Identification of the biologically active liquid chemistry induced by a nonthermal atmospheric pressure plasma jet

Kristian Wende
Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany and Department of Mechanical Engineering, University of Minnesota, 111 Church Street SE, Minneapolis, Minnesota 55455

Paul Williams
Department of Mechanical Engineering, University of Minnesota, 111 Church Street SE, Minneapolis, Minnesota 55455

Joe Dalluge
Department of Mechanical Engineering, University of Minnesota, 207 Pleasant St SE, Minneapolis, Minnesota 55455

Wouter Van Gaens
Department of Chemistry, University of Antwerp, Universiteitsplein 1, BE-2610 Antwerp-Wilrijk, Belgium

Hamada Aboubakr
College of Veterinary Medicine, University of Minnesota, 1333 Gortner Ave., St. Paul, Minnesota 55108 and Food Science and Technology Department, Alexandria University, Aflaton St., El-Shatby, 21545, Alexandria, Egypt

John Bischof
Department of Mechanical Engineering, University of Minnesota, 111 Church Street SE, Minneapolis, Minnesota 55455

Thomas von Woedtke
Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany

Sagar M. Goyal
College of Veterinary Medicine, University of Minnesota, 1333 Gortner Ave., St. Paul, Minnesota 55108

Klaus-Dieter Weltmann
Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany

Anemie Bogaerts
Department of Chemistry, University of Antwerp, Universiteitsplein 1, BE-2610 Antwerp-Wilrijk, Belgium

Kai Masur
Center for Innovation Competence Plasmatis, Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany and Leibniz Institute for Plasma Science and Technology, INP Greifswald e.V., Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany

Peter J. Bruggeman
Department of Mechanical Engineering, University of Minnesota, 111 Church Street SE, Minneapolis, Minnesota 55455

(Received 11 February 2015; accepted 23 April 2015; published 6 May 2015)

The mechanism of interaction of cold nonequilibrium plasma jets with mammalian cells in physiologic liquid is reported. The major biological active species produced by an argon RF plasma jet responsible for cell viability reduction are analyzed by experimental results obtained through physical, biological, and chemical diagnostics. This is complemented with chemical kinetics modeling of the plasma source to assess the dominant reactive gas phase species. Different plasma chemistries are obtained by changing the feed gas composition of the cold argon based RF plasma jet from argon, humidified argon (0.27%), to argon/oxygen (1%) and argon/air (1%) at constant power. A minimal consensus physiologic liquid was used, providing isotonic and isohydric conditions and nutrients but is devoid of scavengers or serum constituents. While argon and humidified argon plasma led to the creation of hydrogen peroxide dominated action on the mammalian cells, argon–oxygen and argon–air plasma created a very different biological action and was characterized by trace amounts of hydrogen peroxide only. In particular, for the argon–oxygen (1%), the authors observed a strong negative effect on mammalian cell proliferation and metabolism. This effect was distance dependent and showed a half life time of 30 min in a
scavenger free physiologic buffer. Neither catalase and mannitol nor superoxide dismutase could rescue the cell proliferation rate. The strong distance dependency of the effect as well as the low water solubility rules out a major role for ozone and singlet oxygen but suggests a dominant role of atomic oxygen. Experimental results suggest that O reacts with chloride, yielding ClO⁻ or ClO₂⁻. These chlorine species have a limited lifetime under physiologic conditions and therefore show a strong time dependent biological activity. The outcomes are compared with an argon MHz plasma jet (kinpen) to assess the differences between these (at least seemingly) similar plasma sources.

© 2015 American Vacuum Society. [http://dx.doi.org/10.1116/1.4919710]

I. INTRODUCTION

Nonequilibrium atmospheric pressure plasma sources have seen an enormous development in the last decade. Recent developments also include the treatment of food to preserve quality or reduce microbial load. Even so, the key processes leading to these beneficial plasma–biological interactions are poorly understood.

A significant amount of knowledge about the gas phase composition and dynamics of nonequilibrium plasma jets is available; nonetheless, information on the respective plasma–liquid interactions is rather scarce in literature although it is a key step in the interaction of plasmas with biological systems surrounded by liquids. It is commonly agreed that reactive oxygen and nitrogen species (ROS and RNS) generated in the active plasma, its afterglow, or at the liquid interface play a major role in creating biological effects of cold plasmas. A multitude of oxygen and nitrogen centered radicals have been detected in the gas phase using highly quantitative and selective spectroscopic methods such as laser induced fluorescence and molecular beam mass spectrometry. The liquid phase chemicals induced by the plasma in the solution are primarily investigated by indirect methods including electron spin resonance, colorimetric or fluorescence techniques, which can disturb the system under investigation and/or be highly nonselective. The combination of the plasma induced complex liquid chemistry and the nonselectivity of many analytical methods poses great challenges to unraveling the chemical processes in the solution phase leading to plasma–bio interactions. Along with these obstacles, plasma related chemical reactions can be obscured or modified in complex body fluids or cell culture media, e.g., by scavenging reactions. Hence, the answer to the question of how a certain biological effect was triggered—and by which species—can be extremely complex. Nonetheless, several species have been suggested to be major in plasma–bio interactions including H₂O₂, O₃, peroxynitrite, NO₂⁻/NO₃⁻, O₂⁻ and singlet oxygen. These conclusions have often been reached through indirect evidence and discrepancies have been attributed to differences in plasma sources, operation mode, cells, and media.

To shed more light on the tunable and transient nature of the interaction of nonequilibrium plasma jets with biological matter (and, in particular, mammalian cells), this report seeks to identify the major biological active species produced by an argon RF plasma jet through analyzing experimental results obtained from physical, biological, and chemical diagnostics complemented with chemical kinetics modeling of the plasma source. Here, different plasma chemistries were created by changing the feed gas composition from argon or humidified argon to argon–oxygen and argon–air mixtures while keeping the dissipated power constant. It was anticipated that increasing the humidity leads to more OH and H₂O₂ production, increasing the air concentration to more NO, increasing the O₂ concentration to more O₃. Using a biological assay, the impact of these different chemistries on mammalian cells was evaluated revealing plasma operational modes producing significantly distinctive biological responses. Subsequently, by modification of the experimental procedure, the use of reactive species scavengers and different buffer systems potential biologically active species were pinpointed and proven further by using chemical or physical detection. The outcomes are compared with the kinpen to assess the differences between (at least seemingly) similar plasma sources.

