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Biology of Human Hair: Know Your Hair to Control It

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Abstract Hair can be engineered at different levels—its structure and surface—through modification of its constituent molecules, in particular proteins, but also the hair follicle (HF) can be genetically altered, in particular with the advent of siRNA-based applications. General aspects of hair biology are reviewed, as well as the most recent contributions to understanding hair pigmentation and the regulation of hair development. Focus will also be placed on the techniques developed specifically for delivering compounds of varying chemical nature to the HF, indicating methods for genetic/biochemical modulation of HF components for the treatment of hair diseases. Finally, hair fiber structure and chemical characteristics will be discussed as targets for keratin surface functionalization.

Keywords Follicular morphogenesis · Hair follicle · Hair life cycle · Keratin

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1 Structure and Morphology of Human Hair

Human hair is mainly composed of fibrous α -keratin proteins. Hair fibres are not continuous in their full length, but rather result from compact groups of cells within the fibre follicle, from which three further basic morphological components of hair structure originate: the multicellular cuticle sheath, the fibrous cortex and the medulla [1, 2].

At the follicular level, a single layer of cells gives rise to the cuticle, a protective layer covering the core of the fibres. It is mainly composed of β -keratins and displays a scaled structure, possessing between seven and ten superimposed layers with the cuticle edges pointing toward the tip of the fibre [3]. The outer surface of the cuticle's scale cells is coated by a thin membrane called the epicuticle, which covers the cysteine-rich exocuticle, a constituent that contains most of the cysteine residues present in the scales [4]. Finally, there is the endocuticle, which is located at the interface of the cortex and is mainly composed of the remaining cell organelles. Endocuticle consists of proteins that, unlike those found in other parts of the hair fibre, have very low sulphur content; thus, it is poor in cysteine, which causes the endocuticle of the scales to swell considerably more in water than the cysteine-rich exocuticle. This might explain the pronounced projection of the scales and the tendency for wool felting in the presence of water [5].

The cuticle tightly encircles the cortex that forms the most voluminous part and the heart of the hair fibre. The cortex is made up of cortical cells, which comprise the macrofibrils, long filaments oriented parallel to the axis of the fibre. Each macrofibril consists of intermediate filaments (IF), known also as microfibrils, and the matrix [6, 7]. It has been established that the molecules that aggregate to form the IFs in keratin fibres are type I and type II keratin chains, arranged parallel to one another and in the axial register. After the formation of the α -helices, it is believed that the two types of chains associate to form a dimer, which then aggregates with another dimer to form a tetramer. Finally, the formation of a pseudo-hexagonal structure (the IFs structure) occurs by the association of seven or eight tetramers. Type I chains are net acidic, with pI values in the range of 4.5–5.5, while type II chains are neutral-basic with pI around 6.5–7.5 [8–11]. As a consequence, the IFs are low in cystine ($\sim 6\%$), whereas the matrix contains up to 20% of total amino acid residues [12–14].

The matrix proteins that surround the IFs through intermolecular disulfide bonds act as a disulfide crosslinker holding the cortical superstructure together and conferring high mechanical strength, inertness and rigidity to keratin fibres. High sulphur proteins, ultra-high sulphur proteins and high glycine-tyrosine proteins are present in matrix proteins (γ -keratins), depending on their cysteine, tyrosine and glycine content [1, 4, 15, 16].

Apart from albinos, all normal humans have melanin hair pigmentation, whatever the colour. Dispersed throughout the structure of the cortex in granular form are the melanin pigment particles. The number, chemical characteristics and distribution pattern of these cells determine the colour of the hair [2]. The actual

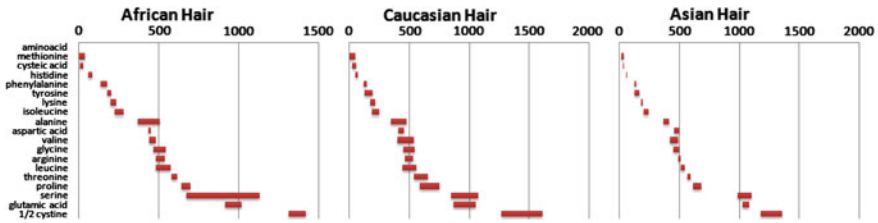


Fig. 1 Amino acid content of human hair of diverse ethnic origins ($\mu\text{M/g}$) (adapted from [1, 2, 18, 19])

shade of colour in each individual depends not only on which melanin is present, but also its quantity and the site, number and shape of pigment granules in the hair cortex (Cx) [17].

