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International Journal of Pharmaceutics 306 (2005) 1–14
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Review

Transfollicular drug delivery—Is it a reality?

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Received 7 June 2005; received in revised form 15 September 2005; accepted 24 September 2005

Abstract

Once regarded as merely evolutionary remnants, the hair follicles and sebaceous glands are increasingly recognised as potentially significant elements in the percutaneous drug delivery paradigm. Interest in pilosebaceous units has been directed towards their use as depots for localised therapy, particularly for the treatment of follicle-related disorders such as acne or the alopecias. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. This paper reviews various key facets of this field including; relevant aspects of pilosebaceous anatomy and physiology, the design and efficacy of follicle-targeting formulations and the emergence of quantitative modeling systems. Several novel developments in this area promise to greatly expand our understanding of this field in the near future.

Keywords: Hair follicles; Pilosebaceous units; Transfollicular; Transdermal; Drug delivery

Contents

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doi:10.1016/j.ijpharm.2005.09.025

1. Introduction

For several decades, researchers working with human skin have questioned the relative importance of drug transport through the continuous stratum corneum versus drug penetration through the follicular shunts of the pilosebaceous units. Although very early work suggested that follicles played a negligible role in facilitating steady state drug penetration (Scheuplein, 1965), the results of subsequent investigations began to cast some doubt on this concept. Specifically, qualitative dye and stain localisation studies provided evidence for penetrant accumulation in the hair follicles (Scheuplein, 1967; Rutherford and Black, 1969) while the greatest absorption of some compounds was observed in regions exhibiting the highest follicular densities (Feldman and Maibach, 1967; Tur et al., 1991). However, these findings subsequently proved difficult to interpret since the most follicle-rich regions were also associated with small corneocyte size, which would influence non-follicular absorption. Despite these caveats and the fact that the nature of pilosebaceous transport mechanisms has yet to be established, recent years have tended to yield progressively more data suggesting that follicular drug penetration could be more significant than previously believed (Lademann et al., 2001; Essa et al., 2002; Grams et al., 2004a). Within this context, it is important to distinguish between two largely distinct drug delivery paradigms. These are pilosebaceous drug depot applications for localised therapeutic targeting versus transfollicular shunt transport for accelerated systemic drug delivery.

After reviewing the anatomic, physiological and site distributions of hair follicles, this paper considers the potential therapeutic rationale of targeting drugs to these structures. Subsequently, there is a discussion of pilosebaceous drug delivery barriers as well as the literature pertaining to distinct drug carrier systems. Finally, emerging methodologies are discussed that should reveal more about this intriguing and complex delivery route.

2. The pilosebaceous units: anatomy and physiology

The pilosebaceous unit is a term used to describe the integrated structure of the hair follicle, hair shaft, adjoining arrector pili muscle and associated sebaceous gland(s). Since the anatomy and biology of these structures have been reviewed extensively in the literature (Whiting, 2000), these details are discussed relatively briefly here.

The hair follicle consists of a hair bulb and shaft enveloped in an inner root sheath, an outer root sheath and an outermost acellular basement membrane termed the glassy membrane. The outer root sheath is a keratinised layer continuous with the epidermis while the inner root sheath ends about halfway up the follicle. Each hair follicle is associated with one or more flask-like sebaceous glands, which are outgrowths of epithelial cells. Ducts join these multilobular holocrine glands to the upper part of the follicular canal. Cells at the hair bulb largely regulate hair growth. Fig. 1 presents a diagram of the pilosebaceous unit.

It is noteworthy that there are actually two types of human hairs—terminal hairs and vellus hairs. Terminal hairs are macroscopically long (>2 cm), thick (>0.03 mm), pigmented and usually contain a medullary cavity (Whiting, 2000). These hairs extend more than 3 mm into the hypodermis. In contrast, the unpigmented vellus hairs are generally short (<2 cm), thin (<0.03 mm) and typically extend just 1 mm into the

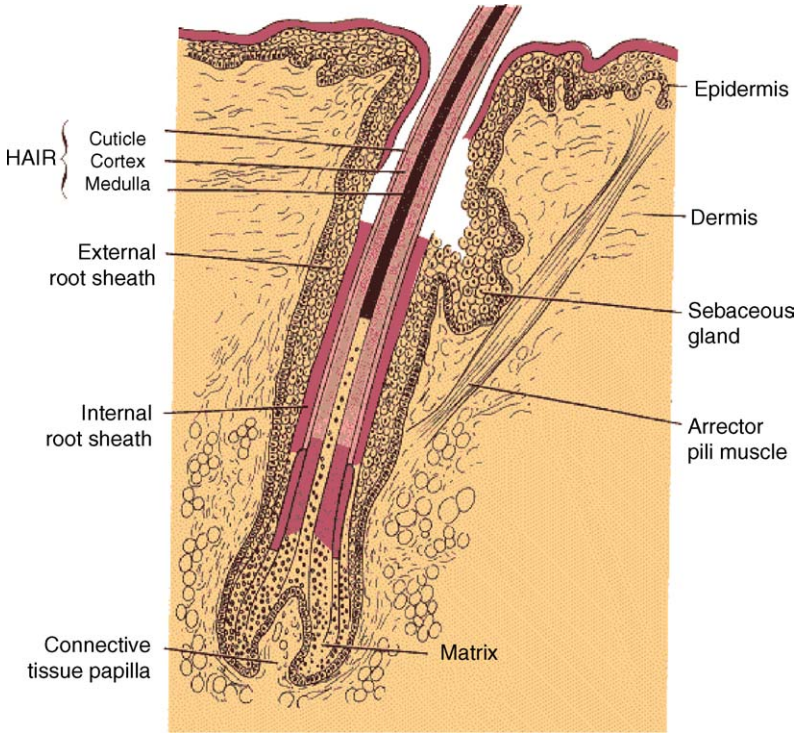

Fig. 1. Cross-sectional diagram of a human hair follicle. Taken from http://www.tgfolk.net/sites/gtg/hair_root.gif.

dermis. Interestingly, some hair follicles can exist in a transitional phase between terminal and vellus forms. In the scalp, the hair follicles typically grow as a unit, each composed of one to four terminal hairs and one to two vellus hairs and encircled by branches from the same arrector pili muscle (Poblet et al., 2002).

