

Acidification and Nitrite/Nitrate Accumulation by Nonthermal Dielectric Barrier Discharge (DBD) Affect Human Dermal Fibroblasts

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ABSTRACT: Reactive species generated by dielectric barrier discharge (DBD) may exert many biological effects including cell toxicity. The reactive nitrogen species nitrogen dioxide hydrolyses in water resulting in acidification and increased osmolality by the formation of nitric acid and nitrous acid. Regarding the small media volumes in which cells were maintained *in vitro* during plasma treatment, here we address the question of whether cell toxicity effects of plasma may also be mediated by changes of pH, osmolality, and nitrite/nitrate concentrations. DBD treatment led to a treatment time–dependent increase of osmolality, acidification, and accumulation of nitrite and nitrate in buffer and cell culture media. In small buffer volumes, DBD treatment rapidly broke down the buffer capacity, and pH fell below the physiological range. The obtained nitrite/nitrate concentrations were considerably higher than those found in blood and tissues. DBD-treated buffer and acidified buffer containing nitrite/nitrate reduced cell viability of human dermal fibroblasts in the same magnitude. The antioxidant sodium ascorbate could not reverse this effect, whereas it protected fibroblasts partially during DBD treatments. Our results indicate that apart from reactive species, DBD-induced chemical and physical changes in the environment of cells may be responsible for many observed biological effects.

KEY WORDS: reactive oxygen species, nitric oxide, antioxidants, buffer capacity viability, osmotic concentration

I. INTRODUCTION

Nonthermal plasma devices operated under atmospheric pressure with ambient air can generate reactive oxygen species such as ozone (O_3), hyperoxide (O_2^-), and hydroxyl radicals (OH^\cdot); and reactive nitrogen species such as nitric oxide (NO) and nitrogen dioxide (NO_2). In particular, NO is an important molecule that has been shown to regulate many processes in human skin physiology.^{1,2} Impaired microcirculation, wound healing,

many pathological skin conditions including psoriasis, and skin tumor formation are all associated with imbalanced NO biosynthesis.^{3–6} Thus, apart from the known decontaminating effects, NO-generating plasma could represent a valuable tool in treating different skin and wound conditions through the modulation of NO bioavailability and NO-dependent pathways, which has already been demonstrated by other research.^{7–9}

However, many groups have shown that the treatment with cold atmospheric pressure plasmas under certain circumstances can induce cell death in a variety of mammalian cells, including endothelial cells, keratinocytes, and lymphocytes, which may represent a drawback in the potential use of plasma in the treatment of chronic and acute wounds or inflammatory skin diseases.^{10–16}

Nonetheless, the induction of apoptotic (programmed) or necrotic cell death in cancer cells, as shown by several groups, makes cold atmospheric pressure plasma an excellent and attractive therapeutic tool in cancer treatment.^{17–25}

In these and other studies many different plasma devices were used to produce atmospheric plasma, namely plasma jets for example the KINpen device^{26, 27} and many different kinds of dielectric barrier discharge devices, generally operated under ambient air.^{11,13,18,20,24,28,29} Independently of the used plasma device, the observed plasma-induced cell toxicity correlates positively with plasma treatment time or plasma dose. In contrast, shorter treatment times showed lesser toxicity but also many interesting biological effects. For example, Kalghatgi *et al.* 2010 observed an enhanced proliferation of porcine endothelial cells by short plasma exposure (DBD; 30 s). This effect was correlated with a significant release of fibroblast growth factor-2. However, a slightly higher exposure time (60s) significantly induced cell death.¹¹ A study of Sensenig *et al.* 2011 could show that DBD induced apoptosis in melanoma cells already after short treatment times (10–30 s).³⁰ In a study by Haertel *et al.* 2012 it was observed that DBD treatment induces a reduction of cell viability in HaCaT keratinocytes and a down regulation of the relevant cell surface molecules E-cadherin and epidermal growth factor receptor. These effects were depended on exposure time (20–120 s) but also the treatment regimen. Here, exposure to plasma-treated media had similar effects as a direct plasma treatment of the cells, whereas a change of medium directly after treatment could partially abrogate the observed effects.¹³ By using a KINpen plasma jet, a treatment of HaCaT (30 s) reduced the same surface molecules and the recovered cell number was equal to the results of Haertel *et al.* No effects were observed after shorter treatment time (10 s).²⁷

