

Drug delivery through the skin barrier enhanced by treatment with tissue-tolerable plasma

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Abstract: Most treatments in dermatology and cosmetology are based on the penetration of topically applied drugs into the skin or through the skin barrier to the target structure in the living tissue. In the case of healthy skin, scarcely 1% of the applied drugs pass the skin barrier, depending on their chemical properties. Therefore, different physical and chemical methods have been developed to stimulate the penetration process. All these methods are based on the partial destruction of the barrier. In this study, an electrical tissue-tolerable plasma (TTP) was used to increase the penetration of a topically applied model drug (fluorescent dye) through the skin barrier. Using laser scanning microscopy, the distribution of the model drug in different depths

of the skin was investigated. It was found that the plasma treatment of the skin is a very efficient process to deliver topically applied substances into the living tissue. In the case of the non-plasma-treated skin, it was found that the fluorescent dye could be detected exclusively on the skin surface. If the dye was applied to the TTP-treated skin, it could be observed in high concentration also in deeper parts of the skin extending down to the stratum basale and the papillary structure.

Key words: laser scanning microscopy – lipid layers – plasma-jet – skin damage – Stratum corneum

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Introduction

The skin is the largest organ of the human organism. The uppermost layer of the skin – the stratum corneum (SC) – represents a barrier to the environment (1,2). It protects the organism against water loss and the body against penetration of harmful substances and micro-organisms from the environment (3,4).

A number of topically applied substances and drugs are only effective if they can pass the skin barrier to reach the living cells. Therefore, several chemical methods like skin hydration, application of ethanol, DMSO and liposomes and various physical methods such as phonophoresis and iontophoresis have been developed to enhance the penetration of topically applied substances through the skin. All these methods are based on a partial disruption of the skin barrier (2,5,6).

Recently, it was reported that cold plasma can be produced by an electrical discharge in a gas stream (7). This effect was used to develop a plasma-jet: a hand-held system, which can be moved in the same manner as a brush over the skin surface (8). In various *in vitro* studies, it could be demonstrated on skin models that the cold plasma is highly efficient for disinfection and antisepsis (8,9). It was demonstrated that the optimal plasma speed on the skin surface for obtaining the most select disinfection is between 5 and 10 mm/s (10). During this plasma treatment, the skin surface temperature ranges between 30 and 50°C. Lademann et al. (11) have shown that the thermal damage because of the plasma in the skin is only superficial inside the upper five layers of corneocytes (10).

The aim of this study was to investigate whether plasma treatment can be used to enhance the penetration of topically applied substances through the skin barrier.

Materials and methods

Tissue samples

The investigations were performed *in vitro* on porcine ear skin, which is a suitable model for human skin (12) as the approval for the *in vivo* application of the plasma-jet is still pending. The porcine ear skin was investigated within 6 h after slaughtering. The pig ears were washed with cold water and carefully dried with paper towels before the experiment started. Three different areas of 3 × 3 cm in size, each, were marked on the skin. One of these skin areas remained untreated, while the other two areas were treated at different speeds of the device movement. Approval for the experiment had been obtained from the Veterinary Board, Berlin-Treptow, Germany.

Plasma treatment

The investigations were performed using the plasma-jet 'kinpen09[®]' (CE certification No. 609.003.1) developed by the Leibniz Institute for Plasma Science and Technology e.V. (INP) produced by Neoplas GmbH, Greifswald (8). The top of the plasma stream acts in the mode of a 'brush' during the treatment of the skin. In Fig. 1, the nozzle of the plasma-jet and the plasma stream is shown. The temperature in the plasma–tissue interaction zones varies between 30 and 45°C, depending on the speed of the device movement on the skin surface. The tissue-tolerable plasma has been described in detail by Lademann et al. (7).

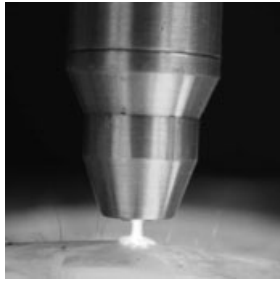


Figure 1. Plasma-jet in operation.

In this study, the skin samples were treated by the plasma-jet at two speeds of 5 mm/s (plasma exposure time 0.6 s) and 10 mm/s (plasma exposure time 0.3 s), each of which is suited for skin antiseptics (10).

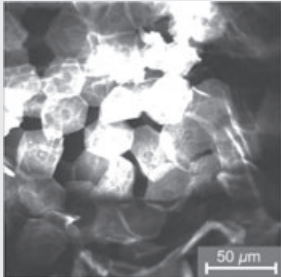
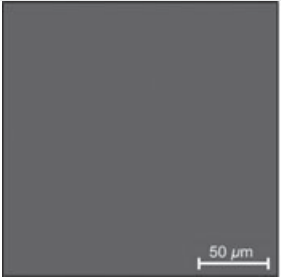

