Plasma Medicine, 3(1–2): 137–152 (2013)

Experimental Evidences on Synergy of Gas Discharge Agents in Bactericidal Activity of Nonthermal Plasma

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ABSTRACT: Nonthermal plasmas (NTPs) represent a new class of sterilizing agents. A full set of NTP bioactive factors includes electric components (E, charged particles and electric field), neutral active particles (R), and UV. A specific construction of the direct current (DC) corona source was used that allowed dissection of NTP bioactive factors and quantitative evaluation of their individual and combined action on bacterial pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. 10 and 120 s were required for the positive and negative coronas, respectively, to kill 10^5 colony-forming units (CFU) *P. aeruginosa*. 10 s was required for both positive and negative coronas to kill 10^5 CFU *S. aureus*. For the positive corona, the bactericidal activity of components decreased as R+UV>E+UV>>UV and E+UV>R+UV>>UV for *P. aeruginosa* and *S. aureus*, respectively. For the negative corona, the bactericidal activity decreased as R+UV>>E+UV=UV=0 and R+UV>>E+UV>UV≈0 for *P. aeruginosa* and *S. aureus*, respectively. Despite low, if any, activity of electric components in the negative corona, the whole plasma effect was much higher than the effect of neutral particles and UV alone. The obtained results demonstrated that different combinations of bioactive plasma components exerted diverse species-specific bactericidal effects. It is synergy among plasma bioactive factors that supplies high bactericidal activity of NTP.

KEY WORDS: nonthermal plasma, sterilization, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

I. INTRODUCTION

Nonthermal atmospheric pressure gaseous plasmas represent a new class of disinfection and sterilization techniques, which are applicable to heat- and chemical-sensitive surfaces including biological tissues.[1–5] The nonthermal plasmas (NTPs) of medical importance are formed at atmospheric pressure and have a temperature close to ambient. Microbicidal activity of NTPs is unspecific: Gram-positive and Gram-negative bacteria, fungi, and spores were shown to be sensitive to NTP.[6–10] Bacterial spores are more resistant to NTP while a relative sensitivity of other microorganisms is dependent on the construction of a plasma source, gas composition, and unidentified intrinsic features of

the microorganisms.[8–12] The ability to achieve microbicidal effects without damaging human tissues enables NTP to be applied directly for disinfection of the skin, mucous, and wound surfaces.[13–16]

The major distinguishing feature of NTP as a sterilizing agent is high microbicidal efficiency at relatively low concentrations of short-lived toxic compounds.[2] In general, active agents of NTP include charged particles, neutral active species, UV, and electric field. Synergy among plasma active compounds was suggested to be an important factor that contributes to total plasma microbiocidal effect.[2,17,18] Nevertheless, experimental evidences of the synergy are still scarce.[19–21]

In this work, positive and negative direct current (DC) coronas in the ambient air were used. Steady-state coronas were produced in the gap between the sharpened metallic pin of a small diameter and the conductive plane (Fig. 1). The DC coronas generate the same set of bioactive agents as "in volume" NTPs: both charged and neutral active particles, electric field, and UV. For a negative corona, generation of the bioactive agents is located only in a small area at the vicinity of the tip of a pin where a strong electric field is created. Another situation takes place in the positive corona where bioactive agents are generated throughout the volume between the pin and the flat electrode. This difference is due to streamers that are present in the positive corona. The streamer is a thin plasma filament (about 100–200 microns in diameter), originating from the pin and rapidly elongating in the direction of a flat electrode. Streamers are randomly distributed in the interelectrode gap and are characterized by a repetition rate up to several kilohertz. The electric field strength is low in the plasma body of streamer but very high in its head. It is the streamer head that is responsible for generation of bioactive agents not only at the pin but everywhere in bulk of the positive corona.

We applied a specific construction that allowed dissection of charged and neutral particles and quantitative evaluation of their individual and combined action to demonstrate that it is synergy among different plasma components that supplies the high bactericidal activity of the DC discharge. The Gram-negative bacterium *Pseudomonas aeruginosa* and Gram-positive bacterium *Staphylococcus aureus* were the objects of our research, because these two pathogens are the most common causative agents of nosocomial infections with high incidence of resistance to antibiotics and ability to colonize abiotic and biological surfaces.[22,23] They demonstrated differed sensitivity that was in line with suggested mechanisms of the synergetic action of the plasma active components.

II. MATERIALS AND METHODS

A. Bacterial Strains

The *Pseudomonas aeruginosa* strain PA103 (commercially available) and the meticillin-resistant *Staphylococcus aureus* (MRSA) strain Sa78 from the collection of Gamaleya Research Institute were used in the study.[8] Bacteria were routinely cultivated on LB (*P. aeruginosa*) or brain heart infusion (BHI) (*S. aureus*) agar at 37°C.

