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Skin Disinfection by Plasma-Tissue Interaction: Comparison of the Effectivity of Tissue-Tolerable Plasma and a Standard Antiseptic

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Key Words
Wound · Skin barrier · Stratum corneum · Hair follicle · Bacteria · Fungi

Abstract
Wound healing disorders frequently occur due to biofilm formation on wound surfaces requiring conscientious wound hygiene. Often, the application of conventional liquid antiseptics is not sufficient and sustainable as (1) the borders and the surrounding of chronic wounds frequently consist of sclerotic skin, impeding an effectual penetration of these products, and (2) the hair follicles representing the reservoir for bacterial recolonization of skin surfaces are not affected. Recently, it has been reported that tissue-tolerable plasma (TTP), which is used at a temperature range between 35 and 45 °C, likewise has disinfecting properties. In the present study, the effectivity of TTP and a standard liquid antiseptic was compared in vitro on porcine skin. The results revealed that TTP was able to reduce the bacterial load by 94%, although the application of the liquid antiseptic remained superior as it reduced the bacteria by almost 99%. For in vivo application, however, TTP offers several advantages. On the one hand, TTP enables the treatment of sclerotic skin as well, and on the other hand, a sustainable disinfection can be realized as, obviously, also the follicular reservoir is affected by TTP.

Introduction

Wounds can occur for many reasons such as injuries and operations, or as a result of diabetes mellitus and chronic venous insufficiency [1, 2]. Generally, wound healing occurs in four stages: hemostasis, inflammation, proliferation or granulation, and matrix formation or remodeling, whereby these stages are not necessarily chronological, but rather a dynamic and integrated coordination of specific processes [3]. The wound healing stages can proceed uninterrupted, as in healing by second intention, or influenced by primary closure and application of medications or topical products.

Wound healing disorders frequently occur in individuals at an advanced age, and basically due to two main reasons: (1) insufficient blood circulation, which means

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that the wound area is not provided with sufficient oxygen and nutrition [4], and (2) the formation of a biofilm containing bacteria and fungi [5]. While most of the bacteria are located on the skin surface, parts have also been detected in deeper areas of the stratum corneum or in hair follicles [6, 7]. In order to prevent critical bacterial colonization, conscientious wound hygiene is essential, including regular wound cleaning with antiseptics. In the case of sclerosis-like skin and scar tissue in and around the wound area, a conventional liquid antiseptic may not efficiently penetrate into the skin [8]. This is particularly the case with patients suffering from diabetes mellitus [9] or chronic venous insufficiency.

Recently, it has been reported that it is possible to produce a cold electrical discharge, which can be applied to biological material and can be used for various therapeutic applications such as skin and wound disinfection [10]. In addition to extremely specific action, tissue-tolerable plasma (TTP) may have a general mechanical effect on the surface of living organisms. In plasma, microorganisms are exposed to intense bombardment by radicals, probably causing surface lesions that the living cell cannot repair sufficiently quickly [11]. The temperature of the TTP ranges from 35 to 45°C [12, 13]. The first experiments concerning a risk assessment of these TTP applications demonstrated that in the case of the plasma being applied to the skin surface at a moving velocity of 10 mm/s, no thermal damage could be observed [12]. The influence of TTP on the antioxidative network of the human skin is still a topic of intensive investigation [12].

In the present study, the disinfection effectivity of TTP and a standard liquid antiseptic (octenidine) was compared. The investigations were conducted on pig ear skin, which is a suitable model of human skin [14] because of the individual structure of the skin layer and hair follicles. Bacterial colonization was investigated prior and subsequent to skin treatment with octenidine and TTP.

Materials and Methods

Tissue Samples

The investigations were conducted on 6 pig ears. The unboiled pig ears had been freshly obtained from a slaughterhouse, and the experiments were performed within 6 h after slaughter. Approval for the experiments had been obtained from the Veterinary Inspection Office Berlin-Treptow.

Skin Disinfection

Three skin areas each of 3 × 3 cm were marked on each pig ear. The first skin area remained untreated (modus A); the second skin area was treated with the standard antiseptic octenidine (Octenisept®; Schuelke & Mayr GmbH, Norderstedt, Germany; modus B). TTP was applied to the third skin area (modus C). The plasma beam was moved at an average velocity of 10 mm/s, which corresponds to an optimal moving velocity of the plasma, as previously reported [12]. The contact time of the plasma with the tissue was approximately 0.3 s.

