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Treatment of Chronic Venous Leg Ulcers with a Hand-Held DBD Plasma Generator

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ABSTRACT: In cold plasma medicine, anti-inflammatory, anti-itch, antimicrobic, ultraviolet, and other therapeutic modalities are combined within one treatment. Two types of cold plasma can be discerned: direct (dielectric barrier discharge [DBD]) and indirect plasma. DBD generates a low-temperature plasma under atmospheric pressure. The PlasmaDerm VU-2010 device is a noninvasive active medical intervention that does not come into direct contact with skin. For our medical application, a nonequilibrium, weakly ionized, physical DBD plasma is generated by the application of high voltages across small gaps; the electrode is covered by a dielectric. The skin itself acts as the second electrode. Chronic leg ulcers are a major problem among the elderly. The prevalence corresponds to 2–4% of the population. Eighty percent of chronic leg ulcers are caused by varicosis. In general, 3 phases of wound healing (cleaning of the wound ground, granulation, and epithelialization) can be discerned as disturbed in chronic venous leg ulcers. Wound debridement, modern wound dressings, and compression hosiery comprise methods of standard care. Despite these measures, leg ulcers often persist. Additional plasma treatment may have the potential to facilitate wound healing by disinfection, stimulation of tissue regeneration and microcirculation, and acidification of the wound environment. We are currently conducting an ongoing clinical trial with the PlasmaDerm VU-2010 device to assess the safety, applicability, and efficacy of plasma treatment for chronic venous leg ulcers. So far, no adverse effects of plasma treatment have been reported, pointing toward a positive outcome of our study.

KEY WORDS: cold atmospheric pressure plasma (CAP), ultraviolet radiation, reactive gas species, electric current, chronic wounds

I. COLD ATMOSPHERIC PRESSURE PLASMA

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

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voltages across small gas-filled gaps results in ionization of, for example, air. Sir William Crookes, a British chemist, first described plasma as radiant matter in 1879.[1] Irving Langmuir introduced the term *plasma* (because of the composition of the ionized gas) in 1928. Plasma indicating an ionized gas may not be confused with blood plasma, the liquid and cell-free component of blood. Plasma, as the fourth and highest energy state of matter, constitutes the by-far dominant state of matter in the universe, for example, relating to the solar corona or solar winds. On earth, under atmospheric pressure, plasma can be generated technically by electric gas discharges in a wide range of parameters such as temperature or density. Air (or any other gas, inert gas, or gas mixture) can be transformed into the plasma state by gaining energy from strong electric fields. Cold atmospheric pressure plasma (CAP) consists of a complex mixture of interreacting and quite short-lived atoms, ions, free electrons, photons, and excited species.[2,3]

On earth, plasma can be generated either at high pressure, low pressure, or under atmospheric pressure. For decades atmospheric pressure plasma has been generated as arcs between 2 metallic electrodes. Unfortunately, because of high collision frequencies and high current densities in such arcs, gas temperatures of hundreds or several thousands degrees Celsius occur as a result of a thermal equilibrium.[4,5] Living tissues like the skin cannot be exposed to such thermal plasmas without becoming necrotic. On the other hand, if tissue removal, cutting, or cauterization is desired, the destructive properties of thermal plasmas can be used medically. Thermal plasmas also are used for sterilization purposes.[6–10] Further developments in the past few years now allow the generation of low-temperature plasmas, which is achieved by particular constructive measures that result in quite homogeneous electrical gas discharges at atmospheric pressure rather than arc formation, resulting in plasmas that do not reach a thermal equilibrium. The temperature of such nonthermal or nonequilibrium plasmas does not exceed body temperature. This allows the direct application of plasma onto living tissue like the skin, named CAP treatment.

In general, 2 types of CAP can be discerned: direct plasma (e.g., via dielectric barrier discharge [DBD]) and indirect plasma. In indirect plasma treatment, the plasma is produced in a remote cavity between 2 electrodes and ejected by gas flow onto the skin in the form of an effluent. The skin is not used as the counter electrode.[11,12] In direct plasma treatment, the skin itself acts as the counter electrode. As early as 1857 von Siemens[13] described direct CAP production applying the DBD technique for the generation of ozone. As noted above, the DBD plasma treatment of biological tissue is quite novel, whereas DBD plasma treatment of technical surfaces has been a standard method for years.[14] When the appropriate technical parameters are selected, a DBD generates a low-temperature plasma under atmospheric pressure, which can be used for the direct treatment of living biological tissue.[15] Advantageous features of direct plasma treatment include higher plasma density as well as induced high-frequency electric current onto the skin (Table 1).