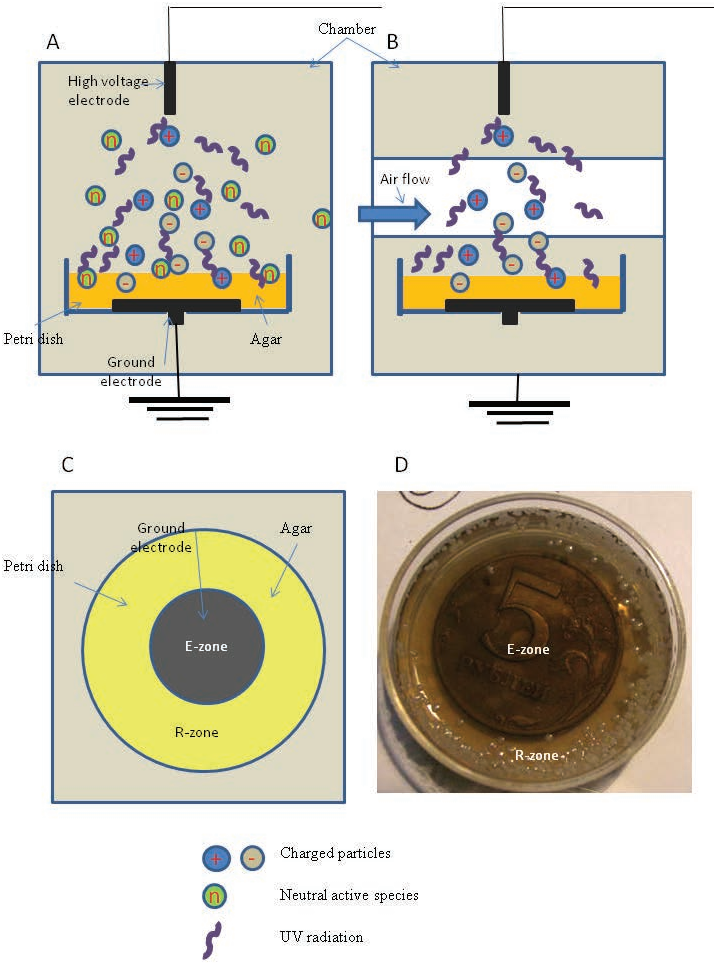

FIG. 1: The construction of The DC corona source used to dissect bioactive plasma components (see details in the text). (A) the isolated chamber; (B) the blown chamber; (C) the scheme of the Petri dish with the ground electrode with the functional E- and R-zones; (D) the top view of the bacterial culture, which was plated on the Petri dish with the ground electrode, treated with the positive DC corona in the isolated chamber for 30 s and then grown for 24 h; note the difference in the amount of surviving bacteria in the E- and R-zones.

B. The DC Corona Source

The DC corona source included a high-voltage rod electrode and a ground flat electrode placed in a Plexiglas cylinder 50 mm high and with a 60-mm inner radius (Fig. 1). The high-voltage electrode consisted of seven metal needles. The ground electrode was a flat thin disk with a diameter of 25 mm and a thickness of 1 mm. It was placed on the bottom of a 36-mm Petri dish and covered with an agar layer. The distance between the tip of the high-voltage electrode and the flat electrode was 15 mm. The Petri dish had

a 5-mm perforation in the bottom to supply contact of the electrode with the electric chain (Fig. 1). The electrode-carrying perforated Petri dishes were made manually and sterilized with UV for 2 h before the nutritive agar was poured into them. The Plexiglas cylinder had holes that could (i) be closed to isolate the chamber from the environment and allowed accumulation of neutral active particles or (ii) be connected to the air source to supply constant air flow through the chamber and to prevent accumulation of neutral active particles. The positive or negative voltage was applied to the rod electrode. The distance between electrodes was chosen in such a way as to minimize edge effects and to make the electric current flow through agar that covered the ground electrode but not through the agar outside of the electrode.

C. Bacterial Treatment and Assessment

Bacteria were grown overnight in the LB or BHI broth, for *P. aeruginosa* and *S. aureus*, respectively, pelleted by centrifugation, washed with PBS, diluted in the same volume of PBS; 30 μl of decimal dilutions ranging from 1×10^2 to 1×10^5 CFU ml^{-1} were plated on electrode-carrying Petri dishes. Plated bacteria were subjected to DC discharge during time intervals from 10 to 300 s. The treatment conditions were chosen to make equal electric currents and the power applied for positive and negative DC discharge. The electric current was 300 μA and the power was about 4 W. Treated bacteria were incubated 24–48 h at 37°C.

Colonies were counted separately for the zone above the electrode (a zone of charged particles; see the Results section) and the zone outside of the electrode (a zone free of charged particles; see the Results section). The area covered with the electrode was about one half of the total Petri dish area (4.9 cm^2 vs 10.2 cm^2) so when colonies were grown uniformly all over the agar, the total number of colonies per plate was halved to calculate the amount of bacteria for each zone. The bactericidal effect was evaluated as a ratio of the amount of surviving CFUs at the corresponding zone to the initial bacterial load for this zone.

