Atmospheric pressure plasma in dermatology: Ulcus treatment and much more


Abstract

Plasma in the sense of ionized gas can be referred to as the fourth state of matter following solids, liquids, and gases in view of their energy content. Application of high voltages across small gas filled spaces results in ionization of the air. Generally, two types of cold plasma can be discerned: direct plasma (e.g. dielectric barrier discharge—DBD) and indirect plasma (plasma torch, plasma jet). In indirect plasma treatment, the plasma is produced in a remote cavity and ejected by gas flow onto the skin in the form of an effluent. In direct plasma treatment, the skin itself acts as the counter electrode. Advantageous features of direct plasma treatment include the higher plasma density as well as the induced high frequency electric current onto the skin. In plasma treatment antiinflammatory, antipruritic, antimicrobial, tissue stimulation, stimulation of microcirculation, and other therapeutic effects are achieved in a single treatment due to the combined action of ultraviolet radiation, reactive oxygen species (e.g. ozone), reactive nitrogen species, and electric fields. In line with other reports, we have already demonstrated the use of direct plasma treatment in skin disinfection, in atopic eczema (superinfected dermatitis), in modulating the epidermal barrier, as well as in chronic wound treatment. We as well as others did not notice any side effects of plasma treatment so far. In summary, cold atmospheric pressure plasma constitutes a new and innovative treatment option especially for superinfected skin diseases. These promising relatively new clinical applications warrant further carefully conducted translational research to delineate the modes of actions of plasma as well as potential long term side effects. This should lead to norms for the technical devices to allow a standardized treatment of given diseases in the mid-term.

1. Cold atmospheric pressure plasma

Plasma was first described by Sir William Crookes, a British chemist, in 1879 as radiant matter [1]. In 1928 the term plasma was introduced by Irving Langmuir due to the composition of the ionized gas. Since then the term plasma indicates an ionized gas in a wide range of parameters such as density and temperature and must not be confused with blood plasma. As the fourth and highest energy state of matter following solids, liquids, and gases, plasma is the by far dominant state of matter in the universe, e.g. solar corona, solar winds. On Earth plasma can be generated technically by electric gas discharges. Due to the energy gained from strong electric fields, air (or any other gas) can be transformed into the plasma state. A complex mixture of inter-reacting and quite short-lived free electrons, ions, atoms, excited species, and photons is generated [2,3].

Low-pressure, high-pressure, and atmospheric pressure plasmas can be discerned. At atmospheric pressure, plasma in the easiest configuration of two metallic electrodes developed in the form of arcs. The drawback of arc plasmas characterized by high current densities and high collision frequencies is a thermal equilibrium resulting in gas temperatures of hundreds or several thousand
degrees Celsius [4,5]. Such thermal plasmas cannot be applied to living tissue like the skin without causing necrosis of cells. However, the thermal plasma energy can be medically used for sterilization or tissue removal, cutting, or cauteryization [6–10]. In contrast, electrical gas discharges at atmospheric pressure can be utilized for the generation of low-temperature plasmas by particular constructive measures and appropriate electrical circuits. Such non-thermal or non-equilibrium plasmas do not exceed body temperature and may be applied to the skin as a cold atmospheric pressure plasma treatment.

Generally, two types of cold atmospheric pressure plasmas can be discerned: direct plasma (dielectric barrier discharge—DBD, corona discharge) and indirect plasma (plasma torch, plasma jet). Indirect plasmas are produced between two electrodes and then transported onto the skin via a constant gas flow. The skin is not used as the counter electrode [11,12]. Direct cold atmospheric pressure plasma production applying the DBD technique was described as early as 1857 by von Siemens for the generation of ozone [13]. While dielectric barrier discharge (DBD) plasma treatment of technical surfaces is a standard method since years, the DBD plasma treatment of biological tissue is quite novel [14]. Operated at appropriate parameters, DBD generates a low temperature plasma under atmospheric pressure and, thus, is a suitable instrument for a non-destructive treatment of biological material [15]. Advantages of direct compared to indirect plasma generation may include a higher plasma density at the treated surface and the electrical current induced in the counter electrode skin (Table 1).