It is important to realise that the pilosebaceous unit is a complex and dynamic three-dimensional structure that regulates various biochemical, immunological and metabolic activities (Rogers, 2004). Furthermore, hair follicles undergo a specific growth cycle of alternating proliferative and rest stages. During the actively growing phase or anagen, the hair matrix cells divide rapidly and migrate upwards to form the hair shaft. Anagen is invariably followed by catagen, a brief period characterised by dramatic morphological changes such as the end of mitosis as well as reabsorption and cell death of the lower follicle segment. The follicle then enters a rest period termed telogen, prior to the hair being shed. Anagen then reoccurs as the hair matrix cells start dividing and the lower follicle redevelops. In most parts of the body the hair cycle lasts for a few months but on the scalp it lasts for 3–8 years. Locally active inhibitors control hair cycling. These chemicals accumulate during anagen and ultimately induce catagen control in a process termed the Chalone hypothesis (Bullough, 1975). It should be noted that the duration of each growth phase as well as the percentage of hair in each growth phases differs markedly between vellus and terminal hairs. Seasonal variations in hair growth are modulated by the endocrine system, under the control of the pineal gland. More specifically, circulating prolactin levels correlate inversely with melatonin concentrations, being elevated during summer and declining in winter.

Another key function of the pilosebaceous units involves the synthesis and release of sebum—a fungistatic and bacteriostatic mixture of short chain fatty acids. The secretion, which is formed by the complete disintegration of the glandular cells, is discharged via ducts into the upper third of the follicular canal (Clarys and Barel, 1995). This creates an environment rich in neutral, non-polar lipids in this region of the follicle. The composition of human sebum (Greene et

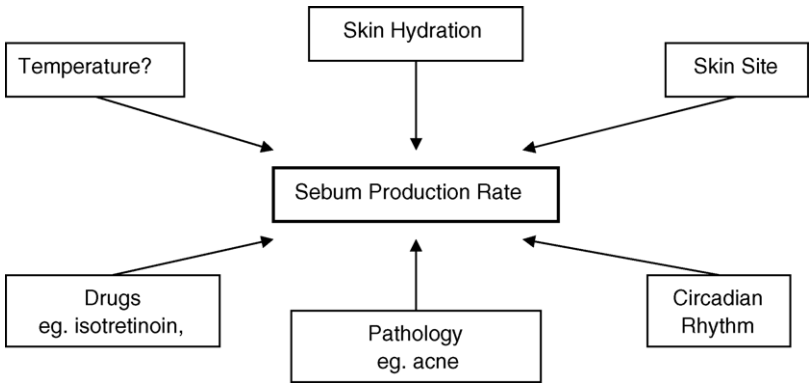

Fig. 2. Scheme depicting the principal factors that influence the sebum production rate in humans.

al., 1970) differs from that of other species in that it contains a high content of triglycerides (57%), the other major constituents being wax esters (26%) and squalene (2%). Sebum production in the glands takes 3 weeks. The ensuing lag time between sebum liberation and its appearance on the skin is 8 days (Downing et al., 1986). Glandular activity is controlled by sex hormones and varies with age. Secretion is absent in infants, accelerated at puberty and reduced in the aged. Nevertheless, typical excretion rates of 0.1 mg/cm^2 of skin/hour have been measured leading to a skin surface content of 0.5 mg/cm^2. Intriguingly, the rate of sebum production is not proportional to the density or size of follicles (Pagnoni et al., 1984), but does follow a circadian rhythm (Clarys and Barel, 1995). There seems to be some controversy over whether sebum secretion is dependent upon temperature. One group has claimed that secretion is constant irrespective of season (Osborne and Hatzenbuhler, 1990) but other evidence suggests that sebum output increases in a warmer environment (Pierard-Franchimont et al., 1990). Fig. 2 lists the factors that can affect sebum production in humans.

3. Regional variations in follicular densities

It is noteworthy that the skin density of pilosebaceous units varies greatly according to body region. On the face and scalp, there are 500–1000 pilosebaceous units per square centimeter with each follicular opening exhibiting a diameter of some 50–100 μm. The combined areas of these orifices may represent as much as 10% of the total surface area of the face and scalp. In other parts of the body, the follicular openings constitute only about 0.1% of the total skin area (Schaefer and Redelmeier, 1996). The sole of the foot, the palm of the hand and the lips do not have hair follicles. A highly detailed human volunteer study was conducted recently by Lademann and co-workers who used a cyanoacrylate surface biopsy method (Otberg et al., 2004b). It was demonstrated that, on average, follicular orifices constituted 1.28% of the available skin surface area on the forehead but only 0.33% of the available surface area on the back. The corresponding values were even smaller for skin areas of the thorax, arm, thigh and calf. However, it should be remembered that the potential area available for topical penetration includes the internal follicular surfaces. When these invaginated areas were taken into account, values of 13.7 and 1% were found for the forehead and forearm, respectively. It is tempting to delineate the extent of follicular transport by simply comparing drug permeation rates through skin regions exhibiting different follicular densities. However, such an approach is unreliable as other site-specific differences such as corneocyte size are also apparent.

There are also regional variations in the distribution and activity of the sebaceous glands. Glandular activity is greatest in the facial region but is absent on the palms of the hands and soles of the feet. Excess sebum from sebum-rich regions tends to flow away in the furrows of the skin microrelief to regions where sebum production is lower. Furthermore, some excess sebum can be reabsorbed into the stratum corneum (Clarys and Barel, 1995).

4. Drug targeting sites

The sebaceous glands, implicated in the aetiology of both androgenetic alopecia (Meidan and Touitou, 2001) and acne (Thiboutot, 2004), represent an obvious therapeutic target site. Considerable effort has been directed towards maximizing the accumulation of various bio-molecules in these androgen-responsive glands. Examples of investigated drugs include adapalene (Rolland et al., 1993), an erythromycin–zinc complex (Morgan et al., 1993), isotretinoin (Tschan et al., 1997), and the anti-androgen RU58841 (Bernard et al., 1997; Munster et al., 2005).

Located just below the sebaceous glands, the mid-follicle bulge represents another attractive targeting zone. This area contains groups of rapidly proliferating cells that play a key role in modulating the growth of the entire hair follicle. As well as the skin mast cell precursors (Kumamoto et al., 2003), the melanocyte stem cells were recently found to be situated in this region (Sharov et al., 2003). The emergence of some skin tumors is probably associated with perturbations occurring at this site.

Other desirable targets include the follicular papilla and hair matrix cells. Situated at the base of the follicle, these structures mediate an important role in controlling hair growth. For instance, the number of matrix cells correlates with the size of the new hair while melanin concentrations in the matrix cells modulate hair pigmentation (Commo et al., 2004). One team showed that hair matrix cells could be transfected with a lac Z reporter gene that had been topically applied to histocultured mouse skin (Li and Hoffman, 1995). Another group successfully delivered pharmacologically relevant doses of antisense oligonucleotides to human hair bulbs in vitro (Lieb et al., 1997). Experiments with a human skin xenograft model demonstrated that plasmid DNA could be effectively transfected into matrix cells (Domashenko et al., 2000). These results have set the stage for further research aimed at modifying either hair growth or pigmentation for cosmetic purposes (Hoffman, 2000, 2005; Saito et al., 2002).

