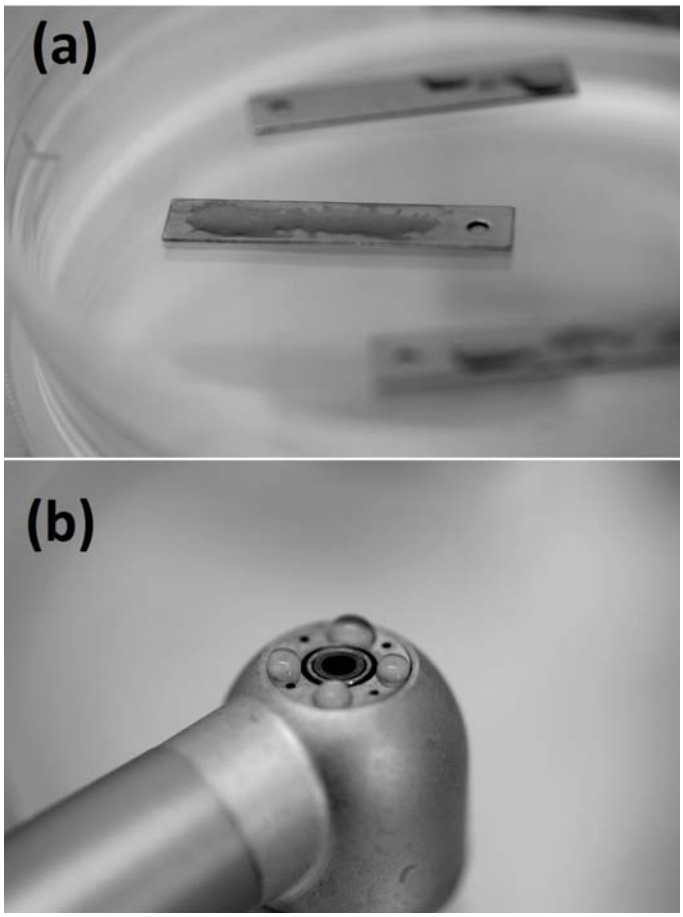

FIG. 3: Bacterial suspension inoculated on a stainless-steel sample (a) and a dental handpiece (b). After inoculation the samples were dried and subsequently treated with plasma.

gas was at room temperature with a relative humidity of ~90 %.

From the survival curve (shown in Fig. 5) three phases can be identified:

1. For plasma treatment times of up to 2 min, no inactivation of *E. mundtii* is observed. This finding agrees with the ozone measurement shown in Fig. 2, where 5 min of plasma treatment is necessary until the ozone is distributed homogenously in the circular plasma system. Thus, it is clear that for treatment times of up 2 min, the concentration of produced reactive plasma species is still too low to inactivate bacteria.
2. The second phase is between 2.5 min and 5 min in treatment time. Within this time frame, the observed inactivation efficacy is relatively fast, with a D value of ~1.5 min.
3. The third phase starts at a treatment time of 5 min. For longer treatment times

the inactivation efficacy is clearly lower, with a D value of ~6.3 min. This large D value or decrease in inactivation efficacy can be explained by inhomogeneous distributions of bacteria on the stainless steel plates. As shown in Fig. 3(a), the bacterial suspension was not distributed fully homogeneously on the stainless-steel plates during the preparation. Thus, the incolculated bacteria can partly form multiple layers on the stainless-steel plates. This stacking of bacteria could lead to a decrease of the inactivation efficacy because the access of the produced plasma species to deeper layers of bacteria is hindered.

For the experiment with dental handpieces, a much lower inactivation efficacy was achieved, as shown in Fig. 5. Within a plasma treatment time of 15 min, less than 2 log inactivation was observed. This can be explained by

1. an increased probability of stacking, i.e., by multiple layers of bacteria due to the hydrophobic nature of the dental handpiece [see Fig. 3(b)];
2. an increased roughness of the dental handpiece, which can lead to lower in activation effects as the rough surface shields the plasma species.[16]

Nevertheless, further experiments on dental handpieces are needed to evaluate the treatment time for achieving the state of disinfection and to understand the factors which influence the degree of in activation.

In summary, the results show that a significant reduction of up to 5 log of bacteria in oculated on stainless steel can be obtained with the circular plasma system within 15 min of treatment time. Based on these results, one can conclude that the produced plasma species are able to pass the small channels and cavities of the dental handpiece in sufficient concentration.

