The Influence of Feed Gas Humidity Versus Ambient Humidity on Atmospheric Pressure Plasma Jet-Effluent Chemistry and Skin Cell Viability

Stephan Reuter, *Member, IEEE*, Jörn Winter, Sylvain Iséni, Ansgar Schmidt-Bleker, Mario Dünnbier, Kai Masur, Kristian Wende, and Klaus-Dieter Weltmann, *Member, IEEE*

***Abstract*— The influence of ambient air species especially humidity is an ever-present challenge for atmospheric pressure plasma jet applications. Especially, where the plasma-induced effects are extremely sensitive to humidity, such as in the field of plasma medicine, an understanding of the influence of ambient species diffusion on plasma chemistry and on reactive component composition is crucial. In this paper, we investigate the influence of ambient humidity versus feed gas humidity on the production of reactive components by atmospheric pressure plasma jets. By the use of a shielding gas curtain, we control the surrounding atmosphere around the active effluent region of the investigated argon RF-plasma jet (kinpen) and control the gas humidity of the ambient gas. By quantum cascade laser absorption spectroscopy and by Fourier transformed infrared (IR) absorption spectroscopy, the effect of diffusing surrounding molecular species on the chemistry of the long-living reactive oxygen species is investigated. Mechanisms of H_2O_2 and O_3 production are studied. In this paper, we have quantified the influence that ambient species, namely, water molecules, have on the reactive species' generation in the gas phase. It is shown that the effect of ambient humidity is important for the long-living species production, feed gas humidity, however, has the much stronger effect. Finally, with the focus of applications in plasma medicine, the cell viability of human skin cells (HaCaT keratinocytes) as a function of feed gas and ambient gas humidity is compared.**

***Index Terms*— Ambient humidity, ambient species, atmospheric pressure plasma jet, cell viability, feed gas humidity, plasma chemistry, plasma medicine.**

I. Introduction

ESPECIALLY in plasma medicine, the use of cold atmospheric pressure plasma jets [1] has become promising within the past decade [2]–[5]. In this field of application, it is vital that plasma and operating conditions are held stable to ensure constant and controllable therapeutic effects. Due to the necessity of plasma operation in ambient air for *in vivo* treatment, potentially uncontrollable conditions arise. It is therefore vital to investigate the influence of these uncontrollable conditions of the ambient surrounding on the reactive species generation mechanisms. Humidity and diffusing air species threaten to be responsible for a distortion of the reactive species composition generated by atmospheric pressure plasma jets.

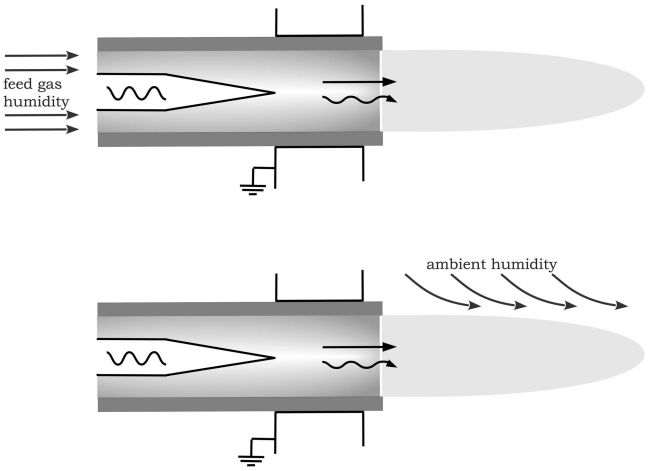

Fig. 1. Schematics of the plasma jet investigated for humidity admixture to the feed gas (top) and humidity admixture to the active effluent region (bottom).

This paper investigates the influence of humidity on long-living reactive species (lifetime > millisecond) generated by an atmospheric pressure argon plasma jet (kinpen [6], [7]) shielded by a gas curtain with a defined gas composition [8], [9]. These reactive species are detected by infrared absorption spectroscopy in the far field of the plasma jet, where no charged or metastable species are expected.

II. Plasma Jet and Gas Curtain

For our study, an atmospheric pressure plasma jet—the kinpen was used, Fig. 1. The plasma jet consists of a centered rod electrode inside a ceramic capillary and a grounded outer ring electrode [6], [10]. To the powered central electrode, a voltage of about 2 kV_{pp} is applied with a frequency in the

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S. Reuter, J. Winter, S. Iséni, A. Schmidt-Bleker, M. Dünnbier, K. Masur, and K. Wende are with the Center for Innovation Competence plasmatis, Leibniz Institute for Plasma Science and Technology, Greifswald 17489, Germany (e-mail: stephan.reuter@inp-greifswald.de; winter@inp-greifswald.de; ansgar.schmidt-bleker@inp-greifswald.de; kai.masur@inp-greifswald.de; kristian.wende@inp-greifswald.de).

K.-D. Weltmann is with the Leibniz Institute for Plasma Science and Technology, Greifswald 17489, Germany (e-mail: weltmann@inp-greifswald.de).