Another application involves exploiting the hair follicles as low resistance shunts for drug delivery to either the viable skin strata or the systemic circulation. As noted above, although the follicular orifices generally occupy no more than about 0.1% of the total skin surface area, the hair follicle represents an invagination of the epidermis extending deep into the dermis, thus providing a greater actual area for potential absorption (Agarwal et al., 2000; Singh et al., 2000). Furthermore, while the surfaces of the follicular openings are initially keratinised, below the ostia of the sebaceous glands there is no mature stratum corneum. In addition, an extensive capillary network associated with the upper dermal vasculature supplies the upper follicle and sebaceous glands with blood while the lower follicle receives its blood supply from the deep dermis and subcutaneous tissues. Under some circumstances, all these features can allow the follicles to function as rapid transport shunts that permit topically applied drugs to bypass the continuous stratum corneum and readily reach either the viable skin layers or the systemic circulation. Recent years have witnessed the emergence of various imaging technologies such as quantitative autoradiography (Touitou et al., 1998), microimaging (Gautier and Bernard, 2001), confocal laser scanning microscopy (Alvarez-Roman et al., 2004a), and combined confocal Raman spectroscopy–confocal microscopy (Caspers et al., 2003). Use of these techniques has confirmed that probe molecules do not only permeate through the intercellular channels of the continuous stratum corneum but also sometimes via the follicles. Both recent experimental findings (Essa et al., 2002; Ogiso et al., 2002; Dokka et al., 2005) and theoretical considerations (Mitragotri, 2003) suggest that this shunt pathway may predominate for hydrophilic and/or high molecular weight bio-molecules. There is some evidence from in vitro human scalp skin studies that the drugs permeate through the junction of the internal and outer root sheaths before diffusing across the outer root sheath into the dermis (Ogiso et al., 2002).

5. Barriers to follicular drug delivery

Although the pilosebaceous units may be desirable as either target sites or shunts for drug delivery, access to these structures can be problematic due to architectural and physicochemical constraints. Table 1 lists these potential barriers and the corresponding resolving strategies.

In the case of particulate delivery systems, the size selectivity of the follicular openings can repre-

Table 1
The potential barriers and resolving strategies associated with pilosebaceous drug delivery

Barrier	Resolving strategy	Examples (reference)
Size selectivity	Optimise microparticle diameter	
	1. 1.5–7.0 μm range	Shaefer et al. (1990), Rolland et al. (1993) and Toll et al. (2004)
	2. 20–40 nm range	Alvarez-Roman et al. (2004b) and Shim et al. (2004)
Sebum	1. Use a lipophilic penetrant	Curcumin (Lademann et al., 2001)
	2. Use a sebum-miscible vehicle	Propylene glycol (Illel, 1997)
Hair cycle	Apply penetrant during anagen	DNA (Domashenko et al., 2000), curcumin (Lademann et al., 2001)

sent a potential barrier. Schaefer's group (Schaefer et al., 1990) found that the follicular deposition of fluorescent polystyrene microbeads in human facial skin was maximised when the beads were 7 μm in diameter. Larger beads remained on the skin surface whilst smaller beads penetrated the superficial layers of the stratum corneum. Very similar size selectivity effects were observed with dansyl chloride-labeled microbeads. Other studies involving polymeric microspheres have identified diameters of 5 μm (Rolland et al., 1993) or 1.5 μm (Toll et al., 2004) as optimal. However, there may well be other size selective processes operating at the nanometer scale. In recent work with porcine skin, Guy's group (Alvarez-Roman et al., 2004b) determined that 20 nm diameter polystyrene particles showed superior follicular deposition than comparable 200 nm diameter particles. Additionally, when minoxidil was encapsulated into microparticles, 40 nm diameter particles were better than 130 nm diameter particles in terms of facilitating transdermal drug penetration through hairy guinea pig skin (Shim et al., 2004). Table 2 lists some of the microparticulate systems described in the literature.

The upward movement of sebum may impede drug transport, particularly in the case of hydrophilic drugs. In vitro work with rodent skin showed that mild heating causes the release of sebum from the sebaceous glands, thus filling the follicles with sebum and blocking the follicular passage of certain hydrophilic drugs (Meidan et al., 1998). It is not known whether this process occurs in human skin. In contrast, the presence of sebum may be a pre-requisite for the follicular uptake of some lipophilic molecules. In this context, the permeation of the moderately lipophilic compound curcumin ($\log K_{o/w} = 3.3$) through the skin of human volunteers was followed by a combination of stripping, staining and laser scanning microscopy (Lademann et al., 2001). The researchers ascertained that follicles tended to be either active or inactive in nature. Active follicles were characterised by sebum production and/or hair growth whilst inactive follicles exhibited neither growth nor sebum production. It was found that curcumin penetrated into the active follicles but not the inactive ones, suggesting that some type of 'pumping' mechanism by the hair follicle shaft was responsible. With regards to sebum in general, it has been proposed that formulating with a suitable wetting agent will ensure that the vehicle makes good contact with the sebum across the vent of the duct (Illel, 1997). However, the role of sebum in drug delivery is

Table 2
Microparticulate systems used in follicular drug delivery research

Microsphere fabrication	Investigated size range (nm)	Penetrant	Skin model(s)	Reference
Polystyrene	1000–24000	Fluorescent label	Human	Schaefer et al. (1990)
Poly(DL-lactic-co-glycolic acid)	5000	Adapalene	Human & rhino mouse	Rolland et al. (1993)
Titanium dioxide	17	Titanium dioxide	Human	Lademann et al. (1999)
Porous nylon	5000	Methylene blue	Hairless rat	Mordon et al. (2003)
Polystyrene	20; 200	Fluorescent label	Pig	Alvarez-Roman et al. (2004b)
Poly(ε-caprolactone)-block-PEG	40; 130	Minoxidil	Hairy guinea pig	Shim et al. (2004)
Polystyrene	750–6000	Fluorescent label	Human	Toll et al. (2004)
Solid lipid nanoparticles	150–500	Nile red & silver	Pig; human regrown epidermis	Munster et al. (2005)

still poorly understood. Significant variations in sebum chemistry between species should be considered before extrapolating data from animal to human studies. The synthesis of artificial human sebum (Motwani et al., 2001; Musial and Kubis, 2003) and the use of differential scanning calorimetry to elucidate the nature of artificial sebum–drug interactions (Motwani et al., 2002; Motwani et al., 2004) may represent the way forward.