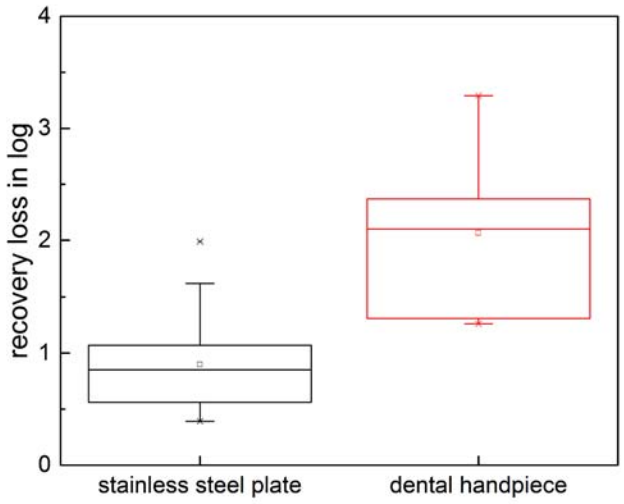

FIG. 4: Recovery loss of *E. mundtii* on stainless steel plates and on dental handpieces

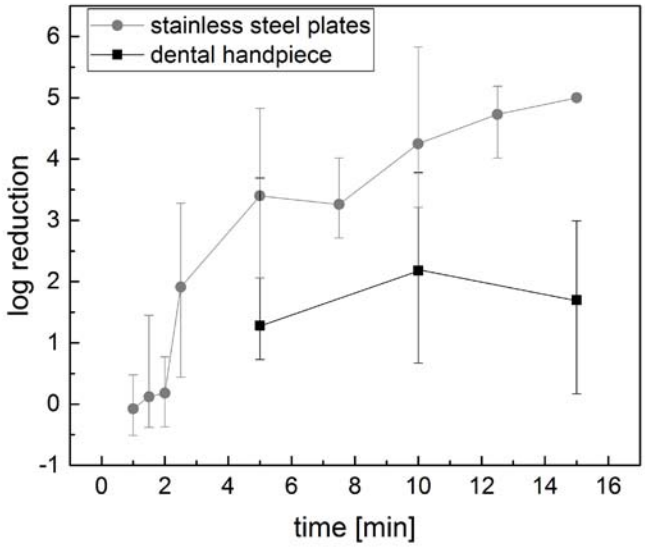

FIG. 5: Inactivation of *E. mundtii* on stainless-steel plates (gray) and dental handpieces (black). The error bars represent the maximum deviation from at least 6 independent measurements.

Figure 6 shows the comparison of the inactivation efficacy of *E. mundtii* on stainless steel using dry air (no humidifier) and humid air (with humidifier). The dry air had a relative humidity of 40%–50%, and in this case the surrounding air was used. The humid air had a relative humidity of 90% and was produced by addition of a humidifier. The data is in accordance with previously published results and clearly show that higher log reductions are obtained for higher humidity.

It is well known that a change in the humidity alters plasma chemistry drastically.[17] It was further reported by Hähnel et al.[18] that a higher relative humidity had a higher inactivation effect on *Bacillus* spores using a dielectric barrier discharge plasma device. Their explanation for the higher inactivation effect was related to the production of hydroxyl radicals.

For our experimental setup, the effect of hydroxyl radicals can be ruled out because the plasma production was separated from the treatment chamber by a certain distance. A possible specie which could explain the higher inactivation efficacy for higher humidites in our setup is H_2O_2. Nevertheless, further research is needed to identify and understand the role of different plasma species and their products in interplay with water in the inactivation of bacteria.

IV. CONCLUSION

In this paper, we investigated the capability of indirect CAP treatment for disinfection

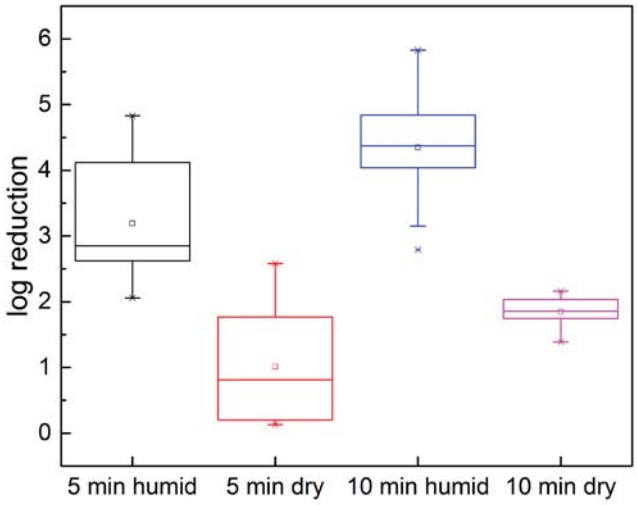

FIG. 6: Inactivation efficacy for two different humidities. Higher humidity results in a higher inactivation effect.

of stainless-steel plates and dental handpieces. The newly developed circular plasma system consists of two SMD electrodes, a treatment chamber, a pump, and a humidifier. Within this circular system, reactive plasma species are produced and transported through the narrow cavities of a dental handpiece into a separate treatment chamber. From this treatment chamber, the species are transported back to the plasma electrode. The plasma species are therefore boosted and confined in the closed volume of the setup. The results on stainless steel showed a 5 log inactivation of *E. mundtii* within 15 min of the plasma treatment. Results on dental handpieces were less promising and showed lower inactivation of up to 2 log within 15 min.

