Contrib. Plasma Phys. **54**, No. 2, 118 – 130 (2014) / **DOI** 10.1002/ctpp.201310061

Plasma Applications: A Dermatological View

R. Tiede[1], **J. Hirschberg**[4], **G. Daeschlein**[2], **T. von Woedtke**[3], **W. Vioel**[4,5], and **S. Emmert**[1]*

[1] Department of Dermatology, Venerology, Allergology, University Medical Center Goettingen, Germany
[2] Department of Dermatology, Ernst Moritz Arndt University of Greifswald
[3] Leibniz Institute for Plasma Science and Technology e.V. (INP Greifswald), Greifswald, Germany
[4] University of Applied Sciences and Arts, Goettingen, Germany
[5] Fraunhofer Institute for Surface Engineering and Thin Films IST Application Center for Plasma and Photonic APP, Goettingen, Germany

Received 15 July 2013, revised 04 December 2013, accepted 12 December 2013
Published online 10 February 2014

Key words Atmospheric pressure plasma, plasma medicine, plasma jet, dielectric barrier discharge, ultraviolet radiation, reactive gas species, electric current, skin diseases.

The ability to produce cold plasma at atmospheric pressure conditions was the basis for the rapid growth of plasma related application areas in biomedicine. Plasma comprises a multitude of active components such as charged particles, electric current, UV radiation, and reactive species which can act synergistically. The anti-itch, antimicrobial, and anti-inflammatory effect was already demonstrated in in vivo and in vitro experiments and until now no resistance of pathogens against plasma treatment was observed. The combination of the different active agents and their broad range of positive effects on various diseases, especially easily accessible skin diseases, render plasma quite attractive for applications in medicine. Hence, plasma medicine as an independent and promising medical field has been emerged recently.

For medical applications two different types of cold plasma are suitable; indirect (plasma jet, plasma torch) and direct plasma sources (dielectric barrier discharge - DBD). So far, no standards and norms are defined for any of these plasma sources. Also, no convenient criteria for standardization of the quality rating of plasma in the view of dermatological applications exist. Although various cold plasma studies have been performed the results are hardly comparable, as physical parameters of the plasma devices, experimental conditions, and organisms used vary greatly. Therefore, standardized risk analyses are necessary for the assessment of different plasma sources. In this review two plasma sources are described and possible risk factors are discussed to estimate the safety of plasma used as a therapeutic tool in dermatology.

1 Introduction

Plasma is ionized gas and in physical terms this is defined as the fourth state of matter with the highest energy rate after solids, liquids, and gases. In general, this is the state in which atoms are split off either completely or only partly due to the impact of a high energy boost, so that ions and electrons of the atoms can move freely. It is believed that more than 99 % of the observable matter in the universe exists in the plasma state. Orbs like the fix stars or our sun consists of plasma. On earth we face plasma in the form of lightings, flames, and auroras.

The transformation of neutral gases into the plasma state requires energy input which can be achieved by thermal excitation, irradiation energy, or electric fields. Plasma on earth can be generated at high-pressure, low-pressure, or under atmospheric pressure. When plasma is produced as arcs between two metallic electrodes under atmospheric pressure the gas temperature can reach hundreds or even thousands of degrees Celsius due to the thermal equilibrium resulting from high collision frequency and high current density in the generated arcs [1, 2]. Such thermal plasmas are used in the industry for waste treatment [3], metal cutting [1, 4], welding [5], or coating (plasma spraying) [6]; only to mention some application areas. Of course, these kinds of plasma are not qualified for the treatment of living organisms or heat sensitive materials.

Nonthermal or cold plasmas can be generated under atmospheric pressure by subjecting a gas to electric discharges. Collisions of the high-energy electrons with the gas molecules lead to the ionization process and,

* Corresponding author. E-mail: semmert@gwdg.de, Tel.: 0551-39 6410, FAX: 0551-39 8983

thus, a complex mixture of biological active agents are created in the plasma, such as charged particles (ions, free electrons, free radicals, and molecules), photons, and neutral atoms [7, 8]. The excited agents can undergo chemical reactions (i.e. oxidation) with the environment and therefore influence or change it.

In general, the use of plasmas in semiconductor etching or thin film deposition is widely studied. With regard to medical applications sterilization and decontamination are probably the most researched areas for the use of cold atmospheric pressure plasma. The antimicrobial effect of plasma has been utilized for decades to sterilize all kinds of tissues and materials [9-19]. In medicine, heat sensitive metallic or plastic surgical equipment and instruments can be sterilized very efficiently by nonthermal plasma application. Ten minutes of exposure to plasma already leads to inactivation of viruses and elimination of high concentrations of spores and bacteria [16]. Moreover, the disinfective use of plasma in dermatology is of great interest since low temperature plasma enables a direct treatment of skin pathogens [8, 14, 20-22]. It was already shown in vitro that different bacteria and fungi can be eliminated in a simulated infected wound environment [23]. In another study the efficient lethal effect of plasma on wound related bacteria was displayed in an ex vivo porcine skin model. Plasma treatment led to a decolonization of the tested bacteria Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *Staphylococcus aureus* and *Escherichia coli* without harming cells of the pig skin sample [22]. Also, the strong reduction power of plasma applications on the bacterial load in wounds in vivo was observed by Isbary et al. [24]. More importantly, plasma was found to act selectively on prokaryotic and eukaryotic cells. While inactivating microorganisms, plasma treatment can even stimulate the proliferation of mammalian cells [25-27]. *With this respect the modes of action of cold atmospheric plasmas, i.e. germ killing, tissue stimulation, anti-inflammatory and anti-itch properties, appear ideal for dermatological applications in wound healing or the treatment of inflammatory skin diseases.* Other promising fields which are of interest for plasma applications are tooth treatment in dental medicine [28, 29], cosmetic treatment of nails [30], or tumor treatment [14, 17, 31-33].

The abundance of potential plasma application areas recently led to the formation of the term plasma medicine as a new and independent medical field. The rapid growth of this promising field led to the development of various plasma sources. They vary greatly in their physical justifications. Since the composition of the agent mixture in plasma (plasma cocktail) depends on the physical parameters of the discharge like flow rate, humidity, or processed gas composition [34], agents' mixtures differ for varying plasma sources. Up to now, there are no standards and norms defined for plasma devices. Also no general guidelines or limits are set up for the safe use of plasma in medical applications. In this review two different plasma sources are characterized. Newer versions of both devices were recently CE certified in Germany as new medical products for dermatological treatment of chronic wounds. Possible risk factors like electric current, UV radiation, reactive species, and heat are analyzed and estimated for the purpose of safety for patients and therapists during plasma treatment.

2 Indirect and direct plasma sources

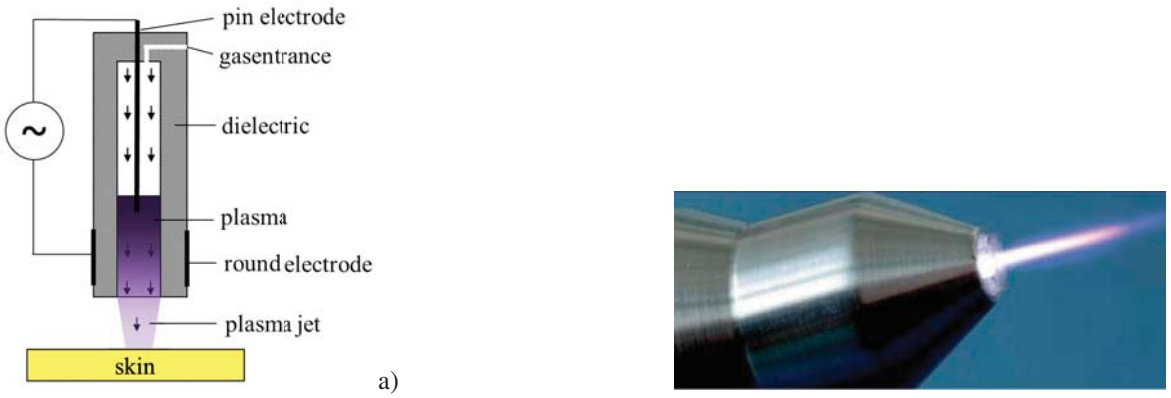

Fig. 1 One possible design of an indirect plasma source is schematically shown in (a) and a concrete device, the kinpen MED, is exemplified in (b).