II. MATERIALS AND METHODS

A. Cell culture and cell viability assay

Cell culture consumables (T75 flasks, 50 ml centrifuge tubes) were purchased from Sarstedt (Newton, NC, USA). Other plastic material (stripettes) came from Corning (Tewksbury, MA, USA) and Nunc Nalgene (Roskilde, Denmark) (96 well plate, flat bottom). Roswell Park Memorial Institute Medium 1640 (RPMI 1640), phosphate buffered saline w/Mg²⁺/Ca²⁺ (PBS), trypsin/ethylendiaminetetraacetic acid (EDTA), and fetal bovine serum (FBS) were purchased from Corning (Tewksbury, MA, USA). Eukaryotic epithelial CRFK cells (Crandell-Reese feline kidney, cortex cells) were obtained from ATCC (CCL-94™).

Complete culture medium was made from RPMI supplemented with penicillin/streptomycin (100 IU/ml penicillin; 100 μg/ml streptomycin), 1.2 μg/ml sodium deoxycholate (Fungizone, Life Technologies, Carlsbad, CA, USA), and 8% heat inactivated FBS. Cells were cultivated in 75 cm² filter cap cell culture flasks at 37 °C/5% CO₂ in a humidified atmosphere and passaged by trypsinization (0.025% trypsin/EDTA) twice a week.

For the cell viability assay, cells were collected as described above. A cell suspension containing 35 000 cells/
ml was prepared in complete medium followed by seeding 100 μl into the inner 60 wells of a clear flat bottom 96-well plate using a multichannel pipette. Cells then were allowed to rest for 24 h in an incubator. Directly before plasma treatment, the medium was discarded and replaced by 100 μl of plasma treated liquid immediately after finishing the plasma treatment (see Sec. II C). After addition of the treated buffer, cells were incubated for 60 min under standard conditions.21 Subsequently, the treated buffer was replaced by fresh complete medium and the cells were incubated for 70 h. The cell viability was determined based on the conversion of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS, Promega, Madison, WI, USA) by metabolically active cells according to the manufacturer’s protocol. After 45 min, optical density was measured at 490 nm using a VMax multiplate reader (Molecular Devices, Sunnyvale, CA, USA). All experiments were carried out in duplicate or triplicate with six parallels each. Data were analyzed using MS EXCEL and GRAPHPAD PRISM 5. To allow a better comparison with results from other labs, the biological impact was expressed as plasma treatment intensity (ms μl⁻¹ cell⁻¹, often given as U). Details and conditions for the use of this unit are discussed elsewhere.21 The 50% effect level (treatment equivalents, TE₅₀) was calculated by sigmoidal regression. The TE₅₀ is defined as the plasma treatment leading to a 50% reduction of the cell division rate. Because we use a separate plasma treatment and dilutions thereof, the term treatment equivalent was preferred over treatment time.

B. Plasma source

Two nonequilibrium atmospheric plasma sources were used for the experiments. One was developed in the laboratory of Professor Bruggeman and has been characterized in significant detail including in situ measurements of the gas temperature, electron density, temperature and reactive species densities such as NO, O, O₂,11,23 Figure 1(a) shows a schematic of the plasma source setup. It consists of an RF driven tungsten needle with a diameter of 1 mm and a sharpened tip surrounded by a quartz tube with an outer and an inner diameter of 3 and 1.9 mm, respectively. A gas flow of 1.5 standard liters per minute (slm) argon is applied through the tube, if desired with admixtures of molecular gases (oxygen or air) or water saturated argon (saturated by bubbling through distilled disinfected water).

Gases were supplied by Matheson Gas (Montgomeryville, PA, USA) with a purity equal to or better than 99.999%. All gas delivery tubing was made from brass or Teflon. The discharge is ignited using a RF frequency of 13.7 MHz generated by a signal generator (Agilent 33220A, 20 MHz Function Generator, Agilent Loveland, CO, USA) connected to a power amplifier (Amplifier Research 75 W, 5–250 MHz, Souderton, PA, USA). An inductance is connected in series between the high voltage output of the power amplifier and the plasma source in order to match the amplifier to the plasma. The plasma dissipated power was measured as described before.11 Alternatively, the so called kinpen was used to perform the plasma treatment. It is an extensively described source in respect to its physical and chemical properties as well as its biological impact (INP Greifswald and ZIK plasmatis). It consists of two electrodes, the 1 mm rod electrode in the center of a ceramic capillary and a grounded electrode surrounding the dielectric capillary [Fig. 1(b)].22 The jet is operated by high frequency of around 1 MHz applied to the centered electrode. As feed gas, 3 slm argon (argon N50, Air Liquide) was used.

C. Plasma treatment and sample preparation

Plasma treatment applying the above mentioned conditions was achieved by the following routine: 3 ml of

---

**Fig. 1.** Schematic of RF plasma jet (a) and *kinpen* (b) (Ref. 24). In both cases, a visible plume extends about 10 mm from the ignited plasma when Ar is used as working gas. Admixtures of molecular gases or water are possible.
physiological liquid (phosphate buffered natural saline with Ca\(^{2+}\)/Mg\(^{2+}\), 1 g/l d-glucose, and pH 7.4 = treatment buffer) was placed into a Corning six-well plate. Using a scissor jack and a digital measuring slide controlled distance treatments were performed. Immediately after treatment was finished (<1 min), 100 \(\mu\)l of the liquid were transferred onto the prepared 96 well cell culture plates and diluted serially within the plate as described before.\(^2\) The procedure is schematically depicted in Fig. 2.

When needed, ROS scavengers were added to the buffer from stock solutions to achieve the respective concentrations: bovine catalase 10 U/ml or bovine erythrocyte superoxide dismutase (SOD) at 100 U/ml. Additionally, the following organic or inorganic compounds were used: mannitol (10 mM), ammonium iron (II) sulfate ((NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\)) 5–1000 \(\mu\)M, and ascorbic acid, l-histidine, or sodium pyruvate at 3 mM. For some experiments, the treated liquid was stored for up to 60 min before it was placed onto the cells (at room temperature, in darkness). Hydrogen peroxide is frequently detected in plasma treated liquids and was therefore used as experimental control (20 \(\mu\)M; \(\approx\)IC\(_{75}\) in CRFK cells).

