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Influence of molecular properties and delivery system design on the transfollicular transport across the skin

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Citation

Grams, Y. Y. (2005, January 12). *Influence of molecular properties and delivery system design on the transfollicular transport across the skin*. Retrieved from <https://hdl.handle.net/1887/598>

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For the treatment of diseases, drugs can be delivered either to the systemic blood circulation or to the local target area. For systemic delivery, various routes are feasible of which the oral route is the most common one. However, the oral route has the disadvantage of the hepatic first-pass effect. Therefore alternative routes of administration are of great interest. One of these routes is the transdermal route. Transdermal delivery has the potential to deliver drugs continuously into the systemic circulation thereby circumventing the first-pass metabolism. However, when focussing on drug delivery to regions in the skin such as the hair follicle, sweat and sebaceous glands, application of the drug on the skin surface also has the potential to increase the drug concentration at the site of action. Additionally the delivery into the systemic circulation might be decreased thereby reducing possible side-effects.

The rationale of topical delivery may be of particular interest for skin diseases such as acne, cancer and alopecia, which originate in the pilosebaceous unit (1-4). Furthermore it may also be of interest for cosmetic products to improve e.g. the hair condition. The latter can be achieved by targeting active ingredients to the pilosebaceous unit. Local delivery can be improved by two approaches. The first approach is the selection of an appropriate formulation, which might contain particulate carriers and additives such as ethanol, surfactants and propylene glycol. A second approach is the selection of a possible drug candidate. The physicochemical parameters of the drug, e.g. size, charge and lipophilicity, can affect the degree of delivery and targeting. In those particular cases where adaptation of the formulation is not feasible, delivery and targeting can only be improved by changing the physicochemical parameters of the penetrant itself.

In order to study local drug delivery to the hair follicle, information on changes in drug accumulation in time in the various skin regions is required. Since the hair follicle can reach a depth of more than 2 mm in the subcutaneous fat located below the dermis (5), a technique is required which can detect not only changes in drug accumulation in time in the superficial layers of the skin, but can also provide information in the deep layers of the skin and subcutaneous fat tissue. Drug accumulation in time can only be obtained with unfixed tissue.

SKIN

Function

The skin is the largest organ of the body weighing more than 10 % of the total body mass (6). Forming the outermost layer of the human body, it has two important functions, namely communication and protection (7). The communicative function is based on neuroreceptors, biochemical transmitted

signals and pigmentation. The protective function prevents loss and penetration of a substance from and into the body (8). On the one hand the skin protects the body from the environment such as physical (radiation, abrasion), biological (microorganisms) or chemical factors (toxic substances). On the other hand, water- and ion-loss from the body is prevented as well (9). Additionally the skin including its sweat glands, hair follicles and systemic circulation enables thermo-regulation to ensure correct functioning of the biochemical apparatus.

Despite of this general function, regional variations in skin morphology occur. Not only the thickness (11) and composition of the stratum corneum varies (12,13) but also the presence of appendages and the number of hair follicles is not constant over the whole body surface (14). These variations are of functional origin giving e.g. the soles higher protection against abrasion.

Structure

The basic skin structure is depicted in Figure 1. The skin is basically composed of two layers (epidermis and dermis) with an adjacent subcutaneous fat tissue. The epidermis forms the outer layer of the body comprising non-viable (stratum corneum) and viable cell layers. The epidermis is generated in the undulating basal cell layer at the epidermal dermal junction (15). On their way to the skin surface the keratinocytes start to differentiate and undergo a number of changes in both structure and composition during migration through the stratum spinosum and stratum granulosum. The final differentiation occurs in the stratum corneum (16). The stratum corneum forms the major barrier for substances to permeate across the epidermis. The stratum corneum consists of dead keratinocytes coated with an impermeable proteinous cornified envelope. The corneocytes are surrounded by lipid matrix (16). This lipid matrix is arranged in multiple layers forming lipid lamellae (17). Due to the impermeable cornified envelope the intercellular lipid matrix forms the main rather tortuous pathway for permeation of substances across the stratum corneum.

The dermis is located beneath the epidermis. The thin upper dermis, which is in direct contact with the undulating epidermis, is the papillary dermis while the thicker main part of the dermis is called reticular dermis. It is a fibrous, filamentous and amorphous connective tissue consisting of collagen, elastin, ground substance and fibroblasts (18-21). Its main function is to provide support for the epidermis and embedded structures (blood vessels, nerves, hair follicles, sweat and sebaceous glands) as well as elasticity of the skin (6). In contrast to the epidermis, this tissue is highly vascularised.

The subcutaneous fat tissue is underlying the dermis. It is an assembly of fat cells linked by collagen fibres thereby creating a thermal barrier, energy storage and mechanical cushion for the body (6,7).

