The Response and Tolerability of a Novel Cold Atmospheric Plasma Wound Dressing for the Healing of Split Skin Graft Donor Sites: A Controlled Pilot Study

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Keywords

Cold atmospheric plasma · Plasma therapy · Plasma medicine · Wound dressing · Acute wound healing · Split skin graft donor site · Hyperspectral imaging

Abstract

Introduction: Cold atmospheric plasma (CAP) has positive effects on wound healing and antimicrobial properties. However, an ongoing challenge is the development of specific modes of application for different clinical indications. **Objectives:** We investigated in a prospective pilot study the response and tolerability of a newly developed CAP wound dressing for the acute healing of split skin graft donor sites compared to conventional therapy. **Methods:** We applied both treatments to each patient ($n = 10$) for 7 days and measured 4 parameters of wound healing every other day (i.e., 1,440 measurements) using a hyperspectral imaging camera. Additionally, we evaluated the clinical appearance and pain levels reported by the patients. **Results:** The CAP wound dressing was superior to the control ($p < 0.001$) in the improvement of 3 wound parameters, that is, deep tissue oxy-

gen saturation, hemoglobin distribution, and tissue water distribution. CAP was well tolerated, and pain levels were lower in CAP-treated wound areas. **Conclusion:** CAP wound dressing is a promising new tool for acute wound healing.

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Introduction

In addition to solid, liquid, and gas, the plasma forms the fourth state of matter, which consists of a mixture of different biologically highly active agents. The plasma contains radical and nonradical reactive oxygen and nitrogen species, electrons and ions, visible light, UV radiation, and electromagnetic fields. The synergistic interaction between these agents is accompanied by immense heat generation [1]. In recent years, much effort has been devoted to developing plasma sources with temperatures below 40°C and comparable properties, that is, cold atmospheric plasma (CAP), which can be applied directly to biological tissues. Over the last decade, CAP has emerged as a new treatment option for different medical

indications. Interdisciplinary research groups demonstrated its great potential in several studies for the use in disinfection, wound therapy, reduction of pruritus, pain, and improvement of specific skin diseases [2–8]. Significant antibacterial, virucidal, fungicidal, and anti-inflammatory effects of the plasma have been reported and supported its use for the sterilization of medical equipment and transplants, and reduction of biofilms on implants. Additionally, CAP significantly reduced the germ load in wounds and dental root canals [4, 9–12]. Importantly, CAP positively influenced the healing process of chronic and acute wounds [9, 13–15]. In the future, the plasma could even reduce treatment costs by eliminating the need for lengthy conventional therapies [6, 10, 16–18].

In our study, we investigated whether a novel and innovative CAP wound dressing could positively affect healing of acute split skin graft donor sites compared to conventional therapy over a 1-week period and how it was tolerated. Every other day, during replacement of wound dressings, we measured deep tissue oxygen saturation (i.e., near-infrared [NIR] perfusion), superficial hemoglobin oxygen saturation (StO₂), hemoglobin distribution (i.e., tissue hemoglobin index [THI]), and tissue water distribution (i.e., tissue water index [TWI]) using a modern hyperspectral imaging (HSI) camera device [19–21]. These parameters provide insights into the biological mechanisms of the healing process and, thus, allow a quantification in the context of the 3 stages of wound healing: inflammation, proliferation, and remodeling [22–24].

Materials and Methods

Study Design

This study was conducted as an investigator-initiated, prospective, longitudinal, controlled, two-armed, monocenter cohort study in patients who received a split skin graft at the Clinic for Dermatology and Venereology of the University Medical Center Rostock, Germany, from December 2018 to June 2020. Exclusion criteria were pregnancy, age below 18 years, implants (especially if electroconductive), arrhythmia, heart failure during the last 6 months, or suffering from epileptic seizures as mentioned by the CINOGY manual [25]. Split skin graft sides were surgically produced by removal of the epidermis and the uppermost layer of the dermis with a dermatome at a thickness of 0.5 mm. Subsequently, the patients remained in the hospital for 7 days and received a total of 18 CAP applications during their stay (3 per day).