The hair growth cycle also appears to influence pilosebaceous drug delivery. It was shown that the liposomal delivery of fluorescent molecules to the follicles was cycle-dependent (Hoffman, 2005). Also, DNA transfection into human hair follicle cells was optimised when performed during the start of anagen (Domashenko et al., 2000). As mentioned above, the follicular deposition of curcumin in human skin was also limited to those follicles in anagen (Lademann et al., 2001). Although the mechanisms remain unclear, identifying the specific cycle phase of hair growth appears to be crucial for standardising experimental conditions (Table 1).

6. Strategies for enhanced follicular delivery

Numerous studies have suggested that the extent of follicular delivery may be modulated by applying certain approaches. Adopted strategies have included; the use of optimised vehicles, microspheres, liposomes, lipoplexes as well as iontophoresis. The literature pertaining to each of these approaches is sequentially discussed below.

6.1. Vehicular optimisation

The extent of follicular drug delivery seems highly dependent upon the vehicle used in the formulation. One suggested strategy for effective pilosebaceous delivery is to use a volatile organic solvent such as ethanol in order to dissolve and draw out sebum from the follicular canal (Illel, 1997). Research with excised rat skin study indicated that the percutaneous absorption of pyridostigmine bromide was maximised when either; ethanol, dimethylsulphoxide or propylene glycol were used (Bamba and Wepierre, 1993). In contrast, the inclusion of the terpene, nerol, or Azone® in the formulation reduced transfollicular penetration at the expense of transport through the continuous stratum corneum. In experiments utilising the hamster ear model, it was shown that salicylic acid deposition in the follicles was maximised when lipophilic rather than hydrophilic vehicles were used (Motwani et al., 2004). In in vitro work with human scalp skin, Bouwstra and co-workers (Grams et al., 2003) determined that propylene glycol–surfactant combinations promoted the follicular accumulation of lipophilic dyes while the use of a 30% ethanolic solution was optimal for less lipophilic dyes (Grams and Bouwstra, 2002). Dokka et al. (2005) analysed the kinetics of modified oligonucleotide deposition in mouse skin. They reported that use of a saline solution resulted in follicular accumulation of oligomer while use of a lipophilic cream trafficked the nucleotide beyond the follicle into the dermis.

Various emulsion systems have been investigated as follicle-targeting vehicles. When plasmid DNA was incorporated into water-in-oil nanoemulsions, topical applications resulted in improved DNA transfection into mouse skin follicular keratinocytes (Wu et al., 2000). Sequential work was directed towards comparing the transdermal penetration of water-soluble penetrants through rat skins exhibiting different follicular densities (Wu et al., 2001). The researchers deduced that significant follicular transport of water-soluble compounds occurred when the compounds were encapsulated within an oil external nanoemulsion droplet. Furthermore, the rate of follicular transport of the model penetrant, inulin, was highly dependent upon the hydrophilic–lipophilic balance (HLB) of the surfactant mixture used in the preparation. Data analysis indicated that lower surfactant HLB values yielded greater transfollicular inulin transport. Mechanistically, the authors proposed that the sebum-miscible external oil phase facilitated follicular penetration of the solubilised penetrant. Zatz's team also found that follicular deposition of salicylic acid could be augmented in a water-in-oil system by increasing the volume of the oil phase (Motwani et al., 2004).

6.2. Use of microparticulate systems

Considerable efforts have been directed towards the design of various microparticulate follicle-targeting systems. Some of this research has been performed using various animal models. For example, one team (Rolland et al., 1993) prepared different topical for-

mulations containing poly(DL-lactic-co-glycolic acid) microspheres. The 5 μm diameter microspheres, were loaded with the antiacne agent, adapalene. Evaluations with the in vivo rhino mouse model indicated that the system was highly comedolytic. More recently, another group (Mordon et al., 2003) tracked the fate of dye-loaded porous nylon microspheres (5 μm diameter) when these were applied on hairless rat skin. Interestingly, 26 h after treatment, the dye was exclusively distributed within the hair follicles and sebaceous glands, diffusing down to a depth of approximately 400 μm below the skin surface. In other experiments (Alvarez-Roman et al., 2004b), fluorescent polystyrene nanoparticles were deposited on to pig skin samples mounted in diffusion cells. Confocal laser scanning microscopy was used to visualise particle penetration through the tissue. Surface imaging revealed that the nanoparticles preferentially accumulated in the follicular openings in a time-dependent manner. In the most recent porcine skin study, specially tailored "solid lipid particles" were shown to preferentially deliver fluorescent molecules to the hair follicles (Munster et al., 2005).

Of course, given the morphological and physiological differences between species, the most relevant studies are those involving human skin. In this context, the topical application of adapalene-loaded poly(DL-lactic-co-glycolic acid) microspheres resulted in the follicle-specific deposition of adapalene in human skin (Rolland et al., 1993). These microsphere formulations exhibited superior efficacy compared to simple aqueous gels of adapalene. Lademann et al. (1999) examined the deposition of coated titanium dioxide microparticles of the type commonly used in commercial sunscreen products. Most of the particles remained on the skin surface or penetrated into the superficial layers of the horny layer. However, less than 1% of the microparticles accumulated in the upper parts of the hair follicles. Toll et al. (2004) applied polystyrene microspheres, in the 0.75–6.0 size range, on to freshly excised human skin samples. Following shock-freezing and slicing into 5 μm sections, the workers used fluorescence microscopy to visualise microparticle deposition. It was found that the microspheres penetrated into the follicles to a maximum depth of 1000 μm below the skin surface. This transport was probably facilitated by the hair shaft 'pumping' mechanism identified by Lademann et al. (2001).

