

# Cold plasma treatment is safe for diabetic foot ulcers and decreases *Staphylococcus aureus* bacterial load

**Aim:** Cold atmospheric plasma (CAP) has antimicrobial properties. We studied the safety of a novel CAP device (PLASOMA prototype; Plasmacure, The Netherlands) that is simple to use and could be applied at a patient's home for the treatment of diabetic foot ulcers (DFUs). Secondary objectives were to investigate the effect of CAP on bacterial load and on ulcer size.

**Method:** We included subjects with non-infected, superficial DFUs and treated them with CAP on a daily basis for 10 days. The primary endpoint was the occurrence of serious adverse device effects (SADE). We defined safety as:  $\leq 10\%$  of patients experiencing a SADE other than infection (non-infectious SADE), and  $\leq 60\%$  of patients developing infection of the foot (infectious serious adverse event (SAE)).

**Results:** We enrolled 20 patients. No SADE occurred, but three infectious SAEs occurred at the site of application within one month

of treatment; three SAEs unrelated to treatment occurred, and 55% of subjects reported transient mild adverse device effects.

*Staphylococcus aureus* bacterial load decreased directly after CAP application ( $p=0.01$ ). The mean decrease of ulcer surface area was 43% (95% confidence interval: 20.2%–65.9%).

**Conclusion:** CAP treatment in DFUs was safe and well tolerated. Ulcer size and *Staphylococcus aureus* colonisation decreased during treatment.

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adverse event • cold plasma treatment • DFU • diabetic foot ulcer • infection • ulcer healing • wound • wound care • wound healing

Foot complications occur in approximately 20% of patients with diabetes. Lower extremity complications account for 25% of the money spent on patients with diabetes, of which the most common is a diabetic foot ulcer (DFU).<sup>1,2</sup> Ulcers are caused by a combination of polyneuropathy, peripheral artery disease and biomechanical changes.

Treatment of DFUs focuses on: optimising arterial vascularisation, biomechanical offloading, glucose management, oedema reduction, treatment and prevention of infection, and local ulcer management. Infection complicates 50% of DFUs, which requires medical and/or surgical treatment.<sup>3</sup> Foot infection and its treatment are associated with amputation, rehospitalisation, acute kidney injury and *Clostridioides difficile*-associated diarrhoea.<sup>4,5</sup> If side effects of antimicrobial treatment occur, these will decrease the quality of life. Furthermore, the use of prolonged courses of antibiotics is associated with an increase in antimicrobial resistance, both in individuals and in the population. Individuals and society would benefit if infections could be treated and prevented by methods other than antibiotics. Plasma medicine is one such alternative.

In the physical sciences, plasma refers to the fourth state of matter, an ionised gas that contains ions and reactive species and produces UV light and electric fields, the plasma 'cocktail'. Over 99% of the visible

matter in the universe (i.e. stars) consists of plasma. Plasma can also be created by man and has many fields of application, e.g., to sterilise medical equipment and for packaging in the food industry.

Plasma medicine is the field of medicine that applies plasma in the treatment of living tissue.<sup>6,7</sup> The so-called cold atmospheric pressure plasmas (CAPs) are used for these applications because they operate at ambient air pressure and at relatively low temperatures, thus avoiding thermal damage to the body. Previous preclinical research has shown the ability of CAPs to inactivate a multitude of microorganisms.<sup>8,9</sup>

At the same time, CAP treatment stimulates mammalian cells to proliferate and to migrate.<sup>10–12</sup> These effects are caused by electric fields and by formation of reactive oxygen and nitrogen species. Several animal studies and clinical trials have demonstrated the potential of CAPs to heal complex

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wounds.<sup>12,13</sup> CAP offers an excellent combination of antimicrobial treatment and direct stimulation of the healing of DFUs.

The plasma devices used in previous clinical trials are generally large, rigid and expensive, and treatment needs to be performed in hospital settings. The device we studied is a small plasma device that is simple to use and, in the future, can be used in a patient's home. The main difference with other similar devices is that this device uses a flexible pad that follows the contours of the skin and that can apply plasma directly to the ulcer.

This study aimed to assess whether CAP with this device is a treatment modality that can safely be applied in patients with a DFU.

## Method

### Participants

Participants were patients with a DFU, present for >2 weeks, attending the outpatient diabetic foot clinic of Amsterdam University Medical Centres, location VUmc. Included were patients with type 1 or 2 diabetes who had a DFU with a maximum depth of 5mm, with or without peripheral vascular disease, but without evidence of bone or joint tissue in the ulcer base and without clinical signs of infection (University of Texas classification 1A/1C/3A/3C).<sup>5</sup> Patients had to be able and willing to comply with the research protocol.

