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FULL PAPER

PLASMA PROCESSES AND POLYMERS

Inactivation of airborne bacteria by plasma treatment and ionic wind for indoor air cleaning

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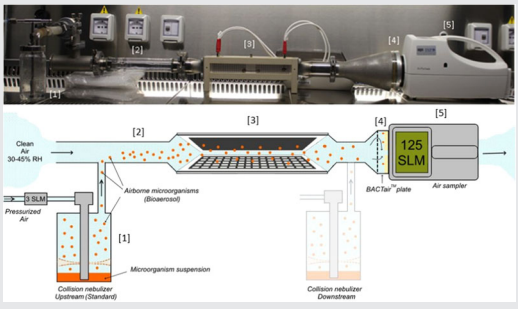
Abstract

Airborne bacteria are a general problem in medical or health care facilities with a high risk for nosocomial infections. Rooms with a continuous airflow, such as operation theaters, are of particular importance due to a possible dissemination and circulation of pathogens including multidrug-resistant microorganisms. In this regard, a cold atmospheric-pressure plasma (CAP) may be a possibility to support usual disinfection procedures due to its decontaminating properties. The aim of this study was to determine the antimicrobial efficacy of a plasma decontamination module that included a dielectric barrier discharge for plasma generation. Experimental parameters such as an airflow velocity of 4.5 m/s and microbial contaminations of approximately 6,000 colony-forming units (cfu)/m^3 were used to simulate practical conditions of a ventilation system in an operating theater. The apathogenic microorganism *Escherichia coli* K12 DSM 11250/NCTC 10538 and the multidrug-resistant strains *E. coli* 21181 and 21182 (isolated from patients) were tested to determine the antimicrobial efficacy. In summary, the number of cfu was reduced by 31–89% for the tested *E. coli* strains, whereby *E. coli* K12 was the most susceptible strain toward inactivation by the designed plasma module. A possible correlation between the number or kind of resistances and susceptibility against plasma was discussed. The inactivation of microorganisms was affected by plasma intensity and size of the plasma treatment area. In addition, the differences of the antimicrobial efficacies caused through the nebulization of microorganisms in front (upstream) or behind (downstream) the plasma source were compared. The presence of ionic wind had no influence on the reduction of the number of cfu for *E. coli* K12, as the airflow velocity was too high for a successful precipitation, which would be a prerequisite for an increased antimicrobial efficacy. The inactivation of the tested microorganisms confirms the potential of CAP for the improvement of air quality. The scale-up of this model system may provide a novel tool for an effective air cleaning process.



KEYWORDS

cold plasma, HEPA filter, hospital-acquired infections, HVAC system, UV irradiation

1 | INTRODUCTION

Nosocomial infections cause more than 2.6 million cases of disease every year in Europe.[1,2] In developed and developing countries, 7 and 10 out of 100 patients, respectively, suffer from at least one nosocomial infection.[3] Examples for nosocomial infections are pneumonia, surgical site infections, urinary tract, and bloodstream infections.[1] As one important transmission path, air—or more specific, bioaerosol—is able to transport biological material such as microorganisms or viruses. Thus, respiration (exhalation) and skin scales of humans are main factors that contribute to the formation of such bioaerosols.[4] In health care facilities, the source for these bioaerosols are obviously the patient itself, staff such as the surgical team in an operating theater, and visitors.[5] Thus, the prevention of nosocomial infections requires not only the disinfection of surfaces such as tables or floors but also the cleaning of air.

This study relates to the air purifying system of a typical surgical operating theater, with the highest demand for clean air (Class Ia), according to the German Standard DIN1946-4:2008-012.[6,7] This system uses a laminar, high volumetric airflow to remove microorganisms, viruses, and particles. Afterward, the contaminated airflow is filtered, air-conditioned, and filtered again inside ventilation ducts (>5 m/s air velocity). To obtain a contamination level ≤1 colony-forming units (cfu)/m^3, high-efficiency particulate air (HEPA) filters with an efficiency more than 99.99% are the common standard.[6] These filters effectively retain particles, such as bacteria, viruses, pollen, mite eggs, and the excrements,[8] but they do not inactivate them. The filters lead to a high back pressure and thereby high costs for replacement and maintenance. Thus, these filters are used only in critical areas, such as operating theaters. Additionally, trapped microorganisms could survive and multiply for a long period in the filters, thus representing a high health risk.

To overcome these drawbacks, an air purifier that uses a cold atmospheric-pressure plasma (CAP) combined with ionic wind has been developed. The electrical characterization, gas diagnostics, and first very simple microbiological investigations of this plasma decontamination module (PDM) have been published previously.[9] The current study represents the next logical step in carrying out a much more profound microbiological examination with various microorganisms (including multidrug-resistant strains) and variations of geometrical, electrical, and physical parameters of the PDM. This should lead to a deeper understanding of the PDM at different operational conditions, which result in the uncovering of the potential for improvement and upscaling.

A CAP is an excited and a conductive gas state with a low temperature (<50°C), which emits electromagnetic radiations such as ultraviolet, visible, and infrared radiations, and other electromagnetic fields, generating charged particles and reactive species.[10–12] The most important species—especially for the antimicrobial efficacy of CAP—are reactive oxygen (ROS) and nitrogen species (RNS).[13] These species include atomic oxygen (O), ozone (O_3), hydrogen peroxide (H_2O_2), nitrite (NO_2^-), nitrate (NO_3^-), nitric oxide ($NO^\bullet$), hydroxyl radical ($HO^\bullet$), superoxide anion radical ($O_2^{\bullet-}$), and singlet oxygen ($^1O_2$).[14,15] Different mechanisms were proposed for the antimicrobial efficacy of CAP in gases. The disruption of the cell envelope or oxidation processes were described, for example, on the cell membrane or other cell components.[10,16]

On the one hand, the antimicrobial efficacy of a CAP increases with increasing treatment time and intensity of the plasma. On the other hand, the intended application as an air purifier inside ventilation ducts allows only for treatment times in the subsecond range. Vaze et al.[17] achieved a reduction of 99.99999% of antibiotic-resistant *Escherichia coli* despite a treatment time of only 0.13 ms by using a CAP with a very high intensity (>40 kJ/L). Despite a much lower intensity of the plasma (4 J/L), Gallagher et al.[18] inactivated 97% of *E. coli* in a contact time of only 1 ms, but even this CAP-based air purifier produced the reactive species ozone with a concentration of 28 ppm. This is a serious problem, as ozone is harmful to human health. The indoor ozone concentration is, therefore, limited by law worldwide and, in particular, inside medical facilities to only 30 ppb.[18] Hence, achieving a high antimicrobial efficacy of a CAP and complying with all regulations and technical requirements seem contrary to each other.