For medical applications two types of plasma can be used; indirect and direct plasma. Indirect plasma is produced when a constant gas flow is directed through a nozzle including one or two electrodes with a voltage coupled to the electrodes [15, 17, 23, 35]. One possible design of such an indirect source containing two electrodes is shown in figure 1a where the gas flow is directed through the gap between a round (outer electrode) and a pin electrode (inner electrode). The ionized gas leaves the device as a jet stream. One advantage of indirect plasma is that different gases can be used (e.g. helium, argon or air) which in turn has an impact on the plasma cocktail. Furthermore, plasma jets comprise the ability of penetrating precisely into small structures like pores. An example of such a plasma device is the kinpen MED from INP Greifswald/neoplas tools GmbH Greifswald, Germany (fig. 1b), the working gas of this source is argon with a flow rate of 5 slm in the continuous working mode at a power of 3 W and a power density of about 300 W/cm^2. The inner electrode is surrounded by a ceramic capillary in which a high voltage of about 2-3 kV is produced with a repetition rate of 2.5 kHz [36]. The basic principle of the plasma device kinpen MED has been described previously in detail for the prototype version kINPen [37].

Direct plasma is generated by the application of high voltages (up to several kV) across a small gap, whereby at least one electrode is covered with a dielectric [38]. The treated material or biological tissue acts as the counter electrode (fig. 2a and b show the skin as counter electrode), so that a multitude of little electric breakdowns, so called microdischarges, emerges between the tissue and the electrode [39]. In comparison to indirect plasma the treated material or tissue is exposed to a higher plasma density and electric current frequency [7]. In Tuemmel et al. [40] our DBD device (fig. 2 b) is characterized. The high voltage electrode is connected to 5-6 kV with a pulse length of 200-300 ns and a repetition rate of 518 Hz resulting in a maximum displacement current of 483 mA and a gas temperature in the discharge of 33 ˚C. The parameters can be adjusted depending on the desired application form. For the treatments of lipids for example a dissipated power of 200 mW (U_{peak} = 7.5 kV, t_{pulse} = 70 μs, f = 300 Hz) was used [41]. In another study a power of 435 mW (U_{peak} = 10.3 kV, t_{pulse} = 90 μs, f = 255 Hz) was applied for reducing the viability of microorganisms [34].

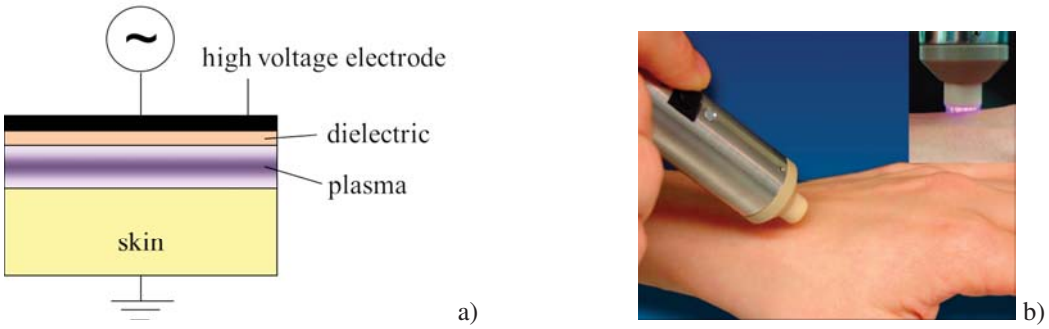

Fig. 2 A schematic design of a direct plasma source is illustrated in (a) and (b) shows a picture of our DBD device.

Further developments of both described plasma sources lead to the CE certification according to the EU directive 93/42/EWG since spring 2013.

The risk factors or working components which are discussed in this review are almost the same for indirect and direct plasma sources. Besides UV radiation, temperature, and reactive species the risk factor electric current plays a role in applications of direct discharges. *The critical analysis of the biological tolerance of the agents in plasma is supported by the existing literature for medical therapies such as phototherapy, electro-physiotherapy, and therapies with ozone [42-48].* In vitro and in vivo application experiments with different plasma sources on living tissue, cell culture, and humans comprise the basis for the estimation of the potential application risk.

3 Temperature

The parameter temperature is of special concern for the characterization of cold atmospheric pressure plasma, especially in regard to therapeutic applications of plasma to heat sensitive tissues. Exposure of the skin to temperatures of 40 ˚C and higher lead to degeneration and destruction processes in cells. Denaturation, loss of function,

and structural alterations of proteins occur at temperatures higher than 45 ° C and resulting in skin burning. However, it was observed that a slight increase of temperature to 38.5 ° C stimulates the proliferation of keratinocytes [49] which could be of advantage in healing processes and regeneration of tissues. For these reasons the thermal impact of the plasmas has to be adjusted very precisely. *This parameter, of course, closely relates to the deposited power of the cold atmospheric plasma device used, i.e. the energy influx into the skin tissue. Others have used this parameter for calorimetric probe measurements* [50,51].

The temperature profile of the plasma jet generated by the kinpen MED device was measured at a gas flow rate of 5 slm and constant input power (3 W). Results show that the maximum temperature at the end of the capillary nozzle is 49 ° C, and, like expected, temperature drops with enlarged distance to the capillary nozzle and with lower power input. In this way temperature can be minimized to 30 ° C (15 mm distance) [52]. In case that the plasma jet is too far away from the treated sample the mode of action may decrease. However, thermal impact to the sample is also a function of contact time, so that moving of the plasma jet over the treated region results in a decrease of a potential heat impact [53].

In DBD devices the microdischarges can reach gas temperatures of 102 ° C. This is the case when lipids of the stratum corneum act as counter electrode and a power input of 200 mW was applied [41]. But this gas temperature exists only during the lifetime of a microdischarge of a few nanoseconds and drops very fast [41, 54]. Also, such high temperatures only occur for a very short time window [38]. Hence, the actual temperature on the treated tissue is much lower. Investigations on the actual gas temperature of a DBD device by Laroussi and Leipold [55] indicate that temperature is independent of the power level. This was shown for a power input in the range of 2-15 W. Also, temperature was observed to be around room temperature [55]. Moreover, treatments with our DBD device of pork skin samples and agar plates revealed no dramatic changes in temperature. Applications of direct plasma treatment (U_{peak} = 12 kV, $t_{pulse} \approx 500$ μs, f = 550 Hz) for 12 s led to a temperature increase on agar plates of only 0.6 ° C [56] and no change was detected after a 20 s application (U_{peak} = 5-6 kV, t_{pulse} = 200-300 ns, f = 518 Hz) to pork skin [40].

Eventually, one has to adjust the appropriate operating parameters for each respective application desired. In principle, burning of the skin or degeneration of biological tissue can be avoided by choosing appropriate working parameters. Therefore, temperature does not present a risk factor in both of the characterized plasma devices.

4 UV radiation

Ultraviolet light is in the range of 100-400 nm and an essential component of sunlight but not visible for the human eye. It is well known that plasma sources generate UV light that has different impact on living organisms. The UV spectrum is divided into UVA (320-400 nm), UVB (280-320 nm), and UVC (100-280 nm). Parts of UVB and all of UVC are blocked by the ozone layer of the earth. Shorter wavelengths are blocked by ordinary air. 95 % of the UV radiation which reaches the earth is UVA. The longer the wavelength, the deeper the light can penetrate into the human skin. But on the other hand the potential danger on biological systems decreases with longer wavelength. UVB and UVA are both able to cross the epidermis, while UVA can even reach the dermis. These wavelengths have the ability to interact with endogenous chromophores and photosensitizers resulting in the generation of reactive oxygen species and causing damage to the DNA, proteins, and lipids. Moreover, UVB can directly interact with the DNA and lead to the generation of dipyrimidine photoproducts [57]. Short-term effects of UV exposure include stimulation of pigment formation and sunburn (erythema). Chronic and excessively high UV irradiation alters the structure of the corneal connective tissue and results in premature skin aging or wrinkles which is mainly induced by UVA radiation. In addition, UVB and UVA irradiation are known to trigger skin cancer [58-60].