D. The Plasma Emission Spectrum Measurements

Figure 2 shows a sketch of the optical scheme for the registration of the UV emission from the corona interelectrode gap. Corona (1) is generated between pin and metal plate separated by a 15-mm gap. Working gas was ambient air. The UV radiation produced by the corona was measured by the method of emission spectroscopy. The optical scheme consisted of a quartz lens (2) with a focal distance of 112 mm, and a monochromator MDR-6 (3) equipped with photomultiplier FEU-100 (4) connected to a PC (5).

To measure the emission intensity distribution along a discharge axis, the electrode system was moved parallel to the discharge axis. A one-to-one image of the object was projected onto the monochromator; the entrance slit played the role of an aperture. The focal depth of our optical system allowed us to collect the light predominantly from the discharge volume of about $2.5\times2.5\times2.5$ mm^3. This volume determines a spatial resolu-

tion in axial distribution of the light emitted by the corona. The size of the entrance slit was 2.5 mm. The spectrum was relatively calibrated but not corrected for the cosine profile of the aperture because we determined the axial distribution, but not the radial one.

The spectra of ultraviolet (UV) radiation in the range of $200 \leq \lambda \leq 300$ nm were recorded with a monochromator MDR-6. UV radiation of coronas is weak in this spectral range. Therefore, we used a highly sensitive photomultiplier (PMT) FEU-100 and signal accumulations over a long time. The spectral resolution is 0.5 nm. The discharge current was measured by an Ampermeter M-2044; the accuracy of measurement is 2 μA. The voltage drop across the discharge gap was measured by a kilovoltmeter C-196; accuracy of measurement is 0.2 kV. Discharge current and PMT waveforms were recorded by a digital oscilloscope, Tektronix TDS-2012. The following parameters were used to measure emission spectra: The discharge current was 200 μA, the voltage at the discharge was 13.3 kV, and the spectral resolution of the monochromator was 0.5 nm. The radiation was collected in a transverse direction to the discharge axis from small volumes located at different distances from a corona electrode.

E. Statistics

All experiments were performed using duplicate samples and repeated at least three times. The mean values and standard errors were calculated with Excel software, a part of the Microsoft Office 2007 package. The paired *t*-test included in the same software was used for assessment of statistical significance.

III. RESULTS

A. Separation of the Plasma Components

The construction of the DC corona plasma source allowed separation of plasma active components as it is described below [Figs. 1(A)–1(D)]. The flat ground electrode was put on the bottom of a Petri dish and covered with a layer of agar. As far as agar has conductivity, the electric current flowed through bacteria that were plated directly above the flat ground electrode and therefore were affected by charged particles and electric field. The zone above the flat electrode was designated as E-zone [Figs. 1(C) and 1(D)]. A zone outside of the area covered with the ground electrode was free of charged particles and electric field and designated as an R-zone. Areas of the E- and R-zones were nearly equal, 4.9 and 5.2 cm^2, respectively.

Besides charged particles, neutral active particles and UV affected plated bacteria. When the chamber was isolated from the environment, the neutral active particles were accumulated uniformly in the bulk of the chamber [Fig. 1(A)]. Then, both E- and R-zones were subjected to the action of neutral particles in the isolated chamber. Analysis of the composition of reactive species generated by positive and negative corona in air was done in our previous paper.[24] When the chamber was blown through with a constant air flow, accumulation of neutral particles was prevented [Fig. 1(B)]. The air flow did not change

the parameters of the electric current. Therefore, concentrations of charged particles, which composed the electric current, were similar for the isolated chamber and the blown chamber. In the blown chamber, both E- and R-zones were free of neutral particles but the E-zone was still affected by charged particles and the electric field. The UV irradiation was similar for both E- and R-zones in isolated as well as in blown chamber conditions. Therefore, depending on whether the chamber was blown or not, bactericidal effects of active plasma components might be assessed in the following combinations: total plasma (an isolated chamber, the E-zone); neutral active particles and UV (an isolated chamber, the R-zone); electric components (charged particles and electric field) and UV (a blown chamber, the E-zone); UV only (a blown chamber; the R-zone).

B. Characterization of UV Emission by the Positive and Negative Coronas

In the negative corona, UV radiation was detected from the pin electrode area only. The average number of UV photons in the negative and positive coronas from the pin area is nearly equal. In contrast, the positive corona was characterized by high levels of UV emission from the whole of the electrode gap due to the presence of the streamers in the interelectrode gap. The current oscillogram and the PMT waveform at a wavelength of 236 nm for positive corona are shown in Fig. 2.

Figure 3 shows the emission intensity distribution at a wavelength of 236 nm along the axis of the positive corona. Reducing the level of the signal with an increase of the distance from the pins is associated with the geometric expansion of the beam of streamers. In our previous paper[24] we have done an estimation of the intensity of UV radiation

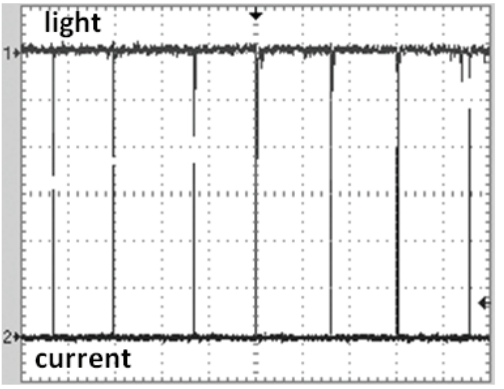

FIG. 2: Time correlation between pulses of light and electric current in a positive streamer corona in air (pumping through discharge chamber is absent). The interelectrode gap is 15 mm. Average discharge current was 200 μA, discharge voltage was 13.3 kV. (1) PMT signal (arbitrary units) recorded for the light emitted in spectral line λ=236 nm. The radiation was collected from the whole discharge volume; time scale *T*=100 μs/div. (2) Current pulses. Each of them corresponds to the shunting of the gap by single streamer. *I*=40 mA/div, time scale *T*=100 μs/div.

