Follicular Targeting–A Promising Tool in Selective Dermatotherapy

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The penetration of topically applied compounds varies considerably in the different regions of the human body. The presence of hair follicles significantly contributes to this effect by an increase in surface area and a disruption of the epidermal barrier towards the lower parts of the hair follicle. The human hair follicle, hereby, serves not only as a reservoir, but also as a major entry point for topically applied compounds. Topical delivery of active compounds to specific targets within the skin may help reduce side-effects caused by unspecific reactions, and may help develop new strategies in the prevention and treatment of skin diseases. Various drug carrier and drug delivery systems are currently being investigated. The aim of these investigational efforts is to direct topically applied compounds to the different types of hair follicles and, ideally, to specific compartments and cell populations within the hair follicles. Follicular targeting offers opportunities for new developments, not only in hair therapy and in the treatment of hair follicle associated diseases but also in gene therapy and immunotherapy.

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The pilosebaceous unit plays an important role in permeation and penetration processes of topically applied compounds. The human hair follicle is not only an important reservoir but also an entry point for topically applied substances and significantly contributes to the transport of drugs into the skin. This is illustrated by the fact that the penetration of corticosteroids is considerably lower in hairless skin, as compared to haired skin (Hueber *et al*, 1994; Tenjarla *et al*, 1999). Similar observations have been made in tissue engineered skin where the insertion of hair follicles significantly increased the penetration rate of hydrocortisone (Michel *et al*, 1999). The follicular penetration of topical compounds can be monitored *in vivo* by laser scan microscopy, a non-invasive technique which produces serial horizontal sections of the skin (Fig 1). This phenomenon may be utilized not only for systemic drug delivery but also for the localized delivery of active compounds to the human hair follicle. Drug delivery systems and formulations designed to selectively target the human hair follicle may allow the conveyance of relevant doses of active compounds into the follicular duct. Possible applications include the treatment of hair growth abnormalities, as well as hair follicle-associated diseases and general skin disorders.

Target Structures in the Human Hair Follicle

The regression of terminal hair follicle (THF) back to vellus hair-like structures in androgenetic alopecia, but also hair overgrowth as it occurs in hirsutism, are common clinical problems. The human hair follicle further plays a central role in the pathogenesis of hair follicle-associated disorders such as acne. Beyond this, the modulation of epithelial and melanocyte stem cells skin may help improve regeneration and pigmentation. Based on these considerations, we have defined three major targets in human hair follicles (Fig 2).

The *follicular infundibulum*: Although the acroinfundibulum of the hair follicle is covered by an intact, rather impermeable stratum corneum, this barrier is interrupted in the lower follicular infundibulum, as the differentiation pattern switches from epidermal differentiation to a tricholemmal differentiation pattern. Only few to little differentiated corneocytes remain and the epidermis must be considered as highly permeable. Due to this increased permeability of the follicular epithelium, epithelial cells and associated cell populations such as antigen-presenting cells, mast cells and others are readily accessible for topically applied compounds.

The *sebaceous gland* is interconnected with the human hair follicle via the sebaceous duct, which opens into the follicular duct in the lower infundibulum. There is evidence that topically applied compounds entrapped in liposomes accumulate not only in the hair follicle itself but also in the sebaceous gland. Bernard *et al* studied the penetration of the antiandrogen RU 58841 in normal hairless rat skin and scarred hairless skin without sebaceous glands. They found that the solution was localized in the stratum corneum, whereas liposome-entrapped RU 58841 was mainly localized in the sebaceous glands (Bernard *et al*, 1997). These findings suggest that follicular targeting may also be a promising tool in topical acne therapy.

The *bulge region* in the outer root sheath, at the insertion level of the M. arrector pili, is well recognized as the reservoir of epithelial stem cells which are capable of repop-

