In Vitro Activity of Atmospheric Pressure Plasma Jet (APPJ) Plasma Against Clinical Isolates of *Demodex Folliculorum*

Georg Daeschlein, Sebastian Scholz, Andreas Arnold, Thomas von Woedtke, Eckhard Kindel, Maria Niggemeier, Klaus-Dieter Weltmann, and Michael Jünger

***Abstract*—Rosacea is a frequent and often disfiguring and chronic dermatologic disease mainly of the midface causing central facial erythema, teleangiectasia, papules, and pustules. In the context of complex pathogenicity, *Demodex folliculorum* plays an important role showing significant density in the skin of patients with papulopustular rosacea. Rosacea belongs to the hard-to-heal diseases, and new approaches for treatment are strongly required. As atmospheric low-temperature plasma [atmospheric pressure plasma jet (APPJ)] proved high efficacy against bacterial and fungal pathogens, we tested its potency as nonantibiotic-based method to inactivate *Demodex folliculorum*. We isolated five parasites of *Demodex folliculorum* from a 54-year-old patient suffering from chronic pustulous rosacea and irradiated the living parasites by APPJ *ex vivo*. The APPJ plasma killed *Demodex folliculorum* after an exposure time of 2 resp. 60 s. Low-temperature atmospheric pressure plasma seems suitable for the treatment of dermatologic and veterinarian diseases caused by *Demodex spp*.**

***Index Terms*—*Demodex folliculorum*, folliculitis, plasma inactivation, skin infection.**

I. Introduction

In THE past few years, plasma medicine has become an important field in medical science; as plasma has proven antiinflammatory, antimicrobial, and antitumor efficacy, most topics are now focusing on immunologic disorders, infections, and tumor therapy. The nonthermal atmospheric pressure plasmas are adjustable in a wide array and can exhibit a multitude of activities depending on the design of the device [1], [2]. The breakthrough of plasma science into medical science came with the introduction of nonthermal plasma sources in the last years. In medical fields, plasma proved antimicrobial efficacy for disinfection, sterilization, and decontamination in a multitude of applications [3]–[7]. Direct killing of pathogens on animal skin by plasma could be demonstrated with mice without affecting the animals [8]. At humans, improved healing of acute and chronic wounds caused by different pathogens was reported [9], [10]. To substantiate the postulated role of antibacterial effects in the healing process described for plasma, we were able to measure high reductive power by the plasma against typical wound bacteria and fungi *in vitro* [11], [12]. With the exception of leishmania (*in vitro*), the knowledge of plasma effects on parasites is rather scant [8]. If plasma also demonstrates efficacy against other parasites, this would be of particular relevance, e.g., in dermatology. Therefore, we investigated the plasma susceptibility of *Demodex folliculorum*, a skin parasite involved in the pathogenesis of rosacea, which is a widespread disease of the facial skin. In this study, we irradiate the clinical isolates of *Demodex folliculorum* from a patient with chronic pustulous rosacea by using low-temperature atmospheric pressure plasma *ex vivo*.

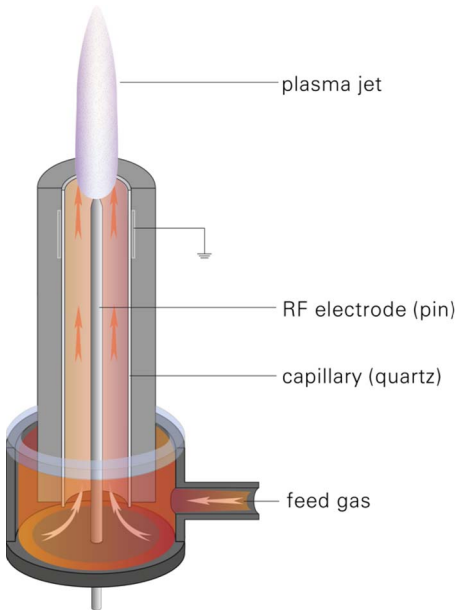

Fig. 1. Schematic setup of the APPJ device [13].

II. Methods

A. APPJ

The schematic setup of the APPJ device used in this study is given in Fig. 1 [13]. It consists of a quartz capillary (inner diameter of 1.6 mm). In the center of the capillary, a pin-type electrode (1-mm diameter) is mounted. Argon, as the

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G. Daeschlein, S. Scholz, A. Arnold, M. Niggemeier, and M. Jünger are with the Department of Dermatology, Ernst Moritz Arndt University, 17489 Greifswald, Germany (e-mail: georg.daeschlein@uni-greifswald.de).