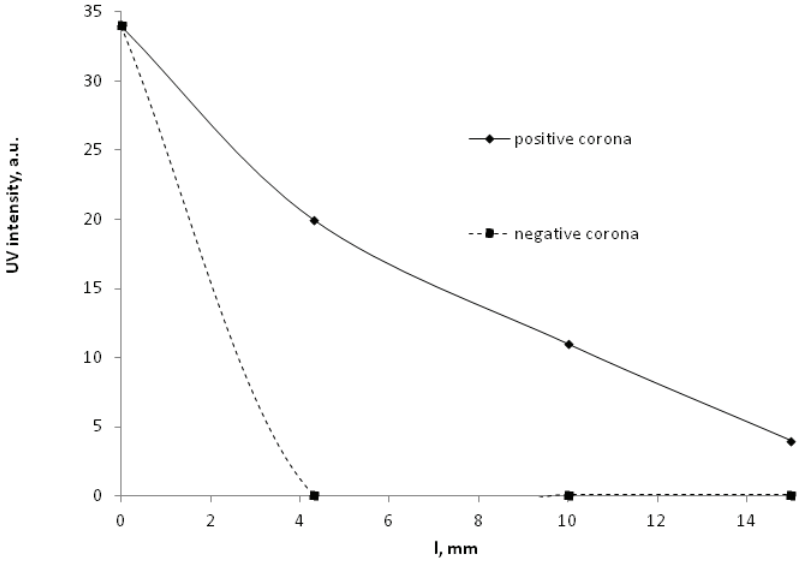

FIG. 3: Distribution of the emission intensity at the wavelength of 236 nm along the axis of the positive corona. Gap was 15 mm. Mean current was 200 μA.

for E- and R-zones. As it turned out, the average intensity for E-zone exceeds that for R-zone twice in a negative corona and approximately three times in a positive corona.

The experimental spectra of positive and negative coronas in the range of $200 \leq \lambda \leq 300$ nm were measured at a small area near the corona electrodes (Fig. 4). The spectra consisted of bands characteristic for the excited molecular nitrogen and the NO and OH radicals. The spectra were normalized to the signal amplitude of molecular nitrogen to show the ratio of NO peaks in the positive and negative coronas [Fig. 4(B)].

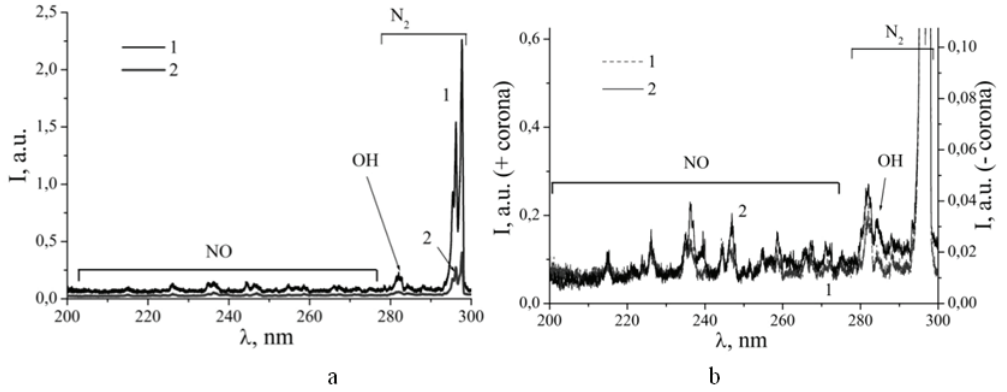

FIG. 4: The spectra of the positive (1) and negative (2) coronas in the air. (A) relative intensities of emission in the positive and negative coronas. (B) zoomed spectra normalized to the signal amplitude of molecular nitrogen demonstrated different shapes in NO emission between positive and negative coronas.

Figure 5(A) demonstrates a significant difference in the intensity of UV radiation near the electrode tip in the positive and negative coronas. One can suggest that the difference increased near the agar surface because UV radiation in the negative corona was spreading from the tip and diminished with the distance, while both the electrode tip and streamer heads were sources of UV radiation in the positive corona (see Fig. 3).

