Alleviation of chronic venous leg ulcers with a hand-held dielectric barrier discharge plasma generator (PlasmaDerm® VU-2010): results of a monocentric, two-armed, open, prospective, randomized and controlled trial (NCT01415622)


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Abstract

Background  Cold atmospheric plasma (CAP, i.e. ionized air) is an innovating promising tool in reducing bacteria.

Objective  We conducted the first clinical trial with the novel PlasmaDerm® VU-2010 device to assess safety and, as secondary endpoints, efficacy and applicability of 45 s/cm² cold atmospheric plasma as add-on therapy against chronic venous leg ulcers.

Methods  From April 2011 to April 2012, 14 patients were randomized to receive standardized modern wound care (n = 7) or plasma in addition to standard care (n = 7) 3 × per week for 8 weeks. The ulcer size was determined weekly (Visitrak®, photodocumentation). Bacterial load (bacterial swabs, contact agar plates) and pain during and between treatments (visual analogue scales) were assessed. Patients and doctors rated the applicability of plasma (questionnaires).

Results  The plasma treatment was safe with 2 SAEs and 77 AEs approximately equally distributed among both groups (P = 0.77 and P = 1.0, Fisher’s exact test). Two AEs probably related to plasma. Plasma treatment resulted in a significant reduction in lesional bacterial load (P = 0.04, Wilcoxon signed-rank test). A more than 50% ulcer size reduction was noted in 5/7 and 4/7 patients in the standard and plasma groups, respectively, and a greater size reduction occurred in the plasma group (plasma – 5.3 cm², standard: – 3.4 cm²) (non-significant, P = 0.42, log-rank test). The only ulcer that closed after 7 weeks received plasma. Patients in the plasma group quoted less pain compared to the control group. The plasma applicability was not rated inferior to standard wound care (P = 0.94, Wilcoxon–Mann–Whitney test). Physicians would recommend (P = 0.06, Wilcoxon–Mann–Whitney test) or repeat (P = 0.08, Wilcoxon–Mann–Whitney test) plasma treatment by trend.

Conclusion  Cold atmospheric plasma displays favourable antibacterial effects. We demonstrated that plasma treatment with the PlasmaDerm® VU-2010 device is safe and effective in patients with chronic venous leg ulcers. Thus, larger controlled trials and the development of devices with larger application surfaces are warranted.

Introduction  High-temperature plasmas have been used for years to sterilize medical devices and implants as well as for tissue cauterization, removal, cutting and coagulation. More recent developments now allow the generation of low-temperature cold atmospheric plasmas (CAP, i.e. ionized air), which have the same benefits as...
high-temperature plasmas but without the enormous heat production and are, therefore, applicable to living tissues like human skin. CAP can be regarded as the fourth state of matter following solids, liquids and gases, and consists of a plethora of active components, such as atoms, ions, free electrons, photons, ultraviolet radiation and excited gas species. Generally, two types of CAP can be distinguished: direct (dielectric barrier discharge, DBD) and indirect plasma. In indirect systems, plasma is produced between two electrodes and a carrier gas transporting the active agents onto the skin is needed. In direct plasma systems, the skin itself acts as the counter electrode. Typically, the distance between the direct plasma device and tissue is 1–5 mm. Advantageous features of direct plasma treatment include the higher plasma density as well as the induced high-frequency electric current within the upper layers of the skin. Here, we utilized a novel DBD plasma device, the PlasmaDerm® (CINOGY GmbH, Duderstadt, Germany) VU-2010 (Fig. 1).

The potential of CAP for reducing bacterial load without relevant side-effects has already been demonstrated in numerous in vivo and in vitro studies. The antibacterial effect of our DBD plasma device has also been confirmed earlier and is mediated by bacterial membrane damage. These disinfectant properties of plasma appear to be the best evaluated medical indication for the usage of CAP today. Current strategies in wound healing aim at the control of bacterial colonization and infections, as both are associated with impaired healing and chronicity of wounds. Plasma can also promote the proliferation of endothelial cells via the stimulation of angiogenic growth factors, another important mechanism for wound healing. In addition, plasma treatment leads to wound acidification. This was shown to exert favourable effects on wound healing.

