TRICOCURE® - HAIR ENERGY BOOST

HAIR GROWTH LOTION



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Company

Mesotech is an innovative company, specialized in providing skin care and beauty solutions from the conception to the manufacturing of products and devices for the aesthetic medical field.

Customer satisfaction and quality are the main priorities for our staff. We develop our range relying on an ongoing dialogue with our customers.

Our Mission

Development of new products from original ideas to a deep research. Original formulations where unique ingredients are selected to restore and correct blemishes and cutaneous.

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TRICOCURE® - HAIR ENERGY BOOST

Since ancient times, a thick and healthy hair, in the collective imagination, is a symbol of beauty, strength and health. Numerous historical testimonies show that the application of oils and ointments for hair care is a procedure that both men and women have been practicing for thousands of years.

The hairloss, up to hundred/day for the thicker hair, should be considered a normal physiological process of hair replacement. Growth, death and regrowth of hair is a cycle that is repeated about 20 times during the existence of an individual. In some people, especially women, the duration of each cycle can reach 6 years. As long as hair loss within the confines of this cycle of regrowth, it should not worry and should be considered completely natural.

Abnormal hair loss can occur in both men and women. It is one of the main skin imperfections and can cause, in those affected, psychological implications that often cause discomfort.

Our product, **TRICOCURE®**, was developed for the purpose of responding to the need of many patients to contrast the main causes responsible for hair loss, without having to resort to invasive surgical procedures.

It is necessary to clarify that there are many causes that can lead to excessive hair loss. Below is a brief analysis of the structure and growth cycle of the hair, as well as the main causes responsible for hair loss. Hair is a unique character found on all mammals but not on other animals. In humans it is a special and cherished feature, especially in females, but its main functions are in protection of the skin from mechanical insults and to facilitate homeothermy1,2; eyebrows and eyelashes, for example, stop things entering the eyes, while scalp hair prevents sunlight, cold, and physical damage to the head and neck.3 It also has a sensory function, increasing the perception of the skin surface for tactile stimuli, and subserves important roles in sexual and social communication

Structure and growth cycle of the hair

Structure

Hair has two separate structures: the **follicle** in the skin and the **hair shaft**, which is visible on the body surface. The **hair shaft** consists of a cortex, cells and, in some cases, a marrow in the central region.

The **hair shaft** consists of a cortex and cuticle cells, and in some cases, a medulla in the central region. The medulla is the central part of the hair, whereas the cortex, which represents the majority of the hair fiber composition and plays an important role in the physical and mechanical properties of hair.

The **follicle** is the essential growth structure of hair. The hair bulb is the portion of the follicle which actively produces the hair. The hair bulb can be divided into two regions: a lower region of undifferentiated cells and an upper region in which the cells became differentiated.



From the matrix, cells move to the upper part of the bulb, where they increase in volume and become elongated vertically.

Hairgrowth cycle

Hair is the cumulative, physical result of a coordinated process of cellular proliferation and differentiation within a hair follicle. The hair follicle cycle is a complex process and entails involvement of cell differentiation, epithelial–mesenchymal interactions, stem cell augmentation, pattern formation, apoptosis (programmed cell death), cell and organ growth



Figura 1 In the adult, three phases of the growth cycle are recognized: a growth phase (anagen), a regressing phase (catagen): and a resting phase (telogen). It is the lower follicle that regenerates at the beginning of each cycle by utilizing intimate and powerful epithelial–mesenchymal interactions of the stem cells in the bulge (B) and the inductive mesenchymal cells of the papilla (P). CTS, connective tissue sheath; E, epidermis; H, hair shaft; IRS, inner root sheath; M, hair matrix; ORS, outer root sheath;

cycles, and pigmentation. By traversing the phases of thecycle (growth, regression, resting, shedding, then growth again), the follicle demonstrates the unusual ability to completely regenerate itself (11). Normal hair follicles cycle between a growth stage (anagen), а degenerative stage (catagen), a resting stage (telogen) and a shedding stage (exogen). The scalp hairs have a relatively long life cycle: the anagen stage ranges from 2 to 5 years, the catagen stage ranges from afew days to a few weeks, and the telogen stage is approximately 3 months (12,13). Hair cycle disturbances have dramatic effects on visible hair growth. If anagen gets prematurely terminated and catagen occurs too early, this must result in effluvium alopecia; and the affected skin region will subsequently sport largely catagen and/or telogen follicles.

