Abstract—Drug-resistant bacteria, prions, and nosocomial infections underline the need of more effective sterilizing technologies. The cold plasma technology is expected to bring a benefit in this context. Six different plasma sources, based on printed circuit boards, were evaluated fourfold. This includes measurements of the power consumption, the ignition behavior by an ICCD-camera, and ozone formation by absorption spectroscopy at 254 nm. To evaluate the biocidal effect, four bacterial test series were performed with Escherichia coli. The entirety of the tests analyzes the plasma inactivation process from the input parameters to the desired biocidal effect. The discharge current and time-resolved ignition behaviors indicated a simultaneous formation of filaments at the beginning of the negative half-cycle. The dynamics of the ozone production showed a saturated exponential growth upon a maximum value of 435 ppm. Additionally, the microbiological test series unveiled differences between the plasma source concepts. A total reduction rate of $10^{-4}$ within a minute was achievable. An air flow through slits within the plasma sources destabilized the plasma. Minor changes of the electrode geometry changed all measured parameters. Hence, to develop a pathogen inactivating plasma source, these results recommend a comb-shaped electrode design, which is laminated on a dielectric.

Index Terms—Absorption spectroscopy, cold atmospheric plasma (CAP), inactivation rate, ozone, power density, time-resolved ignition behavior.

I. INTRODUCTION

THE AMOUNT of nosocomial infections [1] and the rising number of resistant bacteria due to the application of antibiotics [2] emphasize the importance of preventive hygiene in the medical sector. Since there are pathogens which develop a resistance to alcohol-based sterilants [3] or need a longer exposure to heat than it is used in the daily business [4], the technology has to take a step toward more effective sterilization methods. One promising focus of recent research is the plasma sterilization [5], [6].

Plasma consists of electrons, ions, neutral gas, and several reactive molecules. When working with the plasma afterglow of a cold atmospheric plasma (CAP), the charged particles recombine before they can reach a surface by diffusion. Hence, long-living reactive oxygen and nitrogen species (RONS) are considered to cause the main inactivating effect [7], [8]. Low temperature, high inactivation rate, and the absence of plasma resistant pathogens are only a selection of many advantages of the plasma technology. Despite that and the current research, there is still a lack of atmospheric plasma applications for the reprocessing of invasive medical devices in the market. The low temperature of CAP enables the treatment of thermolabile surfaces, such as endoscopes, pharmaceutical goods, or dental instruments [9], [10]. Their reprocessing is very complicated and susceptible to failures, which lead to a high rate of contaminated devices [11]. This emphasizes the urgent development of new applications.

In this article, six new designs of CAP sources are considered to understand the impact of the electrode design on the plasma physics, the plasma composition, and the inactivation capability. Therefore, they become fourfold characterized by their electrical power consumption, time-resolved ignition behavior, ozone production, and inactivation capability. Two of the six sources use an air flow of 1 m$^3$/min to improve the distribution of the RONS. These sources are based on dielectric barrier discharge (DBD), which uses a dielectric to limit the discharge current and to distribute the charges over the surface. A specific setup is the surface micro discharge. Both electrodes (one as a grid) are attached to the dielectric and the plasma is generated within the gaps of the grid [12]. The power consumption drops from volume-, surface-, to coplanar surface-DBDs, which have coplanar electrodes inside of the dielectric [13]. Adapting the coplanar surface-DBD technology, the sources are based on thin printed circuit boards (PCBs) with comb-shaped electrodes [14].

II. EXPERIMENTAL SETUP

The plasma sources were the results of a long development process, which was focused on a scalable and PCB-based design. As a substrate, the standard Flame Retardant FR-4 material, which is a dielectric made of an epoxy resin and a fiberglass inlay, is used. Copper layers are laminated on both sides, which can be etched in every desirable shape. Thus, the advantages are fast prototyping of different electrode geometries and the absence of air between the electrode and the dielectric. A drawback is that the FR-4 does not have a good ozone resistance. Additionally, the dielectric has a thickness of 0.5 mm and a dielectric strength of 20 kV/mm, which results in a maximum supply voltage of 20-kV peak-to-peak (P2P) [15]. Due to these conditions, the shelf life of
these plasma sources was about 15 h. Yet, the improvements of the inactivation capability and the reduction of the power consumption underline the relevance of the plasma research.