Furthermore, Arnd *et al.* 2013 described the toxic effects of a microwave plasma torch on melanoma cells after 2 min treatment. A shorter treatment time (1 min) led to a long-term inhibition of cell proliferation.¹⁸ Moreover, Fridman *et al.* 2007 showed that plasma generated by a floating electrode-DBD device promotes apoptotic behavior in melanoma cells after short treatment times (10–30 s).²⁰

Is it assumed that first of all reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) are responsible for the observed plasma-induced effects in all these mentioned and other *in vitro* studies. Plasma contains a variety of biologically active ROS in the gas phase, including hydrogen peroxide, hydroxyl radical, superoxide and singlet oxygen and provokes intra-cellular oxidative stress.^{9,25,31,32}

Antioxidants could reverse toxic plasma effects significantly but not completely.^{23,30} First, it is assumed that reactive oxygen species (ROS), reactive nitrogen species (RNS), or both are responsible for the observed plasma-induced effects in all the studies just mentioned and other *in vitro* studies. Plasma contains a variety of biologically active ROS in the gas phase, including hydrogen peroxide, hydroxyl radical, superoxide, and singlet oxygen, and provokes intracellular oxidative stress.^{9,25,31,32}

Antioxidants could reverse toxic plasma effects significantly but not completely.^{23,30} However, it is known that plasma treatment can acidify cell culture media, NaCl and PBS and increase nitrite and nitrate concentrations.^{20,25,26,33,34} Regarding the small media volumes (0-200 µl) in which cells were maintained usually during plasma treatment in experimental approaches, we have evaluated the impact of plasma-induced changes of pH, osmolality and nitrite/nitrate concentrations on viability of human skin fibroblasts.

II. MATERIALS AND METHODS

A. Plasma Source

The DBD device consists of only one driven, cylindrical copper electrode covered with aluminum oxide; total diameter is 10 mm. In our experiments, plasma was generated by applying voltage pulses with a maximum of ~14 kV at trigger frequency of 120 Hz. The DBD device was operated in a laminar flow cabinet (EuroFlow E5FE; Clean Air, Woerden, The Netherlands) for treatment of fibroblasts cell cultures.

B. Plasma Characterization

The plasma was characterized using absolutely calibrated optical emission spectroscopy (OES) to determine gas temperature, UV-radiation and plasma parameters like electron density and electron velocity distribution function. Applying OES, the advantage of the well-known photoemission of nitrogen was taken into account. Spectra were measured using a spectrometer (QE 65000, Ocean Optics, Ostfildern, Germany) with a spectral resolution of about 1.3 nm in 200-800 nm wavelength range. The average gas temperature was determined by comparing the measured rotational temperature of nitrogen at 337.1 nm to simulated spectra under same conditions.³⁵ The concentrations of generated nitric oxide (NO) and nitrogen oxides (NO_x), in particular nitrogen dioxide (NO₂) were measured in the gas phase by the NO/NO_x-analyser CLD 822r (Ecophysics, Munich, Germany) (Figure 1) as described elsewhere.³⁶ Using these measured densities the total flux of NO was calculated:

$$\Gamma_{NO} = \frac{1}{4} n_{NO} v_{th,NO}$$

v_{th} is the thermal velocity of neutral species, and n_{NO} is the density of NO. Employing the gas temperature T_G , the Boltzmann constant k_B and the mass of NO m_{NO} , the thermal velocity is:

