

Abstract: Recently, it was reported that a plasma-jet could be efficiently applied for the antiseptics of wounds. In this case, the discharge in an argon gas stream was used to produce a so-called “cold plasma” on the skin surface. The thermal action of the plasma on the skin was investigated in the present study by means of laser scanning microscopy (LSM) and by histological analysis. Consequently, the plasma beam was moved with a definite velocity at an optimal distance over the skin surface. The structural changes of the tissue were analyzed. It was found by LSM that a thermal damage could be detected only in the upper cell layers of the stratum corneum (SC) at moving velocities of the plasma beam, usually applied in clinical practice. Deeper parts of the SC were not damaged. The structural changes were so superficial that they could be detected only by LSM but not by analysis of the histological sections.



Plasma-jet under working conditions

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Application of a plasma-jet for skin antiseptics: analysis of the thermal action of the plasma by laser scanning microscopy

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1. Introduction

The antiseptics of wounds still remains a problem, especially in the case of venous dysfunction, diabetes mellitus, pressure ulcers, and arterial circulatory disorders [1–4]. In this case, the treatment of the wound is often difficult, because of deep infections, as usually, the applied antiseptic

cannot penetrate into the tissue, on account of the occurrence of scabbing and crusting of the wounds [5, 6].

It was reported recently, that cold plasma at a temperature between 30°C and 50°C is well suited to destroy bacteria and fungi on the tissue surface, whereby a plasma beam is produced by an electrical discharge in a gas stream [5, 6].

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Figure 1 Plasma-jet under working conditions

Up to the present time, the investigations of the plasma tissue interactions were performed under *in vitro* conditions [7,8].

For the *in vivo* application of the plasma-jet, a risk assessment needs to be performed. There are four potential risk factors which have to be evaluated, i.e., the electrical safety of the plasma-jet, the produced UV-radiation, the induction of free radicals in the tissue, and the thermal effects. Recently, it was demonstrated that the UV-radiation of the plasma produced in an argon gas flow does not present any danger for the tissue [9]. Also, it could be shown, that the pulsed plasma is applicable without distinct irritation in respect to damage in the HET-CAM test, which opened the applicability on wounds or the eye, possibly due to the negligible thermal effects [5]. To analyze the thermal action on skin, in the present study a CE-certified plasma-jet (electromagnetic compatibility) was used, which fulfils the standards for electrical safety in humans.

The thermal action was investigated by laser scanning microscopy [10–14] and confirmed by histological analysis.

2. Materials and methods

2.1. Tissue samples

Pig ear skin was utilized for the experiments, which is a highly suitable model for human tissue [15]. The pig ears

