Chapter-1

Introduction
Introduction

1.1 Overview of Alopecia

Alopecia is a distressing dermatological disorder characterized by hair loss. Although hair serves no critical physiological function in humans, hair loss can be psychologically devastating and adversely affect the self confidence of the patient [1]. The significance of this effect can be determined from the fact that each year in the US alone, about 60 million people spend approximately $US1.5 billion on various hair regrowth treatments [2]. With increasing longevity adding to the ancient pre-occupation with hair, the desire to extend youthfulness is inevitably fuelled. This ever-increasing fascination with hair-care is reflected in the incessant growth of the hair-care market, already a multi-billion dollar enterprise world-wide.

The recent phenomenal growth of hair care industry has been attributed to the thriving economy, earlier-aging crisis, and the commercialized awareness of organic benefits. Regulatory bodies and pharmaceutical industry all over the world are waking up to the various prospects that indigenous medicines have to offer.

Hair is really one of human biology’s most intriguing structures, although it is commonly dismissed as being of superficial importance and an ornamental feature only [3]. The role of hair in social and sexual communications among humans cannot be undermined. Scalp hair occupies most of the research and attention because of its uniqueness in being thick, long, and pigmented as opposed to the short, fine, unpigmented vellus hair present on the body surface. Hence, any significant reduction or absence of scalp hair leads to significant psychological trauma [1], as observed in cases of chemotherapy-induced alopecia and other naturally occurring alopecia’s.

Traditionally, hair loss was treated with the topical application of different herbal remedies, which were a result of long years of observation and painstaking effort by holistic practitioners. These were overlooked by the new generation of practitioners with the logic that they were outdated and had no clinical significance in the new world where remedies were produced from pure single synthetic molecules. This approach continued for the last one
century in which the newer generation of patients decided that the plethora of serious and irreversible side effects that came in with the xenophobic single and pure molecules were not what they had bargained for. Hence with the advent of the 21st century, there was again a revival of indigenous medicine under the strict eye of regulatory bodies and the accepting public. Indigenous medicine has today gained far more acceptability than it had ever as opposed to modern medicine due to the same reasons that it had been overlooked earlier. It was old, tried and tested for centuries. Today the pharmaceutical industry, the governments of India, China, Africa, S.E. Asia and other countries are in a race to patent their respective indigenous medicine before it gets taken over by the competition. The industry is finally putting money where the tradition is and finding newer avenues to focus its research on.

The holistic approach of involving the complete plant and animal vis-à-vis the modern approach of identifying the single molecule to work upon relies on the facts that extracts from plants and animals are natural products and non-xenophobic to the body. The drug products are formulated as compounds containing multiple components of which all may not necessarily be of therapeutic value. These non-therapeutic excipients may also be effective in another category of disease. They may be synergistic in action or simply inert substances that aid in local delivery and therapeutic action of the aforementioned compound. Another distinction between modern synthetic drugs and traditional medicine is that the amount of drug available in the blood is usually lower for the compound drugs which may decrease the eventuality of overdosing, dose dumping and exceeding the therapeutic window and hence toxicity due to excess of synthetic chemical moieties. Traditional medicine are usually easily metabolized and excreted and reasonably well accepted than the single chemical entity because of the presence of the pharmaceutical aids present in within it.

1.2 Causes of alopecia
Alopecia or hair loss is a common dermatological problem that transcends demographic, economic, racial, gender and age barriers. The etiology of hair loss is uncertain though it has been attributed to many probable factors ranging from hormonal imbalance, chemotherapy, thyroid imbalance, genetic predisposition, abnormal kidney function, abnormal liver function, lupus erythematosus, thermal damage, childbirth, fungal infection, diabetes, vitiligo, stress,
Introduction

trauma, autoimmune diseases, rheumatoid arthritis, use of excessive chemicals on hair such as bleaches, dyes, blow drying, etc.

Hair growth is a result of the proliferative activity of matrix keratinocytes that form the hair shaft and inner root sheath. Matrix keratinocytes are localized in the bulb where they rest on the dermal papilla, which is the condensate of specialized mesenchymal cells with important inductive properties. The surgical removal of the dermal papilla and the lower dermal sheath prevents hair growth, indicating that these specialized mesenchymal cells are the key signalling centres in hair follicles. The fact that a capillary loop is located within the dermal papilla of terminal hair follicles that provides nutrition to these cells; whereas vellus hair follicle dermal papillae typically do not contain capillaries illustrates the significance of nutrition to the papilla and the overlying matrix cells.

1.3 Types of alopecia

Some of the common types of alopecia include alopecia areata, alopecia totalis, alopecia universalis, androgenetic alopecia, cicatricial (scarring) alopecia, trichorrhexis nodosa, telogen effluvium, anagen effluvium & trichotillomania.

Trichotillomania is a common type of alopecia affecting up to 2% of the population. It involves compulsive hair pulling and plucking with the affected individual mostly unaware of their self-destructive actions.

Traction alopecia is hair loss occurring due to inappropriate hair styling such as braiding or corn rows, excessive use of hair stylers, such as rollers or curling irons. Traction alopecia is a very common cause of temporary, reversible, although generally non-scarring and non-inflammatory hair loss.

Telogen effluvium is a form of nonscarring alopecia characterized by diffuse shedding of telogen or club hair, often with an acute onset. It is often caused in response to any metabolic or hormonal stress or by medications. Generally, recovery is spontaneous and occurs within 6 months of hair loss.
Introduction

Anagen effluvium is sudden shedding of anagen hair caused by interruption of active anagen hair-follicle growth by external stresses (chemotherapeutic agents, ingestion of toxic substances etc). Recovery is usually rapid and starts with the removal of stress causing factor.

Alopecia areata (AA) is an organ-specific autoimmune disorder characterized by nonscarring hair loss that may be patchy or generalized. AA has been observed occurring in association with several autoimmune diseases including thyroid and vitiligo and nail abnormalities [4, 5]. Alopecia areata is commonly seen throughout the world. It is estimated that 0.2% to 2% of the US population alone is affected by alopecia areata and the individual lifetime risk is estimated to be approximately 1.7% [6, 7]. About 25% of patients have a genetic history of the disorder, however, various report show familial incidences range from 3-42% [8]. Recently, alopecia areata has been linked with specific HLA class II alleles, especially DQB1*03 and DRB1*1104 [9, 10].

Androgenetic alopecia (AGA) or male/female pattern hair loss attributes to over 95% of hair loss reported worldwide. It is the most common type of alopecia, affecting about 50% of Caucasian males and females beyond age 40 years [11]. The severity of the disease varies from merely accentuated recession of the frontal hairline to loss of all hair except along the temporal and occipital margins. It is a progressive disease resulting in decrease in density of terminal hair and a resulting increase in vellus hairs.

Although etiology of the disease is still unclear, cell culture work by Hibberts and co-workers [12] proposes that increased perception of hair follicles to dihydrotestosterone leads to androgenetic alopecia especially post puberty. Sawaya and Price [13] demonstrated that the concentration of androgen receptor protein within the dermal papilla fibroblasts and outer root sheath are 30% greater in the balding frontal follicles as compared to the non-balding frontal hair follicles. Increased androgen binding at these receptor sites resulted in distinct effects on the physiology of hair follicles.