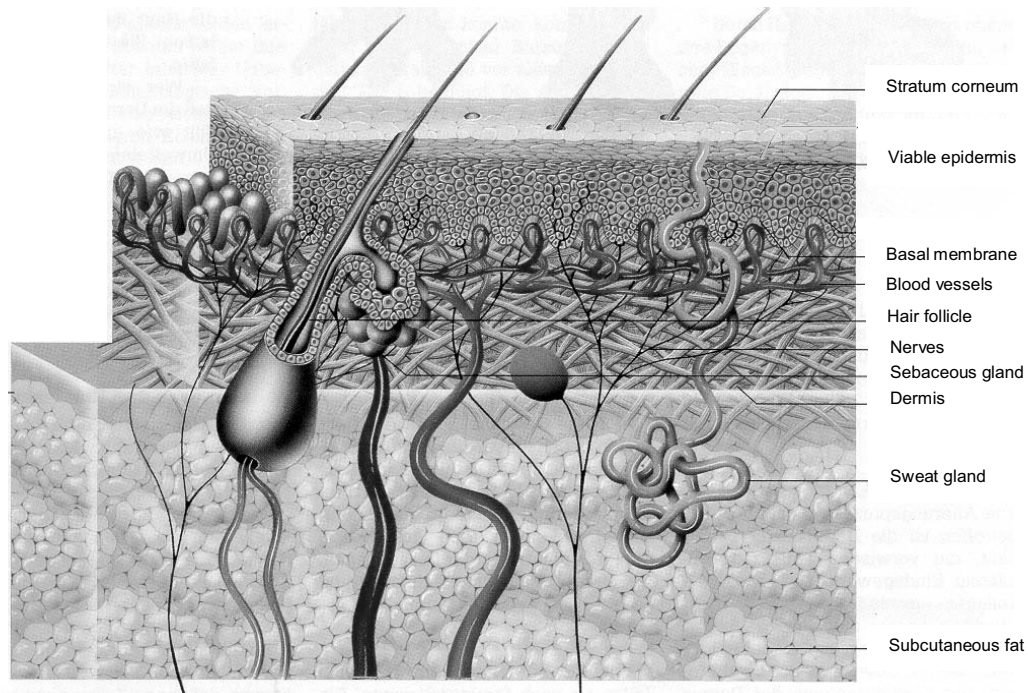


Figure 1. Skin structure of human skin. The main layers are the stratum corneum, the viable epidermis including the basal membrane, the dermis and the subcutaneous fat. Local skin structures are blood vessels, hair follicles, nerves, sebaceous glands and the sweat glands.

Appendages

Appendages are skin structures penetrating the skin and originate either from the dermis or the subcutaneous fat. Their presence varies in different skin regions of the body. Since they emerge from the skin, the appendages form discontinuities in the stratum corneum and can therefore act as potential sites of formulation accumulation and routes of penetration.

Sweat gland

Apocrine and eccrine glands are present in large numbers and distributed over the entire body. Apocrine glands emerge into the follicular duct and are located in the axilla and perianal region in adults. Therefore it has been proposed, that the apocrine glands do not contribute to the thermoregulatory function of the sweat glands but are remnants of the secondary sexual organs (7). Eccrine sweat glands are smaller than the apocrine glands and are spread over the whole body surface except from mucosal tissue. They excrete sweat (hypotonic water) via the sweat duct to the skin surface. These sweat ducts perturb the stratum corneum in a spiral form and straighten in deeper skin layers.

The secretory gland itself is coiled and situated in the lower dermis. Their main function is the thermal regulation of the body (6).

Pilosebaceous unit

The pilosebaceous unit consists of the hair follicle and the sebaceous gland. The hair follicle can be divided into two classes. The smaller vellus hair is rather thin and reaches down into the dermis. The larger terminal hair extends down into the subcutaneous fat (22). The terminal hair occurs mainly in the scalp skin which is the region with the highest density of hair follicles.

Sebaceous glands are located at the whole body surface, however, their density and activity depends on age and sex (23-25). A high density of sebaceous glands is present in scalp and forehead skin, while in palm and sole the sebaceous glands are absent (26). Sebum, mainly consisting of triglycerides, free fatty acids, squalene and waxes, is secreted by the sebaceous gland into the hair duct at a depth of about 500 μm (27-30). This sebum protects the body from microbial infection and prevents water loss from the body (30). Additionally it has the potential to interact with topically applied substances on the skin surface and in the upper part of the follicular duct thereby acting as an additional barrier for permeation of substances across the skin.

