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Contact-free cold atmospheric plasma treatment of *Deinococcus radiodurans*

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Abstract In this study we investigated the sensitivity of *Deinococcus radiodurans* to contact-free cold atmospheric plasma treatment as part of a project to establish new efficient procedures for disinfection of inanimate surfaces. The Gram-positive *D. radiodurans* is one of the most resistant microorganisms worldwide. Stationary phases of *D. radiodurans* were exposed to cold atmospheric plasma for different time intervals or to ultraviolet C (UVC) radiation at dose rates of 0.001–0.0656 J cm^{-2}, respectively. A methicillin-resistant *Staphylococcus aureus* strain (MRSA) served as control for Gram-positive bacteria. The surface microdischarge plasma technology was used for generation of cold atmospheric plasma. A plasma discharge was ignited using ambient air. Surprisingly, *D. radiodurans* was sensitive to the cold atmospheric plasma treatment in the same range as the MRSA strain. Survival of both bacteria decreased with increasing plasma exposure times up to 6 log_{10} cycles (>99.999 %) within 20 s of plasma treatment. In contrast, UVC radiation of both bacteria demonstrated that *D. radiodurans* was more resistant to UVC treatment than MRSA. Cold atmospheric plasma seems to be a promising tool for industrial and clinical purposes where time-saving is a critical point to achieve efficient disinfection of inanimate surfaces and where protection from corrosive materials is needed.

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Abbreviations

CAP	Cold atmospheric plasma
CFU	Colony-forming units
DBD	Dielectric barrier discharge
E. coli	*Escherichia coli*
Gy	Gray
MRSA	Methicillin-resistant *Staphylococcus aureus*
SMD	Surface microdischarge
UV	Ultraviolet

Introduction

Deinococcus radiodurans is one of the most interesting organisms in the microbial world. Resistance against ultraviolet (UV) radiation, oxidation, and desiccation makes it "the world's toughest bacterium," as stated in the *Guinness Book of World Records* [5, 14, 29, 38]. How organisms protect their key macromolecules and how they survive high doses of ionization radiation even up to 5,000 Gy without inactivation is one of the key fundamental questions in basic science. Arthur W. Anderson first identified this red-pigmented, nonmotile, Gram-positive bacterium in 1956 as a contaminant of irradiated canned meat [1]. Since then, the crucial reasons behind the survival strategy of *D. radiodurans* have been studied extensively, and there are plenty of explanations for its resilience.

Under identical conditions, hundreds of genomic double-strand breaks form at the same rate in *E. coli* and *D. radiodurans* during high-level exposure to ionization

radiation. Interestingly, and in contrast to *E. coli*, *D. radiodurans* reassembles accurately before initiation of the next cycle of cell division [11]. The four to ten copies of the genome rather than the usual single copy in a single cell help the organism to recover from radiation exposure. Levin-Zaidman et al. [22] highlighted the condensed nucleoid structure of *D. radiodurans*, which facilitates the DNA repair system by restricting diffusion of DNA fragments. On the other hand, it was mentioned by Daly et al. [16] that the high concentration of Mn(II) ions present in *D. radiodurans* facilitates tolerance of high radiation doses. The involvement of classical repair enzymes with some specialized properties and novel genes were also responsible in nonhomologous end-joining or oxidative stress protection [4, 11, 12, 17, 37, 39].

The organism's efficiency in terms of strong DNA repair, DNA damage export, desiccation, and starvation recovery lead to difficulties in disinfecting *D. radiodurans*. *D. radiodurans* can be found almost everywhere, ranging from organic nutrient-rich environments such as soil, sewage, animal feces, and processed meats to nutrient-poor environments such as dried foods, room dust, medical instruments, and textiles. It can even survive for 6 years in a desiccator with 10 % viability [3]. These facts suggested that scientists consider *D. radiodurans* as a model organism in fields where complete disintegration of microorganisms is required: in the field of medical sciences, where sophisticated instruments and operating tools have to be decontaminated properly without losing their functionality, and in the planetary protection area, where sterilization is very important to prevent both accidental contamination and infection of the outer world with terrestrial organisms, and/or accidental contamination of extraterrestrial samples returned to Earth with terrestrial organisms [40, 41]. Concerning traditional wet or dry sterilization processes, there are plenty of concerns about the loss of properties and functionalities of the diverse set of materials, electronic components, paints, and polymers used for the complex machinery surfaces of spacecraft. For these applications, new sterilization and disinfection methods are needed.