Vacuolated cells may also be present along the axis of coarser α -keratin fibres, forming the medulla. These cells generally constitute only a small percentage of the mass of hair and are believed to contribute negligibly to the mechanical properties of human hair fibres. Physically, the medulla forms the empty space of the fibre [4, 7].

Like all polymeric structures, keratin fibres consist of long, tightly bound molecular chains held together in many different ways from covalent bonds to weaker interactions such as hydrogen bonds, Coloumbic interactions, van der Walls interactions and, when water is present, hydrophobic bonds. Hair reactivity is complex and depends not only on the presence of reactive groups in the fibre, but also on their availability. The latter is significantly affected by fibre morphology and molecular structure [2]. Hair is mostly proteinaceous in nature, while structural lipids and other materials represent only a minor fraction of its constituents.

Human hair is usually categorised ethnically into three major distinct groups: Asian, Caucasian and African. Looking from the perspective of biological variability, environmental effects and diversity of fibre texture, it is remarkable how uniformly the amino acid makeup of protein components is across ethnic groups. The amino acid makeup of the protein components was reviewed by Wolfram and is depicted in Fig. 1 [1, 2, 18, 19].

2 Biology of Human Hair

Hair is an important feature of mammals, where hair shafts fulfill a number of different functions such as thermoregulation, collection of sensory information, protection against environmental trauma, social communication and camouflage. Each of us displays an estimated total number of 5 million hair follicles (HF), of which 80,000–150,000 are located on the scalp [20].

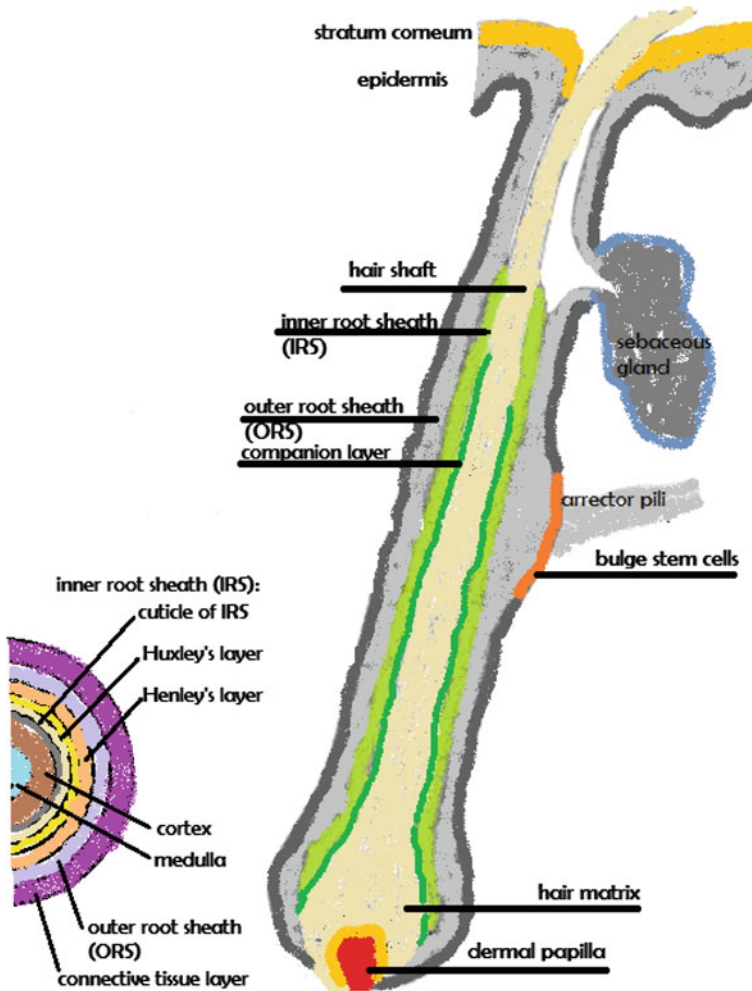


Fig. 2 The human hair follicle: structure, main functional areas and concentrical layers, which constitute the typical hair

The HF (Fig. 2) is one of the most complex mini-organs of the human body with the capacity to reconstitute itself. During postnatal life, HFs show patterns of cyclic activity with periods of active growth and hair production (anagen), apoptosis-driven involution (catagen) and relative resting (telogen) [21]. These cyclic changes involve rapid remodeling of both epithelial and dermal components and suggest the presence of intrinsic stem cells. Stem cells isolated from the bulge area possess high proliferative potential *in vitro* [22] and the capacity to repopulate HFs, sebaceous glands and epidermis *in vivo* [23–27]. These transformations are

regulated by variations in the local milieu, based on changes in expression and/or activity of many cytokines, hormones, enzymes, neurotransmitters and their cognate receptors as well as of transcription factors that have become recognised as key mediators of HF cycling.