Therefore, the therapeutic manipulation of hair follicle cycling is a key challenge in hairloss management.

Effective therapeutic strategies for hair loss in declining order of importance are:

- Inhibition of catagen development in order to prolong anagen.
- Induction of anagen in telogen follicles.
- Inhibition of exogen.

Hair loss and alopecia

Hair loss is a natural daily phenomenon, but this shedding of hair cannot be the main cause of hair loss. Every strand of hair on a human head is genetically programmed to a cycle that includes growth, stabilization, aging and shedding. On average, every day a human head sheds about 50–125 hairs (depending on sex), but most of them will come back after the resting stage as the follicle itself is not destroyed (14). Trouble begins when the loss exceeds re-growth, or the re-growth is weak and unhealthy.

Androgenetic alopecia (AGA) is one of the dermatological conditions most commonly faced by the dermatologist or general physician. The condition affects up to 30% of men under the age of 30 and more than 50% of men over the age of 50. As the condition progresses, scalp hairs and their follicles become progressively miniaturized, and the terminal hair normally found on the adult scalp is replaced by vellus hairs which are shorter, finer and non-pigmented. Concomitantly, the average length of time spent by hairs in anagen (growth phase) decreases, and the proportion of hairs in telogen (resting phase) increases

Baldness or alopecia can be classified as follows: male pattern baldness, female pattern baldness, alopecia areata (an autoimmune disorder causing small, patchy circular bald patches in several parts of the scalp), alopecia totalis (total loss of scalp hair), and alopecia universalis (total loss of hair from the entire body). Another way in which hair loss (alopecia) can be classified is according to factors leading to it. There are two different types of hair loss, known medically as:

- Anagen effluvium: caused by medications taken internally, such as chemotherapy representatives, excessive doses of vitamin A or some hypertension medications.
- *Telogen effluvium*: caused by an increased number of hair follicles entering the latent or rather dead stage. The most common causes of telogen effluvium leading to alopecia could be physical and emotional stress, and thyroid or another hormonal irregularity.

The growth of hairs is affected by various factors, which are listed in Table I (18).

Factors	Description
Major physical emotional stress	Surgery, severe illness, diet or nutrition changes and emotional stress can cause hair loss
Chemotherapy	Cholesterol-lowering drugs, Parkinson medications, anti-ulcer drugs, anticoagulants, agents for gout, anti-arthritic drugs derived from vitamin A, anticonvulsants for epilepsy, antidepressants, beta-blocker drugs, antithyroid agents, antineoplastics, blood thinners, male hormones (anabolic steroids)
Genetic predisposition	Genetic component to androgenetic hair loss exists (polygenic inheritance)
Dihydrotestosterone (DHT)	Increased level of DHT (the testosterone metabolite) shortens the hair cycle and progressively miniaturizes scalp follicles and this may be due to the atherosclerotic process blocking the microvasculature that nourishes the hair follicles
Excessive sebum	Excessive sebum causes a high level of 5-alpha reductase and pore clogging, thus malnutrition of the hair root
Cardiovascular diseases	High levels of LDL in cardiac patients are converted to 5-alpha reductase enzyme, which produces DHT from testosterone, causing hair loss
Smoking	Tobacco smoke damages the lining of blood vessels, leading to less production of nitric oxide and thus inducing hair loss
Endogenous substances	bax, bcl-2 and insulin-like growth factor binding protein-3 (e.g. VEGF) promote hair growth

Table I. Factors leading to hair loss.

Tricocure[®]



TRICOCURE® is the result of the research of Mesotech laboratories. The company mission is always to develop highly effective products, to answer to the demands of careful customers searching more and more performing products, always keeping focussed to the toxicological profile of the raw materials used.