The voltage was supplied from a high-voltage amplifier (Trek 10/40A-HS), which used the initial sine signal from a signal-generator (Rhode & Schwarz HM8150) of 3 kHz. The signal was amplified 1000-times up to a maximum value of 10 kV.

Several tests during the development process, including the evaluation of the discharge behavior and antibacterial capability, lead to six different plasma source designs, which are displayed in Fig. 1.

Since the diffusion of the reactive species to the contaminated surface is an inefficient process, an airflow has been proposed to have three advantages. First, the reactive species will reach the surface faster. Second, more reactive species will be directed toward the substrate, and third, the plasma components with a short lifetime will have a contribution to the biocidal effect, because they reach the surface before dissociation.

The six plasma sources can be grouped in three conceptual geometry designs. The first has displaced electrodes, like (1 & 3). The geometries (2) and (4) have parallel electrodes, which lead to a more uniform electric field and more homogeneous plasma. As Fig. 1 shows, the area of the plasma glow is restricted to the edges of the electrodes. Combining the advantages of both geometries, the plasma concept of geometry (5 & 6) has displaced electrodes without a horizontal distance between the electrodes. The high-voltage electrode was wider than the grounded electrode. Hence, the electrons can accumulate over the dielectric and the plasma glow is larger than that in the case of (2 & 4).

A. Electrical Power Consumption

The electrical measurements focus on the variation of the discharge current and its phase to the voltage. The oscilloscope (Tektronix DPO 2012B) acquired the voltage by the analog output of the high-voltage amplifier and the current with a Rogowski coil, which enables the evaluation of the filaments [16]. From this point, the analysis of the surface power density, power, reactive power, resistivity, and capacitance was possible. The root mean square data of the voltage (RMS), current (RMS), and phase were averaged over 125 000 cycles. Additionally, the surface temperatures of the plasma sources were measured to verify the positive dependency on the surface power density. Based on the findings of the electrical characterization, the operating voltage was chosen for later measurements. The operating frequency was constantly 3 kHz. Since the high-voltage amplifier has a power limit of 400 W and the impedance was influenced by $\frac{1}{\omega C}$, this frequency minimizes the need of the reactive power and, thereby, enlarges the range of possible voltages, while the generated plasma was still homogeneous.

B. Time-Resolved Ignition Behavior

Analyzing the ignition behavior of the filaments requires a very high time resolution. This can be achieved by triggering and shifting the start of the frames. The photons were recorded by the ICCD-camera PI-MAX 4 from Princeton Instruments with an intensifying factor of 100, which was needed because of the weak plasma glow.

To evaluate 100 frames of one sine period with a frequency of 3 kHz, it was necessary to have a gate width of 3333 ns. In every period, one frame was recorded. The gate delay was increased constantly by 3333 ns after each frame. Hence, every frame can be mapped to a defined position within one period, which was fully recorded after 100 frames. This enables the analysis of filaments and their formation.

C. Ozone Production

Besides the other reactive species, ozone was expected to have a major influence on the inactivation performance [17], [18]. Therefore, the ozone concentration was measured for every plasma source at every possible operational voltage by absorption spectroscopy. This revealed the dependencies on power consumption and geometry. The measurement was made within a cubic volume of 5 l.

The principle of the absorption spectroscopy uses UV-light with a wavelength of 254 nm. A deuterium lamp (Ocean
Insight D-2000) generates a wide UV spectrum. The intensity at the wavelength of 254 nm was measured by a spectrometer (OceanOptics HR-4000). This wavelength is in the middle of the Hartley-band of ozone, which has a high photo-absorption cross section of $\sigma_\lambda = 1.15 \times 10^{-21}$ m$^2$ and the other molecules do not interfere there [19]. Hence, only this wavelength becomes absorbed by the ozone in the plasma afterglow. For this purpose, the experimental setup has an absorption path, with a length of $l = 20$ mm. This path was placed 18 mm above the plasma source. Within this defined length, the UV-light is sent through the ozone containing plasma afterglow. At the end of the optical measurement setup, the intensity was monitored before the plasma ignition, during the operation of the plasma source and after the plasma was extinguished. This gives a typical U-shaped sequence, which enables the comparison of the initial reference intensity ($I_0$) and the intensity during plasma operation ($I_1$).