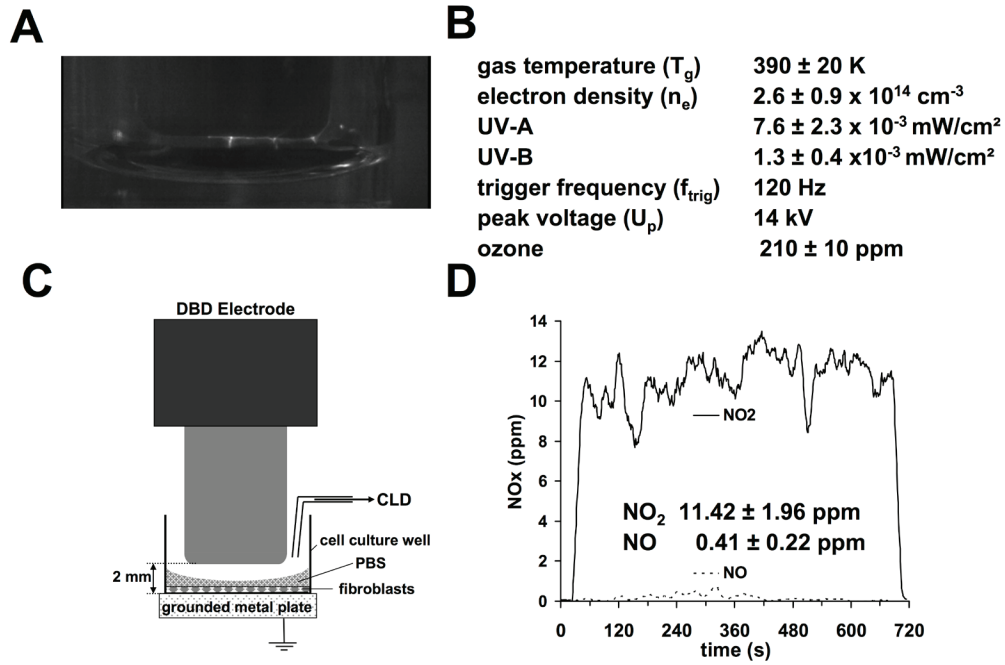


FIG. 1: DBD parameters and experimental setup **A** Picture taken by high-speed camera shows generated plasma and discharge filaments between DBD electrode and media in a single 24-well cell culture plate. Discharge filaments are apparent on the treated surface as well as on the electrode edges. **B** Evaluated plasma parameter and characteristics are given. **C** Experimental set up for DBD treatments and NO/NO₂ measurements. **D** Representative registrations of NO and NO₂ measurements. Given are the mean and standard deviation of five experiments.

$$v_{th,NO} = \sqrt{\frac{8}{\pi} \frac{k_B T_G}{m_{NO}}}$$

The measured amount of 0.41 ppm NO and 11.42 ppm NO₂ yields a flux of $\Gamma_{\text{NONO}_2} = 5 \times 10^{18} \text{ cm}^{-2} \text{ sec}$. At the maximum treatment time of 10 min, the maximum dose of NO/NO₂ is $\Phi_{\text{NONO}_2} = 1.5 \times 10^{21} \text{ cm}^{-2}$, which equals 2.4 mmol.

C. Cell Culture

Primary cultures of human dermal fibroblasts (HDFs) were isolated from skin specimens obtained from 4 female patients and 1 male (35-78 years old, mean 53.8 ± 18.3 years) who had undergone abdominoplasty. Human skin samples were used with donor consent and approval of the Ethics Commission of Düsseldorf University (Study No. 3634). HDFs were prepared, cultivated, pooled and cryoconserved as described

elsewhere.³⁷ For experiments cryoconserved stocks of pooled primary HDFs were thawed and further cultured in T 75 flasks (Cellstar, Greiner Bio-One, Frickenhausen, Germany) under normal culture conditions (5% CO₂, 37°C). For seeding, the cells were detached by rinsing two times with 0.13 M NaCl and 0.01 M sodium phosphate, pH 7.4 (PBS) and incubated with 0.05% trypsin/ 0.02% EDTA/0.9% NaCl solution for 3 to 5 minutes. After the cells had become detached, the remaining trypsin activity was neutralized by addition of 1 ml fetal calf serum (Seraplus, Pan Biotech, Aidenbach, Germany) and subsequently centrifuged (5 min/400 x g). After centrifugation, cells were resuspended in Dulbecco's Modified Eagle's Medium (DMEM, Gibco-Invitrogen, Karlsruhe, Germany supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (PAA, Pasching, Austria) and counted by using a Neubauer counting chamber before the HDFs were seeded at a cell density of 2.5×10^4 in 24-well-plates (0.79 cm²) one day before toxicity/viability experiments. All measurements were performed with HDFs from passages 4-6.