T. von Woedtke, E. Kindel, and K.-D. Weltmann are with the Leibniz Institute of Plasma Science and Technology e.V. (INP) Greifswald, 17489 Greifswald Germany.

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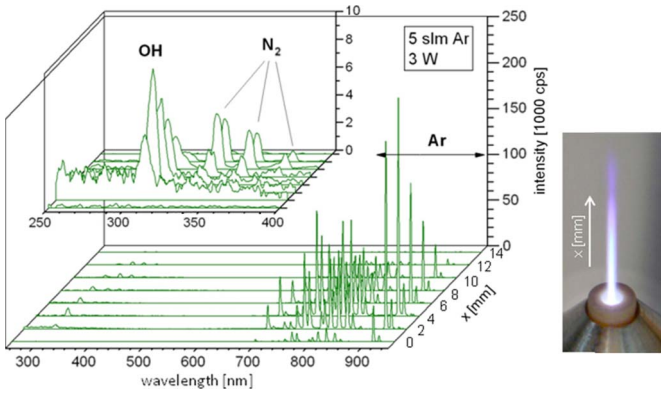

Fig. 2. Optical emission spectra measured at different axial positions of the Ar-plasma jet [13].

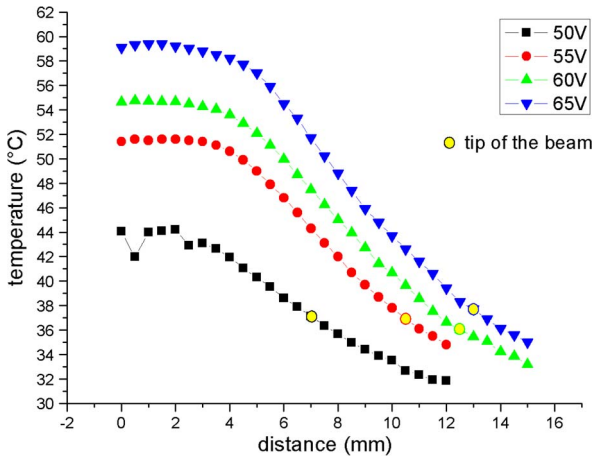

Fig. 3. Gas temperature profile of the plasma jet used in this study.

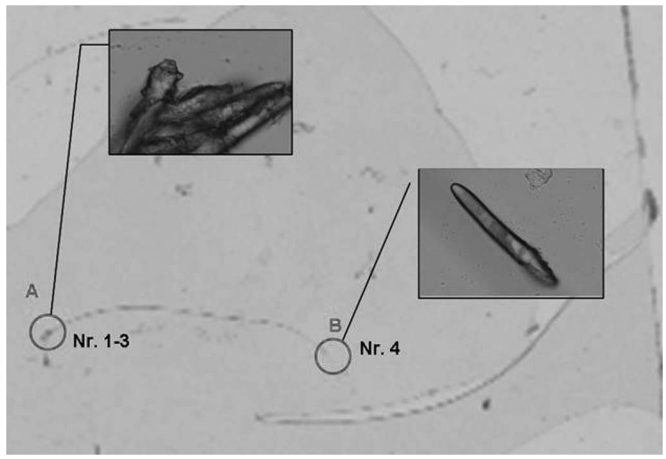

Fig. 4. *Demodex folliculorum in situ* (54-year-old male rosacea patient). Magnification: 20–40×.

feed gas, flows through the capillary. A high-frequency voltage (2–6 kV_{pp}, 1.1 MHz) is coupled to the center electrode. The plasma is generated from the top of the center electrode and expands to the surrounding air outside the nozzle [13]. In our experiments, we used an argon gas flow rate of about 8 L per minute and an APPJ input power for plasma generation of 2.4 W. Depending on the gas flow rate and the applied power, the plasma has a length of up to 12 mm.

Optical emission spectroscopy in the ultraviolet (UV), visible (VIS), and near infrared (NIR) regions of the plasma from 250 to 1000 nm was performed using a fiber spectrometer (StellarNet EPP2000-UVN). Spectra measured at different axial positions of the plasma jet at 3 W and an Ar gas flow rate of 5 slm in the continuous working mode are shown in Fig. 2. In the VIS/NIR region between 700 and 1000 nm, mainly emission lines of excited argon atoms are found. Lines of nitrogen emission have been measured in the UV-A region between 300 and 400 nm. Their relative intensity increases along the plasma jet according to the decrease of argon emission at the tip of the jet because of onward mixing of the feed gas argon with the surrounding ambient air. Most significant is the emission of excited OH radicals at 309 nm. However, there was no detectable emission in the UV-C range between 200 and 280 nm [13].

