

Hair follicles as a target structure for nanoparticles

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For at least two decades, nanoparticles have been investigated for their capability to deliver topically applied substances through the skin barrier. Based on findings that nanoparticles are highly suitable for penetrating the blood–brain barrier, their use for drug delivery through the skin has become a topic of intense research. In spite of the research efforts by academia and industry, a commercial product permitting the nanoparticle-assisted delivery of topically applied drugs has not yet been developed. However, nanoparticles of approximately 600 nm in diameter have been shown to penetrate efficiently into the hair follicles, where they can be stored for several days. The successful loading of nanoparticles with drugs and their triggered release inside the hair follicle may present an ideal method for localized drug delivery. Depending on the particle size, such a method would permit targeting specific structures in the hair follicles such as stem cells or immune cells or blood vessels found in the vicinity of the hair follicles.

Keywords: Skin barrier; penetration; *stratum corneum*; hair follicles; triggered release.

1. Introduction

For more than 20 years, continuous efforts have been made to utilize nanoparticles for the delivery of topically applied drugs through the cutaneous

barrier.^{1–4} These research activities were stimulated by experiments which showed that nanoparticles penetrated efficiently through the blood–brain barrier.⁵ However, a commercial product for

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nanoparticle-assisted delivery of drugs through the cutaneous barrier is still lacking. The first investigations showing that nanoparticles can penetrate into the hair follicles were performed using sunscreens that contained not only chemical UV filters but also physical filters in the form of TiO_2 nanoparticles.⁶ These sunscreens were applied several times a day for a period of two weeks under conditions usually prevailing at the beach. After these two weeks, the *stratum corneum* was removed by tape stripping,^{7,8} and penetration profiles were developed for the TiO_2 .⁶ It turned out that most of the TiO_2 was located on the skin surface, with small amounts of TiO_2 also being detected in deeper layers of the *stratum corneum*. Subsequently, the location of the hair follicles on the stripped tapes could be visualized by staining them with OsO_4 .⁶ It could be shown that in the deeper layers of the *stratum corneum*, the TiO_2 particles were detectable only in the orifices of the hair follicles. As the tape stripping method is unsuitable for taking biopsies from the epidermis, standard biopsies were also taken. From these biopsies, histological sections were prepared and subjected to X-ray fluorescence microscopy to investigate the distribution of the TiO_2 .⁶ The results demonstrated that TiO_2 was detectable in the uppermost layers of the *stratum corneum* as well as in some hair follicles.

2. Open and Closed Hair Follicles

Surrounded by a dense network of capillaries, the hair follicles are interesting target structures. They also host stem cells, which play an essential role in regenerative medicine,⁹ and dendritic cells that are important for immunomodulation.¹⁰ Addressing the exciting topics of nanoparticle penetration into the hair follicles and the existence of open and closed hair follicles, a new study was launched. In the frame of this study, skin areas were marked on different body sites and subsequently photographed. Using the tape stripping method, tapes were removed from the respective skin areas and stained with OsO_4 . In this way, the exact positions of the hair follicles could be determined and were mapped in a follicle map. In addition, sebum production and hair growth were investigated for a period of four weeks, using the sebum tape method and the trichogram technique, respectively. As a result of these

investigations, a specific number was assigned to every hair follicle and its properties were registered in the follicle map. A formulation containing a fluorescent dye was then applied onto the skin surface. By means of cyanoacrylate surface biopsy, the hair follicles were removed noninvasively and subsequently analyzed for the presence of the penetrated dye by fluorescence microscopy. As a result, it turned out that hair follicles that produced sebum or demonstrated hair growth were receptive for penetration, suggesting that a topically applied substance can obviously penetrate into the hair follicles only if a mass flow occurs out of the hair follicle onto the skin surface. While nanoparticles were found to penetrate into the follicular ducts within minutes, both sebum production and hair growth are slow processes extending over several hours or days. This led to the conclusion that the hair follicles must have been blocked by a plug that must be removed before the externally applied substance could penetrate.¹¹ Such a plug was successfully detected using optical coherence tomography, and it was shown that it consisted of desquamated corneocytes and dried sebum.¹² These can be easily removed by washing or mild peeling, so as to render the hair follicles receptive for penetration in the respective skin areas. A typical cyanoacrylate biopsy removed after application of the fluorescent dye is presented in Fig. 1. The fluorescent dye penetrated deeply into the hair follicle where it could be easily detected by excitation using UV light.