Cold atmospheric plasmas have high potential for sterilization, as they are easy to handle, economic, and very efficient [25, 26, 32, 36]. Cold atmospheric plasmas (CAP) consist of charged particles (electrons, positive and negative ions), excited atoms and molecules, reactive species [especially oxygen and nitrogen species (NO, NO_2, O_3, OH, O^*, etc.)], and photons (UV and visible). Recently, Cooper et al. [10] showed that nonthermal dielectric barrier discharge (DBD) plasma was able to inactivate *D. radiodurans* after 30 min of treatment on a dried inanimate surface. In their study, the treatments were done in "direct mode," which means that the bacteria samples and/or sample holder acts as a counterelectrode for the discharge [10].

Here in this study, a new surface microdischarge (SMD) electrode was investigated for contact-free plasma treatment without the necessity for the treated sample to act as a counterelectrode. Such contact-free plasma treatment could facilitate disinfection of bacteria persistent on dry inanimate surfaces in the future.

Materials and methods

Plasma device

A device producing cold atmospheric plasma was used for the treatment of living microorganisms. This plasma device consists of a box made out of plastic (Teflon and polyoxymethylene) incorporating an electrode for plasma production inside, as shown in Fig. 1. On one side of the box, a door is included. By closing the door, the produced plasma gas cannot escape and therefore is confined within. This plasma device is designed for treatment of a 96-well plate (sample), so that the maximum area to treat equals 9×13 cm^2. The plasma electrode is placed above the sample to treat. The distance between the electrode and the sample is adjustable and was set to 6 mm in our experiments. A plasma discharge is ignited by surface microdischarge (SMD) plasma technology using ambient air [25, 35]. This SMD electrode consists of a 0.5-mm-thick Teflon plate sandwiched by a brass planar plate and a stainless-steel mesh grid (line width 2 mm, opening 10 mm, height 1.5 mm). High voltage of 9 kV_{pp} with 1 kHz frequency was applied between the brass plate and the mesh grid, thereby producing plasma on the mesh side of the SMD electrode as shown in Fig. 2. The power consumption for the plasma discharge was approximately 20 W/cm^2 as measured by the Lissajous figure method using a 0.1 μF capacitance.

Since ambient air is used for plasma production, several different components are produced by the plasma discharge and delivered to the sample to treat, i.e., charged particles (electrons, positive and negative ions), reactive oxygen and nitrogen species (NO, NO_2, O_3, OH, O^*, etc.), photons (ultraviolet and visible), and heat. During the experiments, the door of the plasma device was closed; i.e., no gas exchange inside the device could take place. In our study, thermal effects can be ruled out, as the increase in gas temperature in the device for a treatment time of 10 min is at maximum 4 °C. The observed UV radiation between 280 and 420 nm in wavelength (measured with a HAMAMATSU optical spectrometer, TM-UV/VIS C10082CA) mainly results from N_2 molecules (N_2 second positive system) as shown in Fig. 3. The measured UV power

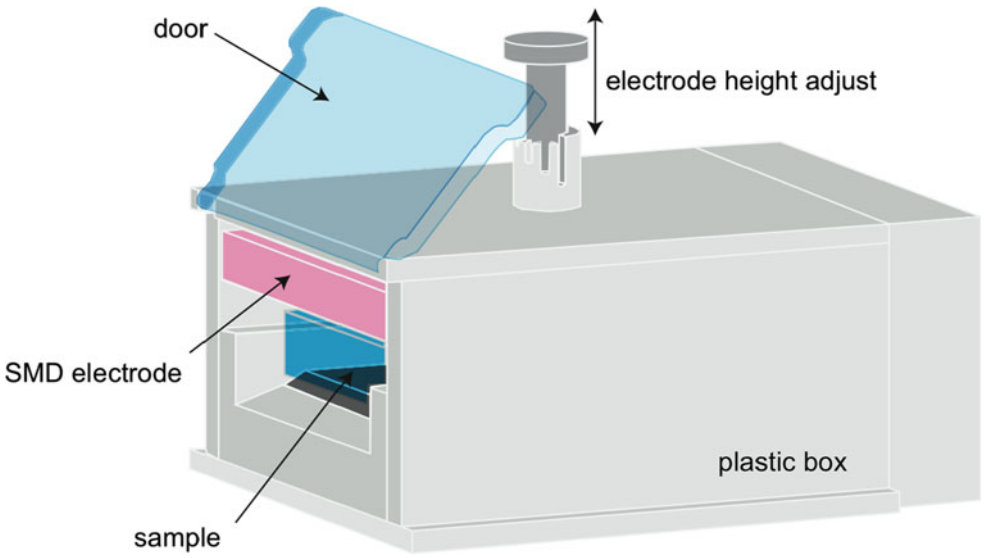

Fig. 1 Sketch of the used plasma device. The device contains one SMD electrode, and the sample to treat is placed below the electrode

