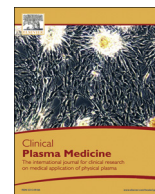




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Decontamination of human skin by low-temperature plasma produced by cometary discharge

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ABSTRACT

Low-temperature plasma produced by DC cometary discharge suppresses bacteria on live human skin of the inner arm side and fingertips. In model experiments, this discharge in open air completely suppressed *Escherichia coli* within 8 min, whereas *Staphylococcus epidermidis* was markedly lowered, but not completely eliminated even after 10 min. After inserting an insulated grid and exposure in an enclosed chamber, *E. coli* was completely suppressed within 2–4 min and *S. epidermidis* within 10 min. This difference suggests the demand to adapt the European Standard describing the disinfectants and antiseptics test method. Similar results were obtained also for natural human skin bacterial microflora, which was completely quenched after 6–10 min exposure to discharge with the inserted grid acting in an enclosed chamber.

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1. Introduction

The rapidly developing field of plasma decontamination and medical applications has previously been reviewed by several authors, e.g. by Laroussi [1], Moreau et al. [2], Kong et al. [3], Laroussi [4], Ehlbeck et al. [5] or Isbary et al. [6]; recently, a book devoted to this topic also appeared [7]. Various reactive particles present in non-thermal plasma, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), were identified as the disinfectant agents; for a review, see [8]. To this end, the plasma was mostly produced in air by dielectric barrier discharges, gliding arc, plasma jets and various corona discharges. In previous research, we compared microbicidal properties of various modes of corona discharges [9,10]. In other papers [11,12], we reported a new type of jet-like point-to-point DC electric discharge produced in atmospheric air and named the cometary discharge. Here, we describe the ability of cold plasma produced by this cometary discharge to inhibit the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria on agar plates. The cometary discharge is similar to that produced by devices called plasma jet, plasma torch, plasma pencil, or plasma needle, which generate low-temperature plasma by radio frequency (RF) discharge in a stream of auxiliary carrier gas [13–16].

The efficiency of cometary discharge may be improved by inserting an electrically insulated metallic grid between the discharge and the exposed object as we reported in [17]. The use of an inserted grid (mesh) was previously reported also by Machala et al. [18], Dobrynin et al. [19] and Timoshkin et al. [20]. In contrast to our arrangement, these studies used the mesh as a grounded auxiliary electrode trapping charged particles (ions) and allowing only neutral particles to reach the target.

The human skin is colonized by a wide variety of bacteria, among which the Gram-positive ones prevail. *S. epidermidis* is the main species, along with other staphylococci, micrococci, *Sarcina* spp., corynebacteria etc.; Gram-negatives, anaerobes and fungi occur, too. Their occurrence and prevalence differs substantially among individuals. Decontamination of skin is important in many medical applications, e.g., in manipulations with blood, where bacterial contamination chiefly results from the resident skin flora [21]. Several methods are available for this purpose, relying on various chemical disinfectants and antiseptics. Validation of new antiseptic must meet the requirements of the European Standard [22]. Attempts to employ low-temperature plasma as a skin antiseptic have also been reported. For example, in model experiments pig skin [23,24] or excisions of skin tumors [25] were exposed to dielectric barrier discharge, plasma jet or surface micro discharge. These studies reported no plasma-induced damage of epidermal and dermal layers. That there was no risk of plasma constituents for humans was also documented by Lademann et al. [26]. Preliminary reports concerning this topic have also appeared also in symposia proceedings [27]. An attempt do decontaminate live human skin on fingertips was recently published by

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Daeschlein et al. [28]. After treatment with a plasma jet or dielectric barrier discharge, the maximum reduction of physiological bacterial microflora was observed after exposures of 210 and 60 s, respectively. The exposure time was 120 and 90 s, respectively, when applied to fingertips artificially contaminated with *S. epidermidis* and *Micrococcus luteus*.

In this paper, we describe the decontamination of live human skin colonized with physiological microflora as compared with the same effect on artificially contaminated skin using DC cometary discharge. Preliminary results were published previously in [29].