6.3. Application of liposomes

Liposomes have been widely used as drug carriers for follicular targeting purposes. Some of the data has been quite promising. For instance, over a decade ago, Balsari et al. (1994) showed that topical applications of a liposome-entrapped monoclonal antibody to doxorubicin completed suppressed doxorubicin-induced alopecia in rats. Other researchers determined that liposomal entrapment of calcein, melanin and high-molecular weight DNA resulted in the accumulation of each penetrant in the hair follicles of histocultured mouse skin (Li and Hoffman, 1997). Crucially, the application of aqueous control solutions of these molecules resulted in no specific intrafollicular accumulation. Other workers experimented with topical applications of non-ionic liposomes that were loaded with either the hydrophilic protein, alpha interferon or the hydrophobic peptide, cyclosporine A (Niemec et al., 1995). In the in vivo hamster ear model, it was found that significant follicular accumulations of both drugs could be achieved. Contrastingly, ionic liposomes proved ineffective as pilosebaceous delivery systems. In later in vivo work in a mouse model, non-ionic liposomes were successfully employed to facilitate the follicular delivery of both minoxidil as well as plasmid DNA (Ciotti and Weiner, 2002). Most recently, fluorescence imaging techniques were used in order to evaluate the follicular deposition of the anticancer agent, adriamycin, in wax-depilated rat skin (Han et al., 2004). The authors reported that, liposomal encapsulation could enhance adriamycin follicular accumulation by well over an order of magnitude, depending upon liposome composition. Finally, Verma and co-workers documented that liposomal formulations of cyclosporine A were effective in inducing hair growth in Dundee experimental bald rats (Verma et al., 2004).

Despite the positive findings listed above, negative reports with liposomes have also been reported. For example, in an in vitro human facial skin study (Tschan et al., 1997), it was determined that use of an ethanolic gel was as efficient as a liposomal or a mixed micellar gel in delivering isotretinoin to the sebaceous glands.

6.4. Topical lipoplex treatment

One promising drug delivery strategy involves using DNA–cationic lipid complexes, termed lipoplexes,

which are designed to facilitate enhanced transfection. The complexes are formed spontaneously when cationic liposomes are mixed with either plasmid DNA or oligonucleotides. It has been shown that the negatively charged nucleotides are electrostatically bound to the cationic lipid (Meidan et al., 2000, 2001). There have been a handful of reports documenting topical lipoplex strategies for follicular targeting. Working with human hair follicles in vitro, one team (Lieb et al., 1997) showed that lipoplex-based formulations enhanced the intrafollicular delivery of oligonucleotides. Approximately 0.5% of the applied dose was delivered to the hair bulbs and the deeper skin strata within 24 h after topical application (Lieb et al., 1997). In a refined study, it was found that it was possible to efficiently transfect the hair follicle progenitor cells of both mouse skin and human xenografts in vivo (Domashenko et al., 2000).

6.5. Iontophoresis

In several studies, the hair follicles have been shown to act as channels, though not usually depots, for the iontophoretic flux of various different molecules (Uitto and White, 2003). Iontophoresis may be particularly useful for the systemic delivery of ionic, polar or high-molecular-weight compounds, which normally undergo slow or negligible passive absorption. Although the importance of the follicular route for iontophoretic flux should not be disregarded, a full discussion of iontophoresis is beyond the scope of this paper, although several excellent reviews are available (Junginger, 2002; Kanikkannan, 2002; Kalia et al., 2004).

7. Emerging follicular methodologies

To date, a major problem in evaluating transfollicular drug delivery has been the lack of a quantitative model system that is truly follicle-free but retains the structural, biochemical and barrier properties of normal skin. Previously employed models such as the Syrian hamster ear, the fuzzy rat, the macaque monkey and the regrown scar tissue system have proved extremely useful for studying follicular penetration (Lauer et al., 1995). However, these systems do not fulfill the criteria stated above. Importantly, two novel quantitative systems have emerged in recent years and these are described below. Furthermore, new technological developments have facilitated ongoing improvements in visual imaging methods and some advances within this context are also reviewed.

7.1. The skin sandwich system

The recently devised and validated in vitro skin sandwich (or composite) system can be used to quantitatively de-convolute the contribution of the hair follicles to total drug penetration (El Maghraby et al., 2001; Barry, 2002; Essa et al., 2002). In this approach, a composite double membrane or sandwich is formed by overlaying an extra stratum corneum membrane onto a human epidermal membrane. It is important that both components of the sandwich originate from the same skin donor. Effectively, the shunt pathway through both parts of the membrane is obstructed, as illustrated in Fig. 3. The theory behind the technique is quite simple. At steady state, passive drug flux through a solid homogenous membrane is inversely proportional to the pathlength taken by the permeant. As the main permeation barrier in human skin resides within the stratum corneum, flux through the sandwich should thus be half that through the single epidermis if the shunts make a negligible contribution to the penetration process. Conversely, if sandwich flux is significantly less than half of single epidermal flux then that would indicate that the shunt route contribution is notable. Similarly, it is also possible to calculate the shunt contribution by measuring lag times since these are proportional to the square of the pathlength.

Application of the skin sandwich system involves making a few reasonable assumptions. Firstly, it is assumed that the shunts represent hair follicles as it is believed that the sweat ducts orifices, with their much smaller dimensions, play a smaller role in drug absorption. Secondly, the small resistance of the nucleated epidermis to permeation is ignored for the sake of simplicity. Furthermore, the system cannot be used for highly lipophilic drugs as the stratum corneum will no longer be the principal barrier to penetration for such compounds. Lastly, the theory assumes no new pores are created during the permeation process. Despite these caveats, the skin sandwich system represents a very powerful new tool in drug delivery research. This was highlighted by a theoretical analysis conducted by

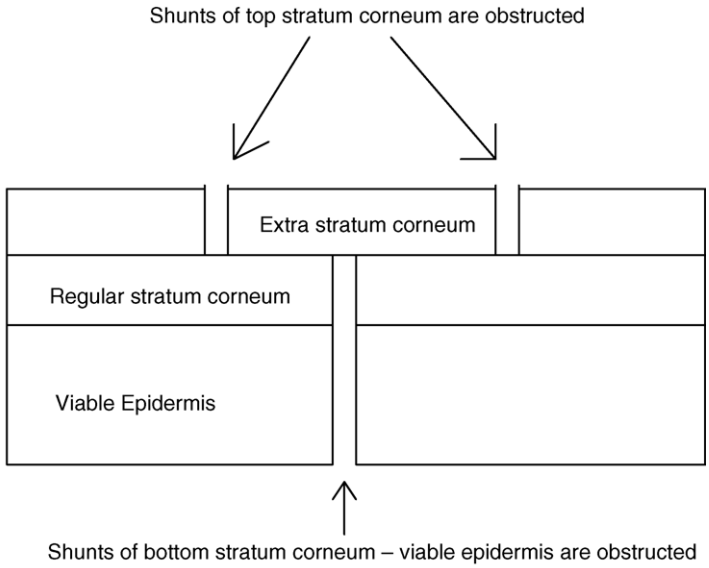

Fig. 3. Schematic representation illustrating the basis of the skin sandwich method.