C. Bactericidal Effects of Positive and Negative DC Corona Plasmas and their Bioactive Agents on *P. aeruginosa*

The positive DC corona reduced the initial number of plated *P. aeruginosa* by a factor of 10^4 for 10 s and resulted in total elimination of 10^5 bacteria within 30 s [Fig. 6(A)]. The negative DC corona was less efficient. Still, it caused elimination of 10^5 CFUs within less than 120 s and therefore was an effective antibacterial agent [Fig. 5(B)].

The next question we addressed was an efficiency of individual plasma components that were separated as described above. Conditions of the isolated chamber were used to compare effects of the whole plasma and the combination of active neutral species and UV. The effect of the total plasma was evaluated as a relative amount of survivors in the E-zone [see Fig. 1(C)], while a relative amount of survivors in the R-zone showed the effectiveness of the combined action of neutral species and UV. An example of differential survival at the E- and R-zones is shown in Fig. 1(D).

For the positive DC, the combination of active neutral species and UV was almost as effective as the whole plasma [Fig. 5(A)]. Total elimination of 10^5 CFUs was observed after 90 s treatment. Interestingly, that reduction in the number of viable CFUs after 30 and 45 s treatments did not differ significantly while the abrupt reduction in the amount of surviving bacteria was observed after minimal treatment for 10 s and a similar abrupt reduction was observed when treatment time exceeded 90 s. For the negative DC corona, the combination of neutral species and UV was the most effective, too [Fig. 5(B)]. Moreover, a nearly similar effectiveness of this combination for negative and positive discharges was observed after 45 s treatment. However, the time required to eliminate 10^5 CFUs was longer for the negative DC corona (about 300 s) and the two-step kinetics was less evident. This suggested different mechanisms underlying the bactericidal effects of the neutral species and UV combined action for positive and negative DC coronas, as discussed below.

The combination of UV with electrical components of the positive DC corona exerted a significant bactericidal effect that caused total elimination of 10^5 CFU within less than 120 s. Bactericidal activity of UV produced by the positive DC corona was considerably lower than the activity of combined action of two or three active agents [Fig. 5(A)]. The 300 s treatment with UV alone decreased concentration of plated bacteria by a factor of 100. That was at least 1000 times lower than bactericidal activity of the combination of UV with any of the other agents.

UV produced by the negative DC corona was not bactericidal [Fig. 5(B)]. Moreover, there were no evidences of a bactericidal activity of UV and electrical components in the negative DC corona even when the treatment was carried out for 10 min, which made a striking difference between the positive and negative DC coronas (data not shown).