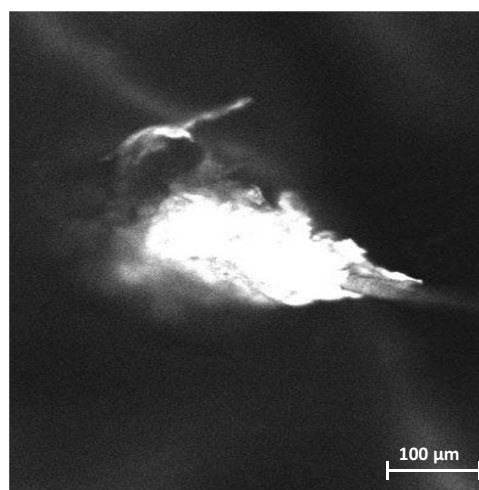


Fig. 1. Cyanoacrylate biopsy removed from the human back skin after topical application of a fluorescent dye.^{12,13}

3. Size Comparison Between the Hair Follicle Reservoir and the *Stratum Corneum* Reservoir

An important factor for an efficient drug delivery to follicular structures is the magnitude of the follicular reservoir. Otberg *et al.*¹⁴ investigated cyanoacrylate tapes stripped from various sites of the human body. With these tapes, the hair follicle contents were removed noninvasively from the skin and could subsequently be analyzed by laser scanning microscopy. In this context, the volumes of the respective hair follicles were determined. The largest follicular volumes were found on the forehead, which accommodates a large number of small vellus hairs, and on the male calf with its numerous terminal hairs.¹⁴ The largest hair follicle reservoir could be determined in the scalp region. Comparing the reservoir of the hair follicles with that of the *stratum corneum*, it becomes apparent that the follicular reservoir on both the forehead and the calf is comparable to the reservoir of the stratum corneum.¹⁴

4. Caffeine Penetration by the Intercellular and Follicular Pathway

Various studies focused on follicular penetration in the past involved mice or rats.^{15–17} Newborn animals, which had not yet developed any hair follicles, were examined for the penetration of topically applied substances. These experiments were repeated

on the same animals once their hair follicles had developed. The findings suggested that follicular penetration had occurred.^{15–17} However, as the *stratum corneum* was modified as animals grew older, a direct comparison between the follicular and the intercellular penetration was not possible. Subsequently, the Center of Experimental and Cutaneous Physiology developed a method permitting the follicular and intercellular penetration to be compared *in vivo*.¹⁸ For this purpose, a 5×5 cm skin area on the male chest was investigated. The hairs in the test area were carefully clipped and the follicular orifices blocked with a lacquer-wax mixture. After the volunteers were subjected to a caffeine diet, a caffeine-containing formulation was applied onto the relevant skin area, and the caffeine concentration in the blood was measured prior to and at various times after application of this formulation.¹⁸ Subsequent to another caffeine diet, the lacquer-wax mixture was applied directly adjacent to the hair follicles in order to ensure that in both experiments the skin surface occluded by the lacquer-wax mixture was of identical size and the caffeine-containing formulation was applied.¹⁹ A comparison of the two experiments disclosed that enhanced caffeine concentrations were detectable in the blood already after 5 min in the open hair follicles, whereas it took up to 20 min until a weak caffeine signal could be detected in the occluded hair follicles. The typical kinetics for one volunteer is demonstrated in Fig. 2.