Excluded were patients with implanted electrical medical devices (e.g., pacemakers), life-threatening cardiac conductivity abnormality, active malignancy, and foot infection requiring antibiotic treatment at enrolment. Also excluded were women who were pregnant or lactating, and women of childbearing age not using contraceptive measures.

### Objective

The primary objective was to assess the safety of CAP treatment of DFUs. The primary endpoint of this study was the occurrence of serious adverse device effects (SADEs), i.e., serious adverse events (SAEs) related to treatment by or use of the device.

Secondary objectives were to investigate the effect of CAP treatment of DFUs on bacterial load, on ulcer healing, on the occurrence of clinically defined infection (according to the International Working Group on the Diabetic Foot (IWGDF)/Infectious Diseases Society of America (IDSA) diabetic foot infection classification<sup>14,15</sup>), clinical outcome (surgical intervention, including amputation, death, ulcer healing, treatment with antibiotics) 3 months $\pm$ 2 weeks after enrolment.

### Outcome measurements

Healing of an ulcer was defined as full re-epithelialisation. Infection was defined clinically according to the IWGDF classification.<sup>14,15</sup> SAEs were defined as events grade 3–5 according to the Common Terminology Criteria for Adverse Events (CTCAE).<sup>16</sup> SADEs were defined as events grade 3–5 if the event occurred while the patient

was participating in the study or within 30 days after the last study intervention, and if the researcher judged the event to be related to the treatment with the device.<sup>16</sup> The treatment was defined as safe if  $\leq$ 10% of patients experienced a SADE other than a foot infection. In persons with DFUs, infection can complicate 50% of foot ulcers.<sup>3</sup> We chose to consider treatment safe if  $\leq$ 60% of patients developed an infection of their ulcer, to avoid misinterpretation due to the relatively small treatment group.

We used a semi-quantitative outcome for the number of colonies on the plate after incubation. The score ranged from 0 (no growth) to 4 (growth in all quadrants). The effect of CAP treatment on bacterial load was considered clinically significant if bacterial load was reduced by 50% at treatment day 10 compared with day 1 as measured by tissue swab, and by 50% directly after cold plasma application on treatment days 1, 5 and 10, compared with immediately before application.

### Sample size calculation

The primary outcome measure was the occurrence of SADEs within 30 days after the last application. We did not expect any in the general population and a maximum of 10% in our study population. For the sample size calculation, we used an incidence of 0.1% in the general population (because the algorithm cannot accept a value of zero), 10% incidence in the study population, an alpha of 0.05 and a power of 80. This resulted in a sample size of 10. For infection, we assumed an incidence of 50% in the general population, a 60% incidence in the study population, an alpha of 0.05 and a power of 80. This resulted in a sample size of 19. With an anticipated dropout of one patient, we considered a sample size of 20 patients adequate.

### Treatment

The medical ethical review board of the Amsterdam University Medical Centres, location VUmc, approved the study and all procedures were performed in compliance with relevant laws and institutional guidelines (approval number: 2015.169). All participants provided written informed consent. We collected demographical and medical data after obtaining informed consent. These data included the prevalence of neuropathy, vascular status, ulcer characteristics, biomechanical plantar pressure and laboratory values.

All patients were treated with CAP at the outpatient diabetic foot clinic of Amsterdam University Medical Centres, location VUmc, on weekdays for 1 minute per application for 2 weeks (a total of 10 treatments), or until their ulcer healed. CAP was applied using the PLASOMA prototype (Plasmacure, The Netherlands).

Weekly sharp debridement and routine wound care protocols, biomechanical offloading of the foot, application of bandages and dressings, and proper metabolic glucose control (aiming at an HbA1c of 53mmol/mol (7.0%)) were conducted. Bacterial load was measured with swab samples obtained after

debridement with the Levine technique<sup>17,18</sup> before and after plasma application on treatment days 1, 5 and 10.

Samples were taken to the laboratory promptly, where routine culture was performed on agar plates. We streaked a plate three times on each quadrant with a new sterilised loop for each quadrant. This classical microbiological streaking technique creates progressive dilutions of the original swab in each quadrant. All plates were incubated under aerobic conditions at 37°C. After 24 hours, the plates were inspected visually and colonies of bacteria counted in each of the four quadrants.

Before and directly after every treatment session, we checked the foot for side effects and signs of inflammation, infection and ulcer healing. After treatment, patients were asked if they experienced any sensations or other possible adverse events, including possible effects of earlier applications. We obtained data on hospitalisation, amputation and antibiotic treatment.

If participants experienced an SAE, we stopped CAP applications until we could rule out a SADE. Patients received necessary medical care, and routine wound care was continued.

#### Data analysis

We used the statistical package SPSS (version 23; IBM Corp, US) to analyse the data. We counted adverse events and SAEs in absolute and relative numbers. For bacterial load before and after treatment, we calculated the statistical effects of treatment with a Wilcoxon matched pairs test. Ulcer healing and occurrence of infection were recorded as absolute and relative numbers.