Chronic leg ulcers represent a major medical problem in the elderly. Up to 80% of such ulcers are caused by venous diseases. Due to the high prevalence and chronicity, leg ulcers constitute an enormous socio-economic burden. About 600–900 million Euros are spent annually on the treatment of venous ulcers in Western Europe alone, which represents 1–2% of the entire health care budget. Standard treatments against venous leg ulcers comprise modern wound dressings keeping the ulcers moist, periodic wound debridement and a continuous compression therapy. Additional plasma treatment may have the potential to facilitate wound healing by disinfection, stimulation of tissue regeneration and acidification of the wound milieu.

In previous experiments, DBD plasma treatment of up to 400 Hz and 120 s revealed no cellular or nuclear damage in human skin biopsies. Likewise, in vivo two-photon microscopy of human epidermis did not show cellular alterations after 120 s of DBD plasma treatment. Further histological assessments of mouse skin after 2-min of DBD plasma treatment did not reveal any alterations. On the basis of these promising preclinical safety data, we now conducted the first clinical trial with the PlasmaDerm® VU-2010 device to assess safety, applicability and efficacy of plasma in the treatment of chronic venous leg ulcers.

**Patients and methods**

**Study design**

We conducted a monocentric, two-armed, open, randomized and controlled clinical trial. The time of recruitment ranged from April 2011 to April 2012. The trial has been performed according to the Declaration of Helsinki principles, approved by all necessary authorities according to German legislation, and is officially listed in trial databases (e.g. NCT01415622 in clinical trials.gov). Written informed consent was obtained from all study participants prior to initiation of the study. The study period per patient amounted 12 weeks, composed of an 8-week treatment period and a 4-week follow-up period. Patients were randomized at a ratio of 1:1 to both study arms by list after screening and confirmation of eligibility.

**Patients**

Patients were included, if they suffered from at least one chronic ulcer of 5–30 cm². The ulcers had to be present for at least
12 weeks. Exclusion criteria were as follows: patients younger than 50 years, non-menopausal women, ulcers of non-venous origin or mixed ulcers, active malignancy, immunosuppressive agents, patients carrying a pacemaker/implantable cardioverter defibrillator, severe cardiac insufficiency, rheumatoid arthritis and non-controlled diabetes mellitus (defined by HbA1c >8%). All patients were examined clinically including Doppler/duplex ultrasound examination of their lower extremities venous system, assessment of ankle-brachial index and blood tests (complete blood count, HbA1c and serum albumin level). Sixteen patients were screened, 15 patients were randomized and 14 patients (eight males and six females) received study interventions and were assessed for trial endpoints in an intention-to-treat collective (Table S1).

Plasma device and configuration
The PlasmaDerm® VU-2010, a DBD cold atmospheric pressure plasma device (CINOGY GmbH, Duderstadt, Germany) generates a non-equilibrium, weakly ionized physical plasma by application of alternating voltage pulses with amplitudes >10 kV and a power density of 120 mW/cm². The high-voltage electrode is covered by a dielectric, which avoids the transition of gas discharge into a hot arc by limiting the current. The skin itself acts as a counter electrode. A contact-free plasma application and constant distance of the DBD electrode to the skin is ensured by a specially constructed and sterile spacer (Fig. 1). Defined physical parameters are determined by the control unit. To avoid any changes of the technical parameters and potential concomitant side-effects, there is only one ‘on’ button on the device. A timer on the display allows treatment duration of exactly 2 × 45 s. Afterwards the PlasmaDerm® VU-2010 switches off automatically and needs to be restarted. The PlasmaDerm® VU-2010 exhibits the following technical parameters – line voltage: 230 VAC, frequency: 50 Hz, maximum power consumption: 8 VA, overall size: 294 mm × 185.2 mm × 76.2 mm, weight: 2.1 kg.

