

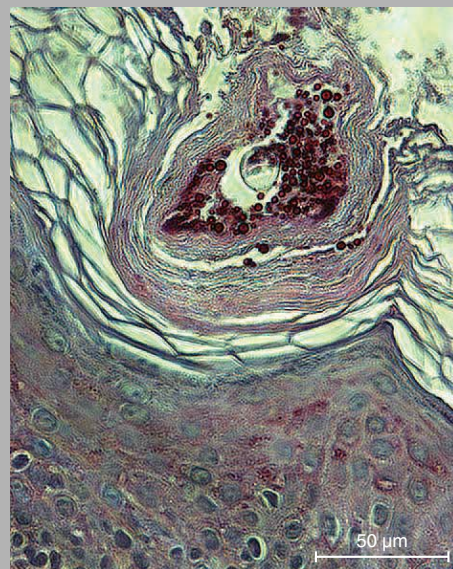
LASER PHYSICS LETTERS

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Abstract: The application of tissue-tolerable electrical plasma (TTP) is highly efficient in skin antisepsis. However, the germs are not only located on the skin surface, but also in the hair follicles, from where they re-colonize the skin surface after antisepsis, e.g. The objective of the present study was to show that plasma is able to reach the follicular reservoir for antisepsis. For this purpose, a solution containing particulate chlorophyll dye had been applied onto porcine skin samples. The fluorescent properties of the dye changed during the plasma tissue interaction. The results demonstrate that TTP penetrates deep into the hair follicles, whereupon the hairs act as a conductor for the plasma. Therefore, it can be concluded that micro-organisms of the follicular reservoir are destroyed more efficiently by the plasma than by conventional liquid antiseptics.



Histological image of a hair follicle colonized with fungi (pityrosporon ovale, PAS staining)

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Antisepsis of the follicular reservoir by treatment with tissue-tolerable plasma (TTP)

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1. Introduction

The formation and presence of biofilms in chronic wounds is a major problem for wound healing, treatment, and bacterial resistance [1,2]. Recently, it has been demonstrated

that on intact skin, the sources for re-emergence of bacterial commensals to levels preset before skin antisepsis are mainly the hair follicles [3,4].

For exemplification, a histological image of a hair follicle colonized with fungi is demonstrated in Fig. 1.

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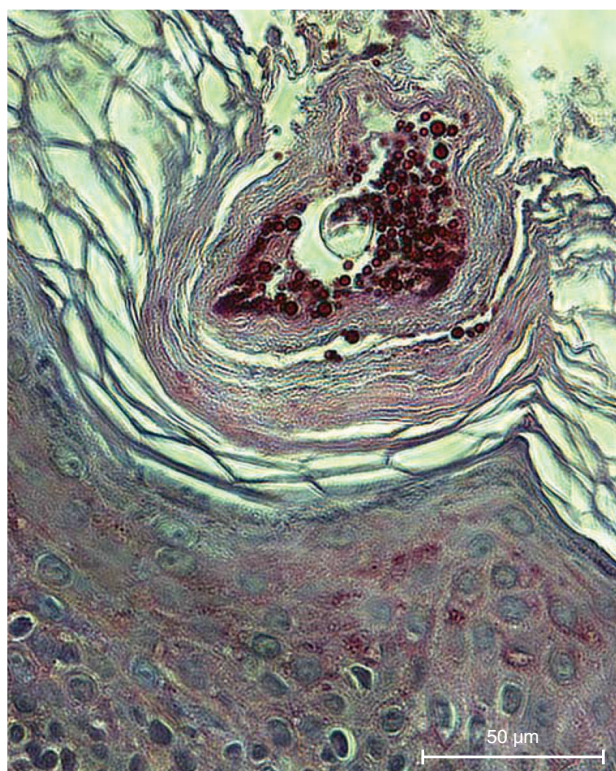


Figure 1 (online color at www.lphys.org) Histological image of a hair follicle colonized with fungi (*pityrosporon ovale*, PAS staining)

In wound healing, the driving force for regeneration and wound closure are stem cells in the remaining hair follicles and the re-epithelization from the wound borders [5]. These mechanisms are disturbed by bacterial and fungal colonization and biofilm formation.