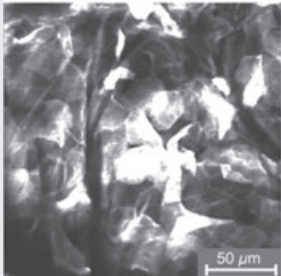
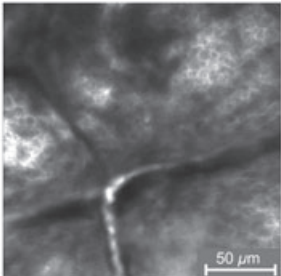
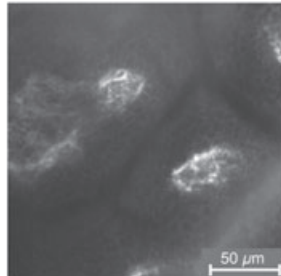
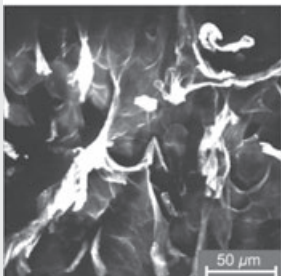
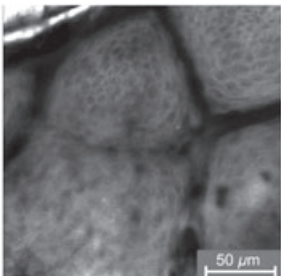
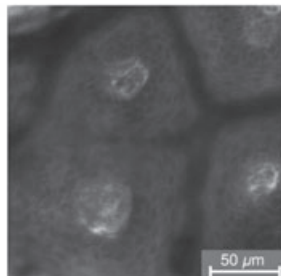
Model drug

A solution of 0.1% of the hydrophilic fluorescent dye, fluorescein, in water was applied onto the non-plasma-treated skin and onto the skin areas 1 min after plasma treatment; $10 \mu\text{l}/\text{cm}^2$ of the solution was applied onto the skin surface using a pipette and was homogeneously distributed with a saturated rubber-gloved finger.

Laser scanning microscopy

Following a penetration time of 15 min, the supernatant of the applied solution was removed by filter tissue. The filter tissue contacted the skin surface for 3 s. Subsequently, the distribution of the dye on the skin surface of the samples and in deeper layers of the skin was analysed by using the *in vivo* laser scanning microscope 'Stratum' (Optilas, Melbourne, Australia). The laser scanning microscope consists of a base, which accommodates the argon laser emitting at a wavelength of 488 nm as well as the control and the detection units. The base is connected by optical

Table 1. Detection of the dye in different depths of the skin without and after plasma treatment

Treatment	Skin surface	Stratum basale	Papillary dermis
Non-treated			
10 mm/s			
5 mm/s			

fibres to a handpiece containing the optical detection system and the focus control unit. The system has been described in detail by Lademann et al. (11).

Results

In Table 1, the distribution of the dye on the skin surface, in the stratum basale and in the papillary dermis is shown for the untreated and plasma-treated skin (speed: 10 and 5 mm/s).

In the case of the non-plasma-treated skin, the dye is only located on the skin surface and in the lipid layers of the uppermost cell layers of the SC. The structure of the corneocytes can be well recognized. In the stratum basale and the papillary dermis, no dye could be detected. When the dye was applied after the plasma treatment of the skin, high amounts of the dye could also be detected in the stratum granulosum, the stratum spinosum, the stratum basale and around the papillary structure for both speeds of the device movement.

When the skin had been treated at a speed of 10 mm/s, the corneocytes on the skin surface showed almost no changes during the plasma treatment, as can be seen in Table 1. When the treatment speed was reduced to 5 mm/s, changes in the structure of the corneocytes could be detected in the first cell layers.

Discussion

The fact that no thermal damage could be observed during the plasma treatment at 10 mm/s is in agreement with the results reported by Lademann et al. (10), who stated that under the conditions applied in the study, no thermal damage to the skin surface and deeper parts of the living epidermis had been detected by laser scanning microscopy or by histological analysis.

Taking into consideration the distribution of the dye in the tissue, it seems that during the plasma treatment of the skin, the lipid layers in the SC had been damaged. Consequently, the skin barrier had become permeable for the topically applied hydrophilic substance. Changes in the lipid structures after heat treat-

ment have been reported previously (13–15). Further investigations are required, however, to establish to what extent the enhanced penetration documented in this study is based on thermal effects, like reaching the transitional temperature, which causes the lipid structure to change, or on the interaction of the lipid structures with the electrical plasma, respectively.

The experiments in the present study were performed under *in vitro* conditions, without the skin barrier regenerating after plasma treatment. Under *in vivo* conditions, such a regeneration process has to be considered. Therefore, it can be expected that there is a limited period of time after plasma treatment, when an efficient penetration of topically applied substances into the skin occurs. This process requires additional investigations.

An application of a formulation onto the skin prior to plasma treatment is not recommended, because chemical changes in the topically applied substances during the interaction with the electrical plasma cannot be excluded.

From earlier studies, it is known that the plasma treatment results in a disinfection of the skin surface (7,8,16). Consequently, the risk of infection, which may be caused by the short-term disruption of the skin barrier, can be neglected.

Summarizing the results of the present study, it can be established that the treatment of the skin surface with TTP is an efficient method for stimulating the penetration of topically applied substances through the skin barrier with a low risk of infection.

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