Treatment protocol
Patients were randomized to receive either a standardized modern wound care or plasma treatment in addition to standard care 3× per week for 8 weeks. Ulcer sizes were determined weekly by Visitrax® (Smith and Nephew Healthcare, Hull, UK) with repeated photo documentation. Patients treated with plasma received a 45 s/cm² plasma application. This corresponded – depending on the ulcer size – to a maximum mean plasma treatment time of 11 min (standard deviation: 12.8 min). If present, fibrin layers were removed using a curette in both groups. All ulcers were cleaned with physiological salt solution, disinfecting measures other than plasma were not allowed. Only two defined wound dressings for standardization purposes were used – Mepitel® (Mölnlycke Health Care, Erkrath-Unterfeldhaus, Germany), a silicon-coated low-adherent wound contact layer for non-exudative ulcers and Mepilex® (Mölnlycke Health Care), an absorbent, silicon-coated polyurethane foam for exudative ulcers (Mölnlycke Health Care, Erkrath-Unterfeldhaus, Germany). Both wound dressings were examined in earlier clinical trials. Finally, a standardized compression therapy was performed with Ulcer X® (Sigvaris, Memmingen, Germany), a class-two compression stocking.

Assessment of safety
The primary endpoint was safety as assessed by the number of adverse (AE) and serious adverse events (SAE) of 45 s/cm² plasma application as add-on therapy in the treatment of chronic venous leg ulcers.

Assessment of bacterial load and bacterial species
At every visit, the bacterial species (swabs taken from the wound) in both groups were determined immediately after dressing removal. The bacterial load was assessed by using contact agar plates that were gently pressed onto the target ulcer before and after plasma application and then incubated at 38°C overnight. Based on photographs of the contact agar plates in the plasma group, the bacteria-free area before and after plasma treatment at baseline were compared in a blinded fashion from a microbiologist not directly involved in the clinical trial procedures (GD in Greifswald).

Assessment of pain and applicability
Pain during and between treatment visits was documented at every visit using a visual analogue scale (VAS) ranging from 0 to 100. Patients (three-item questionnaire) as well as doctors (four-item questionnaire), with five scales each, rated the applicability of plasma at the end of the treatment and follow-up period.

Statistical analysis
All data were analyzed descriptively, stratified to visits and treatment groups. Statistical analysis was performed by the Institut für anwendungsorientierte Forschung und klinische Studien IFS GmbH.

Results
Patient overview
In total, 14 patients were treated and analyzed. There were three females and four males in each study arm. The mean age was 67 years (age range: 51–83 years (standard), 51–85 years (plasma)). The mean duration of ulcers was 34 months ± 38.08 (standard) and 43 months ± 19.48 (plasma). Most ulcers were located on the medial malleolus (standard: n = 3, plasma: n = 4). Nine patients had a recurrent ulcer (standard: n = 3, plasma: n = 6). Except for one patient in the standard arm, all patients showed further ulcers. All co-morbidities were documented. Six of the seven patients in each arm suffered from co-morbidity with hypertension prevailing. Three patients suffered from a post-
thrombotic syndrome (standard: \( n = 1 \), plasma: \( n = 2 \)). There were no differences in weight, height and ankle-brachial index or laboratory findings (complete blood count, HbA1c and serum albumin level) between the two study groups. Three patients terminated the study prematurely: One patient in the standard and one patient in the plasma group dropped out due to ulcer progression and one patient in the plasma group terminated the study in week 7, because his ulcer had healed completely (Table S1).

**Safety of plasma treatment**

In each study arm, one SAE occurred \( (P = 0.77, \text{Fisher’s exact test}) \) (Table S2). The SAE patient in the plasma arm was briefly hospitalized because of backache due to a vertebra shift conditioned by pre-existing osteoporosis. This SAE was labelled unrelated to plasma treatment. In addition, 77 AEs were approximately equally distributed among both arms (standard: 42 AEs, plasma: 35 AEs) (Table S3). All seven patients in the standard group and 6/7 patients in the plasma group exhibited at least one AE \( (P = 1.0, \text{Fisher’s exact test}) \). In two AE cases, a potential relation to plasma treatment could not be excluded (hyperthermia and redness of the leg, pain during one single plasma application). Neither of these two AE led to treatment alteration or study termination. Frequently reported AEs in both arms were ulcer pain and problems with the skin adjacent to the wound (stasis dermatitis, maceration).