Alternatively, to compare direct and indirect RF jet treatment, 100 \(\mu\)l of buffer were treated by the plasma for 1–60 s in 96 well plates, either with CRFK cells attached (= direct treatment) or without (= indirect treatment). In case of indirect treatment, buffer was transferred to 96 well plates containing cells immediately afterwards. After 1 h, buffer was replaced by complete medium in both treatment variants and cell viability determined as described. Effects of carrier gas flow were checked occasionally using identical setup/gas composition without igniting the plasma. For chemical detection of hydrogen peroxide, nitrite/nitrate, and superoxide, identical conditions for treatment were used and the treated liquid was subjected to the respective detection methods after treatment (see below).

D. Determination of hydrogen peroxide and \(\text{pH}\)

Hydrogen peroxide (H\(_2\)O\(_2\)) levels generated by the plasma treatment were measured using the Amplex Red Assay according to the manufacturer’s protocol (Life Technologies, Carlsbad, CA, USA). Differing from it, a 200 mM phosphate buffer in distilled water, \(\text{pH}\) 7.4, was used as assay buffer and diluent. If necessary, the treated liquid was diluted with buffer to keep within the linear detection range of the assay. The measurement was carried out 1 h after reagent addition using a Biotek Synergy HT fluorescence plate reader (filters: excitation 530/25 nm, emission 590/35 nm). Experiments were carried out in triplicate. Calibration was performed in 200 mM phosphate buffer using authentic hydrogen peroxide dilutions (between 0.16 and 10 \(\mu\)M). Alternatively, the hydrogen peroxide concentration was measured via its reaction with titanium (IV) oxysulfate (TiOSO\(_4\) solution, Fluka, Sigma Aldrich, St. Louis, USA). For selected experiments, \(\text{pH}\) was measured three times after 10 min plasma treatment (8 mm distance, 1 ml liquid, Ar or Ar/O\(_2\) RF jet) using a PerpHecT ROSS micro \(\text{pH}\) electrode (Thermo, Beverly, MA, USA).

E. Determination of nitrite/nitrate

Nitrite (NO\(_2^–\)) or nitrate (NO\(_3^–\)) levels were determined by Griess assay according to the manufacturer’s protocol (Life Technologies, Carlsbad, CA, USA). NO\(_3^–\) was reduced using acidic vanadium (III) chloride (VCl\(_3\), 10 mM final

---

**Fig. 2.** Indirect (separate) plasma treatment procedure. A physiologic buffer was treated in polystyrene dishes and transferred to cell monolayers prepared in 96-well plates. A serial dilution within the 96-well plate generates different treatment time equivalents. Columns 2 and 11 are left untreated. The outer wells were left intentionally blank (medium only).
concentration) for 1 h prior to assay. Sodium nitrite dilutions (between 3.13 and 100 μM) were used as calibrator. Optical density versus blank was determined at 548 nm using a Spectronic 20D+ photometer (Thermo Scientific, Waltham, MA, USA).

### F. Determination of tyrosine nitration

Nitration of L-tyrosine to 3-nitrotyrosine was used as measure for superoxide or peroxynitrite chemistry created by plasma liquid interaction depending on the presence or absence of a nitric oxide donor. L-tyrosine (100 μM) was dissolved in 20 mM phosphate buffer at pH 7.3 containing 100 μM sodium diethylene triamine pentaacetic acid. Diethylamine NONOate (Sigma, St. Louis, MO, USA) was used as a nitric oxide donor (10 μM) in some reactions. Subsequently, this mix was exposed to plasma treatment [argon, water saturated argon (0.27% water), argon–oxygen (1%), argon–air (1%), and argon gas flow only] for 10 min at a distance of 8 mm (nozzle-liquid). Immediately after treatment, the solution was snap frozen and stored at −20°C until analysis.

### G. Liquid chromatography-electrospray ionization-tandem mass spectrometry

A waters acquity ultraperformance liquid chromatography (UPLC) coupled to a Waters triple quadrupole mass spectrometer (Acquity TQD) was used for separation and detection of 3-nitrotyrosine. A Waters HSS T3 2.1 × 100 mm column (1.7 μm particles) at 40°C was used during the following 9 min gradient separation with (A): water containing 0.1% formic acid and (B): acetonitrile containing 0.1% formic acid, at a flow rate of 0.6 ml/min: 2% B, 0–1.0 min; 2% B to 18% B, 1.0–4.0 min; 18% B to 97% B, 4.0–5.0 min; 97% B, 5.0–6.0 min; 97% B to 2% B, 6.0–7.0 min; and 2% B, 7.0–9.0 min. By directly interfusing nitrotyrosine and the internal standard [13C6]-nitrotyrosine, cone voltages and collision energies for each selected reaction monitoring transition were optimized. The transitions that produced the highest sensitivity for the determination of each analyte were selected for quantification: Nitrotyrosine: 227.2–181.0; [13C6]-nitrotyrosine: 232.2–187.0. Dwell time for each transition was 0.05 s. For electrospray ionization tandem mass spectrometry (ESI-MS/MS) in positive ionization mode, parameters were as follows: capillary, 3.0 kV; cone, 25 V; extractor, 1 V; rf lens, 0.3 V; source temperature, 120°C; desolvation temperature, 500°C; desolvation flow, 800 l/h; cone gas flow, 20 l/h; low-mass resolution (Q1), 15 V; high-mass resolution (Q1), 15 V; ion energy (Q1), 0.2 V; entrance −1 V; exit, 5 V; collision energy 12 V; low-mass resolution (Q2), 15 V; high-mass resolution (Q2), 15 V; and ion energy (Q2) 3.5 V.

For standardization, five levels of calibration mixtures ranging from 0 to 450 μM were prepared for nitrotyrosine and [13C6]-nitrotyrosine to achieve five different response ratios for nitrotyrosine in the mixtures. These solutions were then analyzed by UPLC-MS/MS, and the data were subjected to a linear least squares analysis with the Waters TARGETLYNX™ software program. The peak area ratios of analyte/internal standard were then used in conjunction with the calibration curves to determine the concentration of 3-nitrotyrosine in the samples.

### H. Plasma chemical kinetics model

The numerical model used in this study is based on the original GlobalKin source code, developed by Kushner. The model is used to simulate a plug flow representation of the jet. We divide this cylinder in segments with lengths that correspond to the distance that such a segment would move along the effluent within one time-step (order of μs) according to the flow speed of the gas at that position. The plasma properties for each cylinder segment are global averages over the volume of that cylinder segment. The model has as input experimentally obtained gas temperature profiles, power distribution derived from emission profiles as described in detail in Ref. 27 and a separately calculated flow field which includes air admixing in the core jet.