Xia et al.[19] investigated a so-called packed-bed CAP device with an application-oriented contact time of 0.25 s and two different intensities. With a low intensity of approximately 0.2 J/L, a reduction of 83% of MS2 bacteriophage, accompanied by only 0.2 ppm ozone, was achieved. The high-intensity operation mode with 0.7 J/L led to a reduction of 99% and an ozone concentration of 2 ppm. By the use of an ozone filter with an arguable back pressure, the ozone concentration was reduced to 10 and 90 ppb for the low- and high-intensity operation mode, respectively.

Ozone filters, which are porous and usually made out of active carbon,[20] are unfortunately not applicable here, as their implementation in ventilation ducts of medical facilities is forbidden (potential breeding ground for microorganisms, DIN1946-4:2008-012[6]). So, the investigated PDM in this contribution uses a combination of a CAP and an inherent electrostatic precipitator to additionally deflect and capture airborne microorganisms. This may drastically reduce the treatment time,

which means that the intensity of the plasma and the concentration of reactive species can be decreased to achieve a sufficient antimicrobial efficacy and meet the concentration limit for ozone.

The present study shows a detailed description of the antimicrobial efficacy of a PDM as ventilation ductwork for air cleaning. The basics of PDM have been described for the first time by Müller et al.[21] A local surface CAP arises through a dielectric barrier discharge (DBD) as the source of ROS/RNS and ions. An additional electric field is supplied perpendicular to this surface CAP by a third, charged electrode, which pulls out ions ("ionic wind"). Therefore, the PDM operated simultaneously as an electrostatic precipitator, as shown by Schmidt et al.[22] Preliminary and very simple microbiological investigations showed that this feature of the PDM reduced the number of airborne *E. coli* by an additional 70%.

In our study, different geometrical, physical, and electrical parameters were varied to improve the antimicrobial efficacy and to simulate application-oriented conditions. Furthermore, the test setup allowed two plasma treatment options. The first one was realized by an upstream (in front of the plasma source) nebulization of the microorganisms, and the second option comprised a downstream (behind the plasma source) nebulization. Although the used plasma is only generated at the surface, a direct contact between microorganisms and plasma is possible. This allowed, at least to some extent, the differentiation of the antimicrobial efficacy caused by direct (realized by upstream nebulization) and indirect (realized by downstream nebulization) plasma treatment. In general, a direct plasma treatment means that the microorganisms were directly in contact with plasma (including thermal and UV/Vis radiation or electromagnetic fields) and at least short-lived reactive species. For the indirect plasma treatment, the microorganisms were influenced only by plasma-generated reactive species or products, such as ozone. Consequently, the contaminated air was not treated directly by plasma, but it came in contact with the plasma-generated long-lived reactive species or their products. The antimicrobial efficacy was tested with the apathogenic strain *E. coli* K12 DSM 11250/NCTC 10538 (DSM—German Collection of Microorganisms and Cell Cultures, NCTC—National Collection of Type Cultures) and two *E. coli* strains 21181 and 21182, belonging to the drug-resistant clade of ST131, which were isolated from two patients.[23] These microorganisms are extraintestinal pathogens with a worldwide dissemination.[24] *E. coli* strains are the dominant isolates from nosocomial urinary tract and bloodstream infections.[24,25] Despite the increasing threat of multidrug-resistant microorganisms, their inactivation by plasma in the air is scarcely reported.[17]

Thus, the introduced PDM may be a useful device for indoor air cleaning of rooms in medical or health care facilities with a high risk for infections. This method might not only be applicable for the inactivation of bacteria, but also for pathogenic viruses, as described for other plasma-based procedures.[26,27]

2 | EXPERIMENTAL SECTION

2.1 | Plasma module

The PDM consisted of a housing (inlet and outlet diameter of 40 mm) with a frame for two plasma plates. The plasma plates were made of ceramics with a size of 90×25 mm (length × width) and a thickness of 1 mm. The plates were covered with a metallic grid (plasma electrode) on one side and with a metallic sheath on the other side (extraction electrode; Figure 1a). The ceramic plates were mounted parallel to each other to form a channel of 20-mm width through which the contaminated air was passed. The plasma was generated on the surface of the plate with the metallic grid when an AC high voltage was applied between grid and metallic sheath on the other side of the plasma plate (refer also to Figure 1b). The second electrode was used as the extraction electrode. In the standard configuration (Figure 1a), the plasma electrode of one ceramic plate was placed opposite to the extraction electrode. At this electrode, a DC high voltage was applied (in this case positive in Figure 1a). By applying the rectified high voltage to the extraction electrode, an additional electric field of one polarity was generated inside the channel. This forced ions of one polarity (negative in Figure 1a) toward the extraction electrode. Thus, the ions collided with neutral air molecules and particles, which induced a so-called ionic wind and the deflection of the charged particles. The resulting trajectory of charged particles was dependent on the airflow velocity, electric field strength, and other parameters.

As the volumetric flow was set to 125 slm, the mean air velocity inside the duct corresponded to 4.5 m/s—a value that is also achieved in commercial air ducts of operating theaters (DIN1946-4:2008-012[6]). Thus, the acquired knowledge of this PDM can be easily transferred to practical applications.