**Bacterial wound colonization**

To assess the bacterial load in ulcers, bacterial growth on ulcer contact agar plates taken before and directly after plasma treatment at the baseline visit was analyzed by measuring the bacteria-free area \( \text{(in mm}^2\text{)} \) on the plates (Fig. 2). Six of the seven plasma patients could be analyzed. There was a significant reduction in bacterial load in ulcers immediately after plasma application as demonstrated by the enlargement of the bacteria-free areas on the plates \( (P = 0.0313; \text{Wilcoxon sign-rank-test}) \). The increase in the bacteria-free area was \( 88.2 \pm 51.09 \text{ mm}^2 \) at the baseline visit. In addition, by taking swabs the bacterial strains and antibiotic resistances were assessed continuously at every visit. Two to four different bacterial strains on average could be identified per ulcer at the beginning. The most common strains identified were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corynebacterium* sp. and *Streptococcus* Gr. C. At the end of the study, the types of bacterial strains did not change nor differ between both treatment arms. In particular, no reduction in bacterial strain numbers in ulcers after 8 weeks of plasma treatment was apparent (data not shown) indicating re-colonization from the wound edges.

**Ulcer size**

At baseline, the median ulcer size was \( 6.9 \text{ cm}^2 \) in the standard and \( 10.9 \text{ cm}^2 \) in the plasma arm (Fig. 3). A more than 50% reduction in ulcer size was noted in 5/7 and 4/7 patients in the standard and plasma group, respectively. However, an ulcer size increase was also noted in 2/7 and 3/7 patients in the standard and plasma group, respectively. There was one drop out due to increase in ulcer size by >20% in both groups. Overall, there was no significant difference regarding ulcer size reduction \( (P = 0.42, \text{log-rank test}) \) between both study arms. Interestingly, in absolute values, a more pronounced ulcer size reduction was noted in the plasma group compared with the standard group until the end of the treatment period at visit 21 (standard: \(-3.4 \text{ cm}^2\) vs. plasma: \(-5.3 \text{ cm}^2\); Hodges–Lehmann estimator: 0.7%; 80% CI: \(-4.0–3.9\)). Also, the only patient whose ulcer closed completely after 7 weeks received plasma (Fig. 3).

In addition, the trial protocol included a 4-week follow-up period to monitor ulcer stability or recurrence. Three of six
patients in the standard arm and two of five patients in
the plasma arm exhibited some increase in ulcer size during
follow-up (data not shown). There was no difference between
the two groups in this regard (P = 1.0, Fisher’s exact test).

Assessment of pain and plasma applicability
Patients in the plasma group quoted less pain during as well as
between the treatments (Fig. 4). The pain processes over time
do not differ from each other [P = 0.5046 during treatments;
P = 0.4753 between treatments, non-parametric analysis of
variance (non-parametric ANOVA)].

The plasma applicability was not rated inferior to standard
wound care by the patients (P = 0.94) as well as the caring doc-
tors (P = 1.0, Wilcoxon–Mann–Whitney test). However, plasma
therapy was rated more time consuming at the end of the treat-
ment period in week 8 (P = 0.22, Wilcoxon–Mann–Whitney
test). When patients quoted their satisfaction with the healing
process some advantages for the additional plasma application
were present (P = 0.23, Wilcoxon–Mann–Whitney test). There
was a clear trend that the caring physicians would again recom-
mand (P = 0.06, Wilcoxon–Mann–Whitney test) as well as
repeat (P = 0.08, Wilcoxon–Mann–Whitney test) the plasma
treatment at the end of the study.

Discussion
This is the first clinical pilot study in patients with the DBD
plasma generator PlasmaDerm® VU-2010 applied as add-on
therapy to modern standard wound care.