Conventional antisepsis of skin surfaces or wounds is regularly performed with liquid antiseptics, which are indeed well distributed on the skin or wound surface and allow a short-term reduction of bacterial and fungal colonization. This measure, however, will not have a direct impact on bacteria located in hair follicles [6] and being protected by biofilms. Consequently, the re-colonization of the skin or wound surface occurs also from the follicular reservoir [7].

Recently, it has been demonstrated that tissue-tolerable electrical plasma (TTP) is an efficient antisepsis method [8]. The plasma is produced inside a plasma-jet. The temperature in the plasma tissue interaction zone is about 45°C. The plasma stream produced by the system can be applied in the same manner as a “brush” for the treatment of the skin surface. O. Lademann et al. [9] showed previously that the optimal moving velocity of the plasma was approximately 10 mm/s. No thermal damage or injury caused by UV radiation of the plasma could be detected.

The efficient antiseptic properties of plasma have been proven by a diversity of different studies being thoroughly comparable to conventional antisepsis [8,10,11]. In addition to extremely specific actions, plasma may have a general mechanical effect on the surface of the living organism. Micro-organisms in plasma are exposed to an intense bombardment by radicals probably provoking surface lesions that the living cell cannot repair sufficiently quickly [10].

A distinct advantage of plasma application is the affection of a broad spectrum of micro-organisms. Furthermore, there are indications that bacteria have neither primary nor secondary resistance to plasma and as a purely physical method, plasma is unlikely to cause allergic or toxic reactions as in the case of conventional antiseptics [12].

The authors hypothesized that a further advantage of plasma antisepsis might be that the plasma also reaches the follicular reservoir leading to a sustainable antisepsis of the skin surface, as the reservoir for re-colonization is likewise destroyed. The aim of the present study was to show that plasma also reaches the follicular reservoir.

2. Materials and methods

2.1. Study design

The investigations were performed on six porcine ears. Two areas A and B were marked on each ear with a size of 3×3 cm². A chlorophyll dye-containing particle solution was applied onto these areas at a concentration of 1 mg/cm². After a penetration time of 30 min, area A remained untreated, while area B was treated with the plasma-jet, using a moving velocity of approximately 10 mm/s. Subsequently, punch biopsies were removed from each marked area. The histological sections obtained from these tissue samples were analyzed concerning the distribution of the fluorescence on the skin surface and in the hair follicles.

2.2. Tissue samples

The investigations were performed on six porcine ears. The non-scalded pig ears had been freshly obtained from the slaughterhouse. Before commencement of the experiments, the pig ears were rinsed under running water and carefully dried with paper towels. The Governmental Veterinary Office, Berlin-Treptow, Germany, had granted permission for these experiments.

2.3. Preparation of fluorescent chlorophyll solution

A plant extract containing high amounts of chlorophyll was filtered for components > 1 μm. Subsequently, the

chlorophyll solution containing only components $< 1 \mu\text{m}$ was centrifuged and the supernatant was removed. The remaining part of the chlorophyll was utilized to prepare a solution containing 80% ethanol and 20% of chlorophyll particles. The chlorophyll contained in the particles showed a strong fluorescence after excitation at 632.8 nm wavelength using a He-Ne laser. A corresponding fluorescence signal was detected in the range between 650 and 750 nm. Preliminary experiments on a microscopic glass plate showed that the fluorescent properties of the chlorophyll solution disappeared after treatment with TTP meaning that the chlorophyll dye was either destroyed or modified by plasma interaction.