TRICOCURE® is the most revolutionary and complete formula to reduce the main hair loss causes. The formula is enriched with numerous active ingredients: *Vascular regrowth factor, Cutaneous microcirculation enhancer, 5-alpha reductase inhibitors and a pool of nutrients and restructuring ingredients* are the **TRICOCURE®** core.

How To Use

The topical application of 3 ml twice a day with the practical applicator allows to bring nutrients and active substances directly on the scalp, thus managing to easily reach the hair follicle.

Main active ingredients of TRICOCURE®

KOPEXYL: Increases the volume of hair in the growth stage by working on the deep structure of the roots.

CG-VEGF: The growth factor VEGF, thanks to its ability to stimulate angiogenesis and the consequent supply of nutrients at the base of the follicle, actively stimulates hair growth.

SAW PALMETTO EXTRACT: Saw Palmetto works by inhibiting the 5- alpha-reductase, an enzyme responsible for converting testosterone into its active molecular form, dihydrotestosterone (DHT). The latter is responsible for the process leading to the atrophy of hair follicles.

BETA-SITOSTEROL: A Natural extract, which is able to simultaneously engage the 5- alpha-reduc tase and the cytosolic receptors of dihydrotestosterone and androstandiol

CAPIXYL: Blend composed of: Biochanin A extracted from red clover, effective inhibitor of $5-\alpha$ -reductase activity (type I and II). Acetyl tetrapeptide-3 is a biomimetic peptide that stimulates tissue remodeling, increasing the size of the hair follicle.

VITAMINS B-COMPLEX: Vitamins essential for maintaining healthy hair. The beneficial action of this group of vitamins results in a good contrast to the processes that lead hair graying and hair loss.

GINSENG ROOT EXTRACT: increases stimulation and improves circulation

Kopexyl



Figura3MolecularstructureDiaminopyrimidine Oxide.

In both men and women hair loss is connected to the deterioration of the roots. Kopexil increases the volume of hair in the growth stage by working on the deep structure of the roots. It rejuvenates the hair roots so that healthy hair growth can persist. Fibrosis condition of the hair roots causes blood vessels to compress and shorten the life span of the hair follicle.

Years of research carried out in numerous clinical institutes worldwide and in Holland have placed Kopexil at the top of the list in the fight against hair loss.

• Hair loss stops

of

• Hair becomes thicker and fuller once you use Kopexil.

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2,4 DPO acts on an enzyme called lysilhydroxilase that is important in the formation of collagen. 2,4 DPO effectively blocks collagen formation (production). There has been a claim that pattern baldness is caused, at least in part, by the build up of collagen around hair follicles. They suggest that collagen build up literally chokes the hair follicle by shutting off the blood supply and reducing the total size of the follicle.

CG-VEGF



Hair growth stimulation through the facilitation of nutrient feeding to hair follicle by the VEGF-induced angiogenesis

Fig.4 Tertiary structure of VEGF

CG-VEGF & Hair-growth



a) Cell Proliferation on Primary Hair Cell

Cell growth assay with primary hair cell after CG-VEGF treatment for 72hrs.

b) Morphological Change of Primary Hair Cell



Control

V

VEGF 50pg/ml

VEGF 500pg/ml

VEGF 5ng/ml

Cell morphology changed after 72hr incubation with CG-VEGF (50pg ~ 5ng/ml) on primary hair cells.

CG-VEGF & Angiogenesis

a) Cell Proliferation on Human Vein Endothelial Cell (HUVEC)



Cell growth assay with human vein endothelial cell line after CG-VEGF treatment for 72hrs.

b) Morphological Change of Human Vein Endothelial Cell (HUVEC)



Significant Morphological change observed on HUVEC cell line with VEGF treatment.

c) Angiogenesis Effect using Ab-CD31



Immuno-cyto chemistry using Ab-CD31 CD31: Specific marker of endothelial cell migration and angiogenesis.

CG-VEGF was treated in HUVEC and used CD31 Ab in immuno-cyto chemistry to see the revelation of CD 31 Ab. It shows CG-VEGF is a strong angiogenic factor.



d) VEGF can Induce Angiogenesis and Enhance Hair Follicle Activity



VEGF transgenic mice showed more and thicker hair compared with wild-type (WT) mice.