Barry (2002) who considered what happens in practice if the two membranes do not adhere tightly together. It might be presumed that this would lead to some lateral aqueous drug transport between the layers. However, use of a Monte Carlo simulation indicated that even assuming incomplete adherence, the distance traveled by a molecule during this lateral diffusion is very long compared with the thickness of the stratum corneum and nucleated epidermis comprising the bottom layer. So, even if adherence in the sandwich is imperfect, the results can still be safely interpreted on the basis of ideal contact behaviour.

The skin sandwich has already been used in several different experimental settings. El Maghraby et al. (2001) first used the technique to investigate if the follicles played a significant role in the transepidermal penetration of estradiol from ultradeformable liposomes. It was found that the follicular route had only a very minor contribution towards estradiol delivery from these formulations. Subsequently, the sandwich technique was employed in order to compare mannitol with oestradiol skin permeation under passive conditions (Essa et al., 2002). Mannitol represented a model hydrophilic permeant while oestradiol represented a model lipophilic drug. It was determined that in the first 8 h of drug penetration, mannitol transport was entirely mediated by follicles while oestradiol penetration was almost entirely non-follicular in nature. A more complex picture emerged when mannitol flux was followed over a longer timescale. Interestingly, encapsulating oestradiol in liposomes did not influence the drug's preference for the non-follicular route. Overall, the skin sandwich system clearly represents a highly effective novel probe, which has yet to be fully exploited in mechanistic skin transport research.

7.2. *The sebum discharge system*

This in vitro methodology relies on the fact that mild heating to 42 °C or low intensity ultrasound application causes sebum to be discharged from the sebaceous glands, thus filling much of the hair follicle shafts (Meidan et al., 1998). The sebum discharge effect has been observed in the skin of Wistar rats as well as guinea pigs. It was demonstrated that lipid deposition in the shafts means that this pathway is blocked for hydrophilic molecules that normally penetrate via this route. In male Wistar rat skin, it was found that the follicular route was responsible for virtually all sucrose and mannitol absorption. Crucially, it has yet to be established whether the phenomenon develops in other species especially human skin. Future investigations would involve further characterisation in order to ascer-

tain as to whether or not the sebum discharge effect occurs in vivo.

7.3. Novel optical imaging systems

As briefly noted above, confocal laser scanning microscopy (CLSM) has become a well-established, non-invasive methodology for deriving high resolution images from skin as well as other biological tissues (Grams et al., 2003; Alvarez-Roman et al., 2004a). Principal advantages of this technology include; its capacity for in vivo application, good time-resolution and the ability to visualise at multiple depths parallel to the sample surface without the need for mechanical sectioning. In a key development, Bouwstra and co-workers recently extended CLSM cross-sectional imaging by allowing on-line visualisation of drug diffusion in non-fixed, fresh human skin (Grams et al., 2004a, 2004b). In vitro work with this variant CSLM approach in human scalp skin showed rapid intrafollicular transport of lipophilic label from an applied aqueous solution (Grams et al., 2004a). The combination use of confocal Raman spectroscopy and confocal microscopy represents another promising tool for analysing follicular drug delivery (Caspers et al., 2003). A further new methodology is optical coherence tomography, which has been employed in conjunction with laser scanning microscopy to visualise follicular orifices (Otberg et al., 2004a). This technology was recently used to differentiate between open hair follicles and those follicles plugged with corneocytes.

8. Conclusions

It can be concluded that transfollicular drug delivery is indeed a reality but that at present its therapeutic value is still under question. It appears that this delivery route is quite complex in nature and that drug transport through the appendages is probably modulated by an array of different variables. To date, most of the work in this field has been undertaken using a multiplicity of; drugs, skin models, application protocols and endpoint evaluation modes. As a result, it has been difficult to discern correlations between penetrant properties, formulation design and the extent of follicular penetration. Clearly, further research needs to be conducted on a more systematic and methodical basis. This should permit identification of the role of the diverse parameters which modulate transfollicular absorption. Once this is achieved, it should be possible to optimise the process.

On a more specific note, there needs to be a clearer distinction made between use of the follicles as shunts for systemic drug delivery as opposed to their use as targets for local therapy. For the former application, the best approach may be to employ the newly available in vitro methodologies i.e. the skin sandwich and sebum discharge systems. These techniques have not yet been extensively used and their quantitative nature will allow us to deconvolute the follicular contribution to transdermal drug transport. Such studies should be used in conjunction with the rapidly improving, novel imaging methodologies described above. However, for both local and systemic follicular applications, in vivo studies will ultimately be necessary since in vitro systems may be compromised by follicular shaft collapse and/or removal of the perifollicular circulation. This was recently evidenced in microsphere studies where particle penetration occurred in vivo but was suppressed ex vivo (Toll et al., 2004).

Another current limitation to our understanding in this field relates to the role of sebum, its physicochemical properties and the nature of sebum–drug interactions. Advances could be made in this area by synthesizing artificial sebum and characterising its properties, especially with regards to the solubilities and diffusion rates of different drug molecules.

References

Agarwal, R., Katare, O.P., Vyas, S.P., 2000. The pilosebaceous unit: a pivotal route for topical drug delivery. Meth. Find. Exp. Clin. Pharmacol. 22, 129–133.

Alvarez-Roman, R., Naik, A., Kalia, Y.N., Fessi, H., Guy, R.H., 2004a. Visualization of skin penetration using confocal laser scanning microscopy. Eur. J. Pharm. Biopharm. 58, 301–316.

Alvarez-Roman, R., Naik, A., Kalia, Y.N., Guy, R.H., Fessi, H., 2004b. Skin penetration and distribution of polymeric nanoparticles. J. Contr. Release 99, 53–62.

Balsari, A.L., Morelli, D., Menard, S., Veronesi, U., Colnagi, M.I., 1994. Protection against doxorubicin-induced alopecia in rats by liposome-entrapped monoclonal antibodies. FASEB J. 8, 226–230.

Bamba, F.L., Wepierre, J., 1993. Role of the appendageal pathway in the percutaneous absorption of pyridostigmine bromide in various vehicles. Eur. J. Drug Metab. Pharmacokinet. 18, 339–348.

Barry, B.W., 2002. Drug delivery routes in skin: a novel approach. Adv. Drug Deliv. Rev. 54, S31–S40.

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

Bullough, W.S., 1975. Chalone control mechanisms. Life Sci. 16, 323–330.

Caspers, P.J., Lucassen, G.W., Puppels, G.J., 2003. Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin. Biophys. J. 85, 572–580.

Ciotti, S.N., Weiner, N., 2002. Follicular liposomal delivery systems. J. Liposome Res. 12, 143–148.

Clarys, P., Barel, A., 1995. Quantitative evaluation of skin surface lipids. Clin. Dermatol. 13, 307–321.