2.1 Hair Follicle Anatomy

The HF results of interactions between epithelial, mesenchymal and neuroectodermal cell populations as well as transient migratory cells.

The epithelium is divided into an upper permanent region, distal to the arrector pili muscle and an inferior region that includes the hair bulb (Fig. 2) [28]. Each HF is composed of nine distinctive epidermal layers: hair matrix (Mx), medulla (M), Cx, hair cuticle (Ce), cuticle of the inner root sheath (Ci), Huxley's layer (Hx), Henle's layer (He), companion layer (Cp) and outer root sheath (ORS), arranged concentrically from core to periphery, as well as two dermal tissues: dermal papilla (DP) and dermal sheath. Among these tissues, only the medulla is optional, given that some hairs have no medulla, whereas in others it is relatively large. The Cx and Ce constitute the major part of the hair shaft that penetrates the skin. Both Cx and Ce tissues undergo heavy keratinization to form the solid hair shaft. The three concentric layers located externally to the shaft constitute the inner root sheath (IRS), which is thought to support the growth and differentiation of the shaft. The innermost layer of the IRS, called the Ci, consists of thin overlapping scales facing the Ce. The Hx layer is the last layer to undergo keratinization. This layer may help other keratinized cells in terms of nutritional and informational support. Importantly, Hx is known to contribute to relieving the distortion caused by uneven keratinization of the shaft, which occurs in curly hair, for example. On the other hand, the He layer keratinizes at a very early phase of hair growth so these keratinized cells are visible at a positionally low level of the HF. He layer provides mechanical support to the most delicate part of the HF in the early stages of its development.

Located within the hair bulb is a population of cells with the highest proliferation rate in the human body: the keratinocytes of the Mx. These can differentiate into trichocytes or cells of the IRS. The hair bulb in the anagen phase functions as a hair shaft producer and provides the hair shaft's trichocytes with characteristic melanin granules. The ORS, Mx and hair shaft derive from the epithelial bulge stem cells [24, 25, 27].

Mesenchymal stem cells in the tissue sheath serve as a reservoir for new DP cells. The DP determines the size of the anagen hair bulb, the duration of anagen and hair shaft diameter [20, 29, 30]. In adult hair, DP maintains the vascular system that provides the nutritional support and hormonal regulation required for hair growth [31].

2.2 Hair Follicle Morphogenesis

Follicle morphogenesis regularly occurs only during embryonic development, so each mammal is born with a fixed number of follicles that normally does not increase afterwards, although folliculoneogenesis can take place during wound healing [32–34].

2.2.1 Hair Follicle Life Cycle

The hair growth cycle describes the changing histological morphology of the shaft and of the follicle over time. Starting with anagen (rapid growth and hair shaft elongation), the follicle and its shaft progress through catagen (involution and apoptosis-driven regression), telogen (resting) and finally exogen (shedding) [20, 21, 35].

In *anagen*, the growth phase of the hair cycle, hair undergoes morphological and molecular events similar to fetal HF morphogenesis [36, 37]. Many key molecular regulators of hair biology not only activate morphogenesis, but also regulate anagen induction and duration [38, 39]. In this phase, epithelial bulge stem cells differentiate into the various cell types that will reconstitute the entire hair shaft [40, 41]. Hair shaft synthesis and melanin production in melanocytes (melanogenesis) [42], only take place in anagen. Pigmentation begins after the initiation of shaft formation and ends before this process is terminated, which causes the shaft to have an unpigmented tip and root. By the 7th week of gestation, melanocytes are already present in the human epidermis where they remain until hair morphogenesis starts, approximately 2 weeks later [43]. During HF morphogenesis, a few melanoblasts leave the epidermis and distribute randomly in the forming HF and in some sebaceous glands [44, 45]. Once the hair fiber is completely formed, melanocytes concentrate near the basal lamina surrounding the DP [44]. Anagen ends with a tightly regulated involution of the HF, which is accompanied with apoptosis and terminal differentiation of cells, a period designated as catagen [46].

The first sign of *catagen* is the cessation of melanin production in the hair bulb and apoptosis of follicular melanocytes, derived from melanocytic stem cells of the secondary hair germ [47]. Programmed cell death of these stem cells seems to play an important role in hair graying [48–50]. In contrast to what happens in the ORS and the Mx, no apoptosis occurs in the DP due to the presence of antiapoptotic protein BCL-2 [37, 46]. During catagen, the DP condenses, moves upward beneath the HF bulge and halts its activity.

After regression, the HF enters *telogen*, a phase of relative quiescence regarding proliferation and biochemical activity. The follicle remains in this stage until it is reactivated by intra- and extrafollicular signals [51].