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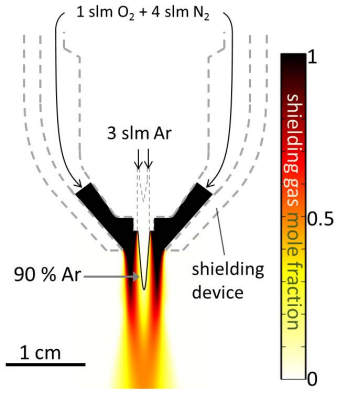

Fig. 2. Effect of gas curtain at 3-slm jet flux and 5-slm curtain gas flux.

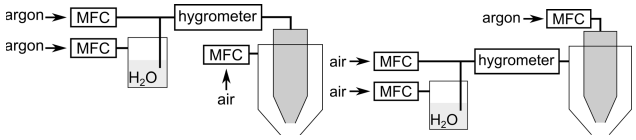

Fig. 3. Feed gas humidification setup (left) and shielding gas humidification (right).

order of 1 MHz. The working gas argon (purity 99.999%) with a gas flow rate of 3 slm is guided directly into the head of the kinpen.

To shield the effusing argon gas from ambient air species, an additional surrounding hull made of glass is constructed around the plasma jet. The operating principle and the efficiency have been shown in [8]. The shielding gas surrounds the active effluent zone. As shielding gas artificial air consisting of 80% nitrogen (N_2 purity 99.999%) and 20% oxygen (O_2 purity 99.995%) with a gas flow rate of 5 slm is used. Fluid simulations (Fig. 2) show the shielding effect of the gas curtain. The simulations are described in detail in [8]. Fig. 2 shows the mole fractions of the shielding gas obtained by the computational fluid dynamics simulations. The contour line, indicating a mole fraction of 90% argon, roughly marks the boundary of the visible effluent. It can be observed that a shielding gas curtain forms around the visible effluent. While the desired interaction of the argon plasma with the shielding gas is enabled, the ambient air is almost completely shielded and reaches only values well below 0.1% in the region of the visible effluent. The varying mass densities for different shielding gases (dry/humid) do not significantly alter the shielding gas distribution. The observed variations of the experimental results are thus associated with the shielding gas composition only.

For the studies, the humidity is varied and determined by a hygrometer (DewMaster, EdgeTech, USA). On the one hand, the humidity is varied from 0 to 2000 ppm in the feed gas at dry artificial air shielding gas conditions. On the other hand, the humidity of artificial air shielding gas is varied in the range between 0 and 20 000 ppm at dry argon feed gas conditions. For the feed gas humidity variation, a bypass argon flux of up to 0.5 slm is fed through a water bubbler (left side of Fig. 3). All gas flows are controlled by mass flow controllers (MKS). For the shielding gas humidification,

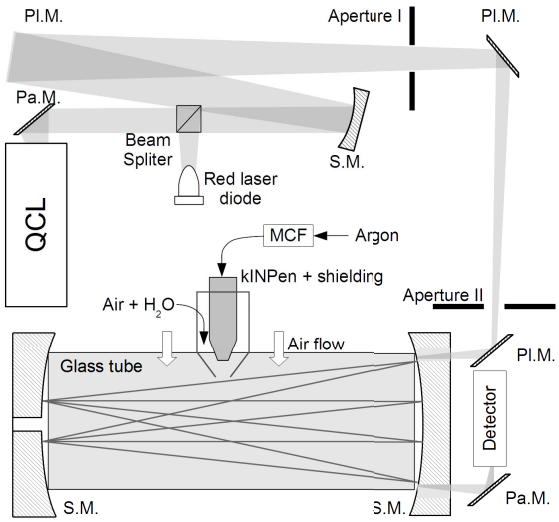

Fig. 4. Schematics of QMACS setup.

the total gas flux (20% oxygen and 80% nitrogen controlled by mass flow controllers) bypassing through the bubbler is regulated by a needle valve (right side of Fig. 3).

III. Diagnostic Setups

In this paper, the long-living species produced by the plasma jets effluent and its interaction with the ambient atmosphere is diagnosed by multipass cell infrared absorption spectroscopy. Using a multipass cell increases the total absorption length and thus the sensitivity of the setup.

A. Optical Emission Spectroscopy

Space-resolved optical emission spectroscopy in the effluent region of the plasma jet is performed using an Andor Shamrock 500 spectrograph with a grating of 2400 lines. The effluent of the plasma jet is imaged onto the entrance slit. The spectra are recorded with an Andor Istar Electron multiplying charge-coupled device. Three maps of the OH(A-X) emission intensity integrated from 305 to 313 nm are recorded, one for dry feed gas and humid shielding gas, one for dry shielding gas and humid feed gas, and one for dry feed gas and dry shielding gas.

