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PILOSEBACEOUS UNIT: ANATOMICAL CONSIDERATIONS AND DRUG DELIVERY OPPORTUNITIES

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SUMMARY Pilosebaceous unit is composed of hair follicle, hair shaft and sebaceous gland. This three-dimensional complex structure present on the surface of mammalian skin has been considered as an important pathway for percutaneous absorption of topically applied drugs and delivery systems. This pathway could be exploited for localized drug delivery for diseases associated with pilosebaceous gland *vis-a-vis* systemic absorption of drugs. A comprehensive overview of pilosebaceous anatomy and surrounding environment is needed for rationale designing of drug delivery systems targeted toward hair follicle. The present communication presents a review of hair follicle anatomy with physiological environment, rationale, opportunities, means and modes for drug targeting to pilosebaceous compartment.

KEY WORDS Hair follicle pilosebaceous unit sebaceous gland topical application targeted drug delivery

1. Introduction

Skin serves as one of the most easily accessible routes of drug administration. However, the presence of stratum corneum on the surface makes it selective towards applied drugs or delivery systems. Although, stratum corneum has been regarded as the major barrier to penetration of substances into and through the skin, it also serves as the main (transepidermal) pathway for permeation and penetration. Indeed, the stratum corneum is comprised of heterogeneous two compartment system of protein enriched cells embedded in lipid domains[1]. The lipid regions have been reported to be possible pathways for transport of lipophilic substances, whereas the corneocytes may represent a hydrophilic pathway[2]. However, recent reports have suggested that in addition to the transepidermal route, pilosebaceous unit, comprising of hair follicle and sebaceous glands (transfollicular) may contribute significantly to transdermal delivery[3-11]. As only about 0.1% of the total skin surface area is occupied by the orifices of hair follicle[12], their role in percutaneous transport appears to be limited. However, the hair follicle is an invagination of the epidermis extending deep into the dermis, thus providing a greater actual area for potential absorption.

The mammalian hair follicle is gaining attraction as a complex, dynamic structure with unique biochemical and immunological reactions, which dictate cyclic growth phase and contribute significantly to passive transport of compounds through skin. Studies[3-11] have suggested that percutaneous absorption of many compounds *via* follicular pathway may be more significant than previously assumed. It has been proved that maximum absorption of some compounds occurred at sites with higher follicular density[9].

A major limitation to date, in elucidating the follicular pathway was lack of an adequate pharmacokinetic model that can clearly distinguish transfollicular from transepidermal percutaneous absorption. However, the concept of targeted drug delivery to hair follicle is a worthwhile consideration in regard to the potential applications for treating conditions like acne, androgenetic alopecia, alopecia areata and some skin cancers. In addition to localized drug delivery to the pilosebaceous compartment, systemic delivery *via* the hair follicle could be appreciated. This review reveals the various structural, functional and therapeutic aspects of transfollicular mode of drug delivery.

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2. Pilosebaceous unit: An integral part of skin

The hair follicle and associated gland comprise the pilosebaceous unit. Human pilosebaceous unit shows an extreme diversity with regard to its variation according to localization along the human body[13]. Targeting specific sites of the hair follicle represents a feasible therapeutic approach, as several dermatological abnormalities are known to originate at the hair follicle.

Skin serves as an efficient barrier against the invasion of various biological, physical and chemical agents. The skin is traditionally divided into three major regions: the stratum corneum, the viable epidermis and the dermis. The outermost of these layers, the stratum corneum serves as a stern barrier against external invaders. Stratum corneum is indeed a formidable, water tight barrier, consisting of a heterogeneous two compartment system of protein enriched cells embedded in lipid domains[1]. The lipids are important for the moisture retaining ability of the stratum corneum[14].

The viable epidermis lies below the stratum corneum and consists of stratified keratinized epithelial cells, whose ultimate function is to produce the stratum corneum. The layer is devoid of blood vessels, and rely on nourishment by cell fluids from the deeper dermis layer. The deepest layer of skin is the dermis, which consists of dense, irregularly arranged connective tissue and it is nourished directly by blood vessels, as shown in Figure 1. Embedded in the skin are eccrine sweat glands, apocrine glands, hair follicles and sebaceous glands. Eccrine sweat glands are simple tubular glands distributed almost all over the human body. Each gland has a secretory part located below the dermis in the subcutaneous tissue and an excretory duct that ultimately opens directly on the skin surface. These glands produce perspiration and are numerous particularly on the palm of the hand. Apocrine glands produce characteristic body odours and are primarily located in the axilla. The secretion of this gland contains cholesterol, steroids and proteinaceous substances. The apocrine gland empties into the hair follicle above the sebaceous gland.

