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Skin and wound decontamination of multidrug-resistant bacteria by cold atmospheric plasma coagulation

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Summary

Background and objectives: Novel concepts to limit the spread of multidrug-resistant bacteria (MDR) are urgently needed. Since treatment with cold atmospheric plasma (CAP) has shown significant antibacterial properties, the purpose of this study was to evaluate the ability of CAP to eliminate MDR- compared to non-MDR-pathogens in chronic wounds.

Methods: Eleven patients with 18 heavily colonized wounds were treated with a CE-certified commercial argon-based CAP source for 10 s/cm² in one session. The antimicrobial efficacy was assessed by calculating the microbial load before and after treatment.

Results: A single CAP treatment reduced MDR in all wounds. In 14 treatments (63.6 %) and for 16 pathogens (66.7 %), a 100 % reduction of the bacterial load was observed. For 11 of 17 (64.7 %) MDR-pathogens and for 5 of 7 (71.4 %) other non-MDR-pathogens, complete eradication was achieved. The remaining 8 treatments showed reductions of 77.5 ± 18.6 % and the remaining pathogens a reduction of 74.8 ± 25.7 %.

Conclusions: As proof of principle, argon-based CAP serves as a potent treatment modality that was shown to limit MDR microbial colonization. The possible role of CAP in clinical MDR decontamination must be evaluated in clinical trials with repeated plasma treatment embedded in a comprehensive hygienic decontamination concept.

Introduction

Cold atmospheric plasma (CAP) provides novel therapeutic options combining potent physical and biological effects via UV, IR, reactive species and has recently been introduced in medical and biological applications [1–4]. Regarding antimicrobial applications, initial results of clinical plasma therapy in the treatment of diverse skin and soft tissue infections, such as bacterial dermatitis or chronic ulcer wounds, have been reported [5–7].

In previous studies, we demonstrated that two different plasma sources, the APPJ (atmospheric pressure plasma jet) and the DBD (dielectric barrier discharge), are highly effective in eliminating bacterial and fungal species (i. e. *Trichophyton interdigitale*, *Trichophyton rubrum*, *Microsporum canis*, *Candida albicans*) [3, 4, 8, 9]. The plasma sources eliminated all treated species on agar from 3- to 30-s exposure time, producing large, distinct inhibition zones depleted in bacterial and fungal growth (bactericidal effect). In accordance with published data by other research groups and additional data from risk assessment [5, 10–13], it can be deduced that CAP may also be effective in wound management to irrigate contaminated, colonized or infected skin and wounds, hence preventing nosocomial infections and supporting hospital hygiene [14]. In this way, plasma could play a relevant role as the initial "physical antiseptic" on skin and chronic wounds, which are well-known risk factors for colonization and distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) and other MDR [15–18]. Accordingly, CAP is being currently investigated as a wound

antiseptic [5–7, 19]. A clinical trial to treat chronic leg ulcers with DBD plasma has been conducted at the Department of Dermatology, Venerology and Allergology in Göttingen, Germany (NCT01415622) [5]. In another study, Isbary et al. found a significant decrease of bacterial load after plasma treatment in chronic ulcer wounds [20]. These results seem to support the role of plasma as the initial physical wound antiseptic, but clinical data regarding focused plasma antisepsis against MDR species are scarce. This study thus investigated the efficacy of a commercial CAP jet – the Maxium® electrosurgery unit with maxium® beamer and beam electrode (Gebrüder Martin GmbH + Co. KG, a company of the KLS Martin Group, KLS Martin Tuttlingen, Germany) – to eradicate these pathogens in chronic ulcer wounds during plasma-assisted wound debridement. To our knowledge, it is the first study examining the antimicrobial efficacy of the above-mentioned device.