During this procedure, it was attempted to move the plasma beam evenly over the entire skin surface in order to prevent residues of untreated skin areas with surviving bacteria. The locations of untreated, antiseptic-treated and plasma-treated skin areas on each pig ear were determined by means of randomization.

Plasma Jet

In the present study, a plasma jet (kinpen 09®) was used, which was developed at the Institute of Plasma Physics, Greifswald, Germany, and manufactured by Neoplas GmbH [13, 15]. The plasma beam had a diameter of 3 mm. Argon was used as carrier gas. During treatment, the temperature of the tissue increased up to 43°C. The plasma stream had a length of 11 mm. The distance between nozzle and tissue was approximately 5 mm. The plasma jet has previously been described in detail [16]. The device kinpen 09 has CE certification for fulfilling the electrical safety standards with humans. Argon gas is used as a charge medium in the plasma jet.

Determination of Disinfection Effectivity

While the first skin area remained untreated (modus A), the second and third skin areas were treated with the antiseptic octenidine (modus B) and TTP (modus C), respectively. Immediately after treatment, the procedure for the determination of the amount of bacteria on the skin surface started according to Williamson's protocol [17]. Therefore, a sterile stainless steel ring with a diameter of 20 mm was applied to each skin area. Inside the ring, 1 ml of a basic solution was applied, consisting of 50% phosphate buffer solution (Dulbecco's PBS; Laboratory GmbH, Graz, Austria) and 50% egg yolk because in the pretest to determine effective neutralization in the recovery medium, only this combination sufficiently inhibited the antiseptic efficacy (data not shown). The solution was evenly distributed inside the ring with an aseptic applicator. The contact time of the basic solution with the tissue was 1 min. Subsequently, 0.5 ml of the supernatant was removed using a pipette. These 0.5 ml were diluted with 4.5 ml of basic solution (diluted solution 1:10). The solution was shaken for 30 s, using a test tube shaker, in order to obtain a homogeneous mixture. Of this solution, 0.5 ml was applied to an agar plate (Merck AG, Darmstadt, Germany) for bacterial growth. The agar plate was kept over a period of 24 h in an incubator at a temperature of 36°C. After 24 h, images were produced of the agar plates, and the bacterial colonies were counted.

Statistics

For statistical analysis, the bacterial colonies were counted; mean values and standard deviations were calculated and compared using the software program Microsoft Excel®. Further statistical analysis was performed with the software program SPSS® 18.0. The Wilcoxon test was utilized to compare the numbers of bacterial colonies after different treatment modi, affording a significance of $p < 0.05$.

Table 1. Bacterial colonization determined after different modi of skin treatment

Tissue sample No.	Number of bacterial colonies					
	modus A (untreated)		modus B (antiseptic-treated)		modus C (plasma-treated)	
	absolute, n	relative, %	absolute, n	relative, %	absolute, n	relative, %
1	73	100	0	0	4	4.7
2	117	100	2	2.3	11	12.9
3	76	100	0	0	5	5.9
4	83	100	1	1.2	2	2.3
5	67	100	0	0	5	5.9
6	96	100	0	0	3	3.5
MV ± SD	85.3 ± 18.4	100	0.5 ± 0.8	0.6 ± 1.0	5 ± 3.2	5.9 ± 3.7

MV = Mean value; SD = standard deviation.

Results

The action of TTP on the skin surface is demonstrated in figure 1. The plasma-tissue interaction zone had a diameter of approximately 2 mm. The plasma beam was moved at an average velocity of 10 mm/s, which corresponds to the optimal moving velocity of the plasma, as reported previously [12].

In table 1, the numbers of bacterial colonies grown after the different treatments are given. In addition to the absolute numbers of bacterial colonies, relative data are given, meaning that the numbers of colonies determined on the untreated skin areas of every tissue sample were standardized to 100% for better comparison of the results.

Both disinfection of the skin surface with octenidine and treatment with the plasma jet led to a significant reduction in bacterial colonization ($p < 0.05$). Differences between octenidine pretreatment and plasma jet treatment were small but significant all the same ($p < 0.05$). The application of octenidine led to an increased reduction of bacteria in comparison with TTP.