For our investigations, a nonequilibrium, weakly ionized physical plasma is generated by the application of alternating voltage pulses with amplitudes >10 kV

TABLE 1. Comparison of Indirect and Direct Cold Atmospheric Pressure Plasma

	Indirect Plasma	Direct Plasma
Mode of generation	Discharge in a device and plasma effluent onto skin via gas flow	Dielectric barrier discharge against the skin
Gas ionized	Noble gas and/or ambient air	Ambient air
Plasma density on skin	Low	High
Distance to skin	Millimeters to centimeters	Millimeters
Gas temperature	Hot at the efflux site	Room temperature
Ultraviolet radiation	Strong in all UV ranges (UVA–UVC)	Weak, predominately UVA and some UVB
Electrical current	No	Yes
Reactive gas species	Produced by mixing plasma with ambient air	Produced in the plasma

UV, ultraviolet.

across air gaps in the low millimeter range. The high-voltage electrode is covered by a dielectric, which constitutes a nonconducting barrier. This dielectric avoids the transition of the gas discharge into a hot arc by limiting the current and is essential in all DBD settings. The counter electrode for the discharge is the biological tissue itself (i.e., the skin). The big advantage of DBD is that the plasma usually propagates in tiny breakdown channels called microdischarges. These microdischarges seem to be stochastically distributed over the electrode area and are cylindrical in shape (radius, 10^{-4} m) (Fig. 1). In the strong oscillating electric field between 2 electrodes, only electrons gain nameable kinetic energies. In the microdischarges a reactive mix of charged particles, ultraviolet (UV) photons, and excited atoms and molecules as well as reactive nitrogen species and reactive oxygen species (ROS) is generated. Ozone, hydroxide, nitrous oxide, and heme-nitric oxide constitute typical species in air plasmas. By choosing different plasma parameters, for example, the type of gas or gas compositions, energy density, local electric fields, gas temperature, or humidity, the composition of reactive species can be modulated.[16]

Fig. 2 depicts our DBD CAP device (CINOGY GmbH, Duderstadt, Germany). Defined physical parameters are determined by the control unit. To avoid any changes in the technical parameters—and thus potential side effects of the plasma treatment—there is only one "on" button. For safety reasons, a timer in the display then allows a treatment duration of exactly 45 seconds. Afterward the device switches off automatically and needs to be restarted by pressing the "on" button. The control unit connects to a hand-held DBD plasma applicator, which carries the electrode covered by a dielectric (1 cm in diameter). Contact-free plasma application as well as a constant

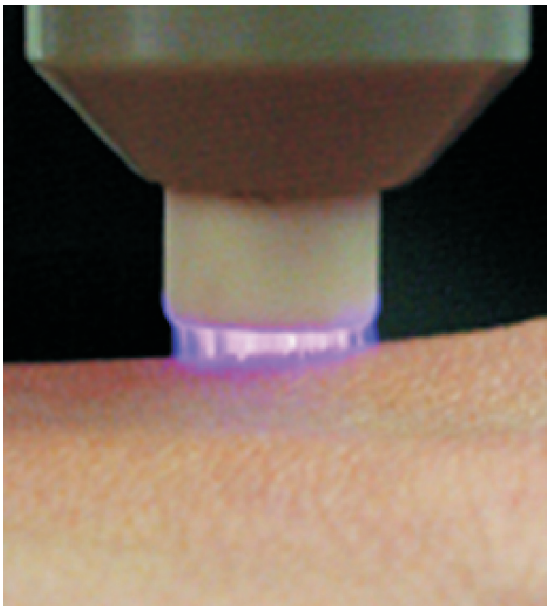

FIG. 1: Random distribution of microdischarges over the electrode area.