B. IR Quantum Cascade Laser Absorption Spectroscopy

The ozone density has been investigated by quantum cascade laser (QCL) spectroscopy in the infrared region [10], [11]. Fig. 4 shows the complete diagnostic setup with the kinpen and the gas shielding device. The measurement system is based on the Q-MACS system developed by neoplas control GmbH, which has been optimized for operation at atmospheric conditions [12]. The midinfrared light source is a pulsed QCL emitting in the spectral range from 1024 to 1030 cm^{-1}. The radiation is controlled by temperature adjustment. The QCL operates in single mode. The system can be tuned within a small range of 0.4 cm^{-1} by an increasing of the operating current. The laser beam divergence is corrected by a parabolic gold-coated off-axis mirror. It is

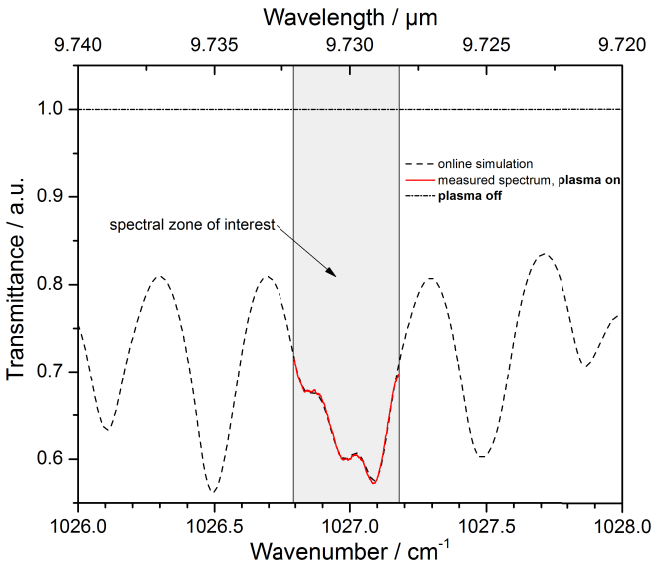

Fig. 5. Absorption spectrum of the Q-MACS IR-measurements.

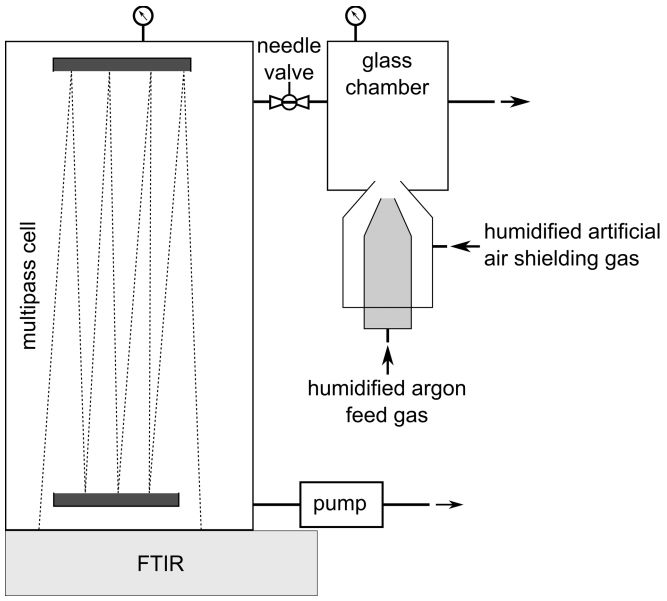

Fig. 6. Schematics of FTIR setup for the case of shielding gas humidification.

then focused onto a spherical mirror with a focal length of 1 m and guided through a 60-cm length multipass white cell. After 28 reflections within the cell, resulting in a total absorption length inside the multipass cell of 16.80 m, the intensity is measured by a fast mercury cadmium telluride detector.

A detailed description of the measurement procedure can be found in [10] and [12]. Briefly, the measurements were performed at atmospheric pressure in a glass tube with openings at each end for the mirrors, where the midinfrared reflection of the beam passes without disturbance. As shown in Fig. 4, the plasma jet is positioned in the middle of the multipass cell, without the laser passing through the plasma itself. The plasma source feed gas is blown directly into the glass chamber. The species distribution is assumed to be homogeneous.

Fig. 5 shows a simulated and a recorded spectrum of the ozone absorption at standard pressure and temperature conditions. The spectral region of interest, corresponding to the maximum tuning range of the QCL source, ranges from 1026.8 to 1027.2 cm^{-1} (shaded area in Fig. 5). The concentration of ozone is calculated from a fit algorithm implemented in the Q-MACSoft Monitor Software, which links the measured spectrum with the simulation [10]. The fit data are taken from the HITRAN database [13]. Fig. 5 shows the perfect agreement of measurement and fit result. For an accurate determination of ozone concentration, a spectrum is recorded every 2 s for approximately 5 min and then averaged. The errors are assumed as twice the standard deviation.

C. Fourier Transformed Infrared Absorption Spectroscopy

For the hydrogen peroxide (H_2O_2) measurement, in this paper, Fourier transformed infrared (FTIR) absorption spectroscopy in a multipass cell using a Bruker Vertex 70 v (Bruker GmbH) was applied. Fig. 6 shows a schematic diagram of the setup. To guide the plasma activated gas into the multipass cell of the FTIR spectrometer, a glass chamber around the effluent of the kinpen was constructed. The gas flow into the multipass cell was regulated with a needle valve. The pressure inside the multipass cell was reduced from the atmosphere and measured with a pressure gauge (Baratron, MKS).

The advantage of the lower pressure is the reduction of the particle collision rate and, as a consequence, a longer lifetime of the investigated species. Nevertheless, only long-living species (>millisecond lifetime) can be detected with this setup. In addition, the pressure broadening of the absorption signal is decreased, thus, a discrimination of the measured signal from the background is easier and thus an attribution of the absorption features to the respective molecules is more specific.