1.4 Conventional modes of treatment

Topical Minoxidil and oral Finasteride are the only approved treatments for alopecia for the past 50 years. These drugs alleviate the symptoms of alopecia, albeit they come with a plethora of serious side effects like transient impotence, hypertrichosis, pruritis, dryness,
Introduction

scaling and local irritation. Also, these treatments have the drawback of high relapse rate and serious side effects like male breast cancer, sexual impotence and aggravated allergies. Another disadvantage is that on cessation of treatment, the regrown hair falls out to pre-treatment or lower levels [11, 14].

Some other drugs under investigation include topical sensitizers/irritants like Squaric Acid Dibutyl Ester (SADBE), Diphenylcyclopropenone (DPCP), hydroxychloroquine, oral, injectable and topical corticosteroids. The FDA has documented use of irritants to elicit mild reactions like itching, erythema, and scaling which are sufficient to induce hair growth [15]. It is hypothesized that these immunosuppressive drugs generate nonspecific suppressor T cells or inhibit pro-inflammatory cytokines thereby inducing hair regrowth.

Sulfasalize, anthralin, FK506, oral cyclosporine [16], phototherapy, psoralen plus ultraviolet A (PUVA) irradiation therapy are also being explored for hair growth promotion effects. Drugs undergoing either preclinical development include dutasteride [17] spironolactone, peptides like Lys-Pro-Val tripeptide [18], glycyl-l-histidyl-l-lysine copper (II) and derivatives [19]. Drugs undergoing clinical trials include Bexarotene, Capsaicin, Plaquenil, and Alefacept [20].

Anagen inducing drugs include FK506 [16, 21,22], norepinephrine-depleting agent [23], estrogen receptor antagonist [24], tretinoin [25], tumor promoter agent (TPA) [25, 26], and various growth factors and neural mediators, such as keratinocyte growth factor (KGF) [27], hepatocyte growth factor (HGF) [28], sonic hedgehog [29], substance P [30], the antagonist parathyroid hormone (PTH) [31, 32], ACTH, and mast cell degranulation [33].

Although these drugs are observed to have a specific mechanistic effect on the hair cycle, the mechanism by which they induce anagen, the molecular signalling they trigger or how exactly do they participate in promoting anagen is still unclear.

1.5 Disadvantages of conventional therapy

Side effects of finasteride include male breast cancer (3.1% to 21.6%), impotence (1.1% to 18.5%), abnormal ejaculation (7.2%), decreased ejaculatory volume (0.9% to 2.8%), abnormal sexual function (2.5%), gynecomastia (2.2%), erectile dysfunction (1.3%),
ejaculation disorder (1.2%) and testicular pain [34-40]. The FDA has added warning to finasteride about an increased risk of high grade prostate cancer [39]. In December 2008, the Swedish Medical Products agency [40] concluded a safety investigation of finasteride and subsequently advised that the use of finasteride may result in irreversible sexual dysfunction [37]. Side effects of minoxidil include severe allergic reactions (rash; hives; breathlessness; swelling of the mouth, face, or tongue); chest pain; dizziness; fainting; tachycardia; sudden, unexplained weight gain; swollen hands or feet weight gain. Pseudo acromegaly cases have been reported due to minoxidil at an unusually high dose [41].

1.6 Indigenous products for the treatment of alopecia

The WHO definition of Traditional Medicine is “the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences of indigenous cultures, whether explicable or not used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness”[42]. The terms "alternative", "complementary", “indigenous”, or "non-conventional medicine" etc are used interchangeably with "traditional medicine" in some countries. Nowadays, indigenous products have become popular as an alternative treatment for various ailments, for boosting the immune system as also for providing nutritional benefits. They have also been incorporated into hair care products because of their myriad beneficial effects, relative safeness, adaptable nature and most of all, their being natural as opposed to being synthetic. The interest of the consuming public is growing manifold day by day, reflected by their increased purchasing of natural products and the colossal growth of the indigenous product industry. This escalating growth in manufacture of hair care products is reflected in the increasing number of natural ingredients used in the various product categories [47] and number of products in each category as reported to the FDA [48]. Some of the chemical classes of ingredients used in hair care products are listed in Table 1.

Table 1. Chemical classes of ingredients used in hair care products [43]

<table>
<thead>
<tr>
<th>Type of hair product</th>
<th>No. of individual ingredient</th>
<th>No. of natural ingredients</th>
<th>Natural ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair dyes</td>
<td>293</td>
<td>2</td>
<td><em>Lawsonia inermis</em> [44, 45]</td>
</tr>
<tr>
<td>Hair conditioning agents</td>
<td>1970</td>
<td>136</td>
<td><em>Ginkgo biloba, Actinidia chinensis</em></td>
</tr>
</tbody>
</table>
Introduction

Hair fixatives

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oryza sativa extract</td>
<td>240</td>
</tr>
<tr>
<td>(kiwi)</td>
<td></td>
</tr>
<tr>
<td>Orchid extract, Adiantum Capillus vaneris leaf extract</td>
<td>47</td>
</tr>
</tbody>
</table>

Hair waving/straightening agents

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oryza sativa extract</td>
<td>4</td>
</tr>
<tr>
<td>(kiwi)</td>
<td></td>
</tr>
</tbody>
</table>

Other cosmetic ingredients used in hair care products include antidandruff agents, antistatic ingredients, fragrances, emulsion stabilizers, preservatives, surfactants, and viscosity controlling agents.

There has been an explosive rise in the use of indigenous products for hair care and treatment in the recent years. Plant and animal sources offer a wide scope of promising active and basic bioactive ingredients that can be used to manufacture drugs as well as cosmetics for hair growth and care (Table 2). The role of these bioactive molecules in alleviating symptoms of disease as well as curing the root of the disease cannot be undermined. Their contribution in providing relief in dermatitis, hair growth, colouring, and various phytotherapies continue to be active areas of research. These are the newest thrust areas of active research to which the world is turning back. An enhanced efficient follicular delivery system and specific targeting to the pilosebaceous units for the bioactive chemicals is needed that would enhance and synergise their effects. The greatest need of the hour, however, is to create safe bioactive products whose therapeutic and cosmetic claims are founded on a good scientific basis and which can be standardized as well as validated like its synthetic pure molecular cousin.