Pilosebaceous unit possesses sebocytes, like epidermal keratinocytes, which express a variety of cytokines, that are implicated in inflammatory and immune responses[15]. Sebocytes have been found to express tumor necrosis factor alpha (TNF-α). Moreover, certain neuropeptides including vascular intestinal peptides and proopiomelanocortin (POMC) peptides as well as their receptors have been localized within the pilosebaceous unit of murine and human skin. POMC exerts important immunoregulatory effects by antagonizing the function of proinflammatory cytokines (*e.g.* interleukin 1, interleukin 6 and TNF-α), induction of immunosuppressive cytokines (like interleukin 10), modulation of costimulatory molecules expression (*e.g.* B7-2) or suppression of macrophage derived nitric oxide.

2.1. Anatomy of hair follicles and associated appendages

Hair follicle along with associated sebaceous glands form pilosebaceous unit. Figure 2 illustrates the hair follicle and associated structures. Hair follicles size range from 10-70 μm depending upon the hair type.

Hair follicle is composed of three primary layers. Outermost layer is continuous with the epidermis and indistinguishable from it. This layer is important for drug delivery purposes. This potentially provides for larger surface area for absorption below the skin.

The function of outer root sheath, a keratinized surface layer surrounding the inner root sheath, is protection and moulding of inner layers. The follicle as a whole is surrounded by an acellular basement membrane, commonly named as glassy membrane.

Sebaceous glands are collaborated to the hair follicle by ducts. The sebaceous glands are well defined in the embryo, remain relatively small during childhood and become drastically enlarged during puberty[16]. In each gland, a common excretory duct is supplied by smaller ducts that originate in the acini of the gland. As the flattened cuboidal peripheral sebaceous cells move toward the center of the gland, lipid synthesis within the cell increases. The cell swells with fat until a 100 to 150 fold increase in the cell volume is attained. The entire cell then ruptures, expelling its contents into the excretory stream of the gland as sebum. This release creates an environment rich in neutral, non-polar lipids, synthesized and discharged every 1-3 weeks. Marked differences are seen in sebum composition among mammals, as shown in Table 1.

Figure 1. Structure of normal skin.

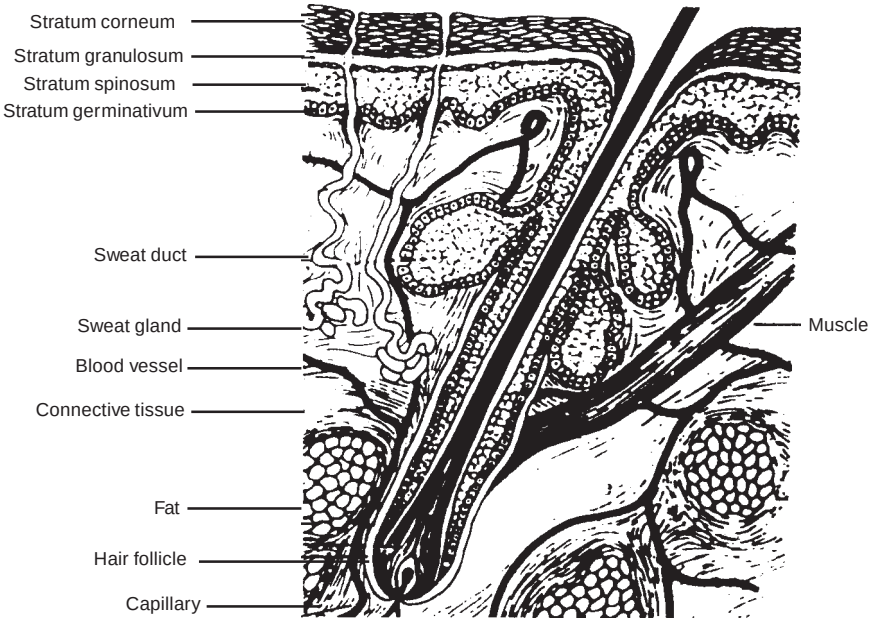


Figure 2. Anatomy of pilosebaceous gland.

