Determination of the cuticula thickness of human and porcine hairs and their potential influence on the penetration of nanoparticles into the hair follicles

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Abstract. An efficient penetration and long-term storage of topically applied substances is important for drug delivery in medical treatment and cosmetics. It has recently become apparent that the hair follicles represent an efficient and long-term reservoir for topically applied substances. It was found that particles sized 300–600 nm penetrate more efficiently into the hair follicles than smaller or larger particles. In the present paper, the hair surface structure of human and porcine hairs was analyzed by electron microscopy. It could be observed that the thickness of the cuticula corresponds to the optimal size of the nanoparticles for penetration into the hair follicles. Additionally, it could be demonstrated that the cuticula of human vellus and terminal hairs were of similar thickness (approx. 530 nm), while the thickness of the cuticula obtained from porcine ear bristles were slightly thinner (approx. 320 nm). © *2009 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.3078813]

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1 Introduction

Drug delivery into and through the human skin is a topic of intense research in pharmacology and cosmetology.[1–3] An evaluation and optimization of drugs and cosmetic products requires knowledge concerning the penetration pathways.[4] In the past, it was assumed that topically applied substances pass through the human skin by intercellular penetration, i.e., along the lipid layers around the corneocytes.[5–7] Recently it was found that the hair follicles also represent efficient penetration pathways.[8–11]

For drug delivery, the hair follicles are of special interest because they are surrounded by a close network of blood capillaries[8] and dendritic cells,[12] and also host stem cells,[13] which are important for immunology and regenerative medicine.

By artificially closing human hair follicles *in vivo* using a special wax/varnish mixture, it was possible to disable follicular penetration.[9] By applying a formulation containing caffeine on the same skin area of volunteers under the conditions of "open" and "closed" hair follicles, it was found that in the case of the "open" hair follicles, caffeine could be detected in the blood much earlier than in the case of "closed" hair follicles.[8,10] In addition to small molecules such as caffeine, particles could also penetrate into and were stored in the hair follicles. Toll et al.[14] investigated the penetration of fluorescing particles sized between 600 and 1.5 μm into the human hair follicles. The investigations were carried out *in vitro* on excised human skin. After application and penetration, biopsies were taken from the tissue samples. The histological sections obtained from these biopsies were analyzed by laser scanning microscopy. It was found that the smallest particles penetrated efficiently into the hair follicles. Lademann et al.[15,16] analyzed the penetration of a fluorescent dye in the particle and nonparticulate form into the hair follicles. Both formulations contained the same amount of the fluorescent dye fluorescein. The particles had a diameter of 320 nm. The experiments were performed on porcine skin, which is a suitable model for human skin. Again, biopsies were taken, and the distribution of the dye in the hair follicles was analyzed in histological sections using laser scanning microscopy.[17,18]

In a first series of experiments,[15] both formulations were applied without massage. In the second part of the experiments, massage was applied using a commercial massage device. In the first case, penetration depths of approx. 300 μm were determined for both formulations. However, when massage was applied, the penetration depths of the nonparticulate formulation increased slightly up to 400 μm, while the penetration of the particulate formulation increased strongly up to 1500 μm. This was surprising, as it was not clear why the particles sized 320 nm penetrated deeper and more efficiently into the hair follicles than the nonparticulate formulation.

Further experiments were performed *in vivo* on human calf

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skin.[16] Because the experiments were conducted *in vivo*, it was not possible to take a high number of biopsies from the same volunteer. Therefore, the penetration and storage of the two formulations were investigated noninvasively using differential stripping.[19] In this method, the stratum corneum (SC) is first removed by tape stripping, and the follicular casts are subsequently removed by cyanoacrylate biopsies as described in detail previously.[19] The amount of dye in the SC and in the hair follicles was determined over a period of 10 days. It was found that the particles were stored longer than 10 days in the hair follicles, while only a small amount of the formulation containing no particles had penetrated into the hair follicles, where it could be detected only for four days.[16] These results confirm the *in vivo* observation that nanoparticles penetrate into the hair follicles more efficiently than nonparticulate formulations.[15] On the other hand, taking the findings of Toll et al.[14] into consideration, particles sized >600 nm showed a less efficient penetration than smaller particles. This suggests that there is an optimal particle size between 300 and 600 nm at which the particles penetrate most efficiently into the hair follicles. It is proposed that this optimal size might be related to the surface structure of the hairs, as efficient penetration and storage could only be achieved under the condition of massage appliance which could induce a moving of the hairs in the hair follicles.

The aim of the present study was to analyze the surface structure of the hairs with electron microscopy in order to determine the thickness of the hair cuticles close to the skin surface and to establish whether a relationship exists between the thickness and the optimal particle size for follicular penetration. As similar results have been observed *in vivo* for human skin and *in vitro* for the model tissue porcine skin, the investigations were carried out with human and porcine hairs.