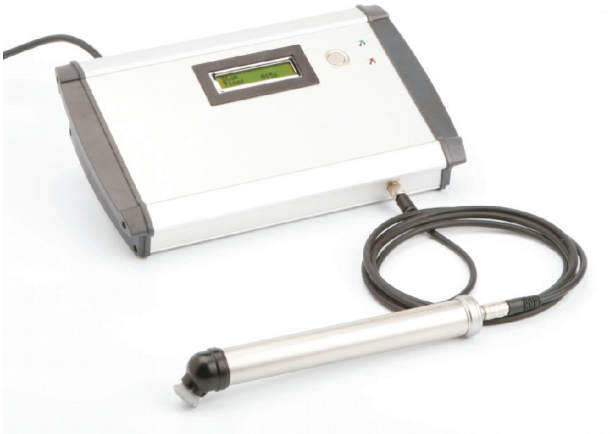

FIG. 2: The PlasmaDerm VU-2010 device constructed by CINOGY GmbH, Duderstadt, Germany, showing the control unit with a start button and timer display and the hand-held plasma applicator.

distance between the DBD electrode and the skin are ensured by applying a specially constructed, sterile, and ready-to-use spacer. If direct contact of the DBD electrode with the skin occurs, the air discharge—and thus the plasma generation above the skin—stop. This is completely harmless.

II. MODES OF ACTION OF CAP

A. Reactive Nitrogen Species

A variety of reactive nitrogen species are produced by CAP on the surface being treated. Applying our plasma device, we measured the generation of nitrite and nitrate on human lipid surfaces.[14] As a result of such nitrogen species there is skin acidification, an effect that is advantageous for skin disinfection as well as proper skin wound healing. Plasma treatment leads to considerable changes in the human skin lipid barrier, as detected during one of our basic research-related projects.[17] We investigated the physiological composition of lipids in human skin and the effects of plasma treatment on the lipid composition by using the latest X-ray photoelectron spectroscopy technology. Skin lipids were stripped off the forearms of healthy volunteers using the cyanoacrylate glue technique, were treated with plasma or not, and then were subjected to detailed X-ray photoelectron spectroscopy analysis. We found that the composition of physiological skin lipids were not different in males and females or in young (25 years) or older (50 years) probands. Significant changes in the stoichiometry of the barrier of lipids in the skin occurred after plasma treatment. The total amount of carbon was significantly reduced and the amount of oxygen increased. Also, a slight increase in nitrogen was noted. Reduced C-C bonds and increased C-O, C=O, C-N, and N-C-O bonds, as well as an increase in C-N and N-C-O bonds, contributed to these findings.[17] With this study we have now the technical means at hand to further investigate whether plasma-induced changes in the skin's lipid barrier may be beneficial in the treatment of ichthyotic or eczematous skin. Others also have applied therapies containing nitrogen species to treat lung diseases.[18]

B. Reactive Oxygen Species (Ozone)

That CAP can induce ROS on the surface of living tissue is well established.[19] Among these ROS, ozone comprises a major component of the reactive gas species produced by our plasma device. The ozone concentration measured using our device is <100 $\mu g/m^3$ at a distance of 5 mm from the electrode.[20] Technical relevant concentration values and maximum inhalative concentration values for ozone are 200 $\mu g/m^3$ for 8 hours and 120 $\mu g/m^3$ for 30 minutes, respectively. For the purposes of wound disinfection and immunomodulation, ozone has been used for 80 years in medicine (60 mg/L ozone for 1 hour).[21] Skin disinfection using ozone and ozone treatment of antimicrobic surfaces are well established as medical procedures.[22] In addition, it is reported that ozone has positive effects on the healing of, for example, diabetic wounds if applied at concentrations of 60 g/m^3 for 1 hour followed by coverage of the lesion with an ozone-containing emulsion.[23] No side effects at these relatively high ozone concentrations were reported by the authors.

C. Alternating Pulsed Electric Current

An alternating, pulsed current is produced by our plasma device, with a pulse length of t_p = 3 µs and pulse repetition rates of about f_p = 300 Hz.[20] The short pulse length ex-

cludes current conduction to inner organs, for example, via nerves that conduct pulses at 30–50 ms. Short-term high currents in combination with actually low current flow until the subsequent initiation of a current pulse (after ~10^{-3} seconds) results in very low root mean square values of approximately 100 µA.