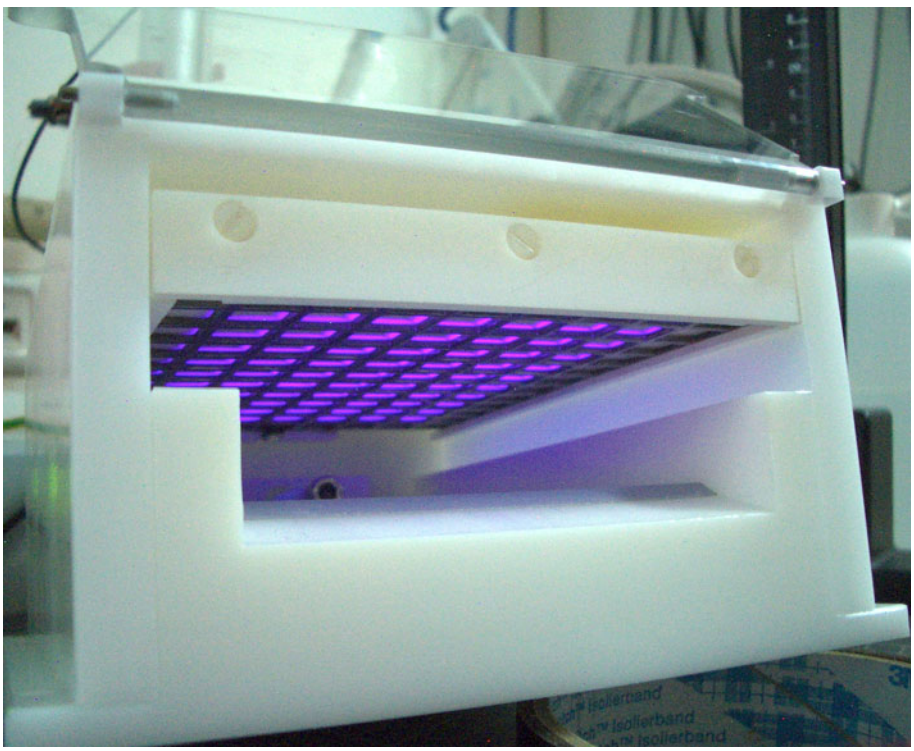

Fig. 2 Plasma discharge on the mesh of the SMD electrode. The SMD electrode is in action; the front door is open

density equals 25 nW/cm^2. Various experiments with bacteria showed that the UV photons alone (achieved by treating a bacteria sample with plasma filtered with a quartz glass) do not show any bactericidal property after 120 s of treatment, since the power was low and the main UV radiation was observed in the UVA region.

From these results, one can conclude that the UV emission produced by the plasma contributes very little to the bactericidal property of the plasma.

Using this SMD plasma device, the main components which contribute to bacterial inactivation are reactive species and charged particles. From the viewpoint of density, the reactive species are more important, since the distance between the SMD electrode and the sample leads to strong reduction of the charged particle density due to excitation, dissociation, attachment, and recombination processes. The measurement of produced ozone (using an UV absorption spectroscopy, model 400E; Teledyne

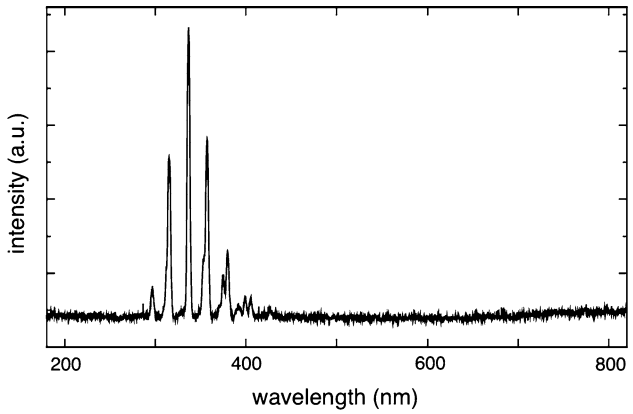

Fig. 3 Spectrum measured for the SMD plasma. Spectrum of the SMD plasma observed in front of the electrode at a distance of 6 mm. Main UV components in the range between 320 and 400 nm are produced from nitrogen molecules excited by electron impact