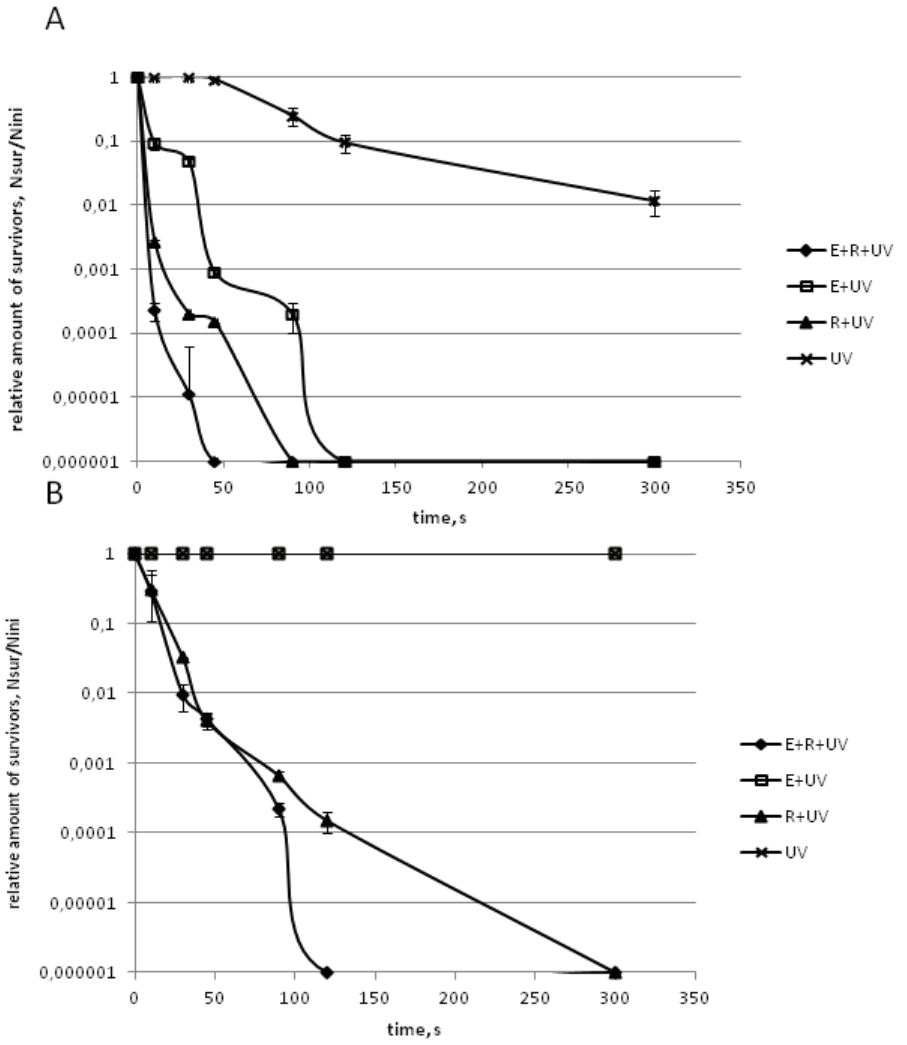

FIG. 5: The bactericidal effects of the positive (A) and negative (B) coronas and their bioactive components on *P. aeruginosa*. Plated bacteria were treated with ultraviolet radiation (UV), neutral active species and UV (R+UV); charge particle and electric field and UV (E+UV); whole plasma (E+R+UV). Relative amount of survivors was calculated as a ratio of the amount of survivors to the initial bacterial load (N_{sur}/N_{ini}). The mean ± standard errors of at least three independent experiments done in duplicate are shown.

D. Comparison of Bactericidal Effects of Plasma and other Agents of Positive and Negative DC Coronas on *S. aureus* and *P. aeruginosa*

The *S. aureus* strain Sa78 and *P. aeruginosa* strain Pa103 were used to compare bactericidal activity of whole plasmas and their components on Gram-positive and Gram-negative bacteria. Previously, the resistance of these strains to argon microwave plasma

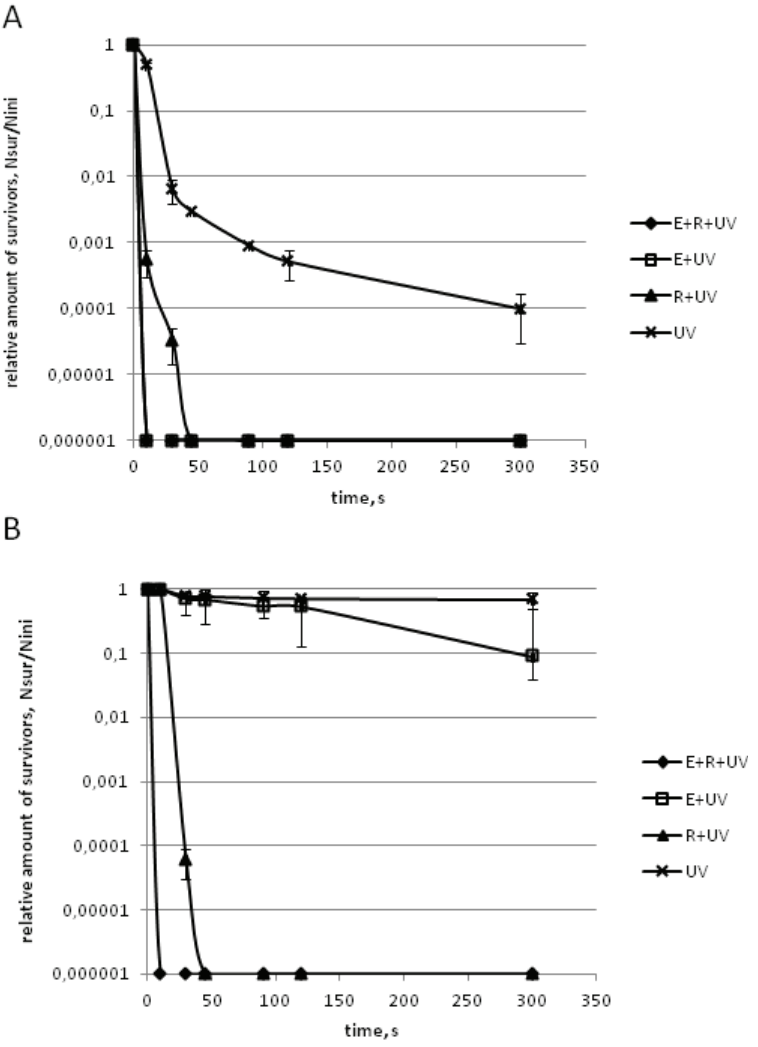

FIG. 6: The bactericidal effects of the positive (A) and negative (B) coronas and their bioactive components on *S. aureus*. Similar designations as in Fig. 6 are used. The means ± standard errors of three independent experiments done in duplicate are shown.

was found to be similar.[8] The positive and negative DC coronas were highly effective against both bacteria, too (Figs. 5 and 6). The resistance of *P. aeruginosa* to DC coronas was higher than the resistance of *S. aureus* (compare Figs. 5 and 6). Total elimination of 10^5 *S. aureus* CFUs required less than 10 s for both positive and negative DC coronas while it took 30 and 120 s for the positive and negative DC coronas, respectively, to reach similar results with *P. aeruginosa*.