A large Ar/N2/O2/H2O reaction set of 85 species, interacting by means of 302 electron impact reactions and 1626 heavy particle reactions is implemented, necessary to calculate the densities of biomedically active species at relatively low concentrations and for a broad parameter range. The reactions with this species are listed in Ref. 28. The modeling results have been compared with in situ measurements of NO, O, and O3 with excellent agreement. Note that the results are obtained for a free jet and local disturbances of the flow by the solution have not been considered. The modeling outcomes should thus be seen as a semiquantitative representation of the actual measurement conditions.

### III. RESULTS AND DISCUSSION

#### A. Motivation of treatment approach

The argon RF plasma jet produces heat, UV radiation, reactive neutral species, electric fields and radicals which can all contribute to the biological interaction. While the gas temperature is higher than room temperature, the temperature of the treated liquid does not increase even at short distances. On the contrary, we observed a decrease of the bulk temperature from 24 to 22.5°C (120 s treatment, 3 ml buffer, 8 mm distance) due to flow enhanced evaporation effects which dominate heat transfer from the active plasma to the liquid. As the plasma is operated in nontouching mode, electric fields at the water surface are moderate and do not penetrate into the liquid due to the high conductivity of the solution. By comparing mass spectrometry measurements of ions and neutrals complemented with the electron density measurements in the plasma, the fluxes of ionic species at a typical distance of 8 mm from the visible plasma plume are approximately five orders of magnitude lower than the flux of neutral reactive species such as NO and O₃.

The plasma emits a considerable fraction of the inserted energy as radiation from the visible down to vacuum UV. Vacuum UV, which is in an argon plasma predominantly

---

Biointerphases, Vol. 10, No. 2, June 2015
due to excimer radiation at 125 nm (Ref. 31) does not penetrate water and triggers photolysis of water molecules at the gas–liquid interface. Thus, reactive oxygen species are created by the homolytic dissociation of H₂O₂, creating hydroxyl radicals and atomic hydrogen. Recombination reactions lead to the formation of gaseous molecular hydrogen and hydrogen peroxide.32 In the case of the argon RF jet, the emitted vacuum ultraviolet light (VUV) triggered creation of small amounts of H₂O₂ when treating distilled water. The impact on CRFK cell proliferation rate was negligible, leading to the assumption that no other bioactive species had been created in significant amounts. In neutral pH (treatment buffer) no effect of VUV radiation on H₂O₂ production or cell proliferation rate could be detected (data not shown). This indicates that photolysis of water does not play a role on the biological effects described in the present work. On the other hand, UV radiation >200 nm can penetrate the liquid and has a broad range of effects in biological systems.33–35 Depending on the wavelength, it allows photochemical reactions in biopolymers, e.g., nucleic acids (formation of cyclobutanes by dimerizing pyrimidine bases). Recently, it was shown that secondary chemistry can be created by the exposure of UV light emitting diodes (LEDs) on plasma treated water.36 The LED luminescence is, however, significantly larger than what is observed for the RF jet and it has been shown that the amount of produced UV was significantly below the biological active threshold.37

In view of the above, for the present conditions, the plasma induced liquid phase chemistry is anticipated mainly through neutral reactive species. Eliminating any direct effect of UV can be achieved by treating the solution without cells and adding the solution to the cell medium as performed in this work. The approach enabled a quick and reproducible treatment of cells in a 96 well plate and allowed an easy correlation with chemical assays. In vitro/ex vivo findings in different model systems of Bekeschus,38 Schmidt,39 and Wende et al.21 further indicated, that a separate treatment of liquids provokes the same biological effect as a direct treatment at least for the kinase. The same effect has been observed for the plasma jet studied in this work (see Fig. 3). No impact of the treatment scheme on cell proliferation rate was detected in case of argon RF jet. Accordingly, comparably long-lived reactive species seem to be the dominant bioactive components in this case. This is in good agreement with work from Kalghatgi,40 Graves,7 and Lukes.41

The impact of (plasma generated) reactive species on cell physiology depends on their concentration and spatial distribution, the composition of the liquid surrounding the cells during treatment, and cell type specific constraints. Mammalian cells are differently well equipped with scavenger systems to control ROS deriving from internal or external sources.42 Because the cell proliferation rate is influenced by a sum of factors, e.g., protein, lipid, and DNA integrity, metabolic activity, and overall cell functionality, it can serve as a measure for the overall biological impact of a chemical, biological, or physical treatment.21 Data are shown as IC₅₀ (concentration of a chemical which reduces the cell proliferation to 50%) or TE₅₀ (plasma treatment equivalents reducing proliferation to 50%). In this study, we used the CRFK feline kidney cells as a well-established mammalian epithelial model. These cells are sensitive to toward exogenous stress signals like ROS as indicated by the IC₅₀ of H₂O₂ (15 ± 2.5 μM, Fig. 4).43 In comparison, human epithelial HaCaT cells are less sensitive and show an IC₅₀ of 23 ± 2 μM (Fig. 4).8,44

As an advantage, CRFK cells compensate growth factor deprivation better than HaCaTs and do not show a cell cycle block after 1 h of incubation in serum free buffers (data not shown).

Because previous experiments revealed a major influence of the liquid present before, during, or after plasma treatment, RPMI 1640 cell culture medium was chosen for its...
low ROS scavenging capacity.\textsuperscript{21,41,45} To reduce the complexity and to facilitate the detection of chemical species, the suitability of minimal buffers was tested against the complete RPMI (standard, Fig. 5). All liquids used showed an acceptable cell performance; however, reproducibility and cell sensitivity varied [see Fig. 5(a)]. When treated in Hanks buffered saline solution (HBSS), with 1 g/l D-glucose, cell sensitivity increased markedly compared to complete RPMI (data not shown). This effect was attributed to the deprivation of CO\textsubscript{2} from the carbonate buffer system present in HBSS causing a nonphysiological pH (>8). The same pH deterioration was observed if RPMI without FBS supplementation was used [Fig. 5(b), filled upright triangles]. In contrast, when using PBS with calcium and magnesium no changes in pH during handling were observed. The liquid showed a good and reproducible performance in the assay, especially when supplemented with 1 g/l D-glucose [Fig. 5(b), diamonds/downright triangles].