2.2 | Electrical and geometrical configurations

The electrical power of the plasma source was modified by two different settings. The low-intensity plasma

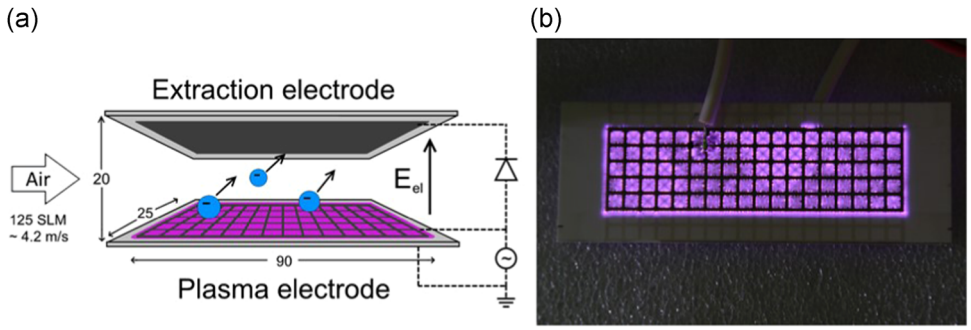


FIGURE 1 (a) The plasma module and its working principle (one-point perspective): at the lattice structure of the lower plate, a surface dielectric barrier discharge developed and charged particles were extracted by ionic wind. The unit of the numbers is millimeters. (b) The photo of the surface dielectric barrier discharge at the plasma electrode at 400 Hz and 14 kV_{pp}

(0.2 W) was excited by applying a sinusoidal voltage of 10 kV_{pp} at 50 Hz, whereas 400 Hz at 14 kV_{pp} formed a high-intensity plasma (1.9 W; Figure 1b). These values corresponded to a specific input energy (SIE) of 0.1 and 0.9 J/L for the low- and high-intensity plasma, respectively (for more information on discharge parameters, refer to Timmermann et al.[9]).

Besides the enhancement of the plasma intensity, different strategies were pursued to further improve the antimicrobial efficacy of the PDM with a high-intensity plasma (14 kV_{pp}, 400 Hz).

Thus, the influence of ionic wind was tested by three different circuits (Figure 2). When the extraction electrode was grounded (Figure 2a), the ions of both polarities just moved back and forth by less than 1 mm, as the half-cycle lasted only 1.25 ms, and the ion velocity was about 0.5 m/s, as measured by Schmidt et al.[22] In this case, no directed ionic wind was induced. Using the circuits in Figure 2b,c, the extraction electrode became either positively (Figure 2b) or negatively (Figure 2c) charged by the amplitude of the applied high voltage of 7 kV. The resulting additional electric field inside the duct was alternating, but unipolar, so a directed ionic wind was induced. These circuits had no effect on the plasma power of 1.9 W (SIE = 0.9 J/L).

In further experiments, the plasma treatment area was varied with respect to size and position (Figure 3). Thus, a doubling of the injected plasma power to 3.8 W (1.8 J/L) was achieved by (a) doubling the plasma treatment area with a "long plasma source" or (b) using a "double-sided plasma source."

2.3 | Experimental setup

The test setup for the microbiological experiments (Figure 4) comprised a nebulizer, a pipe section with an integrated plasma source, and an air sampler. All experiments were performed in a biosafety cabinet (ENVAIR Eco, Emmendingen, Germany) to ensure constant (clean) air quality. The temperature (25–28°C) and humidity (30–45%) were continuously monitored.

The air sampler (MD8; Sartorius AG, Göttingen, Germany) provided a constant airflow of 125 slm through the test setup (Figure 5). The sucked clean airflow was contaminated and mixed with 3-slm bioaerosol produced

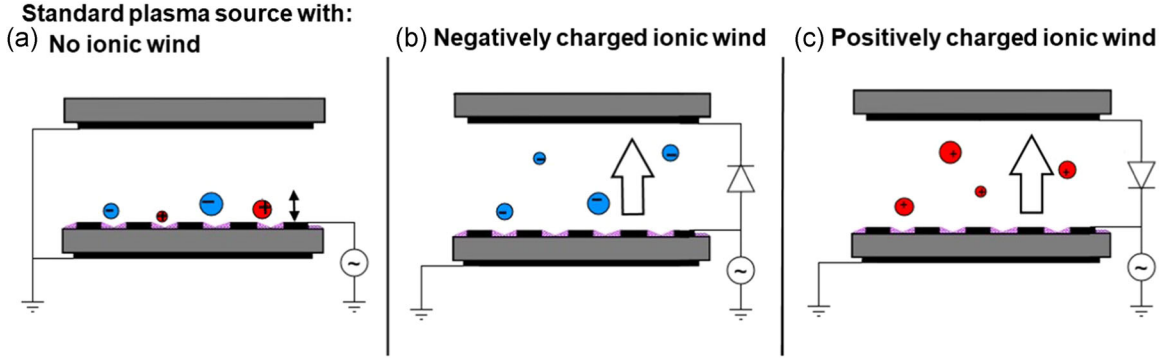


FIGURE 2 Different circuits of the plasma source to form (a) no ionic wind with a grounded extraction electrode, (b) ionic wind with negative ions (due to a positively charged extraction electrode), or (c) ionic wind with positive ions (due to a negatively charged extraction electrode)

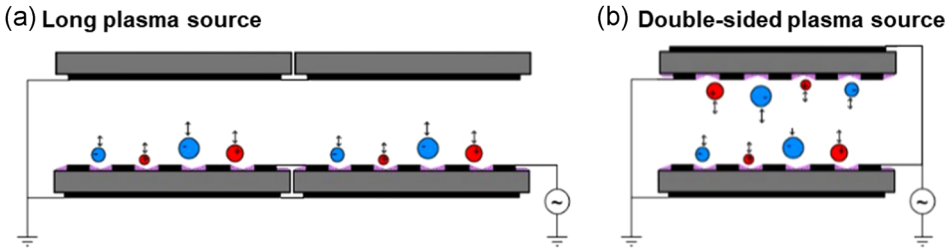


FIGURE 3 The modification of the standard plasma source resulted in (a) doubling the plasma treatment area with a "long plasma source" or (b) double-sided plasma treatment with a "double-sided plasma source." No ionic wind was induced in both modifications

