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Research paper

Penetration and storage of particles in human skin: Perspectives and safety aspects

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ABSTRACT

The application of particles in dermatology and cosmetology represents an emerging field and is closely connected with the question of risk assessment as the potential for, and consequences of, penetration of such particles into the living tissue has not been determined conclusively. In the medical sector, extensive research activities are in progress to develop particles, which can be used as efficient carriers for drug delivery through the skin barrier. In contrast, in cosmetic products, particles are mostly required to remain on the skin surface to fulfill their beneficial effect. Whereas the intercellular penetration of particles seems to be unlikely, the hair follicle has been shown to be a relevant penetration pathway for particles as well as an important long-term reservoir. It has been demonstrated that the penetration depth of the particles can be influenced by their size resulting in the possibility of a differentiated targeting of specific follicular structures. In the present review, the follicular penetration mechanisms and storage properties of particles are discussed.

1. Introduction

Particles such as titanium dioxide (TiO_2) are often used in sunscreens to increase the sun protection factor (SPF). Their absorption properties are in the UV spectral range, provoking the partial reduction of sun radiation reaching the skin. Even more efficient is the protection based on the scattering properties of the TiO_2 particles, which act as nanomirrors on the skin surface and in the upper layers of the stratum corneum. In this case, a disposition of the particles on the skin surface is required; a penetration through the skin barrier would be counterproductive and has not been observed [1,2].

In contrast, for medical applications, particles are considered to be efficient carrier systems for drug delivery through the skin barrier. Especially, the hair follicles exhibit an important reservoir for these particles [3]. The hair follicles are surrounded by a dense network of blood capillaries [4,5], which is essential for drug delivery and ensures the systemic uptake. In addition, hair follicles are targets for regenerative medicine and immunomodulation, being the host of stem cells, surrounded by a high density of dendritic cells [6].

Particles, at a size of some hundreds of nanometers, were shown to penetrate deeply into the hair follicles, where they are stored up to 10 days, which is 10 times longer than in the stratum corneum [7]. In the present paper, the prospects and safety aspects of nanoparticles topically applied in dermatology and cosmetology are discussed.

2. Particles and follicular penetration

Previously, when the intercellular penetration of sunscreens containing TiO_2 particles was investigated by tape stripping, first indications emerged that particles might penetrate deeply into the hair follicles [8]. After long-term application of sunscreens, i.e., twice daily for a period of 2 weeks, the tape strips removed from deeper parts of the stratum corneum still contained TiO_2 particles. After visualizing the hair follicles on the tape strip by staining with OsO_4 and X-ray fluorescence microscopy, it was found that the particles on the first tape strips were localized in the lipid layers around the corneocytes. On the tape strips removed from deeper parts of the horny layer, the TiO_2 particles could be detected exclusively in the orifices of the hair follicles. Subsequently, biopsies were removed to exclude the potential penetration of TiO_2 into the living skin. X-ray fluorescence microscopy confirmed that the particles were localized on the skin surface and in the upper layers of the stratum corneum. In addition, well detectable amounts of TiO_2 could be found in the hair follicles, but not outside the hair follicles in the living epidermis [8].

Surprisingly, not all hair follicles contained particles. For the penetration of topically applied substances, it has to be distinguished between "open" and "closed" hair follicles [9]. The effect of "open" and "closed" hair follicles has been the subject of detailed investigations. For this purpose, the follicle properties were analyzed in vivo on various body sites. Follicle maps were prepared for different skin areas, and the properties of the hair follicles like

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hair growth and sebum production were investigated for a time period of several weeks. In a final step, a dye-containing formulation was applied onto the skin. The penetration of the dye was analyzed non-invasively using the cyanoacrylate surface biopsy technique (Fig. 1). It was found that only those hair follicles were "open" for penetration, which exhibited hair growth and/or sebum production, implying a mass flow out of the hair follicles [9]. These unexpected results were explained by the existence of a hair follicle covering, which is removed by hair growth or sebum production enabling topically applied substances to enter the hair follicles. The covering consisting of desquamated corneocytes and dried sebum could be visualized by optical coherence tomography measurements [10]. Also, external mechanical manipulations such as intensive washing or peeling are able to remove the covering.