2 Material and Methods

2.1 *Hair Samples*

Porcine ear hairs of freshly slaughtered crossbred pigs (Deutsches Edelschwein, Deutsches Hausschwein, and Duroc) were obtained from the Gut Hesterberg (Neuruppin–Lichtenberg, Germany). At the time of slaughtering, the animals were 7–8 months old and weighed approximately 120 kg. Human terminal and vellus hairs were obtained from Caucasian males, who were recruited at the Department of Dermatology. In total, 50 porcine ear hairs, 50 human vellus hairs, and 50 human terminal hairs were investigated.

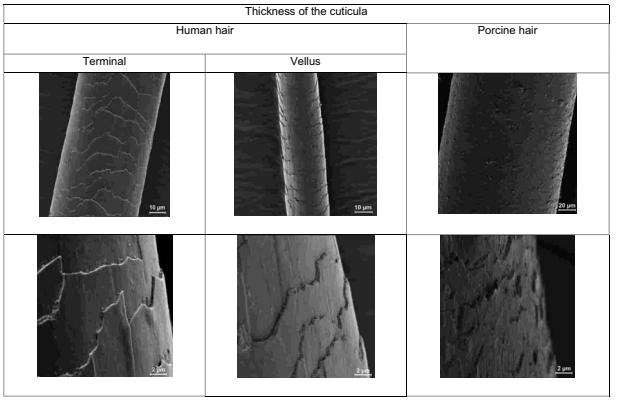
2.2 *Electron Microscopy*

To date, numerous studies have been conducted on the cuticle scale pattern of hair for purposes of diagnostic research as well as forensic science. Light microscopy was commonly used in the past to study scale patterns. This method is very limited however, as these structures are transparent. The recent development of the scanning electron microscope (SEM) has given rise to a more promising method of analysis in which a direct observation of the sample is possible using high-resolution and magnification.

As hairs are of a relatively hard and dry composite, they require little preparation for the SEM. They withstand the vacuum of the coating unit and the examination chamber without overt distortion, and only little damage is caused to the hair cuticle by the electron beam at normal kilovolt ranges. For each individual, the hairs were lined up and mounted with double adhesive labels (Plane, Marburg) onto aluminum specimen plates. The probes were vaporized with gold in a sputter coater (BAL-TEC, SCD 050) for 120 s (specimen coating 30–50 nm). Using the SEM (Zeiss, DSM 950) of the Institute of Veterinary Anatomy of the Free University Berlin, digital photographs were taken of the structure of the hair of each individual. For digital viewing, the computer program Orion (Version 6.60.5) was used.

The photographs were later analyzed by measuring the maximum thickness of the hair cuticles in accordance with the magnification of the SEM so as to provide average values of the thickness of the keratin cells.

Table 1 Electron microscopic images of the surface structure of human vellus and terminal hairs and hairs obtained from porcine skin.



3 Results

Typical electron microscopic images of the surface structure of human vellus and terminal hairs, as well as hairs obtained from porcine skin, are presented in Table 1 in two different magnifications. As demonstrated in the figures, the surface of the hairs represents a zigzag structure formed by the cuticula. The different hair types presented in Fig. 1 are of different diameters. The average thickness of the cuticula and its standard deviation were determined for all hair types. The results are summarized in Fig. 1.

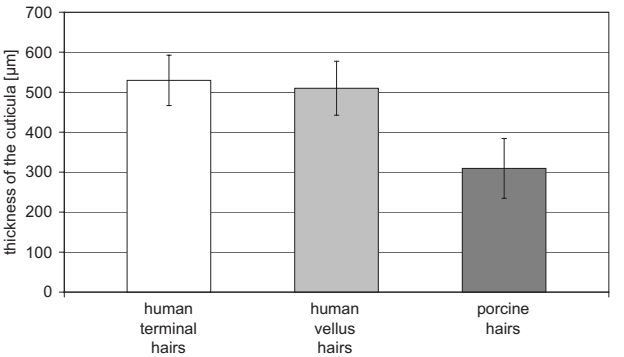


Fig. 1 Average values of the maximum of the thickness of the cuticula and the standard deviations determined for human terminal and vellus hairs, as well as for porcine hairs.

As can be seen in Fig. 1, the thickness of the cuticula of the human vellus and terminal hairs are almost identical (~530 nm thick), while the cuticula of hairs obtained from porcine skin is smaller (~320 nm thick).

4 Discussion

In the past it was assumed that topically applied substances can only pass the barrier of the human skin by the intercellular route.[1,20] Only after the development of optical analytical methods with high spatial resolution such as laser scanning microscopy did it become possible to analyze follicular penetration.[21–23] Otberg et al. demonstrated that the reservoir of the hair follicles is comparable to the reservoir of the SC for topically applied substances in some body sites.[24] The hair follicles represent an interesting target for drug delivery, because they are surrounded by a close network of blood capillaries and dendritic cells; moreover, they host stem cells.[12,13,8]

By *in vivo* as well as *in vitro* investigations, it was found that topically applied particles penetrate efficiently into the hair follicles at a size of several hundred nanometers.[15,16] It seems that the optimal size for follicular penetration of particles should be between 300 and 600 nm, as it was shown that smaller and larger particles penetrate less efficiently into the hair follicles. This penetration process is strongly stimulated by massage application, which might induce movement of the hair in the hair follicle.