The hair follicle

The basic structure of the hair follicle is displayed in Figure 2 (31). The hair follicle can be divided into several sections starting from the skin surface. The infundibulum is the upper part of the hair follicle up to the sebaceous duct. In this area, no tight connection between the hair shaft and the skin is present. Therefore, the hair shaft can move freely within the skin. This gap is filled with sebum of the sebaceous gland. The thickness of the stratum corneum decreases deeper in the infundibulum. This thinned stratum corneum provides a weaker barrier for penetration compared to the stratum corneum at the skin surface (32). The isthmus is located just below the sebaceous duct and up to the area where the arrector pili muscle is attached to the hair follicle. From the isthmus upward the hair follicle is permanent and does not disintegrate during the growth of the hair follicle. However in this region the inner root sheath is disintegrating and disappears between the outer root sheath and the cuticle further to the surface. The bulge area is located where the arrector pili muscle is in contact with the hair follicle. This area is important for regulatory processes during hair growth (33). Below the bulge area starts the lower follicle with the keratogeneous zone. The lowest part of the hair follicle is the hair bulb, where the matrix cells, the basement membrane and the follicular papilla are located. These structures are key features in the regulation of the hair growth (34).

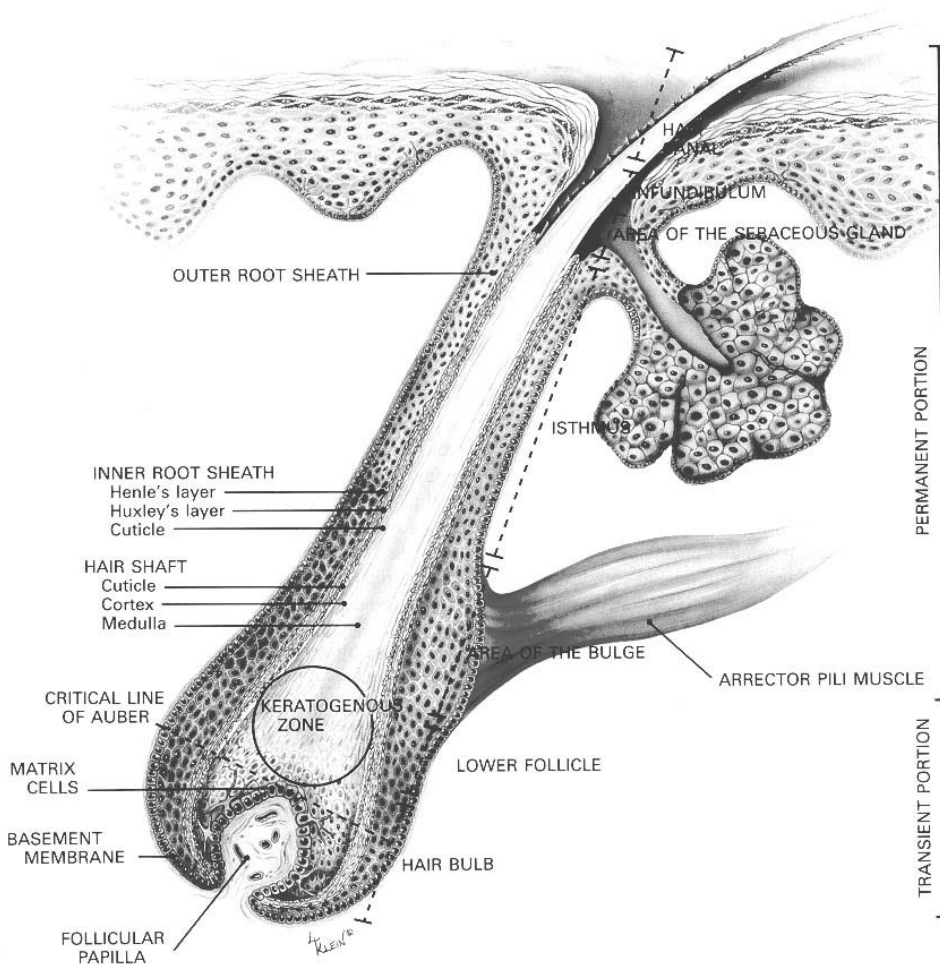


Figure 2. Hair follicle structure (31). The various layers of the hair follicle, described in this thesis are from the dermis to the centre of the hair: Outer root sheath, inner root sheath (Henle's layer, Huxley's layer), cuticle of the inner root sheath, cuticle of the hair shaft and the remaining hair shaft. Furthermore it is depicted that the epidermis is continuous with the outer root sheath and the stratum corneum decreases in thickness in the opening of the follicular duct. The sebaceous gland secretes the sebum into the opening of the follicular duct. The bulge area is located below the duct of the sebaceous gland.