Advanced Pollution Instrumentation) in the closed plasma device after 60 s of plasma production was approximately 500 ppm. The NO_2 concentration was measured as around 3 ppm using a gas detector (Multiwarn II; Dräger AG, Germany).

Bacterial strains

The bacterial strains methicillin-resistant *Staphylococcus aureus* (MRSA: ATCC BAA-44) and *Deinococcus radiodurans* (ATCC BAA-816) were purchased from the American Type Culture Collection (Manassas, VA, USA). MRSA was grown aerobically at 37 °C in Mueller–Hinton broth (Carl Roth GmbH + Co. KG, Karlsruhe, Germany); *D. radiodurans* was grown aerobically at 30 °C using TGY media containing 5 g tryptone, 1 g glucose, 5 g yeast extract, and 1 g K_2HPO_4 per liter. For each bacteria strain, a 5-ml overnight bacteria culture was prepared. When the cultures reached the stationary phase of growth, the bacteria were harvested by centrifugation (200 g, 15 min), washed with 10 mM phosphate-buffered saline (PBS; Biochrom, Berlin, Germany) at pH 7.4 containing 2.7 mM KCl and 0.14 M NaCl, and suspended in PBS at optical density of 0.6 at 600 nm corresponding to 10^7 bacteria ml^{-1}.

Plasma treatment

Prior to plasma treatment, serially diluted aliquots (20 µl) of each bacterium were dropped on Mueller–Hinton agar plates or TGY agar plates using the Miles, Misra, and Iwin technique [23]. Thereafter, the plates were placed in a laminar flow cabinet until visible dryness (45 min). Then, the inoculated agar plates (samples) were placed in the plasma device (Fig. 1) and treated for 20, 40, 60, 120, and 300 s. The number of colony-forming units (CFUs) per milliliter was counted after 24 or 48 h of incubation at 37 or 30 °C for MRSA or *D. radiodurans*, respectively.

UVC treatment

For UVC radiation, a Biometra Transilluminator was used (Biometra FLX-20 M, Goettingen, Germany). The wavelength range of the UV 254 nm tube was narrow banded (254 ± 5 nm). The inoculated agar plates were placed inside the transilluminator without lids and radiated with 1, 5 or 65.6 mJ/cm^2 [4]. Again, the number of CFUs per milliliter was counted after 24 or 48 h of incubation at 37 or 30 °C for MRSA or *D. radiodurans*, respectively.

Statistical methods

All results are shown as medians, including the 25 and 75 % quartiles, which were calculated from the values of at least three independent experiments, each experiment being conducted in triplicate, using Prism 4 for Windows (GraphPad Software Inc., San Diego, CA, USA). The calculation (reduction of CFU/ml) was referred to untreated controls (not plasma treated). A reduction of at least three magnitudes of $\log_{10}$ of viable median numbers of bacteria was stated as "antimicrobial effective," regarding hand hygiene guidelines [7].

Results

As already mentioned in the "Materials and methods" section, ambient air was used for plasma production with the SMD device for treatment of *Deinococcus radiodurans*. This leads to production of several different components which are delivered to the bacteria samples, i.e., charged particles (electrons, positive and negative ions), reactive oxygen and nitrogen species (NO, NO_2, O_3, OH, O^*, etc.), photons (UV and visible), electric field, electrical current, and heat. During the treatment of the bacteria samples, the door of the device was closed; i.e., no gas exchange inside the device took place (Fig. 1). Thermal effects were ruled out, as the increase of the gas temperature in 10 min was at maximum 4 °C. The UV radiation mainly was observed from the N_2 second positive system between 280 and 420 nm in wavelength, as measured with an optical spectrometer (Fig. 2). The main content of the UV radiation belongs to the UVA wavelength range between 320 and 400 nm. The UV power density of the plasma was 25 nW/cm^2. UV photons alone (achieved by treatment of bacteria samples with plasma filtered by a quartz glass) did not show any bactericidal effect after a treatment time of 120 s, since the power was low and the main UV radiation was