#### Results

Between April 2016 and March 2018, we enrolled 20 patients. Two patients did not complete the two-week treatment period; one patient was admitted because of an infection at the ulcer site and one ulcer healed on the fifth day of treatment. Table 1 shows demographic data.

#### Device deficiencies

Technical issues with the device occurred in 31 treatment sessions (16%). In most cases, the technical issue could be solved immediately, thereby ensuring a successful treatment session. Furthermore, no device deficiency led or could have led to a (S)ADE. Device errors were generated when the safety circuit of the device intervened and thus demonstrated the correct functioning of the safety circuit.

#### Adverse events

Table 2 shows data on the occurrence of (S)AEs. Most adverse events were mild (grade 1) and transient. No SADEs occurred. Three patients (15%) experienced an infectious SAE at the site of application within one month of treatment. In those cases, the infection occurred at the site of the plasma application, but it was

**Table 1. Demographic data**

Total number of patients	20
Female/male, n	3/17
Age, mean±SD	61.2±7.3 years
Type 1/type 2 diabetes	5/15
Duration of diabetes, mean±SD (median)	20.4±12.1 years (8 years)
Duration of ulcer, mean±SD (median)	1090±1910 days (140 days)
UT class 1A, n	19
UT class 1C, n	1
PAD, n	8

PAD—history of peripheral artery disease, defined as at least one missing pedal pulsation or if presence of PAD was stated in the subject's medical history; SD—standard deviation; UT class—University of Texas diabetic foot ulcer classification<sup>19</sup>.

**Table 2. Adverse events**

Total number of subjects	20
Subjects experiencing ADE (all grade 1), n (%)	11 (55%)
● Tingling sensation	21*
● Warmth sensation	8*
● Other mild sensation	16*
● Slight pain during/after treatment	5*
SADE	0
Infectious SAE at the foot, n (%)	3 (15%)
SAE at other location, n (%)	3 (15%)

(S)ADE—(serious) adverse device effect; SAE—serious adverse event  
\*Out of 193 treatment sessions

**Table 3. Ulcer surface reduction**

Surface	Size/change
Ulcer surface before plasma application, mean±SD (median)	110.8±149.8mm <sup>2</sup> (41.0mm <sup>2</sup> )
Ulcer surface at treatment day 10, mean±SD (median)	91.9±169.6mm <sup>2</sup> (12.5mm <sup>2</sup> )
Mean ulcer surface decrease in two weeks*, %±SD (median)	43±49% (55%)

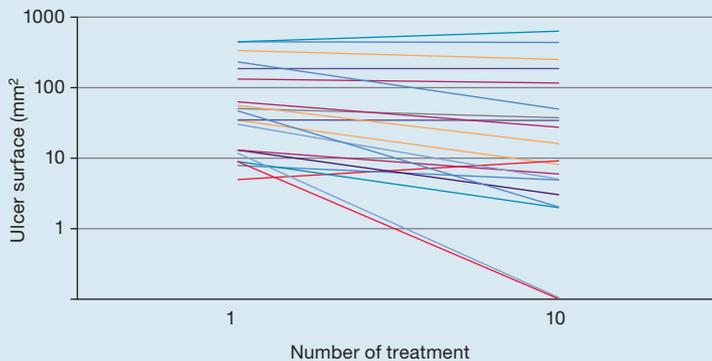
SD—standard deviation; Ulcer surface calculated as ellipse surface ( $\pi \times 0.5 \times \text{length} \times 0.5 \times \text{width}$ ); \*calculated from the individual ulcer surface reductions

unlikely to have been caused directly by the device or the study procedure. SAEs unrelated to the device included pneumonia in one patient; another patient underwent a toe amputation on the contralateral foot, and one patient developed a soft tissue infection of the ipsilateral proximal part of the lower leg.

#### Ulcer healing

Two ulcers (10%) healed and two ulcers increased in size during the 2-week treatment period. Ulcer surface area was reduced by >50% in 11 ulcers in 2 weeks. Median ulcer size reduced significantly within the treatment period ( $p=0.004$ ). The mean decrease of ulcer surface was 43% (95%CI: 20.2%–65.9%). Fig 1 and Table 3 show further data on ulcer surface reduction.

**Fig 1.** Ulcer surface reduction. Changes in ulcer surface per subject. Each line represents an individual subject



### Microbiological results

The median bacterial load (measured on a semi-quantitative scale) was 3 (representing three quadrants with growth using the microbiological streaking method) before the start of treatment and was 3 directly after a single application ( $p=0.18$ , interquartile range (IQR): 3). The mean bacterial load before the start of treatment compared with directly after the last treatment decreased from 2.66 to 2.59 ( $p=0.08$ ). Median *Staphylococcus aureus* bacterial load before application was 3 and decreased significantly to 2 directly after a CAP application ( $p=0.01$ ).

