Hair Loss and the Applied Techniques for Identification of Novel Hair Growth Promoters for Hair Re-Growth

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ABSTRACT

Hairs are the characteristic covering of mammalian skin, originated from embryonic ectoderm. In humans, the main function of the hair is an important facet of appearance. Baldness or commonly called Alopecia is defined as the loss of hair from an area of the body. Hair loss affects millions of people, including over 40% of men over the age of 30 and a significant number of women also. The factors responsible for hair loss are scarring, disease, infection, less blood circulation in the blood capillaries of scalp and sensitivity to the androgen. Although alopecia requires a careful history, close attention to the appearance of the hair loss, and a few simple studies can quickly narrow the potential diagnosis. The alopecia which is caused due to androgen sensitivity is called androgenetic alopecia. Androgenetic alopecia is one of the most common forms of hair loss, usually has a specific pattern of temporal-frontal loss in men and central thinning in women. The importance of hair either hair loss as well as the overgrowth of terminal hair on the body or face, has deleterious effects on self-esteem. Therefore the main focus of this review article is on causes of hair loss and the active ingredients for hair re-growth along with the assessment techniques.

Key words: Alopecia, Hair growth, Natural ingredients, Hair follicle.

INTRODUCTION

Hair is a filamentous biomaterial, which grows approximately 0.3 mm/day or 6 inches per year, while the scalp sheds 100 hairs per day.[1] Hair often refers to two distinct structures: The part beneath the skin, is called the hair bulb. The second part is called hair shaft which is the hard filamentous part that extends above the skin surface.[2] A cross section of the hair shaft may be divided roughly into three zones known as the cuticle which consists of several layers of flat, thin cells laid out overlapping one another as roof shingles. The second innermost layer is called the cortex which consist of the keratin bundles in cell structures that remain roughly rod-like. The innermost layer is called the medulla, a disorganized and open area at the fiber’s centre.[3] There are different types of the hairs produced by the body. The first and foremost hair which is produced in uterus called lanugo hair. Such type of hair is fine, soft, poorly pigmented and has no central medulla.[4] The second one is called Vellus hair, which is non-medullated, fine, and poorly pigmented. The intermediate hair is first observed post-natally as the scalp hair growth subsequent to the initial lanugo hair growth. Intermediate hair is characterized by a relatively rough cuticle, sparse pigmentation and a fragmented or absent medulla.[5] Eyelashes have the largest diameter of all body hair. The specific characteristics of different type of hairs, their growth rate, as well as the estimated numbers of hair follicles, based on the body location are given below in Table-1, 2 and 3 respectively.

HAIR GROWTH AND CYCLE REGULATION

Traditionally, three phases of the growth cycle are recognized: a growth phase (anagen phase I–VI), a regression phase (catagen), and a resting phase (telogen).[6] Two more new phases have been recently identified for hair growth cycle called exogen[7] and kenogen respectively.[8] Under physiological conditions, 85% of the scalp hair is in anagen and approximately 15% is in the telogen phase. The anagen phase of scalp hair follicles typically persists for 2-6 years. The anagen phase of hair follicles of the eyebrows in contrast to scalp hair follicles, are only 70 days, while eyelashes grow for 100-150 days. The duration of body hair follicles are briefly mentioned in table 4.
ACTIVE INGREDIENTS FOR HAIR GROWTH PROMOTERS

Among pharmaceutical agents which dilate the capillaries, directly act on the nervous system, such as Swertia extract (Swertinogen), vitamin E and its derivatives, benzoyl nicotinate, capronium chloride and on the other side those compounds which invigorate the circulation by local stimulation such as tincture of chilly (capsicum annumlinne) and tincture of cantharis (Spanish fly) are given below in table-5.

IMPORTANT NATURAL INGREDIENTS THAT POSSIBLY PROMOTE HAIR GROWTH

There are many hair growth stimulants like vitamins (E, C, A, H and B3), antioxidants, amino acids, proteins, fatty acids and polyphenols. An extract of Asiasari radix showed the potential for hair growth stimulation with increased protein uptake in a mouse study, and an in vitro study of human follicles revealed the expression of vascular endothelial growth factor (VEGF) in human dermal papillae. Proanthocyanidins is a grape seed extract has been shown to promote hair follicle cells and convert the telogen follicle to an anagen follicle In-Vitro. Extract from Ginkgo biloba leaf by an In-Vitro study promoted hair growth through effects on proliferation and inhibition of apoptosis of follicular cells. Aloe vera L or Aloe barbadensis gel has been used traditionally for the treatment of alopecia, exhibiting improvement. Aloenin is the major ingredient.