The axial temperature profile of the plasma was obtained by fiber optic temperature measurement (FOT Lab Kit Fluoroptic Thermometer, Luxotron model 755). A temperature-dependent fluorescent signal of luminescent magnesium fluorogermanate which was excited with a Xe-flash lamp was monitored. To avoid killing effects (and skin damage for future application) by constructional reasons, the temperature at the tip of the plasma jet with a length of up to 12 mm did not surpass 37 °C as a result of the cooling effect by the argon streamed together with the electronic regulation. The tip of the plasma jet is defined as the distal end of the visible pointed discharge. The temperature profile of the plasma jet is shown in Fig. 3.

B. Sampling of the Demodex Parasites

From a 54-year-old rosacea patient who suffered from typical symptoms (teleangiectasia, itching, redness, erosions), hair samples from both temple sites were taken on two different days during ambulant therapy. Single hairs were plucked by forceps to be immediately microscopically analyzed for parasites in the microbiological laboratory of the Department of Dermatology of the Ernst Moritz Arndt University, Greifswald.

C. Visualization and Preanalytics

The hairs were taken from the sample tubes and embedded in sterile saline (0.9%) on a glass slide (76 × 23 mm) under a cover slide (20 × 30 mm) for microscopic examination using 400× (binocular microscope LABOVAL 3 Carl Zeiss Jena) and 20–40× magnification (digital microscope Coolscope, Nikon). Before the irradiation tests, the moving of the parasites was monitored by video recording in order to define the normal pattern of motility under test conditions. The spontaneous motility of parasites was found nearly continuously with slow movements of body and legs (approximately one movement of at least one part of the body/s could be defined as control).

At day one of investigation, two parasites of *Demodex folliculorum* could be isolated from the patient. One did not show any motility during the whole test period and was excluded from the study. At day two (two months later), we were able to detect four parasites of *Demodex folliculorum* on hairs (Fig. 4); two parasites with nearly constant movements near the hair follicle and two parasites embedded in secret-like material

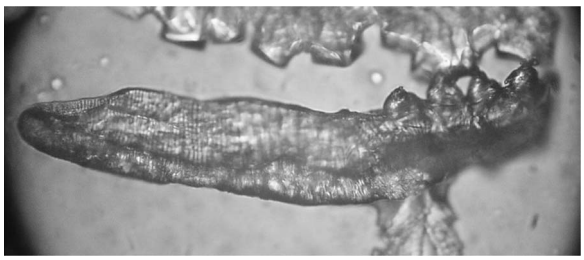

Fig. 5. *Demodex folliculorum* during plasma treatment. Magnification: 400×.

around the hair follicle show slow movements with interruptions of up to 25 s.

All parasites to be treated were transferred from their original slides and hair to a second "treatment" slide with fresh sterile saline where plasma irradiation with APPJ was performed.

D. Plasma Treatment

In total, five parasites were irradiated by APPJ at two investigation days.

Irradiation by the jet was pointed directly against the parasite at first under the cover slide and thereafter without slide with the tip of the jet (visible beam) at a distance from 3 mm to the parasite at an angle of 45°. During irradiation, a lifetime observation with a microscope (LABOVAL 3 Carl Zeiss Jena) was realized. A second person monitored and filmed the motility of the parasites (C-5050 digital camera Olympus, Nikon) under irradiation. Three irradiation periods of 4×15 s were performed with 30 s of interruption. After 3 min, the same irradiation procedure as described was undertaken without a cover slide. At the second investigation day, plasma treatment was performed directly without a cover slide starting with 1, 5, 15, 30, and 60 s of irradiation of every parasite. Up to its own irradiation, every nonirradiated parasite served as control. After each treatment, a period without irradiation of 10 min was introduced to monitor potential delayed plasma effects.

III. Results

In the first experiment, *Demodex* was not killed after 3×60 s of APPJ irradiation through the cover slide, and motility of the parasite only slightly decreased. After removing the cover slide, plasma irradiation of $4 \times 15 = 60$ s of treatment was necessary for definite immobilization of the parasite (monitored with digital camera, Fig. 5). Consecutive control clips were not able to show any movement of the parasite up to 6 h, therefore, we concluded effective killing of the parasite by the APPJ treatment. At the second investigation day, we consecutively treated four parasites of the same patient. This time, we started the irradiation directly without a cover slide and observed complete immobilization after repeated treatments of 1 s with 10 min between the first and second treatment (repetition of 1 s irradiation was included because of significant loss of motility occurring immediately after treatment of 1 s).