Abbreviations: THF, terminal hair follicle; VHF, vellus hair follicle

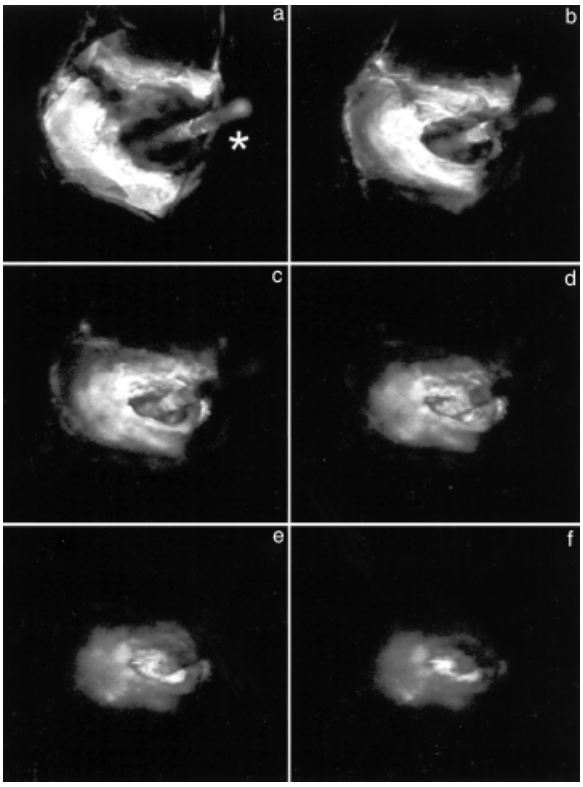

Figure 1
Laser scan microscopy allows to visualize the follicular penetration of topically applied fluorescent compounds such as sodium fluorescein *in vivo*. The device provides an investigated field of vision is 200 × 200 down to a maximum depth of approximately 250 mm. This series of scans illustrates the penetration of topically sodium fluorescein solution (white signal) into a vellus hair infundibulum on the forearm of a healthy volunteer (a–f). Each image shows a horizontal section of the same vellus hair follicle infundibulum at different depths from the skin surface (* hair fiber).

ulating the hair follicle as well as the interfollicular epidermis (Taylor *et al*, 2000). Therapeutic manipulation of stem cells may allow the treatment of hair loss and hair overgrowth, as well as skin diseases. The recent localization of melanocyte stem cells to this lower part of the permanent hair follicle further offers the opportunity to treat pigmentation disorders (Nishimura *et al*, 2002). The bulge region is of particular interest for gene therapeutic approaches. Hair follicles are easily accessible, and Li *et al* (1993) demonstrated *in vitro* that hair follicles can be targeted by liposomes loaded with DNA. Building on these findings Domashenko *et al* (2000) successfully introduced plasmid DNA encoding for the lacZ reporter gene into human hair follicles in a xenograft model). The introduction of genes into follicular stem cells offers wide opportunities for novel treatments of hair and skin diseases.

Hair Follicle Types

The infundibulum, the sebaceous gland and the bulge region are important target structures into THF and vellus hair follicle (VHF). Depending on location and size of the target structure the application protocols have to be modified accordingly. For example, targeting of the bulge region in THF may allow to inactivate stem cells in patients suffering from hypertrichosis. Activation of stem cells in vellus hair-like follicles of androgenetic alopecia patients, in contrast, may help reconvert these hair follicles in strong, pigmented THF. Precise data on the anatomy and the morphology of the different types of hair follicles are essential for the development of targeting protocols. In a large series of investigations, Viragh and Meuli (1995) found that the bulge region in human THF of the scalp is located at a depth of 500–800 μm, whereas the entry level of the sebaceous duct is at

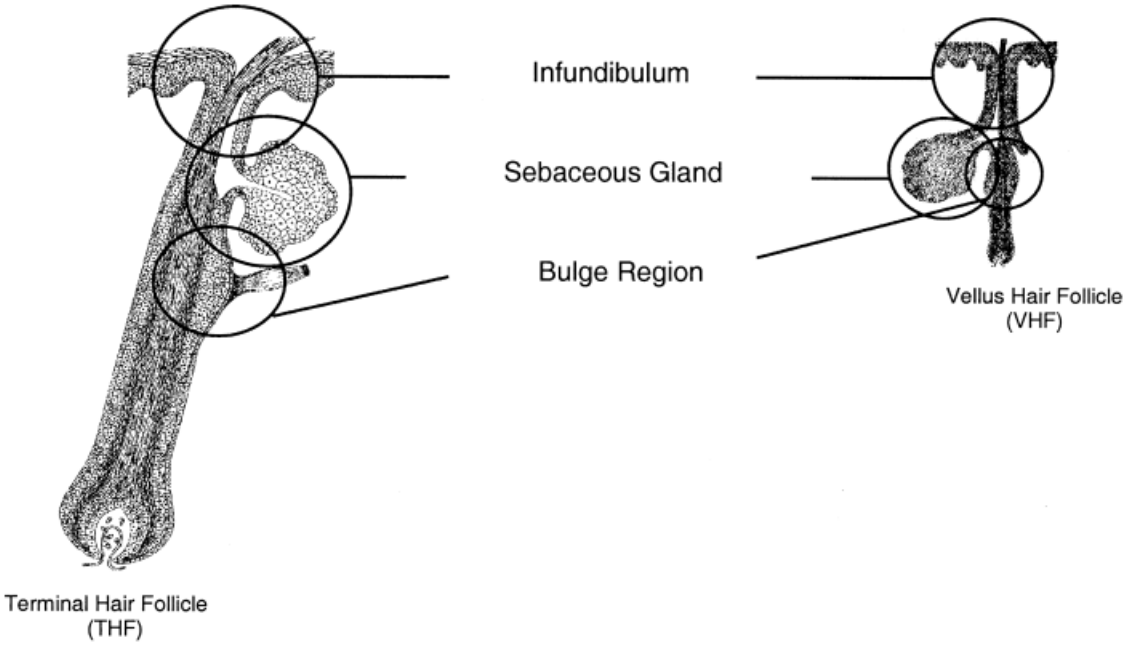

Figure 2
Follicular targeting aims to selectively bring active compounds into human follicles
The infundibulum, the sebaceous gland and the bulge region are important target structures in both, terminal hair follicle and vellus hair follicle.