Once the growth cycle is complete and the phase of telogen reaches the end, the hair shaft will be shed. This process, *exogen* or teloptosis, is independent of a

possible new HF cycle [52]. In fact, it is most common in mammals that a new hair shaft regrows before the resting shaft sheds, assuring the animal is never completely naked. Apart from normal development, in pathologies like trichostasis, where multiple shafts are formed and retained within the same HF, it also supports the thesis that follicle growth and shedding are independent events. Exogen ends when the shaft is released.

Recently, Rebora and Guarrera used the term kenogen to describe the interval in which the HF remains empty after the telogen hair has been shed and before a new anagen hair emerges. During kenogen the HF remains completely empty and possibly inactive. Frequency and duration of kenogen are greater in men and women with androgenic alopecia [53].

All body hairs undergo a similar life cycle, although its extent, the duration of its phases and the length of individual shafts vary between different body areas and between individuals, depending on genetic programming, genre, age and health status.

2.3 Molecular Control of Hair Follicle Development and Cycling

Epithelial-mesenchymal interactions are crucial in HF development and cycling. During normal embryonic development, interactions between epidermal keratinocytes and DP fibroblasts lead to the formation of the hair shaft. The most important factors regulating both developing and anagen follicles include the lymphoid-enhancer factor 1 (Lef1), Notch signaling pathway, Sonic hedgehog (Shh), bone morphogenetic proteins (BMPs), neurotrophins and several members of the Wnt family of proteins.

Bone morphogenetic proteins are expressed during embryonic development and postnatal life of nearly all mammalian organisms and play important roles in the regulation of both cell proliferation and differentiation [54]. BMP signaling inhibits induction of follicle development [35], and the neutralization of BMP-2 and BMP-4 activity by noggin stimulates the initiation of HF development [55, 56]. However, the differentiation of keratinocytes into mature and cuticle cells is severely impaired if levels of BMPs decrease as these proteins restrict the proliferating population to the bulb region [57]. Recent studies reinforce the important role that the BMP signaling network plays in the development and homeostasis of HF structure through the activation and/or maintenance of stem cell populations [58, 59].

The Wnt signaling pathway has been implicated in multiple cellular events including the regulation of cell proliferation, cell fate, polarity, differentiation and pattern formation [60, 61]. Wnts are divided at least into three groups according to their signal transduction pathways: the canonical pathway in which β -catenin stabilization occurs, the planar cell polarity (PCP) pathway and the Wnt/ Ca^{2+}

pathway [62]. Canonical Wnt/ β -catenin signaling plays an important role in HF induction and fate [63–69], and expression of several Wnts, Wnt ligands and inhibitors is specifically elevated in developing and postnatal HFs [61, 70–72]. On the other hand, forced activation of β -catenin signaling promotes HF fate in both embryonic and postnatal skin [67, 73–75]. Recent studies also suggest that another pathway, the ectodysplasin signaling pathway, is critical for the induction and maintenance of placode [76, 77].

In a surprising report, Ito and collaborators revealed that HF can form *de novo* in adult mice after wounding [33], contrary to the belief that follicle neogenesis only occurred during embryonic development. This phenomenon is completely dependent on Wnt signaling, and overexpressing Wnt actually increases the number of HF formed. Interestingly, the cells forming the *new HF* originate in the re-epithelialized interfollicular epidermis and not follicle stem cells.

In vertebrates, Shh is a member of the hedgehog (Hh) family of secreted signaling molecules that play a crucial role in both embryogenesis and organogenesis [78, 79]. Shh is required for HF morphogenesis during embryogenesis and for regulating follicular growth and cycling in the adult. HF formation may start without Shh, but mature HFs fail to develop because hair germs cannot elongate into the dermis to form a hair peg and the DP does not mature [80–82]. In postnatal skin, Shh is relevant for hair cycling, its expression being up-regulated in early anagen. In line with this, treatment with Shh-blocking antibodies is known to cause reversible alopecia, as HF cannot cycle and arrest in telogen [83]. On the other hand, exogenous Hh stimulates the transition from resting (telogen) to growth (anagen) stage [84] and, in some settings, anticipates the start of anagen and promotes hair regrowth in the skin of mice with chemotherapy-induced alopecia [85, 86]. These studies indicate a potential application of Hh modulation in control of hair density.

The Notch signaling pathway is also important for determining cell fate in HF development [87]. Notch signaling acts by blocking cell differentiation, which maintains the competence of undifferentiated cells [88, 89] in both the developing tooth and the HF to respond to inductive signals that determine their developmental fate [87, 90]. Based on expression studies in the HF, Notch may be a competence factor, as its pattern of expression in the HF appears to be restricted to cells that have left the proliferative pool but have yet to terminally differentiate [91, 92].