Therefore, to exclude artifacts and to retain residual physiological conditions, subsequent experiments were conducted in PBS glucose as a minimal consensus physiologic liquid. Performed this way the MTS cell viability assay had a good sensitivity and robustness allowing the intended use of CRFK proliferation as a biosensor.

### B. Argon RF plasma case and comparison with kinpen

Figure 6(a) compares the deposition of H\textsubscript{2}O\textsubscript{2} in treatment buffer by the argon RF plasma jet and kinpen under varying distance between nozzle and liquid. Depending on the distance and plasma source, a 60 s treatment was found to deposit between 15 and 250 $\mu$M H\textsubscript{2}O\textsubscript{2}. Alongside, biological impact increases with decreasing distance in case of the RF jet treatment. There is a transition in plasma liquid interaction between 10 and 11 mm distance were cell proliferation rate jumps down significantly [Fig. 6(b)]. This is a result of the parallel increase in H\textsubscript{2}O\textsubscript{2} deposition. How this step is created is yet undetermined. The major role of H\textsubscript{2}O\textsubscript{2} in RF plasma triggered biological activity becomes evident if the treatment intensity is plotted as $\mu$M H\textsubscript{2}O\textsubscript{2} (from measured values) instead of the time based treatment intensity scale [Fig. 6(c)]. All curves then overlap and an IC\textsubscript{50} of around 10 ± 2.5 $\mu$M was found. This is somewhat lower than expected for authentic H\textsubscript{2}O\textsubscript{2} (15 $\mu$M, Fig. 4). This can be an indication that other ROS may be present after plasma treatment.\textsuperscript{46} However, the 100% rescue observed by the presence of catalase seems to indicate that H\textsubscript{2}O\textsubscript{2} is the sole ROS active under these treatment conditions [Figs. 4 and 8(a)].

Yet, hydrogen peroxide can be seen as a precursor, leading to more active species by secondary reactions (e.g., Haber–Weiss or Fenton reaction), such as OH radicals. This assumption was tested by adding Fe\textsuperscript{2+} to the treatment buffer. However, no increase in the biological effect was observed. In contrast, the cell proliferation rate increased with increasing Fe\textsuperscript{2+} concentration (Fig. 7 and Table I).

This indicates a consumption of free H\textsubscript{2}O\textsubscript{2} by Fenton reaction and in parallel a rapid scavenging of the generated OH radicals by organic molecules present in the treatment buffer (glucose, impurities), leading to a decrease of the observable effect in contrast to the expected increase. Addition of mannitol (10 mM) as an OH radical scavenger did not change the biological impact (Table I). Both observations emphasize the role of the more stable H\textsubscript{2}O\textsubscript{2} for plasma generated effects on mammalian cells.

A similar correlation between H\textsubscript{2}O\textsubscript{2} deposition in liquids and impact on cell proliferation has been reported previously for the kinpen.\textsuperscript{9} In contrast to the RF jet, no distance dependence of typical solution-nozzle spans (7–28 mm) on the biological impact was observed [Figs. 6(b) and 6(d)]. The H\textsubscript{2}O\textsubscript{2} production rate of the kinpen was found almost independent on the distance between nozzle and liquid when compared to the strong distance dependency found in case of the RF jet [Fig. 6(a)]. However, the shortest distance measured (7 mm) deposited significantly less H\textsubscript{2}O\textsubscript{2} than all higher distances, which do not differ from each other. This can be explained by the small amount of ambient species which have traveled into the core effluent at short distance after nozzle.\textsuperscript{47} From
the perspective of biological impact, this difference was not distinctive [Fig. 6(d)].

C. Plasma chemistry triggered by water, oxygen, or air admix

Figure 8 shows the treatment of the CRFK cells with the addition of 1% O2, 1% air, and 0.27% water added to the pure argon feed gas compared to the argon case for a fixed nozzle-solution distance of 8 mm. Catalase is used to assess if other reactive species than H2O2 contribute to the reduction in the observed cell viability. In the case of Ar and Ar + 0.27% H2O plasma treatment the addition of catalase leads to a full recovery of the cell viability [Figs. 8(a) and 8(b)], while this is not the case for Ar/air and Ar/O2 plasma [Figs. 8(c) and 8(d)].

Interestingly, a striking difference is observed between adding air and O2 to the Ar RF jet: adding air does not lead to a 100% reduction in cell viability up to 7700 U treatment equivalents (TE), while adding O2 does for TEs of only 1029 U [Figs. 8(c) and 8(d)].

The calculated densities of the major reactive species in the plasma as a function of axial distance for the four different gas compositions are shown in Fig. 9. The model calculations for the Ar/O2 and Ar/air RF plasma have been compared with gas phase species density measurements.23,27 The plasma conditions are the same as used in Fig. 8; however, the calculations are made for a free jet without the disturbance of the flow field by the liquid. As anticipated, the
OH radical and H$_2$O$_2$ concentrations in the case for adding H$_2$O increase [Fig. 9(b)]. The major differences between argon [Fig. 9(a)] and Ar/air plasma [Fig. 9(c)] are the increased amount of RNS (particularly NO). The major differences between Ar/air and Ar/O$_2$ plasma [Fig. 9(d)] are the significant increase in atomic O density close to the nozzle and the increase of O$_3$ and singlet oxygen. O$_3$ and singlet oxygen do not show a prominent distance dependent variability as is the case for atomic oxygen. These differences seem to trigger significant impact on the plasma induced reactive liquid phase chemistry. Important to note is that the diffusion of surrounding air in the jet effluent leads to RNS (particularly NO) in all cases, even if no air is added to the argon feed gas.

The measurement of H$_2$O$_2$ deposition by the Amplex Red or titanium oxosulfate assay clearly indicates the major role of this ROS not only in the argon but also the argon–water plasma conditions. The addition of H$_2$O to the working gas led to a significant increase in H$_2$O$_2$ concentration in the plasma treated solution [Fig. 9(b)]. For a treatment time of 60 s a H$_2$O$_2$ concentration around 190 µM is found, which is well above the IC$_{50}$ value of the H$_2$O$_2$ control of 15 µM (Fig. 4). In case of the plasma containing air or oxygen admixture, no H$_2$O$_2$ deposition in PBS Glc could be detected by either Amplex Red assay [Fig. 10(a)], nor titanyl sulfate, nor metavanadate reagent (data not shown). The addition of sodium azide to scavenge potentially presents NO$_2^-$ according to Eqs. (1) and (2)

$$\text{NO}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + \text{H}^+,$$

(1)

$$3\text{N}_2^- + \text{NO}_2^- + 4\text{H}^+ \rightarrow 5\text{N}_2 + 2\text{H}_2\text{O},$$

(2)

did not increase H$_2$O$_2$ deposition (data not shown).