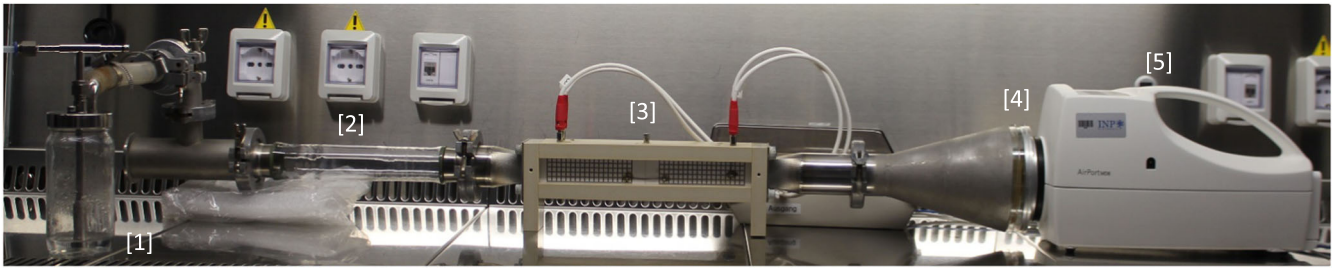


FIGURE 4 The experimental setup. The collision nebulizer (1) sprayed microorganism aerosol in the airflow provided by the air sampler (5). After a mixing zone (2), the (long) plasma source (3) treated the contaminated air. Finally, the airborne microorganisms were collected in the BACTair™ plate (4)

by a collision nebulizer (Mesa Labs, Lakewood). The nebulizer itself was fed with clean, dry air. The resulting concentration of microorganisms in the airflow was in the range of 6,000 cfu/m^3, which corresponded to highly contaminated air.[28]

After a mixing zone of 30 cm, the contaminated air passed through the plasma source. Finally, the microorganisms were collected in BACTair™ plates/filters (116 × 24 mm; impaction velocity: 30 m/s; Sartorius AG). The BACTair™ plates contained tryptic soy agar and were incubated directly after the gathering process at 37°C.

The used test setup allowed the determination of the antimicrobial efficacy during direct plasma treatment or indirect plasma treatment through the possibility to change the position of the collision nebulizer (Figure 5).

2.4 | Microbiological examination

Experiments were carried out using *E. coli* K12 DSM 11250/NCTC 10538 (DSM—German Collection of Microorganisms and Cell Cultures, NCTC—National

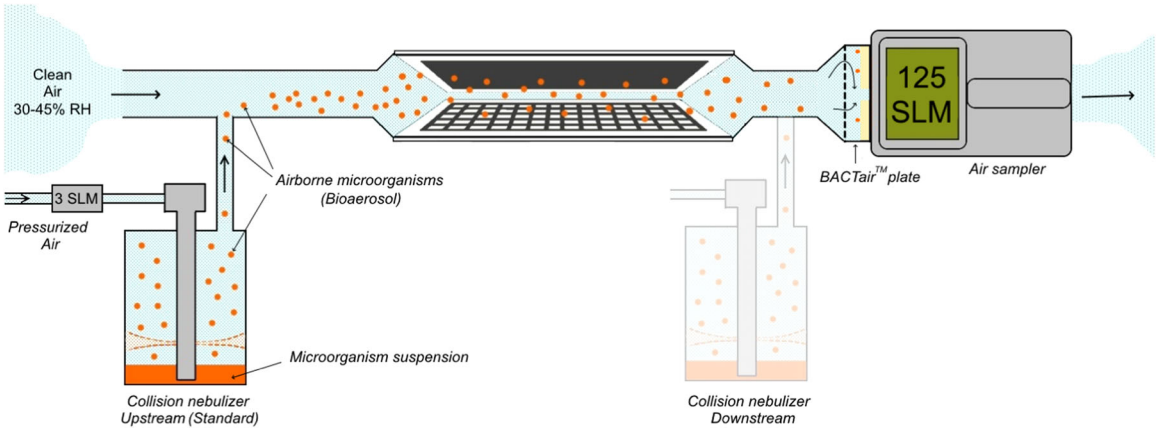


FIGURE 5 The schematic experimental setup. The collision nebulizer placed upstream of the plasma source corresponded to the direct treatment of the microorganisms, whereas when it was placed downstream of the plasma source, it corresponded to indirect treatment. RH, relative humidity

Collection of Type Cultures). Two more *E. coli* strains (21181 and 21182) were isolated from patients, which possessed resistances against different antibiotics. The microorganisms were cultured on tryptic soy agar (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) for 24 hr at 37°C and then stored at 8°C.

To determine the antimicrobial efficacy of the plasma sources, approximately three colonies of the respective strain were transferred to 20-ml tryptic soy broth (Carl Roth GmbH & Co. KG). After an incubation of 24 hr at 37°C, 2 ml of this culture was centrifuged (5 min, 4,500 rpm). The pellet was suspended in a 50-ml saline solution (0.9% NaCl) and adjusted to a final concentration of approximately 10^5 cfu/ml (6,000 cfu/m^3 in air). The nebulizer was filled with 20 ml of the microorganism suspension, and the BACTair™ plate was attached to the air sampler.

The operation sequence (Table 1) contained bioaerosol nebulization, which ensured that for the plasma run, all airborne microorganisms were treated with plasma within 15 s (30–45 s in the plasma run). In the control run, the nebulized microorganisms were not treated by plasma.

Afterward, the BACTair™ plate was subsequently removed and covered. The number of cfu was determined after an incubation time of 24 hr at 37°C (Figure 6: example of a control run).

The operation sequence to test one configuration contained six control and six plasma runs, which were performed alternatingly to ensure the same conditions for each plasma treatment and the respective control. The determined number of cfu was used to calculate the percentage of residual number of microorganisms after plasma treatment in comparison to the cfu of the control.