In a cross-section of the hair follicle below the bulge area, several almost concentric layers can be identified (Figure 2) (31,34). Basically from the outside of the hair follicle to the centre of the follicle the following layers are encountered: the outer root sheath, the inner root sheath including the Henle's layer, the Huxley's layer and the cuticle. The inner root sheath is in direct contact with the hair shaft. The hair shaft itself is composed of a cuticle (outermost layer), a cortex and a medulla (central part). The cuticle of the inner root sheath and of the hair shaft form the connection, along which the hair shaft is moving outward during hair growth.

Keratinisation is important for hair growth and might have a crucial influence on the transport processes. The outer root sheath is in direct contact with the epidermis. Below the duct of the sebaceous gland, the cells of the outer root sheath are only little keratinised or lack keratinisation completely. This allows the moulding of the hair shaft and at the same time provides sufficient protection for the shaft. From the sebaceous duct upwards, the outer root sheath cells are keratinised and resemble more closely the epidermal cells (34). The inner root sheath keratinises deeper in the hair follicle as compared to the outer root sheath with the Henle's layer being the first, followed by the cuticle and the Huxley's layer (34). The cuticle of the inner root sheath keratinises closer to the hair bulb than the cuticle of the hair shaft. Thereby guidance for the emerging hair is provided combined with a loosening of the tight connection between the two cuticles (34).

Growth cycle of the hair

Hair growth varies between different body regions with about 0.21 mm per 24 hours on the female thigh and approximately 0.38 mm per 24 hours on the male chin (7). The hair growth undergoes a repetitive cycle where the anagen phase is followed by the catagen and telogen phase (35) (Figure 3). In the anagen phase, the hair is actively growing while the catagen is characterised by degeneration and resorption of the lower region of the hair follicle. The resting period, where the hair is inactive, is called the telogen phase. After resting, growth of the hair follicle restarts (36).

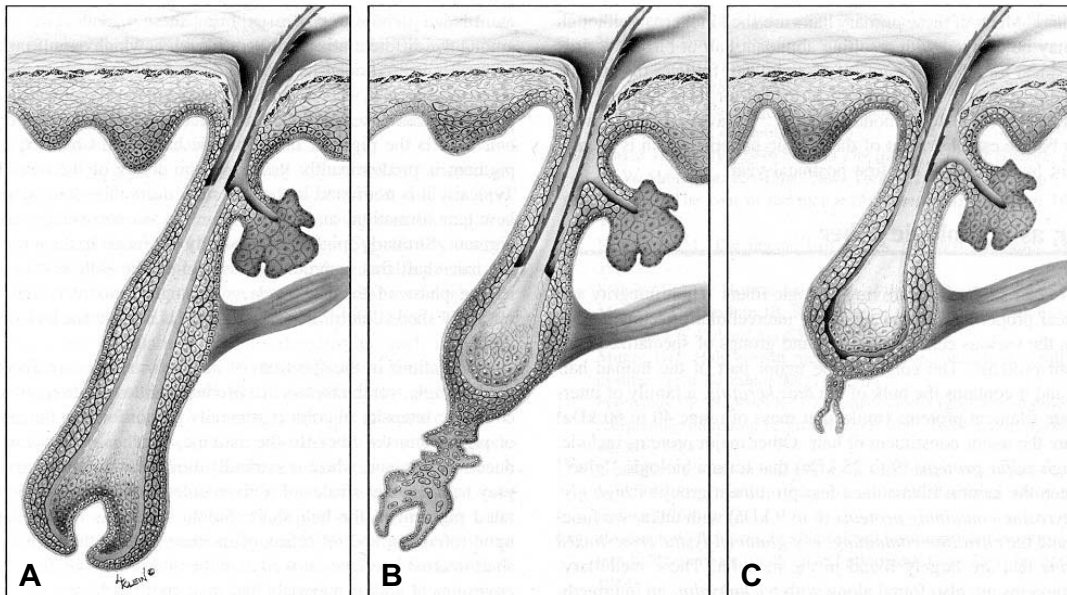


Figure 3. Growth cycle (31). A – Anagen (growth phase), B – Catagen (transition phase), Telogen (resting phase).

Currently it is not clear how the hair growth is initiated. This is reflected by the various discussions and hypotheses regarding the activation and differentiation of stem cells (37,38). However the bulge area and the dermal papilla seem to play a crucial role in the growth process.