observed in the UVA region (data not shown). Therefore, at least the UV produced by the plasma does not contribute to the bactericidal property very much. Furthermore, the produced electric field could cause stress on the cell wall of the bacteria and therefore lead to inactivation. However, from our experience so far, no inactivation of bacteria could be observed by the produced electric field. Concerning the electrical current through living organisms—in our case bacteria—we can conclude that it is negligibly small, because the plasma discharge is terminated in the electrode. Table 1 summarizes the produced components of the SMD discharge relevant for biomedical applications to the best of our knowledge. As listed in Table 1, the ozone concentration in the device after 60 s of plasma production was approximately 500 ppm. The NO_2 concentration was around 3 ppm.

First, the antimicrobial effect of cold atmospheric plasma was tested against *Deinococcus radiodurans* versus MRSA using the SMD device (Fig. 4a). The susceptibility to the plasma treatment was determined by applying plasma from the top to planktonic bacteria samples plated on nutritive agar plates and dried under laminar flow. As a control, the bacteria plates were left untreated. The overall bactericidal effect was determined as the $\log_{10}$ reduction of CFU after treatment with plasma compared with the untreated controls (Fig. 4a). The data indicate that *D. radiodurans* is killed very fast depending on the plasma treatment time. There was a considerable reduction of 5 $\log_{10}$ cycles of *D. radiodurans* within 20 s of plasma treatment, which corresponds to killing efficacy of 99.999 %. By increasing the plasma treatment time up to 5 min, the killing efficacy was even further enhanced. As expected, a plasma treatment time of 20 s was sufficient to achieve killing efficacy of 99.999 % against MRSA, which is in the same range as detected for *D. radiodurans* (Fig. 4a). No antibacterial effect was observed for the untreated controls.

To better understand the effect of cold atmospheric plasma on the bacterial viability, UVC radiation was performed as a control experiment. Both *D. radiodurans* and MRSA were irradiated with different UVC doses of 1, 5 or 65 mJ/cm^2 (Fig. 4b). Radiation at all tested UVC doses showed a dramatic effect on the survival of MRSA. In all cases, killing efficacy of 99.999 % was achieved. In contrast, *D. radiodurans* was still vital for the first two doses. Only the applied dose of 65 mJ/cm^2 induced a bacterial killing of 5 $\log_{10}$ cycles, which is in accordance with already published data [4] (Fig. 4b).

Table 1 Plasma characteristics

Charged particles	Electrons, ions	At the surface of the electrode: $\sim 10^{11}$ cm^{-3}
Reactive species	O_3	$\sim$500 ppm
	NO	<1 ppm
	NO_2	$\sim$3 ppm
	O, OH	Presence according to the literature
Heat		Max. 4 °C above the ambient temperature
Photons	UV, visible	UV power $\sim$25 nW/cm^2 mainly UVA
Static electric field		Max. 10^6 V/m
Electrical current through samples		Negligibly small, below 100 μA

Discussion

Contact-free cold atmospheric plasma and UVC radiation experiments were carried out for two Gram-positive bacteria differing in their DNA repair capacities. The plasma generated in this study led to excellent inactivation of ≥99.999 % for both *D. radiodurans* and MRSA, whereas UVC radiation did not inactivate *D. radiodurans*. These results indicate that a bacterial target other than the DNA of *D. radiodurans* might be damaged by cold atmospheric plasma, since this bacterium can repair DNA damage more efficiently than other bacteria such as MRSA or *E. coli* [11].

The nonequilibrium plasma chemistry is not a single process, but involves over 600 important chemical reactions, resulting in the generation of different reactive oxygen and nitrogen species, atomic O and N, radicals, H_2O_2, and O_3 by interaction with water vapor [20]. Therefore, a multihit process occurs during the plasma treatment, which in turn attacks not only the DNA, but also the outer cell wall areas and the membrane within a short time interval ($\sim$20 s) in order to induce irreversible damage. The energy input onto bacteria from the used cold atmospheric plasma includes several pathways, such as electron–ion recombination, de-excitation of reactive species, and thermal energy transfer by electrons. Since the bacteria samples were placed relatively far from the plasma discharge (6 mm), almost no electrons reach the bacteria. Therefore, the main energy input results from de-excitation of reactive species at the surface. This results in an average energy E released to the surface of bacteria of 5 eV. One-half of the energy is released to the outside of the bacteria, and the other half into the bacteria. Assuming that the density of very active reactive species is in the range of 10 ppb and high-density components such as O_3 are not very reactive, here we have a density of 10^{17} m^{-3}. The flux onto the bacteria with density of 10^{17} m^{-3} and thermal speed of 10^2 m s^{-1} is $F = 10^{17}$ m^{-3} $\times$ 10^2 m s^{-1} $= 10^{19}$ m^{-2} s^{-1}. Therefore, the energy flux Γ on the bacteria is