D. Plasma Treatments of Media and Buffer

Various volumes of buffer or media (100–500 µL) without cells were treated in 24-well cell culture plates placed on a grounded metal plate. Independently from the media volume that was exposed to plasma in the well, the distance between the DBD electrode and the solution surface was kept constant.

E. Determination of Osmolality

The osmolality of plasma-treated PBS was determined with an osmometer (Typ OM, Löser, Berlin, Germany). The measuring principle is based on freezing point measurements of aqueous solutions. The depression of the freezing point relative to that of pure water is a direct measure of the osmotic concentration.

F. Determination of pH Values

The pH values of media and buffers or were measured before and after plasma treatment with a calibrated pH meter (Calimatic 766, Knick, Berlin, Germany) and a pH electrode (InLab-micro, Mettler-Toledo, Giessen, Germany). The buffering capacity of PBS and media were determined by titration with HCl. The obtained data were used to calculate the concentration and accumulation rate of oxonium ions in plasma-treated buffer.

G. Detection of Nitrite and Nitrate

The concentrations of nitrite were quantified by an iodine/iodide-based assay using a NO-analyzer (CLD 88, Ecophysics, Munich, Germany) as described elsewhere.^{38,39} To determine total nitrite and nitrate concentration, samples were added to 0.1 M vanadium (III) chloride in 1 M hydrochloric acid refluxing at 95°C under nitrogen.³⁹

H. Plasma Treatment of HDFs

Before plasma treatment, media was carefully removed, then HDFs were washed once with PBS (500 μL) and covered with PBS (250 μL), with or without sodium ascorbate (1 mM). The cell culture plate was placed on a grounded metal plate under the DBD electrode. The distance between the electrode and the inner bottom of the well was kept at 2 mm (Fig. 1C). Alternatively, HDFs were incubated for 5 min with 250 μL PBS, which was treated with plasma (5 min) directly before application. In addition, HDFs were incubated for 5 min with freshly prepared and acidified PBS (250 μL , pH 6.7) containing sodium nitrite (0.5 mM) and sodium nitrate (1.5 mM).

I. Toxicity and Viability

Directly after plasma treatment of HDFs, treatment buffer was exchanged with PBS containing fluorescein diacetate, Hoechst 33342, and propidium iodide (each dye, 0.5 $\mu\text{g}/\text{mL}$). Living and dead \times cells were visualized and analyzed using a fluorescence microscope (BZ-9000E, Keyence Mechelen, Belgium). For excluding a possible detachment, in control experiments HDFs were observed by a Zeiss light microscope directly after treatment without changing media. Viability of cells and cell numbers relative to the untreated control were determined by a resazurin-based assay (CellTiter-Blue, Promega, Madison, WI) 24 h after plasma treatment in accordance with the manufactures instructions. Briefly, HDFs were incubated for 1 h with resazurin reagent (diluted 1:20 with medium), and fluorescence of the supernatant was quantified using a fluorescence spectrometer (Fluostar Optima, BMG Labtech, Offenburg, Germany) at 540 nm (excitation) and 590 nm (emission).

J. Statistical Analysis

Significant differences were evaluated using either paired two-tailed Student's *t*-test or ANOVA followed by an appropriate post hoc multiple comparison test (Tukey's method). A *p* value less than 0.05 was considered significant.

III. RESULTS

Within the gap between the PBS and the electrode of an operating DBD device in a cell culture well, stochastically distributed filaments under the electrode were observed; even so, there were filaments at the edges of the electrode throughout treatment (Fig. 1A). At a trigger frequency of 120 Hz and peak voltage of 14 kV, the gas temperature was 390 ± 20 K; the calculated intensity of UV-A was $7.6 \pm 2.3 \times 10^{-3}$ mW/cm², and the intensity of UV-B was $1.3 \pm 0.4 \times 10^{-3}$ mW/cm²; the calculated ozone concentration was 270 ± 10 ppm; and the electron density for each filament was $2.6 \pm 0.9 \times 10^{14}$ cm⁻³ (Fig. 1B).