In dermatology phototherapy is already a well-established method for the treatment of eczema and psoriasis [61-64] and the anti-itch, anti-fibrotic as well as anti-inflammatory properties of different UV wavelengths have already been demonstrated [65]. The recommended irradiation doses for phototherapy depend on the wavelength, if a broad or narrow spectrum is used, and on the skin type of the patient (overview of different phototherapy forms can be found in the German Dermatological Society) [66]. A guideline of the US National Institute for Occupational Safety and Health (NIOSH) recommend that unprotected exposure of skin and eyes to UVA should not exceed 1 mW/cm^2 if exposure lasts longer than 1000 seconds and 1000 mW/cm^2 for shorter exposure than

1000 seconds [67]. In addition, the approximate relative erythema efficacy is proposed by the International Commission on Non-Ionized Radiation Protection (ICNIRP): exposure to 180-400 nm should not exceed 3 mJ/cm^2 depending on the skin type [68].

The plasma jet developed in Greifswald is known to emit UV radiation. This UV radiations are suspected to be initiated by $OH^\bullet$ radicals at 309 nm (UVB) and by nitrogen at 350-380 nm (UVA). Very low UVC was detected via optical emission spectroscopy [52, 69]. Therefore, UVC induced DNA damage can be excluded. At typical working distance of about 8 mm the effective UVA and UVB irradiance (280-380 nm) was about 10 μW/cm^2. But the effective irradiance may change dramatically (down to around 1.5 μW/cm^2) by extending the working distance [52]. Other experiments performed in Greifswald showed that the necessary effective doses for UVA and UVB irradiation to damage HaCaT cells are 10-15 J/cm^2 and 2-10 mJ/cm^2, respectively [27]. The delivered radiation doses of UVA and UVB measured were therefore much lower than the doses necessary to damage HaCaT cells [27].

Our DBD device generates predominantly UVA radiation and minor UVB radiation. Emission spectra of discharges ignited in ambient air are dominated by the influence of nitrogen. The second positive system of N_2 shows transitions in the range of UVA at 315.6 nm, 337 nm, 357.6 nm, 375.4 nm and 380 nm. The lower UVB radiation consists of peaks at 295-297 nm and 311-315 nm. Averaged intensity amounts produced by the DBD device and detected in the wavelength range of 250-400 nm are in the range of 0.035-0.04 mW/m^2 [40]. Tuemmel et al. [40] claimed that no UVC radiation was detected in the DBD since it is completely absorbed by O_2 in the plasma volume before it can reach the treated tissue [70]. Moreover, the ICNIRP has suggested a maximum dosage of effective exposure Dmax = 30 J/m^2 per day. Based on intensity parameters of Tuemmel et al. [40] the maximum dosage of UV radiation could not be achieved. Beyond that the typical treatment time is below 1 minute per day. These results are slightly different from optical emission spectroscopy measurements of Awakowicz and coworkers [18] which showed that low levels of UVC occurred in the discharge indicating the existence of excited NO molecules. Also they reveal that UVA and UVB radiation originates from excited N_2 molecules [18].

It has already been demonstrated that UV radiation generated by DBD devices is too weak to produce significant damage on living organisms [14, 26, 40, 55, 71]. In addition, the use of plasma jets on living skin cells was found to be harmless, too [72]. Tape stripped human single corneocyte layers which were treated with a plasma jet display that 25 % of the UV radiation is absorbed. In this study the measured emission spectrum of the plasma jet revealed that the high peak at 310 nm (UVB), which represents a nitrogen band resulting from N_2 in the air, is efficiently absorbed by the stratum corneum. Smaller bands in the range of 325-400 nm (UVA) but no bands lower than 300 nm were detected. Thus, UVA radiation is of minor importance and UVC radiation can be absolutely ruled out. Due to the fact that the stratum corneum of the human skin exists of 15-25 cell layers it is assumed that almost none of the mentioned UV radiations can reach the proliferating living cells [72]. In conclusion, no harmful effects of UV light produced in plasma can be expected.

5 Reactive gas species

Plasma sources produce a number of reactive gas species, especially reactive nitrogen species (RNS) and reactive oxygen species (ROS). In indirect plasma chemical reactions of these species with molecules in the surrounding air can lead to toxic gases like ozone or nitrous gases [37, 38]. Since direct plasma sources work with air which is ionized, these reactive gas species are directly produced during electric discharges [73, 74]. DBD devices are also known to produce an efficient amount of ozone [39]. Furthermore, RNS and ROS are thought to be the most important active agents in plasma for antimicrobial effects [37, 56, 75-77], coagulation of blood [78], or the stimulation of wound healing processes [76, 78].

5.1 Reactive nitrogen species

NO_x species are known to have varying functions in living organisms. In humans NO has an important impact on functions such as blood vessel tone regulation, blood coagulation, the immune system, or apoptosis. Also, the anti-microbial and anti-inflammation properties of exogenic NO have been extensively examined, as well as the proliferation-stimulating effect on cells [79-81]. Therefore it is not surprising that NO-containing plasma is used for stimulating regenerative processes and wound healing [71, 77, 82].

However, NO_x species are also known to produce strong acids in the presence of water. Helmke and coworkers [83] showed that the formation of nitric acids in DBD plasma plays a major role in the acidification of lipid film surfaces. For the experiments they stripped off lipid layers from a human forehead and treated them with DBD plasma. As a result the pH value decreased significantly on the lipid film surface. The transformation of NO_2, which is produced in DBD plasma, into nitrite or nitrous acids is assumed to contribute to the pH changes [83]. This mechanism provides the ability for pH dependent therapies in dermatology, such as treatment of eczema or ichthyosis. Also, the pH decrease could assist the healing process of chronic wounds. These patients suffer from a disturbed healing process due to a strong bacterial colonization [84]. The growth of pathogenic bacteria on the human skin is ideal at a pH value of 6 and bacterial growth is inhibited at lower pH values [85]. Also it is known that the natural body reaction during healing is the acidification of the wound [86]. Consequently, the pH change to an acid environment by DBD plasma treatment would support the healing process of chronic wounds via inhibition of the bacterial growth.

Nevertheless, when inhaled, NO_2 is toxic to some degree. The maximum concentration in the workspace (MAK) is set to 5 ppm or 9 mg/m^3. In case of the kINPen device no NO_2 was found near the plasma jet [37] and therefore no harm is to be expected from NO_2 in indirect plasma.

For this reasons NO_x species in plasma can be of benefit when the settings are selected so that no strong acidification takes place or too much toxic NO_2 is generated. So, these agents rather display an advantage of plasma as a therapeutic tool than being a risk factor.

5.2 Reactive oxygen species

Reactive oxygen species produced in plasma comprise on the one hand free radicals like O_2^-, OH, ROO, RO, atomic oxygen O and on the other hand stable molecules like H_2O_2, ROOH, and O_3. These agents can undergo oxidative reactions with the treated surface or organism. Measurements of intracellular ROS levels after plasma treatment of HaCaT cells revealed that ROS can penetrate through the cell membrane [27]. Also, it was found that the formation of intracellular ROS is the major mechanism by which ionized radiation produces DNA damage [87, 88]. High levels of intracellular ROS can even cause cell death through DNA damage. But low levels were shown to have a stimulating effect on the proliferation of cells [71, 88]. For the therapeutic use of ROS the intracellular concentration has to be controlled, which can be achieved by changing the frequency and voltage waveform of DBD devices as described in Kalghatgi et al. [71].

Ozone may be of particular interest since it is highly reactive in its radical state and has a strong oxidative effect. Too high doses of ozone evoke damage to health, especially in the respiratory system when ozone is inhaled. The maximum inhalation dose of ozone is defined with 120 μg/m^3 for 30 minutes and the maximum concentration in the workplace (MAK) should not exceed 200 μg/m^3 for eight hours [89-91]. In medicine, ozone is already in use for different therapeutic applications [48]. For the purpose of skin disinfection or antimicrobial and antiviral surface treatment special ozone devices like the OzonyTron were developed [92]. Another study described the improved healing process of diabetic feet after ozone treatment applied in a concentration of 60 mg/l over one hour on the skin covered with plastic foil [47].