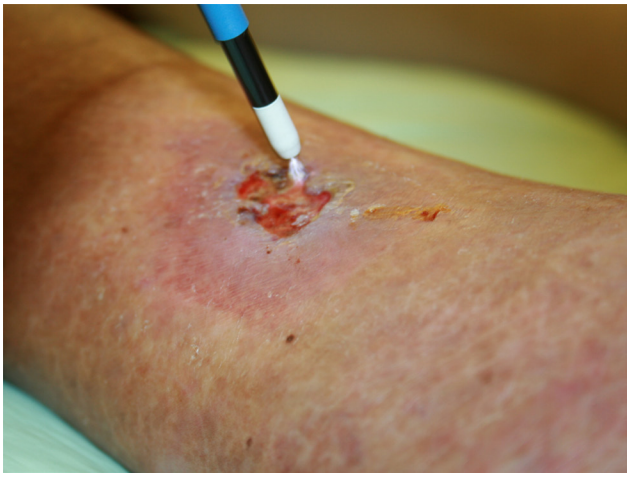

Figure 1 Treatment of wound 9 (patient 4) with Argon assisted plasma (KLS-Martin).

Methods

We used a CE-certified commercial CAP jet, the Maxium® electrosurgery unit with maxium® beamer and beam electrode (Gebrüder Martin GmbH + Co. KG), within its intended use to perform coagulation debridement of chronic leg ulcers. Since the argon beamer includes cold atmospheric plasma known from other devices as a potent antimicrobial agent in vitro, we monitored the antimicrobial efficacy qualitatively and quantitatively in routine wound treatment (quality assessment). Written informed consent was obtained from all participating patients.

A handpiece is electrically connected to the Maxium® and with the maxium® beamer for the argon supply. After starting the high-frequency current, the argon gas is propelled through a window integrated in the feedline and reaches the operating site via the tube electrode in the handpiece. The high voltage of the maxium® allows the ignition of the argon-air mixture at the nozzle of the electrode, ionizing the gas to produce the conductible CAP, which is finally emitted onto the operation field. The settings used were: Cutting and Plasma beam, power 20 W, argon gas-flow rate 6 L/min. The visible diameter of the plasma beam on the target takes approximately 5 mm (corresponding distance between wound surface to tip of device about 0.5 to 1 cm). Temperature at the tip of the device was measured using a laser-assisted infrared thermometer (model VA 6520, Komerci oHG, Ebern, Germany) and averaged 25.1 °C.

Treatment was initiated in 11 patients in our department with a total of 18 wounds highly colonized with pathogens. Wound colonization consisted of 24 pathogens and included 17 (70.8 %) wounds with MDR-pathogens (MDR group) and 7 (29.2 %) with non-MDR-pathogens (non-MDR group). The MDR group (17 MDR-pathogens) included 10 wounds colonized with methicillin-resistant *Staphylococcus aureus*, 3 methicillin-susceptible *Staphylococcus aureus* (MSSA), 3 *Pseudomonas aeruginosa* (PA) (one of which was 4MRGN, Multiresistant Gram Negative Rod) and 1 wound containing *Enterobacter cloacae* (EntC). The non-MDR group (7 non-MDR wounds) comprised MSSA (4 wounds), PA (1 wound), *Serratia marcescens* (SM) (1 wound) and *Streptococcus* Group B (StrepB) (1 wound). A pathogen was defined as MDR if it showed resistance against at least one antibiotic substance of ≥ 3 groups of antibiotics or – in the case of *Staphylococcus aureus* – resistance to oxacillin. Antiseptic wound treatment was performed at least 24 h before CAP treatment. Prior to CAP treatment, any wound fluid and biofilm was removed using sterile compresses. Subsequently, local anesthesia was applied by infiltration and rinsing of the wound with a sterile solution consisting of 0.5 ml of adrenaline (1/1000), 20 ml of ropivacaine hydrochloride (10 %), and 20 ml of lidocaine hydrochloride (2 %). CAP treatment was performed at a distance of 1 to 2 cm above the wound surface by slowly moving the plasma beam over the wound surface in a meandering pattern (Figure 1). Before and directly after treatment, wound swabs were collected for microbiologic analysis (qualitative and quantitative evaluation of microbial growth). A modified Levine technique was used for quantitative swabbing [21].