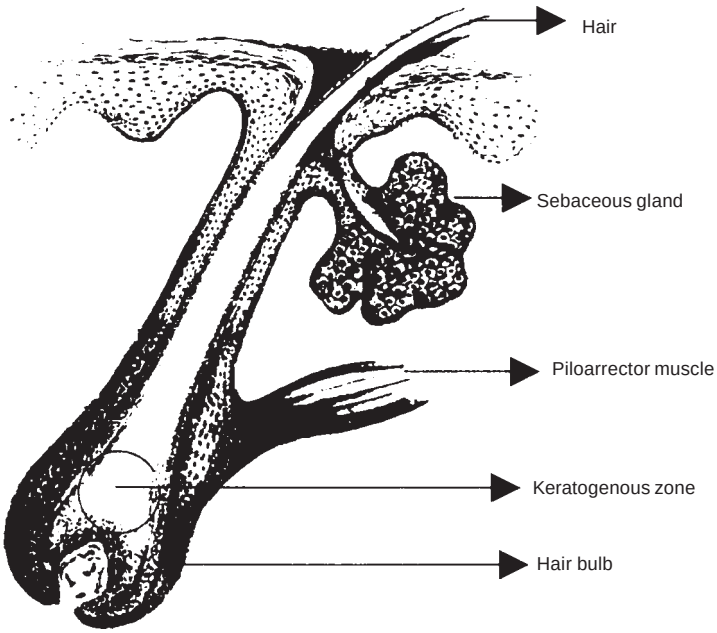
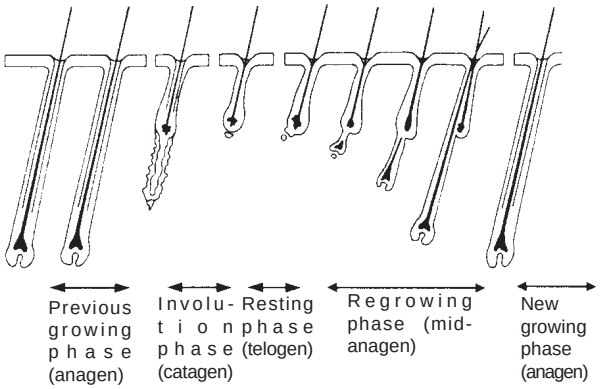


Table 1. Percent composition of some mammalian skin surface lipids.

Component lipids	Man[24]	Rat[25]	Mouse[26]	Guinea pig[27, 28]
Squalene	12	0.5	-	-
Wax esters	26	17	5	-
Sterol ester	3	27	10	33
Wax diesters type I	-	10	} 65	-
Wax diesters type II	-	11		-
Glyceryl ether diesters	-	8	-	28
Triglycerides	57	-	6	-
Free fatty alcohol	-	-	-	6
Free sterols	2	6	13	9

Superscribed numbers refer to reference number.

Figure 3. The growth cycle of the hair follicle.



Triglycerides constitute the principle class of lipids. Squalene, a constituent of human sebum, is the principle surface lipid of the beaver, the kinkajou, and the mole[17,18]. Sterols and sterol esters are frequently present in sebum[19,20]. Similarly, wax esters and various types of wax diesters are also not uncommon[21-29].

2.2. Hair growth cycle

All hair follicles undergo a species specific growth cycle of alternating active growth and rest stages, as illustrated in Figure 3. Knowledge about number of hair follicles in different growth phases are important when studies involve drugs that affect hair growth cycle like minoxidil, which promotes hair growth with an increase in number of hair follicles in anagen phase and doxorubicin, which induces alopecia. Entry of drug delivery systems into pilosebaceous unit is also dictated by presence of hair follicles in different growth phases. Recently presented work with liposome mediated delivery suggests that DNA uptake by hair follicles may be feasible only at specific points in the hair growth cycle [30]. It has been proved that entry of topical liposomes is optimal at the onset of anagen phase. Anagen represents the period of active cell division and upward migration of hair matrix cells to form the hair shaft. Anagen phase is followed by relatively short catagen phase, which results into the cessation of mitosis and resorption of the lower portion of the hair follicle by apoptosis (programmed cell death). Upon completion of catagen, the follicle withdraws from the dermal papilla and the follicle passes into the resting stage known as telogen phase. The cycle then returns to anagen after completion of telogen stage and the lower follicle is reformed. For standardization of experimental conditions, recognition of particular stages of the hair growth cycle may be important[31-34]. Folliculograms, a histogram between percent population of hair follicles and length has been well documented and reflects the effect of bioactives on the hair growth cycle[35].