Wound Therapy and Assessment of Pain Intensity

The split skin graft donor site (rectangle) was divided into two comparably large areas. The distal half was treated with CAP using an innovative, sterile, disposable silicone wound dressing (Plasma-

Derm® Dress, 10 × 10 cm, CINOGY System GmbH, Duderstadt, Germany), which was applied directly to the wound. Plasma-Derm® Dress is a large, square plaster that can be repetitively connected to an electrical power supply. The applied voltage to create the plasma is pulsed in the range of 300 Hz. The patient leakage current is below the threshold defined by IEC 60601-1 and IEC 60601-1-11. The direct barrier discharge source within the plaster allows treatment of larger wound areas without the need for gas supply using ambient air. It consists of 2 electrodes, of which one is shielded by an insulating layer, formed by the dressing. The skin tissue serves as the counter electrode creating a discharge gap filled with atmospheric air containing filamentous micro-discharges (online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000517524). The proximal half served as the control and was treated with our clinic standard practice, a polyhexanide wound gel (Lavanid®, Serag-Wiessner, Neila, Germany) and fatty gauze (Cuticell®, BSN Medical GmbH, Hamburg, Germany). Both dressings were renewed every other day (days 3, 5, and 7; shown in Fig. 1). A flap protruding from the PlasmaDerm® Dress allowed the plasma source to be connected without removing or replacing the dressing. With a distance to the tissue of at least 5 mm, CAP was applied using a single dose of 100–240 V and 1.3–8 VA. We performed the treatment 90 s 3 times a day, with a minimum interval of 5 h, for a total of 7 days in a row. Pain intensity was assessed with a visual analog scale (IVAS; score 0–10; 0, no pain; 10, maximal pain) separately for the proximal and distal wound half at each day after the third treatment.

Hyperspectral Imaging

Every other day, during the dressing replacements, the healing of both wound areas was documented using a HSI camera (TIVITA® Wound, Diaspective Vision GmbH, Pepelow, Germany). Notably, the first HSI measurements on day 1 were performed before the start of CAP treatment. The camera measures wound parameters simultaneously, contact-free, noninvasive, and nonionizing. Multiple HSI camera channels measured the intensity of electromagnetic waves emitted by the wound in the range of 500–1,000 nm. Specific wavelength spikes corresponded to biological and molecular conditions at defined tissue spots and depths, and it provided information on the respective wound healing. This way, the HSI camera measured tissue parameters of wound healing such as oxygen saturation in deeper (4–6 mm) tissue layers (NIR-perfusion; index value: 0–100), hemoglobin oxygen saturation (StO₂, 1 mm deep; in %), hemoglobin distribution in the microcirculatory system (THI; index value: 1–100), and tissue water distribution (TWI; index value: 0–100) [19, 20, 26]. Hyperspectral images were captured in a dark room at a distance of 50 cm from the wound. If a visible fibrin layer was present in the wound, it was debrided with a ring curette before each capture. The HSI camera generated red, green, and blue standard and spectral images, which quantified values of the 4 different wound healing parameters from blue (low values) to red (high values).

In order to perform spectral analysis with the TIVITA® software, we defined certain circular areas (marks) in the red, green, and blue images, and within these areas, the exact values for the 4 specific parameters in their respective spectral images were then calculated by software. We placed 3 marks on each control and CAP-treated wound area, respectively, and additionally 3 on the nonlesional normal thigh skin, as illustrated in Figure 2. Each parameter was quantified independently and without further offset-

Fig. 1. Study design overview. **a** Ten Patients received a split skin removal. The site was divided into 2 comparably large areas after the surgery. The distal half was treated with CAP using an innovative, sterile, disposable silicone wound dressing (PlasmaDerm® Dress), which was activated 3 times a day for 90 s. A flap protruding from the PlasmaDerm® Dress allowed the plasma source to be connected without removing or replacing the dressing. The prox-

imal half served as the control and was treated conventionally with a wound gel (Lavanid®) and fatty gauze (Cuticell®). **b** Both dressings were renewed every 2 days (days 1, 3, 5, and 7). In this process, we measured different wound parameters (superficial and deep blood circulation, TWI, and oxygenation) using a state-of-the-art HSI camera. CAP, cold atmospheric plasma; TWI, tissue water index; HSI, hyperspectral imaging.

ting. Altogether, this resulted in 9 measurement points for each of the 4 parameters and 10 patients on 4 days, that is, a total of 1,440 measurements were performed.

Statistics

IBM SPSS Statistics for Windows (version 25, IBM Corp., Armonk, NY, USA) was used for statistical analysis. A linear mixed model was implemented to compare HSI and VAS values for CAP and conventional therapy. Day of treatment, therapeutic modality, age, anticoagulation therapy, and diabetes were

considered as fixed, whereas the individual patients were used as random effects. Statistical analysis of individual days was performed using the linear mixed model by splitting data and using patients as random, and the treatment as fixed effects. The normal distribution of the error terms in the linear mixed models was checked with the help of Q-Q plots and histograms of the residuals. A p value of $p < 0.05$ was regarded as statistically significant in each method. The p values were not adjusted for multiple testing.

Fig. 2. Images captured by the HSI camera. Spectral images highlighting exact values of the NIR, StO₂, THI, and TWI on a scale from 0 (blue) to 100 (red). NIR, near-infrared; StO₂, superficial hemoglobin oxygen saturation; THI, tissue hemoglobin index; TWI, tissue water index; HSI, hyperspectral imaging.