Table 2. Natural ingredients for hair care.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
<th>Possible mechanism</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asiasari</td>
<td>Asiasari radix</td>
<td>Increased protein uptake, expression of VEGF in human dermal papillae</td>
<td>Not listed as a cosmetic ingredient.</td>
<td>49</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>Vitis vinifera (Grape) seeds</td>
<td>Inhibition of transforming growth factor TGF-β1, conversion of telogen to anagen &amp; promotion of hair follicle cells</td>
<td>2 uses of grape seed extract have been reported.</td>
<td>50</td>
</tr>
<tr>
<td>Cysteine</td>
<td></td>
<td>Thymidine</td>
<td>9 uses reported of which 8 incorporation assays are for hair care products</td>
<td>49</td>
</tr>
</tbody>
</table>

Ph. D Thesis
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Function</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergamot</td>
<td>Increases cutaneous activity of superoxide dismutase, collagen, &amp; decreased malondialdehyde &amp; increased hair growth</td>
<td>Bergamot oil is used in 51 cosmetic products, two of which are hair care products</td>
</tr>
<tr>
<td>Gensenoside-Rb</td>
<td>Demonstrated the potential to promote hair growth on cultured mouse vibrissal hair follicles.</td>
<td>Functions as a skin conditioning agent. Ginseng extract is used in 344 cosmetic products, 118 of which are hair care products</td>
</tr>
<tr>
<td>Extract</td>
<td>In vivo and in vitro studies evaluated petrolatum ether extract of the leaves and flowers of <em>Hibiscus rosa-sinensis</em> for its potential to stimulate hair growth.</td>
<td>Extracts are used as cosmetic ingredients (colorant to skin conditioning agent). There are 34 uses in cosmetic products, 27 of which are in hair care products</td>
</tr>
<tr>
<td>Extract</td>
<td>Suppression of TGF-β, which delays the catagen cycle.</td>
<td>Extract listed as cosmetic ingredients used as skin conditioning agents. There are no reported uses in cosmetic products</td>
</tr>
<tr>
<td>Fruit Extract, Fruit Powder, and Oil</td>
<td>Shikimic acid induced insulin growth factor-1, keratinocyte growth factor, and VEGF in the hair follicle.</td>
<td>Eight uses of anise are reported in cosmetics, none of which are in hair care products</td>
</tr>
<tr>
<td>Dried root extract</td>
<td>Increases growth factors such as insulin-like growth factor-1, keratinocyte growth</td>
<td><em>Sophora Flavescens</em> Root Powder is listed as a cosmetic ingredient with functions ranging from</td>
</tr>
</tbody>
</table>

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Ph. D Thesis
factor in dermal papillae cells, inhibits type II, 5-a-reductase activity.

Acetone extract  
*Boehmeria nippononivea*

Exhibits 5-alpha-reductase inhibition & a hair growth effect.

Extract  
*Rosmarinus officinalis*

used as a tonic or rinse to remove oil (seborrhea), and to add volume and shine to the hair.

Extract  
*Thymus vulgaris L.*

Used to treat dandruff and as a hair growth promoter.

Leaves and fruit  
*Juglans regia L.*

Used to treat skin rashes, acne, alopecia, scalp dermatitis, and seborrheic dermatitis.

A major concern of the industry and regulatory bodies is the lack of chemical characterization of the products used, standardization of extraction method, the presence of contaminants, documentation of ingredients' biological activity on skin and hair [2] and toxicity testing of these drugs. Today, the indigenous products are perceived by the public as being safer than synthetic substances. Various pharmaceutical, clinical and internet publications claim that there is a boom in the clinical as well as short-term usage of natural products, but there is limited information or research regarding the in-vitro and in vivo studies and fewer human studies. Moreover, no large multicenter clinical studies regarding the safety and efficacy of these bioactive ingredients have been conducted [60, 61, 62]. The pharmaceutical industry currently faces the challenge of chemically characterizing the natural constituents, understanding their bioactivity, and hence assures safe and effective use.

While the exact mechanism of action of various hair growth products is unknown, it is
Introduction

suggested that hair growth occurs as a result of the primary actions of accelerated blood flow, activation of the anagen dermal papillae, dihydrotestosterone inhibition, anti-inflammatory activity, and increased nutrition [43].

1.7 Advantages of the indigenous system of medicine

According to an article published in JAMA in 1998, iatrogenic diseases are the fourth leading cause of death in USA and other developed nations [63]. According to another article in the New York Times in 2003, side effects of drugs kill more Americans annually than the World War II and Vietnam War combined. These are two published records highlighting the drawbacks of modern medicine.

Indigenous medicine is easily available and affordable to the common man highlighted by the fact that more than 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care according to World Health Organization (WHO) [42]. The ingredients have strong curative action with little or no side effects and have been proven to be equally efficient in fighting disease as conventional medicine. An added advantage of these medicines is that they treat the root of the disease rather than the symptoms.

Indigenous medicine is centuries old, tried and tested. It is also known as holistic medicine as the drugs contain complete plant or animal product as opposed to synthetic pure molecules and is non-xenophobic or not-alien to the body. Today, guidelines regarding testing safety, efficacy and toxicity of natural and traditional products are easily available to manufacturers as well as consumers and because of this alternate medicine is more acceptable today than it was ever before. The fact that of 750,000 species of plants on earth, only a very small percent has been explored in traditional medicine, and that too not to their complete potential makes it more attractive and promising. Major pharmaceutical companies are conducting extensive research on indigenous medicine and are turning to Ayurveda, unani, homeopathy for leads and blockbuster drugs. The indigenous medicine market is a very promising market and has a place for everyone. If combined with proper pharmaceutical research, analytical processing
Introduction

and formulation development, it could put an end to the suffering of a lot of people without overburdening resources.

1.8 Rationale behind use of indigenous therapies

According to Kunin et al, the annual global trade in animal-based medicinal products accounts for billions of dollars per year [64]. The investigation of traditional medicines has proven a valuable tool in the developing art of bioprospecting for pharmaceutical compounds. Of the 252 essential chemicals that have been selected by the World Health Organization, 11.1% come from plants, and 8.7% from animals [65]. And of the 150 prescription drugs currently in use in the United States of America, 27 have animal origin [66].

Holistic medicine such as Ayurveda, Unani, Siddha provide a variety of age old tried and tested drugs for hair fall, for strengthening follicle roots, curing dandruff, dyeing of hair and other related problems. These cures are in vogue now as increasingly people are becoming aware of the transient nature of relief and side effects associated with modern medicine. The positive and “wholistic” nature of holistic medicine is being acknowledged for the first time. Not only are these drugs natural and non-xenophobic but also cheap, easily available and can be easily modified to suit individual patient needs.

1.9 Anatomy and physiology of the skin

The skin is the outer most covering of the body which provides an interface between the organism and its environment. It is integral to the survival of mammalian life, and serves as an excellent indicator of the health and welfare of an individual. Amongst its varied functions is protection against the vagaries of the environment, thermoregulation, maintenance of homeostasis, metabolism, excretion and to provide an ornamental accessory to the organism.

The skin is the largest organ in humans. It is highly vascular and receives about one-third of the blood circulating through the body. The heterogeneous skin structure consists of epithelial cells forming a stratified epidermis containing keratins which acts as a protective barrier; mesenchymal cells forming the underlying dermis which along with epithelial cells, facilitate
the production of hair follicles and other appendages; hypodermis which regulates energy storage, metabolism and thermostasis; and melanocytes that pigment the skin and hair follicles (Fig. 1)[67]. Specialized appendages derived from the ectoderm and/or mesoderm are present within these layers including sensory nerves, sweat glands and hair follicles having pre-defined functions like thermoregulation, homeostasis, defence, sensory functions, communication, reproduction, etc [68].