2. Materials and methods

2.1. Apparatus

The device producing DC cometary discharge and generating the low-temperature plasma was described in detail in [11,12]. Its improved arrangement containing an insulated grid [17] was also used. Patents [30,31] cover both arrangements. Briefly, cometary discharge is produced between two needle electrodes connected with a stabilization resistor of 10 M Ω to a power supply delivering DC voltage variable from 0 to 10 kV. The electrodes are arranged at an angle of 30°, their tips are 9 mm apart and the tip of the positive electrode is shifted 1 mm above the negative one (see Fig. 1). At 7.7–10 kV and 30–400 μ A, a jet discharge resembling a comet's tail appears between the electrodes. In this study, the voltage was adjusted to 9 kV, the corresponding current was 150 μ A. Various adapters, drawn schematically in Fig. 2, were fitted on the Teflon head of the device body, enabling the following modes of exposure:

- cometary discharge—the electrodes were covered by the plastic safety cover with ventilation openings and the target objects were exposed directly by the discharge jet at ambient air atmosphere (Fig. 2-1).
- with grid—an electrically insulated metallic grid made of the stainless steel net with a mesh size of 1 mm and fixed in a plastic adapter, was inserted between the comet and target objects exposed in a closed chamber (Fig. 2-2). However, in this arrangement free space remained between the adapter border and fingertips.
- with grid and mask—used for exposure of fingertips. An additional mask was inserted between the grid and the exposed fingertip, making a closed chamber of exposure (Fig. 2-3). The distance between the cometary discharge tip and the grid was 1 cm. The same distance was maintained between the discharge and the exposed object, or the grid and the object, respectively. However, these distances were set only approximately because of the concavity of flexible skin.

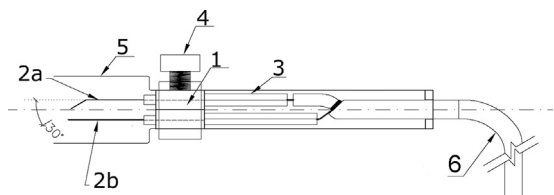


Fig. 1. Scheme of the hand-held device producing the cometary discharge. 1: body of the device (Teflon head); 2a: positive electrode; 2b: negative electrode; 3: holder; 4: screw regulating the electrode distance; 5: additional adapters; 6: coaxial cable to the power supply.

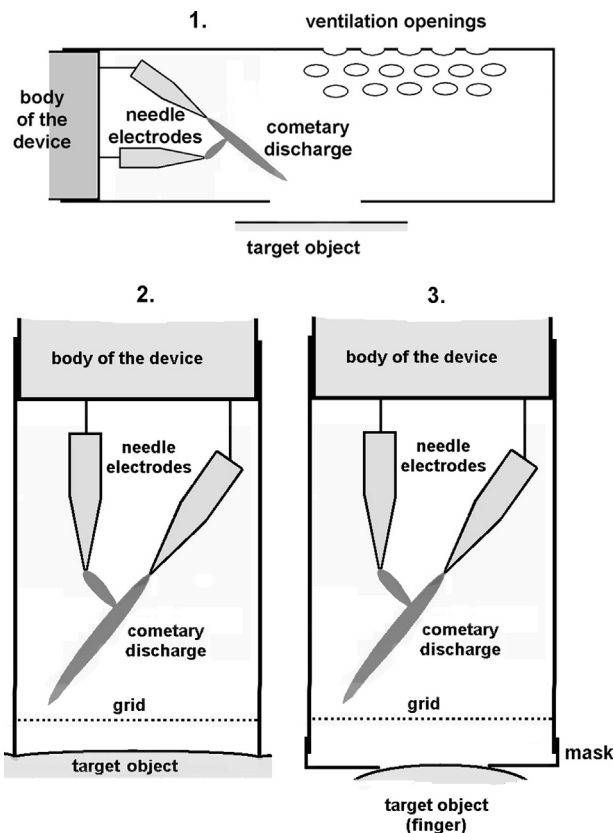


Fig. 2. Adaptors for exposure with cometary discharge: 1. open-air discharge; 2. discharge with a grid; 3. discharge with a grid and a mask.