The data obtained within this study show that the plasma species produced by the two SMD electrodes can be transported through small channels and that sufficient plasma specie concentrations are built up in the treatment chamber for achieving fast disinfection of *E. mundtii* inoculated on stainless steel. From this study, we therefore conclude that CAPs could serve as an alternative method for disinfecting small channels, cavities, etc. of dental or other medical equipment (e.g., endoscopes). Nevertheless, further experiments on different surfaces and within small channels and cavities of dental equipment are needed to evaluate the necessary plasma treatment time for achievement of disinfection.

REFERENCES

1. Rampling A, Wiseman S, Davis L, Hyett A, Walbridge A, Payne G, Cornaby AJ. Evidence that hospital hygiene is important in the control of methicillin-resistant Staphylococcus aureus. J Hosp Infect.

2001;49(2):109–116.
2. Weightman N, Lines L. Problems with the decontamination of dental handpieces and other intra-oral dental equipment in hospitals. J Hosp Infect. 2004;56(1):1–5.
3. Worthington L, Martin M. An investigation of the effect of repeated autoclaving on the speed of some dental turbines in general dental practice. J Dent. 1998;26(1):75–77.
4. Nagai M, Takakuda K. Influence of number of dental autoclave treatment cycles on rotational performance of commercially available air-turbine handpieces. J Med Dent Sci. 2006;53(2):93–101.
5. Kong M, Kroesen G, Morfill G, Nosenko T, Shimizu T, van Dijk J, Zimmermann JL. Plasma medicine: an introductory review. New J Physics. 2009;11(11):115012.
6. Laroussi M. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. IEEE Transactions Plasma Sci. 2002;30(4):1409–1415.
7. Stoffels E. Tissue processing with atmospheric plasmas. Contributions Plasma Physics. 2007;47(1-2):40–48.
8. Fridman G, Friedman G, Gutsol A, Shekhter A, Vasilets V, Fridman A. Applied plasma medicine. Plasma Processes Polymers. 2008;5(6):503–533.
9. Morfill G, Kong M, Zimmermann J. Focus on plasma medicine. New J Physics. 2009;11(11):115011.
10. Zimmermann J, Dumler K, Shimizu T, Morfill G, Wolf A, Boxhammer V, Schlegel J, Gansbacher B. Effects of cold atmospheric plasmas on adenoviruses in solution. J Physics D: Applied Physics. 2011;44(50):505201.
11. Klämpfl T, Isbary G, Shimizu T, Li Y, Zim mermann J, Stolz W, Schlegel J, Morfill GE, Schmidt HU. Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. Applied Environmental Microbiol. 2012;78(15):5077–5082.
12. Weltmann K, von Woedtke T. Plasma medicine current state of research and medical application. Plasma Physics Controlled Fusion. 2016;59(1):014031.
13. Isbary G, Morfill G, Schmidt H, Georgi M, Ramrath K, Heinlin J, Karrer S, Landthaler M, Shimizu T, Steffes B, Bunk W, Monetti R, Zimmermann JL, Pompl R, Stolz W. A first prospective randomized controlled trial to decrease bacterial load using cold atmospheric argon plasma on chronic wounds in patients. Br J Dermatol. 2010;163(1):78–82.
14. Sakiyama Y, Graves D, Chang H, Shimizu T, Morfill G. Plasma chemistry model of surface microdischarge in humid air and dynamics of reactive neutral species. J Physics D: Applied Physics. 2012;45(42):425201.
15. Shimizu T, Sakiyama Y, Graves D, Zimmermann J, Morfill G. The dynamics of ozone generation and mode transition in air surface micro-discharge plasma at atmospheric pressure. New J Physics. 2012;14(10):103028.
16. Pavlovich M, Chen Z, Sakiyama Y, Clark D, Graves D. Effect of discharge parameters and surface characteristics on ambient-gas plasma disinfection. Plasma Processes Polymers. 2013;10(1):69–76.
17. Winter J, Wende K, Masur K, Iseni S, Dünnbier M, Hammer MU, Tresp H, Weltmann K-D, Reuter S. Feed gas humidity: a vital parameter affecting a cold atmospheric-pressure plasma jet and plasma-treated human skin cells. J Physics D: Applied Physics 2013;46(29):295401.
18. Hähnel M, von Woedtke T, Weltmann K. Influence of the air humidity on the reduction of Bacillus spores in a defined environment at atmospheric pressure using a dielectric barrier surface discharge. Plasma Processes Polymers. 2010;7(3-4):244–249. Available from: http://dx.doi.org/10.1002/ppap.200900076.