Stimulation of biological tissue by electrical fields is widely used, for example, in the field of neurology. For dermatological purposes, iontophoresis is applied to treat hyperhidrosis of the palms and soles.[24,25] During iontophoresis, for several minutes daily patients bathe their hands or feet in water through which a constant electric current of 5–10 mA runs. This electric current is well tolerated and significantly reduces sweat production. Iontophoretic transdermal systems comprise other dermatological applications of electric current; a medical substance is delivered into the body systemically through the skin via a constant current of about 170 mA: with such iontophoretic transdermal system technology with 40 µg fentanyl (pain medication) can be delivered transdermally within 10 minutes.[26]

D. Ultraviolet Radiation

The ancient Egyptians used UV radiation therapeutically and practiced photodermatology, which is an unique dermatologic subspecialty.[27] Modern clinical studies in the past three decades clearly established the anti-itch, antifibrotic, and anti-inflammatory properties of different UV wavelengths.[28] For use in dermatology, UV radiation is divided according to its wavelengths into UVA (400–320 nm; UVA1: 400–360 nm), UVB (320–280 nm), and UVC (<280 nm). The adjacent visible light spectrum spans from 400 nm (blue) to 800 nm (red). UVA penetrates deeper into the dermis and exerts ant-itch and antifibrotic properties; this is especially true for the UVA1 therapies with longer wavelengths. UVB, especially monochromatic UVB (311 nm), exerts anti-inflammatory effects. On the other hand, UV radiation also exerts considerable dose-dependent side effects. Sunburn (dermatitis solaris) is a result of an acute overdose of UV (predominately UVB). Accelerated skin aging and wrinkle formation are results of chronic UVA exposure. Chronic UVB exposure may result in skin carcinogenesis, including melanoma and nonmelanoma skin cancer.[27,29] Our CAP DBD generator produces UV emissions as a result of excited molecular nitrogen (second positive system). Predominately UVA (337-, 358-, 375-, and 380-nm peaks) radiation, only minor fractions of UVB radiation (295- to 297-nm and 311- to 315-nm peaks), and effective irradiances of up to 0.4 mW/m^2 between 200 and 850 nm are produced by our device. This value, spectrally weighted according to weighting factors proposed by the International Commission on Non-Ionizing Radiation Protection to indicate the approximate relative erythemal efficacy, is thereby well below the minimal erythemal doses of 30 J/m^2 (sunburn as an acute UV side effect) for treatment of the skin for up to 20 hours/day.[20,30]

E. Disinfection

Several independent groups previously showed the disinfective properties of plasma and its use in the treatment of superinfected skin. This seems to be the best evaluated indica-

tion for CAP usage today. The bactericidal effects of plasma were demonstrated by several *in vitro* and *in vivo* studies. That plasma is active against most germs on wounded skin was recently shown by Daeschlein et al.[31] We have already published reports of the germicidal action of our plasma device.[32,33] The antibacterial effect of our CAP device is mediated by bacterial membrane damage and correlates with a decrease in pH.[33] That plasma treatment exerts disinfective properties *in vivo*, for example, the reduction of bacterial colonization of chronic wounds, was shown by other groups.[34]

F. Skin and Adverse Effects of Plasma Treatment

The amount of skin on a body is about 2 m^2, rendering the skin the biggest human organ. Life would not be possible without our skin. The subcutaneous fat tissue, the dermis (or cutis), and the epidermis constitute the 3 skin layers (Fig. 3). Collagen and elastic fibers are the main components of the dermis and confer elasticity, form wrinkles, and cause skin to age. Hair follicles, sweat glands, and nerve ends comprise other dermal components. A sebaceous gland is connected to each hair follicle. A horizontal capillary network exists in the top of the dermis just below the basal membrane. The purpose of these capillaries is the nourishment of the cells in the epidermis per diffusion. No blood or lymph vessels exist in the epidermis. Thermoregulation is a second important function of this capillary network. The main cellular component of the epidermis is keratinocytes, which undergo a directed differentiation process when they migrate from the stratum basale to the stratum corneum. This differentiation and migration process takes about 30 days. According the differentiation status of the keratinocytes, 4 different epidermal layers can be discerned microscopically. In the stratum corneum, the keratinocytes expel all their organellae and form bricks that are held together by a lipid-rich