During the treatment and 10-min delay time, all controlled parasites continued their physiologic motility until they were also treated and consecutively killed after 2×1 s APPJ in the same way. Additional microscopic observations followed after 20, 30, 60, 75, and 90 min and finally every 60 min to a total time span of 6 h and did not show any more movement of all irradiated four parasites. After 6 h, even gentle pressure of the parasites (via glass slides) did not provoke any vital sign (movement). We conclude that all parasites were killed by APPJ treatment after 2 s of treatment.

IV. Discussion

Rosacea is a common eruptive chronic facial dermatosis with various clinical presentations characterized by intermittent periods of exacerbation and remission on the base of a genetic predisposition. The disease is typically characterized by transient or persistent central facial erythema, teleangiectasia and, often, papules and pustules and can be classified into four subtypes: erythematous teleangiectatic, papulopustular, phymatous, and ocular type. The pathogenetic background of rosacea, yet, is not fully understood. Current hypotheses include potential roles for vascular abnormalities, dermal matrix degeneration, environmental factors, the microorganisms *Propionibacterium acnes* and *Helicobacter pylori* [14], [15]–[17], [19], and the parasite *Demodex folliculorum* [16], [17]. Forton [19] demonstrated a statistically significant relationship between the presence of *Demodex* and perifollicular lymphohistiocytic inflammation in 69 biopsy specimens from rosacea patients. Erbagci [20] demonstrated that the density of *Demodex* was significantly higher in patients with papulopustular rosacea than in age-matched control subjects, where *Demodex* can also be found in hair follicles and sebaceous glands of the face, particularly, forehead, chin, eyelids, nose, and ear canal. These results were supported by further studies by Ruefli *et al.* and Bonnar *et al.* [21], [22]. In conclusion, the simple identification of *Demodex* is, by no means, a proof of causative involvement but needs further support by assessing the density of mites [20], [23].

Mild forms of ocular rosacea respond readily to topical medications and eyelid hygiene, but more severe forms are to be treated with oral antibiotics like tetracyclines and Metronidazole, which is a synthetic nitroimidazole-derived antibacterial and antiprotozoal agent [24]–[26]. The mechanism by which metronidazole reduces inflammatory lesions and erythema in patients with rosacea has not been fully elucidated and, like tetracyclines, it seems likely that the antiinflammatory or immunosuppressive actions of metronidazole account for the therapeutic effect [27], [28]. Metronidazole has few adverse effects, mostly mild, including pruritus, skin irritation, and dry skin but also, in rare cases, epileptiform seizures, encephalopathy, and sensory neuropathy; long-term use of metronidazole has been limited by concerns over adverse systemic effects and toxicity, underlining the need for alternative treatment [29], [30].

In addition, many patients with rosacea and particularly the teleangiectatic subtype are difficult to treat, and the patients respond poorly to topical or oral medications. For this subtype, vascular laser, light therapy, surgery, or laser ablation have been increasingly utilized without consistent success [24], [31], [32].

In conclusion, therapy of rosacea remains unsatisfactory because of the two main causes, the bad responsiveness to therapy and the undesired effects of long-term therapy with antibiotics. Therefore, new therapeutic approaches are most welcome, and

since plasma treatment offers a multitude of biologic effects including antiinflammatory and antimicrobial effects, it could be a promising option in the therapy of rosacea when it proves efficacy against the parasite *Demodex folliculorum*. This could be proven in our study. The data show that plasma (APPJ with Argon as feeding gas) can rapidly inactivate adult *Demodex* parasites *ex vivo*. Surprisingly, we found in one case that 2 resp. and another 60 s of plasma treatment are necessary to kill the mites, but as patients with rosacea in our clinic are rare, the number of tested parasites in this study is low, and the discrepancy of the results has to be clarified and investigated with much more parasites in a dose-dependent manner. Our plasma source generates substantially reactive oxygen species (ROS) which is indicated by significant emission of OH radicals at 309 nm (Fig. 2). There was no UV radiation emission in the UV-C range between 200 and 280 nm [13]. In the UV range between 260 and 360 nm, irradiance between 1 and 2 mW/cm^2 can be measured in the tip region of the plasma jet [13]. Consequently, it can be concluded that the killing of the mites is caused mainly by ROS activity which could be realized in a dual manner: by direct chemical action of OH radicals on the one hand and by additional UV irradiation mainly at 309 nm on the other. However, detailed investigation on the mechanisms of plasma action has to be a matter of future investigations.