Microbiological analysis

All tested bacterial strains were isolated from chronic wounds of participating patients directly before and after plasma treatment. In accordance with German national guidelines for microbiologic diagnostics, samples were processed by culturing on non-selective agar (Columbia blood agar,

Biomérieux, Nürtingen, Germany), selective chromogenic agar (Mast Diagnostica, Reinfeld, Germany) and enrichment broth (caso bouillon, heipha Dr. Müller GmbH, Eppelheim, Germany). Blood agar and chromogenic agar plates were assessed after 24 and 48 hours for typical bacterial colonies. Colonies were counted visually and documented. After 24 h, the broth was streaked onto chromogenic and selective as well as non-selective media to be assessed again after 24 h and 48 h. Cultures were incubated aerobically at 36 °C. Identification and susceptibility testing were performed using the automated VITEK compact system (Biomérieux, Nürtingen, Germany).

Results

Table 1 shows the results of CAP treatment of the 24 pathogens of 11 patients, as well as the microbial loads encountered. The highest load was found in a wound colonized with 1.3 x 10^6 colony forming units (CFU) of PA (4MRGN), and the lowest load was found in a wound colonized with 90.0 CFU of EntC (Table 1). Plasma treatment led to a reduction of bacterial load in all wounds, with a mean relative reduction per wound treatment of 91.8 % ± 15.4 %, a mean reduction factor (RF) per wound treatment of 2.4 ± 1.6, a mean relative reduction per species of 91.6 % ± 18.7 %, and a mean RF per species of 2.5 ± 1.6. The RF ranged between 0.2 for MSSA and PA in wound 15 (treatment 18) and 6.1 for PA in wound 7 (treatment 12) (Table 1). The mean RF did not differ significantly between different species or between the MDR and non-MDR groups (mean RF MRSA: 2.2 ± 1.5, MSSA: 2.1 ± 1.4 PA: 3.4 ± 2.3, SM: 4.6, EntC: 1.8 and StrepB: 3.2; MDR group: 2.3 ± 1.5 and non-MDR group: 2.9 ± 1.8), data not shown. In 14 wound treatments (63.6 %) and for 16 pathogens (66.7 %), a 100 % reduction of the bacterial load was observed. For 11 of 17 (64.7 %) MDR-pathogens (5 MRSA, 3 MSSA, 2 PA, 1 EntC) and for 5 of 7 (71.4 %) other pathogens (2 MSSA, 1 PA, 1 SM, 1 StrepB), complete eradication was achieved (after treatment no bacterial growth was seen on agar plates after 72 h hours). The remaining 8 wound treatments showed reductions of 77.5 ± 18.6 % and the remaining pathogens (5 MRSA, 2 MSSA [normal infection strain], 1 PA) of 74.8 ± 25.7 % (mean).

The eradication efficacy was independent of the grade of resistance and the type of bacterial load (species). The frequency of resistance to the classes of antibiotics tested in the VITEK compact system is shown in Table 1. For SA isolates, resistance was tested against 12 classes of antibiotics, including 15 individual antibiotics. For PA, resistance was tested against 6 classes with 9 individual antibiotics, and for EntC and SM, resistance was tested against 8 classes including 17 individual antibiotics.

The application of CAP was well tolerated in all patients. On a visual analogue scale (range 1–10) for pain assessment, all patients showed scores of 1–3 (data not shown). In the course of the complex wound treatment, healing of all wounds was accomplished between 3 and 6 months after CAP treatment. As we did not conduct a clinical study allowing controls for comparison, a potential contribution of CAP to wound healing should be the subject of further investigation.

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA), like other MDR (e. g. ESBL and carbapenemase-producing enterobacteriaceae), are important causes of skin and soft tissue infection and lead to nosocomial infections such as pneumonia and sepsis [22, 23]. Despite increasing efforts and partial success in reducing MRSA incidence rates in some European countries in the last few years, MRSA infections continue to be a major concern in clinical practice and prevention remains a worldwide challenge for hospitals [22, 24–26]. The highest MRSA rates are typically found in intensive care units, where patients exhibit many risk factors, such as intravascular devices, ongoing antibiotic treatment and multi-morbidity [27]. After decades of intense clinical efforts against MRSA, no real improvement can be observed regarding reliable decontamination of patients and healthy carriers, with the exception of lower infection rates in some European countries. Decontamination procedures themselves, including the current practice of antisepsis, must be critically tested [28]. Therefore, novel antimicrobial strategies are strongly needed and may be provided by CAP, which is now being introduced into biomedical science.