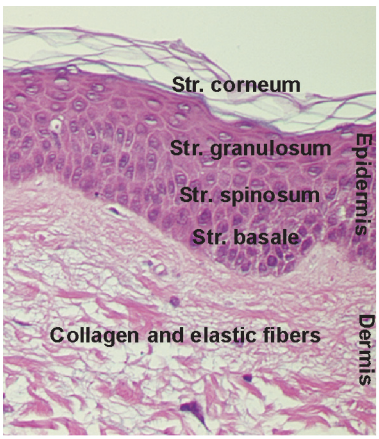

FIG. 3: Histologic skin section (hematoxylin and eosin stain) depicting the different layers of the epidermis, consisting of differentiated keratinocytes (epidermal barrier), as well as the dermis, with its collagen and elastic fibers (red structures).

substance. Mechanical defence obviously is the main function of the epidermis. Another important function of the epidermal barrier is the prevention of water loss. The third most important epidermal function is the prevention of germ invasion. In this respect the epidermis may by considered the biggest immunologic organ. It is conceivable that even small disturbances in this labile balance easily result in skin diseases such as eczema (inflammation), skin infections or superinfections, or skin defects (wounds).[35]

Because of the combined action of reactive nitrogen species, ROS (ozone), electric fields, UV radiation, and possibly other yet unknown modes of action, CAP medicine combines antimicrobic, anti-itch, anti-inflammatory, tissue stimulation, stimulation of microcirculation, and other therapeutic modalities within one application (Table 2).[2,3]

As CAP treatment of skin is a completely new therapeutic modality, potential adverse events have to be seriously investigated. However, to date there are no indications of side effects related to DBD plasma treatment on the skin. No cellular toxicity (e.g., necrosis) was detected in *in vitro* experiments on human cells and *ex vivo* skin models as well as *in vivo* experiments on mouse skin.[20,36] DBD plasma treatment up to 400 Hz and 120 seconds revealed no cellular damage or damage to the cell nucleus in human skin biopsies.[37,38] In addition, no human epidermal skin alterations at the cellular level after 120 seconds of DBD plasma treatment was detected with *in vivo* 2-photon microscopy. Even histological assessments of mouse skin after a 2-minute DBD plasma treatment did not reveal any alterations at all.[39,40]

III. CAP TREATMENT OF CHRONIC VENOUS LEG ULCERS

Chronic wounds are defined as skin tissue defects in diseased skin that show a delayed healing process. Chronic wounds often persist for months or years. By far the most common wounds are ulcers on the lower legs (ulcus cruris) and feet. The elderly especially suffer from such ulcers, and the incidence increases with age. Most patients are within their 60s to 80s.[41] The lifelong risk of developing a leg ulcer is 2%.[42] In 80% of patients, ulcus cruris varicosis and other venous diseases constitute the underlying cause.[42] The 2 other common causes of wounds on the legs or feet are arterial diseases (10%) and

TABLE 2. Clinical Effects and Modes of Action of Cold Atmospheric Pressure Plasma

Clinical Effects	Modes of Action
Anti-inflammation, anti-itch, germicidal	Ultraviolet radiation (UVA > UVB)
Iontophoretic, transdermal resorption, antihydrotic, anti-itch, tissue stimulation	Electric current
Germicidal, tissue stimulation, others	Reactive oxygen species/ozone
Stimulation of cell proliferation and blood microcirculation, acidification, others	Reactive nitrogen species

diabetes (4%). The prevalence of ulcus cruris is estimated between 0.3% and 1.0%; 240,000 to 800,000 patients suffer from ulcus cruris in Germany.[43]

Venous ulcers constitute a considerable socioeconomic burden because of their high prevalence and chronicity. It is estimated that €600 to 900 million in the countries of Western Europe—which is 1–2% of the health care budget—are spent on the treatment of venous ulcers.[44] Per-patient and per-year mean costs are estimated at €9,560.[45] Indirect costs for reduced quality of life or early retirement are not included in this calculation.