3. Size-dependence of the penetration of particles into the hair follicles

The particles found in the hair follicles after sunscreen application were approximately 100 nm in size. Later, Toll et al. [11] investigated the penetration of fluorescence-labeled particles of different sizes between 750 and 6000 nm into the hair follicles by taking biopsies and analyzing histological sections as shown in Fig. 2. It was found that the particles with the smaller diameter penetrated most excellently into the hair follicles. Subsequently, Lademann et al. [7] compared the follicular penetration of the same amount of a fluorescent dye, both in non-particular and particular (320 nm) form, under in vitro conditions. Unexpectedly, the particulate formulation penetrated better into the hair follicles than the formulation without particles. However, this effect was only observed when a massage was applied using an appropriate device. The investigations were performed on porcine ear skin, which represents a suitable model for human skin.

Fig. 1. Cyanoacrylate skin surface biopsy: method for non-invasive removal of the follicular content.

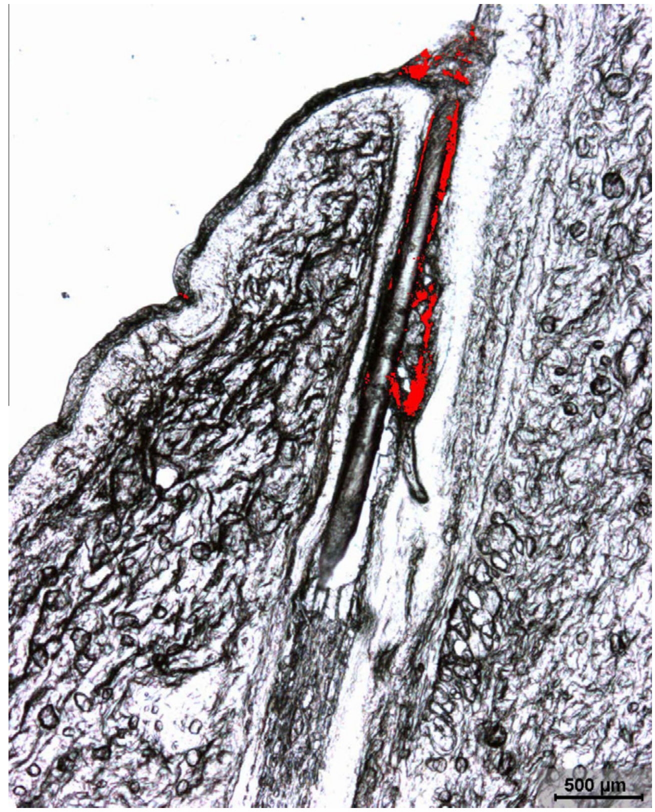

Fig. 2. Follicular penetration. Histological section demonstrating the penetration of fluorescence-labeled particles into a hair follicle.

In the same study, both aforementioned formulations were likewise applied in vivo on the calf of human volunteers. For analytical realization, the non-invasive technique of differential stripping [12] was employed. It was found that the dye in the particle form penetrated better into the hair follicles than the formulation not containing particles. Additionally, the storage time for the particles was significantly increased [7].

Taking these findings into consideration, the optimum size for penetration into the hair follicles was estimated to be between 320 nm and 750 nm. Analyzing various types of particles at different sizes consisting of diverse materials with dissimilar surface properties revealed that particles of approximately 300–600 nm in size showed the deepest penetration into the hair follicles [6], where they were stored significantly longer than in the stratum corneum.

4. Penetration mechanism of particles

Particle penetration in vitro was shown to be increased when massage was applied [7]. Massage seems to induce hair movement, which occurs physiologically under in vivo conditions. The optimum size of the particles, which was around 300–600 nm, corresponded approximately to the size of the cuticula of the hairs, which was found to be in the region of 530 nm in the case of human hairs, and 320 nm in the case of porcine skin [13] (Fig. 3). Based on the information stating that different types of particles consisting of various materials, exhibiting dissimilar surface properties, showed a similar penetration behavior, it was hypothesized that moving hairs act as a geared pump, pushing the particles deeply into the hair follicles. Once penetrated, the particles are entrapped within the hair follicles and are depleted only by means of slow processes, such as hair growth and sebum flow [14].