Table 1. Patient characteristics

Variable	Patients (<i>n</i> = 10)
Age (mean ± SD), years	74.4±8.1
Gender, male/female, <i>n</i>	5/5
Anticoagulation, yes/no, <i>n</i>	6/4
Diabetes, yes/no, <i>n</i>	3/7
Arterial hypertension, yes/no, <i>n</i>	9/1

SD, standard deviation.

Results

Patient Characteristics

Ten patients, who received a split skin removal on the ventral thigh for either covering a tumor excision site, burn wound, or chronic venous leg ulcer, were included in this study (Table 1). The mean age was 74.4 years. Five of them were females and 5 males. One additional patient had to be excluded from the study due to technical reasons and her results were not further considered. The study flow chart and design are depicted in Figure 1.

Hyperspectral Imaging

HSI was used to evaluate the healing of both parts of the skin graft donor site, respectively (shown in Fig. 2). Importantly, on day 1 and before the start of the CAP treatment, no differences were observed in any of the HSI parameters. From day 3 onwards (i.e., the second measurements), deep tissue oxygenation (NIR) and hemoglobin distribution (THI) were significantly higher in the CAP-treated wound areas than the standard-treated areas (shown in Fig. 3a, c). Notably, NIR reached its maximum after 3 days in the CAP and control wound region. THI remained increased in the CAP area on days 5 and 7, while it declined in the control area. The linear mixed regression model calculated a difference of +6.83 for NIR ($p < 0.001$; [4.9, 8.8]) and +19.93 for THI ($p < 0.001$; [14.8, 25.1]) in CAP-treated areas compared to control areas. TWI decreased by 6.47 ($p < 0.001$; [−8.1, −4.8]) in the CAP area showing the lowest values after 3 days (shown in Fig. 3d). StO₂ was reduced by 4.14 ($p = 0.002$; [−6.8, −1.5]; shown in Fig. 3b).

Pain Assessment

Pain levels were assessed daily and separately for the CAP and conventionally treated areas using VAS (shown in Fig. 4). A significant difference was observed on day 2,

Fig. 3. a–d Effect of plasma treatment over 7 days on the 4 HSI parameters (NIR, StO₂, THI, and TWI) of acute skin wounds. Patients with acute split skin graft donor site wounds were treated for 7 days with a conventional wound dressing and a CAP applying dressing on comparably large areas of the wound. Every other day, HSI measured parameter indices (scale 1–100) on both areas. Statistics were analyzed using a linear mixed model on each day separately (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). CAP, cold atmospheric plasma; HSI, hyperspectral imaging; NIR, near-infrared perfusion; StO₂, superficial hemoglobin oxygen saturation; THI, tissue hemoglobin index; TWI, tissue water index.

with a reduction of 0.7 VAS points ($p = 0.025$; [0.11, 1.29]). Notably, the first 2 dressing changes (at days 3 and 5) led to a transient increase in pain levels in both wound areas. As expected for the healing of acute wounds, the lowest values of pain in both areas were documented on day 7.

Clinical Evaluation

All patients in this study showed no signs of irritation, infection, or inflammation in either wound area. In the

conventionally treated wound halves, hair follicles were more prominent, and little to no exudate was visible. The CAP-treated area appeared to be more exudative and bloodier, and in some patients, a thick plate of blood clots formed, which was carefully removed with a curette during dressing changes. Nevertheless, wound edges treated with CAP showed earlier signs of reepithelialization than the control area.

Fig. 4. Reported pain levels by the patients according to VAS. Statistics were analyzed using a linear mixed model on each day separately. Score 0–10; 0, no pain; 10, maximal pain. * $p < 0.05$. VAS, visual analog scale.

Association with Anticoagulant Therapy and Other Patient Parameters

Six patients received anticoagulation therapies before and during the study and 3 patients suffered from type 1 or 2 diabetes (Table 1). Statistical evaluation revealed an association only for age (-0.45 per year; $p = 0.018$; $[-0.79; -0.11]$) and anticoagulation therapy ($+5.95$; $p = 0.043$; $[-0.25; 11.65]$) on NIR. Diabetes did not show association with the HSI parameters, and the VAS score was not affected at all (data not shown).

Discussion

We conducted a clinical pilot study with CAP using an innovative wound dressing (PlasmaDerm® Dress) for the acute healing of split skin removal sites on the thigh and revealed that CAP wound dressings significantly increased NIR and THI, and reduced TWI, indicating improved healing of acute skin wounds. Measurements of early wound healing parameters were performed by HSI, using novel spectral analysis software (TIVITA®). HSI provides a standardized, comparable, noninvasive, and fast method to measure tissue parameters and has already been used in other studies for the assessment of the wound healing process [27–29].