For our investigations, a non-equilibrium, weakly ionized physical plasma is generated by the application of alternating voltage pulses with amplitudes > 10 kV across air gaps in the low mm range, whereas the high voltage electrode is covered by a dielectric. This non-conducting barrier, which is essential in DBD settings, avoids the transition of the gas discharge into a hot arc by limiting the current. The biological tissue itself (i.e. skin) acts as the counter electrode. The plasma usually propagates in tiny breakdown channels, the so called microdischarges. These are of cylindrical shape (radius of 10^-4 m) and appear stochastically distributed over the electrode area (Fig. 1). Only electrons gain nameable kinetic energies in the strong oscillating electric field between two electrodes. A reactive mix of excited atoms and molecules, UV photons, charged particles as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS) is formed in the microdischarges. Typical species in air-plasmas are O_3, OH, N_2O, and HNO_2. The composition of species can be modulated depending on the plasma parameters, e.g., process gas composition, humidity, gas temperature, energy density and local electric fields [16]. Our DBD cold atmospheric pressure plasma device is depicted in Fig. 2 (CINOGENCY GmbH Duderstadt). The control unit (Fig. 2a) ensures defined physical parameters. Only one “on” button allows the start of treatment for exactly 45 s (timer in the display). Then the device switches off automatically and needs to be re-started. The hand held applicator (Fig. 2b) is connected to the control unit and carries the DBD electrode covered by a dielectric (1 cm in diameter). A specially constructed spacer (sterile, ready to use) ensures a contact-free plasma application as well as a constant distance of the DBD electrode to the skin. Direct skin contact of the DBD electrode is harmless except that the discharge stops and, thus, the plasma generation above the skin.

2. Effective components of plasma skin treatment

The skin comprises the biggest human organ. Its size is about 1.5–2 m². Without the skin life would not be possible. Generally, the skin consists of three layers, the epidermis, the dermis (or cutis), and the subcutaneous fat tissue (Fig. 3). The main components of the dermis are collagen and elastic fibres giving skin elasticity and, if damaged, wrinkle formation and skin ageing. Other components of skin are nerve ends, sweat glands, and hair follicles. A sebaceous gland empties into each hair follicle and a small erector pili muscle connected to the hair follicle results in “goose skin”, if contracted. In the top of the dermis just below the

Table 1
Comparison of direct and indirect cold atmospheric pressure plasma generation.

<table>
<thead>
<tr>
<th>Mode of generation</th>
<th>Direct plasma</th>
<th>Indirect plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas ionized</td>
<td>Dielectric barrier discharge against the skin</td>
<td>Discharge in a device and plasma efflux onto skin via gas flow</td>
</tr>
<tr>
<td>Plasma density on skin</td>
<td>Ambient air</td>
<td>Noble gas and/or ambient air</td>
</tr>
<tr>
<td>Distance to skin</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Gas temperature</td>
<td>Millimetre</td>
<td>Millimetre–Centimetre</td>
</tr>
<tr>
<td>Ultraviolet radiation</td>
<td>Room temperature</td>
<td>Hot at the efflux site</td>
</tr>
<tr>
<td>Electrical current</td>
<td>Weak, predominately UVA and some UVB</td>
<td>Strong in all UV ranges (UVA–UVC) [57]</td>
</tr>
<tr>
<td>Reactive gas species</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Produced in the plasma</td>
<td>Produced by mixing plasma with ambient air</td>
</tr>
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</table>
basal membrane a horizontal capillary network exists which nourishes the cells in the epidermis per diffusion. No blood or lymph vessels exist in the epidermis. A second important function of this capillary network is thermoregulation. The epidermis consists of four layers all formed by a sequential differentiation of keratinocytes. It takes about 4 weeks for a keratinocyte to migrate from the stratum basale to the stratum corneum. The main function of the epidermis lies in the mechanical defence. In the stratum corneum the keratinocytes have expelled their organelles and form the bricks held together by a lipid-rich substance. This epidermal barrier prevents from water loss. Further, the epidermis can be viewed as an immunologic organ preventing germ invasion. If this labile balance is disturbed, skin diseases like eczema (inflammation), infections or superinfections, dry skin (ichthyoses) or skin defects (wounds) may occur [17].

In cold atmospheric pressure plasma medicine antiinflammatory, antipruritic, antimicrobial, tissue stimulation, stimulation of microcirculation, and other therapeutic modalities are combined within one application due to the combined action of ultraviolet radiation, electric fields, reactive oxygen species (ozone), reactive nitrogen species, and possibly others, yet unknown (Table 2) [2,3].