2.2.1.Control of hair growth cycle

Synchrony between hair growth cycle has been observed in many animals in the neonatal period, including man[36]. Seasonal changes in hair growth are controlled by the endocrine system under the coordinating influence of the pineal gland, which transduces the environmental signals. Circulating prolactin levels correlate inversely with melatonin levels, being raised during the summer and falling during the winter, and it has been proved that prolactin, under pineal control is partly responsible for regulating seasonal changes. In rats, estradiol, testosterone and adrenal steroids delay the onset of anagen and gonodectomy and adrenalectomy have the reverse effect. Thyroid hormones accelerate the onset of follicular activity, whereas thyroidectomy or treatment with propylthiouracil delays it.

It has been known for many years that plucking of resting hairs from telogen follicles advances the onset of anagen. This led to the idea that the hair cycle

Table 2. Studies involving drug delivery to pilosebaceous compartment.

Delivery system	Drug	Animal model	Comment	Ref.
Liposomes	Carboxyfluorescein	Hamster ear model	Significant targeting was observed	67
Liposomes	Calcein, Melanin	-	Significant drug targeting observed	68
Liposomes	RU 58841 (anti androgen)	Scar formation after burning	Considerable levels of drug targeting observed when drug levels compared in normal and scar skin	69
Liposomes	Monoclonal antibodies against doxorubicin	-	Complete prevention of doxorubicin induced alopecia was recorded	70
Liposomes	T4 endonuclease	-	Appreciable amount of enzyme detected in pilosebaceous units	71
Liposomes	DNA	*In vitro* mouse skin histocultures	High concentration of DNA reached in pilosebaceous units	72
Liposomes	Lac-Z reporter gene	Hairless mouse	Highly selective gene therapy was achieved	73
Liposomes	Melanin, genes, proteins and small molecules	-	Significant pilosebaceous targeting was observed	77
Novasome I	α-interferon, Cyclosporin	Hamster ear model	Novasome I showed great levels of drug compared with liposomes	81
Non-ionic liposomes	Cimetidine	Hamster ear model	Non-ionic liposomes showed significant drug targeting compared with liposome with no systemic pharmacological effect	82

is controlled by a locally active inhibitors that accumulates during anagen causing entry into catagen, when present in sufficient concentration (the Chalone hypothesis)[37,38].

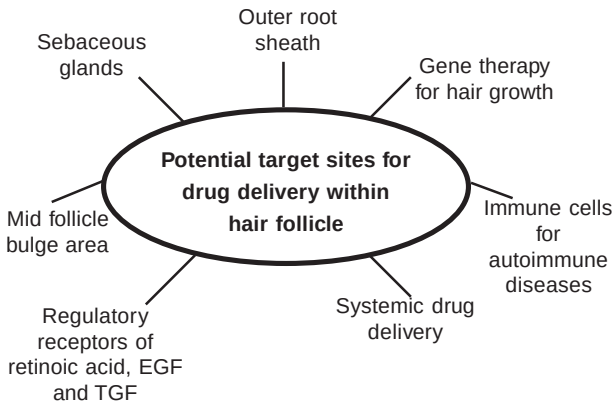
The Chalone hypothesis is based on the current concepts of paracrine and autocrine regulation of cell growth and differentiation. Studies revealed that levels of tissue growth factor (TGF), *i.e.,* TGFβ-1, TGFβ-2, and TGFα remained constant during the cycle, whereas the signals for TGF β-3 and bFGF (basic fibroblast growth factor) were present in anagen[39]. More variable epidermal growth factor (EGF) levels were observed. EGF induces a catagen like changes in cultured human hair follicle[40].

Some hormonal drugs like androgens cause abnormal hair growth (hirsutism). Hypertrichosis occurs due to chronic medication with non-hormonal drugs like minoxidil, diazoxide and phenytoin[41-45].

2.3. The dermal environment of hair follicle

Hair follicle base is embedded in the dermis. In rats, follicular cycles are synchronized and changes in vascularity, permeability, *etc.,* of the dermis regulate activity. Skin with active follicles is more richly vascularized than skin with resting follicles. Water content, fat content and total collagen content have been shown to fluctuate in phase with spontaneous follicular cycles, both in untreated rats and in ones whose spontaneous hair cycles have been accelerated or retarded by appropriate hormonal treatment[46]. Both soluble and insoluble collagens remain constant during anagen, they increase in early telogen and decrease during later[47]. They also found that the elastin content increases during anagen and falls during telogen. Other dermal constituents which have been found to change in amount during a follicular cycle include histamine, serotonin, mast cells[48] and

Figure 4. Potential target sites for drug delivery within hair follicle.



catecholamine[49]. Moretti *et al*[50] and Mackawa[51] found that amount of dermal glycosaminoglycans are elevated in anagen and decreased in telogen.