2.5 | Ozone measurements

As the SIE of the plasma source is relatively low, a plasma chemistry dominated by ozone as the main long-lived species was assumed. Therefore, the ozone concentration was measured in preliminary tests 30 cm downstream of the plasma source via an ozonometer (AP 360; HORIBA Europe GmbH, Dresden, Germany) with dependence on the relative humidity of the feed gas.

TABLE 1 Operation sequences for the experiments with plasma (plasma run) and without (control run)

Starting point (s)	Plasma run	Control run
0	Air sampler on	Air sampler on
15	Plasma source on	–
30	Nebulizer on	Nebulizer on
45	Nebulizer off	Nebulizer off
60	Plasma source off	–
75	Air sampler off	Air sampler off

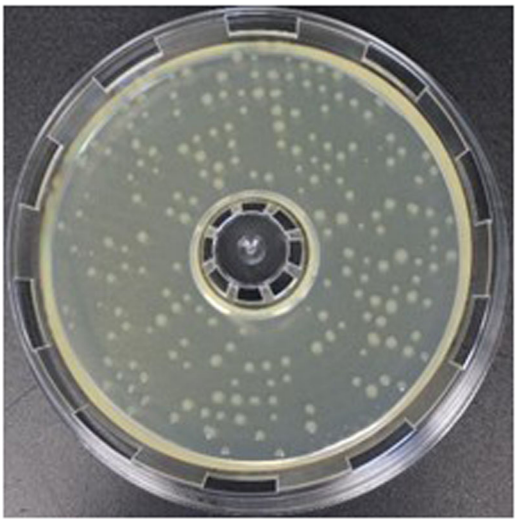


FIGURE 6 BACTair™ plate with colony-forming units of *Escherichia coli* K12 DSM 11250/NCTC 10538 after sampling of 125 slm air containing 3 slm bioaerosol for 15 s without plasma treatment and incubation for 24 hr at 37°C

3 | RESULTS

3.1 | General observations

The antibacterial efficacy of plasma was determined against *E. coli* K12 DSM 11250/NCTC 10538 and two *E. coli* isolates 21181 and 21182. Among them *E. coli* K12 was the most susceptible strain toward inactivation by the designed PDM.

The intensity of plasma defined by frequency and voltage, as well as by size and position of the plasma treatment area, influenced the inactivation of *E. coli*. Furthermore, the impact of the presence of ionic wind and that of plasma-generated species was determined.

A plasma treatment time of 15 s and a bacteria concentration of 10^5 cfu/ml were used for all experiments.

3.2 | Inactivation of *E. coli* K12

3.2.1 | The influence of plasma intensity and ionic wind

The adjustment of the plasma intensity was achieved by the variation of the following physical parameters: frequency and voltage.

The low-intensity plasma (50 Hz, 10 kV_{pp}) resulted in no reduction of the viable count for *E. coli* K12 (Figure 7), whereas a reduction of 72% was achieved with the high-intensity plasma (400 Hz, 14 kV_{pp}; without ionic wind). Thus, all further experiments were performed solely with the high-intensity operation mode.

The three different circuits (refer to Figure 2) that resulted in no, positive or negative ionic wind were also tested for their antimicrobial efficacy.

The experiments without ionic wind (high intensity; 400 Hz, 14 kV_{pp}) reduced the viable count by 72%. The additional ionic wind, with 72% reduction of the cfu for the positive and 66% for the negative mode, had no significant influence on the cfu reduction of *E. coli* K12.

3.2.2 | The influence of the plasma treatment area

To double the plasma treatment area, the standard plasma source was modified, resulting in a long plasma source and a double-sided plasma source.

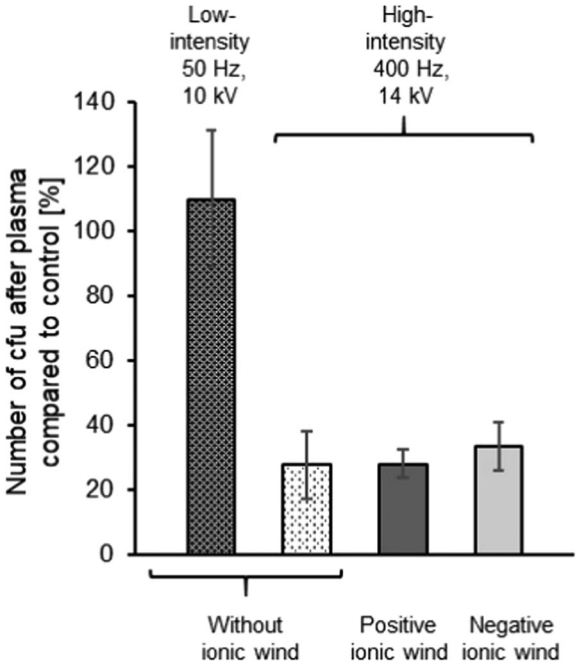


FIGURE 7 The standard plasma source. The number of colony-forming units (cfu) of *Escherichia coli* K12 DSM 11250/NCTC 10538 after the application of a low-intensity (50 Hz, 10 kV_{pp}) or high-intensity (400 Hz, 14 kV_{pp}) plasma without positive and negative ionic wind, compared with cfu of the control run (%)

The reduction of the number of cfu for *E. coli* K12 increased with both plasma sources (Figure 8).

The double-sided plasma source achieved a reduction of 76%—an increase of 9%, compared with the standard plasma source. The long plasma source reduced the viable count by 89%, an even larger increase of 22%. Therefore, doubling the length of the plasma treatment area seems to be the most effective strategy for improving the antimicrobial efficacy of the plasma source.

The ozone concentration was determined to be 5–10 ppm for the standard plasma source at a high intensity, whereas a doubling of the plasma treatment area in the two modified plasma sources doubled the ozone concentration as well.