Local target areas

In the pilosebaceous unit various target areas for topically applied drugs are of interest. One of the potential target areas is the sebaceous gland due to its direct connection with the follicular duct. In case of dysfunction of the sebaceous gland, the topical application has the potential to deliver drugs directly to the target area with limited systemic delivery (39,40). In case of toxic active ingredients, efficient targeting is essential to limit the toxic side effects such as for isotretinoin in the treatment of acne (41). Within the hair follicle, interesting target areas are the bulge area, the bulb and the various concentric layers in the follicle (39). Regulatory receptors for various molecules (e.g. epidermal growth factor) have also been identified (42). Several research groups propose that the bulge area initiate the hair growth, although the exact mechanism is yet unknown. Another reason for targeting to the bulge region is that various forms of skin cancer have been thought to originate from this region (43). In the bulb, melanocytes are present, which are responsible for the hair colour and thus for greying of the hair. Treatment of these melanocytes in albino mice has demonstrated that it is possible to grow pigmented hair (44). Furthermore the bulb contains a proliferating population of cells in the germinal matrix, which are responsible for new cell formation and therefore for hair growth. Sufficient nutrients for the growth process in the bulb area are provided by extensive vascularisation (45). Various attempts for the treatment of hair loss are also of great interest and under discussion (46).

(TRANS)DERMAL DELIVERY

Routes of penetration

When drugs are applied on the skin surface, penetration into and through the skin can occur via various routes. Drugs penetrate either via the stratum corneum (transepidermal) or via the appendages (transappendageal) (Figure 4) (8,47). During penetration through the stratum corneum, two possible routes can be distinguished, i) penetration alternating through the corneocytes and the lipid lamellae (transcellular route) and ii) penetration along the tortuous pathway along the lipid lamellae (intercellular route). Generally it is accepted that the predominant route of penetration through the stratum corneum is the intercellular route. This is mainly caused by the densely cross-linked cornified envelope

coating the keratinocytes. However transcellular transport for small hydrophilic molecules such as water cannot completely be excluded (6).

The appendageal route or shunt route includes either the duct of the eccrine sweat glands or the follicular duct. The content of the eccrine sweat glands is mainly hydrophilic, while the content of the follicular duct is lipophilic. This is mainly due to the sebum excreted into the opening of the follicular duct.

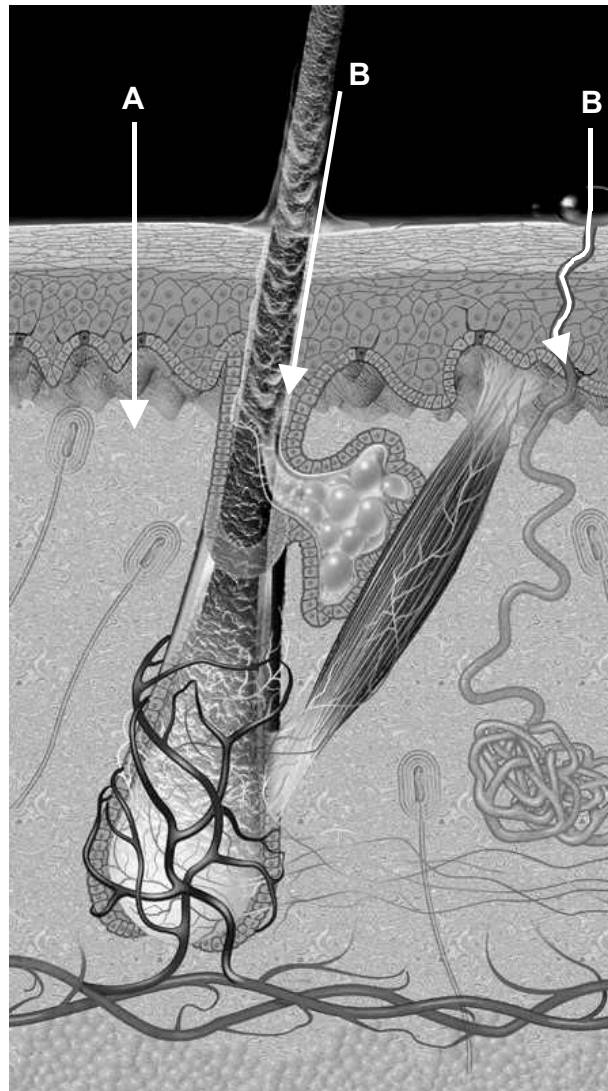


Figure 4. Transepidermal (A) and transappendageal route of transport into the skin. The transappendageal route (B) includes diffusion via the hair follicle and the sweat gland.

It is generally accepted that due to its large surface area, passive skin permeation mainly occurs through intact stratum corneum. Since the appendages cover approximately 0.1 % of the total skin surface, it is discussed that the permeation along appendages contributes only slightly to the overall

passive transdermal penetration (48,49). However Scheuplein (49) has calculated that in the initial phase of diffusion, the influence of the shunt route is very important. Investigations of several other groups provided additional data indicating that the contribution of the follicular pathway might be underestimated. They discovered an accumulation of the selected penetrant in the follicular region (50-53). These results are of great interest for drugs used in the treatment of follicle related skin diseases.