Measurements on our DBD device showed that the ozone concentration in a distance of 5 mm away from the discharge is below 0.1 ppm (< 100 μg/m^3). Nevertheless, higher concentrations are reached in the target location [40]. The maximum ozone concentration generated around the plasma jet of the kinpen MED device is 180 μg/m^3 at a distance of about 20 cm and 120 μg/m^3 at a distance of more than 30 cm [52].

In summary, all defined thresholds are easily met by both plasmas; indirect and direct. Therefore, no danger is to be expected from the use of both plasma sources regarding inhalation of ozone.

6 Electric current

Electric current can influence biological tissue and has been utilized for therapeutic purposes in human medicine for decades. Medical drugs can be efficiently delivered into the body by application of low currents. Such devices are called iontophoretic transdermal systems (ITS). A continuous current of 170 mA is applied to the skin which acts as the cathode. The drug molecules are then entrained into the blood vessels with the flowing current. With this method 40 μg of the analgesic drug Fentanyl can be delivered into the body in about 10 minutes [45, 46]. As a long-term therapy iontophoresis is used for the treatment of palmoplantar hyperhidrosis on feet and hands

which reduce sweat production. For this purpose, hands or feet are lying in a flat chamber with water and a constant electric current of 5-10 mA is running through the water. It is assumed that H^+ ions are concentrated at the end of the sweat duct and, thus, lead to the inhibition of perspiration [44, 93, 94]. These currents are well tolerable for humans.

Our DBD device was utilized in several studies with a pulsed high-voltage power supply including pulse repetition rates of 255-500 Hz and pulse durations of 0.2-500 μs [34, 40, 41, 56]. These very short pulse lengths prevent that body cells are negatively influenced by the current. Even neurons are not affected by such short pulse durations. The device produces very low rms (root mean square) discharge currents in the range of several μA which results from high but short pulses [95]. As a consequence we can assume that no risks are to expect during therapeutic applications of the DBD device.

7 Treatment of skin diseases

There are a number of different skin diseases and the degree of the severity of a skin disease may range from harmless (i.e. senile wart), over disturbing (i.e. minor eczema or ichtyosis) and painful (i.e. infected chronic wounds) to lethal (i.e. malignant melanoma). Lighter forms of inflammatory skin diseases can be treated with topical crèmes comprising disinfected substances and steroids. In more severe forms the treatment with antibiotics is unavoidable, either topically applied as a component in a crème or systemically. Especially in the case of superinfected chronic skin diseases patients suffer from painful therapeutic treatments and side effects of drugs.

Since in vitro plasma experiments revealed the strong antimicrobial effect and the ability to stimulate proliferation of mammalian cells, it is not surprising that plasma therapy was already applied to patients with different skin diseases that are difficult to treat. But which dermatological diseases may be most promising regarding plasma therapy? For standardization purposes we divide the disease into diseases with intact epidermis or wounded epidermis either slightly or heavily colonized by bacteria.

7.1 Epithelialized skin diseases only slightly germ-contaminated

7.1.1 Treatment of cancer cells with plasma

Fridman et al. [78] showed that plasma treatment lead to apoptosis of melanoma cancer cells. Even days after treatment the lethal effect of plasma treatment still remained. At low doses of plasma (5 s) the cells are not immediately damaged but develop apoptosis hours after the treatment as observed during a 24 h period [78]. Tumor cells die without affecting neighboring healthy cells which is of advantage in comparison to cell death via necrosis. Inflammation and damage to neighboring cells occur when intracellular enzymes and cell breakdown products are released from cells in their necrosis procedure, whereas during apoptotic processes the cell membrane remains intact so that no pro-inflammatory substances can leak the cell [96, 97]. They also showed that high doses treatment with a FE-DBD (floating electrode DBD; 15 s with 1.4 W/cm^2) caused cell death due to necrosis of the cancer cells. However, this high level is still under the threshold for the damage of healthy tissue [98,99]. Another in vitro study on different melanoma cell lines but with the direct plasma device miniFlatPlaSter revealed similar results [23]. 2 minutes of plasma treatment were sufficient for the inactivation of the cells via induction of apoptosis. In addition, they observed that lower doses and only 1 minute of treatment with plasma led to almost no apoptotic effects but to long-term inhibition of the proliferation of the cells.

7.1.2 Plasma skin regeneration in cosmetic medicine

Foster and others [95] 105 introduced the first plasma system for commercial usage; the Portrait® PSR, and summarized first results of in vivo treatments. The Portrait® PSR is basically a radio frequency plasma jet generated in nitrogen. High energy treatment (3-4 J) led to a controlled damage of the skin. Already after ten days the epidermis completely regenerated. They also confirmed an ongoing collagen production, reduction of elastosis, and progressive skin rejuvenation beyond one year after the treatment. The patients reported a 60 % improvement of their skin texture including wrinkle reduction and skin tone improvements [100-102].

7.2 Epithelialized skin diseases heavily germ-contaminated

7.2.1 Treatment of atopic eczema

Atopic eczema is a form of eczema and a very widespread disease (3-5 % of the population is affected). Symptoms include redness, swelling, itching, and dryness of the skin. Usually, patients are treated with a moisturizing crème followed by topical anti-inflammatory and antimicrobic treatments. Mertens and coworkers [103] presented a case study in 2009 on atopic eczema with one patient. In this study the left upper arm of the patient was exposed to plasma (fig. 3) whereas the right upper arm was treated with a moisturizing crème. They used our DBD device with a power of 0.2 W. Plasma treatment time was 1 min daily over 30 days. The daily energy density can be calculated to about 1 J/cm^2.

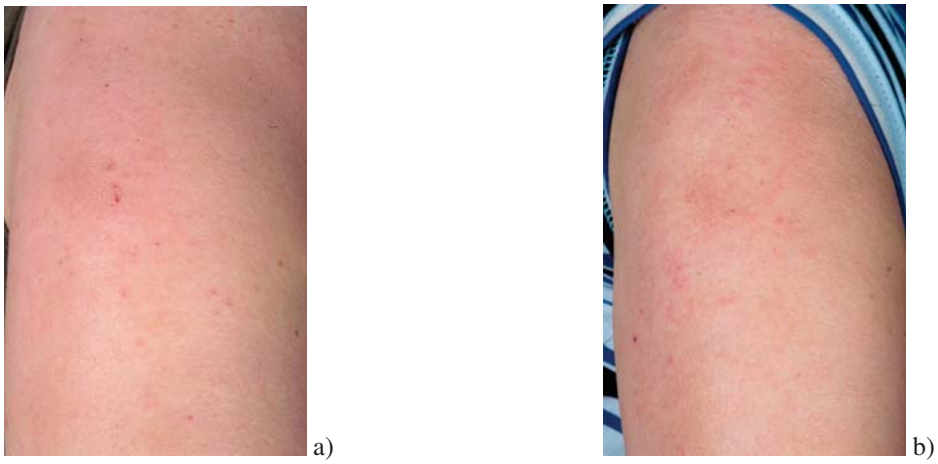
a) b)

Fig. 3 Pictures show the left upper arm of the patient with atopic eczema before (a) and right after (b) plasma treatment. A reduction of the redness and swelling can be observed in (b).

After 30 days a reduction of some symptoms like swelling and redness on the upper arm which was exposed to plasma could be observed (fig. 3). Furthermore, the patient declared a reduction of the itch from 8 to 3 points; the point scale was ranged from 0 to 10, with 10 being the strongest itch. The eczema overall improved by two points (scale from -5 to 0 to 5 points, representing complete healing and strong worsening of the eczema). No side effects during and after the study were observed [98, 103]. In addition agar plates were pressed onto the skin of the patients and results revealed a 1 log step reduction of the bacterial load of the plasma-treated skin after two days (*Staphylococcus aureus*).