In the study presented here, we were able to demonstrate the potent killing of *Demodex folliculorum* by plasma *ex vivo*. Therefore, the treatment of *Demodex* in the lesions of patients with rosacea becomes realistic, and clinical effects can be expected. In conclusion, plasma could be an alternative in the often frustrating therapy of rosacea since it provides antiinflammatory, antiproliferative, broad antibacterial, and also antiparasitic efficacy as we were able to demonstrate in this *ex vivo study*.

V. Conclusion

Plasma irradiation was able to kill *Demodex folliculorum* from clinical samples *ex vivo*. The potent and fast (2 resp. 60 s) antiparasitic efficacy of plasma *ex vivo* may encourage *in vivo* treatment of diseases with involvement of *Demodex spp*. like rosacea and canine demodicosis in veterinary medicine.

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Georg Daeschlein was born in 1959. He received the M.D. degree from Christian-Albrechts-Universität Kiel, Kiel, Germany, in 1985 and specializations in laboratory medicine, in microbiology and epidemiology, and in hygiene and environmental medicine from Ernst-Moritz-Arndt University, Greifswald, Germany, in 1992, 1995, and 2005, respectively.

Since 2006, he has been the Registrar and Head of the Dermatologic Laboratory, Department of Dermatology, Ernst-Moritz-Arndt University of Greifswald (with Director Prof. Dr. med. M. Jünger).

Sebastian Scholz was born in 1977. He received the Dipl. Ing.-agr. degree in agricultural sciences from Martin-Luther-University Halle-Wittenberg, Halle, Germany, in 2009.

Since 2009, he has been a Scientist with the Department of Dermatology, Ernst-Moritz-Arndt University of Greifswald, Greifswald. Germany.

Andreas Arnold was born in 1968. He received the Doctorate of Medicine (M.D.) degree from Westfälische Ruhr-University Münster, Münster, Germany, in 2000. In 2005, he had his specialization in dermatology and venereology from Ernst Moritz Arndt University, Greifswald, Germany.

Since 2006 and 2007, he has been a Specialist Registrar and an Assistant Medical Director, respectively, with the Department of Dermatology, Ernst Moritz Arndt University, Greifswald. In 2008, he received a certificate from ICH/GCP. He was the Leader of the Skin Tumor Centre (TZH) of the Ernst Moritz Arndt University, Greifswald.

Thomas von Woedtke was born in 1962. He received the Dr. rer. nat. degree and the State Doctorate degree (Habilitation) from Ernst-Moritz-Arndt University of Greifswald, Greifswald. Germany, in 1996 and 2005, respectively, both in pharmaceutical technology.

Since 2005, he has been with Leibniz Institute of Plasma Science and Technology e.V. (INP Greifswald), Greifswald, where he has been the Programme Manager in experimental plasma medicine since 2008.

Eckhard Kindel born in 1949. He received the Dipl.-Phys. degree in physics and the Dr. rer. nat. degree in experimental physics from Ernst-Moritz-Arndt University of Greifswald, Greifswald, Germany, in 1973 and 1979, respectively.

Since 1992, he has been with Leibniz Institute for Plasma Science and Technology e.V. (INP Greifswald), Greifswald.

Maria Niggemeier was born in 1961. She received the technical laboratory assistance degree from the Hygiene Institute Gelsenkirchen, Gelsenkirchen, Germany, in 1984.

Since 2006, she has been with the Microbiologic and Mycologic Laboratory, Department of Dermatology, Ernst-Moritz-Arndt University of Greifswald, Greifswald, Germany (with Director Prof. Dr. med. M. Jünger).

Klaus-Dieter Weltmann was born in 1962. He received the M.S. degree in electronics and the Dr. rer. nat. degree in physics from Ernst-Moritz-Arndt University of Greifswald, Greifswald, Germany, in 1989 and 1993, respectively.

In 2002, he became Business Uni R&D Manager. Since 2003, he has been the Director and Chair of the Board of Leibniz Institute of Plasma Science and Technology e.V. (INP Greifswald), Greifswald.

Michael Jünger was born in 1957. He received the M.D. degree from Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany, in 1984; the Habilitation from Eberhard Karls University Tübingen, Tübingen, Germany, and specialization in dermatology and venereology from Eberhard Karls Universität Tübingen in 1991 and 1997, respectively; and specializations in phlebology, in allergology, in environmental medicine and medical quality management, and the M.S. degree in health care management from Ernst-Moritz-Arndt University of Greifswald, Greifswald, Germany, in 1994, 1996, 1999, and 2004, respectively.

Since 2001, he has been the Chair and Director of the Department of Dermatology, University Hospital, Ernst-Moritz-Arndt University of Greifswald.