3.2.3 | The influence of direct and indirect plasma treatment

As mentioned above, the upstream nebulization of microorganisms allowed, to some extent, a direct treatment, which is described by a direct contact of plasma and all generated RONS with the microorganisms when passing through the plasma source. The downstream nebulization of microorganisms is characterized by an indirect treatment, whereby only the formed reactive species have an effect on the microorganisms. These plasma products

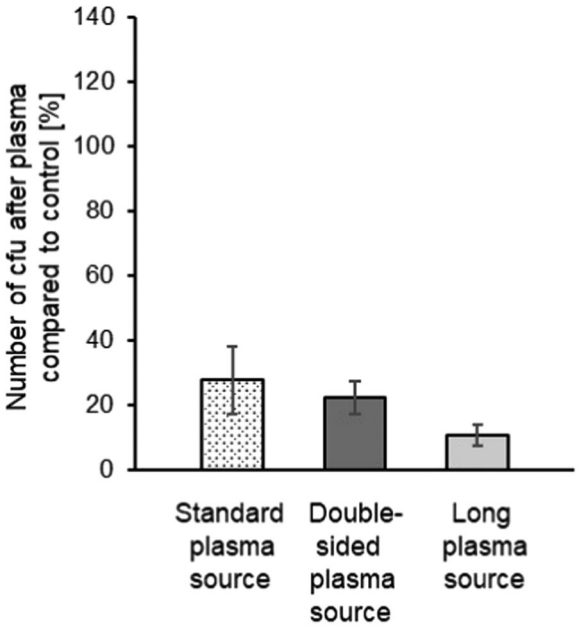


FIGURE 8 The standard, double-sided and long plasma sources. The number of colony-forming units (cfu) of *Escherichia coli* K12 DSM 11250/NCTC 10538 after the application of a high-intensity plasma (400 Hz, 14 kV_{pp}), compared with cfu of the control run (%)

are still present after switching off the plasma source, which may lead to an inactivation of the microorganisms. To evaluate the influence of both treatment possibilities, the test setup was changed. For the direct plasma treatment, the nebulizer was placed in front of the plasma source and for the indirect one, behind the source.

The direct plasma treatment reduced the number of cfu for *E. coli* K12 by 72%, whereas the indirect plasma treatment resulted in 31% inactivation (Figure 9).

This led to the assumption that passing through the plasma source (direct treatment) resulted in a reduction of 41%, and that the residual 31% was the result of the plasma products that were still present after the plasma treatment (indirect plasma treatment).

3.2.4 | Inactivation of multidrug-resistant *E. coli* strains 21181 and 21182

The long plasma source was examined for the efficacy to inactivate two multidrug-resistant *E. coli* strains (Figure 10).

The *E. coli* strains 21181 and 21182 were inactivated by 40% and 36%, respectively. Both of the tested strains were less susceptible against plasma treatment than *E. coli* K12 (89% reduction of the cfu; also refer to Figure 8).

4 | DISCUSSION

In our study, we described the efficacy of different plasma configurations for the inactivation of the apathogenic *E. coli* K12 and two multidrug-resistant *E. coli* strains

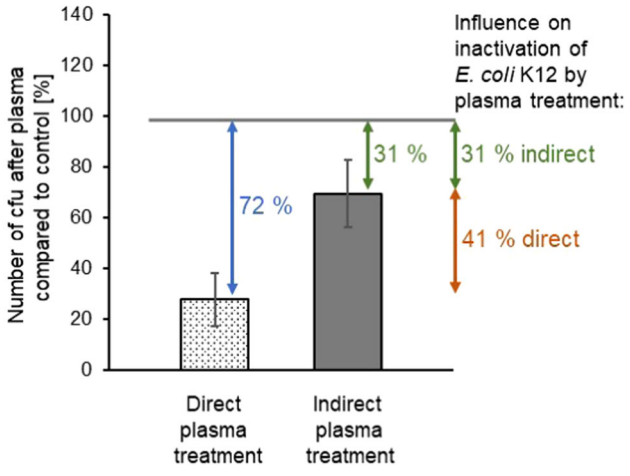


FIGURE 9 The standard plasma source. The number of colony-forming units (cfu) of *Escherichia coli* K12 DSM 11250/NCTC 10538 after the direct or indirect application of a high-intensity plasma (400 Hz, 14 kV_{pp}), compared with cfu of the control run (%)

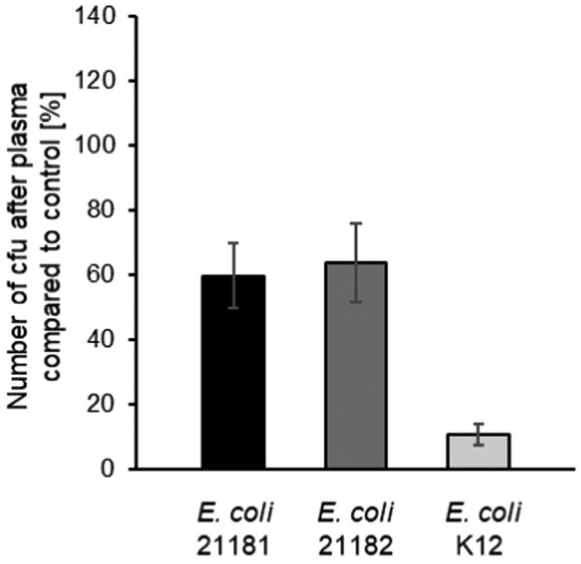


FIGURE 10 The long plasma source. The number of colony-forming units (cfu) of *Escherichia coli* 21181, 21182, and *E. coli* K12 DSM 11250/NCTC 10538 after the application of a high-intensity plasma (400 Hz, 14 kV_{pp}), compared with cfu of the control run (%)

(21181 and 21182) for the utilization in health care facilities. The effect of the PDM was dependent on the tested *E. coli* strain, as well as the intensity and size of the plasma source.