Differences in both treatments were observed from day 3 to 7, which are part of the second phase of wound healing [22]. This phase lasts from day 2 to 10 after injury and is characterized by the formation of a new tissue and especially new blood vessels, called angiogenesis,

stimulated by growth factors such as FGF2 or VEGF, which additionally promote proliferation, migration, lymphangiogenesis, and cell survival. CAP contains reactive oxygen species, such as H_2O_2 , OH , and ROO^- , which have been shown to stimulate the release of growth hormones and cytokines, including FGF2 [22, 30–34]. Additionally, multiple studies have demonstrated that CAP activates the endothelial nitric oxide synthase increasing the synthesis of nitric oxide, which has stimulatory effects on wound healing by promoting angiogenesis [35–37]. Accordingly, previous studies demonstrated increased porcine endothelial cell proliferation and elevated reepithelialization and neovascularization in mice after CAP treatment [21, 38]. These mechanisms are likely reflected by increased NIR and THI in our study.

We also observed a reduction of TWI in the CAP wound dressing treated areas, that is, a lower amount of water in the deeper tissues, which has been associated with increased lymphatic drainage. The removal of tissue water from the wound ground and lymphangiogenesis is a representative of improved wound healing, possibly due to the removal of old and unneeded materials [39–41].

StO₂ was significantly lower in the CAP-treated areas. This observation is contradictory to the results of another study, which showed increased postcapillary blood oxygenation (1–2 mm depth) in the healthy skin after CAP treatment with the PlasmaDerm® FLEX9060-device, that applies no relevant pressure to the upper skin surface [42]. Presumably, due to the superficial skin pressure of the new wound dressing made of a nonabsorbent plastic

layer, diminished drainage of wound exudate and reduced StO₂ resulted. Further development of the CAP wound dressing composition is warranted to reduce superficial pressure points and might, thus, be also beneficial to enhance StO₂ in the future.

As a control treatment, we used an antiseptic wound gel and a fatty gauze, which represents the standard treatment at our clinic for acute split skin graft donor sites. Because of the distinct compositions of the control and CAP wound dressing, the observed differences cannot undoubtedly be attributed to the CAP application itself. However, it is unlikely that the plastic layer of the CAP wound dressing per se had considerable positive effects on the measured HSI parameters.

CAP treatment was well tolerated. Pain decreased in both wound areas during the study treatment and was significantly lower in the CAP-treated areas on day 2. These results are consistent with previous studies, in which CAP was able to reduce pain in skin infections [43].

Remarkably, the novel CAP wound dressing is compact, portable, easily applicable, and could even be performed by patients themselves at home. This kind of treatment can be applied many times over several days without removing the wound dressing and thereby possibly disturbing the healing process.

Although a faster reepithelialization of the wound margins in CAP-treated areas was subjectively noted, our study lasted only 7 days and is therefore not able to quantify changes in the wound size. However, the positive role of CAP on different early wound healing parameters in our study is consistent with other studies, which have evaluated the effect of CAP on acute and chronic wounds and have shown predominately beneficial results [6, 9, 10, 14, 44–47].

Limitations of our study include its small size and design as a monocentric, prospective, longitudinal cohort study. This study provides positive results of CAP for the healing of acute wounds after surgery as applied through a novel, innovative wound dressing, which is easily applicable and patient-friendly. Further studies with a larger sample size and longer duration are necessary to confirm our findings and thereby further promote the implementation of CAP in clinical practice for the treatment of acute wounds.

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Statement of Ethics

This study was approved by the Ethics Committee of the Medical faculty, University of Rostock (study reference: 2018-011 6). Written informed consent was obtained from each patient before participation, and the principles of the Helsinki Declaration were applied. This cohort study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline [48].

Conflict of Interest Statement

The hyperspectral camera used for the measurements in this publication was developed by Diaspective Vision GmbH. Philip Wahl is an employee of this company. Diaspective Vision GmbH had no influence in the study design and analysis or interpretation of the data. The other authors have no conflicts of interest to declare.

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Author Contributions

The study was conceived and designed by S.E. A.W., S.R., J.T., P.W., and S.E. performed the study. A.W., M.H., F.W., L.B., S.E., and A.T. were responsible for the data analysis and interpretation. A.W., M.H., and F.W. performed the statistical analysis. Administrative, technical, and material support was provided by A.W., P.W., S.R., J.T., S.E., and A.T. A.W. provided the first manuscript draft. M.H. and A.T. reviewed and edited the script. All authors have participated in the critical revision of the manuscript with regard to important intellectual content.

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