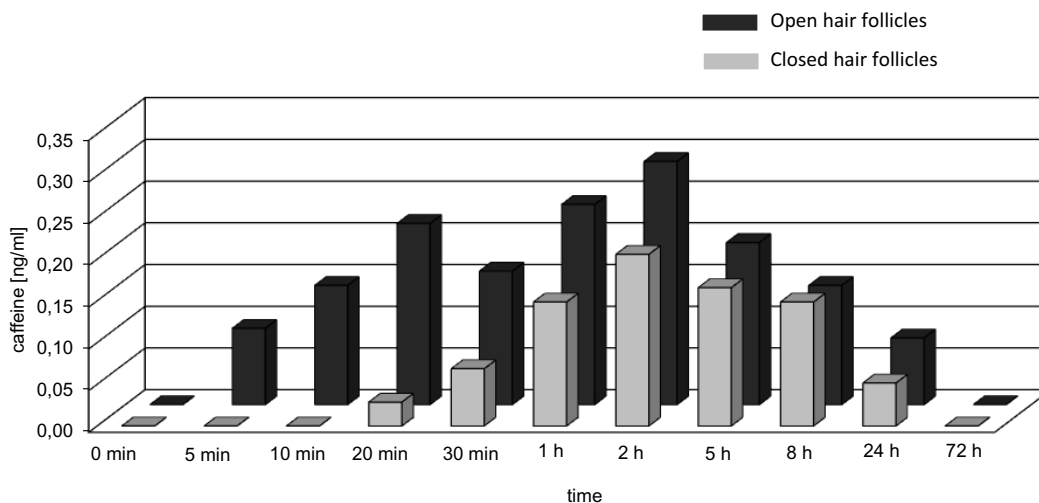


Fig. 2. Kinetics of the caffeine penetration via the intercellular and follicular pathways through the skin barrier and into the blood.¹⁹

As a result of these experiments, it could be evidenced for the first time that the hair follicles serve as a reservoir for topically applied substances and may permit penetration into viable tissues. However, the caffeine concentrations measured in the blood were extremely low ranging in the ng/mL blood scale.

5. Nanoparticle Penetration into the Hair Follicles

Based on the already described TiO₂ investigations, further investigations were conducted to determine the optimal nanoparticle sizes for penetration into the hair follicles. As these experiments required a large number of biopsies, they could not be performed *in vivo*. Therefore, porcine ear skin was implemented for the experiments. Porcine ear is a commonly used model for human skin that is optimally suited for studying the follicular penetration.²⁰ as — contrary to human skin — porcine ear skin does not contract after excision.²¹ In these *in vitro* investigations, two different formulations were applied that contained the same amount of dye, once in non particulate form and once in particulate (particle size 320 nm) form.²² The formulations were applied onto porcine ear skin and were allowed to penetrate. After a penetration time of 60 min, the porcine ears were biopsied. Subsequently, histological sections were taken from the biopsies and investigated for dye penetration into the hair follicles. Unexpectedly, no differences were found in the penetration depths of the particulate and non-particulate formulations; in both cases the dyes penetrated up to approximately 300 μm in depth.²² In order to simulate the *in vivo* situation, the *in vitro* experiments were repeated. This time, porcine ear skin was treated with a massage device for 1 min after topical application of the formulations. It was found that the particulate dye penetrated significantly deeper into the hair follicles than the nonparticulate dye. For the particulate formulation, a penetration depth of 1500 μm was determined, whereas the nonparticulate formulation penetrated to a depth of only 400 μm into the hair follicles.²²

Further experiments were performed on the calves of human volunteers using the differential stripping method instead of biopsies that were impracticable for *in vivo* investigations.²³ Subsequent to the topical application of a substance and an

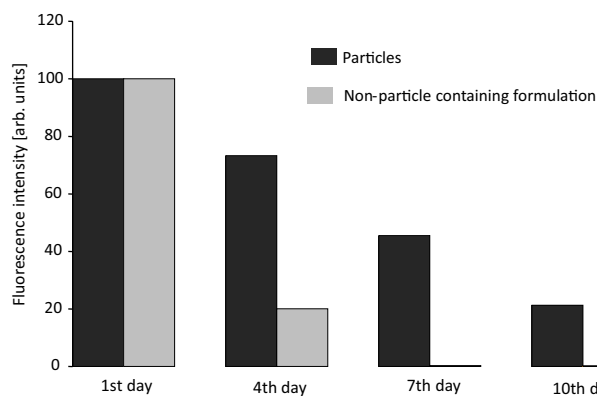


Fig. 3. Kinetics of the same concentration of a fluorescent dye in nonparticulate and particulate forms *in vivo* on human skin.²³

appropriate penetration time, the *stratum corneum* was removed by tape stripping. Thereafter the follicular contents were removed from the tapes by cyanoacrylate surface biopsies. Both samples were analyzed separately for their specific contents. The diagram in Fig. 3 shows the storage of the particulate and nonparticulate dyes in the hair follicle over a period of 10 days.²³ As early as on day 4, only low concentrations of the nonparticulate dye were detectable in the hair follicle, while the particulate dye was still visible on day 10. Consequently, these *in vivo* experiments confirmed the results of the previous *in vitro* investigations on porcine ear skin.