The last result is consistent with another study on the inactivation efficiency of plasma treatment performed by Daeschlein and others [23] with a plasma jet. The plasma jet was generated with argon gas and a voltage supply of 1-5 kV with a frequency of 1.5 MHz. Among other bacteria, which could be isolated from wounds, they exposed *Staphylococcus aureus* colonies grown on agar plates to the plasma jet. Observations showed an antimicrobial reduction of this pathogen by 2.7 log steps (RF 2.7) after 2 minutes of plasma treatment. The inactivation efficiency of plasma varied for the five different species tested in this study indicating a viability of the plasma doses needed for elimination of different wound pathogens. But still the lowest reduction factor of 1.9 log steps, which was found for *Enterococcus faecium*, represents a pronounced inactivation of the bacterial load [23], which seems suited for the inactivation of most kind of realistic in vivo occurring microbial contamination, colonization and also infection. Plasma was also shown to significantly reduce multidrug resistant pathogens in vitro, i.e. *Pseudomonas aeruginosa*, Extended spectrum β-lactamase (ESBL)*Escherichia coli*, Methicillin resistant *Staphylococcus aureus* (MRSA), Methicillin resistant *Staphylococcus epidermidis* (MRSE), Vancomycin resistant *enterococci* (VRE) and High level gentamycin resistant *enterococci* (HLGR) offering new horizons regarding hygienic sanitization and preoperative skin antisepsis besides wound decontamination. Antifungal treatment is another scope for plasma treatment in dermatology since excellent plasma susceptibility of the most important clinical fungal species in vitro could be demonstrated [104]. Furthermore dermatologic diseases with parasitic involvement like demodicosis could benefit from plasma treatment since strong killing efficacy was observed against *Demodex folliculorum* [105].

We propose that the following biological parameters may be assessed in developing plasma devices for the treatment of epithelialized skin [106, 107]: transepidermal water loss (TEWL), pH and skin moisture, which preferably are measured using a combined analyzing tool. Most references were measured with the Multiprobe Adapter Systems MPA® including the Tewameter® TM 300 and Corneometer® CM 825 (Courage and Khazaka, Cologne, Germany) [108]. It is important that all subjects to be tested acclimatize at least during 20 minutes at room temperature prior to a measurement and to better compare results, conditions like temperature and relative humidity should not differ significantly during different tests.

7.3 Skin diseases with wounded epidermis and germ-contaminated

7.3.1 Plasma treatment of chronic wounds

Many people suffer from chronic wounds on the lower legs (ulcus cruris), the main cause of which are varicosis and other venous diseases (80 %) besides arterial diseases (15 %) or diabetes (5 %). Especially old people are affected by venous ulcers and their treatment take up a sizeable proportion of the health care budget [8, 109] which is why there is a need of cost effective alternative ulcer therapies. Plasma has the capability of being such an alternative not only because it can be generated with minimum effort but also because it is easy in handling. Furthermore plasma treatment combines some mechanisms of action which operate positively on the wound healing process: i) the strong antimicrobial effect would restrict the bacterial load in the wound and therefore prevent the delayed healing process due to intense colonization [84]; ii) plasma is known to stimulate the proliferation of endothelial cells [26, 110] and iii) plasma treatment leads to a decrease of the pH value which also would support the healing process since acidification of wounds is also the natural response of the body [86].

We conducted successful clinical trials for the treatment of ulcers on the lower legs with our DBD device as well as the kinpen MED that led to the certification of the two plasma devices, respectively. Other studies also revealed very similar beneficial effects of plasma therapy on chronic wounds. Fetykov and coworkers [111] demonstrated the two fold faster healing process of wounds after treatments with the indirect plasma device Plasmafon and also observed a pain reduction of their patients within 5 days.

More extensively, Isbary and coworkers explored the effects of argon plasma on infected wounds in in vivo experiments [24, 112, 113]. 2010 they published results of the first clinical trial with the indirect plasma device MicroPlaSter α (ADTEC Plasma Technology Co Ltd, Hiroshima, Japan). 36 patients with 38 ulcers (mostly due to venous causes, but also traumatic, arterial and diabetic causes) were daily treated with the plasma for 5 minutes in addition to the standard wound care. The control areas were only treated with the standard wound care and were of about 3 cm^2 in diameter whereby patients act as their own controls. 291 treatments were performed and results display a high significant reduction of the bacterial load in the wounds of 34 % ($p < 10^{-6}$) [112]. One year later another study of Isbary and others revealed the beneficial effect of plasma applications on the genetic disorder Morbus Hailey-Hailey [115]. Severe outbreaks of this disease are characterized by the formation of blisters and rashes which then often lead to chronic infected wounds when they break open. In the case study one patient with Hailey-Hailey was treated with a newer version of the MicroPlaSter device, called MicroPlaSter β which was more comfortable to handle because of its smaller size and a flexible four-joint treatment arm [24]. The torch was held 5 minutes and 2 cm away from the target area. As a result, the plasma applications significantly improved the healing process in both treated areas of the patient; the right axilla and the groin. In addition, a persistent positive effect after plasma therapy was observed; the patient remained asymptotic for several months. In the most recent clinical study on chronic wounds of the same working group they compared 2 minute treatments of both MicroPlaSter devices (α and β) with the same experimental conditions (except of treatment duration) with each other [24]. Again, plasma treatments of the patient resulted in a significant reduction of bacterial load in the wounds. When using the MicroPlaSter α a significant reduction of the bacterial load of 40 % ($p < 0.016$) were observed in the wounds and a reduction of 23.5 % ($p < 0.008$) when using the MicroPlaSter β. In all these studies of Isbary and coworkers no side effects occurred and the applications were well tolerable for the patients.

For all these studies on chronic wounds it can be certainly assumed that not only the wound pathogens were directly exposed to plasma but also cells of deeper layers in the skin, like the keratinocytes in the proliferative basal layer or fibroblasts in the dermis. Direct microbiological effects of plasma to eukaryotic cells are not yet fully understood and are of major interest. To absolutely ensure that in the long run no damage or side effects occur possible genotoxic effects of plasma treatment has to be genetically investigated. For that purpose, risk

analysis will be performed for both plasma devices (kinpen MED and our DBD device) comprising the investigation of cell damage at the level of DNA and cell membrane. For this, common gentoxicity and cytotoxicity tests will be performed like the Ames test and different host cell reactivation assays. The Ames test can be used for the determination of the mutagenic potential of chemical substances (in our case this would be plasma). The test uses different strains of *Salmonella typhimurium* with a mutation in the histidine biosynthesis so that the auxotroph mutants require addition of histidine for their growth. The treatment with the mutagen substance can cause revertants which are then able to grow on histidine free medium and thus can be separated from the auxotroph mutants. This method was already successfully used for the detection of mutagenity of different substances which are metabolized via the cytochrome P450 enzyme system [114]. Two plasmid DNA vector test systems are planned as methods for the replacement of animal experiments: The host cell reactivation assay (HCR) is based on reporter genes which are used to quantitatively analyze the DNA repair rate of cells [115, 116]. Here, the non-replicating reporter gene plasmid coding for an enzyme will be treated with plasma and transfected into host cells. In case the plasma treatment would lead to DNA lesions and the host cells would repair them this would be then displayed in the rate of enzyme expression of the reporter gene. So, the enzymatic expression would be an indirect evidence for the mutagenic potential of plasma. Another test system is the Plasmid-Shuttle-Vector-Mutagenesis-Assay which was already successfully used to detect the age-related DNA repair capacity in different cells [117]. This assay is based on a plasmid which carries the supF gene (bacterial suppressor tRNA gene of *Escherichia coli*) as a marker for mutagenesis [118]. The plasmid DNA (pSP189) will be treated with plasma, transfected into host cells, isolated after some days and transformed into bacteria. Colonies which are light blue or white indicate a mutation in the supF gene and therefore the amount of these colonies display the mutation frequency. The mutated plasmids can be further used for sequence and mutation spectrum analysis. In conclusion, these assays present valuable of tolls for the characterization of the two different plasma sources and the standardization of experimental parameters and criteria in respect to medical applications.

