Int. J. Surg. Wound Care
Vol. 3, 33-36, 2022
ISSN 2435-2128

Original Article

Using Plasma to Treat Chronic Foot Ulcer Infections

Yuta Terabe, MD, PhD, Nobuhito Kaneko, MD, Keisuke Nakabayashi, MD, PhD, and Hiroshi Ando, MD, PhD

Limb Salvage Center, Kasukabe Chuo General Hospital, Japan

ABSTRACT

Introduction: Cold atmospheric plasma (CAP) is an effective disinfection and wound-care tool. We conducted the first clinical trial for plasma care® in Japan to evaluate bacterial load reduction.
Methods: Seven patients with chronic foot ulcers were treated with plasma care® for one week. Plasma care® was used once daily during dressing changes. Bacterial load was measured before and after treatment.
Results: Treatment with plasma care® reduced bacterial load in five patients, while two patients showed no changes. The treatment was safe with no adverse events. Although CAP exerted antibacterial effects, we observed no clear reduction in wound size during the observation period.
Conclusion: Plasma care® is safe and effective for bioburden reduction in patients with chronic foot ulcers.

Key words : bacterial reduction, bioburden, chronic foot ulcer, cold atmospheric plasma

Introduction

Nonthermal physical plasma has long been used for surface modification, as well as for cleaning and disinfecting objects such as microelectronic components and implants. In recent years, cold nonthermal plasma (temperature below 313.5 K/40°C) has been developed for therapeutic purposes. Several potential medical applications for cold atmospheric plasma (CAP) have been successfully examined.

CAP exhibits antibacterial, antiviral, and antitumor properties. The antibacterial effect, in particular, has been reported worldwide, and numerous studies on plasma therapy in diabetic foot ulcers (DFU) have been reported[1].

CAP

Plasma, one of the four states of matter, is a (partially) ionized gas that can conduct electricity, as it consists of ions, free electrons, free radicals, excited molecules, photons, and other components. The addition of energy transforms the gas into plasma; thus, it is a high-energy aggregate state. Plasma is defined as cold when the temperature increases only marginally during formation and when normal pressure conditions are sufficient for its generation.

On applying energy, ambient air can be transformed into a CAP, comprising reactive oxygen and nitrogen species (RONS), among other components. Thus, it can be utilized in several medical settings.

The effects of CAP are mediated by several factors, including RONS and reactive nitrogen species (RNS), free radicals, ions, electric fields, electrons, and ultraviolet photons[2, 3]. These CAP-mediated factors do not require high-energy dosage and afford safe clinical treatment. Moreover, these factors mediate the antimicrobial applications of CAP-induced bacteriocidic effects, which can be attributed to membrane oxidation and cell wall and DNA disintegration[4].

Infection control

The incidence of chronic foot ulcers, such as DFU and chronic limb-threatening ischemia (CLTI), is increasing globally[5, 6]. Treatment can be challenging for most clinicians, owing to several factors that impede the healing process. Infection is one of the most challenging problems in wound treatment. Multidrug-resistant bacteria and the high cost of antibiotic therapy remain considerable obstacles. Plasma treatment could resolve these issues[7]; thus, we included cold plasma therapy to treat chronic foot ulcers. Herein, the objective was to improve critical colonization.

Corresponding author: Yuta Terabe, MD, PhD
Limb Salvage Center, Kasukabe Chuo General Hospital, 5-9-4 Midori, Kasukabe, Saitama 344-0063, Japan
Received: 2, November 2021 / Accepted: 3, March 2022
E-mail: k.sk.tera@gmail.com