6. Optimal Particle Size for Penetration into the Hair Follicles

Unexpectedly, the results of the described investigations showed that particles of 320 nm in size penetrated much more efficiently into the hair follicles than nonparticulate substances. To determine the optimal particle size for follicular penetration into the hair follicles, the penetration of particles of various compositions and sizes were investigated. Toll *et al.* compared nanospheres ranging from 750 nm to 6 μm in diameter for their penetration into the hair follicles.²⁴ These investigations were also conducted on porcine ear skin. Again, biopsies were taken and cut into histological sections. This study revealed that the smallest particles of 750 nm penetrated most efficiently into the hair follicles.

Next, six different sizes of differently composed particles were analyzed for their penetration capacity into the hair follicles.²⁵ The results of this

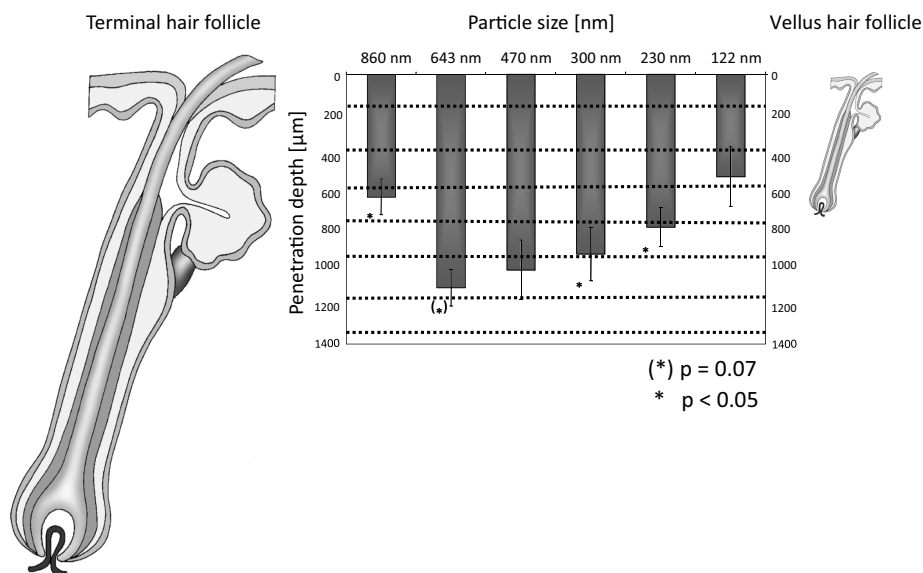


Fig. 4. Penetration of different sizes of particles into the hair follicles.²⁵

analysis are shown in Fig. 4. As can be seen from this figure, particles of approximately 600–700 nm penetrated most efficiently into the hair follicles. In addition, the figure reveals that the penetration depth of the particles could be influenced by the particle size. Almost no differences were observed in terms of the materials used and the surface structure of the particles. However, such effects were only noticed if the topical application of the formulation was followed by massage.²⁵ The efficient penetration of particles sized approximately 600 nm could be explained by examining the hair surface structure.²⁶ Figure 5 shows the cuticles of a hair shaft. The cuticle thickness amounts to approximately 600 nm. Consequently, the moving hair acts like a

geared pump that delivers the nanoparticles directly into the hair follicles.

So far, only the penetration of nanoparticles into the hair follicles has been reported, however, with the exception of caffeine, no penetration was observed into the viable skin. The successful penetration of nanoparticles larger than 20 nm through the intact follicular barrier could not yet be demonstrated.^{27,28} The caffeine study revealed that small molecules, such as caffeine, are capable of penetrating through the barrier of the hair follicle, but do not penetrate efficiently into the hair follicles.^{29,30} Summarizing these results, nanoparticles have been identified as efficient carrier systems into the hair follicles. For drug delivery applications, they must be loaded with a drug that will be released inside the follicle close to the target structures. The released drug must then penetrate the follicular barrier.