VEGF overexpressing hair follicles at days 12 and 15, accelerated hair growth and increased hair size were a consequence of VEGF mediated angiogenesis.

Scale bars = 100um

Ref. Klichiro Y. et al., 2001 J. Clin. Invest. 107: 409-417

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Saw Palmetto (Serenoa Repens)

Saw palmetto (Serenoa repens) is a small, low-growing, dwarf-palm tree, native to Southeastern North America, particularly Florida. The American Indians of this region used the berries medicinally and as a food source, long before the arrival of the Europeans. Preparations of saw palmetto are made of the ripe, partially dried olive size berries.

Usually, the extract contains 70–95% free fatty acids like capric, caproic, caprylic, lauric, myristic, oleic, linoleic, linolenic, stearic and palmitic acids with their ethyl esters and glycerides that occur most likely during the extraction process. Further phytosterols like β -sitosterol and β -sitosterol 3-O-D-glucoside, campesterol, and stigmasterol are found with about 0.1% of the total mass of the berries. Other constituents are carbohydrates such as mannitol and polysaccharides with galactose and arabinose, triterpenes, aromatic acids like ferulic and vanillic acid, β -carotens, the vitamine E derivates γ -tocopherol and δ -tocopherols, mono-acyl glycerides like 1-monolaurin and 1-monomyristin, and the monoamine tyramine. A combination of several factors has been implicated as potential mode of action for saw palmetto. The main properties to consider are its anti-androgenic, pro-apoptotic and anti-inflammatory effects.

S. repens extract contains β -sitosterol. The latter can reduce steroid hormone synthesis, especially testosterone. A recent study has also demonstrated that its extract can inhibit both types of 5- α reductase. S. repens, when used topically, could increase hair number and hair weight in patients with AGA, as shown by hair clipping method.

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A study conducted by Wessagowit et al. showed that S. repens was shown to significantly increase the numbers of total hair and especially that of terminal hair. This translated into clinical benefits, as confirmed by investigators' evaluation showing the change in Norwood– Hamilton AGA grading and an increase hair growth in the photographic assessment. Volunteers also reported limited hair growth and increased satisfaction with the appearance of their hair, with no serious side-effects.

Capixil™

Capixyl[™] is composed of a high tech patented four amino acids biomimetic peptide with a red clover extract rich in Biochanin A.

- **Red Clover Extract** Red clover (Trifolium Pretense) flowers have been found throughout central and northern Europe and Asia. Red clover was traditionally used to treat asthma, cancer, gout, and various inflammatory skin disorders like eczema and psoriasis.
- Biochanin A is the major isoflavone in red clover. Biochanin A is known to be an effective inhibitor of 5α-reductase type I & II activity18. Researches have demonstrated that Biochanin A modulates chronic inflammation and that red clover's isoflavones may also function as important antioxidants, limiting free radical damage to the skin and scalp.

- Acetyl Tetrapeptide-3 Biomimetic Peptide

This unique patented peptide is a stimulator of extracellular matrix proteins favoring a better hair anchoring. It is derived from a signal peptide which has potent tissue protective

properties and stimulates tissue remodeling after the initial phase of wound healing. The peptide has a direct effect on hair follicle. The remodeling signal will increase the size of hair follicle for better hair number and vitality.

Capixyl[™] efficacy is based on the combined action of its two ingredients, allowing direction action on DHT preventing the hair cycle to shorten and hair follicle miniaturization, improving the ECM proteins in dermal papilla surrounding hair papilla for better anchoring and promoting an increase of follicle size. Capixyl[™] also inflammation which is a contributing factor in hair loss.



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CAPIXYL: CLINICAL STUDY ON HAIR LOSS

OBJECTIVES

The goal of this clinical study was to evaluate the efficacy of Capixyl[™] in vivo as a hair care active ingredient to prevent hair loss and to promote hair growth under normal use conditions. Furthermore, acceptability of the product upon normal use was also evaluated.