Table 1. Results of the plasma care® treatment for one week

Case	Wound area	Pre-treatment	Post-treatment
1	Medial heel	*Escherichia coli* (ESBL) 1+	no
2	post-Chopart amputation	*Corynebacterium* spp 1+	no
3	Post-Lisfranc amputation	MRSA 1+ *Corynebacterium* spp 1+ *Escherichia coli* 1+ *Pseudomonas aeruginosa* 1+	MRSA 1+ *Corynebacterium* spp 1+ *Escherichia coli* 1+ *Pseudomonas aeruginosa* 1+
4	II-IV toe amputation	CNS1+ *Streptococcus constellatus* 1+	no
5	Plantar	*Pseudomonas aeruginosa* 1+ *Citrobacter freundii* 1+ *Proteus mirabilis* (ESBL) 1+	no
6	Post-Transmetatarsal amputation	*Stenotrophomonas maltophilia* 1+	*Stenotrophomonas maltophilia* 1+
7	Ankle	*Proteus mirabilis* 1+ *Pseudomonas aeruginosa* 2+ *Corynebacterium* spp 1+ Group G *Streptococcus* 2+	no

*no: There is no bacteria l growth in culture plate.
**CNS: Coagulase negative *Staphylococcus*, ESBL: Extended-spectrum beta-lactamases, MRSA: Methicillin-resistant *Staphylococcus aureus*

Methods

Study design

Herein, we present the first report examining the application of plasma care® in Japan. We conducted a single-armed, open-label case study. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Research Ethics Committee of our hospital (Permission No.2007-4). The study period was one week per patient. All participants provided written informed consent before study initiation.

Patients

We enrolled seven patients who had CLTI-induced foot ulcers. The minimum size was 4 cm^2. The patients underwent debridement after endovascular treatment. Granulation tissue was collected after debridement, and a wound specimen was obtained after plasma treatment to assess the bioburden. Patients received standard care along with daily plasma care® for one week.

Assessment of the bioburden

Granulation tissues were cultivated in the laboratory. A bacterial identification test was then performed. The results were presented as n, +1, +2, and +3 (n, +1, +2, and +3 indicate that there was no, less than one-third, from one-third to two-thirds, more than two-thirds bacterial development in culture medium, respectively). Bacterial identification was confirmed before and after plasma treatment. Table 1 lists all identified bacteria. The bioburden of wounds before and after plasma treatment was compared.

Standard care

Standard care was based on the concept of wound hygiene. The concept includes cleansing, debridement, refashioning, and dressing every day. Gauze and ointment (petrolatum) were used for dressing wounds. The patients did not receive local or systematic antibiotic therapy during cold plasma therapy.

Plasma treatment device (Fig. 1)

Plasma care® is a cold plasma medical device used for treatment and is approximately the size and weight of an old telephone handset. Plasma care® is used in combination with an individually packaged, sterile attachment (plasma care®spacer) to prevent cross-contamination.

CAP was formed from the air within the spacer. Plasma care® is equipped with surface microdischarge technology, ensuring no electrical current runs through the patient. The skin and wound surface only come in contact with therapeutically active, durable plasma components, ascertaining that healthy tissue remains undamaged.

The treatment area of the plasma care® is 13 cm^2. The plasma care® spacer can be used several times (up to six applications per spacer) on an individual patient within one treatment session. The treatment time was 60 s per wound area.

Results

Patient overview (Table 2)

In total, 7 patients were included in the present study, including 3 males and 4 females. The mean patient age was 75 years (standard deviation, 4.69). The average ulcer area was 48.4 cm^2 (4.84−113.46 cm^2). All comorbidities were