Some other differences between *S. aureus* and *P. aeruginosa* were revealed when effects of combinations of bioactive agents were studied. The most important differ-

ence was observed when relative bactericidal activities were compared for the following combinations: UV and electrical components, and UV and neutral species. While the combined action of UV and neutral species was more effective against *P. aeruginosa*, it was less effective against *S. aureus* when compared to the action of UV and electrical components [compare Figs. 5(A) and 6(A)]. For the positive DC, it took less than 10 s, and about 45 s, to eliminate 10^5 *S. aureus* CFUs with the combinations of UV and electrical components, and UV and active species, respectively [Fig. 6(A)]. To eliminate 10^5 *P. aeruginosa* CFUs, it required about 120 and 90 s for the combination of UV and electrical components, and the combination of UV and active species, respectively [Fig. 5(A)].

The difference between Gram-positive and Gram-negative bacteria in resistance to the combinations of UV with other bioactive agents was even more evident for the negative DC corona. While *P. aeruginosa* was 100% resistant to the action of negative DC corona UV and electrical components, *S. aureus* demonstrated dose-dependent loss of viability under the same conditions that resulted in tenfold the number of viable bacteria after 300 s treatment [Figs. 5(B) and 6(B)].

S. aureus was more sensitive to UV produced by the positive DC corona. The treatment for 300 s decreased the number of viable bacteria by a factor of 10^4 and 10^2 for *S. aureus* and *P. aeruginosa*, respectively [Figs. 5(A) and 6(A)].

E. Synergy of the Bactericidal Action among Gas Discharge Active Agents

The obtained results suggest that there is a synergy among gas discharge active agents that brings about high bactericidal activity of the whole plasma. To make this synergy evident, we presented in the same diagram the experimental data obtained for *P. aeruginosa* and the sum of bactericidal effects of the components that would have been in the case if the synergy was absent. To construct the hypothetical curves of the whole plasma effect without synergy of plasma components we summed the number of bacteria killed by UV and electrical components (E+UV) and bacteria killed by UV and neutral species (R+UV) and subtracted the number of bacteria killed by UV alone, i.e., (E+UV)+(R+UV)–UV=E+R+UV. As one can see, the experimental curves demonstrated much faster decrease in the number of surviving bacteria than could be suggested on the base of assumption of independent plasma component actions (Fig. 7).

IV. DISCUSSION

Here an examination of the bactericidal activity for separated DC corona bioactive agents was performed. The obtained results support the view that it is a synergy between NTP components that supplies its high bactericidal activity. Some differences in resistance to combinations of the bioactive agents between Gram-positive and Gram-negative bacteria were revealed. Positive and negative DC air coronas were used. The coronas were equalized by the current and the power but they differed in other properties. The most important distinctions between positive and negative coronas were the way in which charged particles spread from the high-voltage electrode to the ground

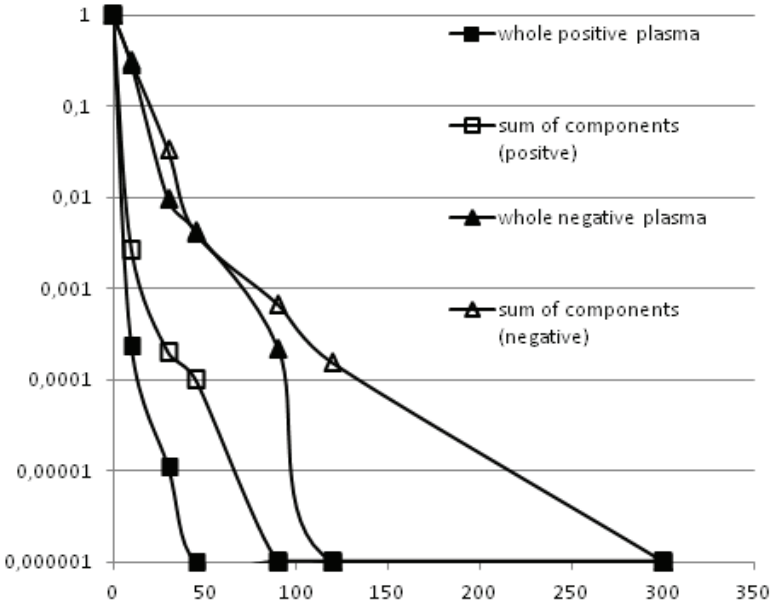

FIG. 7: The comparison of the effect of the positive and negative coronas and the arithmetic sum of the effects of the separated components on *P. aeruginosa*.

electrode and the intensity of UV radiation. The positive corona was represented by distinct streamers, which are separate flows of charged particles born at the high-voltage electrode which move to the ground electrode.[25] Upon reaching the ground electrode and establishing a contact between electrodes, the streamer decays within a few microseconds. The diameter of the streamer is about 100–200 μm. Streamers are formed chaotically with a frequency of about several kH. Therefore, each fragment of agar undergoes multiple streamer attacks for the duration of treatment time. The head of the streamer is characterized by high values of electric field strength (up to 1.5×10^5 V cm^{-1}) that result in several important consequences. Firstly, UV and active particles are delivered by the streamer head to the vicinity of the treated surface (particularly agar and plated bacteria). Secondly, the high voltage emerges between the coming streamer head and the target. The theoretical evaluation of the electric field strength between the streamer head and the bacterial surface gave a value of more than 10^6 V cm^{-1} which is enough to cause an electroporation of the bacterial membrane.[26] At last, the streamer head delivers a positive charge that might accumulate on the bacterial surface. The theoretical prediction gives the charge value of 10^{-15}–10^{-14} C per bacterial cell with the characteristic time of the charge draining of several tens of μs. The accumulated charge might contribute to electroporation of the bacterial cell.