Based on the law of Beer–Lambert, this intensity ratio ($I_0/I_1$) can be correlated to the absorption events/ozone concentration ($c$)

$$\log_{10}(I_0/I_1) = \sigma_\lambda \cdot c \cdot l.$$  \hspace{1cm} (1)

The plasma was ignited for minimum 15 min to evaluate the dependencies of the ozone dynamics on the heating and quenching effect. The quenching effect is the production of nitrogen species instead of ozone [17]. After extinguishing the plasma, the ongoing measurement proofs if the reference intensity was reached after the dissociation of the ozone.

To achieve a good signal to noise ratio, an integration time of 400 ms for each data point was used in every measurement. Also, an electric dark correction was applied to reduce the offset of the sensor.

D. Inactivation Capability

Based on the measurements of the electrical characterization, the operational voltage was chosen, respectively, to the power surface density. The power density of the four sources (1)–(4) was set to 0.427 W/cm$^2$, because with these settings, (2) produced the highest ozone concentration. Due to the restricted operational voltage of 6 kV for geometry (6), the power density of the third plasma source concept (5 & 6) was set to 0.288 W/cm$^2$. For the evaluation of the inactivation performance, a microbiological study on nonpathogenic strain K-12 of Escherichia coli (E. coli) was performed. To prepare the experiment, the bacteria were transferred from an agar plate to 10 ml of the nutrient medium lysogeny broth (LB) via an inoculation loop. After mixing on a test tube shaker (Genie Vortex 1), the solution was incubated at 37°C for 24 h. Then, the optical density ($OD_{600}$) was set to 1 by diluting the culture with pure LB medium. The OD is the relative optical transmission of the bacteria culture to the pure medium at a wavelength of 600 nm.

The resulting solution was diluted down by $10^{-4}$ for the exposition to plasma and down by $10^{-6}$ for the control measurement. Four series of tests were applied to every plasma source. The bacteria were spread over the agar plates with a Drigalski cell spreader. Each test series contained of 108 agar plates with 18 per plasma source. This number contains of two dilutions at three exposure times with three agar plates per case. Fig. 2 shows the experimental setup for the plasma exposure of the inoculated agar plates. The plasma source was placed onto a U-shaped frame 2 cm above the petri dish. The resulting volume around the petri dish is 0.21 l, where the ozone saturation was reached after 3 s. Hence, the effect of an increasing concentration during the exposition can be neglected.

Every colony forming unit (CFU) was counted manually after 24 and 48 h of incubation. This enables the differentiation of fast growing colonies in the first step and the identification of slow growing colonies in the second step. However, the relative difference of both measurements has never exceeded 10%.

III. RESULTS

A. Electrical Power Consumption

The operational voltage was set as a P2P-value at the sine generator. With voltage, current, and phase, the active power, capacitance, and resistance were derived. With a frequency of 3 kHz, the plasma ignited homogeneously and the reactive power was reduced, which limited the combinations of the available voltages and frequencies.

Since the current was defined by the plasma source, only voltage and frequency were the input parameters. Therefore, the power was proportional to the square of the voltage, which was verified for every plasma source.

The free electrons in the plasma influenced the systems’ capacity and resistance, which were evaluated. Additionally, the ratio of the active power to the surface gives the surface (active) power density, which can be chosen from 0.05 to 0.57 W/cm$^2$ with respect to the plasma source concept. The surface power density was expected to be a predictor of the ozone concentration [17], [18].

Increasing supply voltages lead to a higher capacity and lower resistance, because the number of free electrons increased. Except in case of plasma source (5), which shows an increasing resistance. Due to resistive heating, the temperature of the plasma sources reached up to 64°C with increasing supply voltages.

Fig. 3 shows the operational voltage (black) with 14-kV P2P at 3 kHz and the discharge current (blue). Its course was representative for all plasma sources and shows two plasma ignitions during one period of the voltage. Increasing
Fig. 3. Operational voltage (black) and discharge current (blue) of plasma source (1) at 3 kHz and 14 kV; averaged over 4 (left) and 32 (right) cycles.