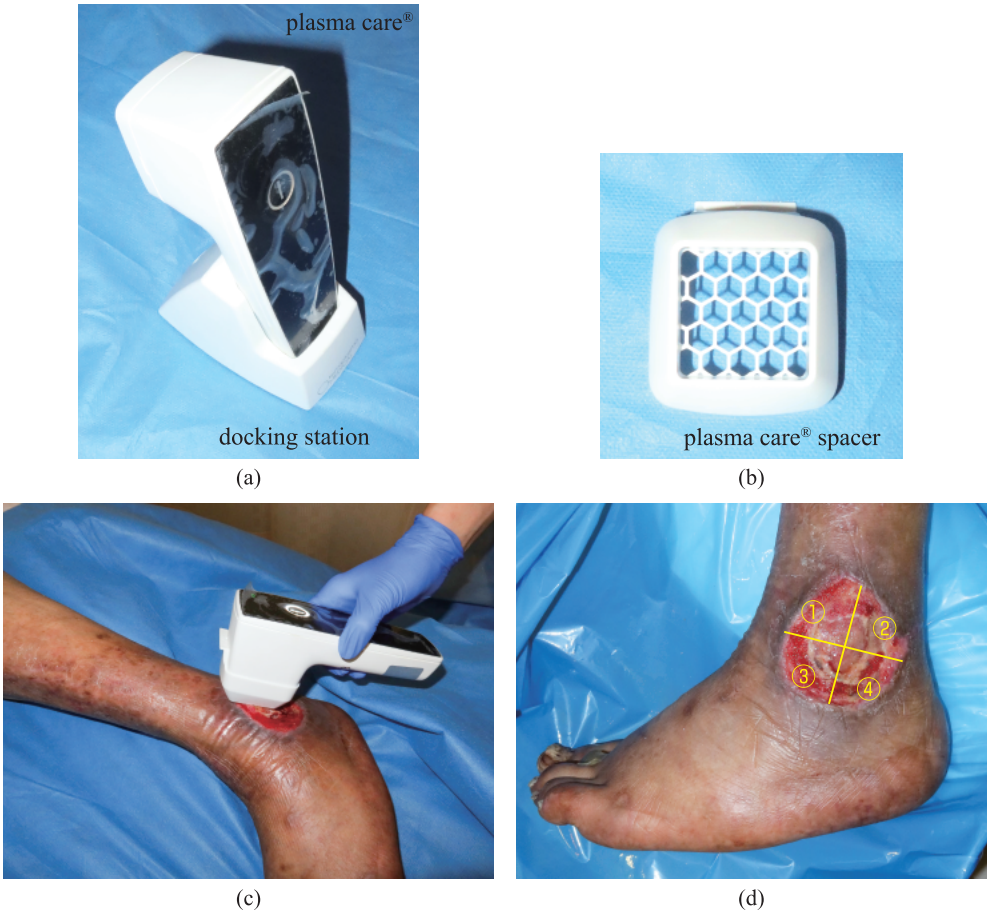

Fig. 1. How to use plasma care®.
(a) The indications are given in the table. The charging process of the plasma care® is inductive at a docking station.
(b) Plasma care® is used in combination with plasma care® spacer.
(c) The spacer is gently placed onto the wound without causing additional pain due to pressure.
(d) The treatment time is only 60 seconds per wound are. Above the figure is 4 area. Total 240 seconds (60 × 4).

Table 2. Patients characteristic, laboratory data, and comorbidities

case (number)	1	2	3	4	5	6	7	average
age (years)	73	76	67	75	80	79	80	72.75 (4.69)
sex (men: women)	M	F	M	M	F	F	F	3 : 4
white blood cell (10^2/μL)	100	57	46	99	80	85	48	73.57 (23.1)
hemoglobin (g/dL)	12.3	9.6	9.5	10.1	9.3	10.4	10.7	10.27 (1.03)
albumin (g/dL)	3.5	3.2	3.5	3.3	2.4	3.3	2.8	3.14 (0.4)
c-reaction protein (mg/dL)	1.13	1.53	0.27	1.4	4.59	1.94	0.34	1.6 (1.45)
ABI	1.13	0.8	1.18	1.57	0.83	0.99	1.35	1.12 (0.28)
DM (n)	+	+	+	+	+	+	−	6
CKD (G5d) (n)	−	−	−	+	+	+	−	3
CVD (n)	+	+	−	−	−	−	−	2
CAD (n)	−	+	−	+	+	+	+	5

*ABI: ankle-brachial index, DM: diabetic mellitus, CKD: chronic kidney disease, CVD: cerebrovascular disease, CAD: coronary artery disease
**average (standard deviation)

documented. Laboratory data (white blood cell count and hemoglobin, albumin, and C-reactive protein levels) and ankle-brachial index were determined.