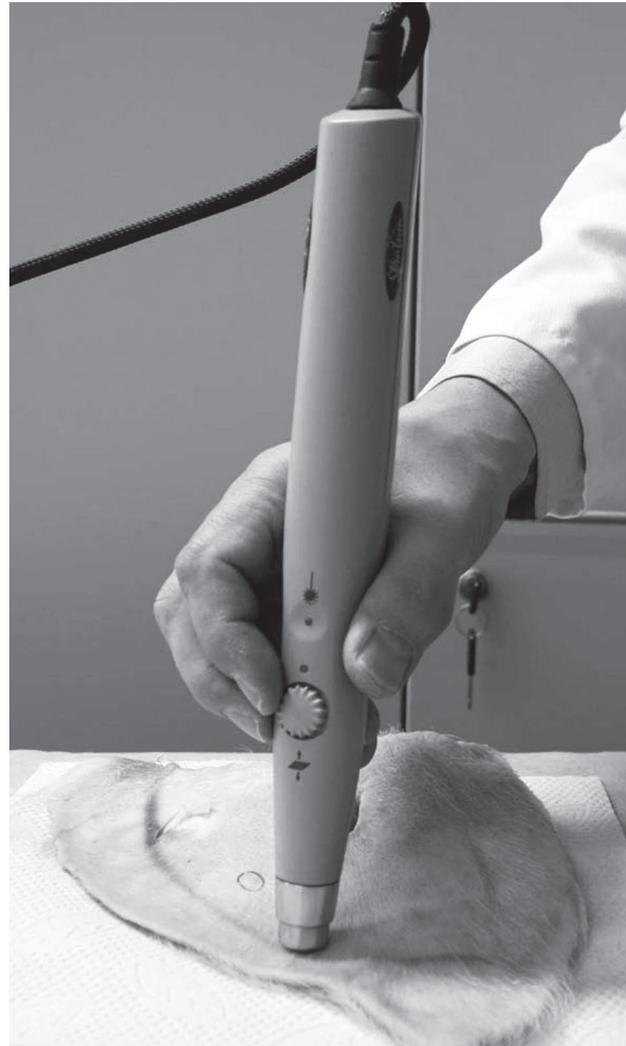


Figure 2 Analysis of the skin surface with the laser scanning microscope “Stratum”

had been freshly obtained from the local butcher and were investigated within 6 hours after slaughtering. Permission for the experiments had been granted by the Veterinary Medical Board of the District of Berlin–Treptow.

2.2. Plasma-jet

In the present study, the HF plasma-jet “kinpen09[®]” (CE certification No. 609.003.1) was used, developed by the Leibniz Institute for Plasma Science and Technology e.V. (INP) and manufactured by neoplas GmbH Greifswald.

The used plasma-jet fulfils the physical demands for an ideal interventional instrument in anti-infectious dermatotherapy [5].

Parameters such as UV radiation, temperature, generation of radicals, charged particles and electromagnetic

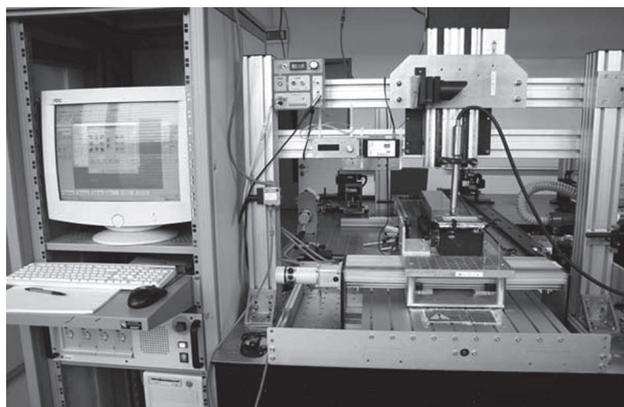


Figure 3 Experimental setup. The tissue is fixed onto a table, which could be moved in two directions with a control velocity

fields can be controlled in their composition offering different applications in medicine. Especially UV emission of atmospheric pressure plasma is a well known phenomenon [16, 17].

The used compact miniaturized atmospheric plasma exhibits a very promising technological potential for different surface treatments. The contracted and comparably cold plasma allows a treatment, even on objects sensitive to heat, such as tissue and cells with temperature loads to the surface between 30°C and 65°C [5, 9]. Argon gas was used as a discharge medium in the plasma-jet. A photograph of the plasma-jet under working conditions is presented in Fig. 1.

2.3. Laser scanning microscopy

The laser scanning microscope “Stratum” (Optilas, Melbourne, Australia) was used to analyze the tissue structure before and after treatment of the tissue with the plasma-jet. The Laser scanning microscope consists of a base station containing the argon laser ($\lambda = 488$ nm), the control and the detection unit. The base station is connected by optical fibers to a handpiece, containing the optical system and the focus control unit (Fig. 2). The measuring area was $250 \times 250 \mu\text{m}^2$ and the laser scanning microscope was used in the fluorescent mode [18]. Therefore, a solvent containing 0.1% of the fluorescent dye, fluorescein, was applied onto the skin surface, making the structure of the tissue visible.