After turning on the DBD device, a steady state of 11.42 ± 1.96 ppm NO₂ and 0.41 ± 0.22 ppm NO could be measured in the cell culture well above the PBS (Fig. 1C and

1D). By calculating the flux, it can be estimated that 2.4 mmol of NO/NO₂ reached the treated PBS within 10 min of plasma treatment.

Preliminary experiments have shown that if the buffer level in a cell culture well (24-well plate) was below 215 μ L during the plasma treatment, a direct toxic effect on cells was observed in the center of the well (Fig. 2A). This effect occurred with even shorter plasma treatment times (15 sec). No direct toxic effects were observed when the media volume was greater than 215 μ L PBS. Using 250 μ L PBS for cell culture treatment, a reduction of cell viability could be observed after longer treatment times. For example, a 300-sec plasma treatment significantly reduced cell viability to $68.2 \pm 14.1\%$ measured 24 h after treatment (Fig. 2B).

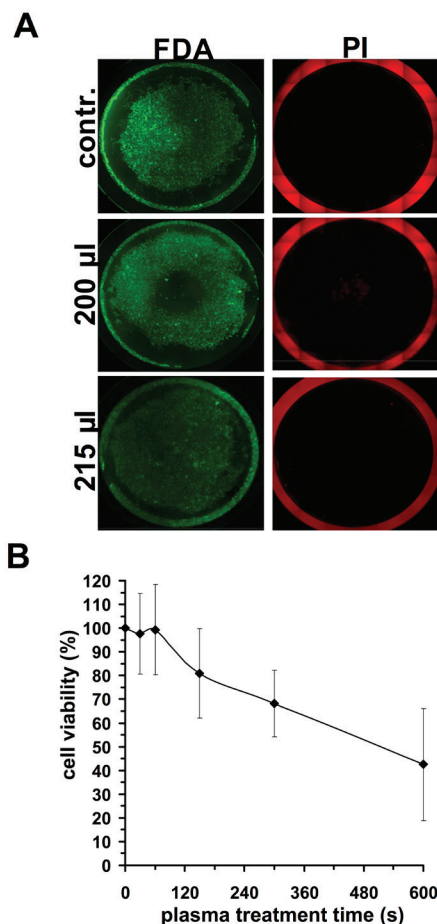


FIG. 2: Plasma effects on cell viability **A** Microphotographs of human dermal fibroblast cultivated in 24-well cell culture plates. Live/dead staining with fluorescein diacetate (FDA) and propidium iodide (PI) directly after DBD treatment (1 min) in media (PBS) volumes as indicated. **B** Cell viability of human dermal fibroblasts was measured 24 h after plasma treatment by a resazurin-based assay (mean \pm SD, $n = 4$).

To investigate plasma-induced chemical and physical changes, osmolality, pH values, and nitrite/nitrate concentrations were measured in different volumes of PBS after plasma treatment. We observed that plasma-induced acidification was a function of treatment time and buffer volume. In a volume of 100 μL , the buffer capacity of PBS breaks down after a treatment time of approximately 180 sec, and in 250 μL , after more than 600 sec (Fig. 3B). Based on the titration curve for PBS (Fig. 3A), the oxonium concentration and accumulation rate could be calculated by the obtained pH values. Under our conditions, the oxonium accumulation rate was 0.89 ± 0.15 nmol/sec, which would theoretically acidify smaller volumes of 25 and 50 μL PBS much faster (Fig. 3B). Here a breakdown of buffer capacity would be estimated after plasma treatment times of 60 sec and 100 sec, respectively. Furthermore, the osmolality of PBS increased as a result of plasma treatment. After plasma treatment (10 min) the osmolality of 400 μL PBS was 287 mOsm/kg H_2O ; of 200 μL PBS, 291 mOsm/kg H_2O ; and of 100 μL PBS, 305 mOsm/kg H_2O , compared to 284 mOsm/kg H_2O (PBS untreated). We found that nitrate and nitrite concentrations of PBS were increased after plasma treatment (Fig. 3C). For example, nitrate concentrations of 0.88 ± 0.21 mM were measured after 5 min and 2.16 ± 0.68 mM after 10 min plasma treatment of 250 μL PBS. In parallel, nitrite concentrations increased to 0.28 ± 0.08 mM and 0.32 ± 0.09 mM after 5 and 10 min, respectively. The accumulation rate of NO and NO_2 together was 0.99 ± 0.03 nmol/s.