Fick's law of diffusion

Diffusion processes in human skin are very complex due to the inhomogeneous and layered structure of the skin and the presence of appendages interrupting the intact stratum corneum. In order to model diffusion processes and compare the diffusion of various drugs, a simplified model of the skin has to be considered. In Fick's first law (equation 1) the skin is considered to be homogeneous and the acceptor phase provides sink conditions for the penetrant. It describes that the transported mass dm [g] per time unit dt [h] through a defined area A [cm²] is directly proportional to the diffusion coefficient D [cm²/s], the size of the area and the concentration gradient [g/cm³]. However it is reverse proportional to the distance dx [cm] of the transported mass.

$$\frac{dm}{dt} = -DA \frac{dc}{dx} \quad \text{Equation 1}$$

The diffusion coefficient D [cm²/s] is the proportional factor and depends on physico-chemical characteristics of the medium and the penetrant. If diffusion occurs from a liquid donor phase into the skin, the partition coefficient (K) of the model penetrant is introduced. This partition coefficient links the concentration in the donor phase (c_d) to the concentration in the skin layer exposed to the donor phase. In a steady state situation a constant gradient is present in the membrane. Assuming the concentration in the acceptor phase is negligible, Fick's law can be described as:

$$\frac{dm}{dt} = -DKA \frac{c_d}{dx} \quad \text{Equation 2}$$

Plotting the cumulative amount of penetrated drug against time, a linear dependency is present in steady state conditions. The steady state flux (J_{ss}) can be determined directly from the slope (equation 3).

$$J_{ss} = \frac{dm}{dt} * \frac{1}{A} \quad \text{Equation 3}$$

FOLLICULAR DELIVERY

General aspects of follicular delivery

The follicular duct forms an intrusion in the stratum corneum. This interruption of the stratum corneum barrier can serve as a route for drug delivery. Additionally, the orifice of the hair follicle is a site where dermatological formulations may accumulate and deposit, thereby forming a depot for long-term delivery. In the past, the transappendageal route has long been considered of minor importance, since the orifices account for only 0.1 % of the total skin surface. However the density of hair follicles on scalp and face can reach as much as 10 % of the total skin surface, creating a higher local surface area (54) and allow greater absorption by this route (55). The hair follicle openings are continuous with the epidermis but possess a much thinner layer of stratum corneum (39), which terminates at a depth of about 200 μm in the duct. The pilosebaceous unit is also closely surrounded by a high density of blood vessels, having the potential of high absorption of permeating substances into the systemic circulation. Nevertheless, parameters governing the follicular delivery are not clear yet.

Lieb et al. (54) demonstrated that permeation into hair follicles depends on size and charge of the permeant and the formulation in which the permeant is applied. Other chemical properties, like lipophilicity might also play a significant role in follicular penetration. Additionally, a certain degree of lipophilicity for compounds entering the hair follicle might be necessary due to the presence of sebum in the follicular duct from the sebaceous gland (39).

Based on theoretical considerations, it has already been proposed by Scheuplein et al. (49) that the follicular route plays an important role in the initial period of the transport process, but that at a later stage penetration occurs predominantly via the stratum corneum. In fact, only little information is available on the contribution of the hair follicles to the total transport across the skin and even less is known about the effect of formulations and the chemical properties of the penetrant itself on this contribution.

Analysis methods for follicular delivery

Skin models

In skin research, various skin models are used to investigate penetration of drugs in general. Human skin from cosmetic surgery is most favourable (e.g. abdominal and mamma skin). Some researchers use cadaver skin (56) with the danger of skin disintegration once the skin is available. Furthermore pig skin has been reported to have similar barrier properties as human skin and therefore is suggested to be an acceptable model for human skin (57). Due to low availability

of human skin, mouse and rat skin is frequently used in screening experiments. However it has been shown, that permeability of mouse skin is sometimes several orders of magnitude higher than of human skin (58,59) while rat skin is only slightly more permeable (60).

The correlation between the results obtained *in vitro* and *in vivo* is of great importance for the development of new drugs and formulations. Various research groups have studied the *in vitro* - *in vivo* correlation within one skin model however also comparing results between animal models and human skin. Several authors (61-64) have demonstrated a good correlation for *in vitro* and *in vivo* data in rodents (rats and rabbits). Also for the monkey model, a correlation between *in vitro* and *in vivo* data has been reported (65). Even from a comparison of *in vitro* data obtained from porcine skin compared to *in vivo* data obtained from humans, literature demonstrates a correlation of the percutaneous absorption (66,67). Other researchers showed a good *in vitro* - *in vivo* correlation in deeper layers of human skin. However, due to clinical practice of disinfecting procedure, poor correlation at the skin surface has been reported (68). While *in vitro* data in human skin have been shown to overestimate the *in vivo* results (69), the low-frequency ultrasound technique had a higher effect *in vivo* than *in vitro* (70). Therefore various parameters such as skin model, used method of penetration enhancement, penetrant and skin treatment during analysis has to be considered when attempting *in vitro* - *in vivo* extrapolation of diffusion data.