3. Barriers within hair follicle against drug delivery

Hair follicle appears to possess potentially significant target sites for drug delivery. However access to these sites is very difficult due to structural aspects of hair follicle and its chemical environment. The keratinous layers of the inner and outer root sheaths and the glassy membrane surrounding the entire follicle may restrict passage of molecules deep within the follicle.

Sebum is a secretion composed of neutral and nonpolar lipids. It flows in upward direction and may interrupt the passage of drug. Sebum discharge into the follicle is constant, regardless of seasons or amount of lipid already present in the follicle or on the skin[52]. Effective drug delivery and pharmacological effects depend upon the interactions between drug and sebum and also on the physicochemical properties of the vehicle.

Size of the particles regulates the entry of delivery system into the hair follicle[53] as orifice of hair follicle is very small. Size range of particles between 5-7 μm is optimum for the passage into the hair follicle, whereas smaller and large particles are likely to be localized in the stratum corneum and skin surface.

4. Potential target sites for drug delivery within the hair follicle

Several target sites within the hair follicle may be accessible for topical delivery of compounds (Figure 4). Outer root sheath has been the major target site, which is in continuation with epidermis.

The sebaceous glands are desirable target site in that the aetiology of some skin disorders, such as acne and androgenetic alopecia, are believed to be associated with sebaceous gland activity[30]. Regulatory receptors for retinoic acid, epidermal growth factor and transforming growth factor have also been identified within the hair follicle, and many feasible target sites for drug delivery[54]. The mid-follicle bulge area may also be considered as a potentially significant site for targeted drug delivery. This population of cells, found just below the sebaceous gland, possesses one of the fastest rates of cell division in mammals[34]. Some kinds of skin cancer have been thought to be linked to this highly proliferative area of the hair follicle, particularly during telogen phase. Studies have proved the involvement of immunocompetent cell in hair growth, which encourage new therapeutic approaches to target these cells within the hair follicle. Etiologically alopecia areata has been shown to be an autoimmune disease, which may lead to immunotherapeutic targeting of immune cells[55]. Gene therapy may also have great potential as many genes which control hair growth are identified.

Hair follicle could also be exploited for systemic drug delivery in addition to localized targets. A bunch of capillaries surrounding hair follicles and sebaceous glands may facilitate systemic absorption through the hair follicle.

5. Models and quantitative assessment of the follicular pathway

The specific role of the hair follicle in percutaneous transport remains difficult to elucidate due to the lack of an appropriate animal model to distinguish transfollicular from transepidermal percutaneous absorption. Stump-tailed macaque monkeys have been extensively used for *in vivo* study of hair growth[35,56]. The macaque exhibits a species specific frontal scalp baldness that coincides with puberty in both sexes. The hormonal and genetic factors that cause alopecia in the macaque have been proved to be

identical to those in human androgenetic alopecia[56]. Thus, macaque alopecia became a pertinent animal model for studies of human androgenetic alopecia.

Models for Quantitative assessment of the follicular pathway

1. Stump-tailed macaque monkeys
2. Syrian hamster ear
3. Rodent models: (a) Fussy Rat, (b) Follicle free rat (Scar) skin

Human sebaceous gland related studies are normally performed on Syrian hamster ear. The ventral side of the Syrian hamster ear is rich in sebaceous glands and resembling human sebaceous glands which is large and androgen-sensitive. The hamster ear could be stratified into compartments for assay of sebaceous gland contents after scraping the ventral side[57].

Aside from these models, a number of rodent models have been explored in order to obtain easy accessibility, for primary screening of the drug effect[58,59]. A genetic mutant of the albino rat has been developed with short vellus hairs in the whole body; thus it is named as *fussy rat* [60]. The short vellus follicles in the adult rat showed asynchronized cyclic phases and the size of individual anagen follicles varied over a wide range. This model has been widely used for studies involving follicular delivery of drugs. However, this model suffers from drawback that it cannot represent exclusively the transepidermal pathway of absorption since it is not completely follicle free.