Furthermore, cell culture experiments demonstrate that cell viability of HDFs after plasma treatment (5 min; plasma direct) was significantly higher in the presence of the antioxidant sodium ascorbate than without ($87.0 \pm 12.7\%$ vs. $67.0 \pm 6.3\%$). The incubation of HDFs with freshly plasma-treated (5 min) PBS (indirect plasma) for 5 min reduced cell viability down to $76.5 \pm 13.2\%$; but in this approach, addition of sodium ascorbate could not attenuate this reduction in cell viability ($76.9 \pm 16.5\%$). The incubation of HDFs with freshly acidified PBS (pH 6.7) containing nitrite (0.5 mM) and nitrate (1.2 mM) for 5 min also reduced cell viability to $81.5 \pm 12.4\%$. Analogously to indirect plasma treatment, sodium ascorbate had no significant protective effect ($79.3 \pm 11.3\%$). Under these conditions the 5-min exposure of HDFs to an acidic pH of 5.5 significantly decreased HDF viability ($80.5 \pm 11.5\%$); whereas in solutions with pH values of 6.5–6.8, viability was still 87–92% (Fig. 3D).

IV. DISCUSSION

It is widely accepted that one important mechanism of how cold atmospheric-pressure plasmas exert biological effects, including cell toxicity, bases on the generation of reactive oxygen and nitrogen species. However, the survival and functionality of cells depends on a wet environment with a stable physiological pH, osmolality, temperature and as well as an adequate oxygen and nutrient supply. Plasma exposure of cells in cell culture and in tissues could essentially influence one or more of the mentioned parameters, which in turn may have effects on the physiological functionality of the treated biological samples. For example, plasma jets *in vitro* and *in vivo* leads often to drying-off effects by using relative high gas flows (up to 6 l/min). Using DBD as a plasma source drying-off