As expected, the high-intensity (400 Hz, 14 kV_{pp}) plasma resulted in an increased inactivation of microorganisms, compared with the low-intensity (50 Hz, 10 kV_{pp}) plasma. This tendency was also reported by Timmermann et al.[9] who determined a 10% and 25% reduced number of the cfu for *E. coli* K12 with a low-intensity (50 Hz, 10 kV_{pp}) and high-intensity (400 Hz, 14 kV_{pp}) plasma, respectively. Nevertheless, there is a difference between the 72% reduction of the cfu, compared with the 25% reduction determined by Timmermann et al.,[9] even though the same plasma source was used. Most likely, this could be the result of the different sampling methods (BACTair™ filters instead of gelatin filters). The BACTair™ filters/plates contained tryptic soy agar, whereas the gelatin filters were dry and were transferred after the sampling process to tryptic soy agar. To achieve a similar number of sampled cfu in the control runs, the nebulized solution had to contain a 400 times higher bacterial load in case of the gelatin filter.[9] This, in turn, means that the gelatin filter method suffers from parasitic inactivation, probably due to dehydration on the dry surface. Therefore, these results are not comparable with the results of the present work.

In contrast, the improved efficiency of the BACTair™ filters could theoretically be attributed to a parasitic

interaction of the plasma-generated RONS with the wet surface on the agar plates, but the high amount of scavenging components such as amino acids and proteins in the agar probably prevent this, as such scavengers strictly limit the reaction time and thus the antimicrobial efficacy of plasma-treated solutions.[29] The experiments with the nebulizer positioned upstream and downstream of the plasma source at least showed that 31% of the reduced number of cfu can be attributed to long-lived species. Nevertheless, attempts with an even more differentiative perspective for parasitic inactivation effects, similar to Nayak et al.[27] using the impinger method, can be considered for future works.

The size and position of the plasma-generating surface also influenced the antimicrobial efficacy of the PDM. Thus, doubling the plasma treatment length was more effective than using a double-sided treatment, which hardly led to improvements. Therefore, increasing the direct treatment time seems to be an effective instrument.

The influence of the ionic wind was determined by different circuits. Thus, the ionic wind had no significant influence on the reduction of *E. coli*. In contrast, the experiments of Timmermann et al.[9] resulted in an improved cfu reduction of 70%, which can be attributed to a 2.5-fold lower airflow velocity (1.8 m/s instead of 4.2 m/s in our study). By comparing length (90 mm) and width (20 mm) of the air duct inside the plasma system, it can be shown that the airflow velocity has to be only 4.5-fold higher than the speed of the ionic wind (0.5 m/s[22]), so that particles can be deflected at all. This means that the induced ionic wind had no precipitating effect above an airflow velocity of 2.25 m/s, similar to the case for our study. A reduced distance or an increased electric field between the plates could be a possible improvement, but the prevailing turbulent flow conditions (Reynolds number of 7,000) are also usually not desirable in electrostatic filters. Therefore, a successful implementation of an electrostatic filter effect will probably be achieved only by a reduced flow rate.

The antimicrobial effect of plasma-generated reactive species, such as ozone or hydroxyl radicals, can be concurrent with the direct plasma treatment. Thus, the indirect effect is still present after the plasma is switched off. It was at least in part possible to separate the two treatment options by different experimental setups. As a consequence, 41% of the antimicrobial effect can be due to the direct plasma treatment, passing through the plasma source, which includes not only RONS but also factors like radiation or electromagnetic fields.

In general, the reactive species can be divided into short-lived and long-lived species. The long-lived reactive species alone are responsible for an inactivation of *E. coli* by 31%. One of these species is ozone, which has a well-known antimicrobial effect.[30,31] However, the doubling of the plasma treatment zone and thus ozone concentration resulted in an increase of the antibacterial effect by only 9% or 22% (double-sided or long plasma source compared with the standard plasma source). In contrast, a correlation between the antimicrobial efficacy against *E. coli* and the ozone concentration was determined for a saline solution treated with air plasma indirectly.[32] They described ozone as the main factor for microbial inactivation, which is not the case for the present study. Thus, other RONS are also important for an antimicrobial effect.[33,34] Nayak et al.[35] described the formation of NO_x by a surface DBD. These reactive species may also be responsible for the antibacterial effect, for example, due to the formation of peroxynitrous acid[30] in the anticipated acidified solution droplets. As the plasma source in the present study had 50 times less SIE, accompanied by almost no temperature increase, it is highly likely that NO_x played no significant role.[36] This led to the short-lived reactive species. These are ions, electrons, and radicals such as hydroxyl radicals or superoxide anion radicals. In particular, the hydroxyl radical has a strong oxidative property. In general, hydroxyl radicals, superoxide anion radicals, singlet oxygen, or nitric oxide are important precursors in the peroxynitrous acid chemistry.[15,27]

The herein tested PDM was able to inactivate also multidrug-resistant *E. coli* strains to a different extent. The *E. coli* strains 21181 and 21182 were resistant against β-lactam antibiotics (such as penicillins and cephalosporins), tetracyclines, chloramphenicol, and fluoroquinolones. The strain 21182 also possesses a resistance against aminoglycosides, sulfonamides, and trimethoprim. Thus, *E. coli* strain 21182—with resistances against additional three classes of antibiotics, compared with 21181—was 16% less susceptible to plasma than strain 21181. Both strains were less susceptible than *E. coli* K12.

This supports the assumption that the degree of plasma-mediated inactivation may be connected with the susceptibility of microorganisms toward antibiotics, for which reliable data are missing. Thus, Daeschlein et al.[37] treated different skin- and wound-relevant pathogens (194 isolates) with kINPen 09 (INP, Greifswald, Germany), a CAP jet, or a DBD (CINOGY GmbH, Duderstadt, Germany). In these in vitro susceptibility tests, the microorganisms were spread on blood agar. Afterward, the microorganisms were treated with the respective plasma source. The resulting inhibition zones (defined as area without visible growth of microorganisms; named in

accordance with agar disc diffusion assay)—as measure for the susceptibility against plasma—increased for DBD and decreased for jet plasma, with increasing number of antibiotic resistances of the respective microorganisms. Thus, *E. coli* strains with a resistance against one to seven classes of antibiotics were more susceptible toward DBD plasma treatment than the extended spectrum β-lactamases (ESBL)-producing *E. coli* strains with 3–10 antibiotic resistances. The kind of resistances and the resulting changed physiology or morphology were not described. A separation of the results for Gram-positive and Gram-negative microorganisms showed a rather diminished susceptibility, with an increasing antibiotic resistance independent of the used plasma source, which was in accordance with our study for the different *E. coli* strains. Napp et al.[38] compared methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* isolates. The resistant isolates were less susceptible against DBD plasma treatment than the susceptible ones; in contrast, with a CAP jet, no differences were determined. The authors assumed that the rigidity of the cell wall could be a reason for the differences.