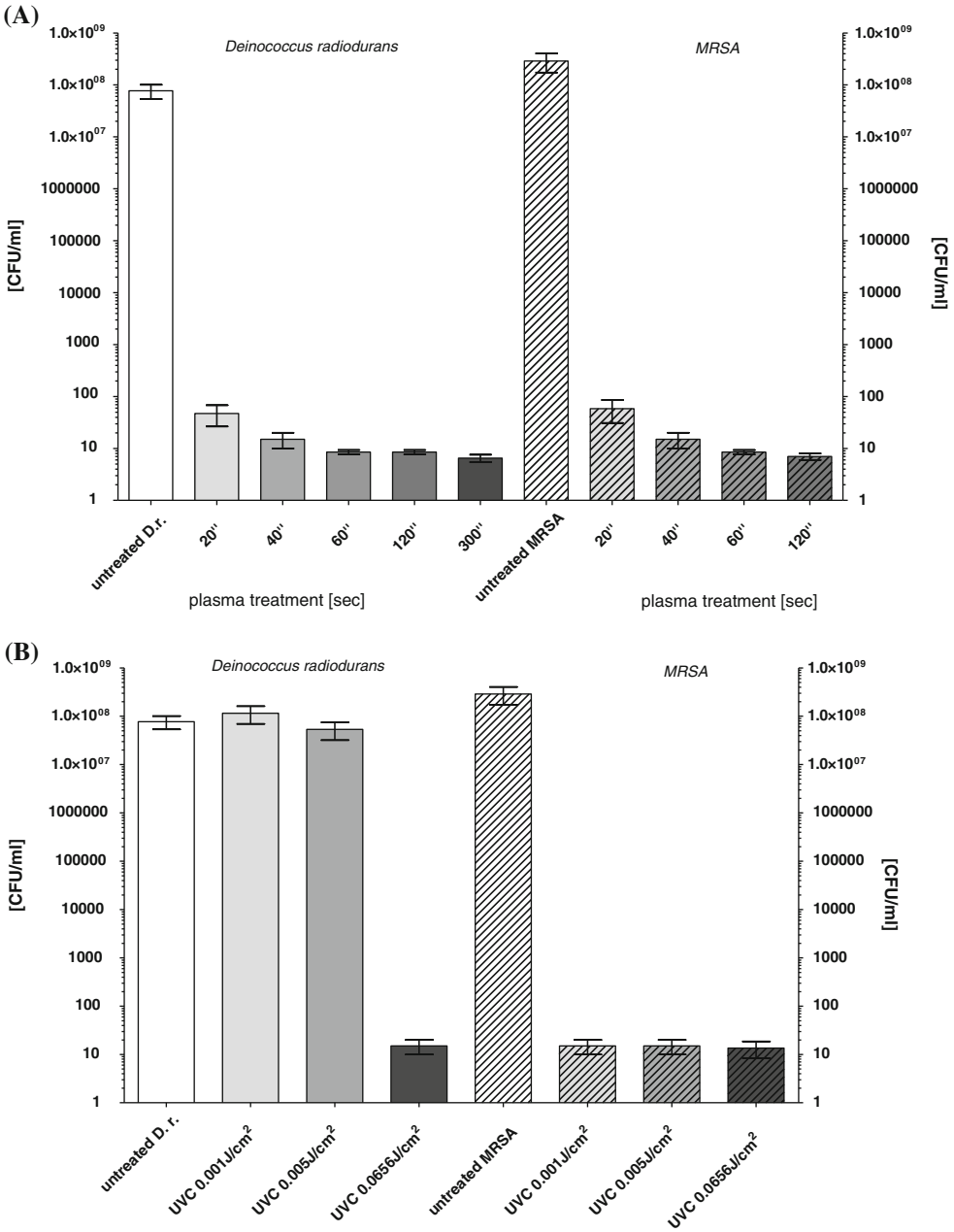

Fig. 4 **a** Plasma treatment of *D. radiodurans* and MRSA: 20-µl serial-diluted free-floating *D. radiodurans* or MRSA suspensions according to the Miles and Misra technique were applied to agar plates and dried for 45 min. **a** Cold atmospheric plasma treatment of *D. radiodurans* or MRSA was done using different time intervals. **b** UVC radiation of *D. radiodurans* or MRSA. Surviving colonies were counted 24 h later. White bar and white cross-hatched bar: untreated samples of *D. radiodurans* (D.r.) or MRSA. *Grey bars*: plasma-treated *D. radiodurans*; *grey cross-hatched*: plasma-treated MRSA. ($n = 3$, median ± interquartile range). **b** UVC radiation of *D. radiodurans* and MRSA

$\Gamma = E \times F = 0.5 \times 5 \times 1.6 \times 10^{-19}$ J $\times 10^{19}$ m^{-2} s^{-1} = 4×10^{-4} W cm^{-2}. In this study, treatment times of 20, 40, 60, 120, and 300 s were used. Therefore, the energy inputs can be estimated as follows: 20 s → 8; 40 s → 16; 60 s → 24; 120 s → 48; 300 s → 120 mJ cm^{-2}. This means that the same or lower total doses of plasma were applied to the bacteria to induce irreversible damage compared with the UV radiation experiments (65.6 mJ cm^{-2} UVC) or other new approaches such as the "photodynamic reaction" (range between 10 and 100 J cm^{-2}), which only produces reactive oxygen species, such as singlet oxygen, to kill microorganisms [4, 13]. Furthermore, the photodynamic process, where a dye is activated by light to generate reactive oxygen species, also showed high sensitivity of *D. radiodurans* to photodynamically produced singlet oxygen [34]. Schäfer et al. [34] could demonstrate that *D. radiodurans* could not repair oxidative damage to such an extent as compared with a single UV radiation or ionizing radiation, reflecting the high sensitivity of *D. radiodurans* to reactive oxygen species. Therefore, direct damage of the DNA by the plasma treatment does not seem to be the primary mechanism of action to kill *D. radiodurans*. However, on alteration of the bacterial membrane by an oxidative burst during the plasma treatment, a rapid loss of membrane potential can occur, which is critical for *D. radiodurans* survival. Furthermore, atomic force microscopy (AFM) experiments on plasma-treated *E. coli*