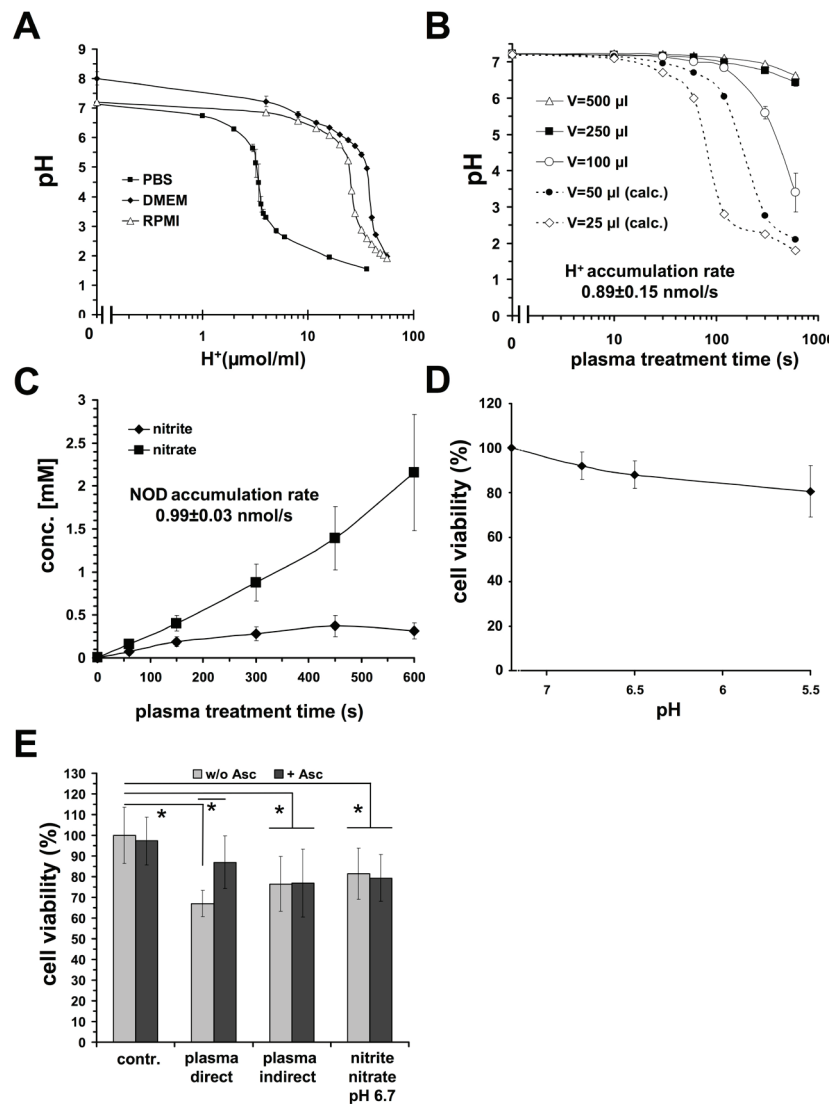


FIG. 3: Plasma-induced changes of treatment media affect cell viability **A** Titration curves of cell culture media and PBS buffer under ambient air conditions (mean \pm SD, $n = 3$). **B** Measured and calculated pH values of different volumes of PBS after plasma treatment. Given is the calculated oxonium (H^+) accumulation rate (mean \pm SD, $n = 4$) **C** Measured nitrite and nitrate concentration in 250 μL PBS after plasma treatment (mean \pm SD, $n = 6$). Given is the accumulation rate of nitrite + nitrate (nitric oxide derivatives; NOD). **D** Cell viability of human dermal fibroblasts (HDFs) 24 h after incubation for 5 min in acidified PBS **E** Cell viability of HDFs was measured 24 h after plasma treatment (5 min; plasma direct), incubation of fresh plasma-treated PBS (5 min, 250 μL ; plasma indirect) and freshly acidified PBS (pH 6.7) containing nitrite (1 mM) and nitrate (1.2 mM) with or without sodium ascorbate (1 mM; ASC) (mean \pm SD, $n = 7$).

effects by gas flow can be avoided; however, DBD may affect living cells by interfering with the well balanced wet environment required for cell survival. As demonstrated in our experiments, an immediate toxicity is induced by DBD treatment if cells are not covered sufficiently by media. Due to the hydrophilic properties of plastic, there is weak capillary action in a cell culture well, leading to a concave meniscus as pictured in figure 1C. Therefore, in the center of the cell culture well, where cell toxicity occurred, the layer of water is notably thinner than towards the edges. This observed effect indicates that the direct interaction of plasma with cells leads to a physical disruption of cell membranes and consequently to cell death, possibly by direct discharges. Furthermore, DBD treatment can acidify water, NaCl solution and even buffered cell culture media and PBS.^{20,33} Applying DBD on PBS, we observed a fast breakdown of buffer capacity (pH-shift >1), which was naturally more pronounced in small PBS volumes. The physiological pH 7.2-7.4 is well balanced in organisms and already minor deviations have severe clinical consequences. In cell culture experiments a pH-value of 7.1 inhibited proliferation of fibroblasts.⁴¹ Nevertheless, in normal wound healing initially an acidic pH stimulates platelet degranulation and the release of platelet-derived growth factor, which in turn stimulates fibroblast proliferation in higher levels.^{42,43} Cell culture media such as DMEM or RPMI has more buffer capacity and due to the bicarbonate buffering system the tendency to become more basic in the open air counteracting against plasma-induced acidification. However, cell culture media contains many organic components, which can react with reactive species for example to organic peroxides, which in turn mediate delayed biological effects and cell toxicity.⁴⁴ Thus, more and more studies are investigating the indirect effects of plasma or the plasma-treated medium which reveals plasma-like effects e.g. the induction of apoptosis and cell injury in cancer cells.⁴⁵⁻⁴⁷