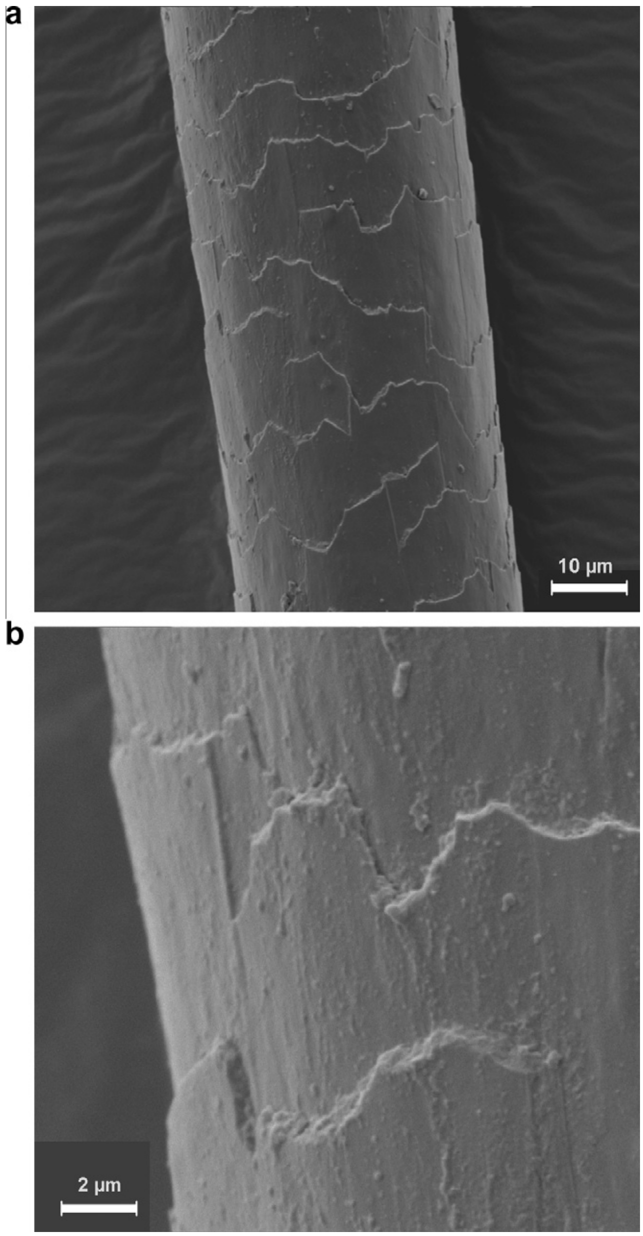

Fig. 3. Hair surface structure. The surface structure of a human terminal hair is determined by the cuticula (two different magnifications).

5. Hair follicle size

Next to the penetration depths of particles, likewise, the size of the follicular reservoir, in comparison with the reservoir of the stratum corneum, has become a topic of interest. In this context, Otberg et al. [15] investigated the volume of hair follicles of different volunteers on various body sites using the cyanoacrylate skin surface biopsy method. The highest follicular volume could be observed on the forehead and on the calf region, in comparison with the back, thorax, upper arm, forearm and the thigh. On the forehead, a high density of small (vellus) hair follicles is developed, whereas on the calf, a low density of large (terminal) hair follicles is present. The volume of the hair follicles on both these body sites was comparable to the reservoir of the stratum corneum. The volume of the hair follicles on the scalp was not closely determined, but is most likely to be even larger than the volume of the forehead and the calf.

In the light of these results, the hair follicles have to be classified as important and long-term storage target structures in the skin.

6. Safety aspects of particles

In the focus of public discussion, the safety aspect of particles, in general, has changed. In this context, the question arises whether topically applied particles are able to overcome the skin barrier. The hair follicles represent interruptions in an otherwise highly potent skin barrier and may act as pitfalls for topically applied particles, which are not able to overcome the skin barrier via intercellular penetration. Topically applied substances can follow different routes once they penetrate into a hair follicle. Small molecule drugs can penetrate through the follicular epithelium into the living tissue, where they may be taken up by the blood circulation. Substances that are too large for penetration, such as particles, are entrapped within the hair follicles, where they are depleted only by slow processes such as hair growth and sebum flow [6].