2.1. Ultraviolet radiation (UV)

Photodermatology is a unique dermatological subspecialty [18]. The therapeutic use of UV irradiation dates back as long as to ancient Egypt where photochemotherapy was already practiced. Today, the anti-inflammatory, anti-pruritic, and anti-fibrotic properties of different UV wavelengths are well established [19]. UV radiation in medicine is divided into UVA (UVA2: 320–340 nm; UVA1: 340–400 nm), UVB (320–280 nm), and UVC (below 280 nm). UVA is adjacent to the visible light spectrum (blue: 400 nm–red: 800 nm). UVA, especially UVA1, exerts anti-pruritic and anti-fibrotic properties as it penetrates deeper into the dermis whereas UVB, especially monochromatic UVB 311 nm, exerts anti-inflammatory properties. On the other side of the chart, UV light also has considerable, dose-dependent side effects. Acute over-dosage results in sunburn (dermatitis solaris). Chronic UV exposure may result in accelerated skin ageing and wrinkle formation (predominately UVA-induced) as well as in skin carcinogenesis (predominately UVB-induced) [18,20]. Our cold plasma generator produces emission from excited molecular nitrogen (Second Positive System). Minor UVB (295–297 nm and 311–315 nm peaks) and predominately UVA (337, 358, 375 and 380 nm peaks) radiation at effective irradiances of up to $E_{\text{eff}} = 0.4 \text{mW/m}^2$ between 200 nm and 850 nm. This value is spectrally weighted according to weighing factors proposed by the International Comission on Non-Ionizing Radiation Protection (ICNIRP) to indicate the approximate relative erythemal efficacy and is thereby well below the minimal erythemal doses of $H_{\text{eff}} = 30 \text{J/m}^2$ (sunburn as an acute UV side effect) for skin treatment of up to 20 h per day [21,22].

2.2. Electric current

The plasma device produces an alternating, pulsed current with pulse repetition rate of about $f_p = 300 \text{Hz}$ and a pulse length of $t_p = 3 \mu\text{s}$ [21]. This excludes current conduction to inner organs e.g. via nerves, as the nerve conduction velocity is much slower (30–50 ms). In-vivo investigations revealed a skin current with instantaneous amplitude values of up to 420 mA [21]. Yet, these rather high current amplitudes are extremely transient events and only last for some $10^{-5}$ to $10^{-6}$ s during one pulse length whereby the energy transfer per current pulse is inherently restricted. Briefly said, short-term high currents in combination with actually now current flow until the subsequent initiation of a

<table>
<thead>
<tr>
<th>Modes of action</th>
<th>Clinical effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraviolet radiation</td>
<td>Antiinflammation, antiitch, germicidal</td>
</tr>
<tr>
<td>(UVA &gt; UVB)</td>
<td></td>
</tr>
<tr>
<td>Electric current</td>
<td>Iontophoretic, transdermal resorption, anti-hydrotic,</td>
</tr>
<tr>
<td></td>
<td>ant itch, tissue stimulation</td>
</tr>
<tr>
<td>Reactive oxygen</td>
<td>Germicidal, tissue stimulation, others</td>
</tr>
<tr>
<td>species/ozone</td>
<td></td>
</tr>
<tr>
<td>Reactive nitrogen</td>
<td>Stimulation of cell proliferation and blood</td>
</tr>
<tr>
<td>species</td>
<td>microcirculation, acidification, others</td>
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current pulse (after $10^{-3}$ s) results in very low rms (root mean square) values in the range of 100 $\mu$A.

Electrical fields can stimulate and act on biological tissue. In dermatology, iontophoresis is applied to treat hyperhidrosis of the palms and soles [23,24]. Patients wet their hands or feet in water for several minutes daily. A constant electric current of 5–10 mA runs through the water, reduces sweat production, and is well tolerated. Other dermatological applications of electric current are iontophoretic transdermal systems (ITS). Here, a constant current of about 170 $\mu$A can deliver a medication into the body via the skin. For example, 40 $\mu$g Fentanyl (anaesthetic) can be delivered transdermally within 10 min [25]. These established medical applications indicate that a plasma application creating an average of 100 $\mu$A (rms value) can be considered safe.

2.3. Reactive oxygen species (ozone)

It is well established that plasmas operated in air induce reactive oxygen species on the surface of living tissue [26]. A major component of the reactive gas species produced by the device is ozone. The TRK (technical relevant concentration) value for ozone is 200 $\mu$g/m$^3$ for 8 h. The MIK (max inhalative concentration) value is 120 $\mu$g/m$^3$ for 30 min. The ozone concentration measured at a distance of 5 mm from the electrode is $<100$ $\mu$g/m$^3$ [21]. Ozone is used for 80 years for wound disinfection and immunomodulation in medicine (60 mg/l ozone for 1 h) [27]. Ozone skin disinfection and antimicrobial surface ozone treatment is already in use [28]. In addition, positive effects of ozone on wound healing, e.g. on diabetic wounds at concentrations of 60 g/m$^2$ applied for 1 h followed by a coverage of the lesion with an ozone containing emulsion, were reported [29]. The authors found no side effects at these relatively high ozone concentrations.