Although Notch activation is not required for HF formation in the embryo, blocking Notch signaling in postnatal epidermis results in conversion of HF into interfollicular epidermal cysts. In the absence of Notch signaling, the hair shaft still forms and contains appropriately positioned cells expressing markers for each fate; however, because IRS cells fail to adhere to each other, the follicular architecture cannot be sustained, which leads to the transformation of these aberrant HF into epidermal cysts [92–95]. Very recently, Demehri and Kopan [96] proposed that Notch acting on bi-potential bulge stem cells, or their uncommitted migratory descendants, plays an inhibitory role in preventing bulge stem cells from differentiating into epidermal cells, thus ensuring the follicular fate.

It was also reported that Notch signaling, via its target gene *Hes1*, is essential for the maintenance of melanoblasts and adult melanocyte stem cells [97, 98]. Moreover, mice with a conditional deletion of RBP-J κ (Notch transcription mediator) display, soon after birth, impaired hair pigmentation and subsequent progression of hair graying. Specifically, Notch1 and Notch2 targeted deletion in melanocytes led to inactivation of the *RBP-J κ* gene in a dose-dependent manner, and three intact alleles of *Notch1* and *Notch2* are required for preventing precocious hair graying [98]. Notch 3 and 4 are not implicated in this phenotype [99, 100].

Hormones, particularly androgens, are among the most important, although non-essential, modulators of hair growth. Androgens have paradoxically different effects on human HF depending on their body site. Androgens can stimulate hair growth on the face (beard), axilla, pubis and chest, where they are necessary for conversion of vellus hair to terminal hair, while they simultaneously can inhibit HF on parts of the scalp, converting terminal hair to vellus hair, causing balding in genetically susceptible individuals (for reviews, see [101, 102]).

Androgens, except for pubic and axillary follicles, require the intracellular enzyme 5 α -reductase to metabolize testosterone to its more potent metabolite 5 α -dihydrotestosterone (DHT). DHT then interacts with intracellular androgen receptors in the HF cells to modulate target gene transcription. The exact mechanism is not fully established, but Randal proposed the DP as the primary target [103, 104]. In this model, androgens act directly on DP cells where they bind to androgen receptors and then initiate altered gene expression of regulatory factors modulating indirectly the period of time when hair is growing, size and activity of the DP, keratinocytes and melanocytes. The factors produced by these populations may be soluble paracrine factors and/or extracellular matrix factors, including insulin-like growth factor-1 (IGF-1) in growth stimulation [105], stem cell factor in altered pigmentation [102] and transforming growth factor- β [106, 107] and dickkopf 1 [108] in inhibition. The opposite effects of androgens on beard and scalp follicles probably reflect differences in androgen receptor content, which is higher in beard DP than in occipital hair DP [109] and/or 5 α -reductase activity, which is higher in beard than in scalp [110]. Recent observations suggest that the dermal sheath can also respond to androgens without the DP acting as an intermediary [111].

Several other hormones are implicated in the regulation of the hair growth cycle, including melatonin, prolactin, melanocyte-stimulating hormone (MSH), thyroid hormones and oestrogens. Prolactin is implicated in hair growth regulation in both mice and human [112–114]. Similarly, melatonin has been reported to promote hair growth in animals and in humans, where topical application of melatonin induced hair, probably through induction of anagen [115].

Human hair is deeply affected by the level of unbound thyroid hormones that have been described to prolong the duration of anagen, up-regulate the proliferation of Mx keratinocytes and also stimulate intrafollicular melanin synthesis [101, 116, 117]. On the other hand, several studies reveal that a significant percentage of patients presenting hair loss were diagnosed as suffering from hypothyroidism [118, 119].

Estrogens, namely 17β -estradiol, act mainly as hair growth inhibitors. Topical application to mice skin of 17β -estradiol inhibits hair growth and accelerates catagen, while antiestrogens promote early anagen [120, 121]. A similar inhibitory effect on hair growth was observed in humans [122, 123]. Nevertheless, the exact role of estrogens in the regulation of the HF life cycle is still under debate.

Very recently, mammalian circadian clock genes such as *Clock* and *Bmal1* were reported to be involved in the regulation of HF cycling [124], suggesting that modulation of hair growth may also be achieved by modulating circadian genes through control of the cell cycle.

Taken together, these studies suggest that the described genes and signaling pathways regulating HF morphogenesis and cycling can be reactivated and modulated in adult skin, creating new strategies for hair regeneration.