DBD treatment increases osmolality of liquids, probably by increasing nitrite and nitrate concentrations. Osmotic stress is known to induce many signal pathways. One example is the induction of terminal differentiation in cultured keratinocytes.⁴⁷ Interestingly, osmotic changes have not been measured in any plasma studies to date. This could be a critical point, in particular, when osmotically sensitive cell types such as keratinocytes or epithelial cells are treated with plasma jets in small media volumes or even under physiological conditions *in vivo*. In our experiments, osmolality was still in the physiological range after all treatment times. DBD produced high amounts of ozone, which may react rapidly ($k_1 = 9.6 \times 10^9 \text{ cm}^3 \text{ mol}^{-1} \text{ sec}^{-1}$) with NO to produce NO₂, O₂, and photons.⁴⁸ In our study, we found high NO₂ (~11 ppm) and low NO (~0.4 ppm) concentrations in the gas phase during plasma treatment, which confirmed this assumption. Although NO has low water solubility, NO₂ rapidly hydrolyses into nitric acid (HNO₂) and nitrous acid (HNO₃), which subsequently acidified aqueous solutions such as PBS. Indeed, we found similar accumulation rates for oxonium and nitrite/nitrate ions in plasma-treated PBS, confirming that acidification is the consequence of nitrous acid (HNO₂) and nitric acid (HNO₃) formation by NO₂. These results are in accordance with previous findings of Oengen et al.³³ The obtained nitrite and nitrate concentrations in our experiments demonstrate that the physiological nitrite and nitrate ranges can be

easily exceeded by plasma treatment, especially when small volumes of liquids are used. For example, we obtained $\sim 200 \mu\text{M}$ nitrite and $\sim 1200 \mu\text{M}$ nitrate after a 5-min plasma treatment of $250 \mu\text{L}$ PBS. For comparison, blood plasma of healthy volunteers contains $0.1\text{--}0.3 \mu\text{M}$ nitrite and $14.4\text{--}30 \mu\text{M}$ nitrate, whereas skin tissue contains $5.1\text{--}8.4 \mu\text{M}$ nitrite and $190\text{--}278 \mu\text{M}$ nitrate.⁴⁹

The exposure of HDFs to an acidic pH of 6.7 decreased cell viability, which was further decreased by the addition of nitrite and nitrate. Besides possible osmotic effects, nitrite in particular participates in several signaling events along the physiological and pathophysiological oxygen gradient. These include hypoxic signaling events such as vasodilation, modulation of mitochondrial respiration, and cytoprotection following ischemic insult.⁵⁰

Acidified nitrite in higher concentrations can induce inflammatory and cytotoxic effects, probably due to the release of nitric oxide.⁵¹ However, nitrite and acidified nitrite have antimicrobial properties and can inhibit biofilm formation of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Candida albicans*, and other human pathogens.^{52–55} In a recent study, DBD treatment of chronic venous ulcers significantly reduced lesional bacterial load without visible hazardous side effects.⁵⁶

V. CONCLUSION

Investigation of plasma-induced effects has some experimental challenges. Many studies in this field have focused on the possible biological effects of plasma-generated ROS. However, volumes of media or buffer were used for plasma-treatment of cells were often small, ignoring the impact of plasma on pH, osmolality, and nitrite/nitrate concentrations. Our data implicate how important it is to characterize plasma-induced chemical and physical changes of buffer/media, which may be responsible for many observed biological effects. In particular, acidification and higher nitrite levels can explain antibacterial effects but also could represent an important mechanism affecting many cell types. Theoretically, a possible delayed release of nitric oxide in plasma-treated wounds may improve wound healing and dermal microcirculation. However, further studies are necessary to maximize beneficial and minimize hazardous effects of plasma treatment.

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