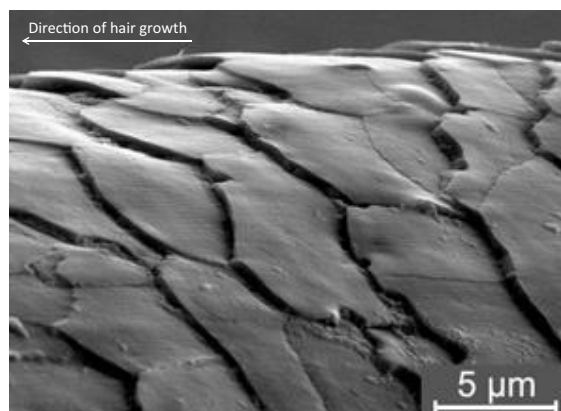


Fig. 5. Cuticular structure of the human terminal hair.²⁶

7. Triggered Release of Drugs from Nanoparticles

Based on the described results, a new strategy was developed using nanoparticles as efficient drug carriers for triggered release inside the hair follicles.³¹ For this purpose, nanoparticles comprised of bovine serum albumin (BSA) were loaded with a fluorescent model drug. Subsequently, the nanoparticles were degraded within 60 min by the addition of a

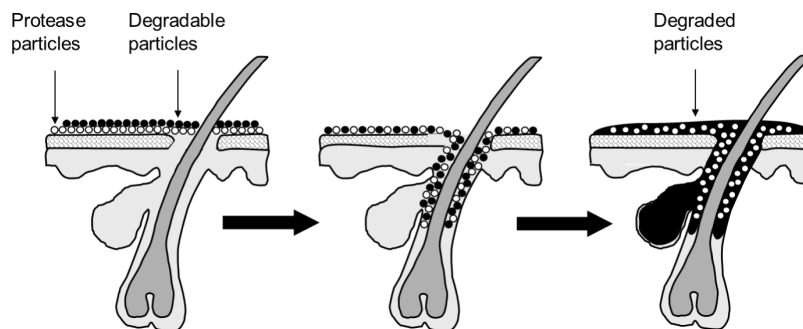


Fig. 6. Modified schematic diagram of penetration and release concept.³²

protease. When the particles and the protease were applied successively, the model drug was released only in the upper region of the hair follicles into which the protease had penetrated, as visualized by detecting fluorescence. The release of the model drug could be demonstrated by increased fluorescence intensity as the dye on the particles was only weakly fluorescent due to quenching processes.³¹ Although the principle of triggered release of drugs from nanoparticles could be shown using this method, a two-step approach may be impractical for patients in a clinical setting. In a further experiment which is schematically depicted in Fig. 6, the protease was tagged with rhodamine and included in CaCO₃ porous silicon particles that were applied together with BSA particles loaded with the model drug fluorescein isothiocyanate on to porcine ear skin.³² This enabled the detection of the efficient release of the model drug inside the hair follicle down to a depth of 900 μm . Thus, this two-component system proved to be an efficient tool for transferring highly concentrated drugs into the hair follicles and their localized release.³² A third experiment focused on the release of the model drug by an external trigger signal. For this purpose, the drug-loaded particles were coated with gold. Subsequent to penetration, the skin was exposed to water-filtered infrared radiation,³³ which was absorbed by the gold-coated particles. This caused a heating of the nanoparticles, which in turn led to their degradation and enabled the drug release. In this case, penetration depths between 900 μm and 1000 μm were also achieved.

8. Summary

The use of nanoparticles for drug delivery through the human skin could not be implemented as

originally intended as the cellular and intercellular barriers of healthy skin prove to be very strong, preventing the penetration of nanoparticles into viable tissue. On the other hand, it could be shown that the hair follicles represent an efficient reservoir, particularly for particles of approximately 600 nm in size. However, these particles cannot penetrate through the follicular barrier without assistance, while small molecules such as drugs, once localized within the hair follicle, may penetrate the follicular barrier as shown for caffeine. Therefore, a concept of triggered release of drugs from nanoparticles was developed by loading the specific drugs onto particles. The particles were subsequently applied in such a manner that they penetrated into the hair follicles. Finally, the model drugs were released from the particles by means of an external trigger signal. Once released, the model drugs were capable of penetrating through the barrier of the hair follicle without assistance. Thus, a new concept of triggered drug delivery via nanoparticles was established which requires further intensive research in the years to come.

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References

1. J. Lademann, H. Richter, M. C. Meinke, B. Lange-Asschenfeldt, C. Antoniou, W. C. Mak *et al.*, “Drug delivery with topically applied nanoparticles: Science fiction or reality,” *Skin Pharmacol. Physiol.* **26**(4–6), 227–233 (2013).

