Microbial Inactivation by Electric Discharge with Metallic Grid

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The area of microbial inactivation by the low-temperature plasma produced by DC electric cometary discharge is increased by insertion of an electrically insulated metallic grid between the discharge and the target object. Gram-negative bacteria are almost fully inactivated; an additional zone of incomplete inactivation appears for Gram-positive bacteria and yeasts.

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1. Introduction

The rapidly developing field of plasma decontamination and medical applications was previously reviewed many times, e.g. in [1–5]; a paragraph devoted to action to bacteria is involved also in [6]; recently, a book devoted to this topic was also edited [7]. The non-thermal plasma for this purpose was mostly produced in air by dielectric barrier discharges, gliding arc, plasma torches and various corona discharges; we compared microbicidal properties of various corona discharges in [8]. In other papers [9, 10] we reported a new type of jet-like point-to-point DC electric discharge produced in atmospheric air and named cometary. We also described here the ability of cold plasma produced by this discharge to inhibit the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria on agar plates and on human skin. The cometary discharge is similar to this one produced by devices called plasma jet torch, plasma pencil, or plasma needle, which generate the low-temperature plasma by RF in a stream of carrier gas [11, 12].

In the study [13], the mechanism of decontamination was studied using the grounded mesh electrode inserted between the positive electric discharge and the target sample, here a contaminated agar plate. This electrode (metallic grid) shielded the electric field and trapped the charged particles, but allowed the neutral particles and UV light to reach the agar surface. The experimental arrangement used in this study is similar, except the use of cometary discharge and the inserting of electrically insulated metallic grid.

2. Experimental arrangement

The low-temperature plasma was produced by the device described in detail in [9] and [10]. Briefly, it consists of two needle electrodes connected to the power supply delivering DC voltage variable from 0 to 10 kV. The electrodes were arranged at an angle of 30°, their tips were 9 mm apart and the tip of the positive electrode was shifted 1 mm above the negative one. Under these conditions, the cometary discharge appears between the electrodes at 7.7–10 kV and 30–400 μA. This discharge is a pulseless one, as measured by oscilloscope EO 213 (RFT).

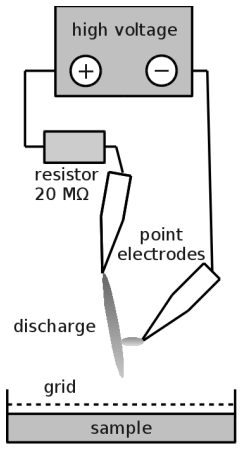

Fig. 1. Schematic arrangement of the device producing the cometary discharge with the grid. Objects are not in scale.

In the device reported here, the electrically insulated metallic grid was inserted between the discharge and the exposed object. The grid consisted of stainless steel wire 0.1 mm in diameter forming the net with a mesh size of 1 mm. The net was mounted into polyethylene collar; four screws used as a stand enabled setting the distance between the grid and the exposed plane, e.g. an agar cultivation plate. This arrangement is drawn schematically in Fig. 1 and depicted in Fig. 2. We also developed a hand-held device, which may be used for exposure of various objects including human skin; its properties and

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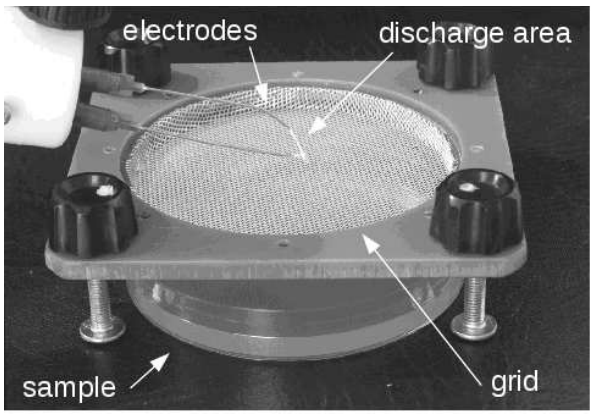

Fig. 2. The arrangement of the device producing the cometary discharge with inserted grid.

abilities are similar to those of the basic arrangement and will be described in detail elsewhere.

During exposure to the cometary discharge, the insulated grid is rapidly charged to the positive electrostatic potential of *ca.* 4–6 kV as measured by Kolbe's electroscope.

