

HAIR LOSS AND REGENERATION PERFORMED ON ANIMAL MODELS

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Abstract

Research in the field of reversal hair loss remains a challenging subject. As Minoxidil 2% or 5% and Finasteride are so far the only FDA approved topical treatments for inducing hair regrowth, research is necessary in order to improve therapeutical approach in alopecia. In vitro studies have focused on cultures of a cell type - dermal papilla or organ culture of isolated cell follicles. In vivo research on this topic was performed on mice, rats, hamsters, rabbits, sheep and monkeys, taking into consideration the advantages and disadvantages of each animal model and the depilation options. Further studies are required not only to compare the efficiency of different therapies but more importantly to establish their long term safety.

Keywords: hair regrowth, animal models, research in vivo, alopecia

The physical appearance of both males and females is under society pressure, due to the interest in the general aspect of the individual regarding body shape, hair, make-up and clothing [1-5].

Hair brings an important contribution to the outlook of human body [6,7] playing an interesting part in social and sexual communication, as many hair loss studies show [8].

This idea is also supported by the fact in some cultures young women are asked to cover their hair, which indirectly suggests its role in the overall beauty and attraction.

Functions of hair

Hair represents a defining characteristic of mammals, with an important thermoregulatory function. Also it has camouflage purposes, hair follicles being able to modify their type and density during seasonal coat changes [9]. Besides offering protection, hair also has a sensory function, extending the sense of touch that occurs at skin level [10]. In animals, the hair/fur is often used

for signaling or communication and sometimes provides defensive functions [11].

Hair can also modify its color over the time, both in animals and humans: from vellus hairs in babies (uncolored, soft hair) into final hair in adult males (colored beard, thick and strong hair) [12]. Along the aging process it loses its pigment and becomes white.

Although human hair has lost its main thermoregulatory function, hair growing on the head has an important role in heat insulation and cooling by evaporating sweat from soaked hair [13,14]. It also protects the scalp against ultra-violet radiation by acting like a sunscreen [15,16].

Structure of hair

Hair is deriving from the ectoderm of the skin and represents an accessory structure of the integument along with the sebaceous glands, sweat glands and nails [8,12]. The word "hair" usually refers to two distinct structures: the hair follicle (beneath the skin) and the shaft of the hair (hard filamentous part that extends above the skin surface). In a cross section evaluation the shaft consists of

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three layers, starting from the outside: the cuticle (having several layers of flat, thin cells, overlapping one another), the cortex (containing the keratin bundles in rod-like cell structures) and the medulla (a disorganized and open area at the fiber's center) [17,18].

On the other hand, in the dermis, we find the bulb, which maintains stem cells that not only re-grow the hair after it falls out, but are also recruited to repair skin after a wound [9].

The hair follicle pigmentary unit provides the hair shaft melanin components, due to precise interactions between follicular melanocytes, keratinocytes and specialized dermal papilla fibroblasts (also involved in wound healing) [19,20]. The dermal papilla has an important role in hair formation, growth and cycling [21]. The blood vessels from the dermal papillae nourish all hair follicles and offer nutrients and oxygen to epidermal cells in the lower layers.

In the human skin, the dermal papillae are small extensions of the dermis into the epidermis and at the surface of the hands and feet, they appear as epidermal or papillary ridges, also called fingerprints [22].

Biology of the hair loss and hair regrowth

Human hair is different from hair grown by mammals, due to the unsynchronized growth cycles. Even if there is a certain seasonal co-ordination, each human hair follicle works independently [23,24,25]. This mosaic human pattern consists of hair in different stages: 90% anagen (growth phase), 1-2% catagen (regression phase) and 8-9% telogen (resting phase). [26,27]. The cyclic changes from anagen to telogen via catagen involve rapid remodelling of both the epithelial and dermal components of hair follicles [28,29]. In animals, as in humans, the hair cycle is influenced by stimulatory and inhibitory factors, such as hormones, growth factors, cytokines, neuropeptides and pharmaceutical products [26,30,31].

The dermal papilla, as the main mesenchymal component, induces the new hair follicles formation and maintains hair growth [32]. In telogen phase the old hair is lost, but the follicle will be regenerated in early anagen, when a new hair grows up. In order for this to happen, the dermal papilla cells support an increased cell division and growth rate that also require a good supply of nutrients and a toxin-free environment for the growing cells. If these requirements are not fulfilled the follicles will remain in the telogen phase [33].

Two factors usually determine terminal hair miniaturization leading to hair loss. The first is **the shortening of the anagen**, within an abnormal hair cycle and the anagen: telogen rate shifting from 6:1 to 2:1. The consequence of this process would be a shortening of hairs, shaft loss and an increased number of hairs in telogen phase. The second factor with a negative impact on hair growth is **the small size dermal papilla or the hair matrix** leading to hair modification both in diameter and aspect. Final hair

(thick and pigmented) turns into vellus (thin and white). Scientists have also discovered that in hair loss, the scalp suffers of vasoconstriction and hypoxia [34,35,36].