2. J. L. Jenita, V. Chocalingam, B. Wilson, "Albumin nanoparticles coated with polysorbate 80 as a novel drug carrier for the delivery of antiretroviral drug-Efavirenz," *Int. J. Pharm. Investig.* **4**(3), 142–148 (2014).
3. S. Scalia, E. Franceschinis, D. Bertelli, V. Iannucelli, "Comparative evaluation of the effect of permeation enhancers, lipid nanoparticles and colloidal silica on *in vivo* human skin penetration of quercetin," *Skin Pharmacol. Physiol.* **26**(2), 57–67 (2013).
4. M. B. Delgado-Charro, "Richard Guy and his collaborators: 'Crackling' the skin code," *Skin Pharmacol. Physiol.* **26**(4–6), 302–312 (2013).
5. Y. Cheng, Q. Dai, R. A. Morshed, X. Fan, M. L. Wegscheid, D. A. Wainwright *et al.*, "Blood-Brain Barrier permeable gold nanoparticles: An efficient delivery platform for enhanced malignant glioma therapy and imaging," *Small* **12**(8), 73–77 (2014).
6. J. Lademann, H. J. Weigmann, C. Rickmeyer, H. Barthelmes, H. Schaefer, G. Mueller *et al.*, "Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice," *Skin Pharmacol. Appl.* **12**(5), 247–256 (1999).
7. F. Kluschke, H. J. Weigmann, S. Schanzer, M. Meinke, T. Vergou, W. Sterry *et al.*, "Gain or loss? Sunscreen efficiency after cosmetic pretreatment of the skin," *Skin Pharmacol. Physiol.* **27**(2), 82–89 (2014).
8. O. Vogt, J. Lademann, F. Rancan, M. C. Meinke, S. Schanzer, E. Stockfleth *et al.*, "Photoprotective properties of the fluorescent europium complex in UV-irradiated skin," *Skin Pharmacol. Physiol.* **26**(2), 76–84 (2013).
9. W. R. Heath, S. N. Mueller, "Hair follicles: Gatekeepers to the epidermis," *Nat. Immunol.* **13**(8), 715–717 (2012).
10. A. Kosykh, A. Ngamjariyawat, S. Vasylovska, N. Konig, C. Trolle, J. Lau *et al.*, "Neural crest stem cells from hair follicles and boundary cap have different effects on pancreatic islets *in vitro*," *Int. J. Neurosci.* **19**(9) 1–21 (2014).
11. N. Otberg, H. Richter, H. Schaefer, U. Blume-Peytavi, W. Sterry, J. Lademann, "Visualization of topically applied fluorescent dyes in hair follicles by laser scanning microscopy," *Laser Phys.* **13**(5), 761–764 (2003).
12. N. Otberg, H. Richter, A. Knuttel, H. Schaefer, W. Sterry, J. Lademann, "Laser spectroscopic methods for the characterization of open and closed follicles," *Laser Phys. Lett.* **1**(1), 46–49 (2004).
13. J. Lademann, N. Otberg, U. Jacobi, R. M. Hoffman, U. Blume-Peytavi, "Follicular penetration and targeting," *J. Investig. Dermatol. Symp. Proc.* **10**(3), 301–303 (2005).
14. N. Otberg, H. Richter, H. Schaefer, U. Blume-Peytavi, W. Sterry, J. Lademann, "Variations of hair follicle size and distribution in different body sites," *J. Investig. Dermatol.* **122**(1), 14–19 (2004).
15. X. J. Xing, L. Yang, Y. You, B. Y. Zhong, Q. H. Song, J. Deng *et al.*, "Study of the biological function and penetration pathways of the mouse epidermal growth factor ethosomal delivery system," *Exp. Dermatol.* **20**(11), 945–947 (2011).
16. B. Mahe, A. Vogt, C. Liard, D. Duffy, V. Abadie, O. Bonduelle *et al.*, "Nanoparticle-based targeting of vaccine compounds to skin antigen-presenting cells by hair follicles and their transport in mice," *J. Investig. Dermatol.* **129**(5), 1156–1164 (2009).
17. S. Dokka, S. R. Cooper, S. Kelly, G. E. Hardee, J. G. Karras, "Dermal delivery of topically applied oligonucleotides via follicular transport in mouse skin," *J. Investig. Dermatol.* **124**(5), 971–975 (2005).
18. A. Teichmann, N. Otberg, U. Jacobi, W. Sterry, J. Lademann, "Follicular penetration: Development of a method to block the follicles selectively against the penetration of topically applied substances," *Skin Pharmacol. Physiol.* **19**(4), 216–223 (2006).
19. N. Otberg, A. Patzelt, U. Rasulev, T. Hagemester, M. Linscheid, R. Sinkgraven *et al.*, "The role of hair follicles in the percutaneous absorption of caffeine," *Br. J. Clin. Pharmacol.* **65**(4), 488–492 (2008).
20. A. Patzelt, H. Richter, R. Buettemeyer, H. J. Huber, U. Blume-Peytavi, W. Sterry *et al.*, "Differential stripping demonstrates a significant reduction of the hair follicle reservoir *in vitro* compared to *in vivo*," *Eur. J. Pharm. Biopharm.* **70**(1), 234–238 (2008).
21. J. Lademann, A. Patzelt, H. Richter, S. Schanzer, W. Sterry, A. Filbry *et al.*, "Comparison of two *in vitro* models for the analysis of follicular penetration and its prevention by barrier emulsions," *Eur. J. Pharm. Biopharm.* **72**(3), 600–604 (2009).
22. J. Lademann, H. Richter, A. Teichmann, N. Otberg, U. Blume-Peytavi, J. Luengo *et al.*, "Nanoparticles — An efficient carrier for drug delivery into the hair follicles," *Eur. J. Pharm. Biopharm.* **66**(2), 159–164 (2007).
23. J. Lademann, H. Richter, U. F. Schaefer, U. Blume-Peytavi, A. Teichmann, N. Otberg *et al.*, "Hair follicles — A long-term reservoir for drug delivery," *Skin Pharmacol. Physiol.* **19**(4), 232–236 (2006).
24. R. Toll, U. Jacobi, H. Richter, J. Lademann, H. Schaefer, U. Blume-Peytavi, "Penetration profile of microspheres in follicular targeting of terminal hair follicles," *J. Investig. Dermatol.* **123**(1), 168–176 (2004).