3. Methods

The microbicidal effect of the above-described device was stated on microorganisms inoculated on the surface of agar cultivation media, where the inhibition of growth appeared as zones of inhibition. Bacteria *Staphylococcus epidermidis* and *Escherichia coli* and a yeast *Candida albicans* were employed. All microorganisms were "wild" strains isolated from clinical cases at the Institute of Immunology and Microbiology; the same strains were employed also in our previous studies. Microorganisms were suspended in the physiological saline in the concentration of 10^6 cfu (colony forming units) ml^{-1} and 1 ml of these suspensions was spread on the surface of Mueller–Hinton (MH) agar (bacteria) or Sabouraud agar (yeast). Standard Petri dishes of diameter 9 cm appeared in preliminary experiments to be too small to contain the large inhibition zones, so that the large Petri dishes of 19 cm in diameter were inoculated with 1 ml of suspensions and used for quantitative evaluation. The dishes were exposed for 10 min to the cometary discharge at voltage 9 kV and current 200 μA, the distance from the tip of working electrode to the grid was 15 mm, and the distance from the grid to agar surface was 3 mm. The exposed dishes were incubated for 24 h at 37 °C (bacteria) or 48 h at 30 °C (yeast). The areas of inhibition zones in microbial coat were measured manually using the millimeter paper grid. The zones of full inhibition, containing < 1 cfu cm^{-2}, and zones of incomplete inhibition were distinguished. The incomplete inhibition zones, containing more than 1 residual isolated cfu cm^{-2}, appeared as a ring surrounding the zones of full inhibition. Whereas the borders of continual growth were mostly sharply delineated, the borderlines between incomplete and complete inhibition were fuzzy and hard to estimate, thus, were only approximately assessed.

4. Results

The difference in the microbicidal effect demonstrates Fig. 3 on *Candida albicans* as an example. The left and middle dishes were exposed directly to the cometary discharge without grid, whereas the right plate was exposed through the inserted grid. The direct exposure from various distances between discharge and plate surface yielded comparable zone sizes, but the much larger zone of mostly full inhibition, exceeding the area of 9 cm Petri dish, is apparent after grid insertion. An illustrative example of *Staphylococcus epidermidis* exposed on 19 cm plate to the cometary discharge with inserted grid is shown in Fig. 4; the complete inhibition zone is only indistinctly apparent near to the discharge tip.

Fig. 3. Exposure of *C. albicans* on 9 cm dishes to various modes of cometary discharge. Inhibition zones appeared as pale areas within the continual coat of *C. albicans* on the Sabouraud agar. From left: direct exposure to cometary discharge from the distance of 15 mm; the same, but from the distance of 8 mm; insulated grid inserted 15 mm below the discharge. The points on bars indicate the position of discharge tip.

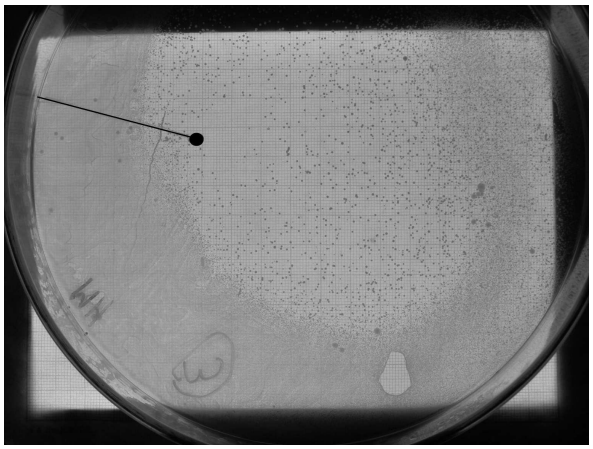

Fig. 4. Exposure of *Staphylococcus epidermidis* on 19 cm dish to cometary discharge with inserted grid. Note the fuzzy border of indistinctly pronounced complete inhibition zone in the vicinity of discharge tip.

TABLE

Areas (in cm^2) of full and incomplete inhibition of microorganisms after direct exposure to cometary discharge and after exposure to the same discharge with inserted grid.

Inhibition	Full	Incomplete	Total
Candida albicans on Sabouraud agar			
direct exposure	1	1	2
with grid	26	21	47
Escherichia coli on MH agar			
direct exposure	25	1	26
with grid	81	1	82
Staphylococcus epidermidis on MH agar			
direct exposure	15	176	191
with grid	73	177	250

The quantitative evaluation of results obtained on 19 cm Petri dishes for *Candida albicans*, *Escherichia coli* and *Staphylococcus epidermidis* is summarized in Table. The figures represent average values from three-time repeated exposures, the parallel results did not differ more than 10%. Due to the small number of experiments, no further statistical evaluation of results was performed.