The sensitive response to androgen is the second feature characterizing human hair in contrast to the hair grown by mammals. An excess of androgens generates hair miniaturization and thinning, followed by hair fall and loss of pigment, mostly in the vertex and the crown-frontal area of the scalp [37,38].

There are some characteristics of the hair depending on localization in a specific site of the body: beard, axially and pubic hair react differently than hair from the scalp, as they are androgen-sensitive [12]. The metabolism of the testosterone into 5-alpha-dihydrotestosterone has also been of great research interest. Results show that good metabolism limits the length of hair growth (beard) and the 5 alpha-reductase deficiency produces strong thick hair (axilla and pubic area) [27,39,40,41,42].

On the other hand, androgens act on the hair via the dermal papilla and stimulate the production of terminal hairs after puberty, but have no hair growth effect on eyelashes and the occipital area [43,44]. Androgens seem to alter the production of regulatory factors (soluble paracrine factors and extracellular matrix components) by the dermal papilla cells [21].

Consequences of hair loss

Although a natural part of the aging process, hair loss represents a great concern for the patient, who suffers from anxiety and distress more severely than expected [45]. Several studies already showed that this common dermatological condition generates adverse psychosocial sequelae [46]. Cash et al found that the psychological effects of hair loss on women are far more severe than in male subjects. Surveys have shown that around 40% of women with alopecia have had marital problems and around 63% claimed to have career related problems [47].

Hair loss is a stressful experience for both sexes, but substantially more distressing for women, as they do not accept the disease, neither do they cope with it as easily as men [48]. Stress functions as a cause and risk factor in the development of the disease, but it is also a consequence of hair loss. Alopecia determines a poor quality of life by the physical, psychological and social consequences it produces. It causes low self-esteem, depression and distorts social perception and psychosocial functioning [49,50].

A large variety of over the counter products claim to treat hair loss pathology. This multibillion dollar, worldwide market of hair tonics, hair balms, hair masks, shampoos, leave in conditioners, topical solutions or foams function as potential anti-hair loss agents [51]. However, in most of the cases, clinical studies do not prove how these doubtful hair growth-promoters exert their expected effects of ceasing hair loss and enhancing hair regrowth [51].

As a consequence, clinically speaking, there is an increased number of patients with "great expectations"

who, encounter plenty of disappointments after the treatment [52,53].

Evaluation of hair loss and hair regrowth

Unfortunately, researchers do not have a standardized method to assess hair regrowth *in vivo*. Possible tools include scales of measuring hair regrowth based on percentage evaluations that can be performed with the naked eye (visualization and photographs of the area of interest) or by trichoscopy [49]. These qualitative assessments are limited in number, therefore, new, accurate and minimally invasive procedures are still needed.

To explore the efficiency of a topic substance in inducing hair growth, *in vivo*, researchers usually analyze the hair growing pattern in animal groups treated with different substances, compared to control by using the macroscopic aspect. The skin of the animals, usually the dorsal part, is observed and photographed either every 3 days or at specific time intervals (day 1, 7, 14 and 21 after depilation) in order to notice the start of hair regrowth period and the hair regrowth pattern. There are several hair regrowth potential scores, but one easy to use was described by Matsuda et al: 0 = no hair growth, 1 = less than 20% of hair growth, 2=20-39% of hair growth, 3=40-59% of hair regrowth, 4=60-79% of hair regrowth, 5=80-100% of hair regrowth [54]. Researchers also use self-designed scales of hair regrowth, such as: Type 4 (high hair density, full, thick fur), Type 3 (moderate hair density with no visible skin area), Type 2 (low hair density, with the visualization of the skin), Type 1 (uneven hair growth on the test area, skin easily seen) [55].

Trichoscopy is performed with a hand held device called dermatoscope, with polarized light to magnify and allow inspection of the skin. A decrease of hair diameter up to ten times or diameter variations can be detected by trichoscopy, so a correct hair regrowth evaluation can be performed.

As far as quantitative methods are concerned, an area of 1 cm² of skin with regrown hair is cut and weighed with an analytical balance for hair weight determination [56].

To further investigate the hair growth promoting effect of a certain substance, the histopathological examination offers a precise and necessary evaluation tool for hair regrowth. Usually, at the end of the treatment period, the rats are sacrificed and a skin biopsy is isolated in order to examine the histological features. The skin thickness and hair follicles localization in the dermis/subcutis can be evaluated by microscopic photograph (magnification x400). Taking into consideration the existent knowledge about hair cyclicity, the anagen induction is calculated with the formula: (number of follicles in subcutis) x 100 / (number of follicles in dermis). Previous microscopical studies on animals showed an association of increasing skin thickness, follicle count and macroscopic development of skin pigmentation with anagen induction [26,31].