The measurement procedure is as follows. First, the gas supply of the kinpen and the shielding device are switched on. Second, the initially evacuated multipass cell is filled with the working gas up to the desired pressure (100 and 600 mbar in our study). Then, the background is measured with the FTIR. Immediately afterward, the plasma jet is switched on and the signal is measured for 15 min until it reaches a constant value. Finally, the plasma jet is switched off and the subsequent set of parameter is adjusted. After flushing the multipass cell for at least 15 min with the new gas mixture, the next measurement is started. Fig. 7 shows an exemplary FTIR spectrum of a gas shielded atmospheric pressure argon plasma jet operated with humid feed gas (3-slm argon and humidity concentration: 1890 ppm) and dry shielding gas (compressed air). The multipass cell pressure and absorption length were 100 mbar and 19.2 m, respectively. For clarity, only every fifth measurement data point is displayed. The spectrum is fitted by Q-MACSoft HT using spectroscopic data of O_3 and H_2O_2 from the HITRAN database [13]. For the quantification of the H_2O_2 production, metallic surfaces in the experimental setup were reduced as much as possible, but could not be completely avoided. However, due to the large volume of the multipass cell and the resulting high volume to surface ratio, as well as the reduced pressure, the interaction of the molecules with

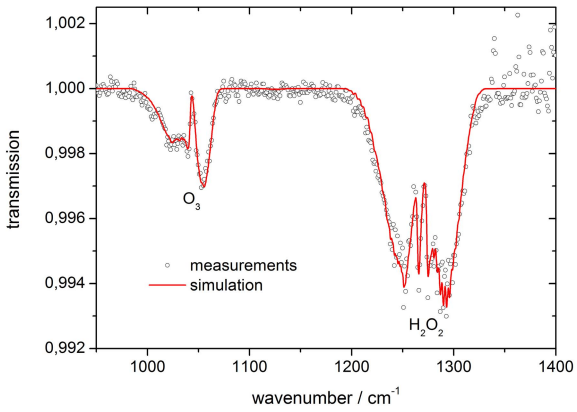

Fig. 7. Fitted FTIR spectrum at humid feed gas and dry air shielding gas conditions.

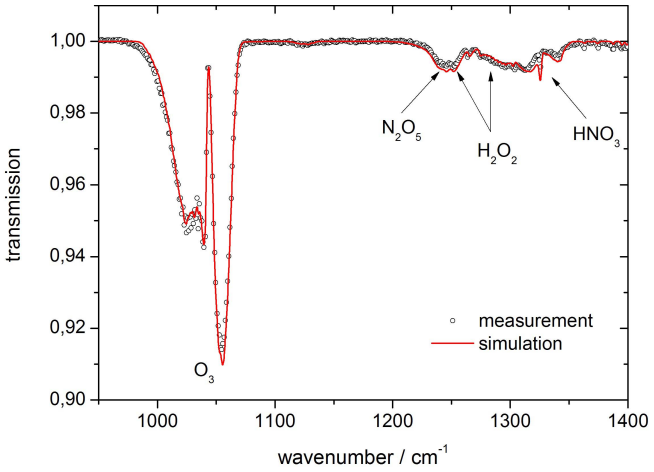

Fig. 8. Fitted FTIR spectrum of at dry feed gas and humid shielding gas conditions.

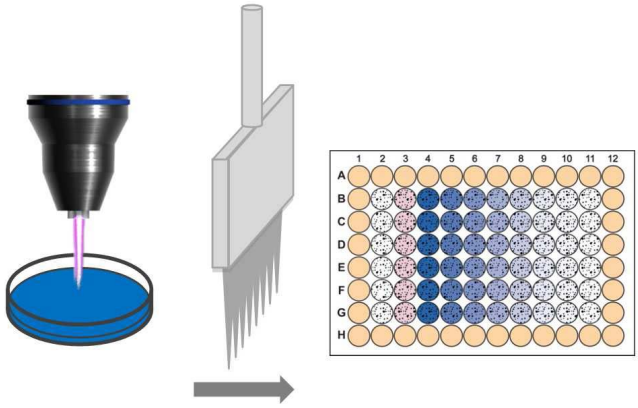

Fig. 9. Treatment procedure for separate treatment of HaCaT cells with variation of feed gas humidity where cell culture medium is treated and then pipetted into a cell containing 96 well plate (based on [14]).

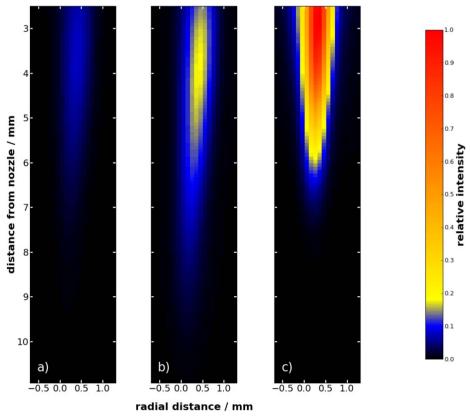

Fig. 10. 2-D emission profile of the OH(A-X) emission band for (a) almost dry conditions (<20 ppm), (b) ambient humidity (10 000 ppm), and (c) feed gas humidity (490 ppm).

the surface, including the metallic one is considered not to dominate the chemical processes.