Wound pathogen cultivation

Table 1 presents bacteria identified before plasma treatment. No bacteria were detected after plasma treatment in patients 1, 2, 4, 5, and 7.

Safety of plasma treatment

Plasma treatment was performed daily and induced no adverse events.

Discussion

In the present study, all patients had critically colonized (non-infected) wounds. The plasma care® device was used effectively against wound pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and Methicillin-resistant *Staphylococcus aureus* (MRSA), confirming preclinical findings. No significant difference was observed in bacterial sensitivity to CAP, and no resistance was noted. Wounds were colonized with multidrug-resistant bacteria, such as MRSA and extended-spectrum β-lactamase bacteria. Plasma treatment has shown some efficacy against these bacteria[8, 9].

Previous case reports have demonstrated that plasma care® could potentially improve the treatment of critically colonized ulcers. However, we observed no improvement in ulcer size during the brief one-week observation period. It has been reported that plasma treatment can positively impact the reduction in ulcer size[10]. Thus, increasing the study duration might reduce ulcer size. Previous studies have reported that CAP stimulates the secretion of growth factors[11]. Consequently, wound healing may improve with plasma therapy[12]. Long-term treatment effects need to be examined in future studies.

Conclusion

Plasma care® is easy to implement and can be instantly employed by most patients to initiate treatment. Given that the present study is limited by the small sample size, we plan to expand the application of plasma care® and perform in-depth assessments.

Acknowledgments

The authors would like to thank Enago (www.enago.jp) for the English review.

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors declare no conflicts of interest associated with this manuscript.

References

1) Mirpour S, Fathollah S, Mansouri P, et al: Cold atmospheric plasma as an effective method to treat diabetic foot ulcers: a randomized clinical trial. *Sci Rep* 2020; 10: 10440.
2) Leduc M, Guay D, Leask RL, Coulombe S: Cell permeabilization using a non-thermal plasma. *New J Phys* 2009; 11: 115021.
3) Yonson S, Coulombe S, Léveillé V, Leask RL: Cell treatment and surface functionalization using a miniature atmospheric pressure glow discharge plasma torch. *J Phys D: Appl Phys* 2006; 39: 3508-13.
4) Arndt S, Landthaler M, Zimmermann JL, et al: Effects of cold atmospheric plasma (CAP) on ß-defensins, inflammatory cytokines, and apoptosis-related molecules in keratinocytes *in vitro* and *in vivo. PLoS ONE* 2015; 10: e0120041.
5) Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y: Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. *Ann Med* 2017; 49: 106-16.
6) Conte MS, Bradbury AW, Kolh P, et al: Global vascular guidelines on the management of chronic limb-threatening ischemia. *Eur J Vasc Endovasc Surg* 2019; 58: S1-109.e33.
7) Daeschlein G, Napp M, Lutze S, et al: Skin and wound decontamination of multidrug-resistant bacteria by cold atmospheric plasma coagulation. *J Dtsch Dermatol Ges* 2015; 13: 143-50.
8) Maisch T, Shimizu T, Isbary G, et al: Contact-free inactivation of *Candida albicans* biofilms by cold atmospheric air plasma. *Appl Environ Microbiol* 2012; 78: 4242-7.
9) Maisch T, Shimizu T, Li YF, et al: Decolonisation of MRSA, *S. aureus* and *E. coli* by cold-atmospheric plasma using a porcine skin model *in vitro*. *PLoS One* 2012; 7: e34610.
10) Brehmer F, Haenssle HA, Daeschlein G, et al: 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). *J Eur Acad Dermatol Venereol* 2015; 29: 148-55.
11) Kalghatgi SU, Fridman G, Fridman A, Friedman G, Clyne AM: Non-thermal dielectric barrier discharge plasma treatment of endothelial cells. *Annu Int Conf IEEE Eng Med Biol Soc* 2008; 2008: 3578-81.
12) Helmke A, Hoffmeister D, Mertens N, Emmert S, Schuette J, Vioel W: The acidification of lipid film surfaces by non-thermal DBD at atmospheric pressure in air. *New J Phys* 2009; 11: 115025.