2.4. Reactive nitrogen species

Plasma also induces a variety of reactive nitrogen species on the treatment surface. For example, we measured the generation of nitrate ($NO_3^-$) and nitrite ($NO_2^-$) on human lipid surfaces with our DBD device [14]. Such nitrogen species probably relate to skin acidification, an effect mediating disinfection as well as proper wound healing. We already found in a basic research-related project that plasma treatment leads to considerable changes in the human skin lipid barrier [30]. By using the latest X-Ray Photoelectron Spectroscopy (XPS) technology we investigated the physiologic skin lipid composition of human skin and the effects of plasma treatment on the lipid composition. Skin lipids were stripped off over the forearms of healthy volunteers using the cyanoacrylate glue technique, plasma treated or not, and then subjected to detailed XPS analysis. We found that the human lipid skin barrier consisted of 84.4% carbon (+0.2 sem%), 10.8% oxygen (+0.3 sem%) and 4.8% nitrogen (+0.5 sem%). The compositions of physiological skin lipids were not different in males and females. Plasma treatment resulted in significant changes in skin barrier lipid stoichiometry. The total carbon amount was reduced to 76.7% and the oxygen amount increased to 16.5%. There was also a slight increase in nitrogen to 6.8%. These changes could be attributed to reduced C–C bonds and increased C–O, C=O, C–N, and N–C–O bonds. The moderate increase in nitrogen was caused by an increase in C–N and N–C–O bonds. Our proof of principle investigations established the technical means to analyse, if plasma-induced skin lipid barrier changes may be beneficial in the treatment of ichthyotic or eczematous skin. In addition, nitrogen species-containing therapies have been applied in the past to treat lung diseases [31].

2.5. Side effects

Up to now there is no indication that there are DBD plasma treatment-related side effects on the skin. In-vitro experiments on human cells and ex-vivo skin models as well in-vivo experiments on mouse skin revealed no cellular toxicity, e.g. necrosis [21,32]. Using human skin biopsies DBD plasma treatment of up to 400 Hz and 120 s revealed no cellular damage or damage of the cell nucleus [33,34]. In addition, in-vivo two photon microscopy revealed no human epidermal skin alterations on the cellular level after 120 s of DBD plasma treatment. Histological evaluations of mouse skin after 2 min DBD plasma treatment also showed no alterations at all [35,36].

3. Treatment of skin infections/disinfection

The disinfective properties of plasma and its use in the treatment of superinfected skin appears to be the best evaluated indication for cold atmospheric plasma usage today, as shown by several groups independently. Several in-vitro and in-vivo studies demonstrated the bactericidal effects of plasma. Däschlein et al. showed that plasma is active against most germs on wounded skin [37]. We also detected a germicidal action of the plasma device [38,39]. We found that the antibacterial effect correlates with a decrease in pH and is mediated by bacterial membrane damage [39]. Other groups showed the disinfective properties of plasma treatment in-vivo, e.g. in reduction of bacterial colonization of chronic wounds [40].

4. Treatment of eczema

Atopic eczema is a very common chronic medical condition throughout the population (3–5%). Predisposition sites include the great flexures on the arms and legs. Usually a combinatory treatment of eczema is performed including a moisturizing treatment as a basic treatment, followed by topical antiinflammatory (e.g. corticosteroids) and antimicrobic treatments. Due to therapeutic resistance this is often combined with UV-phototherapy. The long-term adequate combination of these different topical treatments is often difficult to pursue for the patient at home and the combination of several treatments per day is laborious [41].

We treated an adult patient with atopic eczema for 30 days and for 1 min/d with the DBD device on a defined area on the external aspect of the left upper arm. The right side served as a control. A moisturizing basic emollient treatment was allowed for the body via the skin. For example, 40 $\mu$g Fentanyl (anaesthetic) can be delivered transdermally within 10 min [25]. These established medical applications indicate that a plasma application creating an average of 100 $\mu$A (rms value) can be considered safe.

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and led to significant improvement of the disease after 11 treatments.