3 Strategies for Cosmetic and Clinical Purposes

3.1 *Treatments of Hair Growth Disorders: Room for Improvement*

The most common types of hair growth disorders are caused by aberrant HF cycling. One such disorder is androgenetic alopecia, characterized by a shortening of the anagen phase and a prolongation of telogen, combined with miniaturization of HF [125]. Hirsutism, on the other hand, is defined as the presence of excess terminal hairs in females in an adult male typical pattern [126]. To control the amount of hair produced for clinical purposes, the main strategy is to alter anagen duration either by shortening it in cases of hirsutism or by increasing it to correct alopecia.

The first-generation drugs used for hair growth control—topical minoxidil solution and oral finasteride—are the only FDA-approved drugs for this application. Minoxidil prolongs the anagen phase, and promotes growth and enlargement of follicles in telogen phase [30, 127]. The effects of minoxidil on human HF are nevertheless very contradictory. Some studies describe that, at low concentrations, minoxidil stimulates anagen in human follicles [128–130], while it can cause growth inhibition at high concentrations [128]. Other authors reported no effect at all [131].

Nevertheless, it is assumed that treatment with minoxidil lengthens and thickens the small vellus hairs and decreases shedding. Minoxidil is a potassium channel opener that causes vasorelaxation [132] and stimulates cutaneous blood flow to the scalp [133]. Minoxidil sulfate, a metabolite of minoxidil, is a potent vasodilator. Uptake and conversion of minoxidil to minoxidil sulfate occurs within the HF, suggesting a direct action on the follicle [134]. The most probable site of action of minoxidil is the DP [135], and the mechanism of action has been linked to its effects on the Kir6.1/SUR2B potassium channel expressed by the derma papilla [136–138].

Finasteride inhibits 5 α -reductase type II and decreases both serum and cutaneous dihydrotestosterone concentrations, thus inhibiting androgen-dependent miniaturization of HF [139]. The DP is probably also the target of finasteride. This drug is not effective in treating androgenetic alopecia in women, but it can be beneficial for women with hirsutism. Finasteride must be nevertheless used very cautiously because of its potential feminizing effects on male fetuses [104] and even in adult male patients where some cases of gynecomastia have been reported [140].

Latanoprost has also been found to reverse alopecia and induce increased growth of the eyelashes [141]. A beneficial role in reversing androgenetic alopecia has not, however, been found, and detailed studies regarding whether latanoprost or another prostaglandin analog could be clinically used are still lacking.

Additional current pharmacological therapies for hair disorders include a range of antiandrogens that block the intracellular androgen receptors [142]. The mechanisms by which all these compounds trigger hair cycle changes are not clear and justify further studies.

In recent years, the molecular characterization and isolation of living bulge stem cells along with the studies that revealed their high proliferative capacity and multipotency opened up new directions for their utilization in cutaneous (hair and skin) regenerative medicine [24, 25, 143, 144]. This new approach suggests the control of hair characteristics from the living interior of the fiber. For this purpose, profound knowledge of genes and signaling pathways involved in hair disorders will permit specific modulation using, for example, RNA interference. miRNAs, which play a critical role in skin morphogenesis, were reported to be involved in the regulation of HFs development and cycling, but neither their expression nor their roles have been characterized yet [145]. This elucidation is crucial to develop new efficient treatments for hair disorders that do not cause hair damage or skin injuries, possibly using RNA interference for highly specific modulation of genes involved in these processes.

As proof of principle, we have achieved effective siRNA delivery and efficient modulation of a ubiquitous, highly expressed KRT1 gene in the epidermis of DBA/2J mice with a topical treatment (Araujo et al., submitted data).

Interestingly, topical application of cationic nanoliposomes loaded with specific siRNA has been tested as an effective approach for control of cutaneous melanomas [146], and siRNAs conjugated to cationized gelatin showed a positive effect on symptoms of alopecia in C3H/HeJ mice [147]. A promising line of research has been developed by Kerner and colleagues that proposes modulating androgen receptor expression with RNAi for hair and skin therapy [148, 149]. Nevertheless, this is still a very incipient area that promises to bring new and highly targeted strategies for skin and hair diseases.

HF as well as sweat glands are ideal targets for drug delivery and may represent an alternative to the intercellular route of skin permeation. HF, in contrast to the stratum corneum, represent an efficient long-term reservoir (up to 10 days) for topically applied substances, as their depletion occurs only through the slow processes of sebum production and hair growth [150–152]. The molecules that

penetrate HF can also access the tissues surrounding the follicle and reach the blood circulation through the dense network of blood capillaries, thereby avoiding the stratum corneum barrier. For example, it was shown that when caffeine was included in a shampoo formulation, it was detectable in blood just 5 min after application [153]. Therefore, HF may serve not only as a major entry point, but also as a reservoir for dermally applied substances. In addition, HF also contained multiple target structures for innovative therapeutic approaches. These include specific cell populations in and around the HF, such as immune cells, stem cells and melanocytes, sebaceous glands and perifollicular blood vessels [154–156].