A truly follicle free skin has been created by immersion of dorsal side of the rat in 60ºC water for one minute[61], the epidermis was removed and allowed to redevelop over several weeks into truly follicle free skin. However, this model must be further characterized to ensure that the resultant skin is not different in other respects from skin with hair follicles.

Furthermore, inappropriate assessment techniques have also made elucidation of the follicular route difficult. In tape stripping studies, incomplete stripping may cause high detection of compounds in the skin and if follicular contents are stripped away it may result in under estimation of follicular deposition.

In microscopic visualization studies, skin should be carefully sectioned and minimally fixed to prevent cross-contamination of sections. Fluorescence microscopic studies, require a careful observation and logical interpretation. Autoradiography has also been established as an important technique for demonstration of changes taking place during hair growth cycle.

6. Diseases associated with hair follicle

6.1. Alopecia

The term alopecia signifies loss of hair, resulting in decreased density of hair. Male pattern alopecia is a condition in which terminal hair on the scalp are progressively replaced by cosmetically unsatisfactory vellus hair. Ultimately, some follicles about a third of the total, may disappear. Male pattern alopecia or alopecia androgenetica is inherited as an autosomal dominant trait, but is manifested only in the presence of androgens[62,63]. In case of alopecia areata, areas of patchy baldness are observed on an otherwise normal scalp; other body hair are not affected. Complete scalp hair loss is called alopecia totalis and loss of terminal hair from whole body known as alopecia universalis.

6.2. Hirsutism

Hirsutism is defined as the growth of coarse terminal hair in female, in part or in whole of the adult male pattern. Excessive hair growth is found on the face, arms, legs and trunk, often resulting in severe psychological disturbance. Most patients have no definable endocrine disease but minor hormonal changes observed.

6.3. Hypertrichosis

Excessive growth of long, often apigmented hair can occur in a variety of systemic disorders. It may occur in either sex, where the hair distribution is not of the male type, though the face is often affected. Hypertrichosis may occur due to disseminated neoplasia, visceral carcinoid syndrome, anorexia nervosa and drugs *e.g.*, antiepileptics (phenytoin), antihypertensive (diazoxide, minoxidil) and topical corticosteroids.

6.4. Acne

It is a disorder of the pilosebaceous unit and usually manifests in adolescence. It is generally characterized by seborrhoea and comedone formation, and inflammatory lesions such as papules, pustules, nodules and cysts may develop. Acne is probably the result of four main interactions; sebaceous gland

hyperplasia producing seborrhoea, pilosebaceous canal obstruction; bacterial enzyme activity and biochemical changes in lipids.

6.5. Cancer

Localized cancer may be observed in pilosebaceous unit. The unit possesses bulge area, a highly proliferative group of cells, which plays a critical role in hair follicle growth and development. This bulge area is usually involved in uncontrolled growth (cancer) associated with pilosebaceous unit.

7. Pilosebaceous targeting using delivery systems

Drug delivery and targeting using transepidermal pathway has been a major area of research for last few decades[64,65]. However, follicular targeting is an emerging area in the field of drug delivery. Till date limited reports are available involving pilosebaceous targeting and few delivery systems have been exploited (Table 2)[53,66,67]. However, emphasis has been laid on the exploitation of follicular targeting of drugs/genes, rather than attempt to enhance the percutaneous absorption. Liposomes represent a promising drug delivery module. However, many disadvantages like cost, stability *etc.,* associated with it switched over the paradigm to niosomes, which are thought to be better candidates for topical drug delivery as compared to liposomes. Neimec *et al.*[53] have reported pilosebaceous targeting by polymeric microspheres, however narrow range of particles *i.e.,* 3-6 µm limits their future prospective.

7.1. Pilosebaceous targeting by liposomes

Liposomal formulations provide several advantages over non-liposomal formulations. A major advantage being the amphipathic nature of liposomes, which allows incorporation of a wide variety of hydrophilic and hydrophobic drugs. Physicochemical characteristics and the lipid compositions dictate the exact location of a drug in liposome. Hydrophilic molecules possess greater affinity for the hydrophilic head groups and aqueous core, whereas hydrophobic molecules tend to be intercalated into the fatty acyl chains of the lipid bilayer. Recent studies have shown that liposomes serve as efficient carrier for topical delivery of small hydrophilic compounds, and that the pilosebaceous unit may act as a primary reservoir [66].