### Discussion

The aim of this study was to assess the safety of CAP treatment with a novel device in DFUs. We did not observe any SAEs. Occurrence of an ulcer infection was lower than expected. Observed AEs were low-graded and transient; CAP treatment with this novel device was well tolerated. No patients discontinued the study because of the burden of the intervention. Technical problems with the device were uncommon and never led to discontinuation of treatment. The results of this study indicate that CAP produced by the PLASOMA prototype is safe in the treatment of patients with DFUs.

Although it was not a placebo-controlled study, it is remarkable that 10% of these hard-to-heal and persistent ulcers healed, that all but two ulcers decreased in size, and that 55% of patients experienced a reduction of ulcer surface area of >50% during the 2-week treatment period.

These observations are in line with other reports, that suggest that plasma might accelerate ulcer healing by stimulating growth of mammalian cells.<sup>12</sup> These changes could be directly due to cell stimulation, or indirectly through change in number, type or diversity of ulcer surface bacteria. These results can be used to plan further studies into efficacy and efficiency of CAP treatment in DFUs and other wound types.

The antimicrobial features of plasma treatment are

important. In a world of increasing prevalence of antimicrobial resistance, CAP could offer an alternative to other treatment options for ulcer pathogens, including *Staphylococcus aureus*, such as systemic or topical antibiotic treatment or antiseptic dressings. In contrast to other studies with plasma, we did not observe a significant decrease in overall bacterial load.<sup>7,12,13</sup> The load of *Staphylococcus aureus*, however, did decrease after a single treatment session. That is important, as *Staphylococcus aureus* and beta haemolytic streptococci are the most important pathogens in diabetic foot infection.<sup>4</sup>

The factors that influence the antimicrobial effect of CAP in DFUs are unknown. Such factors could be related to the patient, the bacteria or the device. Patient-related factors include a high glucose concentration in the ulcer bed and macrovascular and microvascular induced ischaemia. Certain bacterial species could be inherently more sensitive to plasma application and to exposure to reactive oxygen species. Such increased sensitivity could be due to the structure of the peptidoglycan wall or the relative superficial habitat of an organism in the wound, leaving it more exposed to plasma and its byproducts.

We used standardised methods to obtain wound samples and to perform microbiological analyses. Measuring the bacterial load in an uninfected wound is difficult as the quantities are usually small. The sensitivity of culture techniques might be too low to detect relatively small changes in bacterial load. Furthermore, since the wounds were uninfected at the start of the study, no large reduction in bacterial load during the treatment period was expected. Specific device settings (e.g., change in frequency or amplitude of the voltage that ignites the plasma) and shorter or longer duration of treatment could theoretically influence the antibacterial effect on different species. Longer duration of application is more lethal to bacteria in general, but could lead to tissue damage. Settings were chosen to optimise antibacterial effect while avoiding harm to the patients' tissue. Further research is needed to identify the factors that influence bactericidal activity of CAP.

### Limitations

The present study has certain limitations. One of these is that there was no placebo control group. The effect of daily inspection at the outpatient clinic, with weekly wound care, could increase the placebo effect. Another limitation is that possible individual trends towards ulcer healing before inclusion were unknown. Although the median duration of the ulcers at inclusion was long (140 days), it is theoretically possible that we included ulcers that already showed a healing trend before patients were enrolled in the study.

We chose not to include patients with clinical signs of inflammation, necessitating the initiation of systemic antimicrobial treatment, or patients who

were already being treated with systemic antimicrobials. Such patient groups, however, could potentially benefit from the combination of topical bactericidal effect of CAP with systemic antibiotics. Including patients treated with antibiotics would have made it impossible to assess any additional effect of CAP on the occurrence of infection or SA(D)E, or on changes in bacterial load; these changes could in theory all be attributable to the antimicrobial treatment, and not to CAP application. For future studies into the efficacy and effectiveness of CAP, we suggest to include a placebo arm and to also include patients with infection treated with systemic antimicrobials in both arms to eliminate these forms of bias.

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## Reflective questions

- Explain whether cold atmospheric plasma (CAP) would be safe for treating patients with infected ulcers?
- Explain what effect CAP might have on ulcer healing?
- Explain what effect CAP might have on bacterial load?

## Conclusion

CAP produced by PLASOMA is safe in non-infected DFUs. Its application is well tolerated and semi-quantitative culture measures reveal a possible bactericidal effect, specifically on *Staphylococcus aureus* bacterial load. Therefore, it might be a valuable addition to the armamentarium for the treatment of DFUs. **JWC**

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