2.4. Plasma-jet

In the present study, the plasma-jet “kINPen09®” (CE Certification No. 609.003.1) was used. The system had been developed by the Leibniz-Institut für Plasmaforschung und Technologie e.V. (INP Greifswald e.V.) and manufactured by neoplas GmbH, Greifswald, Germany [13]. Argon gas was used as a discharge medium in this system. The plasma tissue interaction zone had a diameter of approximately 2 mm and was investigated visually. A detailed description of the system was provided by O. Lademann et al. [9].

2.5. Histological sections

3 mm punch biopsies were removed from the non-treated and plasma-treated porcine skin samples, after the application and penetration of the particle-containing solution.

2.6. Laser scanning microscopy (LSM)

The laser scanning microscope LSM 2000 (Carl Zeiss, Jena, Germany) was used to analyze the histological sections obtained from the punch biopsies [14–16]. The samples were measured both in the transmission and fluorescent modes. Superposition of the images allowed the investigation of the distribution of the fluorescent dye inside the hair follicles [17,18]. 20 hair follicles from each marked skin area were investigated. The fluorescent dye was excited at 632.8 nm using a He-Ne laser. The fluorescence signal was detected at wavelengths $> 670 \text{ nm}$.

3. Results

In Fig. 2, the superposition of transmission and fluorescence images is presented. The histological section in Fig. 2a originates from a control tissue sample (area A,

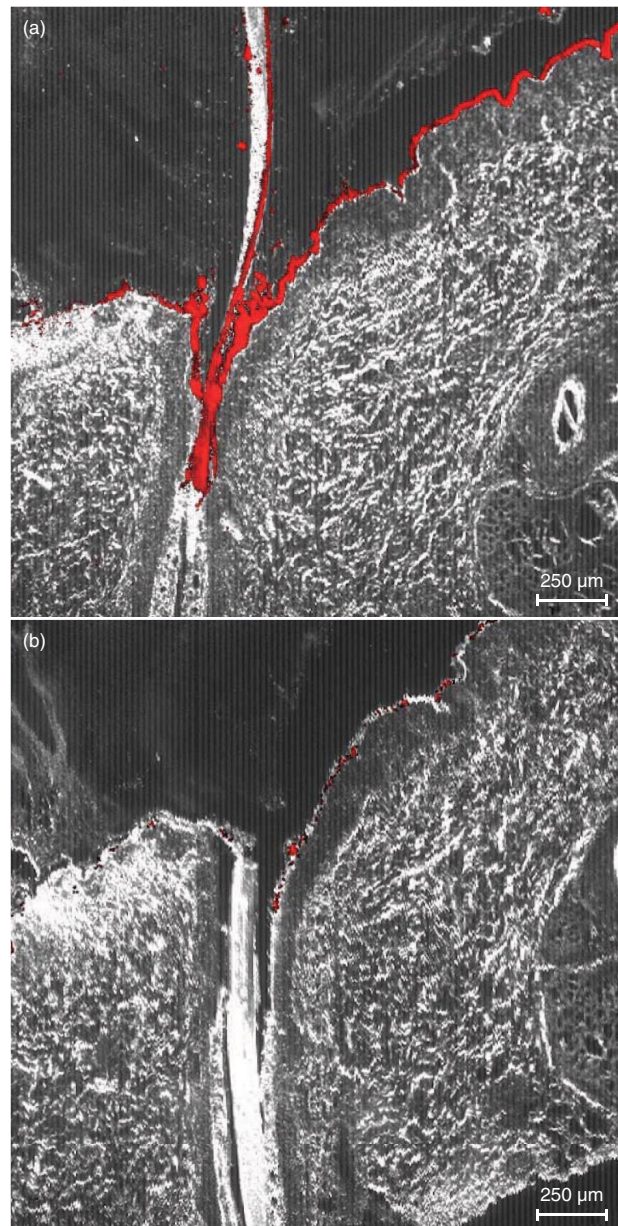


Figure 2 (online color at www.lphys.org) Histological images of hair follicles pre-treated with the fluorescent chlorophyll solution obtained by laser scanning microscopy (superposition of transmission and fluorescent mode). (a) – without plasma treatment (area A) and (b) – after plasma treatment (area B)

without plasma treatment). The red fluorescence corresponds to the distribution of the chlorophyll solution and is located on the skin surface and within the hair follicles.