DERMAL COMPONENTS OF HAIR FOLLICLE

The dermal portion of the hair follicle can be divided into two compartments, the dermal papilla (DP) and dermal sheath (DS). The DP is located at the base of the hair follicle. The potent inductive ability along with secretory power of the dermal papilla determine the size of the anagen bulb, subsequently the diameter of the hair shaft produced by the hair bulb and the rate of hair growth, respectively.

CAUSES OF HAIR LOSS

There are many factors associated with hair loss (Alopecia), but overall causes for hair loss are attributed to male hormones (Androgens), genetic factors and age. The causing factors for hair loss are listed below.

- Reduction in hair follicle function due to male hormones
- Reduction in metabolic functions of hair follicles and hair bulbs
- Reduction in scalp physiological functions
- Local impairment of the circulation due to tension in the scalp

HAIR GROWTH PROMOTERS

Hair growth promoters are preparations made by adding various pharmaceutical agents or natural ingredients to an alcohol water solution which is applied to the scalp to normalize its function. By increasing the blood circulation in the scalp or inhibiting the activity of 5-α-reductase enzyme activity; the promoters improve hair follicle function which promotes hair growth and prevents hair loss.
**Table 5: Natural and Pharmaceutical agents used as hair growth promoters**

<table>
<thead>
<tr>
<th>Action</th>
<th>Pharmaceutical agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulation improvement (Vasodilator)</td>
<td>Swertia extract, Vitamin E and its derivatives, Benzoyl nicotinate, γ-oriزانol, Cepharanthine &amp; Minoxidil</td>
</tr>
<tr>
<td>5α reductase II inhibitors</td>
<td>Finasteride, Dutasteride or G198745, Lamin or preziade copper, folign or copper chloride, MK-386 or β-dimethyl-4 aza-5-cholestan-3-one, turosteride, essential fatty acid like γ-linolenic acid, palmitoleic acid, oleic acid, linoleic acid, evening primrose oil, emu oil etc.</td>
</tr>
<tr>
<td>Dihydrotestosterone receptor blocker</td>
<td>Flutamide, Spironolactone, Cyproterone acetate, Citimidine and β sitosterol.</td>
</tr>
<tr>
<td>Local stimulation</td>
<td>Tincture of chily (capsaicin) and cantharis, camphor, vanilyl amide nonylate, nicotinic acid benzyl ester etc.</td>
</tr>
<tr>
<td>Hair root activating agents (Nourishing agent) Amino Acids &amp; vitamins</td>
<td>Placenta extract, pantothenic acid and its derivatives, allantoin and quaternium-73 Cystine, cysteine, methionine, serine, leucine and tryptophan</td>
</tr>
<tr>
<td>Anti seborrhea</td>
<td>Vitamins A, B1, B2, B6, E and its derivatives, Pantotheinic acid and its derivatives and Biotin (Vitamin H) Sulpir, thioroxine and Vitamin B6</td>
</tr>
</tbody>
</table>

**NATURAL PRODUCTS FOR HAIR CARE AND TREATMENT**

Cysteine is a major amino acid; enhanced hair growth in a mouse screening study was used to evaluate the hair-growth-promoting effects of plant extracts. The extracts were painted on the backs of mice for 30-45 days and protein synthesis was measured using the cysteine assay, using cultured murine vibrissae follicles. Bergamot and boxthorn applied topically increased the cutaneous activity of superoxide dismutase, collagen, and decreased malondialdehyde with an observable increase hair growth. The Chinese herb extract “Dabao” was applied topically and resulted in modest hair growth as compared to the placebo (42% compared to 37%). The main ingredient of Ginseng is gensenoside-Rb (1) or G-Rb (1) shows hair growth activity while other extracts are ineffective. The leaves and flowers of *Hibiscus rosa-sinensis* for its potential to stimulate hair growth. Topical preparations were applied to the backs of albino rats and to cell cultures of hair follicles from albino rat neonates. From the study it was evident that, compared to the flower, the leaf extract was more potent as hair growth promoter. The extracts of *Hydrangea macrophylla* extract promotes hair growth through the suppression of TGF-beta, which delays the catagen cycle. The mechanism may be accounted by the fact that TGF-beta is activated by caspase in the lower portion of the follicle and the outer layer of the outer root sheath. This mouse study suggests that TGF beta suppression could be used to treat alopecia. *Illicium anisatum* has been shown to increase blood flow in a mouse model. In an in vitro study of mouse vibrissae follicles, a water-soluble extract of *Illicium anisatum* leaves, fruits, and roots (shikimic acid and glycosides, and polysaccharides) produced better growth than controls. Similar acetone extracts inhibited the growth of hair follicles. Shikimic acid induced insulin growth factor-1, keratinocyte growth factor, and VEGF in the hair follicle. The results of this study suggest that *Illicium anisatum* water extract could be a useful additive to hair growth products. In a mouse study, the topical application of an extract of *Sophora flavescens* dried root induced increases in growth factors such as insulin-like growth factor-1 (IGF-1) and keratinocyte growth factor (KGF) in dermal papillae cells and inhibited type II, 5α-reductase activity. This result suggests that sorphora has potential as a natural hair growth promoter. A number of natural fatty acids include gamma-linolenic, linoleic, palmitic, elaidic, oleic, and stearic acids primarily having the 5-alpha-reductase inhibition activity. In a mouse study, an acetone extract of *Boehmeria nipononivea* exhibited 5-alpha-reductase inhibition and a hair growth effect. In a double-blind clinical trial, six out of ten patients with androgenetic alopecia have been shown improvement when treated with Saw palmetto, a liposterolic extract of *Serenoa repens*, and beta-sitosterol.