Griess assay showed the deposition of nitrite/nitrate depending on dose and distance in the case of argon or Ar/H$_2$O plasma [Fig. 11(a)]. This is an expected result and correlates well with the known chemistry of jet based plasmas and their interaction with liquids.$^{37,48}$ However, the impact of these nitrogen species on cell proliferation rate is limited. Sodium nitrite did not affect HaCaT cell proliferation rates up to 2000 µM under test conditions [Fig. 12(a)]. In combination of NaNO$_2$ (125 µM) with H$_2$O$_2$, the effect was solely determined by the H$_2$O$_2$ concentration [Fig. 12(b)].
Interestingly, neither nitrite nor nitrate deposition could be measured by the Griess assay for the Ar/O2 and Ar/air condition. This is contradictory to the gas phase reactive species densities which indicate the presence of significant amounts of NO, NO2, and NO3 even if no nitrogen or air was admixed to the working gas [Figs. 9(c) and 9(d)]. An interpretation of this observation could be the impact of gas flow mechanics in confined spaces, which reduced the amount of surrounding air diffusing into the jet effluent zone, and had not been taken into account in the modeling approach. Another possible explanation could be the creation of peroxynitrite, which cannot be detected by the Griess assay. This RNS can be created by the following different chemical reactions:49

$$\text{O}_2^- + \text{NO} \rightarrow \text{ONOO}^\cdot$$  \hspace{1cm} (3)

$$\text{NO}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{O} = \text{NOOH} + \text{H}_2\text{O}.$$  \hspace{1cm} (4)

The presence of superoxide would be mandatory for reaction (3). The use of superoxide dismutase to rescue cell viability after argon–oxygen RF jet treatment was not effective.

**TABLE I. Array of the tested scavengers and their effect on the cell proliferation rate.**

<table>
<thead>
<tr>
<th></th>
<th>Fe²⁺</th>
<th>Catalase</th>
<th>SOD/catalase</th>
<th>Mannitol</th>
<th>Histidin</th>
<th>Ascorbic acid</th>
<th>Sodium pyruvate</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon</td>
<td>+</td>
<td>+++</td>
<td>+++*</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Humidified argon</td>
<td>+</td>
<td>+++</td>
<td>+++*</td>
<td>–</td>
<td>nd</td>
<td>nd</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Argon—1% O2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>nd</td>
</tr>
<tr>
<td>Argon—1% air</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>nd</td>
</tr>
</tbody>
</table>

*Effect of catalase

Note: (ability to interfere with plasma impact on cell viability: +++ very strong, no observable effect of plasma treatment; ++ strong, observable effect clearly diminished; + weak, slight reduction of observable effect; - no effect, effectivity of plasma treatment not affected; and nd, not measured). Fe²⁺ is the iron ions, SOD is the superoxide dismutase, and GSH is the glutathione. Treatment conditions: RF jet with 2.5 W, 8 mm distance, 1.5 slm argon w/or w/o admixture.
(see Table I). Also, the creation of superoxide from solvated electrons and dissolved oxygen in the buffer is unlikely since the amount of electrons and negative ions in the afterglow of the plasma in Ar/O2 was very low (noncontact treatment conditions). According to reaction (4), an acidic environment is necessary to create peroxyxynitrous acid, which is not present in the physiologic buffer systems (pH ≈ 7.4) and the reaction would be slow.

If formed, peroxynitrite is very reactive, is not stable under neutral pH conditions, and reacts with carbon dioxide yielding carbon centered radicals [Eq. (5)].\(^{50}\)

\[
\begin{align*}
\text{ONO}_2^- + \text{CO}_2 & \rightarrow \cdot\text{NO}_2 + \cdot\text{CO}_3^-
\end{align*}
\]
\[k = 4.6 \times 10^4 \text{M}^{-1}\text{s}^{-1} \text{ (pH 7.4).} \tag{5}\]

This is a moderately fast reaction, which can easily occur in cell culture medium. However, Carballal et al. indicated the parallel creation of NO2 under these conditions [see Eq. (5)].\(^{51}\) Being the anhydride of HNO3 it would be detectable by the Griess assay after reduction by VCl3.

The deposition of RNS is often accompanied by the creation of protons in the treated liquids. An acidic environment may have an impact on cell viability by itself. However, no changes in pH values were detected in PBS (pH 7.46) even after 10 min treatment (pH 7.46) with argon RF jet, w/or w/o 1% oxygen. In contrast, using distilled H2O (pH 5.91), a pH drop was measured (pH 3.80) under identical conditions. Accordingly, the RF plasma induces the creation of protons but these are scavenged by the buffer. We assume, therefore,
that chemical reactions or biological effects under the experimental conditions are not induced by pH changes.

In an alternative approach, the presence of RNS (or superoxide) was investigated using tyrosine as a chemical sensor according to Sawa et al.\textsuperscript{25} If either reactive species is created by the plasma treatment peroxynitrite can be formed in the liquid under specific experimental conditions. Its reaction with tyrosine would yield a nitrogen adduct (3-nitrosotyrosine, 3-nitrotyrosine) which could then be detected by UPLC/mass spectrometry. The optional presence of an NO donor (diethylamine NONOate) allows distinguishing between RNS plasma chemistry and ROS plasma chemistry. The results of this assay are shown in Fig. 11(b). Highest yields of 3-nitrotyrosine (3-NT) were detected for argon–air and pure argon RF plasmas, indicating the deposition of RNS by the plasma. An additional supplementation of a NO donor only increased 3-NT generation in the case of the pure argon RF plasma (Fig. 11, column Ar w/NO donor). Only small amounts of 3-NT could be measured for Ar/O\textsubscript{2} RF plasma even in the presence of the NO donor (columns ArO w/NO) regardless of distance (8 or 12 mm). This observation suggests that there is a role of RNS but it might be negligible under Ar/O\textsubscript{2} conditions and rather points at the presence of low amounts of ROS.