Also, the skin is highly innervated, and contains large amounts of specialized cells like dendritic Langerhans cells, Merkel mechanoreceptor cells (form complexes with sensory axons), and mast cells (produce histamine). These cells of diverse origins undergo extensive interactions, relocation, propagation, and differentiation during embryonic development to come to their present state [67].

Fig. 1. Skin layers of both hairy and hairless skin [adapted from Wikipedia].

The average adult has over 3,000 square inches of skin surface area and the fat-free skin accounts for almost 9kg or at least 6 percent of an individual's total weight. The location decides the density of structures in the skin, but on average one square centimeter of skin contains about 10 hair follicles and 15 sebaceous glands, 100 sweat glands, half a meter of blood vessels, 2 meters of nerves, with 3,000 sensory cells at the ends of nerve fibers, 200
nerve endings to record pain, 25 pressure receptors for the perception of tactile stimuli, 2 sensory receptors for cold, and 12 sensory receptors for heat [69].

Skin appendages like hair, nails, claws, horns, salivary glands, sebaceous glands and mammary glands are topological transformations of originally flat epithelia into specialized structures that are either protruding outwards such as the hair, or invaginating inward as for a gland [70]. They then diverge into more complex skin appendages e.g. claws, nails, hair from ex-vaginating bud and sweat glands, scent glands from the invaginating bud.

1.10 Hair

Hair is a threadlike keratinized epidermal structure about 0.1mm thick, developing from a hair follicle sunk in the dermis, produced only by mammals and characteristic of a particular group of animals. Hair and hair follicles occur in a wide diversity all over the body except on the palms, soles, glabrous foreskin, and the lip vermillion. Four different hair follicles produce the four categories of hair; lanugo hair-producing hair follicles, vellus hair follicles, intermediate hair follicles, and terminal hair follicles.

Hair consists of a permanent, superficial portion from the bulge upward which is known as the hair shaft, and a lower, inferior portion which is the root (Fig. 2), that cycles between anagen, catagen, and telogen in order to produce new hairs.

1.11 The hair follicle

The hair follicle (HF) is a multifarious mini-organ in its own right. Each individual hair follicle is formed by multiple mesenchymal and epithelial cell layers, together comprising more than 20 different cell populations. Relevant anatomical divisions can be made between the permanent, superficial structure and the transient cycling component of the hair follicle including the bulb. The morphological dividing line between these two components lies
Introduction

below the bulge region, the putative site of epithelial stem cells, mast cells and Langerhans cells. Hair growth results from the proliferative matrix of keratinocytes that reside in the bulb, where they sit on the dermal cells with important inductive properties.

Hair cycling is traditionally divided into a growth phase (anagen I-VI), a regression phase (catagen), and a resting phase (telogen). The shedding of hair fibre has recently been identified as an active process of its own (exogen). The events of morphogenesis and cycling are controlled by a complex network of sequential activation and inactivation of autocrine, paracrine and endocrine signalling pathways. The hair follicle is surrounded by a dense meshwork of blood vessels and nerve endings. Multiple specialized cell populations can be found associated with the different compartments of the hair follicle, including melanocytes, neuroendocrine cells and immune cells.

Hair follicles are formed primarily during embryonic development (morphogenesis) of the organism. It is rare for HFs to be formed after birth, although individual HFs can change drastically over time being influenced by androgens. The human body is covered with about 5 million HFs at birth, of these 80,000 to 150,000 are to be found on the scalp.

Although the exact mechanism that initiates follicular formation and regulates the cycle is yet to be understood, it is known that transition from one phase to another is based on the
signalling and expression of various transcription factors, cytokines, neurotransmitters, growth factors, hormones and enzymes [71, 72, 73]. An accurate understanding of the influence of these factors on the hair cycle is important since it presents the various scenarios where hair growth can be regulated and manipulated [74].

Length of hair is determined by the duration of the anagen phase. Anagen in the human scalp hair lasts for anywhere in between 2 and 6 years while it is considerably shorter for places other than the scalp.

1.12 Anatomy of the hair follicle

The mature anagen hair follicle is divided into vertical or concentric (horizontal) compartments. The three major vertical compartments of the hair (from superficial to deep) are (Fig. 2):

i. The upper follicle comprising of the infundibulum and the isthmus
ii. The middle portion of the hair follicle consisting of the bulge
iii. The lower follicle containing of the suprabulbar and the bulbar areas

Although the upper and middle parts of the follicle are permanent, the lower follicle regenerates itself with each turn through the hair follicle cycle.

The major parts of the hair follicle from outermost to innermost are connective tissue sheath, outer root sheath, inner root sheath, cuticle, hair shaft cortex and medulla.

The inner root sheath

The inner root sheath extends from the bulb to the isthmus and is situated between the outer root sheath and the shaft of the hair. The IRS is composed of the middle cylinders of the follicle [75]. The IRS contains three layers: the cuticle of IRS, Huxley's layer, and Henle's layer. The cuticle of the IRS is made of distally pointing scales interlocking with similar but opposing scales hence making up the cuticle of the hair shaft surface whose scales point proximally.
This combined interlocked cuticle structure allows the hair shaft and IRS to simultaneously move during the anagen phase. Henle’s layer keratinizes first in the anagen follicle [74, 76]. It surrounds the shaft sheath structure and interfaces with the stationary ORS. It is inherently strong and appears to be tightly attached to the ORS. The hair shaft and the IRS complement each other in shape and hence together they form a solid core of hardened tissue with a nearly circular cross-section [75].

The hair shaft

The part of hair which can be seen above the scalp is known as the hair shaft. It comprises of dead cells, keratins, binding material and small amounts of water. It is made of three parts: the cuticle, the cortex, and the medulla.

The hair follicle bulb

The bulb is the deep, spherical portion of follicle surrounding the dermal papilla. It consists of matrix cells which are living, actively proliferating group of cells, which later differentiate and keratinize to form the hair cortex. The rate of proliferation of matrix cells is the highest in the body. As they multiply and enlarge, these cells progressively shove the formerly produced cells upwards. When these cells reach the upper part of the bulb, they begin to modify and organize into six cylindrical layers, each succeeding the other with the three inner layers producing the actual hair.

The dermal papilla

The dermal papilla (DP) directs and determines the generation of an embryonic hair follicle. It is made up of highly active cells that induce follicular development from the epidermis. DP contains spindle-shaped cells, fibroblasts, collagen bundles, stroma, nerve fibers and a single capillary loop. It is contiguous with the perifollicular or dermal sheath of connective tissue that encloses the lower follicle.