Fig. 8 shows an exemplary FTIR spectrum of a gas shielded atmospheric pressure argon plasma jet operated with dry feed gas (3-slm argon) and humid shielding gas (humid air, humidity concentration: 3655 ppm). The multipass cell pressure and absorption length were 600 mbar and 32 m, respectively. For clarity, only every fifth measurement data point is displayed. The spectrum is fitted using spectroscopic data of O_3, N_2O_5, H_2O_2, and HNO_3 from HITRAN database via Q-MACSoft HT [13]. From the measurements, an HNO_3 concentration of roughly $3 \cdot 10^{12}$ cm^{-3} can be estimated.

D. HaCaT Skin Cell Treatment and Cell Viability Assay

For the investigation of biological effects, Human Keratinocyte cells (HaCaT) are treated indirectly with the plasma jet. For this procedure, the plasma jet is moved with an xyz stage in a meandering circular shape across a 60-mm petri dish with 5-ml Roswell Park Memorial Institute cell culture medium (Fig. 9). After the given treatment time, the plasma treated medium was pipetted into a 96 well plate (qualitative sketch in Fig. 9). Afterward, a dilution series is started according to [14]. Cell viability was assessed after 72 h by resazurin conversion assay (50 μm and 1 h), indicating the impact of the plasma treatment on cell proliferation rate.

IV. Results and Discussion

A. Influence of H_2O Admixture on the Spatial OH Emission Profile

As a first insight into the influence of water admixture on the reactive species generation, space-resolved optical emission spectroscopy was performed for various dry and humid parameter sets. The wavelength-integrated space-resolved OH(A-X) emission band is shown in Fig. 10. The various parameters shown are wet feed gas and dry shielding gas, wet shielding gas and dry feed gas, as well as dry feed gas and shielding gas.

A very intense emission can be observed for the case of humid feed gas. It decreases monotonously from the nozzle.

For the case of humid shielding gas, a maximum OH emission can be observed outside the jet nozzle. Due to limited excitation outside the jet, this maximum can be attributed to the precursor inflow from ambient water molecules.

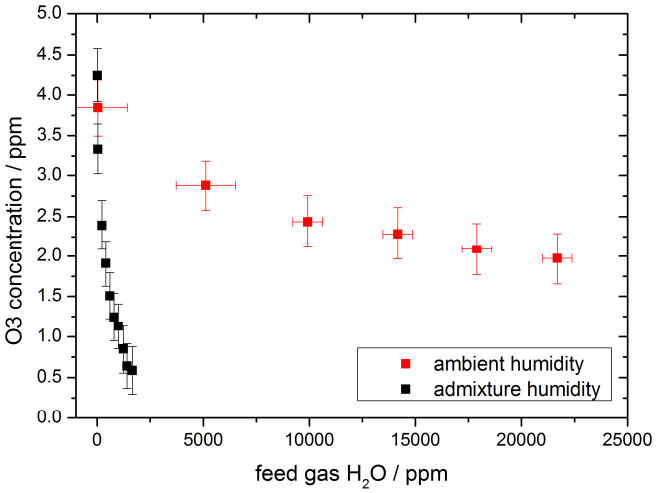

Fig. 11. Effect of feed gas humidity versus ambient humidity on ozone concentration in the multipass cell.

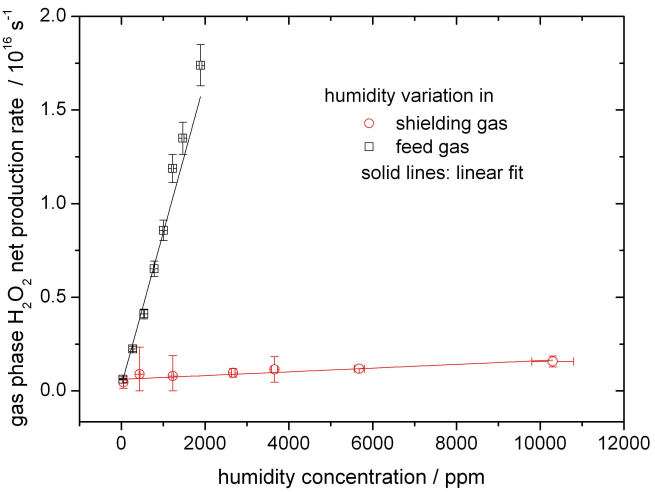

Fig. 12. Gas phase H_2O_2 concentration derived from FTIR measurements in the multipass cell for different humidity concentrations either in the feed gas (data taken from [2]) or in the shielding gas.

Near dry conditions exhibit a very low OH emission with only a slight maximum outside the nozzle. This seems to be an overlay of both effects.

The interesting fact is that a high OH emission signal achieved for feed gas humidity is reached at water admixture of 10 times lower than that of outside humidity. This agrees with the investigations performed in [2].

These results lead to the assumption that humidity related processes in the feed gas have greater impact than ambient humidity.