The data from Griess assay and from the tyrosine nitration assay show that RNS clearly plays a role in the case of pure argon RF jet and its humidified version as seen by nitrite deposition and 3-nitrotyrosine production. The role of RNS in the Ar/air and Ar/O\textsubscript{2} RF plasma triggered chemistry is less obvious as seen by a negative Griess assay and discrepant 3-NT production. Yet unraveled mechanisms may interfere with RNS detection or speed their decomposition. Their role in the liquid chemistry hence remains to be specified and thoroughly investigated in future work.

D. Time related effects of Argon–oxygen RF plasma

Effect of delay (1–60 min) between plasma treatment and transfer of the liquid onto cells is shown in Fig. 13. With a distance of 12 mm between nozzle and liquid surface [Fig. 13(a)], the average cell viability was unaffected by treatment with Ar/air plasma (360 s, \textapprox 95\% residual cell viability) or Ar/O\textsubscript{2} plasma (360 s, \textapprox 92\% residual cell viability). After argon RF plasma treatment (120 s) a residual cell viability of about 58\% remains throughout the observation period. This shows that active species generated by the argon plasma were stable for at least 60 min. This is in good agreement with H\textsubscript{2}O\textsubscript{2} centered chemistry as shown in Figs. 6(a), 6(b), 8(a), and 10(a). If the distance is reduced to 8 mm, the observed biological impact increases [Fig. 13(b)].

Argon RF plasma treatment (60 s treatment) showed a residual average cell viability close to 30\% throughout the observed time. A similar observation was made for Ar/H\textsubscript{2}O plasma (30 s treatment, \geq 20\% cell viability). In both cases, the increased deposition of H\textsubscript{2}O\textsubscript{2} with reduced distance between nozzle and liquid explains the observation [Fig. 6(a)]. When using Ar/air RF plasma jet no effect on cell viability was detected even after 360 s treatment (\textapprox 95\% residual cell viability, no effect of delay time). In contrast to all three cases, there is a strong dependence of the biological effect on delay time in the case of Ar/O\textsubscript{2} plasma (180 s treatment). A very profound impact on CRFK viability was detected if buffer was added immediately to the cells (0 min delay). This impact degrades with increasing delay time between treatment and addition to the cells; after 60 min delay, no effect on cell viability could be detected. The half-life time of this effect was approximately 30 min. In contrast, H\textsubscript{2}O\textsubscript{2} is stable under the experimental conditions for at least 60 min (in treatment buffer, a 3.5\% decay of plasma H\textsubscript{2}O\textsubscript{2} was observed within 90 min). Concentrations <500 \textmu M markedly reduce the cell proliferation rate by inducing a cell cycle block but do not lead to necrosis and total loss of cell viability within the 1 h incubation according the experimental procedure (Fig. 4). Hence, even after long RF argon plasma treatment (>1000 U), inducing H\textsubscript{2}O\textsubscript{2} chemistry, a residual cellular metabolism is detectable (Fig. 4). As this is not the case for Ar/O\textsubscript{2} RF plasma [Fig. 13(b)], this treatment creates a different chemistry.
To facilitate identification, the reactive species created by the RF plasma variants, different scavengers have been added to the treatment buffer (PBS + 1 g/l glucose). The results are summarized in Table I. Neither catalase nor the small molecule H$_2$O$_2$ scavenger sodium pyruvate did attenuate cell viability reduction triggered by argon–air or argon–oxygen RF plasma, but both were effective in the argon and argon–water case. This is in agreement with the chemical detection of the H$_2$O$_2$ presence [Fig. 10(a)]. Catalase reacts in an enzymatic reaction with H$_2$O$_2$. The reaction is very fast ($2 \times 10^{-7}$ M$^{-1}$s$^{-1}$), yielding oxygen and water. Pyruvate nonenzymatically reacts with H$_2$O$_2$, creating CO$_2$, acetic acid, and water.

\[ \text{CH}_3\text{COCOO}^- + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{CH}_3\text{COO}^- + \text{H}_2\text{O}. \]  

The scavenging effect of peroxynitrite with pyruvate has also been proposed ($k \approx 88$ M$^{-1}$s$^{-1}$ at pH 7.4$^5$). No effect on cell viability was found upon the addition of SOD and SOD-catalase mixture in the case of argon–oxygen RF plasma. This seems to indicate that the effect on cell viability reduction in the effluent remains constant [see Fig. 9(d)]. Atomic oxygen is the only neutral species that seems to have significant distance dependence in the effluent (Fig. 9). This is consistent with the distance dependent effect observed in the cell proliferation study (Fig. 6). Secondary chemistry induced by O in the liquid phase with a longer lifetime seems to be responsible for the effect. Unfortunately, the O chemistry in liquids is not studied in detail and actually only recently gained interest in the biological community.$^5$9

To check the hypothesis of atomic oxygen induced chemistry, we investigated the decomposition of H$_2$O$_2$ by the plasma treatment (Fig. 14). Under some conditions, H$_2$O$_2$ reacts quickly with atomic oxygen (1.6 $\times$ 10$^9$ M$^{-1}$s$^{-1}$, pH > 8.7) but much slower with ozone O$_3$ (0.065 M$^{-1}$s$^{-1}$). Indeed, a reduction of the H$_2$O$_2$ concentration was found for 8 mm, while this was not the case for 14 mm [Fig. 13(a)], indicating that the O chemistry might indeed be important for the distance effects observed in the argon–oxygen RF plasma jet.

The O chemistry could lead by reaction with dissolved O$_2$ in the liquid phase to form O$_3$ and in principle induce the same chemistry as O$_3$.$^5$1 Nonetheless, we found that the presence of chloride ions (Cl$^-$) is critical to obtain the depletion in H$_2$O$_2$ concentration [Fig. 13(b)]. We believe that this could be due to the known effect of scavenging of active chlorine compounds by H$_2$O$_2$.$^5$2

The following reaction mechanism is proposed:

\[ \text{Cl}^- + \text{O} \rightarrow \text{OCl}^- . \]  

However, to the authors’ knowledge, reaction rates of this reaction are not reported in literature. It is similar to
ClO
\(^{-}\) will react with H\(_2\)O\(_2\) forming Cl\(^{-}\), O\(_2\), and H\(_2\)O, as is as a standard performed as a dechlorination process [Eq. (9)] (Refs. 65 and 66)

\[
\text{OCl}^- + \text{H}_2\text{O}_2 \rightarrow \text{Cl}^- + \text{H}_2\text{O} + \text{O}_2, \quad (9)
\]

\[
\text{H}^+ + \text{ClO}^- \rightarrow \text{HOCI}; \quad p\text{Ka} = 7.8. \quad (10)
\]

ClO\(^{-}\), being in equilibrium with its corresponding acid [Eq. (10)] has a strong impact on cell viability if present (>10 \(\mu\)M, Fig. 4). As indicated by the decomposition of 200 \(\mu\)M H\(_2\)O\(_2\) within 100 s plasma treatment, these concentrations can be easily reached [Fig. 13(b)]. Hypochlorite is a strong oxidant and has intense antimicrobial properties but holds no genotoxic potential. In humans, it plays an important role in unspecific immunity as the product of the enzyme myeloperoxidase, which is found in neutrophils.