The negative DC corona is different as it is represented by a constant flow of negatively charged particles from the high-voltage electrode to the ground electrode.[25] The average current was the same for the negative and positive DC coronas in our experi-

ments. Therefore, the immediate strength of a constant flow of charged particles in the negative discharge was lower than the strength of a streamer of the positive discharge. As a consequence, the negative DC corona produced UV only near the high-voltage electrode tip, and UV acts from a much longer distance (see Fig. 3). The large distance decreased UV radiation near the agar surface in the negative corona if compared with the positive corona where UV was produced at the head of the moving streamer. Results of UV measurement are in a good agreement with biological effects: a bactericidal effect of UV was negligible for the negative DC corona but not for the positive DC corona for both bacterial species. A region responsible for generation of the neutral reactive species correlated with the area, where the electric field strength has a maximum value. In the case of a negative corona, this area locates in the vicinity around the tip of a high-voltage pin. In the case of a positive corona, this area locates at the head of each streamer originating from the tip of a pin and moving towards the plane electrode. Because the streamers are chaotically distributed in space and time, bioactive agents are generated throughout the volume.

The low intensity of the instant current in the negative DC corona determines low values of the electric field strength near agar where bacteria were situated, and of the electric charge that accumulated on the bacterial surface. Indeed, cell damage determined by electric components did not cause *P. aeruginosa* death and exerted a statistically insignificant bactericidal effect in *S. aureus*. Nevertheless, the electric component significantly increased the effectiveness of the bactericidal action of neutral active species of the negative DC corona that resulted in a considerably higher bactericidal effect of the whole plasma in comparison with the action of neutral species. The synergy of the electric component and the neutral species might be based on the membrane damage caused by electroporation that would provide easier access of the active species to internal cellular structures. Still, the electric field strength of the negative DC corona near the agar surface had relatively low values (about 10^4 V sm^{-1})[25] that are about ten times lower than the intrinsic electric field strength of the membrane and lower than the theoretical prediction.[26] Therefore, the electric field did not seem to be strong enough to cause direct damage of the bacterial cell. Alternatively, the effect of the electric component might be due to cell polarization under the stationary electric field of the negative DC corona. Redistribution of the negative and positive charges along the cell surface for a prolonged time might disturb functioning of the cellular processes based on the proton force energy. While these processes are not lethal themselves they did not allow the cell to repair damage caused by neutral active species. The obtained data did not allow deducing whether UV added to the whole effect of the negative DC corona. Still, we suggest the effect of UV to be negligible for the negative corona because of the low intensity of UV radiation near the agar surface. The observed difference in kinetics of bacterial killing by UV/neutral particles for the positive and negative DC coronas (see Fig. 2) might be due to the absence of the UV component in the negative DC corona.

Our results are comparable with ones obtained by Bruggeman and colleagues.[27] Using a radio-frequency atmospheric plasma jet for inactivation of *P.aeruginosa* in

solutions, they showed that active plasma components, such as UV radiation, ozone, $ONOO^-$, HNO_2, H_2O_2, and other products of plasma-chemical reactions in gas-solution phase could achieve the bactericidal amounts, but the observed strong antibacterial effect of plasma could be explained by synergistic interactions of its active components.

The obtained results demonstrated that *P. aeruginosa* was more resistant than *S. aureus* to both positive and negative DC coronas. Higher sensitivity of the used *S. aureus* strain to UV radiation produced by the DC coronas might be responsible for the higher sensitivity to both plasmas. Beside this, *S. aureus* was more sensitive to the synergetic input of the electric component into the combined action of other active components. This effect was observed for both positive and negative coronas. When the relative sensitivity to different combinations of positive discharge active plasma components was compared, *S. aureus* was more sensitive to the combined action of UV with the electric component than with the neutral species while relative sensitivity of *P. aeruginosa* was the opposite; it was more sensitive to the UV with neutral species than to UV with electric components. Moreover, *P. aeruginosa* was totally resistant to electric components and UV of the negative DC corona while *S. aureus* was sensitive. The thick cell wall of the Gram-positive *S. aureus* had to be more resistant to the mechanical damage caused by electric field than the thin cell wall of *P. aeruginosa*. Therefore other effects of the electric components might be responsible for the higher sensitivity of the *S. aureus*. The above-suggested distortion of the intrinsic membrane electric field due to cell polarization might be one of them.

Taken together, the obtained results clearly evidence the importance of synergy of plasma bioactive components for the whole bactericidal effect of the nonthermal plasma. This synergy allows effective surface sterilization in the presence of relatively low concentrations of toxic components such as UV, and active neutral species of oxygen and nitrogen, which is particularly important for applications associated with decontamination of biological objects, e.g., skin, wounds, mucous surfaces, etc.

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