B. Influence of H_2O Admixture on Ozone and H_2O_2 Concentration

A vital role in plasma chemistry is played by H_2O [2]. However, the mechanism and consequence of humidity diffusing into the active effluent have not been deeply investigated so far. Since atmospheric pressure plasma jets are usually operated in ambient air at different humidity levels, a thorough humidity influence investigation is necessary, especially for these kinds of plasma sources.

Fig. 11 shows the influence of different shielding gas humidity concentrations on ozone. Since only H_2O is admixed to the feed gas (humid feed gas conditions), the observed O_3 concentrations for the case of humid feed gas mostly, and for the case of dry feed gas only, originate from reactions of indiffusing oxygen and H_2O molecules from the shielding gas.

For the ozone concentration, a strong decrease is observed with increasing humidity concentration. It can be observed that water reduces the concentration of ozone in the multipass cell by a factor of two for the case of shielding gas admixture and by a factor of three for the case of feed gas admixture.

The ozone reduction is either due to a reduced production or an increased destruction of ozone.

The major ozone production process is the reaction of molecular oxygen with atomic oxygen according to

$$O_2 + O + M \rightarrow O_3 + M \tag{1}$$

with a reaction coefficient $5.92 \cdot 10^{-34}$ $cm^6/mol^2 \cdot s$ at 298 K. In the present case, it is considered that the production of O or O_3 by UV radiation is minor and less than 5% compared with the chemical reactions [15]. Therefore, ozone results only from the reactions of molecular oxygen and atomic oxygen. Obviously, the production of ozone can be highly influenced by an admixture of molecular oxygen within the feed gas [10].

Since the concentration of the collision partner M and the molecular oxygen in (1) are independent of the humidity concentration, only a humidity-induced decrease of atomic oxygen would explain a decrease in the production of ozone. This can be attributed to the loss of electrons due to the molecular admixture. This loss of electrons will reduce excited or ionized argon species and reduce the dissociation of oxygen from air and water.

On the other hand, the destruction of ozone can generally occur for our conditions via the following channels:

$$O_3 + AB \rightarrow AO_2 + BO \tag{2}$$

$$O_3 + A \rightarrow AO + O_2 \tag{3}$$

$$O_3 + A \rightarrow O + O_2 + A \tag{4}$$

where A and B are nitrogen, oxygen and/or hydrogen reaction partners. Since the concentration of the most reactants A and AB depend on H_2O concentration the destruction of O_3 itself is humidity dependent. Presumably, the observed O_3 decline is not only a reduced production or increased destruction processes but also a superposition of both.

From Fig. 11, it can be observed that both for the admixture of water to the feed gas and to the shielding gas, the ozone density decreases monotonously. The shape of ozone concentration reduction is almost the same for both cases, and the quantity is different by a factor of 20.

Fig. 12 shows the development of H_2O_2 concentration as a function of humidity admixture both for the case of H_2O admixture to the feed gas and to the shielding gas.

Clearly, a linear increase can be observed as a function of humidity both for the feed gas and shielding gas

humidity admixture. To analyze the measurements, three different plasma regions need to be considered for the production and destruction of H_2O_2, namely, the core plasma region with electron and excited species dominating the processes, the effluent plasma region, where additionally ambient species chemistry is present, and the far field region, where plasma species play no dominant role and only chemical reactions occur. Since only argon and water molecules are feed into the core plasma zone H_2O_2 is produced by dissociation of water via the production of OH according to

$$P + H_2O \rightarrow OH + H + P \tag{5}$$

where P is a plasma species, namely, electrons or excited argon species. OH recombines in a three-body collision with a collision partner M to H_2O_2

$$OH + OH + M \rightarrow H_2O_2 + M.$$

If ambient humidity is present, this process also occurs in the second region—the effluent region. Since the electron density and temperature are much lower in this region, the amount of H_2O_2 produced is lower compared with the core plasma region. In addition to the H_2O_2 production via OH, the abundance of ambient O_2 in that region opens a second way of generating H_2O_2

$$H + O_2 + M \rightarrow HO_2 + M \tag{6}$$

where atomic hydrogen reacts with molecular oxygen to form the perhydroxyl radical. Subsequently, two perhydroxyl radicals can finally react to H_2O_2

$$2HO_2 + M \rightarrow H_2O_2 + O_2 + M. \tag{7}$$

However, perhydroxyl also reacts with remaining OH radicals according to

$$HO_2 + OH \rightarrow H_2O + O_2 \tag{8}$$

yielding in a self-destruction of educts necessary for H_2O_2 generation.

From the results, the relevance of the respective production processes can be deduced. Since adding water to the feed gas yields the most efficient way to generate H_2O_2, the dissociation of H_2O by plasma species to generate OH is the dominant reaction pathway, and (6) and (7) provide only minor contribution to the hydrogen peroxide generation.

It can be observed that as for the ozone generation, the H_2O_2 concentration requires a more than tenfold higher humidity admixture in the shielding gas compared with the admixture to the feed gas to have the same effect.

C. Influence on Cell Viability

Fig. 13 shows the cellular viability of HaCaT skin cells treated by the procedure described above after 72 h of incubation. The viability is shown as a function of feed gas humidity (black squares) and of shielding gas humidity (open circles). It can be clearly observed that the cell viability decreases with higher humidity (for the case of feed gas admixture) or remains unchanged for the case of shielding gas humidity.