The basic processes of wound healing can be divided into 3 phases: the cleaning phase, granulation phase, and epithelialization phase.[46] During each of these phases a variety of different processes are active, including blood coagulation, inflammation, cell proliferation, matrix synthesis, fibrosis, angiogenesis, and remodeling.[47] The wound is cleaned of dirt, bacteria, and cell detritus during the first phase of healing. During the second (granulation) phase, dermal tissue growth and angiogenesis occur, which start to close the wound from its depth. During the last phase of wound healing, epithelialization, keratinocytes from the epidermis proliferate from the edges, cover the dermal granulation tissue, and finally close the wound. It should be stressed that all these phases of the healing process are far from being understood by themselves and are being intensively investigated at the moment. In any phase of wound healing, delayed processes can occur, and chronic wounds often result from a delay of multiple processes in multiple healing phases.

As a logical consequence of the lack of understanding the exact molecular mechanisms of wound healing, the strategies used to treat chronic wounds—especially venous leg ulcers—are largely symptomatic. The only kind of causative treatment measure in the modern therapy for venous ulcers is compression therapy. Venous blood flow back through the body and, as a result, decreased venous stasis is supported by the use of elastic bandages or stockings. As a second treatment measure, adequate wound dressings are applied. The current paradigm of modern wound treatment is to keep the wound moist. On the other hand, the dressing should also have the capacity to take on wound exudates without drying the wound. Because of these characteristics, modern wound dressings need to be changed only every 2–4 days. In addition, several other properties are demanded of modern wound dressings: They should allow air exchange, control bacterial colonization, protect the wound against mechanical stress, and reduce pain. Moreover, the wound dressing should consist of hypoallergenic and nonirritating material. Because patients often change dressings themselves, another prerequisite for modern wound dressings is ease of handling. As an optional procedure surgical, debridement may be performed as needed.[48]

The disinfective property of CAP treatment seems to be the best established property and has been demonstrated in several *in vitro* as well as *in vivo* studies, as outlined above. Also, in modern wound healing strategies, control against bacterial supercolonization is a major goal because bacterial colonization delays wound healing and fosters chronic wounds.[49] Proliferation of endothelial cells via the stimulation of growth factors for angiogenesis is another result of plasma treatment[50] and also constitutes an important

mechanism in wound healing. Third, plasma treatment decreases pH, leading to wound acidification, as we and others found in previous studies.[14,51] Wound acidification is a physiological process that occurs during the normal healing process.[52] Based on this, we can conclude that our cold plasma device combines several single positive effects on wound healing within one application, which should be beneficial in the treatment of a delayed wound healing process (Table 2).

On the basis of these theoretical considerations, we initiated a clinical trial to investigate the safety, efficacy, and applicability of DBD plasma treatment of chronic venous leg ulcers. Inclusion and exclusion criteria were strictly selected. The trial has been certified by all necessary authorities according to actual German legislation. It seems that our trial applying direct cold atmospheric plasma (using the PlasmaDerm VU-2010 device) in wound treatment is the first clinical trial officially listed in trial databases (e.g., NCT01415622 at clinical trials.gov) worldwide. Because pathomechanisms seem to be similar, the results obtained from this trial for the plasma treatment of chronic venous leg ulcers may also be transferred to the plasma treatment of other wounds caused by arterial or diabetic diseases or for mixed ulcera,[53] as well as wounds due to skin injuries.[54]

IV. CONCLUSIONS

CAP combines several modes of action within one treatment application and seems to be a novel and promising new therapeutic strategy in medicine. In plasma treatment, anti-inflammatory, anti-itch, antimicrobic, tissue stimulation, stimulation of microcirculation, and other therapeutic modalities are combined within one application through the combined action of, for example, UV radiation, ROS (e.g., ozone), reactive nitrogen species, and electric fields. *In vitro* and *in vivo* investigations have clearly established the germicidal property of plasma. Furthermore, positive results in the treatment of superinfected wounds have been reported in initial clinical studies. So far, no side effects of plasma treatment were reported. In summary, CAP constitutes a new and innovative treatment option, especially for superinfected skin diseases. These promising first 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 technical devices to allow a standardized treatment of given diseases in the midterm.

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