**METHODS FOR EVALUATING HAIR GROWTH PROMOTERS**

Methods for the evaluation of Hair growth promoters can be broadly classified in to three categories.

- **Tissue culture**
- **In-Vivo study**
- **On human volunteers**

**Tissue culture based screening**

Jahoda and Oliver were the first two scientists who initiate to grow the dermal papillary cells for the evaluation of hair growth promoters. The quantification of the cells proliferation done by MTT tetrazolium salt assay. The most common primary and established cell lines used for the hair growth studies are dermal papillary and HaCat cell line.


**In-Vivo study**

Various species of animals like mice, rats, sheep and monkeys are widely used for the hair studies. The mice model is most widely reported for hair growth promotion studies and the most common parameters are qualitative hair growth study (Hair growth initiation and completion time) as well as quantitative hair growth study by measuring hair length.

**On human volunteers**

The final evaluation of hair growth promoters need to be completed on human volunteers. During testing on humans; there are many problems which have to solved, for example Individual differences, variation between different locations, the difficulty of controlling the people undergoing the tests and the poor understanding of daily and seasonal fluctuations. However in human volunteers study, the quantitative data can be obtained more easily but the costs are high and subject management requires a lot of efforts. The list of hair growth evaluation techniques are given below in Table-6.

**CONCLUSION**

In summary a number of reasons for hair loss and wide range of natural and synthetic chemical entities are available in the markets, so called hair growth promoting oils or tonic for hair re-growth. An ideal molecule for hair growth has yet to be developed. The most commonly used hair growth promoters like Minoxidil is one of them showing number of side effects include burning or irritation of the eye; itching; redness or irritation at the treated area; unwanted hair growth elsewhere on the body, and headache along with reversible hair loss, upon discontinuation of medication and its effectiveness has largely been demonstrated in younger men (18 to 41 years of age); while Finasteride or Propecia show a no. of different type of side effects include impotence, abnormal ejaculation, decreased ejaculatory volume, abnormal sexual function, gynecomastia, erectile dysfunction, ejaculation disorder and testicular pain. Therefore now the hair biologist are trying to come up with some new novel molecules which should be free from these side effects and can be widely used in all types of hair loss or alopecia including AGA.

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**Table 6: Methods for evaluating hair growth promoters**

| 1) Evaluation using cells or tissue culture | Culturing of Dermal papillary cells and measurement of the cell proliferation by MTT assay.  
Culturing of HaCat cell line and measurement of the cell proliferation by MTT assay.  
Culturing of Normal human epidermal keratinocytes and measurement of the cell proliferation by MTT assay.  
Immunofluorescence assay for Ki-67 as a (Proliferation marker).  
*In-Vitro* Hair culture. |
|---|---|
| 2) Evaluation using Animals | **Mice**  
Measure hair length  
Measure hair initiation and completion time |
| | **Rabbits**  
Measure hair length and weight  
Record of start growth  
Vascular Permeability test |
| | **Hamsters**  
Measure size of sebaceous gland (*In-Vivo*)  
Measure inhibition of 5α reductase (*In-Vitro*)  
Measure inhibition of DHT receptor (*In-Vitro*) |
| | **Monkeys**  
Measure hair growth at front of head in red faced monkey.  
Photographic evaluation method, observe hair growth situation.  
Measure inhibition of 5α reductase (hair root). |
| 3) Evaluation using humans | **Hair wash test**  
**Measure blood flow**  
**Trichogram method**  
**Unit area trichogram**  
**Phototrichogram (PTG)**  
**Hair pull test**  
**Hair Weighing** |
REFERENCES