8 Conclusion

The primary purpose of this paper is to provide evidence that the treatment with non-thermal atmospheric pressure plasmas is harmless in regard to the previously mentioned potential risk factors (temperature, UV-irradiation, reactive nitrogen species, reactive oxygen species, ozone, and current conduction). The ability to influence most of the factors which are potentially dangerous by changing physical parameters of the plasma sources and settings of the experimental procedures ensure that thresholds for safety of the treated patients and therapists can be easily adhered to. This is further demonstrated by the presented plasma applications used in the treatment of various skin diseases and their beneficial effects. Ultimately, due to these safety and efficacy issued both plasma devices, our DBD device as well as the kinpen MED recently obtained a CE certificate. From the dermatological point of view we propose not only adherence of newly developed plasma devices to physical parameters, but also to biological parameters including genotoxicity tests, TEWL, skin pH measurements, corneometry and others. *For this purpose we have recently developed a first standard to which plasma devices should adhere (DIN SPEC). This standard has been developed together with the German Institute for Standardization (DIN e.V.) and is published (DIN SPEC 91315; http://www.spec.din.de/cmd?level=tpl-proj-detailansicht&committeeid=0&artid=188349886&languageid=de& bcrumblevel=1).* The fact that so far no side effects, no bacterial resistance, and no allergic reactions during plasma treatment were observed, reinforces the assumption that cold atmospheric pressure plasma is one of the most promising therapeutic treatment options emerging right know. *With this respect, clinical studies will have to be performed in the future to demonstrate the efficacy and safety of cold atmospheric plasma in the treatment of specific diseases. In dermatology these include chronic wounds, psoriasis, atopic eczema, and in the future probably also skin cancer.*

Acknowledgements The financial support of the German Federal Ministry of Economics and Technology (grants no. 01FS12001, 13N9773, 13N9779, 01FS12003) and the DFG (EM/63 8-1) is gratefully acknowledged

References

[1] S. Ramakrishnan and M.W. Rogozinski, J. Phys. D: Appl. Phys. **30**, 636-644 (1997).

[2] S. Semenov and B. Cetegen, J Therm Spray Techn. **10**, 326-336 (2001).
[3] E. Gomez, D. Amutha Rani, C.R. Cheeseman, D. Deegan, M. Wise, and A.R. Boccaccini, J. Hazard. Mater. **161**, 614-626 (2009).
[4] V.A. Nemchinsky and W.S. Severance, J. Phys. D: Appl. Phys. **39**, 423-438 (2006).
[5] A.B. Murphy, M. Tanaka, K. Yamamoto, S. Tashiro, T. Sato, and J.J. Lowke, J. Phys. D: Appl. Phys. **42**, 194006 (2009).
[6] R. Suryanarayan, in: Plasma Spraying: Theory and Applications, R. Suryanarayan (World Scientific Publishing Co Pte Ltd, Singapore, 1993).
[7] J. Heinlin, G. Morfill, M. Landthaler, W. Stolz, G. Isbary, and J.L. Zimmermann, J. Dtsch. Dermatol Ges. **8**, 968-976 (2010).
[8] S. Emmert, F. Brehmer, H. Haenßle, A. Helmke, N. Mertens, R. Ahmed, D. Simon, D. Wandke, W. Maus-Friedrichs, G. Daeschlein, M.P. Schoen, W. Vioel, Clinic. Plasma Med. **1**, 24-26 (2013).
[9] K. Kelly-Wintenberg, T.C. Montie, C. Brickman, J.R. Roth, A.K. Carr, K. Sorge, L.C. Wadsworth, and P.P.Y. Tsai, J. Ind. Microbiol. Biotechnol. **20**, 69-74 (1998).
[10] M. Moisan, J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian, and L'H Yahia, Int. J. Pharm. **226**, 1-21 (2001).
[11] M. Moisan, J. Barbeau, M.-C. Crevier, J. Pelletier, N. Philip, and B. Saoudi, Pure Apll. Chem. **74**, 349-358 (2002).
[12] M. Laroussi, Plasma Process. Polym. **2**, 391-400 (2005).
[13] R. Brandenburg, J. Ehlbeck, M. Stieber, T. von Woedtke, J. Zeymer, O. Schlueter, and K.D. Weltmann, Contrib. Plasma Phys. **47**, 72-79 (2007).
[14] G. Fridman, A.D. Brooks, M. Balasubramanian, A. Fridman, A. Gutsol, V.N. Vasilets, H. Ayan, and G. Friedman, Plasma Process. Polym. **4**, 370-375 (2007).
[15] T. Shimizu, B. Steffes, R. Pompl, F. Jamitzky, W. Bunk, K. Ramrath, M. Georgi, W. Stolz, H.U. Schmidt, T. Urayama, S. Fujii, G.E. Morfill, Plasma Process. Polym., **5**, 577-582 (2008).
[16] R.A. Venezia, M. Orrico, E. Houston, S.M. Yin, Y.Y. Naumova, Infect. Control. Hosp. Epidemiol., **29**, 5, 430-436 (2008).
[17] K.D. Weltmann, R. Brandenburg, T. von Woedtke, J. Ehlbeck, R. Foest, M. Stieber, E. Kindel, J. Phys. D: Appl. Phys. **41**, 194008 (2008).
[18] P. Awakowicz, N. Bibinov, M. Born, B. Busse, R. Gesche, A. Helmke, A. Kaemling, V. KolbBachofen, R. Kovacs, S. Kuehn, J. Liebmann, N. Mertens, U. Niemann, C. Oplaender, H.E. Porteanu, J. Scherer, C. Suschek, W. Vioel, and D. Wandke, Contrib. Plasma Phys. **49**, 9, 641-647 (2009).
[19] J. Ehlbeck, U. Schnabel, M. Polak, J. Winter, T. von Woedtke, R. Brandenburg, T. von dem Hagen, and K.D. Weltmann, J. Phys. D: Appl. Phys. **44**, 013002 (2011).
[20] E. Stoffels, Contrib. Plasma Phys. **47**, 40-48 (2007).
[21] G.E. Morfill, T. Shimizu, B. Steffes, and H.U. Schmidt, New J. Phys., **11**, 115019 (2009).
[22] T. Maisch, T. Shimizu, Y.F. Li, J. Heinlin, S. Karrer, G. Morfill, and J.L. Zimmermann, Plos One **7**, 34610 (2012).
[23] G. Daeschlein, T. von Woedtke, E. Kindel, R. Brandenburg, K.D. Weltmann, and M. Juenger, Plasma Process. Polym. **7**, 224-230 (2010).
[24] G. Isbary, J. Heinlin, T. Shimizu, J.L. Zimmermann, G. Morfill, H.U. Schmidt, R. Monetti, B. Steffes, W. Bunk, Y. Li, T. Klaempfl, S. Karrer, M. Landthaler, and W. Stolz, Brit. J. Dermatol. **167**, 404-410 (2012).
[25] E.A. Sosnin, E. Stoffels, M.V. Erofeev, I.E. Kieft, and S.E. Kunts, IEEE Trans. Plasma Sci. **32**, 1544-1550 (2004).
[26] D. Dobrynin, G. Fridman, G. Friedman, and A. Fridman, New J. Phys. **11**, 115020 (2009).
[27] K. Wende, K. Landsberg, U. Lindequist, K.D. Weltmann, and T. von Woedtke, IEEE Trans. Plasma Sci. **38**, 2479 (2010).
[28] R.E. Sladek, E. Stoffels, R. Walraven, P.J.A. Tielbeek, and R.A. Koolhoven, IEEE Trans. Plasma Sci. **32**, 1540-1543 (2004).
[29] H.W. Lee, S.H. Nam, A.-A.H. Mohamed, G.C. Kim, and J.K. Lee, Plasma Process. Polym. **7**, 274-280 (2010).
[30] C. Kaemling, A. Kaemling, S. Tuemmel, and W. Vioel, Plasma Surf. Engin., **200**, 668-671 (2005).
[31] X. Zhang, M. Li, R. Zhou, K. Feng, and S. Yang, Appl. Phys. Lett. **93**, 021502 (2008).
[32] G. Kim, H. Lee, and C. Shon, J. Korean Physical Society **54**, 628-632 (2009).
[33] S. Arndt, E. Wacker, Y.F. Li, T. Shimizu, H.M. Thomas, G.R. Morfill, S. Karrer, J.L. Zimmermann, and A.K. Bosserhoff, Exp. Dermatol. **22**, 284-289 (2013).
[34] A. Helmke, P. Gruenig, U.-M. Fritz, D. Wandke, S. Emmert, K. Petersen, and W. Vioel, Recent Pat. Antiinfect. Drug. Discov. **7**, 223-230 (2012).
[35] R.E. Sladek and E. Stoffels, J. Phys. D.: Appl. Phys., **38**, 1716-1721 (2005).
[36] Gebrauchsanweisung kinpen MED, INP Greifswald/neoplas tools GmbH Greifswald, Germany 2012.
[37] K.D. Weltmann, E. Kindel, R. Brandenburg, C. Meyer, R. Bussiahn, C. Wilke, and T. von Woedtke, Contrib. Plasma Phys. **49**, 631-640 (2009).
[38] P. Rajasekaran, P. Mertmann, N. Bibinov, D. Wandke, W. Vioel, and P. Awakowicz, J. Phys. D: Appl. Phys. **42**, 225201 (2009).
[39] U. Kogelschatz, Plasma Chem. Plasma P. **23**, 1-46 (2003).
[40] S. Tuemmel, N. Mertens, J. Wang, and W. Vioel, Plasma Process. Polym., **4**, 465-469 (2007).
[41] J. Hirschberg, T. Omairi, N. Mertens, A. Helmke, S. Emmert, and W. Vioel, J. Phys. D: Appl. Phys. **46**, 165201 (2013).