5. Discussion

The data presented in Sect. 4 show that the inserted grid improves the efficiency of inhibition as measured by the inhibition zone area. Various microbes displayed different sensitivity to the action of cometary discharge: the yeast *Candida albicans* appeared to be less sensitive than bacteria, but displayed the greatest improving of inhibition after the grid insertion. For bacteria, the grid insertion caused three- to fourfold improving of inhibition, flagrant despite the lack of statistical treatment. An interesting phenomenon was the formation of diffuse edges of inhibition zones, i.e. appearance of zones of incomplete inhibition surrounding the zone of full inhibition; this effect was pronounced especially for *Staphylococcus epidermidis* and is probably caused by the different cell wall structure of used microbes.

Recently [14], we studied the sensitivity of various microbes to the action of cold plasma produced under various conditions. Using the same microbial strains cultivated at the same conditions as in the present study, we exposed the microbes on an agar surface to the negative corona discharge: the yeast appeared to be most sensitive and *E. coli* and *S. epidermidis* displayed comparable but lesser zones and thus a lower sensitivity to produced plasma. On the contrary, exposure of these microbes in aqueous suspension revealed the highest sensitivity of *E. coli*, medium sensitivity of *S. epidermidis* and the lowest sensitivity for *C. albicans*; the complete inhibition of these organisms occurred after *ca.* 75 s, 4 min, and 30 min, respectively. The sensitivity of the same organisms to the cometary discharge described here increased in the order *C. albicans* $<$ *E. coli* $\leq$ *S. epidermidis* as ordered according to full inhibition zone areas. Nevertheless, the Gram-positive *S. epidermidis* exhibited the additional large zone of incomplete inhibition, negligible at Gram-negative *E. coli*. All these observations imply different mechanisms of inhibition in different organisms, thus, a different nature of particles produced and acting in different discharges and in different ambient medium (i.e., in air or water). The cell walls erosion and/or lysis (disruption) of cells proved by scanning electron microscopy (SEM) micrographs is one of bacterial inactivation mechanisms. Hence, the thick peptidoglycan cell wall of *S. epidermidis* may play a role in its partial protection from reactive particles of cold plasma, but other mechanisms are undoubtedly also significant (cf. [4]). The above-mentioned difference between the effect of negative corona and cometary discharge is probably caused by the opposite polarity of these discharges, because the comet is in fact a positive discharge.

Many authors studied the mechanism of inactivation and sterilization action of low-temperature plasmas. In general, this effect is mediated by ionic and non-ionic reactive particles produced in air, in lesser extent also by UV radiation. The reactive particles may be unstable and short-lived ions and radicals, namely atomic oxygen O, superoxide $O^{2\bullet-}$, $OH^{\bullet}$, OH^{-}, $O_2H^{\bullet}$, $^1O_2$ and others, as well as stable molecules as ozone O_3, hydrogen peroxide H_2O_2 or nitrogen oxides NO_x. The nature of reactive particles in low-temperature plasmas was extensively studied many times (see, e.g., [15] or [16]), modeling and simulation of low-temperature atmospheric pressure plasmas in interactions with living cells was reviewed in [17]. Recently, the nature and bactericidal action of various active particles in low-temperature plasma was discussed, e.g., in [18–20] or [21]. In our still unpublished study, we compared the action of corona discharge and dielectric barrier discharge (DBD) on microbes: at comparable conditions, we observed that the inactivation effect of corona discharge is more powerful to bacteria in water suspension, whereas the DBD acts better on bacterial layer onto the agar plate. Despite all these (sometimes controversial) findings, it seems that the mechanism of inactivation depends strongly on the experimental arrangement and conditions.

The cause of enlarging the effectively exposed area is not clear, but the scattering of the reactive particle beam on the grid may be a possible explanation. An additional effect may also be the accumulation of charged particles on the grid, which is charged to the electrostatic potential of 4–6 kV. The rest of particles are carried of by ion wind to the target, whereas the charged grid acts as a secondary electrode with discharges in its nodes distributed uniformly over the exposed area. These secondary discharges are not visible and weaker than the primary beam, but their synergic action may be sufficient for causing the microbial inhibition. The weaker effect of secondary discharges may explain the formation of zones of incomplete inhibition, apparent mainly in *Staphylococcus epidermidis*.

6. Conclusion

Insertion of an insulated metallic grid between the cometary discharge and the target object increases the area where microbes are inactivated by the low-temperature plasma. Almost complete inactivation occurs for Gram-negative *Escherichia coli*, whereas a large zone of incomplete inactivation appears for the Gram-positive *Staphylococcus epidermidis*. Yeast displays somewhat lesser but still good inactivation.

Acknowledgments

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