confirmed rupture of the cell wall and release of cell plasma to the outside [30].

Furthermore, by destroying the stable attachment of DNA to membrane areas, the plasma treatment may have a more negative, although indirect, impact on DNA synthesis and bacteria division than DNA strand breaks alone [19, 28, 33]. Furthermore, the lethal effect of ionizing radiation is mediated as well through reactive oxygen species generated from water in the irradiated microorganisms. Recently, a new mechanism of action was postulated for the protective role of accumulated Mn^{2+} ions in *D. radiodurans* [9, 15, 18]. Replacement of divalent cations with Mn^{2+} as a cofactor in enzymes provided protection against protein degradation by reactive oxygen species, especially superoxide anions and H_2O_2. Both catalytic removal of superoxide anion and catalytic decomposition of H_2O_2 by enzyme complexes containing Mn^{2+} induce high sensitivity of *D. radiodurans* to ionizing radiation [2, 6, 8]. Barnese et al. [2] could demonstrate that manganous phosphate acts as a superoxide dismutase to protect cells against oxidative stress and ionizing radiation. In case of plasma, more than 600 important chemical reactions occur during and after treatment. Therefore, the formation of scavenging Mn^{2+} complexes might be insufficient to protect against the resulting amount of reactive oxygen species for survival of *D. radiodurans*. Furthermore, the combination of O_2 gas + cold plasma induces DNA strand scissions (decrease of supercoiled DNA) [24]. In the same study, a custom-built plasma device was used, emitting lower intensities of oxygen than the O_2 plasma but higher intensities of UVB, which induces as well DNA strand breaks. In our study, the used SMD device generates as well UV components in the range between 280 and 400 nm, resulting from nitrogen molecules excited by electron impact, which might induce DNA strand breaks. From all reports and experiments carried out so far, one can therefore conclude that the known composition of reactive species generated by different plasmas is of interest in the near future to design appropriate plasma sources for special applications for sterilization and removal of pathogens from the environment.