1.13 Morphogenesis of hair

Morphogenesis of hair initiates at 8 weeks in the fetus with the manifestation of placodes in the epidermal basal layer, above the dermal packs of mesothelial cells [67, 71, 77, 78].
Introduction

Epidermal pegs grow below and enclose these dermal papillary cells, (Fig.3). When development of the inferior section and formation of the follicle are concluded, hair growth commences with the initial growth completing by 22 weeks. The first coat of hair is fine lanugo hair that develops as an advancing wave from the frontal to the occipital scalp and is shed by 36 weeks' gestation. Later, a second coat of lanugo hair appears which is shed in a synchronized wave pattern 3–4 months post birth. The bare occipital scalp patch often seen in infants is physiological manifestation, resulting from synchronized shedding of the final wave of lanugo telogen hairs prior to their replacement by normal scalp hairs [79, 80]. The maximum number of hair follicles is present at birth; with follicular density being highest in neonates and lessening progressively during childhood and adolescence as the scalp stretches over the growing skull until it stabilizes in adults (250–350 hairs per cm²) [81].

Synchronized wave like follicular cycling is lost in humans one year post birth to be replaced by a random or mosaic pattern of asynchronous hair cycling. The newly created hair follicles then continue to cycle incessantly during their life span through stages of growth, rest, shedding, and regrowth [82, 83, 84].

Hair on the scalp is made of large terminal hairs and small vellus hairs with the average ratio of terminal to vellus hairs being 7:1. Terminal hairs are prominent and exceed 0.03 mm in diameter and 1 cm in length, and are melanised and medullated. Grading of terminal hairs based on hair shaft diameter is done as small (0.031–0.06 mm), medium (0.061–0.09 mm), or large (greater than 0.091 mm). Vellus hairs are not noticeable and are 0.03 mm or less in diameter and often less than 1 cm in length and lack melanin and medulla [85]. Vellus-like hairs are terminal hairs miniaturized to vellus hair proportions. Terminal hairs are rooted in subcutaneous tissue or deep dermis, whereas vellus hairs are rooted in the upper dermis (Fig. 6) [43].

Activation of catagen or the intermediate phase marks the termination of the growing or anagen phase. During this phase, the hair shaft shrinks back upwards and the outer root sheath recedes by individual cell death, also known as apoptosis. Here, the inferior part of the follicle fades away leaving behind an angiofibrotic strand or streamer (stela) signifying the former position of the anagen root. The subsequent telogen phase lasts an average of 3
months before a new anagen hair develops. During telogen, the resting club root is located at the “bulge” level, where the arrector pili muscle enters into the hair follicle [86]. The telogen bulb lacks pigment and an inner root sheath. The hair shaft is surrounded by trichilemmal keratin and the trichilemma (outer root sheath). Finally, the telogen hair is shed in the exogen phase, which occurs in either late telogen or early anagen. Usually, there are 100,000 scalp hairs in a normal adult with 10% hair in telogen; hence, the average hair loss equals 100 per day. The subsequent anagen cycle initiates with swelling of the dermal papilla at the “bulge” level and the development of a new anagen bulb [86], which starts expanding down the follicular stela towards the point of origin in the subcutaneous tissue.

Substance P has been shown to stimulate epidermal keratinocyte proliferation in organ-cultured mouse skin with telogen HF [87].

1.14 The Hair Cycle

The hair follicle growth cycle illustrates the varying morphology of the shaft, grossly, and the follicle, histologically with time [88] (Fig. 3). All body hairs undergo this cycle, albeit the duration of cycle, of the individual phases, as well as the length of the individual shafts differs enormously from location to location [89, 90]. Every follicle has its own inherent pattern, and hence is asynchronous with the neighbouring follicle in humans and guinea pigs. Whereas in most rodents, synchronous follicle growth occurs in waves that sweep nuchal-caudally and is affected by the neighbouring follicles [91] and general stimuli.

It is unclear as to how the hair cycle spreads in waves, though research suggests that these growth waves are controlled by intrinsic factors of the hair follicle group in a “reaction-diffusion” system [92, 93, 94]. Neighbouring follicles and systemic (e.g., endocrine) stimuli influence the intrinsic rhythm [91, 90], since the waves of hair growth become synchronized with time in parabiotic rats [96]. Hence the cycle is essentially autonomous and has an inherent pattern; it is also influenced by environmental systemic and local factors [95]. Follicular morphogenesis (Fig. 3) and the first postnatal catagen, telogen, and anagen development of the hair follicle follow a precise time-scale [97] (Fig. 4). However, the genetic
Introduction

background (strain), the sex (e.g., prolonged telogen in the female), environmental factors (time of the year, temperature, light periods) and nutritional factors also play an important role.

Since follicular melanogenesis and HF cycling are synchronized with each other it is evident

![Diagram of hair follicle cycling phases]

Fig. 3 Schematic representation of the increasing and decreasing length of the HF and the localization of the most proximal part of the HF in correlation with the panniculus carnosus and the border between the dermis and subcutis. Arrows between panniculus carnosus and the border dermis/subcutis indicate the hair cycle-associated changes in the thickness of the subcutis. The approximate duration of each phase is indicated in brackets. Note changes of the DP shape and size throughout the cycle as well as the increasing size of the SG during anagen IV-VI. Reprinted by permission from Macmillan Publishers Ltd: [JOURNAL OF INVESTIGATIVE DERMATOLOGY] (Muller-Rover S, Handjiski B, van der Veen C, Eichmuller S, Foitzik K, McKay IA, Stem K, Paus R. A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. pp 117:3-15) copyright (2001) [74].
that anagen development is associated with distinctive changes in skin pigmentation in pigmented C57BL/6 mice (Fig. 5) [98, 99, 100]. Also, the time synchronized HF cycling induces profound alterations in the histomorphology and thickness of the skin, which are most evident proofs of the various phases of the hair cycle (Fig. 5) [76, 101, 102].
mice. This difference becomes morphologically indistinguishable nine days after depilation, when the induced anagen HF reach their maximal length [97, 99, 102, 103]. The spontaneously developing anagen wave moves as a caudal-nuchal wave, however the spontaneously developing catagen waves moves in the opposite direction of nuchal-caudal. The other difference between depilation induced anagen development and spontaneous anagen is that anagen is induced synchronously all over the back in the depilated mice whereas it progresses in a wave like form caudal-nuchal in spontaneous anagen [74]. Catagenic changes in HF histomorphology are initially observed on day 17 after depilation in the neck region, and are recognizable visually by a change in skin color from black to gray-pink. This catagenic wave reaches the tail region 2 days later on day 19-20 [74, 76, 97, 104] (Fig. 5).

1.14.1 Anagen

The active growth phase of the hair cycle where metabolically active and dividing cells above and around the dermal papilla of the follicle grow upward to form the hair shaft is known as anagen. The rate of proliferation is intense and is observed in all hair follicle cells and epithelial compartments with matrix cells exhibiting the highest intensity. The duration of anagen is predetermined genetically and varies with the size and location of the hair follicle. It may last several years, as seen in terminal HF's on the scalp, or may persist only for few weeks as observed in HF in the extremities.