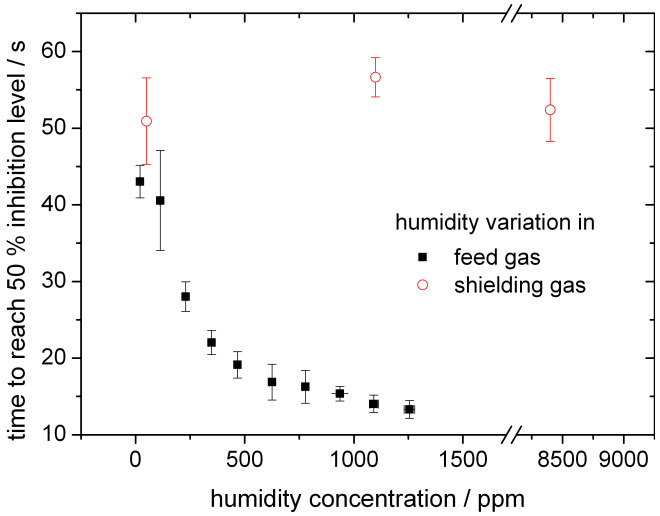

Fig. 13. Cell viability of HaCaT skin cells for the treatment with humidity added to the feed gas (black squares) and to the shielding gas (open circles).

From the species measurements, we can observe that for increasing feed gas humidity admixture, viability decreases, and ozone production also decreases. For the shielding gas humidity variation, the viability remains constant while the ozone concentration drops by a third.

More interestingly, the H_2O_2 concentration increases with humidity admixture, which correlates with a reduced cell viability. In earlier work, the effect of humidity was investigated on the cell viability [2]. Already, the assumption was made that the viability correlates with the H_2O_2 dynamics. This is confirmed by this paper, but more studies are needed to verify the assumption.

Finally, the shielding gas humidity variation shows little effect on the H_2O_2 concentration, which is reflected in the cell viability response.

V. Conclusion

In this paper, the influence of ambient and feed gas humidity on reactive species generation by plasma and their effect on cell viability was investigated. It could be shown that for feed gas humidity variation, the ozone concentration rapidly decreased by a factor of three. For humidity variation in the shielding gas, also a decrease of ozone was detected, but the effect was 20 times lower than for the feed gas humidity variation.

The H_2O_2 concentration increased linearly by almost an order of magnitude, while again, for an ambient humidity variation, the effects were more subtle and the H_2O_2 concentration increases only slightly.

From this paper, it could be shown that electron or energetic plasma species-based dissociation of water to the OH molecule is the relevant production pathway for the generation of H_2O_2.

The cell viability reaction to the plasma treatment rather followed the development of the H_2O_2 species than the ozone species. Further investigations will ensure the effect of H_2O_2 in indirect plasma treatment of skin cells.

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Stephan Reuter (M'12) received the M.E. (Dipl.-Phys.Ing.) and M.Sc.(Dipl.-Phys.) degrees in plasma physics from the University of Duisburg-Essen, Essen, Germany, and the Ph.D. (Dr.rer.nat.) degree in 2007 at the University of Duisburg-Essen for the investigation of oxygen formation mechanisms in atmospheric pressure plasma jets.

He became a Research Fellow at the Centre for Plasma Physics, Queen's University Belfast, Belfast, U.K., in 2008. He is currently the Head of the Extracellular Effects Junior Research Group with the German Federal Ministry of Education and Research-Funded Centre for Innovation Competence Plasmatis, Leibniz Institute for Plasma Science and Technology, Greifswald, Germany, where he performs research on controlling the interaction of atmospheric pressure plasmas with biological liquids in the field of plasma medicine. The focus of his research group lies on optical diagnostics and modeling of atmospheric pressure plasma jets interacting with liquids.

Dr. Reuter is a member of the German Physical Society, the International Society for Plasma Medicine, the Nationales Zentrum für Plasmamedizin, and the International Society for Plasma Chemistry.

Jörn Winter received the Diploma degree in physics and the Ph.D. (Dr.rer.nat.) degree in experimental physics from the University of Greifswald, Greifswald, Germany, in 2005 and 2009, respectively.

He became a Scientific Staff Member at the Leibniz Institute for Plasma Science and Technology, Greifswald, in 2006, where he was involved in mercury-free low-pressure gas discharges for lighting, and plasma diagnostics. In 2008, he joined the Department of Research and Development, Webeco GmbH and Company KG., Selmsdorf, Germany, where he was involved in plasma generation for the sterilization of endoscopes. Since 2010, he has held a post-doctoral position with the German Federal Ministry of Education and Research-Funded Centre for Innovation Competence Plasmatis, Leibniz Institute for Plasma Science and Technology, where he is investigating plasma-cell interactions with a focus on plasma diagnostic.