Quantitative deposition of a low molecular weight fluorescent hydrophilic dye, carboxyfluorescein (CF), into the pilosebaceous unit has been studied[67]. Deposition of carboxyfluorescein from phospholipid liposomes was enhanced almost 8-fold that of the aqueous solution. A maximum of 2-3 fold enhancement over the aqueous solution was attained from other vehicles.

Li *et al.* [68] prepared hair follicle delivery system of phosphatidylcholine liposomes entrapping fluorescent dye calcein and pigment melanin and applied topically to mice. They observed negligible amounts of delivered molecules enter the dermis, epidermis and blood stream, thereby demonstrating the enrichment of follicle delivery.

Target effect of liposomes has been demonstrated using antiandrogen (RU 58841) entrapped liposomes[69]. RU 58841 was dissolved in an alcoholic solution and encapsulated in liposomes for comparison. After 24 hr cumulative percentage of RU 58841 absorbed *in vitro* was 3-4 fold higher in normal skin; in the case of liposomes, the accumulation of the drug in the normal dermis was significantly higher than in the scar one. In the *in vivo* cutaneous distribution, the epidermis and dermis of the normal skin contained significantly higher amounts of RU 58841 than the scar skin. It was concluded that alcoholic solution encouraged the localization of drug into the stratum corneum, whereas liposomes targeted the sebaceous glands.

The topical application of a liposome-entrapped monoclonal antibody to doxorubicin completely prevented doxorubicin-induced alopecia in rats[70]. Multilamellar phospholipid based liposomes labeled with a fluorescent, lipophilic dye have been utilized for delivery of 16000 Da DNA repair enzyme, T4 endonuclease V[71]. An appreciable amount of enzyme was detected in pilosebaceous unit. Delivery of DNA repair enzymes into the hair follicle may have a number of applications, including prevention of carcinogenesis originated from the very site. Follicular delivery of liposomally entrapped high molecular weight DNA have been reported[72], using mouse skin histocultures complete with hair follicles. The DNA was labelled with ^{35}S-dATP and entrapped in phosphatidylcholine-based liposomes. Autoradiograms indicated specific high radioactive labelling in the cell membranes and cytoplasm of hair follicle cells in samples applied with liposomes as compared with application of DNA.

Li *et al.*[73] has shown that liposomes can selectively target hair follicles for delivery of small and large molecules. They selectively targeted the lac-Z reporter gene to the hair follicles in mice after topical application of the gene entrapped in liposomes. They demonstrated that highly selective, safe gene therapy for the hair process is feasible.

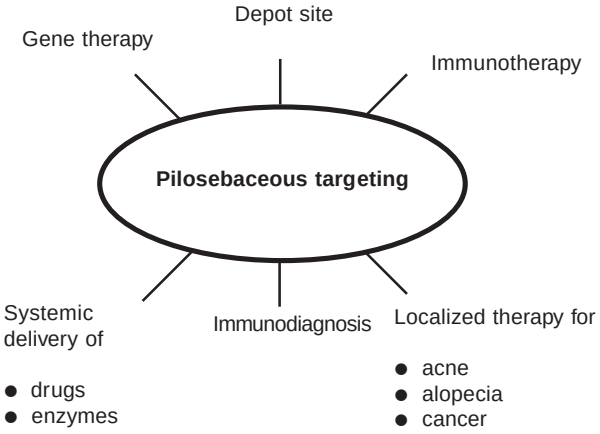
Liposome entrapped melanins, proteins, genes and small molecules have been selectively targeted to the hair follicle[74]. However, liposomal delivery of these molecules was found to be time-dependent. Topical liposomal delivery selectively to hair follicles has demonstrated the ability to colour hair with melanin *vis-à-vis* the delivery of the active lac-Z gene to hair matrix cells and delivery of proteins have also been achieved using topical liposomal dispersion. Liposomes have been reported to show high potential in selective hair follicle targeting of large and small molecules, including genes. This has opened the field of gene therapy and other molecular therapies of the hair processes to restore hair growth, physiologically restore or alter hair pigment and to prevent or accelerate hair loss.

A novel method for isolation and maintenance of the human pilosebaceous unit has been documented[75]. This method makes it possible to obtain viable human pilosebaceous units by microdissection, and to maintain them *in vitro* for up to 7 days with apparently full retention of hair follicle function, but only partial retention of sebaceous gland. This method provides a means for successful *in vitro* studies related with human pilosebaceous units.