1.14.2 Catagen

Catagen is a short period initiating from late anagen and concluding in early telogen. It is characterised by a fundamental restructuring of the extracellular matrix, involution of the hair follicle, and a cessation of protein and pigment production. Massive apoptosis in the transient, inferior bulbular portion of the hair follicle leads to regression of the HF and formation of a fibrous streamer. Onset of this apoptosis is predetermined and finely orchestrated, resulting in degeneration of two thirds of the HF (Fig. 6). The club hair is formed when the part of the hair follicle in contact with the lower portion of the hair becomes attached to the hair shaft. Catagen is the first module of the initial hair cycle occurring after morphogenesis.
1.14.3 Telogen

In telogen, the hair follicle regresses to about half of its previous size and does not extend beyond the upper dermis (Fig. 6). The dermal papilla is no longer enveloped by surrounding epithelial cells and sits as a small band of cells in close association with the epithelial cell finger which do not show any significant DNA or RNA synthesis, nor is there any synthesis of proteins such as trichohyalin and the hair cortical keratins. Notably however, keratin 14 (K14) synthesis does continue in the epithelial sac to which the telogen hair fibre anchors. In telogen follicles, the volume of the dermal papilla extracellular matrix is much reduced, and dermal papilla cells have scant cytoplasm and are relatively quiescent. The telogen hair shaft, the club hair, can be retained for months in this epithelial sac.

1.14.4 Exogen

Exogen is a specific process of its own in hair follicle cycling [105]. The exogen root, in contrast, is made of very few cells and these cells are separated at their outer edge by intercellular cleavage as compared to the telogen root which is made of packed nucleated cells which show intracytoplasmic fractures surrounding a cornified core making up the shaft. The morphology of the hair root suggests that the exogen process involves a proteolytic event that occurs between the moving cells of the telogen shaft base. A possible role of desmoglein and proteolytic events are suggested [106].

1.14.5 Kenogen

The interval of the hair follicle cycle in which the hair follicle remains empty after shedding of the hair fiber and the telogen hair has been extruded and before a new anagen hair emerges is termed as Kenogen. The frequency and duration have been reported to be greater in men and women with androgenetic alopecia [107].

1.15 Duration of the hair cycle

The length of the various phases depends on the type and localization of the hair follicle. Under physiological conditions, 85% of the scalp hair is in anagen and approximately 15% in telogen [108, 109]. Anagen is generally observed anywhere from 2 to 6 years in scalp hair
although some individuals may have much longer anagen leading to exceptionally long hair. Hair length is primarily determined by the duration of anagen. The hair follicles of the body are characterized by an increased telogen frequency and duration as compared to scalp hair. Under physiological conditions, each hair follicle continues to cycle throughout life.

Throughout anagen (Fig. 3), an entire hair shaft from tip to root is produced, while two thirds of the cycling portion of the HF undergoes apoptosis during catagen (Fig. 6). Extensive research have been performed on the three major phases of the HF cycle in humans [83], as well as in many animal strains particularly mice [110, 111, 112]. A comprehensive guide on murine (mouse) HF cycling was established by Paus and colleagues in 2001 [74].

Fig.6. Schematic representation of the increasing and decreasing length of the HF and the localization of the most proximal part of the HF in correlation with the panniculus carnosus and the border between the dermis and subcutis. Arrows between panniculus carnosus and the border dermis/subcutis indicate the hair cycle-associated changes in the thickness of the subcutis. The approximate duration of each phase is indicated in brackets. Reprinted by permission from Macmillan Publishers Ltd: [JOURNAL OF INVESTIGATIVE DERMATOLOGY] (Muller-Rover S, Handjiski B, van der Veen C, Eichmuller S, Foitzik K, McKay IA, Stenn KS, Paus R. A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. pp 117:3-15) copyright (2001) [74]. This guide summarizes criteria for recognizing distinct stages of the murine hair cycle based on previous staging guides [3, 82]

1.16 Initiation of hair growth cycling

It is generally postulated that the first signal for hair follicle formation (i.e., at morphogenesis) comes from the mesenchymal cells [98, 113, 114]. Although the stimulus
that initiates this transformation of anagen to telogen in mature hair follicles is still unknown, it is theorised that it could arise from either the resting papilla itself, or the resting epithelial germ, the adjacent epidermis, or, in theory, even the supportive vessels, nerves, lymphatics, and resident dermal hematopoietic cells of the region.

Various in vivo and tissue culture studies have implied this signal to arise independent of central organized neural elements and vascular or endocrine signals [115, 116].

1.17 Theories of Hair Follicle Cycling

Six theories proposing the regulation of hair follicle cycling have been presented in the year 1999 [100] (Table 3). These theories include the cyclic characteristics, interactions between the epithelium-mesenchyme, site to site variability, effect of various extra follicular growth modulating signals, and dietary changes amongst others.

Earlier, theories in favour of both a stimulatory and inhibitory control of spontaneous hair growth induction have been presented [117, 118]. Chase [76], Argyris et al and Jahoda et al [119, 120] proposed that initiation of anagen was a result of down-regulation of an inhibitor or loss of inhibitor release mechanism, (the inhibition-disinhibition theory). Although no specific evidence is available, telogen epidermis has been reported to contain an inhibitor to hair growth induction which is not present in anagen epidermis [121]. Thus, it can be safely said that the recent presentation that follicle formation is at least in part controlled by an inhibitor release mechanism (bone morphogenic protein-4/noggin complex) [113] supports Chase’s inhibitor-release hypothesis for mature cycle initiation.

Table 3. Theories of hair follicle cycling

<table>
<thead>
<tr>
<th>Theory</th>
<th>Concept</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial theory</td>
<td>Gradually cycling cells in the bulge of hair follicle, conceal a second cycle that coordinates the follicle cycle Hair cycle is synchronized with the release of a cycle of growth morphogens by papilla cells which secrete morphogens only during the G0/G1 phase. When concentration of the secreted morphogens exceeds a critical threshold, it stimulates anagen.</td>
<td>100</td>
</tr>
<tr>
<td>Papilla morphogen theory</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ph. D Thesis
Introduction

The cycle is precipitated by the stem cells in bulge region of the papilla. The newly developed cells created from the stem cells can undergo only a limited number of mitoses, thus establishing duration of anagen and the onset of catagen.

Controls of the hair cycle do not dwell in a single cell compartment but rather reverberate a command set up by the tissues, i.e., disseminating and answering responses by morphogens of a defined tissue space.

Telogen cells accommodate an oscillation (e.g., transcription factor levels inside the cells), that triggers the residual movement of the follicle into anagen.

Hair cycling occurs in response to an inherent cycle clock, established during embryogenesis and which continues all through life.

The epithelial hair bulb harbours an accumulating endogenous mitotic inhibitor during each anagen phase.

Follicular cell growth ceases when a certain threshold level is reached. When the activity decreases to a level of disinhibition in telogen, anagen starts again.

1.18 Techniques for assaying Hair Growth

Hair is an ornamental feature of the human body, especially in social and sexual interactions. It represents beauty, youth, health, vitality etc to the wearer as well as the onlooker. Hair loss, alopecia, hypertrichosis, hirsutism are all common ailments in clinical dermatology, but patients seeking assistance are not actually completely bald or excessively hairy. The onus of diagnosing the actual disorder that afflicts the particular patient lies on the physician. It is imperative thus, to be able to distinguish between an actual disorder and a subjective complaint and to analyze the underlying pathogenesis. Ascertaining the accurate diagnosis is the key facet of effectively managing a hair patient.