The sebaceous glands represent an important therapeutic site for follicular targeting since they are implicated in the aetiology of acne and androgenetic alopecia [157], as well as in other sebaceous gland dysfunctions [158]. Hueber et al. [157] suggested that the sebaceous glands specifically promoted the penetration of hydrocortisone and testosterone into the skin. Evidence suggests that topically applied compounds entrapped in liposomes accumulated not only in the HF, but also in the sebaceous glands [159]. While the presence of lipophilic sebum may favor follicular uptake of lipophilic molecules, sebum production may, however, moderate drug transport, especially for hydrophilic drugs, functioning as a physical and a chemical barrier for drug penetration [160].

More efficient drug delivery vehicles are therefore being sought. Among the newly emerging concepts, drug delivery systems based on nano- and microparticles, which efficiently penetrate via the follicular route, are highly promising approaches. Nanospheres of different chemical natures are being tested for their capacity to facilitate the transport of substances to deeper layers of the skin, with obvious potential in topical delivery applications.

Another attractive targeting area within the HF is the bulge region. This region, including Mx cells, controls hair growth and pigmentation [161] and is responsible for follicle reconstitution due to the presence of stem cells with a high proliferative capacity and multipotency. The bulge region, in the ORS, has been described as the reservoir for keratinocyte stem cells in both humans and rodents [24, 26, 143, 162–164]. These cells are the target for gene delivery to facilitate long-term gene correction of congenital hair disorders or genetic skin disorders. Targeting of HF stem cells offers unique therapeutic options for genetic hair and skin therapy and regenerative medicine.

3.2 Modifications of Hair Fiber Surface and Structure

Hair's chemical and biological properties make it an ideal material for undergoing an infinite number of changes and treatments, from cosmetic to clinical applications.

Healthy and beautiful hair is desired, and the need for products that improve the look and feel of the hair surface has created a huge industry for hair care. Products such as shampoos and conditioners, along with damaging processes such as

chemical dyeing and permanent wave or relaxing treatments, alter many hair properties, which results in damage to the hair fibre [165–167]. In recent years, many innovations have taken place, and new approaches for hair treatments have been reported in the literature to overcome this problem.

Unaltered human hair (virgin hair) has an isoelectric point near 3.67 [168]. Hence, during a normal hair washing procedure performed at neutral pH ($\text{pH} > \text{pI}$), the surface of hair acquires negative charges. For this reason, most conditioning shampoos possess cationic polymers in order to counteract these negative charges, thereby improving hair texture and feel. Electrostatic interactions are believed to play a crucial role in the adsorption mechanism of such compounds [168]. However, these products are a small part of the polymer-based cosmetic products. Polymer-containing compositions represent the second-most common ingredient in cosmetic and personal care products. A diverse range of polymers is applied in this segment as film formers, fixatives, rheology modifiers, associative thickeners, emulsifiers, stimuli-responsive agents, foam stabilizers and destabilizers, beneficial skin feel agents and antimicrobials [169].

Studies on protein-based formulations to treat hair fibres have also been widely explored. Several patents disclose compositions capable of restoring hair health by providing excellent finishing effects. Applications of proteins such as a water-soluble compound derived from a vegetable protein derivate [170], non-naturally occurring keratin proteins [171], a mixture of a hydrolyzed protein and an amino acid with an aliphatic side chain [172], and other hydrolyzed proteins [173, 174] are also examples within this category.

Several studies have been published regarding how to improve the structure of the keratin hair shaft mainly for cosmetic purposes. These studies take into account the keratinous structure of hair fibres and explore the potential benefit effects of amino acids and peptides in hair care applications [175–177]. Silva et al. [178] described the importance of peptide structure in hair penetration using conventional fluorescent microscopy. We have been investigating the protein disulfide isomerase (PDI) for the functionalization of keratinaceous surfaces with cysteine-containing compounds (CCC) (Fernandes et al., submitted data). PDI is a multi-functional enzyme that catalyses formation and isomerization of disulfide bonds (disulfide shuffling) in a wide range of substrates. In vivo, PDI promotes the correct formation of disulfide bonds in proteins (oxidative folding), leading them to the native state. The CCCs were shown to penetrate inside the hair shaft and attach to the cortex without damaging hair. This approach can represent a promising strategy for the development of new hair care formulations with the ability to dye and restore the integrity of damaged hair (Fernandes et al., submitted data).