The potential for penetration of nanoparticles into the living tissue has not been determined conclusively. Most investigations so far have been carried out in vitro, the number of in vivo human studies being few. Several recent studies concluded that metal oxide nanoparticles are not able to penetrate the stratum corneum [16–19]. In contrast, Gulson et al. [20] described the absorption of zinc from zinc oxide particles (19 nm and ⩾100 nm) in sunscreens through human skin admitting that the zinc detected in the blood and urine was not necessarily in the form of ZnO (nanoparticles) as ZnO particles can partially dissolve. Vogt et al. [21] reported that nanoparticles of 40 nm in size, which were topically applied to excised human skin, could be detected in Langerhans cells. However, it has to be considered that the particles were applied to a previously interrupted skin barrier, as cyanoacrylate skin surface biopsies had been removed beforehand. Several further studies likewise demonstrated that nanoparticles were able to pass the skin barrier; however, in many cases, the skin barrier had been manipulated. Rouse et al. [22] found a penetration of fullerenes on dermatomed porcine skin only after skin flexing. Other authors utilized diffusion cell experiments [23,24]. Zhang et al. [25] found quantum dots only penetrating into the upper stratum corneum layers in intact skin, whereas the quantum dots reached the living cells, when the skin had been damaged previously.

Taking into consideration the state of the art, there are no indications that particles of ⩾100 nm in size can pass the healthy skin barrier.

For nanoparticles of ⩽100 nm in size, the investigations are still continuing, but it can be expected that also nanoparticles of ⩾40 nm in diameter do not penetrate through the intact skin barrier.

7. Prospects of drug delivery using particles

The results obtained in various studies demonstrate that the particles applied in sunscreens are safe, as they remain on the skin surface or around the upper cell layers of the stratum corneum protecting the skin against UV radiation of the sun.

Nevertheless, the particles can likewise be applied as drug carrier systems due to their effective follicular penetration behavior. Within the hair follicles, at least 4 target sites of interest have been recently defined (see Fig. 4) [26]. The sebaceous gland (1) and the bulge area (2) are of particular interest. The sebaceous gland is associated with the aetiology of androgenetic alopecia, acne and other sebaceous gland dysfunctions [6]. The bulge region is the host of the epithelial stem cells and provides a high proliferate

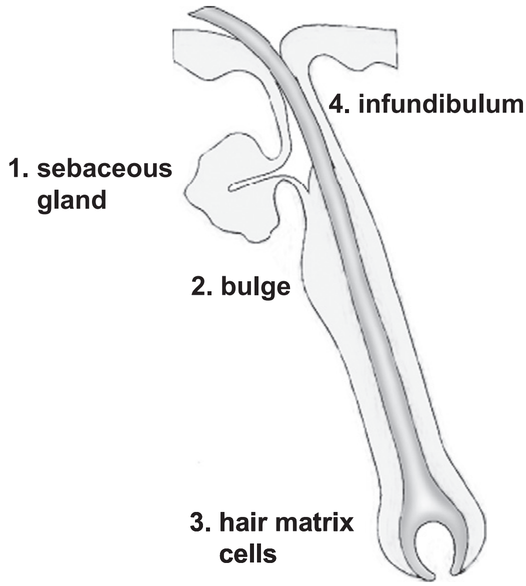

Fig. 4. Follicular targeting. Four different target structures of interest within the hair follicle.

capacity and multipotency [27]. The hair matrix cells (3) may also represent prospective target sites in terms of hair growth control [28]. Around the infundibulum region (4), a high density of immune competent cells is located. Thus, the hair follicle provides important target structures for drug delivery, regenerative medicine and immunomodulation.

Advantages of particulate carrier systems are, on the one hand, the deep and preferred penetration into the hair follicle and, on the other hand, the long storage time within the hair follicle [29]. Drug-loaded particles with release mechanisms should be preferred in comparison with drug carrier systems, as the particle is only the transporter bringing the drug exactly to the specific target site within the follicle. After being released, the drug must penetrate independently.

Whilst the process of particle transport into the hair follicles has been well investigated, the release of the drugs from the nanoparticles onto the correct target site is still a topic of intensive investigation.

Acknowledgements

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