25. A. Patzelt, H. Richter, F. Knorr, U. Schafer, C. M. Lehr, L. Dahne *et al.*, "Selective follicular targeting by modification of the particle sizes," *J. Control. Release* **150**(1), 45–48 (2011).
26. M. Ossadnik, H. Richter, A. Teichmann, S. Koch, U. Schafer, R. Wepf *et al.*, "Investigation of differences in follicular penetration of particle- and nonparticle-containing emulsions by laser scanning microscopy," *Laser Phys.* **16**(5), 747–750 (2006).
27. A. Melero, A. Ferreira Ourique, S. S. Guterres, A. R. Pohlmann, C. M. Lehr, R. C. R. Beck *et al.*, "Nanoencapsulation in lipid-core nanocapsules controls mometasone furoate skin permeability rate and its penetration to the deeper skin layers," *Skin Pharmacol. Physiol.* **27**(4), 217 (2014).
28. K. Heinrich, U. Heinrich, H. Tronnier, "Influence of different cosmetic formulations on the human skin barrier," *Skin Pharmacol. Physiol.* **27**(3), 141–147 (2014).
29. S. Mutalik, H. S. Parekh, Y. G. Anissimov, J. E. Grice, M. S. Roberts, "Iontophoresis-mediated transdermal permeation of peptide dendrimers across human epidermis," *Skin Pharmacol. Physiol.* **26**(3), 127–138 (2013).
30. S. Naumann, S. Lange, G. Polak, V. Kalhoelfer, L. Motlagh, A. Goebel *et al.*, "Penetration studies of an extremely lipophilic active model substance from an oil-in-water emulsion: Influence of the lipophilicity of the formulation in human skin – part 2," *Skin Pharmacol. Physiol.* **27**(2), 97–105 (2014).
31. W. C. Mak, H. Richter, A. Patzelt, W. Sterry, K. K. Lai, R. Renneberg *et al.*, "Drug delivery into the skin by degradable particles," *Eur. J. Pharm. Biopharm.* **79**(1), 23–27 (2011).
32. W. C. Mak, A. Patzelt, H. Richter, R. Renneberg, K. K. Lai, E. Ruhl *et al.*, "Triggering of drug release of particles in hair follicles," *J. Control. Release* **160**(3), 509–514 (2012).
33. J. W. Fluhr, P. Caspers, J. A. van der Pol, H. Richter, W. Sterry, J. Lademann *et al.*, "Kinetics of carotenoid distribution in human skin *in vivo* after exogenous stress: Disinfectant and wIRA-induced carotenoid depletion recovers from outside to inside," *J. Biomed. Opt.* **16**(3), 035002 (2011).