The results obtained in the present study demonstrate that the hair surface consists of a zigzag structure formed by the cuticula. The thickness of the cuticula (approx. 530 nm) is similar in human vellus and terminal hairs, while in the case of hairs obtained from porcine skin, this value is significantly smaller (approx. 320 nm) ($p < 0.05$).

Relating the results obtained in penetration studies of nanoparticles (optimal particle size between 300 and 600 nm under *in vivo* conditions or under massage application) to the results of the present study (cuticula thickness of approx. 530 nm in human hairs versus approx. 320 nm in porcine hairs), it can be concluded that the cuticula thickness might have an influence on the penetration of nanoparticles. It is suggested that the moving hair acts as a geared pump, pushing the nanoparticles deeply into the hair follicles. In this context it has to be taken into consideration that the corneocytes of the stratum corneum are extended deep into the orifices of the hair follicles. These corneocytes have a thickness similar to the cuticula of the hairs and are structured in the same way. Therefore, not only the hairs but also the orifices of the hair follicle show this typical zigzag structure.

Hair movement occurs naturally *in vivo* and can be stimulated *in vitro* by massage. This suggests that the penetration process of nanoparticles may be strongly influenced by mechanical forces. The penetration process of the nanoparticles into the hair follicles stimulated by the moving hair is assumed to take place as long as there is a gradient of concentration from the skin surface to deeper parts of the hair follicle. If the concentration of the particles on the skin surface and in the orifice of the hair follicles is reduced by washing, textile contact, and desquamation, the concentration gradient disappears. In this case, the particles stored in the hair follicles will be transported by sebum flow out of the hair follicle back to the skin surface, which is a slow process. This hypothesis is confirmed by the kinetics of the storage process of the particles and the hair follicles, because it was found that after application, the concentration of the particles in the hair follicles decreases over a period of 10 days.

The penetration of particles sized between 300 and 600 nm through the hair follicles into the living tissue can be neglected, as the hair follicle is also protected by a barrier. In the upper part of the hair follicle, the barrier is similar to the structure of the SC, while in deeper parts of the hair follicles, a barrier is represented by tight junctions. Consequently, the best strategy for drug delivery into the hair follicles will be to use the particles as carrier systems for drugs. This means that nanoparticles must be loaded with active substances which penetrate and can be stored together with the nanoparticles in the hair follicles for several days. In the hair follicles, drugs should be released from the nanoparticles and should pass the barrier of the hair follicles independently. This concept of drug delivery is partly realized by the application of liposomes in cosmetics and pharmaceuticals.

The results obtained in this study emphasize that follicular penetration should be investigated under *in vivo* conditions or using model tissue with the application of massage. Because of the similar surface structure of human hairs and porcine hairs, investigations concerning the optimal size of particles for penetration and storage in the hair follicles and also for dermatopharmacokinetics can be carried out on porcine skin as a suitable model[25] for human skin. In this case, it must be taken into consideration that the optimal size of the nanoparticles applied to porcine skin is smaller than in the *in vivo* experiments on humans because of the differences in the thickness of the cuticula. In contrast to the penetration process, the release process of nanoparticles out of the hair follicles can be investigated only under *in vivo* conditions, because in this case not only the moving hair, but also the sebum production, may influence this process.

Based on the results obtained in this paper, further studies must be carried out to demonstrate that in the case of the porcine skin, the optimal size of the nanoparticles is in the region of 300 nm, in contrast to human skin, where the optimal size should be in the region of 500 nm. The penetration of drug carriers into the hair follicles is not only interesting for the treatment of humans, but also for the field of veterinary medicine. Most mammals have a thick coat and a higher concentration of hair follicles per square centimeter than humans. It is of interest to investigate whether different animal species of animals require a special drug carrier size.

Summarizing these results, it has to be assumed that based on the zigzag structure of the hair surface, the moving hair can be considered as a geared pump for pushing nanoparticles into the hair follicles. The typical thickness of the cuticula of human hairs is approx. 530 nm and in the case of porcine hair approx. 320 nm. Optimal penetration and storage occurs if the topically applied nanoparticles have similar dimensions as the cuticula. Particles sized several hundreds of nanometers can efficiently penetrate into the hair follicles, but they cannot pass through the hair follicles into the living tissue. Therefore, for drug delivery strategies, it would be helpful to use the nanoparticles as carrier and storage systems in the hair follicles. These particles should be loaded with an active drug. After transport of the drug into the hair follicles, the drug

should be released to reach the target structures, such as the blood capillaries, the dendritic cells, or the stem cells. As the moving hairs seem to be essential for follicular penetration, studies in dermatopharmacokinetics must be performed under *in vivo* conditions or on model tissue, such as porcine skin, where the hairs should be moved artificially. Nevertheless, of the differences in the thickness of the cuticula of human and porcine skin, pig ears are a suitable model for follicular penetration studies.

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