Sylvain Iséni was born in France in 1988. He received the M.Sc. degree in physics from the University of Orléans, Orléans, France, in 2011, the M.Eng. degree in optics, laser, and plasma physics and processes and the Dipl.Eng.M.Sc. degree from the École Polytechnique de l'Université d'Orléans, Orléans in 2011. In 2011, he performed his master's thesis at the Eindhoven University of Technology, Eindhoven, The Netherlands, and the Leibniz Institute for Plasma Science and Technology, Greifswald, Germany, with the topic of diagnostics of atmospheric pressure plasma jets applied for biomedical applications. He is currently pursuing the Ph.D. degree with the German Federal Ministry of Education and Research-Funded Center for Innovation Competence Plasmatis, Leibniz Institute for Plasma Science and Technology, with a focus on electrical characterization and laser diagnostics of atmospheric plasma microdischarges.

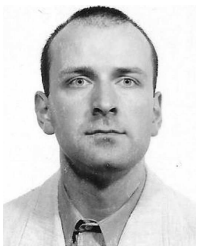
Mr. Iséni has been a member of the International Society for Plasma Medicine since 2012.

Ansgar Schmidt-Bleker received the Diploma degree in physics from RWTH Aachen University, Aachen, Germany, in 2010. He is currently pursuing the German Federal Ministry of Education and Research-Funded Centre for Innovation Competence Plasmatis, Leibniz Institute for Plasma Science and Technology, Greifswald, Germany, with a focus on simulations for biomedical applications of plasmas.

He investigated nonlinear effects in quantum plasmas with the Institute for Theory of Statistical Physics until 2011 as a scientific employee with RWTH Aachen University.

Mario Dünnbier received the B.Sc. and M.Sc. degrees in plasma physics from the University of Greifswald, Greifswald, Germany, in 2009 and 2011, respectively. He is currently pursuing the Ph.D. degree with the German Federal Ministry of Education and Research-Funded Centre for Innovation Competence Plasmatis, Leibniz Institute for Plasma Science and Technology, Greifswald, with a focus on investigating atmospheric pressure plasmas in the field of medical applications.

Mr. Dünnbier has been a member of the International Society for Plasma Medicine since 2012.



Kai Masur was born in Germany in 1974. He received the Diploma degree in biochemistry in 1998, and the Ph.D. (Dr.rer.nat.) degree from the University of Witten/Herdecke, Witten, Germany, in 2001, with a focus on the signal transduction of metastasizing tumor cells.

He was involved in signal transduction of pancreatic beta cells with the New England Medical Centre, Tufts University, Boston, MA, USA, from 2001 to 2003. From 2003 to 2009, he was a Principal Investigator with the University of Witten/Herdecke, where he established his own work group by combining diabetes research and oncology. Since 2009, he has been the Head of the Cellular Effects Junior Research Group with the German Federal Ministry of Education and Research-Funded Centre for Innovation Competence Plasmatis, Leibniz Institute for Plasma Science and Technology, Greifswald, Germany, where his group is investigating the interplay of nonthermal plasma with living cells and tissues—focusing on the alterations of the genome and proteome—to stimulate cellular activities. His current research interests include cellular signal transduction cascades and how to manipulate those signals.

Dr. Masur is a member of the German Society for Biochemistry and Molecular Biology, the International Society for Plasma Medicine, and the Society for Signal Transduction.

Kristian Wende received the Diploma and Ph.D. (Dr.rer.nat.) degrees from the University of Greifswald, Greifswald, Germany, in 1998 and 2003, respectively.

He was involved in the phytochemical composition of plants and different analytical techniques from 1998 and 2003. From 2004 to 2009, he investigated the interaction of marine/terrestrial natural products, UV light, and nonthermal plasmas with different cellular models with the Institute for Pharmacy, Greifswald, as a Group Leader of Cell Biology. Since 2010, he has been a member of the German Federal Ministry of Education and Research-Funded Centre for Innovation Competence Plasmatis with the Leibniz Institute for Plasma Science and Technology, Greifswald, Germany. His current research interests include eukaryotic cell responses after exogenic stimuli.

Dr. Wende is a member of the International Society of Plasma Medicine and the German Pharmaceutical Society.

Klaus-Dieter Weltmann (M'95) received the Diploma degree in electronics and the Ph.D. (Dr.rer.nat) degree in applied physics from the University of Greifswald, Greifswald, Germany, in 1989 and 1993, respectively.

He was involved in nonlinear dynamics in low-temperature plasmas and plasma diagnostics. In 1994, he was a Visiting Scientist with the Plasma Physics Laboratory, West Virginia University, Morgantown, WV, USA. In 1995, he joined ABB Corporate Research Ltd., Baden-Dättwil, Switzerland, where he was involved in the research and development of HV and MV switchgear. In 1998, he became the Head of the High Voltage Systems Group, ABB Corporate Research Ltd. In 2000, he was appointed to lead Research and Development of Gas Insulated Switchgear (GIS) at ABB High Voltage Technologies Ltd., Zurich, Switzerland, where he became the Business Unit Research and Development Manager GIS in 2002. Since 2003, he has been the Director and Chairman of the Board of the Leibniz Institute for Plasma Science and Technology, Greifswald, and a Professor of Experimental Physics with the University of Greifswald. His current research interests include switchgears, arc physics, atmospheric plasmas, modeling and simulation, plasma-medicine and plasma decontamination.

Prof. Weltmann is the President of the International Society for Plasma Medicine, and a member of the German Physical Society and several consulting committees in industry and research. He is an initiator of three spinoff companies.