PDI has also been used for treatment of wool [179] and hair fibres [180]. King and Brockway [179] showed that PDI was able to restore part of the original properties on aged or harshly treated wool. The same enzyme was used by Brockway [180] to perform a curling, waving or straightening treatment safely under mild condition.

A method to gently and permanently relax or straighten hair was also attained by Presti [181] using a protease, kerA. This enzyme was found to cleave inter-

peptide bonds, allowing the hair fiber to be relaxed or straightened with less damage to the fiber than would have occurred using traditional or existing straightening methods [181].

Humidity is an important factor when considering hair beauty and styling. Air humidity affects hair form and structure at the level of hydrogen bonds. A humidity increase of 30–70% will augment by twofold the water content of hair, thereby increasing its volume by more than 20%. This influx of water eventually causes the hair fibres to swell, which results in friction between fibres and an additional increase in volume and frizz, changing hair appearance [182]. To overcome this issue, scientists from Massachusetts Institute of Technology (MIT) developed a technology that reduces hair frizz using a polyfluoroester, a molecule smaller than the traditional ones used for frizz control. Because of its chemical nature, the formulation adheres tightly to the hair, promoting long-lasting resistance to moisture. Because of its low surface energy, this technology repels most other materials like water and oils. As an additional benefit, the low refractive index of the coating produces a unique, long-lasting shine and pop in the colour of the hair [183].

Today, people in ever-greater numbers alter their hair colour and appearance. However, the aggressiveness of the available techniques poses a big drawback to hair colouring. Commonly used hair dyeing compositions are driven by a mechanism of diffusion of small molecules into the hair fiber [184]. There are three different types of hair colouring agents: permanent, semi-permanent and temporary colourants. The permanent hair dyes are constituted of small dye precursors, able to penetrate into hair, which develop the colour within the hair shaft in the presence of high alkalinity and oxidative conditions. These dyes provide the best colouring results, but cause significant hair damage. The semi-permanent and temporary dyes are molecules too large to diffuse into the hair, therefore acting on the exterior of the fiber at the cuticle. This process does not harm the fiber because of the absence of alkaline oxidative conditions, but fails in terms of colour durability [166]. For this reason, the development of a colouring agent that provides the durability of the permanent hair dyes without the use of oxidizing agents that damage hair is highly desirable.

It is generally accepted that penetration of chemicals into hair occurs through intercellular diffusion, i.e., by adsorption onto the keratin substrate. Faucher and Goddard [185] have shown that the amount of polymer adsorbed on the hair surface increased with decreasing molecular weight. Similarly, low molecular weight compounds might also penetrate the hair shaft, at the cortex level, since the diffusion process is greatly facilitated when hair is exposed to water. Low molecular weight compounds are, however, only retained while the hair is dry because further contact with water opens the cuticle scales, facilitating their escaping. Recent solutions to this problem rely on the use of hair-binding peptides coupled to dyes or pigments that are able to penetrate into the hair shaft, although they lack the required durability for long lasting colour effects. Huang et al. [186] have tested a hair-binding peptide coupled to carbon black and the use of chemically functionalized carbon nanotubes that provided an enhanced interaction with

the hair, resulting in a more durable hair colouring effect. Nevertheless, more durable hair colourants are still needed.

For this reason, there have been attempts to enhance the binding of the cosmetic agent to hair. Richardson et al. [187] describe the covalent attachment of cosmetic agents to hair using transglutaminases. These enzymes promote the crosslinking of the cosmetic agent's amine to the glutamine residues in hair. Similarly, Green et al. [188] describe the use of the enzyme lysine oxidase to covalently attach cosmetic agents to hair.

Despite all the research for cosmetic applications, conventional products are still being used, with small improvements. Formulation requirements imply very strict criteria for dye/product selection. The remaining problem is achieving perfect compatibility in all respects between the dyes/products and the various other constituents of the products.

4 Final Remarks

Hirsutism, graying, alopecia and other disorders of human HFs have dramatic effects on the appearance, socio-cultural status and self-esteem of the affected individuals. The most current treatments available are very aggressive to hair and potentially to human health. In order to solve these problems, new and more efficient approaches are required.

Much progress has been made in understanding the genetic basis and molecular pathways activated during HF embryogenesis, cycling, disorders and the ageing process. HFs are a rich source of stem cells. The recent advances in the isolation, gene expression profile and propagation of stem cells could lead to the modulation of these cells with therapeutic purposes in the treatment of alopecia, wounds, burns, carcinogenesis and even ageing.

The potential specific targeting of different HF-associated cells, namely DP and immune cells, by bioactive compounds, along with effective follicular delivery systems will allow the development of home-based procedures for the maintenance and treatment of hair and scalp disorders.

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