CAP offers a unique package of biopotent species including UV, IR, electrons, reactive oxidative species, NO, electrical fields, currents, ions and particles [1–3]. In previous studies, we showed that two different CAP sources, the APPJ and the DBD (as well as a "historical" violet wand plasma [unpublished data]), were highly effective in reducing bacterial and fungal load in vitro [3, 8, 9] in a short treatment time span from 3 s to 30 s [3, 8, 9]. Integrated in a modern wound management approach, including antisepsis, debridement and proper dressing change, argon plasma-based wound coagulation/debridement proved highly efficacious in killing the most important clinical skin- and wound-pathogens (*Staphylococcus aureus* and *Pseudomonas aeruginosa)* as well as other relevant pathogens. This method cleared (100 % germ reduction) 14 of 22 wounds per treatment, eradicated 16 of 24 pathogenic species in one treatment session, and diminished substantial bioburden in all other wounds. A total of 8 wounds

Table 1 Results of cold plasma treatment of 18 wounds with 24 pathogens.

Treatment No.	Wound area	Location	Diagnosis	Patient (wound)	Species	n resistant classes of antibiotics/ tested classes of antibiotics*	CFU before treatment	CFU after treatment	Reduction factor
1	2.0 cm²	lower leg right	Granulomatosis disciformis chronica et progressiva	1 (1)	MSSA	MDR (3/12)	2.2e2	0	2.3
2	2.3 cm²	lower leg right	Granulomatosis disciformis chronica et progressiva	1 (2)	MSSA	MDR (3/12)	2.4e2	0	2.4
3	0.3 cm²	lower leg right	Granulomatosis disciformis chronica et progressiva	1 (3)	MSSA	MDR (3/12)	2.0e2	0	2.3
4**	1.0 cm²	lower leg left	Ulcus cruris postthromboticum + CVI	2 (4)	**MRSA**	MDR (4/12)	3.4e2	4.0e1	0.9
5***	2.0 cm²	lower leg left	Ulcus cruris postthromboticum + CVI	2 (5)	**MRSA**	MDR (4/12)	2.6e4	1.3e3	1.3
6	1.0 cm²	lower leg left	Ulcus cruris venosum	3 (6)	PA	MDR (4/6)	1.8e2	3.0e1	0.8
7****	6.3 cm²	lower leg left	Ulcus cruris venosum	3 (7)	PA	MDR (4/6)	2.1e2	0	2.3
8**	1.0 cm²	lower leg left	Ulcus cruris postthromboticum + CVI	2 (4)	**MRSA**	MDR (4/12)	9.0e1	0	2.0
9***	2.0 cm²	lower leg left	Ulcus cruris postthromboticum + CVI	2 (5)	**MRSA**	MDR (4/12)	6.6e3	4.2e2	1.2
10	1.0 cm²	lower leg left	Ulcus cruris postthromboticum + CVI	2 (8)	**MRSA**	MDR (4/12)	3.2e3	5.4e2	0.8
11	4.0 cm²	lower leg right	Ulcus cruris venosum	4 (9)	MSSA	(1/12)	6.7e4	1.9e4	0.6
12****	6.3 cm²	lower leg left	Ulcus cruris venosum	3 (7)	PA (4MRGN)	MDR (5/6)	1.3e6	0	6.1
13	25.0 cm²	lower leg left	Hypergranulation	5 (10)	**MRSA**	MDR (4/12)	1.2e4	0	4.1

Table1 Continued.