2.4. Histological analysis

Biopsies with a diameter of 3 mm were taken from the tissue, before and after treatment with the plasma-jet. The biopsies were fixed in 4% buffered formaldehyde and embedded in wax. Histological sections (thickness of $4 \mu\text{m}$, hematoxylin & eosin staining) obtained from the biopsies

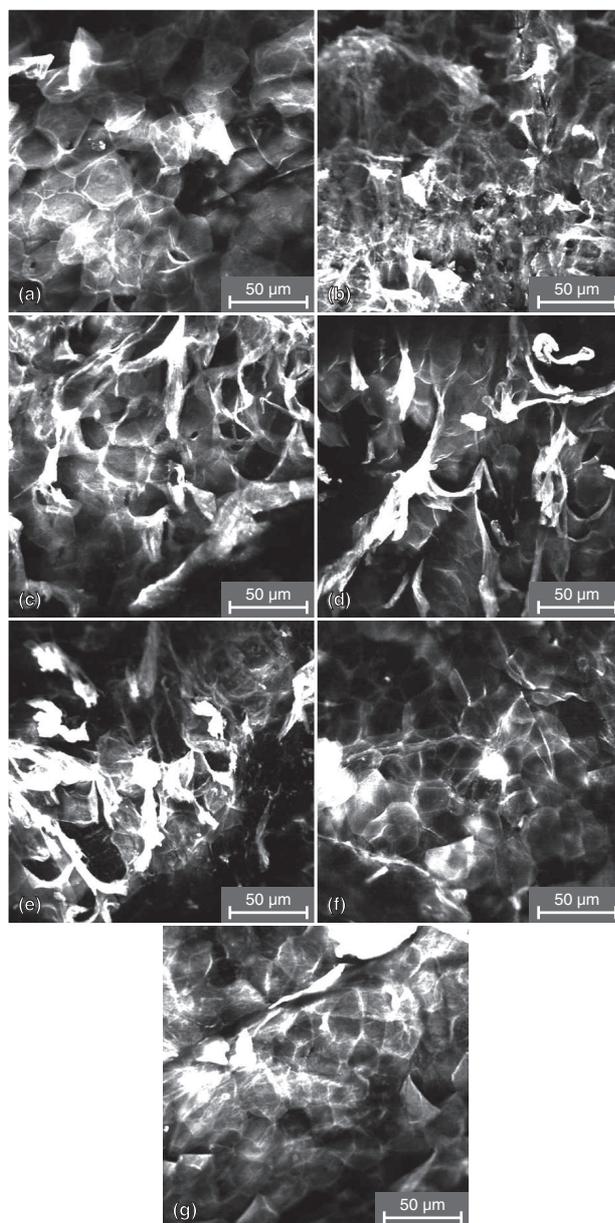


Figure 4 Typical LSM images of the skin surface before and after treatment of the skin with the plasma-jet at different moving velocities: (a) – non-treated skin, (b) – 2 mm/sec, (c) – 4 mm/sec, (d) – 6 mm/sec, (e) – 8 mm/sec, (f) – 10 mm/sec, and (g) – 12 mm/sec

were analyzed, concerning the possible thermal damage, with a standard light microscope BX 60, Olympus, Germany, at a 40-fold magnification.

2.5. Study design

The pig ears were cleaned under cold running water without addition of detergents and dried with paper towels.

Afterwards, they were fixed onto a table, which could be moved in two directions (X-Y-movement) with controlled velocities (Fig. 3). Six pig ears were treated and analyzed in the study. The plasma beam was moved with the following velocities on the pig ear: 2 mm/sec, 4 mm/sec, 6 mm/sec, 8 mm/sec, 10 mm/sec, and 12 mm/sec.

The plasma tissue interaction zone was 5 mm in diameter.

3. Results and discussion

Laser scanning microscopy is well suited to analyze the skin surface structure, noninvasively. The experiments in the present study were carried out on pig ear skin, as the structure of this tissue is very similar to human skin [19, 20]. The undamaged skin surface is characterized by a homogeneous arrangement of the corneocytes of the SC. These cells, surrounded by lipid layers, form the barrier of the body to the environment. In Fig. 4a, a typical image of such a skin surface is presented. The corneocytes can be well recognized; they form a homogeneous honeycomb structure. In preliminary experiments, it was found that the optimal moving velocity of the plasma beam on the skin surface should be between 5 and 8 mm/sec, in order to obtain the best antiseptic properties [9]. If the velocity in the experiments of the present study was reduced to 2 mm/sec, thermal damage of the skin surface could be detected, as demonstrated in Fig. 4b. The lipid layers were melted and the corneocytes thermally damaged. This destruction can be observed only on the skin surface, on the five layers of corneocytes. The SC consists of approximately 20 cell layers; consequently, the cell damage is only superficial.