2.2. Bacterial cultures

S. epidermidis and *E. coli* bacteria were employed, both were “wild” strains isolated from clinical cases at the Institute of Immunology and Microbiology. They were incubated on Mueller–Hinton (MH) nutrient agar for 24 h at 37 °C, a loopful of bacterial biomass was harvested, suspended in physiological buffered saline (PBS) and their concentration adjusted to approx. 10⁶ cfu (colony forming units) ml⁻¹. These suspensions were used in experiments with artificially contaminated skin.

2.3. Artificially contaminated skin

These experiments were performed according to the European Standard [22] with slight modifications: The examined skin area of experimental persons was decontaminated with commercial disinfectant Spitaderm[®] (0.50 g chlorhexidylgluconate and 0.45 g hydrogen peroxide in 70 g of isopropyl alcohol). The disinfectant was carefully washed off with ethyl alcohol. After drying, the fingertips were immersed into the bacterial suspension and after drying they were imprinted (blotted) by pressing for 10 s against the surface of MH agar in a Petri dish (as in forensic fingerprints). In addition to *E. coli* required by the European Standard [22], *S. epidermidis* was also used as a tested bacterium. A different technique, adopted according to recommendation in [32], was used for taking samples from inner arm side skin, namely palm (*vola manus*), wrist (*carpus*), forearm (*antebrachium*) and inner elbow (*fossa cubitalis*). In these cases, the skin was contaminated using a swab immersed in bacterial suspension. For taking imprints, molten MH agar was aspirated into a 20 ml plastic syringe and after cooling and solidification of the agar, the syringe tip was cut off. 5 mm of agar was extruded from the syringe, the surface of the agar column was then pressed for 10 s against the

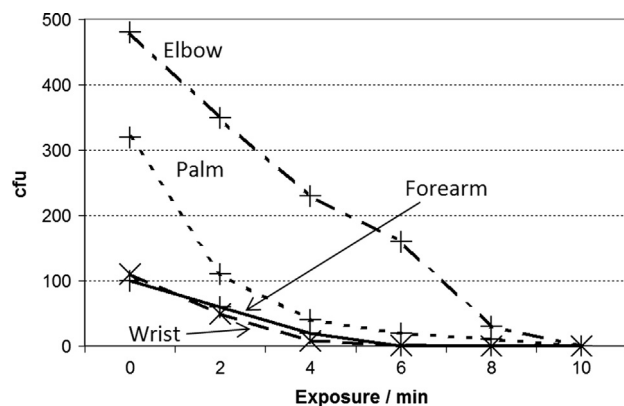


Fig. 3. Time dependence of skin physiological microflora decrease during exposure to cometary discharge with a grid (a typical example).

examined skin, a slice was cut by a sterile knife and placed in an empty Petri dish. The area of agar slices was 3.5 cm². These experiments yielded control numbers of colony forming units (cfu), the same technique was used for imprinting of skin exposed to low-temperature plasma. After blotting, the Petri dishes and slices were incubated as above and the number of colonies (cfu) was counted manually under a magnifying lens. A dense and almost continual bacterial growth with overlapping colonies sometimes appeared; in these cases, the number of cfu was evaluated as 1000.

2.4. Physiological microflora

The same methods and experimental parameters as mentioned above were used for taking samples from the skin covered with untreated natural microflora. The authors were experimental persons in all cases working on their own risk, so that no approval of experiments was asked.