Color version available online

Fig. 1. Application of the plasma jet to the skin surface.

Discussion

Octenidine represents a very effective standard antiseptic which is frequently utilized for wound cleaning purposes both under clinical and domestic conditions. In the present study, the bacterial load was significantly reduced in all tissue samples after application of octenidine, although 100% disinfection was only achieved in 4 of 6 tissue samples. In 2 of the 6 tissue samples, 1 bacterial colony and 2 bacterial colonies were found, respectively. Insufficient disinfection as well as contamination of the agar plate or of the basic solution – potentially from the follicular orifices – have to be considered. The harvesting procedure used in the present study allows for a quantitative analysis of bacterial colonies in contrast to the swab sampling method.

The present investigation revealed that the application of a plasma jet also led to a significant reduction in bacterial colonies. These results are in concordance with those of previous studies which described a significant reduction of the bacterial load after plasma application [10, 18]. In addition to very specific action, plasma may have a general mechanical effect on the surface of a living organisms. In plasma, microorganisms are exposed to intense bombardment by radicals, probably causing surface lesions that the living cell cannot repair sufficiently quickly [11]. Thus, a distinct advantage of plasma application is that a broad spectrum of microorganisms is affected. Various studies have shown the efficacy of plasma against Gram-positive and Gram-negative bacteria, biofilm-producing bacteria, viruses, fungi and spores [10]. Furthermore, there are indications that bacteria have neither primary nor secondary resistance to plasma [10] and that, as a purely physical method, plasma is unlikely to cause allergic or toxic reactions [10] as much as conventional disinfectants might do. Additionally, it might be of advantage that plasma is also able to reduce the bacterial load of the follicular reservoir, as pre-investigations have demonstrated (unpublished results). Previously, Ruppert [7] has shown that hair follicles represent a reservoir for bacteria which, on the one hand, is protected against topical antiseptics and, on the other hand, is depleted via sebum flow under in vivo conditions, which means that recolonization of the skin surface occurs from the follicular reservoir. In the present study, due to the in vitro situation, sebum flow and thus recolonization via hair follicles did not occur. This has to be considered in further studies when the long-term effectivity of antiseptic methods is compared.

The application of TTP likewise revealed highly effective disinfection, but TTP was significantly less effective than the standard antiseptic octenidine. In all likelihood, the disinfection effectivity of TTP could be enhanced by increasing the plasma diameter of the plasma jet. Due to the current, small plasma beam of 3 mm, thorough and homogeneous disinfection of the complete skin surface seems difficult. It cannot be excluded that small residues of the skin surface were not affected by the plasma stream during treatment. Consequently, in all probability, bacteria may have survived in these skin areas, leading to an increased number of bacterial colonies.

The investigations in the present study were conducted in vitro on pig ear skin, which is a suitable model of human skin [14]. It has to be expected that under in vivo conditions, the effectivity of TTP in skin disinfection is superior to that under in vitro conditions, especially concerning long-term investigations. In vitro, sebum flow and, therefore, bacterial recolonization from the follicular reservoir do not occur. On the contrary, in vivo, TTP seems to affect the follicular reservoir as well, which should result in sustainable disinfection. Also the condition of the skin might have an influence on the effectivity of both disinfection methods. Occasionally, the borders and surrounding area of problematic wounds consist of callous tissue similar to scleroderma [19]. This type of tissue has extremely strong barrier properties. Therefore, conventional liquid antiseptics remain on the skin surface as they are not able to penetrate into the upper layers of the stratum corneum. Thus, the efficacy of disinfection of such wounds with liquid antiseptics is relatively low, as has been demonstrated in different studies [16, 20, 21]. Especially in these cases, the application of the plasma jet might be of benefit since the direction of plasma issuing affects not only the skin surface, but also deeper parts of the stratum corneum, obviously also including the hair follicles.

Conclusions

TTP is an innovative technique which has recently been introduced into biology and medicine. The first results obtained by this technique have demonstrated that TTP is able to realize a highly effective disinfection of skin surfaces. It can be expected that under clinical conditions such as in the case of treatment of problematic wounds, the plasma technique might become a favored method of disinfection.

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