Such morphological changes of microorganisms may obviously result in a changed susceptibility against plasma, but many resistances against antibiotics can also be ascribed to physiological changes, which is also the case for the *E. coli* strains 21181 and 21182. The ESBL-producing microorganisms are resistant against different antibiotics, and different genes are responsible for these multiple resistances. Thus, the *E. coli* strains 21181 and 21182 produce β-lactamases, which are encoded by bla_{CTX}, bla_{TEM} and $bla_{\text{CTX-M15}}$, $bla_{\text{OXA-1}}$, respectively. β-Lactamases are responsible for the resistance against β-lactam antibiotics such as penicillins and cephalosporins.[39] Both *E. coli* strains were resistant against tetracyclines and chloramphenicol. The resistance against tetracyclines is caused by the formation of an efflux pump in the membrane (gene: *tet*A), which result in a decreased accumulation of the antibiotic in the microbial cell.[40] The gene *cat*B encodes chloramphenicol acetyltransferase, which acetylates chloramphenicol. The resulting product cannot bind to the microbial target. Both *E. coli* strains possess mutations (genes for 21181, 21182: *par*C p.S80I, *par*C p.E84V; gene 21182: *par*E p.I529) in the binding site for fluoroquinolones during DNA supercoiling. The strain 21182 has also different mutations in genes for gyrases/topoisomerases II (genes: *gyr*A p.S83L, *gyr*A p.D87N) and a protection protein (gene: *qnr*B19) against fluoroquinolones.[41] The strain 21182 is also resistant against aminoglycosides (genes: aac(6′)-Ib-vr, *aad*A5) through an acetylation of the antibiotic and an alternative folic acid metabolism (*sul*1/*dfr*A), which is responsible for the resistance against sulfonamides and trimethoprim.[42]

Although the proof for a direct connection of the antibiotic resistance and the susceptibility against plasma is missing, some speculations must be allowed and may lead to further research in this field. Thus, the mutations in the genes *par*C, *par*E, and *gyr*A may be of special importance for the antibiotic resistance as well as for the reduced efficacy of plasma treatment. In these cases, the formed proteins are, in particular, involved in the replication and repair of the nucleic acid—genetic information—which may be an essential part in surviving the attack of plasma-generated reactive species. Thus, gyrases/topoisomerase II (gene: *gyr*A) is responsible for the negatively supercoiling of double-stranded DNA.[43] Afterward, the topoisomerase IV (genes: *par*C, *par*E) can unlink the DNA during replication.[44] These enzymes are involved in DNA replication and also in DNA repair, which consequently may enable the compensation of plasma-induced DNA damages.

The strain 21182 possesses the gene *dfr*A such as *dfr*A12 encodes a dihydrofolate reductase, which is an enzyme in folic acid metabolism and is, thus, also responsible for the synthesis of amino acids or DNA precursors. The protection and repair of the DNA must be of special importance for microorganisms during or after plasma treatment. Thus, in a study with plasma-sensitive mutants, 87 genes were identified that possessed a protective function against a plasma that was applied for the treatment of bacteria-containing solutions.[45] These genes encoded, for example, transcriptional factors to prevent DNA alkylation and initiate DNA repair mechanisms. Other genes encoded structural proteins involved in motility or biofilm formation as well as iron-containing proteins involved in pathways such as respiratory electron transport chain.[45] In general, DNA strand breaks or DNA lesions possibly caused by modified nucleobases can be formed by (V)UV radiation or reactive species such as ozone or hydroxyl radicals.[46,47] Such effects were also described for plasma treatments using plasmid-containing solutions[48] or plasmid solutions dried on glass carriers.[49] The induction of DNA repair mechanisms after plasma treatment of bacteria-containing solutions was described for *Bacillus subtilis* by Winter et al.[50] In consequence, microorganisms with functional and optimized DNA repair processes may be less sensitive toward plasma treatment, which may explain, at least in part, the lower inactivation of the *E. coli* strains 21181 and 21182 (compared with *E. coli* K12).

5 | CONCLUSION

Indoor air quality is an important issue in medical or health care facilities; in particular, the dissemination of pathogenic microorganisms has to be prevented. In this regard, the developed PDM using a CAP as an antimicrobial agent may be a novel tool for an effective air cleaning process.

The achieved inactivation of the tested microorganisms by 89% confirms the potential of CAP for decontamination. Thus, the apathogenic *E. coli* K12 was inactivated by 89%, whereas the multidrug-resistant *E. coli* strains 21181 and 21182 were reduced by 40% and 36%, respectively. The differences may be attributed to the varied susceptibility of the tested microorganisms toward antibiotics due to morphological or physiological changes.

The inactivation of microorganisms was affected by plasma intensity and size of the plasma treatment area. Thus, doubling the plasma treatment length was more effective than using a double-sided treatment. The presence of ionic wind had no influence on the cfu reduction of *E. coli*. In addition, the influence of an upstream (in front of plasma source) and downstream (behind plasma source) nebulization of the microorganisms showed that passing through the plasma source (direct treatment) led to a higher antibacterial efficacy than the indirect treatment (whereby a clear differentiation between direct and indirect treatment was not possible under the chosen test conditions).

The introduced technology enables the inactivation of microorganisms including multidrug-resistant strains to improve air quality and to reduce the risk for infections. A further development of the plasma source is necessary to increase the antimicrobial efficacy. Thus, a plasma generation not only as a surface plasma but also directly in the airflow may allow an enhanced treatment and thus an inactivation of more microorganisms. Additionally, the efficacy against viruses should be determined.

Beyond the utilization of the plasma module in medical settings, an application in the food-producing industry, such as in stables to improve animal health, is also possible.

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