Efficacy was objectively evaluated by using instrumental measurements (digital trichogram with TrichoScan[™]). Measurements from a group of 15 Capixyl[™]-treated volunteers were compared with those of 15 Placebo-treated ones. Every one of the volunteers evaluated acceptability of the product on a daily basis.

The assay was carried out on volunteers suffering AGA, who had been chosen using the following criteria: 200 hairs or less on digital trichogram hair count, and/or less than 70% hairs in the anagen phase.

PRINCIPLE OF TRICHOSCAN

TrichoScan is suitable for the analysis of human scalp hair in AGA. TrichoScan is a non-invasive method, combining standard epiluminescence microscopy with automatic digital image analysis, for the measurement of human hair. The most important advantages are:

- Total hair counts can be analyzed within the same day

- The same target site can be used to calculate the number of telogen and anagen hairs.

Determination of total hair density

- 1. A shaving mask is positioned on the volunteer head in order to shave a 1.8 cm2 area on the zone or zones to be studied.
- 2. Three days later, as hairs do not normally contrast well enough with the scalp (due to gray or fair hairs) skin for digital photography, hairs must be dyed and subsequently cleaned with alcohol.
- 3. Images were recorded with the equipment camera in order to evaluate the anagen and telogen phases.

Patients were asked not to wash their hair for two days prior to the evaluation with TrichoScan.

Recording the images

After acquisition, the digital images are transmitted to specific software for the analysis of the total hair density (anagen + telogen).

- 1. Original image
- 2. Detection of hair by the software
- 3. Specific marked hair
- 4. Detection of hair in anagen and telogen phases:
 - Red: telogen phase;
 - Green: anagen phase;



- Yellow: hair is touching the edge of the picture, grouping follows via a special statistical procedure.

Marked hairs

Anagen/Telogen hairs

PROTOCOL

Biological Material

• 30 healthy volunteers suffering from AGA, (average age 46).

• Patients were clinically evaluated and individual case histories were recorded in order to rule out possible pathologies as iron deficiency anemia, thyroid related conditions or other possible pathologies.

• Twenty (20) drops of the tested products were applied in the evening and gently distributed with the fingertips on the experimental area during 4 consecutive months.

• Digital trichogram was performed using professional TrichoScan. The number of hairs per sample had to be 200 or less and/or the proportion of anagen hairs had to be less than 70%.

• Every week, patients were given a plastic bag, where they had to collect all the hairs on their pillows, combs and clothes on a daily basis; they had to bring the bag to the laboratory for hairs to be counted.

Evaluation of activity

The TrichoScan[™] software quantifies the number of hairs in the studied area and the proportion of these hairs in the anagen and the telogen phase. This software is calibrated on the basis of 0.3 mm hair growth per day during the anagen phase and no hair growth during the telogen phase. Two measurements – one at the beginning and one at the end of the study – were taken to each volunteer.

Results analysis

Anagen hair density (n/cm2): in the definition of the TrichoScan procedure, an anagen hair is a hair which is detectable three days after complete hair shaving. Within this time only anagen hairs should grow significantly.

Telogen hair density (n/cm2): by definition a terminal hair will not grow whereas anagen hairs will. When images are taken three days after clipping, growing hairs can be differentiated from non-growing hairs based on different hair length. TrichoScan identifies non-growing hairs as telogen hairs and growing hairs as anagen hairs.

Ratio A/T: Comparison of the numbers of anagen and telogen hair, which is an indication of the percentage of active hair follicles.

 \uparrow A/T ratio = activation of hair growth

 \downarrow A/T ratio = loss of hair growth activity

 \rightarrow efficacy of treatment

 \rightarrow alopecia continues

RESULTS

Determination of anagen hair count

The TrichoScan software defines anagen hairs based on the knowledge that anagen grows at approximately 0.3 mm/day whereas telogen and catagen hairs do not grow.

During successful treatment, the anagen hair count should increase and therefore this approach can be used to monitor a treatment response.

Determination of telogen hair count

In the software sense a telogen hair is a nongrowing hair.