In follicular research, the influence of hair follicles on the penetration and accumulation in the follicular area has been studied using several models. The first model is hairless rodent skin. Although hairless rodents lack the actual hair, the hair follicles are still present. These follicles are different and underdeveloped when comparing them to regular follicles (71). Penetration studies using hairless skin might therefore result in an underestimation of the contribution of the follicular pathway to the overall diffusion. Another model used in follicular research is scarred skin. The scar formation is obtained after short burning of the skin resulting in absence of hair follicles. In this model hair follicles are absent. However the skin has undergone severe stress and exhibits different histology (52). Therefore also results obtained using this scarred skin model might result in over- or underestimation of the contribution of the follicular pathway. A third model used in follicular research is the skin behind the ear of a guinea pig, which is totally follicle free (72). Because the histology of the skin of the hairy part of the animal is comparable to the non-hairy part, a comparison of penetration values within one animal is possible. However depending on the study, different results were obtained for the *in vitro* and *in vivo* set-up. A fourth model, the ear of a Syrian hamster (73) was used to study the sebaceous gland. In the hamster ear, a high density of sebaceous glands is present which resembles the sebaceous glands of the humans. This model has not been used with a focus on the hair itself. Another skin model is pig skin. It is generally accepted to be the best model

for human skin when interested in penetration of drugs. Pig skin deviates from human skin in its appendages. It is void of sweat glands and the hair bulb of the follicle is situated in the dermis. In human skin, the hair follicle reaches into the subcutaneous fat up to a depth of approximately 2-3 mm. Therefore pigskin is not a realistic model for examining transport processes into deeper regions of the hair follicle. Human scalp skin from cosmetic surgery is currently the best skin material to investigate *in vitro* diffusion processes. The only draw back is its limited availability and the difficulties to perform *in vivo* studies using human scalp skin with the currently available techniques.

Quantification

Quantitative access to deposition of substances in the follicular region is a challenging task, since the hair follicle of human skin extends down into the subcutaneous fat. Most studies have been carried out either using fluorophores or radioactive agents as model penetrants. The distribution profiles of these labels were mostly determined by quantification of model penetrants in stratum corneum tape strips in combination with quantification in biopsies (74,75), immunohistochemical analysis (54,76), isolation and dissolution of the various skin regions including hair follicles (76,77), hair plugging and follicle dissection (54). However, when isolating hair follicles, cell structures can be destroyed thereby bearing the danger of delocalisation of the dye resulting in artefacts. Recently, techniques used for relative quantification of penetrant distribution within the skin have been reviewed elsewhere (75). Turner and Guy (78) have published relative quantification data of cryo-fixed hairless mouse skin samples. The authors focused on the fluorophore distribution after iontophoresis *ex vivo*. As far as *in vivo* quantification is involved, particularly non-invasive methods of quantification, such as in depth-resolved near-IR spectroscopy, have still an insufficient resolution (31 μm) for visualisation of the various regions in the hair follicle (79). Furthermore, absolute quantification in unfixed tissue has not yet been reported.

Visualisation

Visualisation in fixed skin

Visualisation methods in skin research have been summarised elsewhere (80). This review provides an excellent overview of advantages and disadvantages of the various available methods (80) to depict penetrated substances. While in conventional light microscopy a penetrant with a strong contrast has to be used, autoradiography requires radioactive labelling of the permeant. Electron microscopy has the advantage of providing images with a very high resolution. However, in most cases the choice of the permeation agent

is limited to substances with high electron density or substances linked to an electron dense marker, such as gold. All of these techniques require fixation, which can be obtained for example by chemical embedding or cryo-fixation of the skin. This fixation procedure in combination with subsequent slicing of the object can introduce artefacts, such as delocalisation of the label. Therefore fixation by chemicals or by cryo-fixation should be avoided.

Visualisation in non-fixed skin

Visualisation techniques which do not require embedding and freezing of the object are magnetic resonance imaging (81-83), video microscopy (84-87), ultrasound backscatter microscopy (88) and confocal laser scanning microscopy (89-95). The combination of the latter technique with confocal raman spectroscopy (96) even enables to obtain a molecular composition of selected spots in the skin with high spatial resolution (97). For these techniques model drugs with adequate characteristics such as raman active substances, dipole structures or fluorescent dyes have to be selected. Confocal raman spectroscopy has already been applied *in vivo* in humans. Magnetic resonance imaging has the advantage that it can be used *in vivo* very deep in the skin and subcutaneous tissue however; the resolution is limited compared to confocal raman or confocal laser scanning microscopy. The resolution of video and ultrasound microscopy is even worse and can therefore not be used for visualisation of substances in hair follicles.