2.5. Exposure

Artificially contaminated or physiologically colonized skin areas were exposed to low temperature plasma produced by the methods described in the Apparatus section. The tip of first finger (thumb) was exposed for 2 min, the second for 4 min, the third for 6 min, the fourth for 8 min and the fifth for 10 min. The sites of the inner arm skin were exposed consecutively for 2, 4, 6, 8 and 10 min, and imprints were taken from the same site after each exposure interval. Due to the variability of initial cfu numbers, imprints were taken from pertinent sites before exposure and used as individual controls (100%) for each site and exposure. The cfu numbers quoted in Supplementary data tables represent a rounded average from triplicate reproduction, from which the mean percentages were calculated. Due to a large dispersion of controls, no further statistical treatment was performed.

3. Results

3.1. Artificially contaminated skin

The results of all artificially contaminated skin areas decontamination acc. to the European Standard [22] are shown under Supplementary data, Tables S1-S12. It is apparent that the complete disappearance of *E. coli* occurred after 6–8 min of exposure to cometary discharge at ambient atmosphere (Table S1), and after 4–6 min of exposure with an inserted grid (Table S2). Exposure of skin contaminated with *S. epidermidis*, which is not required by

the Standard, showed, that *S. epidermidis* did not disappear even after 10 min of exposure to open air discharge. Nearly complete disappearance occurred after 10 min of exposure to discharge with a grid. The residual cfu numbers of *S. epidermidis* at lower exposure times were also markedly higher than for *E. coli*.

Similar results were obtained also for fingertips. Exposure to open-air discharge (Table S3) reduced the number of *E. coli* cfu to zero within 8 min, but considerable amounts of *S. epidermidis* remained even after 10 min. Exposure to discharge with a grid (Table S4) quenched *E. coli* to zero after 4 min, whereas *S. epidermidis* needed 10 min for the same effect. After exposure to discharge with grid and mask (Table S5), *E. coli* disappeared already after 2 min, but *S. epidermidis* took 6 min.

3.2. Physiological microflora

The variability of natural skin microflora is documented under Supplementary data, Table S6, where the counts of bacteria taken from various body sites of one experimental person in one-day intervals are shown. For example, consecutive imprints of the inner elbow (*fossa cubitalis*) yielded from 220 to 520 cfu (mean value 340 cfu, standard deviation 128). Lower cfu numbers were found on other sites, but with similar dispersions. This expresses the quantitative differences in skin colonization of the experimental person. On the other hand, only slight decrease (if any) of bacterial number was observed in consecutive imprints taken from the same site in 10 min intervals (Table S7).

The time dependence of the physiological skin microflora decrease during exposure to cometary discharge with a grid is illustrated in Fig. 3. The detailed results are documented in Tables S8–S12 as a percentage of residual bacteria. In the case of a skin exposed to open-air cometary discharge (Table S8), complete disappearance of bacteria was not achieved, except for palm exposure. After exposure to cometary discharge with a grid (Table S9), bacteria disappeared after 8–10 min of exposure. On fingertips, exposure with open-air discharge (Table S10) and discharge with a grid (Table S11) retained some living bacteria even after 10 min exposure, although the number was considerably lower in the second case. During exposure with cometary discharge with a grid and a mask (Table S12), a complete disappearance of bacteria was achieved after exposure for 6 min.

4. Discussion

The examination of natural skin microflora is affected by its variability both among persons and in one human being. The cfu number is usually higher in the crinkle of inner elbow, as well as on the thumb, where it is caused by the greater area of its tip as compared with other fingers. From the data given under Results, it follows that it is necessary to take individual control cfu numbers for each exposure experiment in order to quantitatively express the decrease in the bacterial count. This makes it difficult to evaluate the time course of bacterial suppression among different persons and/or different body sites. Nevertheless, a complete quenching of bacteria on the particular body site occurred at reproducible exposure times. The time to reach sterility seems to be dependent on the initial bacterial count; it is conspicuous namely in the densely populated *fossa cubitalis*.

It may be concluded that cometary discharge may be a suitable source of low-temperature plasma for skin decontamination. Although the decontamination was studied previously on various skin excisions (see Section 1), we did not find comparable results obtained on live human skin except [28]. In this work, the well-established plasma jet and dielectric barrier discharge were used and beside the shorter exposure times, the observed effect was