Assessment of scalp hair requires sensitive objective techniques that can evaluate fundamental factors such as hair quality (density, elasticity, strength, and fragility), diameter of hair fiber, proportion of actively growing to resting hair, linear growth rate, and to differentiate hair shaft anomalies. This information is essential for determining normal hair...
morphology, for perceiving transformations developing from the disease, and for deciding the proper line of treatment.

Qualitative and quantitative methods are necessary for independently appraising hair growth activity. Clinical studies require standardized techniques to be followed for evaluating products that inhibit or promote hair growth. Hair density, hair width, and global photography evaluation are globally accepted parameters for judging hair volume, hair growth, and hair loss.

Several techniques have been reported in literature to analyse the rate of hair growth [122, 123]. These methods can be classified as invasive (e.g., biopsies [124, 125]), semi-invasive (trichogram [126, 127], unit area trichogram [128]) or noninvasive (e.g., global hair counts [129] and phototrichograms (PTG) methods [130, 131, 132, 133, 134, 135]). Thus, such quantitative methods for the evaluation of human hair growth and hair loss are essential to determine the efficacy of hair-promoting drugs.

118.1. Hair Pull Test

The hair pull test determines the ongoing hair growth activity and severity of any kind of hair loss.

Procedure
A bunch of about 50-60 hairs are held between the thumb, index finger, and middle finger from the base near the scalp. The hair is firmly, but not forcibly, tugged away from the scalp as fingers slide along the hair shaft [136]. Another method by using both hands, a clump of hair is grasped between two fingers of one hand and pulled at with the other [137]. Later, the number of extracted hairs is counted and occasionally examined under the microscope for diagnosis. If more than 10% of grasped hairs, or six hairs, are pulled away from the scalp, this implies a positive pull test and signifies active hair shedding (Table 4). If lesser than six hairs are easily pulled out, this is deemed as normal physiologic shedding [136].

Table 4. Results of Pull test (PT) and the corresponding hair disease [43, 136, 138, 139]

<table>
<thead>
<tr>
<th>Disease</th>
<th>Range</th>
<th>Test Value</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0-5 hairs = normal; =6 = positive</td>
<td>Positive</td>
<td>Normal physiological shedding</td>
</tr>
<tr>
<td>Alopecia Areata</td>
<td>=6 hairs</td>
<td>Positive</td>
<td>Light microscopy shows dystrophic</td>
</tr>
</tbody>
</table>

Ph. D Thesis
Introduction

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hairs</th>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgenetic alopecia</td>
<td>6</td>
<td>Mostly normal</td>
<td>Anagen and telogen stage Active AGA; positive on top of scalp; negative in occipital area</td>
</tr>
<tr>
<td>Acute anagen or telogen effluvium</td>
<td>6</td>
<td>Positive in active phases</td>
<td>Increased number of anagen or telogen hairs</td>
</tr>
<tr>
<td>Chronic telogen effluvium (CTE)</td>
<td>6-8</td>
<td>Positive only in active phases</td>
<td>Hair are examined under microscope &amp; observed to be usually telogen hairs</td>
</tr>
<tr>
<td>Trichotillomania</td>
<td>0</td>
<td>Negative</td>
<td>No pluckable hair</td>
</tr>
<tr>
<td>Loose anagen syndrome</td>
<td>6</td>
<td>Highly positive (upto 100%)</td>
<td>When hair are examined under microscope, it is seen that anagen hair root mostly lack hair sheath (anagen dysplastic)</td>
</tr>
</tbody>
</table>

1.18.2. Global Photography

Global photography is an important means in assessing hair growth and disorders. It is a standardized method for evaluating hair growth and volume using a stereotactic device with subsequent blinded evaluation by an expert comparing photos taken before and after treatment. Lederle in 1987 first standardized global hair photograph for hair growth research. Clinical trials for finasteride in 1992 led to the successful widespread usage of this as a secondary end point in clinical studies [140]. They are also used as a follow up approach for long term patients.

Technical Procedure

The patient's chin and forehead are fixed on a stereotactic positioning device, on which a camera with a flash device are also mounted, provided that the view, magnification, and lighting are reproducible at consecutive visits, hence allowing standardized study and follow up of the same area of interest. The researcher can transfer the camera to the region of interest with vertex, mid-pattern, frontal, and temporal views [129]. The standardized photographs are then subjected to evaluation by a panel of dermatologists whose reproducibility and inter observer agreement of their assessments are very high [140].
1.18.3. Unit Area Trichogram
The unit area trichogram is a semi-invasive (plucking) quantitative method for estimating three of the four main hair growth parameters: hair follicle density, proportion of anagen fibres, and hair shaft diameter in scalp hair.
It can be used for follow-up of changes in hair in a study group, for analysing hair growth cycling, and for tracking topical or systemic drug effects [141, 142].

Technical Procedure
The basis of the unit area trichogram is plucking hair in a defined area (usually >30 mm²) which has been identified prior to depilation. This area is initially degreased with an acetone: isopropanol (60:40, v: v) mixture to remove surface lipids. The sample area can also be quantitatively measured from an enlarged black and white photograph containing a scale bar or a digital computer image. A swift, single, smooth action in the direction of growth in order to minimize hair root trauma is used to epilate single hairs. Consequently the plucked hairs are mounted on double-sided tape and organized by length. Microscopic analysis facilitates differentiation between the hair growth phases and measurement of hair length. Each hair is measured in its largest (major axis) and smallest (minor axis) to determine the hair shaft diameter. The diameter of each hair is assessed at the average of these two dimensions [143]. Roughly only 10-15% hairs are telogen hairs. In telogen effluvium 30-60% hairs are in telogen whereas in anagen effluvium, 100% telogen hairs.

1.18.4. Trichogram
The trichogram is a semi-invasive (plucking) microscopic method for evaluation of hair root and phase of hair cycle. Scott et al in 1957 first described the morphological examination of hair roots [109], though the word “trichogram” was coined by Pecoraro in 1965 [144], who illustrated further trichometric parameters such as hair shaft diameter, hair growth and telogen rate. The trichogram is based on the hair cycle and quantifies hair follicles in their different growth phases.
The trichogram is used to diagnose and differentiate between the different types of hair, hair shedding and alopecia through hair root pattern [143].
Technical Procedure

A rubber-armed forceps is used to pluck 60–80 hairs at two specific scalp locations depending on the hair disorder. In AGA, in diffuse effluvium and in loose anagen hair, the first site is 2 cm behind the frontal line and 2 cm from the midline, and the second site is on the occipital region, 2 cm lateral from the protuberans occipitalis [143]. In alopecia areata the first site is at the border of the alopecia patch and the second on the contralateral, clinically unaffected, side. The instrument is closed tightly over the hairs at about 0.5 cm above the scalp and rotated to ensure a firm grasp.

Hairs are removed with a rapid, forceful pull perpendicular to the scalp along the direction of hair growth. Hair roots are evaluated under a magnifying lens or a low-power microscope to determine the number of hairs in the different phases of the hair cycle and results are given as a percentage of the total number of plucked hairs [143].