In hair follicle research high resolution of the visualised area is necessary paired with reaching deep layers of the skin for the visualisation of the hair bulb. As pointed out above, the current *in vivo* visualisation techniques are either limited in resolution or depth penetration. Therefore, it was decided to primarily focus on a promising *in vitro* technique fulfilling the requirements of resolution and depth penetration. Therefore confocal laser scanning microscopy is the method of choice, if fixation of the skin is circumvented by alternative methods and if the skin is sliced perpendicular to the skin surface. This immediately limits the confocal method for visualisation in deeper follicular regions to the *ex vivo* situation. Once the confocal method is set up, the *ex vivo* results might be investigated *in vivo* using either confocal raman spectroscopy in combination with confocal laser scanning microscopy or magnetic resonance imaging accepting limited resolution or limited depth visualisation. Although these drawbacks have to be accepted, first *in vitro/in vivo* correlation of penetration into the human hair follicle might be accessible.

THIS THESIS

Objective

Local administration of drugs to the skin with the aim of high accumulation in the follicular area is of great interest. Especially the pharmaceutical industry in case of local drug targeting and the industry in the field of personal care products forms a potentially interested group. However, the influence of factors such as physico-chemical properties of the permeant and the effect of the vehicle is not clearly understood.

Extending the cleansing function of shampoo formulations by providing supplementary nutrients to the hair follicle would be very desirable. However from a formulation point of view, liposomes which have already been reported to improve accumulation of a drug in the follicular duct (98-100) are not applicable in shampoo formulations. The liposomal structure is not expected to be stable in the presence of the surfactants of the shampoo. Therefore the efficiency of alternative approaches has to be studied for their potential to increase accumulation of the active ingredient in the hair follicle.

Furthermore, to study drug accumulation in the human hair follicle, thick skin pieces have to be used since the hair bulb reaches down into the subcutaneous fat. In order to investigate the delivery of drugs to the hair follicle, a powerful method has to be developed. This method has to access the hair bulb in deep skin layers, has to have sufficient resolution to visualise the various layers of the hair follicle, has to limit the danger of artefact formation and has to have the potential to visualise diffusion processes on-line.

From these aspects, the following objectives are formulated:

- A. *Develop a method that allows the comparison of degrees of accumulation in the various layers of the hair follicle, the dermis, the viable epidermis and the stratum corneum.*
- B. *Examine the influence of permeant lipophilicity on the accumulation in the various follicular layers, the viable epidermis, dermis and stratum corneum.*
- C. *Examine the influence of formulation composition on the accumulation in the various follicular layers, the viable epidermis, dermis and stratum corneum.*
- D. *Design a method to visualise the diffusion process **on-line** and examine the diffusion pathways of the permeant in the various regions of the skin, including the gap, along the entire hair follicle to reach the hair bulb. Only in this way information about the permeant penetration pathways can be achieved.*

Organisation

Chapter II: A new method of relative quantification was developed using confocal images of cross-sections perpendicular and parallel to the skin surface. This method enabled the comparison of the dye distribution in the various regions of the skin including the hair follicle after diffusion of the dye over a selected time period. Therefore information was provided about the relative accumulation of dyes in the hair follicle regions.

Chapter III: Diffusion studies with dyes of increasing lipophilicity were performed. Diffusion studies were carried out, after which the fluorophore was visualised in the skin, including the various regions in the hair follicle. Using the method described in chapter II, the relative accumulation of the dyes in the various skin regions was determined and the influence of permeant lipophilicity was accessed.

Chapter IV: Subsequently the influence of the vehicle composition, i.e. the presence of a basic shampoo formulation with and without propylene glycol was accessed. The penetrated amount of the various dyes and the degree of accumulation in the various follicular layers and the non-follicular regions were determined using different formulations.

Chapter V: In the studies described in this chapter a new method was developed in which transport of a lipophilic dye was visualised in real-time (during transport) for a period of 8 hours. In these studies the main focus was to visualise the permeation process in the stratum corneum, epidermis and dermis.

Chapter VI: In a subsequent series of studies, on-line diffusion was examined with a lipophilic fluorescent dye. The diffusion process was visualised close to the opening of the hair follicle (the gap) to a depth of approximately 400 μm . The diffusion process was followed over a time period of 16 hours.

Chapter VII: In order to study the diffusion process along the entire hair follicle, the diffusion process of the lipophilic dye was visualised in several sections at selected depths. This included the diffusion of the dye to the hair bulb.

Chapter VIII summarises the studies described in this thesis. In addition the future perspectives of the on-line method and the use of basic shampoo formulations for local targeting to the hair follicle are discussed.

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