The hair growth cyclicity (anagen, catagen, and telogen phases) can be used as a diagnosis tool for the hair growth condition and also as a treatment assessment method for the hair growth promoting agent.

Hair regrowth treatment

The insufficient insight into the basic mechanism leading to alopecia together with therapies that failed to cure it, determined scientists to developed large research programs to overcome hair loss [57]. Despite the many treatment alternatives that have been tested, hair loss continues to remain a frequent dermatological condition.

Up to the present, pharmaceutical hair loss management includes only two FDA approved hair loss drugs: Finasteride and Minoxidil, both commonly used in clinical practice [46,51,58].

The hair growing effect of **Minoxidil** has been accidentally discovered, as this antihypertensive oral drug, caused side effects such as increasing hair growth on the scalp or even darkening the fine body hairs. The 2% topical formulation was approved by the FDA in the 1990s for use in treating androgenetic alopecia in men (for central/vertex hair loss only) and in females as well (in female pattern hair loss) [59]. The 5% formulation is allowed only for males and the foam version is associated with 70% self-reported improvement.

Minoxidil slows or stops hair loss and promotes hair growth because it is a vasodilator that increases the cutaneous blood flow to the scalp [60]. It is also a potassium channel opener, causing hyperpolarization of cell membranes, allowing more oxygen, blood and nutrients to reach the follicle [61]. Minoxidil contains an N-oxide group able to release NO, and besides being a vasodilator [62], it also acts as a nitric oxide agonist. However, it has no therapeutic action on the hormonal and genetic causes of hair loss.

Minoxidil usual dosage is 1 mg per day, applied topically, with slight massage on the affected area of the scalp and no contact with water allowed for at least 4 hours after application [63,64]. Minoxidil must be used as a continuous support for the hair follicles, otherwise the hair regrowth will ceases and hair loss will begin again in 30 to 60 days if Minoxidil treatment is stopped for more than 6 months [65]. Several studies have shown that the efficacy of Minoxidil ranges from 20-40%, causing discontinuity of treatment in the majority of patients [66].

Finasteride is considered a dihydrotestosterone-suppressing 5 alfa-reductase inhibitor, recommended for male use only, in the treatment of androgenetic alopecia. It acts by decreasing the serum levels of dihydrotestosterone, stopping hair fall (in 48% of the cases) and stimulating hair regrowth (in 51% of the cases). Studies showed that 1 mg Finasterid oral treatment has a similar efficacy as daily topical Minoxidil application on the scalp, but their efficacy is seen after at least 4 months of daily usage [67,68]. These two treatments can be combined in order to boost the hair

follicles, but both treatments must be performed a-la-longue, if not, the hair regrowth effect will cease. Adverse reactions are rare, still sexual dysfunctions were reported in 4% of the cases [69,70].

Given the temporary efficacy of Finasteride and Minoxidil and the limited number of treatments available in alopecia, it is a challenge to discover new therapies in order to prevent hair loss and enhance hair regrowth [69,70]. Some substances diminish the physiopathological processes that induce hair loss, but clinical studies in this respect are lacking.

Arginin stimulates microcirculation, bringing in essential nutrients for hair bulb growth.

Aminexil 1.5% diminishes the rate of hair loss as it reduces the accelerated aging of the roots by fighting the process of fibrosis. It also maintains the elasticity of the tissue surrounding the hair root and prevents the stiffening of collagen sheets, in order to fasten the hair root within the scalp [52,53].

SP94 peptide is captured by the root and turned into the hair constructive elements for building up the fiber from root to tip.

B5 and B6 Vitamins function as cellular nutrients that nourish the hair roots and help generating beautiful, shiny hair, that becomes thicker and stronger from within [65].

Thermal Spring Water that contains anti-free radical Selenium, enhances the therapeutic action of **Madecassoside**, which inhibits the local micro-irritation, preventing the spreading to the capillary bulb [65].

Low Level Laser Therapy (LLLT) provides a promising treatment option for patients, this photobiomodulation produces a shifting of the follicles from telogen (resting phase) to anagen (active phase), preventing premature catagen development (hair falling phase) [71-73]. LLLT, through its low power coherent monochromatic red light, produces vasodilation and increases ATP production, also determining a modulation of Reactive Oxygen Species (ROS) and inflammatory mediators [74-76].

Hair regrowth studies in vitro

Human hair follicles are not proper research material due to ethical problems, invasive methods and limited amount of follicles that can be extracted for testing [77,78]. Cotsarelis et al., discovered that bald areas have the same number of stem cells as the normal scalp and also noticed that hair follicles decrease in size, but do not disappear. In alopecia, one of the major issues would represent the activation of stem cells converting to progenitor cells in the affected areas of the scalp [79].