This result has been confirmed by the analysis of the histological sections obtained from the plasma tissue interaction zone (Fig. 5a). No thermal damage could be detected in this case, as the changes were too superficial.

If the moving velocity of the plasma beam had been increased, the thermal damage of the skin surface was reduced, as can be seen in Fig. 4b–Fig. 4g. Again, no thermal damage could be detected on the corresponding histological sections (Fig. 5b–Fig. 5f). Furthermore, when the velocity was increased to > 10 mm/sec, no structural changes could be observed on the skin surface, also in the case of laser scanning microscopy (Fig. 4g). The corneocytes showed the same homogeneous structure, which is characteristic for an undamaged skin.

Taking into consideration the results obtained in previous experiments [9], the temperature in the plasma tissue interaction zone, at a moving velocity of the plasma, at $v = 5$ mm/sec, is in the range of 60°C, whilst at $v = 10$ mm/sec, the temperature is reduced down to 40°C. These temperature changes correspond to the thermal damage of the skin surface. At velocities between 5 mm/sec and 8 mm/sec, which are used in the practical application of the plasma-jet for antiseptics, thermal damage was observed only on the skin surface but not in deeper layers of the SC.

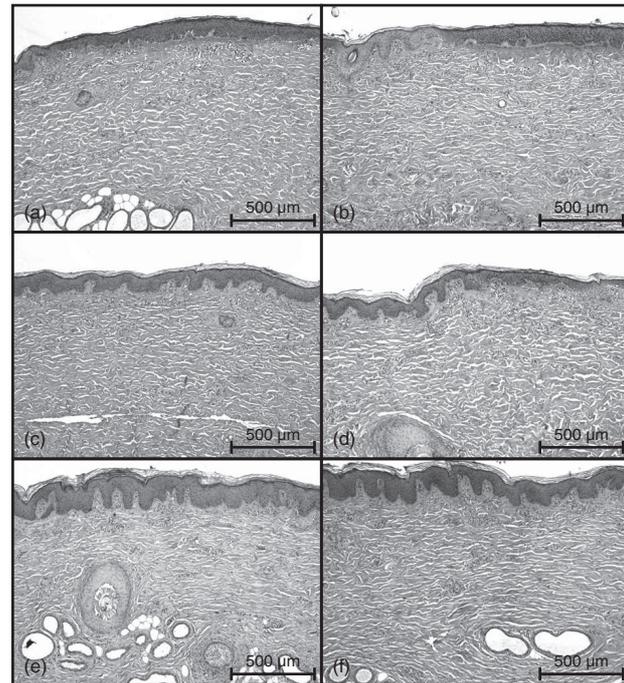


Figure 5 Typical histological sections obtained from biopsies removed after treatment of the skin with the plasma-jet at different moving velocities: (a) – 2 mm/sec, (b) – 4 mm/sec, (c) – 6 mm/sec, (d) – 8 mm/sec, (e) – 10 mm/sec, (f) – 12 mm/sec; H&E staining, 40-fold magnification

Therefore, it is clear that the temperature increase of the tissue during plasma treatment is not the only reason for the antiseptic properties of the plasma-jet. On the other hand, the single UV-radiation of the plasma is not efficient enough to sufficiently destroy the bacteria and fungi on the skin surface, as shown in the past [9]. Thus it seems that the high amount of radicals produced on the surface of the tissue in connection with temperature and UV radiation is responsible for the efficient antiseptic action.

Summarizing the results of the present study, it can be established that during the plasma tissue interaction under real conditions on skin, thermal damage of the tissue was only detected on the skin surface but not in deeper parts of the dead horny layer. Additionally, it could be demonstrated that laser scanning microscopy is better suited to analyze the surface effects of the skin than the classical histological analysis for investigation of superficial structural changes in the skin.

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