[42] Dermatological Phototherapy and Photodiagnostic Methods, J. Krutmann, H. Hoenigsmann, C.A. Elmets, and P.R. Bergsstresser, (Springer-Verlag Berlin Heidelberg New York, Germany, 2001).
[43] J. Heinlin, J. Schiffner-Rohe, R. Schiffner, B. Einsele-Kraemer, M. Landthaler, A. Klein, F. Zeman, W. Stolz, and S. Karrer, JEADV **25**, 765-773 (2011).
[44] O.P. Kreyden, J. Cosmet. Dermatol. **3**, 211-214 (2004).
[45] C.M. Herdon, Pharmacotherapy **27**, 745-754 (2007).
[46] I. Power, Br. J. Anaesth. **98**, 1, 4-11 (2007).
[47] G. Martínez-Sánchez, S.M. Al-Dalain, S. Menéndez, L. Re, A. Giuliani, E. Candelario-Jalil, H. Álvarez, J.I. Fernández-Montequín, and O.S. León, Eur. J. Pharmacol. **523**, 151-161 (2005).
[48] C.G. Nogales, P.H. Ferrari, E.O. Kantorovich, and J.L. Lage-Marques, J. Contemp. Dent. Pract. **9**, 1-9 (2008).
[49] P. Boukamp, R.T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, and N.E. Fusenig, J. Cell. Biol. **106**, 761-771 (1988).
[50] H. Kersten, H. Deutsch, H. Steffen, G.M.W. Kroesen, and and R. Hippler, Vacuum **63**, 385-431 (2001).
[51] S. Bornholdt, M. Wolter, and H. Kersten, EPJD, **60**, 653-660 (2010).
[52] R. Bussiahn, N. Lembke, R. Gesche, T. von Woedtke, and K.D. Weltmann, Hyg. Med. **38**, 212-216 (2013).
[53] O. Lademann, H. Richter, A. Patzelt, A. Alborova, D. Humme, K.D. Weltmann, B. Hartmann, P. Hinz, A. Kramer, and S. Koch, Laser Phys. Lett. **7**, 458-462 (2010).
[54] M. Kuchenbecker, N. Bibinov, A. Kaemling, D. Wandke, P. Awakowicz, and W. Vioel, J. Phys. D: Appl. Phys. **42**, 045212 (2009).
[55] M. Laroussi and F. Leipold, Int. J. Mass Spectrom. **233**, 81-86 (2004).
[56] A. Helmke, D. Hoffmeister, F. Berge, S. Emmert, P. Laspe, and W. Vioel, Plasma Process. Polym. **8**, 278-286 (2011).
[57] J. Cadet and E. Sage, T. Douki, Mutat. Res. **571**, 3-17 (2005).
[58] J.H. Rabe, A.J. Mamelak, P.J.S. McElgunn, W.L. Morison, and D.N. Sauder, J. Am. Acad. Dermatol. **55** 1-19 (2006).
[59] S. Mouret, C. Baudouin, M. Charveron, A. Favier, J. Cadet, and T. Douki, Proc. Natl. Acad. Sci. **103**, 13765-13770 (2006).
[60] SCCP/0949/05 Scientific Committee on Consumer Products (SCCP). Opinion on biological effects of ultraviolet radiation relevant to health with particular reference to sundbeds for cosmetic purpose. European Commission Health & Consumer Protection Directorate-General, June 2006.
[61] E. Hoelzle, in: Dermatologische Qualitätssicherung: Leitlinien und Empfehlungen, H.C. Korting, R. Callies, M. Reusch, M. Schlaeger, and W. Sterry (ABW Wissenschaftsverlag GmbH, Berlin, Germany, 2003).
[62] K. Degitz, C. Berking, P. Kaudewitz, M. Roecken, J. Simon, M. Kollmann, and H. Hoenigsmann, in: Dermatologische Qualitätssicherung: Leitlinien und Empfehlungen, H.C. Korting, R. Callies, M. Reusch, M. Schlaeger, W. Sterry (ABW Wissenschaftsverlag GmbH, Berlin, Germany, chapter 19, 2005).
[63] T. Gambichler, Arch. Dermatol. **143**, 647-649 (2007).
[64] J. Heinlin, G. Isbary, W. Stolz, G. Morfill, M. Landthaler, and T. Shimizu, J. Eur. Acad. Dermatol. **25**, 1-11 (2011).
[65] D.Y.M. Leung, M. Boguniewicz, M.D. Howell, I. Nomura, and Q.A. Hamid, J. Clin. Invest. **113**, 651-657 (2004).
[66] German Dermatological Society; http://www.awmf.org/uploads/tx_szleitlinien/013-029.pdf
[67] NIOSH; http://www.cdc.gov/niosh/hcwold5e.html
[68] International Commission on Non-Ionized Radiation Protection (ICNIRP), Health. Phys. **87**, 171-186 (2004).
[69] H.R. Metelmann, T. von Woedtke, R. Bussiahn, K.D. Weltmann, M. Rieck, R. Khalil, F. Podmelle, and P.D. Waite, Am. J. Cosmetic Surg. **29**, 52-56 (2012).
[70] W. Vioel, J. Hackmann, and J. Uhlenbusch, in: Proceedings of the 7^{th} International Symposium on Plasma Chemistry, C.J. Timmermans (Eindhoven University of Technology, The Netherlands, pages 333-338 1985).
[71] S. Kalghatgi, C.M. Kelly, E. Cerchar, B. Torabi, O. Alekseev, A. Fridman, and G. Friedman, J. Azizkhan-Clifford, Plos One **6**, 16270 (2011).
[72] J. Lademann, H. Richter, A. Alborova, D. Humme, A. Patzelt, A. Kramer, K.D. Weltmann, B. Hartmann, C. Ottomann, J.W. Fluhr, P. Hinz, G. Hubner, and O. Lademann, J. Biomed. Opt. **14**, 054025 (2009).
[73] I.A. Kossyi, A.Y. Kostinsky, A.A. Matveyev, and V.P. Silakov, Plasma Sources Sci. T. **1**, 207-220 (1992).
[74] R. Dorai and M. J. Kushner, J. Phys. D: Appl. Phys. **36**, 666-686 (2003).
[75] L.F. Gaunt, C.B. Beggs, and G.E. Georghiou, IEEE Trans. Plasma Sci. **34**, 1257-1269 (2006).
[76] E. Stoffels, Y. Sakiyama, and D.B. Graves, IEEE Trans. Plasma Sci. **36**, 1441-1457 (2008).
[77] K. Oehmigen, M. Haehnel, R. Brandenburg, C. Wilke, K.D. Weltmann, and T. von Woedtke, Plasma Process.Polym. **7**, 250-257 (2010).
[78] G. Fridman, G. Friedman, A. Gutsol, A.B. Shekhter, V.N. Vasilets, and A. Fridman, Plasma Process. Polym. **5**, 503-533 (2008).
[79] J. Stadler, T.R. Billiar, R.D. Curran, D.J. Stuehr, J.B. Ochoa, and R.L. Simmons, Am. J. Physiol. **260**, 910-916 (1991).
[80] A.B. Shekhter, R.K. Kabisov, A.V. Pekshev, N.P. Kozlov, and Y.L. Perov, Byull. Eksp. Biol. Med. **126**, 829-834 (1998).
[81] A. Ghaffari, D.H. Neil, A. Ardakani, J. Road, A. Ghahary, and C.C. Miller, Nitric Oxide Biol. Chem. **12**, 129-140 (2005).
[82] A.B. Shekhter V.A. Serezhenkov, T.G. Rudenko, A.V. Pekshev, and A.F. Vanin, Nitric Oxide **12**, 210-219 (2005).