1.19 Techniques for assaying hair growth in mice

Hair growth assessment in mice is done via assays which describe hair follicle morphogenesis; hair follicle cell differentiation leading to shaft and sheath formation; hair follicle cycling including anagen, catagen, telogen, and exogen; hair follicle heterogeneity; hair follicle switch from the vellus to the terminal state; and hair shaft pigmentation.

Experimental mice models are popular since they offer quick, pertinent, simple and economical ways of testing new drugs. Although to assess whether the system used has actually measured hair growth and not a nonspecific or irrelevant biochemical/physiological pathway is a challenge in itself.

The use of pure molecules or cells for measuring hair cycle is limited by the simple fact that the unique cellular and molecular pathways controlling this cycle are hereto unknown or ill-defined. For proper experimental interpretation, it is essential that parameters like donor animal type, age, site of follicle origin etc are well established.

Cell cultures including Dermal Papilla Cells (DPC) are also being used to imitate the hair growth cycle in vitro. However, in vitro growth pattern [145] and expression of certain genes
differs from those occurring in vivo [146, 147]. Also in vitro cultures have restricted usage due to the difficulties of preparation, variability and viability [148].

1.19.1 Whole Animal Systems

Whole animal systems include are the most relevant but also the most difficult to control, quantify, and analyze. Animals commonly used include mice [76, 101], rats [118], sheep [149], and monkeys [150], although research has also been conducted on other mammals including the cat [151], horse [152], rabbit [153], opossum [154], guinea pig [95], prairie vole [155], and hamster [156]. It may be noted here that no evident proof is there to point that these basic controls of hair follicle cycling are different among different mammalian species. Although the most relevant model in assessment of hair growth disorders in humans is still the human, macaques are considered to be the next best. The use of macaques is difficult due to the rarity of availability, expense, ethical considerations and general housing and handling issues. The macaque male and female have both displayed the patterned baldness syndrome.

Pigmented C57BL/6 and C3H mice are the most commonly used laboratory mouse for hair studies [74, 102, 157]. These mice are chosen especially because their truncal epidermal melanocytes and truncal pigmentation is completely dependent on their follicular melanocytes. This coupled with the fact that melanogenesis is active only during the active follicular growth makes it especially attractive to researchers, since the truncal skin grows from pink in telogen to grey in catagen and finally black in anagen phase. Thus the simplicity of assessing hair growth in these mice is done on the basis of melanogenesis. Also the anagen phase in these mice can be synchronized thereby allowing for isolating and consequent analysis of follicles in specific phases of hair growth post depilation by plucking [103, 118]. Hair growth can be synchronized by use of depilatory creams, use of chemotherapeutic agents, and shaving etc. Regrowth can be therefore, induced and checked by various test drugs in comparison to standard drugs like minoxidil. Lastly, the extensive database of genes
and the availability of specific mutants lead to increased possibility of successful transgenic manipulation to generate desired hair mutations in these mice models [162].

Recently, successful gene delivery to hair follicles of mice has been effected [159-162].

1.19.2 Ex Vivo Systems

These are a combination of both in-vitro and in vivo methods. Recently, researchers have used follicular cells and tissues in situ and transplanted these on the skin of immune compromised animals [156, 163, 164] or beneath the kidney capsule of a syngeneic living animal [165]. According to the highly sensitive Lichti system [163, 164], follicular epithelium of the newborn and mixed or cloned [145] follicular papilla cells are initially grown in culture and then transplanted to immunodeficient mice. Lichti system can be used to investigate folliculo-neogenesis and skin organ regeneration as also follicular cell lineages [166] and the effect of specific cellular and genetic manipulations on follicle growth [167].

1.20 Topical drug delivery system as an effective means for combating alopecia

The skin is an example of an extraordinary evolutionary achievement in the mammal. Skin not only physically envelopes the living being and provides a multifaceted interface between the being and its micro environment, but also, is continually occupied in the working of an extremely efficient homeostatic barrier to the outward loss of water. Consequently, it supplies an equally efficient membrane that further limits two way molecular transport from and to the body. The purpose of topical drug delivery is essentially to overcome and subjugate this barrier and to mould it according to requirements.

Topical administration of drugs via the skin is of two general categories, those applied for local action and those for systemic effects. Local action occurs on or at the surface of skin, and generally these drugs exert their action on the stratum corneum, and modulate the function of the epidermis and/or the dermis. Common local acting products include creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, and solutions. Creams, ointments, and gels generally are referred to as semisolid dosage forms. Transdermal drug delivery systems (self adhesive) or transdermal patches are the most common drug products applied to the skin for systemic effects.
The U.S.P. definition of gel is “a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid”. The inorganic constituents develop into a three-dimensional “house of cards” assembly. A gel is made up of a two-phase system in which inorganic constituents are dispersed throughout the continuous phase whereas the organic constituents are dissolved in the continuous phase, randomly coiled in the flexible chains.

1.20.1 Advantages of Topical Drug Delivery Systems [168-172]

1. Efficacy is achieved with lower total daily dosage of drug by continuous drug input.
2. Fluctuation in drug levels and hence inter- and intra-patient variations are prevented.
3. Area available for application is relatively large in comparison with buccal or nasal cavity.
4. The delivery system is localized and selective on target site thereby reducing unnecessary exposure to other parts.
5. It delivers constant plasma levels for drugs thereby improving physiological and pharmacological response.
6. The delivery system offers ease of access and is suitable for self-medication.
7. Since constant plasma levels of drugs is maintained, drugs with a narrow therapeutic window and those with short biological half life, have the advantage of minimizing the risk of toxic side effects or lack of efficacy.
8. They avoid variables that affect the gastrointestinal absorption of the medication, such as pH, enzymatic activity and drug-food interactions.
9. First-pass metabolism is bypassed; hence drug inactivation by digestive and liver enzymes is avoided.
10. Multiday therapy is possible with a single application.
11. Dosage form can be easily removed in the event of toxicity
12. It is a good alternative route for compromised patients e.g., nauseated and unconscious patients.
1.20.2 The primary disadvantages of topical drug delivery systems include the following:

1. Skin irritation or contact dermatitis is a strong possibility due to the drug and/or excipients.
2. Dose dumping may occur due to the large reservoir available.
3. Poor permeability of certain drugs through the skin.
4. Possibility of allergic reactions.
5. Can be used only for drugs which require very small plasma concentration for action.
6. Denaturation of drug may be a possibility from epidermal enzymes.
7. Large particle sized drugs are not easily absorbed through the skin.
8. Therapy is suitable for chronic conditions like hypertension, angina, smoking cessation, etc.
9. Variations in lag time can lead to different plasma concentration.
11. Development and manufacture of transdermal patches may be expensive and complex as compared to other delivery systems.

Gels were selected as the preferred formulation for the novel indigenous drugs since they have the added advantage of patient compliance. Gels allow the drug to remain in contact with the skin for longer duration leading to better penetration and hence more effective hair growth. Also hair gels are amenable to additives like perfumes and colours which further their appeal as styling aids.