Representative discharge current for all plasma sources.

the voltage causes stronger discharges, which unveil in higher currents. The spikes indicate that the plasma is filamentary [20]. Even if the negative current has a stronger gradient and spikes, it was not possible to clarify, if this was caused by stronger and/or more filaments. Therefore, images of the time-resolved ignition behavior with the ICCD-camera were made. Due to the high variation of spikes, the data has to be averaged for the trigger. Fig. 3 compares two different averages of the same object and operational parameters. The smoothing effect of an average over 32 cycles is displayed. This shows that statistical and systematical effects influence the formation of the filaments.

The feed through led to higher discharge currents, especially during the ignition phase of the plasma. Since the air flow did not influence the discharge current at all, differences of the power consumption were a result of the geometries. Even though the visible inspection, displayed in Fig. 6, shows a destabilization of the plasma, which shows the reduction of filaments, when the airflow was applied, this was not recognizable in the measured currents.

B. Time-Resolved Ignition Behavior

The results were 1024 × 1024 matrices with an intensity value for each pixel. These data were plotted as false-color images. Black areas indicate no emissions from the plasma and violet to white areas represent the detection of photons. Those were caused by the emission of the plasma glow.

Figs. 4 and 5 show the most relevant frames of the plasma sources in the negative half-cycle, which show the filaments and their systematical distinctions in dependency of the geometry. To see the dynamics, other frames and results can be requested from the authors. Due to the distance between the electrodes, the plasma of geometry (1) forms arc-like filaments. In the left part of Fig. 4, several ionization events unite to one streamer toward the grounded electrode. Another frame (not shown here) shows the opposite direction of the electrons. The electrons distribute above the dielectric-covered high-voltage electrode.

Figs. 4 and 5 show the simultaneous discharge of the filaments at the zero-crossings of the voltage. Due to the different geometries and the resulting different electric fields, the exact timing varies between the plasma sources.

Fig. 4. False color images of filaments within the negative half-cycle, ground electrode in orange, high-voltage electrode in red (left), grounded electrode is on top of the high-voltage electrode (right), and gate-width: 3333 ns.

Fig. 5 shows the simultaneous discharge of the filaments at the zero-crossings of the voltage. Due to the different geometries and the resulting different electric fields, the exact timing varies between the plasma sources.

The characteristic for concept (2) was the 1-mm wide areas at the edges of the electrodes, where the plasma ignites. There were only few filaments, which reach up to the dark space between the small lines of the plasma glow.

One of the most interesting frames was displayed in the left part of Fig. 5 for geometry (3). The ignition behavior of this plasma source was remarkable. First, the plasma ignites above the dielectric surface and higher voltages lead to a plasma formation within the slits in the dielectric. Fig. 6 shows the plasma source (3) with and without an applied airflow, which causes a weaker plasma and spaces without discharges.

Since the positions of the filaments were the same, the airflow does not affect the ignition behavior of geometry (4), because its filaments does not span over the slits. Based on the first two plasma sources, the electrode concept (5 & 6) shows the desired ignition behavior. Since the edges of the high-voltage and grounded electrodes do not have a horizontal distance, the plasma ignites as homogeneously as plasma source (2). The 1.5-mm wide dielectric-covered electrodes spread the plasma uniformly over the entire dielectric surface. The length of the filaments in the right part of Fig. 5 indicates that they were longer than the half of the high-voltage electrode width. Increasing this width to 3 mm leads to the formation of a darker area in the middle.
C. Ozone Production

With the results of the electrical power consumption, the ozone concentration was measured at all available operational voltages. During all measurements, the reference intensity was reached, when the plasma was shut off, which is visible in Fig. 7. There the black line indicates the reference concentration, based on the initial intensity \(I_0\) in Beer–Lambert’s law, see (1). There were three different dynamics of the ozone concentration. As Fig. 7 shows, the concentration is constant after reaching a saturation level (red). But when a plasma source heats itself up, there was a negative linear trend (blue). Since the air flow was an active cooling, the thermal effects were stronger without it. The destabilization of the plasma by the air flow leads to a lower ozone production after few minutes, because of the small number of filaments. In the case of the wide high-voltage electrodes (5 & 6), an exponential drop of the concentration occurs (green). To display the dynamics of the ozone formation in dependency of the power density and time, Fig. 8 shows the saturation concentrations and the values after 15 min. This indicates a transition from production to reduction depending on the surface power density and the electrode geometry. The most tremendous decrease shows geometries (3) and (5), which reduce the concentration down to a nonsignificant level. The difference of those sources to geometry (2) is visible in Fig. 8. The ozone concentration is reduced at lower surface power densities than it is the case for geometry (2). On the contrary, geometry (4) did not show any significant changes at all. Therefore, the influence of the geometry cannot be neglected.