[83] A. Helmke, D. Hoffmeister, N. Mertens, S. Emmert, J. Schuette, and W. Vioel, New J. Phys. **11**, 115025 (2009).
[84] R. Edwards and K.G. Harding, Curr. Opin. Infect. Dis. **17**, 91-96 (2004).
[85] P.S. Stewart, Int. J. Med. Microbiol. **292**, 107-113 (2002).
[86] L.A. Schneider, A. Korber, and S. Grabbe, J. Dissmond, Arch. Dermatol. Res. **298**, 413-420 (2007).
[87] B.E. Lehnert and R. Iyer, Hum. Exp. Toxicol. **21**, 65-69 (2002).
[88] B.A. Olofsson, C.M. Kelly, and J. Kim, Mol. Cancer Res. **5**, 1319-1330 (2007).
[89] The MAK Collection for Occupational Health and Safety, WILEY-VCH (2006); http://www3.interscience.wiley.com/cgi-bin/mrwhome/104554790/HOME.
[90] GESTIS-Stoffdatenbank; http://www.dguv.de/ifa/de/gestis/stoffdb/index.jsp#.
[91] Directive 2002/3/EC of the European Parliament and of the Council of 12 February 2002 relating to ozone in ambient air. Official Journal of the European Communities, 9.3.2002.
[92] P. Skomro, K. Opalko, J Gadomska-Krasny, D. Lietz-Kijak, and M. Perzanowska-Stefanska, in: Annales Academiae Medicae Stetinensis, (Państwowy Zakład Wydawnictw Lekarskich, edition **51**, pages 39-42 2005).
[93] C. Raulin, S. Rosing, and D. Petzoldt, Hautarzt **39**, 504-508 (1988).
[94] T. Vorkamp, F.J. Foo, S. Khan, J.D. Schmitto, and P. Wilson, The Surgeon **8**, 287-292 (2010).
[95] S. Emmert, F. Brehmer, H. Haenßle, A. Helmke, N. Mertens, R. Ahmed, D. Simon, D. Wandke, M.P. Schoen, W. Maus-Friedrichs, W. Vioel, and G. Daeschlein, in press in Plasma Medicine (2014).
[96] D. Kanduc, A. Mittelman, R. Serpico, E. Sinigaglia, A.A. Sinha, C. Natale, R. Santacroce, M.G. Di Corcia, A. Lucchese, L. Dini, P. Pani, S. Santacroce, S. Simone, R. Bucci, and E. Farber, Int. J. Oncol. **21**, 165-170 (2002).
[97] M.G. Kong, G. Kroesen, G. Morfill, T. Nosenko, T. Shimizu, J. van Dijk, and J.L. Zimmermann, New J. Phys. **11**, 115012 (2009).
[98] G. Fridman, M. Peddinghaus, H. Ayan, A. Fridman, M. Balasubramanian, A. Gutsol, A. Brooks, and G. Friedman, Plasma Chem. Plasma P. **26**, 425 (2006).
[99] G. Fridman, A. Shereshevsky, M. Peddinghaus, A. Gutsol, V. Vasilets, A. Brooks, M. Balasubramanian, G. Friedman, and A. Fridman, 37th AIAA Plasmadynamics and Lasers Conference, June 5th-7th, 2006, San Francisco, California, USA.
[100] K.W. Foster, R.L. Moy, E.F. Fincher, and J. Cosmet. Dermatol. **7**, 169-179 (2008).
[101] B. Kronemyer, in: European Aesthetic Buyers Guide, Spring, 2006; http://www.miinews.com/pdf/eabg_Rhytec_0306.pdf
[102] M.A. Bogle, K.A. Arndt, and J.S. Dover, Arch. Dermatol. **143**, 168-174 (2007).
[103] N. Mertens, A. Helmke, A. Goppold,S: Emmert, A. Kaemling, and D. Wandke, Second International Conference for Plasma Medicine, March 16th-20th, 2009, San Antonio, Texas, USA.
[104] G. Daeschlein, S. Scholz, T. von Woedtke, M. Niggemeier, E. Kindel, R. Brandenburg, K.D. Weltmann, and M. Juenger, IEEE Trans. Plasma Sci. **39**, 815-821 (2011).
[105] G. Daeschlein, S. Scholz, A. Arnold, T. von Woedtke, E. Kindel, M. Niggemeier, K.D. Weltmann, and M. Juenger, IEEE Trans. Plasma Sci. **38**, 2969-2973 (2010).
[106] G. Daeschlein, S. Scholz, R. Ahmed, A. Majumdar, T. von Woedtke, H. Haase, M. Niggemeier, E. Kindel, R. Brandenburg, K.D. Weltmann, and M. Juenger, J. Dtsch. Dermatol. Ges. **10**, 509-15 (2012).
[107] G. Daeschlein, S. Scholz, R. Ahmed, T. von Woedtke, H. Haase, M. Niggemeier, E. Kindel, R. Brandenburg, K.D. Weltmann, and M. Juenger, J. Hosp. Infect. **81**, 177-83 (2012).
[108] U. Heinreich, U. Koop, M.C. Leneveau-Duchemin, K. Osterrider, S. Bielfeldt, C. Chkarnat, J. Degwert, D. Haentschel, S. Jaspers, H.P. Nissen, M. Rohr, G. Schneider, and H. Tronnier, J. Cosmet. Sci. **25**, 45-53 (2003).
[109] C.N. Etufugh and T.J. Phillips, Clin. Dermatol. **25**, 121-130 (2007).
[110] S.U. Kalghatgi, G. Fridman, A. Fridman, G. Friedman, and A.M. Clyne, Conf. Proc. IEEE Eng. Med. Biol. Soc. **2008**, 3578-3581 (2008).
[111] A. Fetykov, E. Avdeeva, and J. Fulton, Second International Conference for Plasma Medicine, March 16th-20th, 2009, San Antonio, Texas, USA.
[112] G. Isbary, G. Morfill, H.U. Schmidt, M. Georgi, K. Ramrath, J. Heinlin, S. Karrer, M. Landthaler, T. Shimizu, B. Steffes, W. Bunk, R. Monetti, J.L. Zimmermann, R. Pompl, and W. Stolz, Brit. J. Dermatol. **163**, 78-82 (2010).
[113] G. Isbary, G. Morfill, J.L. Zimmermann, T. Shimizu, and W. Stolz, Arch. Med. Dermatol. **147**, 388-390 (2011).
[114] B. Emmert, J. Buenger, K. Keuch, M. Müller, S. Emmert, E. Hallier, and G.A. Westphal, Toxicology **228**, 66-76 (2006).
[115] K.M. Thoms, J. Baesecke, B. Emmert, J. Hermann, T. Roedling, P. Laspe, D. Leibeling, L. Truemper and S. Emmert, Scand. J. Clin. Lab. Invest. **67**, 580-588 (2007).
[116] K.M. Thoms, C. Kuschal, E. Oetjen, T. Mori, N. Kobayashi, P. Laspe, L. Boekmann, M.P. Schoen, and S. Emmert, Exp. Dermatol. **20**, 232-236 (2010).
[117] S.I. Moriwaki, S. Ray, R.E. Tarone, K.H. Kraemer, and L. Grossman, Mutat. Res. **364**, 117-123 (1996).
[118] C.N. Parris and M.M. Seidman, Gene **117**, 1-5 (1992).