Treatment No.	Wound area	Location	Diagnosis	Patient (wound)	Species	n resistant classes of antibiotics/ tested classes of antibiotics*	CFU before treatment	CFU after treatment	Reduction factor
14*****	9.0 cm²	lower leg left	Ulcus cruris venosum	6 (11)	**MSSA**	(0/12)	1.2e3	0	3.1
15	280.0 cm²	lower leg left	Ulcus cruris not elsewhere classified	7 (12)	MRSA	MDR (4/12)	1.6e2	0	2.2
16	3.0 cm²	lower leg left	Ulcus varicosis (both legs) + venous thrombosis	8 (13)	SM	(3/8)	3.7e4	0	4.6
17	26.0 cm²	lower leg left	Ulcus cruris postthromboticum	9 (14)	MSSA	(0/12)	9.0e3	0	4.0
18	25.0 cm²	ankle left	Ulcus cruris mixta, CVI IIIb, PTS; PAOD; arthrogenic congestive syndrome	10 (15)	MSSA	(2/12)	9.0e4	1.2e5 → 7.6e4	7.6e4 → 0.1
					PA	(4/6)	3.3e4	0	4.5 (0.2)
19*****	6.0 cm²	lower leg left	Ulcus cruris venosum	6 (11)	EntC	MDR (3/8)	6.5e1	1.8e3 → 0	0 → 1.8
					StrepB	-	1.8e3	0	3.2 (3.3)
20	100.0 cm²	lower leg left distal	Ulcera crurum with livedo vasculitis	11 (16)	**MRSA**	MDR (4/12)	2.7e4	0	4.4
21	100.0 cm²	lower leg left latera-lis	Ulcera crurum with livedo vasculitis	11 (17)	**MRSA**	MDR (4/12)	1.5e4	0	4.2
22	120.0 cm²	lower leg left tibial	Ulcera crurum with livedo vasculitis	11 (18)	**MRSA**	MDR (4/12)	3.2e3	1.1e3	0.5

Abbr.: CFU, colony forming units; CVI, chronic venous insufficiency; PTS, post thrombotic syndrome; PAOD, peripheral arterial occlusive disease; MSSA, methicillin-susceptible *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; EntC, *Enterobacter cloacae*; SM, *Serratia marcescens*; StrepB, *Streptococcus* Group B; MDR, multidrug-resistant bacteria; 4MRGN, multiresistant Gram-negatives being resistant to 4 of the 4 classes of antibiotics

*Tested in the VITEK compact system, **4 id to 8 (5 weeks with no treatment), ***5 id to 9 (5 weeks with no treatment), ****7 id to 12 (10 weeks with no treatment), *****14 id to 19 (5 1/2 months with no treatment)

were not disinfected, but in the light of the high rate of total clearance (14 wounds) by one treatment, a significant potentialization of repeated use can be expected. Classical decontamination needs at least 5 days (known for MRSA), and 5 days of plasma decontamination with one treatment per day seems realistic. Since conventional decontamination procedures against MRSA are highly unreliable [28] and the efficacy of those against MRGN and VRE is inconclusive, plasma treatment, being completely different from conventional techniques, seems promising. In addition, our data show that plasma may also play a relevant adjunctive role in the eradication of "conventional" (non-MDR) bacterial bioburden during extended surgical wound debridement, i. e. during wound shaving, where conventional antisepsis is unable to prevent relevant wound germ transmission during the intervention [unpublished data]. Although the mean RF did not differ significantly between different species or between MDR and non-MDR groups, the reduction seemed more pronounced for gram-negative germs (PA, SM); thus, these data need confirmation using a larger sample size. Indeed, we found less susceptibility in gram-positive bacteria in another trial [9]. The origin of this difference has yet to be determined.

The present study provides initial systematic in vivo decontamination data as a base for the optimization of plasma treatment of MDR and other wound and skin-pathogens in chronic wounds as an alternative to conventional, ultimately unsatisfactory antiseptic decontamination approaches. This could be the key to further progress in "zero tolerance" strategies against MDR, MRSA, and the newly emerging MRGN in infection prevention.

Of course, our results from a single treatment as proof of principle need further confirmation and definitive estimation of efficacy, but it seems clear that most decontamination approaches warrant repeated plasma treatments.

Conclusion

In the search for new antimicrobial methods, it is of crucial importance to find principles not related to conventional drugs and techniques. This entails overcoming the problems of classical resistance development, such as plasmid transfer and induced overproduction of compromising enzymes (e. g. beta lactamases and carbapenemases). Cold plasma may be such a novel principle, as it demonstrates high efficacy against different MDR species in chronic ulcer wounds, and could thus be a new alternative in the worldwide fight against multidrug-resistant pathogens.

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