approx. 100–500 μm. Only few data are available on VHF. Although structurally similar to THF there is evidence for a characteristic histomorphology and histochemistry of VHF. VHF are smaller than THF and produce fine, silky hair with an average diameter of 30 μm, which is generally unmedullated and unpigmented. Blume *et al* (1991) first determined the density of VHF on different body sites by phototrichogramm. In our group, we measured the infundibular volume of VHF from different body sites by cyanoacrylate stripping. VHF of the forehead are among the smallest hair follicles of the human body, whereas the largest VHF are found in the calf region. Due to the high density of approx. 290 to more than 400 VHF per cm^2 on the forehead as compared approx. 14 VHF per cm^2 on the calf, however, the forehead holds the highest overall infundibular volume (Otberg *et al*, 2004). Besides these anatomic characteristics, a wide variety of factors including the drug carrier system, the formulation, temperature, humidity, and pretreatment techniques of the skin influence the degree of drug deposition in the pilosebaceous unit. Our research focus is to develop standardized application protocols, which allow to specifically target compartments such as the infundibulum or the bulge region in the different types of hair follicles.

Drug Carrier Systems

It is well recognized that for certain drug delivery systems, hair follicles are privileged penetration pathways. They enter faster into these shunts than through the stratum corneum and hereby offer the possibility to create high local concentrations of the active compounds within the follicular duct. Among the various drug delivery systems, which are currently under investigation for topical therapy, liposomes, and microspheres are most widely used. Successful delivery of liposomal DNA and protein into hair follicles has been demonstrated in animal models and human skin. Lieb *et al* (1992) delivered calcein by topically applied multilamellar liposomes to pilosebaceous units in the hamster ear *in vitro*). Li *et al* (1993) demonstrated that hair follicles can be targeted by liposomes loaded with DNA. Balsari *et al* (1994) reported that liposome can deliver monoclonal antibodies into hair follicles of rats for protection against doxorubicin-induced alopecia. Topically applied melanin entrapped in phosphatidylcholine liposomes entered into the hair follicle cells and the hair shafts forming pigmented hair fibers, whereas compounds in solution did not enter below the stratum corneum (Li and Hoffman, 1997). Building on these findings, Domashenko *et al* (2000) successfully introduced plasmid DNA encoding for the lacZ reporter gene into human hair follicles in a xenograft model.

Compared to liposomes, microspheres offer several advantages. They display good stability and may allow a controlled release of the active compound (Fig 3). Loaded with fluorescent dye for experimental studies, the microspheres can be conveniently followed by fluorescence microscopy. The penetration properties of the microspheres in the skin depend on the the size of the particles. Rolland *et al* (1993) reported that microspheres of 3–10 μm topically applied to human skin aggregated in the follicular orifices whereas particles larger than 10 μm remained on the skin surface. Particles <1 μm spread widely on intact skin and also

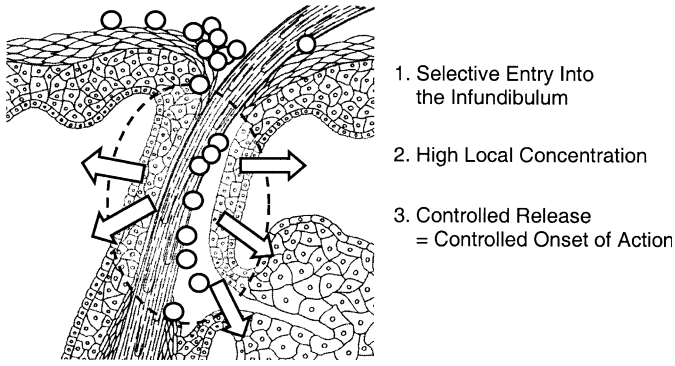

Figure 3
Microparticles enter selectively into the hair follicle and may yield high local concentrations of the active compound. The controlled destabilization of the microspheres may allow a controlled release of the compounds. The penetrations depth depends on the size of the particles and the size of the target.