Commo, S., Gaillard, O., Bernard, B.A., 2004. Human hair graying is linked to a specific depletion of hair follicle melanocytes affecting both the bulb and the outer root sheath. Br. J. Dermatol. 150, 435–443.

Dokka, S., Cooper, S.R., Kelly, S., Hardee, G.E., Karras, J.G., 2005. Dermal delivery of topically applied oligonucleotides via follicular transport in mouse skin. J. Invest. Dermatol. 124, 971–975.

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

Downing, D.T., Stewart, M.E., Strauss, J.S., 1986. Changes in sebum secretion and the sebaceous gland. Dermatol. Clin. 4, 419–423.

El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2001. Skin hydration and possible shunt route penetration in controlled oestradiol delivery from ultradeformable liposomes. J. Pharm. Pharmacol. 53, 1311–1322.

Essa, E.A., Bonner, M.C., Barry, B.W., 2002. Human skin sandwich for assessing shunt route penetration during passive and iontophoretic drug and liposome delivery. J. Pharm. Pharmacol. 54, 1481–1490.

Feldman, R.J., Maibach, H.I., 1967. Regional variation in percutaneous penetration of cortisol in man. J. Invest. Dermatol. 48, 181–183.

Gautier, B., Bernard, B.A., 2001. On the use of micro-imager to directly visualize drug distribution in human skin. Skin Pharmacol. Appl. Skin Physiol. 14, 41–45.

Grams, Y.Y., Alaruikka, S., Lashley, L., Whitehead, L., Bouwstra, J.A., 2003. Permeant lipophilicity and vehicle composition influence accumulation of dyes in hair follicles of human skin. Eur. J. Pharm. Sci. 18, 329–336.

Grams, Y.Y., Bouwstra, J.A., 2002. Penetration and distribution of three lipophilic probes in vitro in human skin focusing on the hair follicle. J. Contr. Release 83, 253–262.

Grams, Y.Y., Whitehead, L., Cornwell, P., Bouwstra, J.A., 2004a. Time and depth resolved visualization of the diffusion of a lipophilic dye into the hair follicle of fresh unfixed human scalp skin. J. Contr. Release 98, 367–378.

Grams, Y.Y., Whitehead, L., Cornwell, P., Li, G., Bouwstra, J.A., 2004b. On-line visualization of dye diffusion in fresh unfixed human skin. Pharm. Res. 21, 851–859.

Greene, R.S., Downing, D.T., Pochi, P.E., Strauss, J.S., 1970. Anatomical variation in the amount and composition of human skin surface lipid. J. Invest. Dermatol. 54, 240–247.

Han, I., Kim, M., Kim, J., 2004. Enhanced transfollicular delivery of adriamycin with a liposome and iontophoresis. Exp. Dermatol. 13, 86–92.

Hoffman, R.M., 2000. The hair follicle as a gene therapy target. Nat. Biotechnol. 18, 20–21.

Hoffman, R.M., 2005. Gene and stem cell therapy of the hair follicle. Meth. Mol. Biol. 189, 437–448.

Illel, B., 1997. Formulations for transfollicular drug administration: some recent advances. Crit. Rev. Ther. Drug Syst. 14, 207–217.

Junginger, H.E., 2002. Iontophoretic modeling of apomorphine: from in vitro modeling to the Parkinson patient. Adv. Drug Deliv. Rev. 54S1, S57–S75.

Kalia, Y.N., Naik, A., Garrison, J., Guy, R.H., 2004. Iontophoretic drug delivery. Adv. Drug Deliv. Rev. 56, 619–658.

Kanikkannan, N., 2002. Iontophoresis-based transdermal delivery systems. BioDrugs 16, 339–347.

Kumamoto, T., Shalhevet, D., Matsue, H., Mummert, M.E., Ward, B.R., Jester, J.V., Takashima, A., 2003. Hair follicle serves as a local reservoir of skin mast cell precursors. Blood 102, 1654–1660.

Lademann, J., Otberg, N., Richter, H., Weigman, H.J., Lindemann, U., Schaefer, H., Sterry, W., 2001. Investigation of follicular penetration of topically applied substances. Skin Pharmacol. Appl. Skin Physiol. 14, 17–22.

Lademann, J., Weigmann, H.J., Rickmeyer, C., Barthelmes, H., Schaefer, H., Mueller, G., Sterry, W., 1999. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol. Appl. Skin Physiol. 12, 247–256.

Lauer, A.C., Lieb, L.M., Ramachandran, C., Flynn, G.L., Weiner, N.D., 1995. Transfollicular drug delivery. Pharm. Res. 12, 179–186.

Li, L., Hoffman, R.M., 1995. Model of selective gene therapy of hair growth: liposome targeting of the active Lac-Z gene to hair follicles of histocultured skin. In Vitro Cell Dev. Biol. Anim. 31, 11–13.

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

Lieb, M.L., Limatta, A.P., Bryan, R.N., Brown, B.D., Krueger, G.G., 1997. Description of the intrafollicular delivery of large molecular weight molecules to follicles of human scalp skin in vitro. J. Pharm. Sci. 86, 1022–1029.

Meidan, V.M., Docker, M., Walmsley, A.D., Irwin, W.J., 1998. Low intensity ultrasound as a probe to elucidate the relative follicular contribution to total transdermal absorption. Pharm. Res. 15, 85–92.

Meidan, V.M., Touitou, E., 2001. Treatments for androgenetic alopecia and alopecia areata: current options and future prospects. Drugs 61, 53–69.

Meidan, V.M., Cohen, J.S., Amariglio, N., Hirsch-Lerner, D., Barenholz, Y., 2000. Interaction of oligonucleotides with cationic lipids: the relationship between electrostatics, hydration and state of aggregation. Biochim. Biophys. Acta 1464, 251–261.

Meidan, V.M., Glezer, J., Amariglio, N., Cohen, J.S., Barenholz, Y., 2001. Oligonucleotide lipoplexes: the influence of oligonucleotide composition on complexation. Biochim. Biophys. Acta 1568, 177–182.

Mitragotri, S., 2003. Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. J. Contr. Release 86, 69–92.

Mordon, S., Sumian, C., Devoiselle, J.M., 2003. Site-specific methylene blue delivery to pilosebaceous structures using highly porous nylon microspheres: an experimental evaluation. Lasers Surg. Med. 33, 119–125.

Morgan, A.J., Lewis, G., Van den Hoden, W.E., Akerboom, P.J., 1993. The effect of zinc in the form of erythromycin–zinc complex (Zineryt lotion) and zinc acetate on metallothionein expression and distribution in hamster skin. Br. J. Dermatol. 129, 563–570.