Nevertheless, a multihit process occurs during cold atmospheric plasma treatment, consisting of a "cocktail" of charged particles, excited atoms and molecules, reactive oxygen and nitrogen species, and photons, inducing an oxidative burst of the bacteria cell wall components immediately during treatment. Furthermore, here we also want to point out that we cannot completely exclude DNA damage from the plasma treatment, especially if the generated plasma components induce synergistic effects when destroying the repair mechanisms, which in turn do not protect DNA. Recently, Muranyi et al. [27] reported about DNA damage in vegetative cells after a few seconds of treatment. However, a different dielectric barrier discharge (DBD) plasma device was developed and used for these studies, combining generation of intensive monochromatic UV light (282 nm) with chemically reactive plasma to induce DNA damage. Concerning the safety assessment, DNA damage might not be the relevant factor for inactivation in our case, because *Deinococcus radiodurans* did not survive the cold atmospheric plasma treatment, which produced a minimum of UVC radiation of less than 50 nW/cm^2. This is an advantage regarding the prevention of mutations and regarding resistance build-up upon plasma treatment.

Hence, the effects of plasma treatment and UV radiation on *D. radiodurans* will be different, even if they both contain the same amount of applied energy. In accordance with previously published results, it can be concluded that cell wall, membrane damage, and protein damage and not DNA breakage are the major causes for *D. radiodurans* death upon contract-free cold atmospheric plasma treatment [10, 21, 31]. Roth et al. [31] reported a 6 $\log_{10}$ reduction in 60 s using microwave plasma under low pressure (50 Pa, 4 kW). In this low-pressure plasma, the UV component including vacuum UV is high. This is due to less absorption of photons by neutrals. The charged particle density around the bacteria is also much higher than in our study. This is due to the much lower collision frequency and much higher power input for the plasma production. This means that, for this low-pressure plasma, the main reactive agents are charged particles, reactive species, and photons. In our case, due to the ambient pressure, mainly reactive species affect the bacteria. Furthermore, Cooper et al. used cold atmospheric plasma to treat *D. radiodurans* [10]. The plasma device that Cooper et al. [36] used—a dielectric barrier discharge (DBD) device—also produces cold plasma at atmospheric pressure and therefore is similar to the SMD plasma device we used. They achieved 4 $\log_{10}$ reduction of *D. radiodurans* in 15 s when the samples were wet. These results are similar to our fast reduction efficacies of viable bacteria numbers applied as dried samples. The main difference between their DBD plasma device compared with our plasma device setup is that the bacteria samples were immersed in the DBD plasma discharge. This means that the bacteria samples were surrounded by a much higher density of charged particles. In our study, the main agents to inactivate *D. radiodurans* are presumably reactive oxygen species, which react with the primary targets, such as the cell wall or the membrane areas, as stated above. However, charged particles do not seem to play a major role, because there is no large difference between our findings and the report by Cooper et al. in the overall reduction speed of *D. radiodurans* inactivation [10]. In addition, Cooper et al. reported that wet conditions led to better reduction efficacy than

dried bacteria samples on inanimate surfaces. This implies that the direct impact of charged particles on bacteria is not crucial. In contrast, the reactive species produced with water, e.g., OH radicals, could be more important. We have tested the inactivation of *E. coli* and *E. faecalis* under different humidity conditions [36]. No large differences in the bactericidal property using a similar SMD plasma device were detectable for relative humidity between 20 and 80 %. However, this is an important and future topic to investigate the inactivation mechanisms of the used plasma device. In our study, we achieved an inactivation rate of 6 $\log_{10}$ within 20 s for dried bacteria samples, whereas Copper et al. needed 30 min to achieve the same killing efficacy. Of course, synergetic effects of charged particles and radicals cannot be ruled out. Therefore, further investigation concerning the mechanism of action of *D. radiodurans* inactivation by plasma is needed.

Conclusions

Overall, contact-free treatment of inanimate surfaces by cold atmospheric plasma seems to be an effective approach to eliminate extremophile and multiresistant microorganisms, such as *Deinococcus radiodurans* or MRSA, in the future. In this study, *Deinococcus radiodurans* was efficiently inactivated by plasma but not by UVC radiation. These results emphasize the advantageous potential of plasma for disinfection of materials where heat or wet conditions must be avoided.

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