5. Treatment of chronic wounds

Chronic wounds comprise skin tissue defects in diseased skin. Ulcers on the lower legs (ulcus cruris) and feet are the most common wounds from which especially elderly suffer. Varicosis and other venous diseases are the underlying cause in 80% of patients with ulcer cruris [44]. Arterial diseases (10%) and diabetes (4%) constitute the two other common causes for wounds on the legs or feet. The prevalence of ulcer cruris is estimated between 0.3 and 1.0%. In Germany 240,000–800,000 patients suffer from ulcer cruris [45]. The risk for venous ulcers increases with age and most patients are within their 60s to 80s [46]. The lifelong risk to develop a leg ulcer is 2% [44].

Due to the chronicity and high prevalence venous ulcers constitute a considerable socioeconomic burden. It is estimated that 1–2% of the health care budget is consumed by the treatment of venous ulcers which is 600–900 million Euros in the countries of Western Europe [47]. Mean costs per patient are estimated at 9,560 Euro per patient per year [48]. This does not include indirect costs for early retirement or reduced quality of life.

Basically, wound healing can be divided into three phases: a cleaning phase, a granulation phase, and an epithelialisation phase [49]. A plethora of different processes is active in these phases and include blood coagulation, inflammation, matrix synthesis, angiogenesis, fibrosis, cell proliferation, and remodeling [50]. During the first phase the wound is cleaned from cell detritus, dirt, and bacteria. Then angiogenesis and granulation of dermal tissue occurs closing the wound from the depth. During the final phase keratinocytes from the epidermis proliferate over the granulation tissue and finally close the wound. Delayed healing processes can occur in any phase and often include multiple processes in multiple phases. These processes are far from being understood and are intensively investigated.

As the exact molecular mechanisms of wound healing are largely unknown, therapeutic strategies to treat venous ulcers are largely symptomatic. Modern wound care of venous ulcers includes compression therapy. The use of elastic bandages or stockings is suggested to support the venous blood flow and decrease venous stasis. Second, adequate wound dressings are recommended. The paradigm is to keep the wound moist, yet the dressing should have the capacity to take on wound exudates to allow a change of the dressing only every 2–4 days. Modern wound dressings should allow air exchange, reduce pain, control bacterial colonisation, and protect the wound against mechanical stress. Moreover, the material of the wound dressing should be hypoallergenic and non-irritative. Ease of handling is another prerequisite as the patients often change the dressings themselves. Additional procedures like surgical debridement may be indicated [51].

As outlined above the disinfective property of cold atmospheric plasma treatment has been demonstrated in several in-vitro as well as in-vivo studies. The control against bacterial supercolisation is one goal in modern wound healing strategies as bacterial colonisation delays wound healing and fosters chronic wounds [52]. Secondly, cold plasma leads to proliferation of endothelial cells via the stimulation of growth factors for angiogenesis [53]. This constitutes another important mechanism in wound healing. Third, we and others found that plasma decreases the pH leading to wound acidification [14,54]. Therapeutically induced wound acidification can positively affect the healing process [55]. Thus, our cold plasma device combines several single positive effects on wound healing within one application (Table 2).

Due to these reasons we are currently conducting a clinical trial to investigate the safety, efficacy, and applicability of DBD plasma treatment of chronic venous wounds. We strictly selected inclusion and exclusion criteria. The trial is certified according to actual legislation in Germany by all necessary authorities. It is the very first clinical trial applying direct cold atmospheric plasma (PlasmaDerm® VU-2010 device) in wound treatment officially listed in trial databases (e.g. NCT01415622 in clinicaltrials.gov) world-wide. We envisage that our results obtained in this trial for the plasma treatment of chronic venous leg ulcers may also be transferred for wound plasma treatment of arterial or diabetic causes as pathomechanisms appear similar [56].

6. Conclusions

Cold atmospheric pressure plasma appears as a novel and promising new therapeutic strategy in medicine as several modes of action are combined within one treatment application. UV irradiation, electric current, reactive oxygen species, ozone, and reactive nitrogen species confer antiinflammatory, antitoxic, antimicrobial, tissue stimulation, stimulation of microcirculation, and potentially other therapeutic properties. The germicidal property of plasma is clearly established and first clinical reports show positive results in the treatment of superinfected wounds or superinfected dermatitis of different causes. So far, no side effects of plasma treatment were reported. These results should foster broader translational research efforts to further the understanding of plasma treatment benefits as well as potential side effects. With this respect the mid-term goal would be to standardize technical parameters for the treatment of a given disease.

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