Alternatively, the following reaction could be responsible for the decay of H\(_2\)O\(_2\) in the presence of Cl\(^{-}\):62,69

\[
\text{Cl}^- + \cdot\text{OH} \rightarrow \text{HClO}^-; \quad k = 3 \times 10^9 \text{M}^{-1}\text{s}^{-1}, \quad (8)
\]

which is a very fast reaction and an equivalent reaction is proposed in biology.64

\[
\text{HClO}^- \rightarrow \text{Cl}^- + \cdot\text{OH}, \quad (8a)
\]

The ClO\(^{-}\) will react with H\(_2\)O\(_2\) forming Cl\(^{-}\), O\(_2\), and H\(_2\)O, as is as a standard performed as a dechlorination process [Eq. (9)] (Refs. 65 and 66)

\[
\text{ClO}^- + \cdot\text{OH} \rightarrow \text{HClO}^-; \quad k = 3 \times 10^9 \text{M}^{-1}\text{s}^{-1}, \quad (8)
\]
able to detect the deposition of NO$_2^-$/NO$_3^-$ [Fig. 11(a)] after treatment with Ar/air or Ar/O$_2$ plasma. This observation highlights again a possible role of RNS, which have not yet been thoroughly understood.

In Figs. 14(c) and 14(d), the residual impact of a hydrogen peroxide solution in physiological buffer (138 vs 1 mM chloride) on mammalian cells after Ar/O$_2$ RF jet treatment is shown. A significant difference between treated (apparent IC$_{50}$) and untreated PBS-H$_2$O$_2$ solution (IC$_{50}$ = 15.5 μM) was detected [Fig. 14(e)]. This difference is attributed to the reduction of H$_2$O$_2$ in the solution according to Eqs. (9) and (11). In contrast, no such effect could be detected when the chloride depleted sucrose buffer is used [Fig. 14(d)]. Apparent and authentic IC$_{50}$ are almost identical (16.8 vs 15.5 μM). This conclusion is further supported by using a chemical quantification of H$_2$O$_2$ [Fig. 10(b)]. Hydrogen peroxide accumulation is only seen when chloride ions are (almost) absent (chloride –). In high chloride conditions (chloride +), roughly 100 μM of H$_2$O$_2$ are consumed within 40 s of Ar/O$_2$ treatment, which is then reflected in the increase of the apparent IC$_{50}$. These observations proof the assumed reaction (7) yielding OCl$^-$ under Ar/O$_2$ RF treatment and underline the modifying role of chloride ions in plasma liquid chemistry. ClO$^-$ is thermodynamically unstable and it is in equilibrium with Cl$_2$ through:

$$2H^+ + ClO^- + Cl^- \rightarrow Cl_2 + H_2O.$$  \hspace{1cm} (14)

The formed chlorine gas can be released from solution which could account for the limited lifetime of a RF Ar/O$_2$ plasma treated solution in terms of biological impact [Fig. 13(b)]. The presence of other RNS and ROS could enhance this effect. A similar observation has not been made for the kinpen due to the lower O density produced. The lower density is most likely due to the fact that the kinpen does not operate in RF mode (13.7 MHz) but at lower frequency (around 1 MHz). Transitions from typical DBD modes (Townsend discharge, glow discharge) to RF modes have been found by Massines et al. at 1.3 MHz in parallel plate geometries consistent with such a finding.

IV. CONCLUSIONS

An RF plasma jet operating with four different working gases (argon, argon/water, argon/air, and argon/oxygen) was investigated with respect to its biological impact, its distance dependency and the effect of time between plasma–liquid and liquid–cell contact. We found remarkable differences in the magnitude of the biological impact: Ar/water $\gg$ Ar $>$ Ar/O$_2$ $>$ Ar/air plasma (distance nozzle–liquid 12 mm, no time delay after plasma treatment). In this case, effects could be attributed to hydrogen peroxide. However, if the distance was reduced to 8 mm, Ar/O$_2$ RF plasma showed a massive impact on cell proliferation. This distance dependent effect wore off with increasing delay time between treatment of the liquid and its cell contact. Using modeling data, chemical detection, comparison with the kinpen, and literature, we propose that in case of Ar/O$_2$ RF plasma, hydrogen peroxide plays no prominent role when standard physiologic buffer or body fluids are treated; instead, the plasma chemistry is driven by atomic oxygen. Via reaction with chloride yielding Cl$_2^-$ or ClO$^-$, any hydrogen peroxide produced by the plasma itself is decomposed. These chlorine species have a limited lifetime under physiologic conditions and therefore show a strong time dependent biological activity. They interact with biological macromolecules, show an antimicrobial effect, and could hence be attributed to be responsible for the strong effects of oxygen admixtures in the biological impact of many nonthermal atmospheric pressure plasma sources. In addition, the results show clear differences in the biological action between two seemingly similar plasma jets, i.e., RF plasma jet and kinpen, which is attributed to strong differences in reactive species densities and different geometries. Hence, a distance dependency of species concentrations occurs in the RF jet, which is not found in the kinpen. While the importance of RNS for certain plasma conditions has been shown, the detailed chemical mechanism remains unclear.

ACKNOWLEDGMENTS

This work was founded, in part, by the German Federal Ministry of Education and Research (Grant No. 03Z2DN11), the Department of Energy Plasma Science Center through the U.S. Department of Energy, office of Fusion Energy Sciences (Contract No. DE-SC0001939), the University of Minnesota and the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT Flanders). W.V.G. and A.B. thank Mark Kushner (University of Michigan) for providing the numerical code.

5S. Perni, G. Shama, and M. G. Kong, J. Food Prot. 71, 1619 (2008).


S. Chemicals, in Dechlorination with Hydrogen Peroxide (Solvay Chemicals, 2005), Vol. H7-166.