The software will measure the length of hair and by statistical analysis will discriminate between growing versus non-growing hairs. (catagen and exogen hairs will be judged as non-growing hairs).





Capixyl[™] induces a clear increase in the anagen hair density in comparison with placebo, demonstrating an efficacy of the treatment for hair growth after 4 months application.

Capixyl[™] also induces a strong reduction in the telogen hair density in comparison with the placebo, which clearly indicates a reduction of hair loss with Capixyl[™] treatment.

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Determination of Anagen/Telogen ratio (A/T)

The A/T ratio is a comparison of the numbers of anagen and telogen hair follicles, which is an indication of the percentage of active hair follicles. The graphic shows the averaged beginning-ofstudy and end-of-study measurements for the Placebo-treated and the Capixyl[™]-treated groups. Capixyl[™] statistically increases the Anagen/Telogen ratio (which represents an increase in the number of hair in the growing phase with a reduction of the number of hair in



the resting phase) of 46% compared to a reduction of -33% for the placebo.



Capixyl[™] increases the A/T ratio of 46% compared to a reduction of -33% for the placebo attesting the efficacy for stimulating hair growth and reducing hair loss

Before & After Pictures

Ginseng Extract



Ginseng has been used as a prophylactic and restorative agent to replenish vital energy in traditional Chinese medicine. From a morphological point of view, ginseng can be separated into three parts: the main root, the fibrous root and the rhizome. The major ginsenoside, which possesses an oleanane - type aglycone (Matsuda et al., 2010).

The medical use of ginseng is not only restricted to the improvement of general wellness, but also extended to the treatment of organ-specific pathological conditions. In the field of dermatology, ginseng and ginsenosides have been shown to regulate the expression and activity of major proteins involved in hair-cycling phases. The promotion of hair growth and prevention of hair loss by ginseng and its metabolites are associated with the induction of anagen and delaying of catagen phases. Although the underlying mechanisms by which ginseng and its metabolites regulate hair cycling have been explored to a limited extent, further studies, especially focusing on extended human trials, are required to establish this natural remedy for hair loss. Alopecia, originating from a variety of causes, including hyperactivation of androgenic signals, exposure to chemotherapeutics, aging, or skin photodamage, is considered as a skin pathology and has great psychosocial impact. Thus, it would be a plausible approach to develop hair growth-stimulating formulations, either as FDA-approved therapeutics or as cosmeceuticals, by using the index component of red ginseng (Table 1).

A study conducted by Murata et al. showed the hair re-growth effects of ginseng. The inhibitory activities of ginseng rhizomes and roots, as well as purified ginsenosides, against testosterone 5 α - reductase (5 α R) were investigated. 5 α - Reductase catalysing the reduction of testosterone to dihydrotestosterone (DHT) is a key enzyme in androgenetic alopecia (Rathnayake and Sinclair, 2010), and the effects of topical application of ginseng rhizomes and ginsenoside on testosterone - treated C57BL/6 mice were thus investigated. Furthermore, the inhibitory activities of Panax Japonicus Rhizome and American ginseng were evaluated.

The inhibitory activity of therhizome and the main root extracts from RG, WG and CG against 5aR were tested and the results are shown in Fig. 2. The rhizome extracts showed more potent inhibitory activity against 5αR than those of the main root in all plant samples tested (Fig. 2). The rhizome showed 44.2% inhibition at 1000 µg/mL, while the rhizome of WG showed the most potent activity among the samples tested (68.9% inhibition at the same concentration). The inhibitory activity of the CG rhizome was the lowest among the samples tested (35% inhibition at 1000 µg/mL).



Figure 2. Inhibitory activities of RG, WG and CG against 5α R. Values represent mean ± SE of triplicates. Significantly different from control group at *p<0.05, **p<0.01. Significantly different from each sample at ##p<0.01. C, conversion rate (%) of testosterone to DHT; for sample groups (Csample), Csample=r of test sample - r of control - 30 min, but for control group (Ccontrol), Ccontrol=r of control - 0 min - r of control - 30 min (r=testosterone/I.S.)

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