Motwani, M.R., Rhein, L.D., Zatz, J.L., 2001. Differential scanning calorimetry studies of sebum models. J. Cosmet. Sci. 52, 211–224.

Motwani, M.R., Rhein, L.D., Zatz, J.L., 2002. Influence of vehicles on the phase transitions of model sebum. J. Cosmet. Sci. 53, 35–42.

Motwani, M.R., Rhein, L.D., Zatz, J.L., 2004. Deposition of salicylic acid into hamster sebaceous glands. J. Cosmet. Sci. 655, 519–531.

Munster, U., Nakamura, C., Haberland, A., Jores, K., Mehnert, W., Rummel, S., Schaller, M., Korting, H.C., Zhouboulis, C.C., Blume-Peytavi, U., Schafer-Korting, M., 2005. RU 58841-myristate—prodrug development for topical treatment of acne and androgenetic alopecia. Pharmazie 60, 8–12.

Musial, W., Kubis, A., 2003. Preliminary assessment of alginic acid as a factor buffering triethanolamine interacting with artificial skin sebum. Eur. J. Pharm. Biopharm. 55, 237–240.

Niemec, S., Ramachandran, C., Weiner, N., 1995. Influence of nonionic liposomal composition on topical delivery of peptide drugs into pilosebaceous units: an in vivo study using the hamster ear model. Pharm. Res. 12, 1184–1188.

Ogiso, T., Shiraki, T., Okajima, K., Tanino, T., Iwaki, M., Wada, T., 2002. Transfollicular drug delivery: penetration of drugs through human scalp skin and comparison of penetration between scalp and abdominal skins in vitro. J. Drug Target. 10, 369–378.

Osborne, D.W., Hatzenbuhler, D.A., 1990. The influence of skin surface lipids on topical formulations. In: Osborne, D.W., Amann, A.H. (Eds.), Topical Drug Delivery Formulations. Marcel Dekker, New York, pp. 69–86.

Otberg, N., Richter, H., Knuttel, A., Schaefer, H., Sterry, W., Lademann, J., 2004a. Laser spectroscopic methods for the characterization of open and closed follicles. Laser Phys. Lett. 1, 46–49.

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

Pagnoni, A., Kligman, A.M., el Gammal, S., Stoudemayer, T., 1984. Determination of density of follicles on various regions of the face by cyanoacrylate biopsy: correlation with sebum output. Br. J. Dermatol. 131, 862–865.

Pierard-Franchimont, C., Pierard, G.E., Klihman, A.C., 1990. Seasonal modulation of sebum excretion. Dermatologica 181, 21–22.

Poblet, E., Ortega, F., Jimenez, F., 2002. The arrector pili muscle and the follicular unit of the scalp: a microscopic anatomy study. Dermatol. Surg. 28, 800–803.

Rogers, G.E., 2004. Hair follicle differentiation and regulation. Int. J. Dev. Biol. 48, 163–170.

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

Rutherford, T., Black, J.G., 1969. The use of autoradiography to study the localization of germicides in skin. Br. J. Dermatol. 81, 75–87.

Saito, N., Zhao, M., Li, L., Baranov, E., Yang, M., Ohta, Y., Katsuoka, K., Penman, S., Hoffman, R.S., 2002. High efficiency genetic modification of hair follicles and growing hair shafts. Proc. Natl. Acad. Sci. U.S.A. 99, 13120–13124.

Schaefer, H., Redelmeier, T.E., 1996. Skin Barrier: Principles of Percutaneous Absorption. Karger, Basel.

Schaefer, H., Watts, F., Brod, J., Illel, B., 1990. Follicular penetration. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Prediction of Percutaneous Penetration: Methods, Measurements, and Modelling. IBC Technical Services, London, pp. 405–441.

Scheuplein, R.J., 1965. Mechanism of percutaneous absorption. I. Rate of penetration and solubility. J. Invest. Dermatol. 45, 334–346.

Scheuplein, R.J., 1967. Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration. J. Invest. Dermatol. 48, 63–70.

Sharov, A.A., Li, G.Z., Palkina, T.N., Sharova, T.Y., Gilchrest, B.A., Botchkarev, V.A., 2003. Fas and c-kit are involved in the control of hair follicle melanocyte apoptosis and migration in chemotherapy-induced hair loss. J. Invest. Dermatol. 120, 27–35.

Shim, J., Seok Hang, H., Park, W.S., Han, S.H., Kim, J., Chang, I.S., 2004. Transdermal delivery of minoxidil with block copolymer microparticles. J. Contr. Release 97, 477–484.

Singh, P., Sihorkar, V., Jaitely, V., Kanaujia, P., Vyas, S.P., 2000. Pilosebaceous unit: anatomical considerations and drug delivery opportunities. Ind. J. Pharmacol. 32, 269–281.

Thiboutot, D., 2004. Regulation of human sebaceous glands. J. Invest. Dermatol. 123, 1–12.

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

Touitou, E., Meidan, V.M., Horwitz, E., 1998. Methods for the quantitative determination of drug localized in the skin. J. Contr. Release 56, 7–21.

Tschan, T., Steffen, H., Supersaxo, A., 1997. Sebaceous-gland deposition of isotretinoin after topical application: an in vitro study using human facial skin. Skin Pharmacol. 10, 126–134.

Tur, E., Maibach, H.I., Guy, R.H., 1991. Percutaneous penetration of methyl nicotinate at three anatomic sites: evidence for an appendageal contribution to transport. Skin Pharmacol. 4, 230–234.

Uitto, O.D., White, H.S., 2003. Electroosmotic pore transport in human skin. Pharm. Res. 20, 646–652.

Verma, D.D., Verma, S., McElwee, K.J., Freyschmidt-Paul, P., Hoffman, R., Fahr, A., 2004. Treatment of alopecia areata in the DEBR model using cyclosporine A lipid vesicles. Eur. J. Dermatol. 14, 332–338.

Whiting, D.A., 2000. Histology of normal hair. In: Hordinsky, M.K., Sawaya, M.E., Scher, R.K. (Eds.), Atlas of Hair and Nails. Churchill Livingstone, Philadelphia, p. 918.

Wu, H., Ramachandran, C., Weiner, N., Roessler, B.J., 2001. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int. J. Pharm. 220, 63–75.

Wu, H., Ramachandran, C., Bielinska, A.U., Kingzett, K., Sun, R., Einer, N.D., Roessler, B.J., 2000. Topical transfection using plasmid DNA in a water-in-oil nanoemulsion. Int. J. Pharm. 221, 23–34.