Overall, the application of plasma was well-tolerated. Only
two patients stated some pain and a feeling of hyperthermia at
one of the 23 study visits in the plasma group. No SAE related
to plasma occurred. These observations are in line with earlier
in vitro plasma safety assessments.22 Recently, safe and effective
5-min as well as 2-min plasma treatments with other indirectly
acting plasma devices called MicroPlaSter® (Max-Planck Insti-
tute for Extraterrestrial Physics, Garching, Germany; Adtec
Plasma Technology Co. Ltd., Hiroshima, Japan) could be dem-
onstrated in patients with chronic infected wounds.23,24 The
authors also demonstrated a significant reduction in bacterial
loads in plasma-treated wounds. In general, DBD plasma is
able to achieve a higher plasma density above the wound com-
pared with indirect acting plasmas and bacterial inactivation.
appears much faster as charged particles contact bacteria directly. The benefit of the MicroPlaSter® plasma treatment is that a larger area of up to 6 cm² can be treated in a single application, whereas our hand-held DBD plasma generator could only treat a small area of 1 cm² at the same time and had to be moved across the ulcer to achieve a 45 s/cm² treatment of the complete surface. This renders the PlasmaDerm® VU-2010 technique more time consuming, because larger electrodes are not available at this time. Nevertheless, our data confirmed that the applicability was not rated inferior to conservative standard wound care alone.

The distance between the ulcer and device of <1 mm is ensured by a sterile spacer. Due to the fact that the device is hand-held, the spacer may accidentally come into contact with the wound. In this case a carry-over of bacteria cannot be excluded, regardless of whether the plasma treatment is performed spirally starting at the ulcer border in circling movements towards the middle of the ulcer (analogous to bacterial swabs ‘Essener Kreisel/Essen Rotary’) or in collateral rows. Bacterial re-colonization from the wound borders between study visits (the wound edges and adjacent skin were not plasma treated) is suggested by our findings that markedly reduced bacterial colonization of the ulcer was seen only directly after plasma treatment (plates taken at baseline) but not over the entire study period (swabs taken at each visit). Further technical developments, such as larger and flexible DBD plasma electrodes that adapt to the skin and ulcer surfaces, may improve the treatment of larger ulcers and adjacent skin.

Our pilot study is characterized by a very homogeneous patient population. We decided to include only patients with chronic ulcers due to chronic venous insufficiency to reduce confounding factors as much as possible. Nevertheless, our results may be applicable to chronic wounds of other causes, such as arterial or diabetic foot ulcers, as there is strong evidence that these ulcers share some biological processes with venous ulcers. In line with this observation, it was recently showed that plasma treatment seems to support the healing process of laser-induced skin lesions and resulted in superior aesthetic scar formation.

Although the ulcer sizes could be reduced by approximately 50% in both study arms we found evidence of a greater absolute size reduction with additional plasma application, especially after first three study weeks. Furthermore, the only patient whose ulcer closed after 7 weeks received plasma. In addition, only 3/7 patients in the standard group but 6/7 patients in the plasma group had a recurrent ulcer as target ulcer at baseline, which are generally more difficult to treat than newly occurring ulcers.

Other research groups could demonstrate that plasma promotes the proliferation of endothelial cells via stimulation of angiogenic growth factors and acidifies wounds, both of which have favourable effects on wound healing. Arndt et al. reported that plasma can stimulate wound healing by several factors including gene expression of relevant cytokines and growth factors, keratinocyte migration, fibroblast activation, promoting matrix deposition and activation of macrophages. In line with
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this Isbary et al. showed that especially patients with chronic venous ulcers could profit from additional plasma applications. Moreover, the modes of action of CAP as known today may not only confer efficacy in wound treatment but also in the treatment of inflammatory or itching skin diseases and epidermal barrier defects.

Despite apparent limitations of our study due to the low number of patients, we demonstrated that plasma treatment with the PlasmaDerm® VU-2010 device can be considered safe and effective to reduce the bacterial load in patients with chronic venous leg ulcers. Thus, larger controlled trials and the development of devices with larger application surfaces are warranted.

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Author contribution
FB, SE and HAH performed the study; GD analyzed data; RA, SP, AG, DW and DS assisted in medical writing, regulatory affairs, data analyses and performed study monitoring; MPS, FB and SE designed the study, had access to all data and wrote the manuscript.

References

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Supporting information
Additional Supporting Information may be found in the online version of this article:

Table S1. PlasmaDerm pilot study CONSORT study flow chart.
Table S2. Distribution of severe adverse events (SAEs) in the study arms.
Table S3. Distribution of adverse events (AEs) in the study arms.