Besides liposome, hair follicles and sebaceous glands can be privileged pathways for some formulations, which enter faster into these shunts than, they do through the stratum corneum[76].

Lieb *et al.*[77] studied the elements that govern the intra-follicular delivery of large molecules to follicles of human scalp skin *in vitro*. Effect of size, charge and formulation on intra-follicular disposition of drug was observed. Fluorescein covalently linked to anti-sense oligonucleotides and rhodamine-conjugated dextrans were topically applied to fresh human scalp skin *in vitro*. They concluded that topically applied agents of relatively large molecular weight, in properly formulated delivery vehicles, have the potential to reach at the hair bulb in pharmacologically active concentration.

Figure 5. Potential applications of pilosebaceous targeting.



The topical administration of liposome-DNA mixtures (lipoplex) to mouse skin and to human skin xenografts resulted in efficient *in vivo* transfection of hair follicle cells[78]. Transfection depended on liposome composition, and occurred only at the onset of a new growing stage (anagen phase) of the hair cycle. Manipulating the hair follicle cycle with depilation and retinoic acid treatment resulted in nearly 50% transfection efficiency-defined as the proportion of transfected, newly growing follicles within the xenograft. Transgenes administered in this fashion are selectively expressed in hair progenitor cells and therefore have the potential to affect the characteristics of the follicle. These findings laid down the foundation for the future use of topical lipoplex applications to alter hair follicle phenotype and treat diseases of the hair and skin.

7.2. Pilosebaceous targeting by niosomes

Non-ionic surfactant based vesicles (niosomes) are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures. The assembly into closed bilayers is rarely spontaneous[79] and usually involves some input of energy such as physical agitation or heat. The result is an assembly in which the hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups enjoy maximum contact with same. These structures are analogous to phospholipid vesicles (liposomes) and are

able to encapsulate aqueous solutes and serve as drug carriers. The low cost, greater stability and ease of storage of non-ionic surfactants[80] has presented these vesicles as an alternative to phospholipids. Non-ionic surfactants overwhelm the problem of natural variability of phospholipids and are reported to follow the pilosebaceous route for entry into systemic circulation when vesicles based on these surfactants are applied topically[81,82].

Niosomes formation involves a particular class of amphiphiles and aqueous solvent[83-85]. In some cases, cholesterol is required for vesicle formation and vesicle aggregation may be overcome by inclusion of molecules that stabilize the system against the formation of aggregates by repulsive steric or electrostatic effects.

The *in vivo* hamster ear model has been used to quantitate pilosebaceous deposition of a predominantly hydrophilic peptide, α-interferon, and a predominantly hydrophobic peptide, cyclosporin[81], deposition from non-ionic liposome, egg phosphatidylcholine based liposome, aqueous interferon, and hydro alcoholic cyclosporin solution was assessed. It was shown that greatest drug level resulted after application of a non-ionic liposome formulation denoted as Novasome I. It consists of glyceryl dilaurate, cholesterol and polyoxyethylene 10-stearyl ether at a weight percent ratio of 57:15:28, respectively.

A correlation has been established between *in vivo* deposition of cimetidine and its anti-androgenic effect[82]. Appreciable deposition of ^{3}H-cimetidine was attained into the pilosebaceous unit of the hamster ear after topical application of 50% ethanol solution (pH 7.4), glyceryl dilaurate-based non-ionic liposomes (pH 5.5) and egg phosphatidylcholine based liposomes (pH 5.5). It has been shown that hydro-alcoholic and non-ionic liposome solutions appeared equipotent in suppressing sebaceous gland growth, whereas phospholipid liposomes had almost no pharmacological effect. The reason behind the inactivity of the phospholipid liposome formulation was explained as being an ion-pairing phenomenon at the pH of the formulation. These interesting results made the formulation factors, particularly charge effects, as influenced by pH, an important concern.

8. Future prospects

Topically applied liposomes and niosomes are capable of targeting wide range of drugs, including macromolecules into the hair follicle. Potential applications of pilosebaceous targeting are illustrated in Figure 5. Targeted drug delivery to the pilosebaceous compartment may have profound therapeutic applications for treating several hair follicle associated disease states. Besides localized delivery, systemic delivery *via* the hair follicle may be achieved. Thus, the transfollicular pathway could serve as the major paradigm in targeted delivery of bioactives in forthcoming future.

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