Researchers insist in finding new natural and chemical agents, which may convert stem cells into progenitor cells and generate terminal hair [79,80].

In vivo models (experiments performed on natural animals and genetically manipulated models) as well in

vitro research (cultures of a cell type - dermal papilla or organ culture of isolated cell follicles) have been used successfully, to obtain the data that we now possess about the function of the hair follicle in health or under disease [81-86].

Scientists may choose one of the two types of experimental studies taking into consideration the purpose of the research, the advantages and disadvantages it offers.

Hair regrowth studies in vivo

Animal models

For a better understanding of the physiopathological processes involved in hair loss and regeneration, animal models have been used in hair research since 1950 [87].

In vivo research on hair loss and regrowth was performed so far on mice, rats, hamsters, rabbits and sheep in laboratory conditions [88,89,90,91]. Lately, the interest in hair growth promoting agents has grown considerably and in the attempt to discover an ideal therapy for alopecia, new treatments have been studied even in stump-tailed macaque [92]. Still, researchers must take into consideration the differences between species regarding the follicular function and limited androgen-sensitive models [93]. The periodic intervals of rodent hair cycles, particularly the duration of the anagen phase are much more consistent and less susceptible to iatrogenic influences [94].

The normal hair cycling, including growth waves and hormonal control were studied on Wistar Bratislava rats and mice [95]. The black mouse C57BL/6 was used for the skin-free pigment and early visible pigmented tips of new anagen regrowth [91,96]. The C3H mouse model was the most widely reported for hair growth promotion, even though the increased hair density of the animal and the wave pattern hair cycle progression presented disadvantages [95,96,97]. Laser therapy applied in C3H mouse, 20 second daily, 3 times per week, induced a much longer growth phase, after only 2 weeks of treatment, with most of the follicles from the tested area being in anagen hair growth phase [98].

The androgen action upon the hair follicles has been studied on spontaneous and genetically engineered nude mutant mice. Immunodeficient mice (with T and B cells deficiencies) were used as models for autoimmune disease mechanisms and androgenetic alopecia studies [96]. Also, by inhibiting the rejection of foreign skin, human skin grafts were applied and even rat dermal papillas continued to produce hair after reimplantation in vivo on a rat model [99,100,101].

Recently scientists discovered that a certain progenitor cell population in mice is analogous to the human cells. These mature cells were tested by injection on immunodeficient mice animal model and the results showed the development of new hair follicles and increased hair regrowth [98].

The *Mesocricetus auratus* (Golden hamster) was used for macroscopic assessment (hair density

dermatoscopy analysis) and microscopic evaluation (hair diameter analysis) as an animal model for hair regrowth [102].

Since Minoxidil 2% is thought to be the gold standard treatment for hair loss, scientists consider the validation of Minoxidil treatment on an animal model as being very important. Several studies have shown that topical Minoxidil affects the normal hair cycle by shortening telogen, causing premature entry of the resting follicles into anagen phase which will lead to an increased hair follicle size [103,104].

Housing conditions

Usually animals of either sex and weight were used in the studies. They were acclimatized to the experimental room at a temperature of 23 degrees Celsius, in controlled humidity conditions with a 12:12 h light and dark cycle for at least 7-14 days prior to the experiment. Usually, there was an individual housing or maximum 2 animals per cage (to avoid licking) with access to standard laboratory diet and water ad libitum. Following the experiment they were euthanized according to the current regulations. Some experiments were performed in triplicate for accurate results.

Depilation methods

Experimental designs may ask for the whole back or body of the animal to be shaved, or there may be only some specific areas to be denuded for testing.

In Dr. Mester's study, before each successive treatment, the skin was depilated by shaving, procedure that may induce mechanic stimulation of hair growth, as previously reported in the literature. An experiment done on adult rats proved that after the fur was dyed and shaved, concluded that the regrowing hairs on the rat skin formed a system of linear loops, that were closely correlated with the shaving process [74,75].

In order to avoid this effect, some scientists do not shave the tested area of the animal model before each daily therapy. It has been also proved that physical factors influence the hair regrowth process: temperature triggers fast regrowth after shaving.

Depilation-induced hair cycle has a strict course: 9 days after depilation, the hair follicles enter the final stage of the growth cycle (anagen VI). Around day 17 the follicles enter the regression stage (catagen) and around day 20 after depilation, follicles get to the resting stage (telogen) [30].

Besides shaving, there are also other methods for depilation, such as a raisin mixture or a hair removal cream [91, 105].

Conclusion

As a conclusion, we tend to refine our knowledge on human hair diseases and hair regrowth by using proper animal models. Hair research provides further insights into the physiopathological pathways, genetic and cell biochemical mechanisms that could promise the cure of hair loss.

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