D. Inactivation Capability

The biocidal effect is the desired capability of the RONS produced by the plasma. To understand the implications of the electrode geometry for the inactivation, a microbiological study was conducted to evaluate the differences of inactivation capability between the plasma sources. Therefore, the D-values can be estimated and the best plasma source can be identified. But a much wider theoretical contribution can be made by the correlation of these results to the other measurements.

Before the inactivation performance was estimated, the data were screened. Plates with too much CFU (≥300) or outliers were removed from the statistics. Then, for every test series, the initial bacteria load was evaluated with the control plates. The counted CFUs divided by the initial CFUs gives the inactivation performance, which was estimated for every agar plate.

Therefore, Fig. 9 shows the means of the inactivation performance for every plasma source [(3) and (4) with the air flow] and exposure time. Similar to the gas composition in Fig. 8, Fig. 9 reveals differences of the inactivation capability, even when the power consumption was constant. The difference of the sources (1)/(5) to (2) are interesting. Since the ozone is expected to be a predictor of the inactivation capability, plasma source (2) reaches higher concentrations but the inactivation rate of (1) and (5) is better [12]. They inactivate up to \(10^4\) CFUs within a minute, which corresponds to a D-value of 15 s. The values, which represent decreasing
Fig. 8. Dynamics of ozone production for all plasma sources without an air flow. Ozone concentration at the saturation level (left) and ozone concentration after 15 min (right).

Fig. 9. Inactivation rates of all plasma sources, (3) and (4) with airflow.

...inactivation rates with increasing exposition times, as (3) and (6) show, were not theoretically supported and were a result of statistical fluctuations.

E. Air Flow Analysis

All data are analyzed with respect to the air flow (1 m³/h). The electrical parameters were not influenced by an applied air flow through the slits. A much different behavior unveil the results of the time-resolved ignition behavior. The slits bring up virtual electrodes and the air flow destabilizes the formation of filaments within the slits. This is visible in Fig. 6. During the first 10 s after the air flow was applied, the plasma becomes weaker and does not ignite homogeneously. Either the reason is a deformation of the filaments or a higher recombination due to the higher pressure by the air flow. The air flow changes the formation of filaments and the ozone production. Therefore, the microbiological studies reflect those results by the inactivation capability, which was reduced three to four orders of magnitude.

IV. CONCLUSION

This work supported the applicability of thin PCBs as plasma sources. The resulting advantages are the fast prototyping compared to dielectrics, such as glass or ceramics and the lower power consumption compared to volume DBDs [13]. Additionally, PCBs have laminated electrodes, therefore, it is not possible that gases can be between the electrodes and the dielectric.

The electrical analyses showed that an increasing voltage causes stronger discharges, which results in higher currents. As Table I shows, surface power densities of 0.05 to 0.57 W/cm² were achievable. The spikes in Fig. 3 indicated the formation of filaments in the plasma [20]. The strong smoothing effect of an average over 32 cycles was recognizable. The statistical distribution of the filaments was caused by locally increased electric fields, which are influenced by previous streamer formation, supply voltage, and electrode geometry [20]. It was also visible that the discharge starts close to the zero crossing of the voltage and the simultaneous ignition of the filaments is not a statistical effect. The shape of the measured discharge current revealed the dynamic of the filaments and was associated to the frames of the time-resolved ignition analysis. This measurement clarifies that the higher discharge current in the negative half-cycle was caused by more coincident filaments. Their first occurrence is influenced by the gap-width of the electrodes and the amount of the residual charges on the dielectric, because they increased the electric field. These factors lead to strong differences of the filaments’ shape, which are summarized in Table I. Longer discharge gaps lead to arc-like filaments and due to the lower electric field, also to a shorter duration of the plasma ignition. All plasma sources have in common, that the discharge at the beginning of the negative half-cycle was the strongest and consists of simultaneously igniting filaments. The combination of a short electrode distance and residual electrodes can lead to a plasma ignition before the zero-crossing of the voltage.