penetrate into the upper layers of the stratum corneum of interfollicular epidermis, but no penetration into viable epidermis was observed. Specific delivery and controlled release of adapalene into the hair follicles for the 5 μm microspheres were demonstrated *in vitro* and *in vivo* on hairless rats and on human skin. Similarly, rhodamine-6G-loaded 5 μm microspheres dispersed into silicone entered into follicular duct without penetration within the stratum corneum (Sumian *et al*, 1999). Methylene blue-loaded 5 μm microspheres penetrated into the follicular duct and in sebaceous glands structures of hairless rat skin without penetration into the stratum corneum (Mordon *et al*, 2003). Building on these findings our group recently performed a large series of investigations, on the penetration profiles of microparticles from 6.0 μm down to 0.75 μm on freshly excised human scalp skin. 6.0 μm particles aggregated in the infundibular region of terminal hair follicles and penetrated down to approx. 500 μm, which corresponds approximately to the entry level of the sebaceous duct (Toll *et al*, 2004). Smaller particles with a diameter of 1.5 or 0.75 μm penetrated deeper with 40% of terminal hair follicles targeted down to depth of the bulge region at approx. 800 μm. These data suggest, that microparticles of different sizes allow to target compartments within human hair follicles, e.g., the infundibulum or the bulge region.

Conclusion

In summary, the work of multiple groups proves that the localized delivery of drugs to the human hair follicle is a feasible approach. Recent data from our own group suggest that it is moreover possible to predominantly reach certain compartments within the hair follicles. Hence, follicular targeting offers remarkable opportunities for new developments not only in general dermatotherapy but also in fields such as gene therapy and immunotherapy.

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References

Balsari AL, Morellis D, Menard S, Veronesi U, Colnaghi MI: Protection against doxorubicin-induced alopecia in rats by liposome-entrapped monoclonal antibodies. FASEB J 8:226–230, 1994

Bernard E, Dubois J-L, Wepierre J: Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 86:573–578, 1997

Blume U, Ferracin J, Verschoore M, Czernielewski JM, Schaefer H: Physiology of the vellus hair follicle: Hair growth and sebum secretion. Br J Dermatol 124:21–28, 1991

Domashenko A, Gupta S, Cotsarelis G: Efficient delivery of transgenes to human hair follicle progenitor cells using topical lipoplex. Nat Biotechnol 18:420–423, 2000

Hueber F, Besnard M, Schaefer H, Wepierre J: Percutaneous absorption of estradiol and progesterone in normal and appendage-free skin of the hairless rat: Lack of importance of nutritional blood flow. Skin Pharmacol 7:245–256, 1994

Li L, Hoffman RM: Topical liposome delivery of molecules to hair follicles in mice. J Dermatol Sci 14:101–108, 1997

Li L, Lishko V, Hoffman RM: Liposome targeting of high molecular weight DNA to the hair follicles of histocultured skin: A model for gene therapy of the hair growth processes. *In Vitro* Cell Dev Biol Anim 29a:258–260, 1993

Lieb LM, Ramachandran C, Egbaria K, Weiner N: Topical delivery enhancement with multilamellar liposomes into pilosebaceous units: *In vitro* evaluation using flurescent techniques with the hamster ear model. J Invest Dermatol 99:108–113, 1992

Michel M, L'Heureux N, Pouliot R, Xy W, Auger FA, Germain L: Characterization of a new tissue-engineered human skin equivalent with hair. *In Vitro* Cell Dev Biol Anim 35:318–326, 1999

Mordon S, Sumina C, Devoisselle JM: Site-specific methylene blue delivery to pilosebaceous structures using highly porous nylon microspheres: An experimental evaluation. Lasers Surg Med 33:119–125, 2003

Nishimura EK, Jordan SA, Oshima H, *et al*: Dominant role of the niche in melanocyte stem-cell fate determination. Nature 416:854–859, 2002

Otberg N, Richter H, Schaefer H, Blume-Peytavi U, Sterry W, Lademann J: Variations of hair follicle size and distribution in different body sites. J Invest Dermatol 122:14–19, 2004

Rolland A, Wagner N, Chatelus A, Shroot B, Schaefer H: Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. Pharm Res 10:1738–1744, 1993

Sumian CC, Pitre FB, Gauthier BE, Bouclier M, Mordon SR: A new method to improve penetration depth of dyes into the follicular duct: Potential application for laser hair removal. J Am Acad Dermatol 41:172–175, 1999

Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM: Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102:451–461, 2000

Tenjarla SN, Kasina R, Puranajoti P, Omar MS, Harris WT: Synthesis and evaluation of *N*-acetylprolinate esters–novel skin penetration enhancers. Int J Pharm 19:147–158, 1999

Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U: Penetration profile of microspheres in follicular targeting of terminal hair follicles. J Invest Dermatol 123:168–176, 2004

Viragh de PA, Meuli M: Human scalp hair follicle development from birth to adulthood: Statistical study with special regard to putative stem cells in the bulge and proliferating cells in the matrix. Arch Dermatol Res 287:279–284, 1995