In contrast, the histological section in Fig. 2b removed from area B (after plasma treatment) showed small spots of fluorescence on the skin surface, whereas no fluorescent

signal was detectable within the hair follicles. Analogical results were found for all 6 porcine samples.

During the application of the plasma jet, the tissue interaction zone was investigated visually. It was observed that the hairs act as a conductor for the plasma penetrating into the hair follicles.

4. Discussion

Laser scanning microscopy is a well suited method for the analysis of the skin structure and the investigation of the penetration of topically applied substances into and through the skin barrier [4,17,19]. The hair follicles possess a considerable amount of reservoir properties not only for topically applied substances, but also for micro-organisms, thus enabling re-colonization after antisepsis of the skin surface. Today, several efficient antiseptics are commercially available, all of which are applied in liquid form. Disadvantage of the liquid antiseptics is that they penetrate only into the orifices of the hair follicles and do not reach deeper parts, where the micro-organisms are likewise located [7,20]. In the present study, it could be visualized that the hairs act as a conductor transporting the plasma deep into the hair follicles, and probably, enabling a local antisepsis of the follicular reservoir. This hypothesis has been substantiated by further investigations analyzing the fluorescence behavior of a chlorophyll-containing particle solution, with and without plasma treatment by fluorescence laser scanning microscopy. A particle solution was chosen as the particulate structure allows a deeper and a more efficient penetration into the hair follicle than a non-particulate substance [20]. After plasma treatment, even in deeper parts of the hair follicles, the fluorescence signal had disappeared completely, probably due to the destruction or modification of the fluorescent dye chlorophyll during the plasma - dye interaction. Thermal destruction of the dye seems improbable as TTP produced by "kINPen09®" at a moving velocity of 10 mm/s does not lead to thermal damage of the tissue [9].

It is supposed that in addition to extremely specific effects, the mode of action of the plasma is based on the formation of highly reactive free radicals provoking surface lesions of micro-organisms. Additionally, synergistic effects of radical formation, the increase in temperature and the UV radiation generated by the plasma have to be considered.

The results of the present study clearly indicate that plasma is able to enter the follicular reservoir and thus can be considered as an effective antiseptic of the latter. The ability of follicle reservoir antisepsis represents a unique property and a distinct advantage in comparison to conventional antiseptics, which were shown, however, to be slightly more effective than plasma application. The marginal inferior power of plasma might be increased by utilizing a plasma stream of a greater diameter ensuring a homogeneous plasma treatment, which is not warranted

when utilizing a plasma stream of 2 mm in diameter as in the present study. Here, small residues of the skin remain untreated, as clearly demonstrated by the small spots of dye detectable on the skin surface (Fig. 2b). An additional promising possibility to increase the efficiency of the TTP is the parallel application of a chemical skin antiseptic.

As an application field for TTP, primarily, antisepsis of chronic wounds has to be considered, as TTP is well suited for application on distinct areas. Further advantages are probably a retarded re-colonization due to antisepsis of the follicular reservoir, absent resistances and improbable allergic or toxic reactions.

5. Conclusions

In summarizing these results, it was demonstrated that tissue-tolerable plasma is able to act both on the skin surface and also in the hair follicles, as the hair represents a conductor for the plasma. The ability to reach the follicular reservoir represents a distinct advantage in comparison to liquid antiseptics, which are located mainly on the skin surface or in the orifices of the hair follicles. The fact that the plasma tissue interaction zone of the applied plasma-jet had only a diameter of approximately 2 mm is obviously the reason why small residues of fluorescence could be detected on the skin surface. It can be expected that in the near future, the development of new improved systems with a greater diameter and a higher efficiency can be achieved.

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