Additionally, this analysis verifies the concept of the virtual electrodes, which lead to a discharge above the dielectric and not in the slits at low voltages. A higher electric field leads to accumulation of electrons at the edges of and filaments over the slits. The displayed frames unveil that this behavior was a result of electron accumulation at the sides of the slits. These edges act like virtual electrodes. After increasing the voltage, the electrons reach to the opposite edge of the slits and form a plasma glow, which is shown in the left part of Fig. 5.

| Geo. | Power density range [W/cm²] | Power density shape | Ozone concentration [ppm] | Log-reduction |
|------|-----------------------------|--------------------|---------------------------|---------------|
| (1)  | 0.32 - 0.55 | 0.43 | arc-like | 212 | 5 |
| (2)  | 0.06 - 0.57 | 0.43 | short | 435 | 4 |
| (3)  | 0.43 | 0.43 | virtual electr. | 376 | 1 |
| (4)  | 0.05 - 0.45 | 0.43 | virtual electr. | 125 | 2 |
| (5)  | 0.07 - 0.57 | 0.29 | curved | 82 | 5 |
| (6)  | 0.29 | 0.29 | curved | 184 | 4 |
The transition from ozone to nitrogen mode was expected to be constantly around 0.1 W/cm² [17], the measurements indicate that there was a strong influence of the plasma source concept. On the one hand, (1) and (2) can reach 0.32 W/cm² without a drop of the ozone density. On the other hand, even low surface power densities of 0.05 W/cm² can lead to a decrease of the concentration in the case of geometry (5), which is visible in Fig. 8. After 15 min, higher power densities have lowered the concentration to a nonsignificant level for plasma source (3) and (5). This unveils that the transition mode had no constant power surface density value and depends on the geometric parameters of the plasma sources. Hence, the power density is not the only predictor of the ozone concentration.

Geometry (2) produces the highest ozone concentration of 435 ppm. The ozone density shows a positive dependency on the supply voltage as well as the ozone production. In the case of the wide high-voltage electrodes (5 & 6), an exponential drop of the concentration occurs, which is displayed in Fig. 7 (green). This leads to the assumption, that RNS are quenching of the reactive oxygen species with higher power in line with previous findings [17]. The formation of curved filaments occurs only with the plasma concept (5 & 6). The linkage of the filaments’ shape to the gas composition will become addressed by further research.

Table I shows the remarkable differences regarding filaments’ shape, ozone production, and inactivation capability of the plasma sources (1), (5), to (2). Even though (2) has a higher ozone concentration, (1) inactivates up to 10⁶ CFUs within a minute, which corresponds to a D-value of 15 s. This capability is comparable to plasma source (5), which uses a lower surface power density but should reach the quenching effect. Those plasma sources have inactivation rates of 10⁻⁴–10⁻⁵, which were the best results of all geometries. The RNS of (5) was expected to cause a higher inactivation performance [8]. Yet, Fig. 8 does not show an inactivation from minute 2 to 3, when the quenching effect produces RNS.

The air flow in this case destablizes the plasma and extinguishes the filaments. Due to that, the worst case (3) shows a drop of the inactivation performance of three to four orders of magnitude. Table I emphasizes this behavior. It is proposed that the streamers were deformed and extinguished, as Fig. 6 shows. The suggested improvement of the RONS’ distribution by this air flow compared to diffusion has to be neglected.

Finally, it is to state that the electrode geometry influences the plasma chemistry and inactivation capability. Therefore, it should be defined with respect to the scope of application. The results recommend displaced electrodes to improve the inactivation. Yet, ozone formation increased, when parallel electrodes and spaces with a darker plasma glow are used.

The PCB-technology is a promising process for plasma source development. Yet, epoxy resin-based dielectrics have a limited lifetime of 15 h. Therefore, further development effort will be invested in PCB-based DBDs with other dielectric materials. This should make the plasma inactivation the next disinfection technology of the future, which should reduce nosocomial infections and durations of reprocessing cycles.