LUCASMEYER COSMETICS

ACTIVE INGREDIENTS

TECHNICAL FILE

CAPIXYL™

ANTI-AGING HAIR CARE COMPLEX

Biomimetic peptide combined with a red clover extract

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For stronger and thicker hair and lashes

PRESERVATIVE FREE

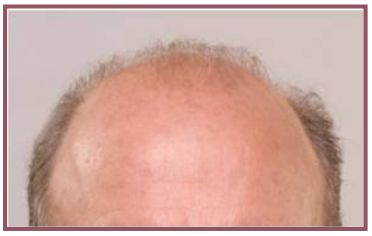
TABLE OF CONTENT

Capixyl™

SUMMARY

INCI NAME **CAS** **EINECS**	Butylene Glycol (1) Water (and) (2) (and) Dextran (3) (and) Acetyl Tetrapeptide-3 (4) (and) Trifolium Pratense (Clover) Flower Extract (5) 107-88-0 (1), 7732-18-5 (2), 9004-54-0 (3), 827306-88-7 (4), 85085-25-2 (5) 203-529-7 (1), 231-791-2 (2), 232-677-5 (3), - (4). 285-356-7 (5)
ORIGIN	Biomimetic peptide combined with red clover extract rich in Biochanin A
COSMETIC PROPERTIES	• Improves ECM proteins renewal for optimal hair follicle anchoring • Reduces IL-8 pro-inflammatory cytokine • Reduces 5-α-reductase enzyme activity
SKIN BENEFITS / POTENTIAL CLAIMS	• Reduces hair loss • Stimulates / Boosts hair growth • Improves hair follicle anchoring by maintaining proteins matrix support and integrity • Reduces inflammation in the scalp
APPLICATIONS	• Anti-hair loss • Hair regrowth • Eye lashes • Leave-on • Hair treatment for menopaused women • Scalp treatment • Lotions • Tonics • Anti-aging hair care product • Treatment for seasonal hair loss • Eyelash mascara • Eyelash serum • Eyelash conditioner • Eyelash treatment • Active makeup
RECOMMENDED DOSAGE	• **Intensive treatment: 5%** • **Preventive care: 0.5 – 2.5%**
USAGE pH RANGE	pH: 4 – 8
INCORPORATION	At the end of the formulation (< 40˚C)
INCOMPATIBILITIES	Not known
PATENTS	**WO 2005/009456 – US7057719**

INTRODUCTION



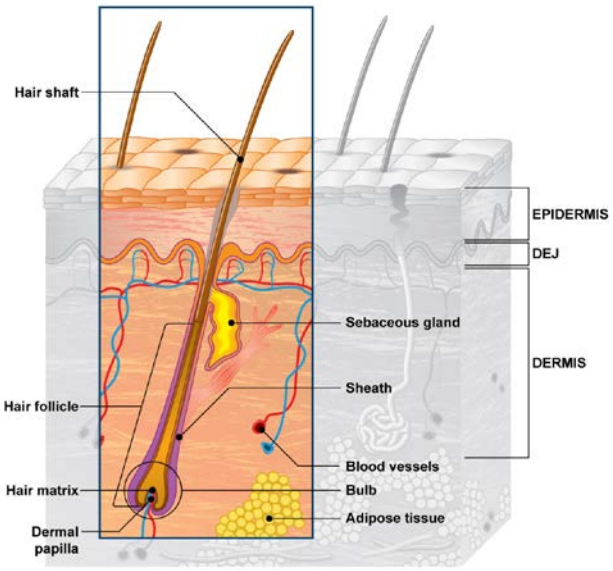
The importance of hair in our lives cannot be overstated. Whether men or women lose their hair, they lose much more than their natural, youthful appearance. People also lose their self-esteem and self-confidence associated with healthy looking hair. Hair loss also called alopecia is a common problem affecting both men and women. There are six major types of hair loss, but the most common one is Androgenetic/Androgenic alopecia which seems to represent 95% of all hair loss.

Androgenetic alopecia is said to affect roughly 50% of men and perhaps as many women older than 40 years. Androgenetic alopecia (AGA) is also called male pattern baldness (MPB). By the age of 35 two-thirds of American men will experience some degree of appreciable hair loss. Approximately 25% of men who suffer with MPB begin the painful process before they reach the age of twenty-one. AGA affects an estimated 35 million men in the United States and about 21 million women. In France 10 million of people are affected by hair loss, which represents 2 men out of 3 and one woman out of 5. In Japan, 30% of the male population experiences balding, usually later in life after 45 years of age. The prevalence of AGA in Chinese men is 21.3% and 6% for women, these rations are lower than in Caucasians but similar to that in Koreans. Approximately 13% of premenopausal women reportedly have some evidence of AGA. However, the incidence of AGA increases greatly in women following menopause[1], and it may affect 75% of women older than 65 years[2].

According to The Washington Post, American hair loss sufferers spend more than 3.5 billion dollars a year attempting to treat their hair loss. There are numerous products on the market addressing this condition. The most popular are Minoxidil (Regain®) an OTC vasodilator medication known for its ability to slow or stop hair loss and promote hair regrowth, Finasteride (Propecia®) also a drug that acts by inhibiting the enzyme that converts testosterone to dihydrotestosterone (DHT) in AGA and Aminexil® a patented molecule by l'Oréal[3].

In today's image-conscious society, people are looking for an affordable cosmetic hair loss solution that delivers on its promises. This presents an opportunity for high performance, clinically proven anti-hair loss products.

Hair Science

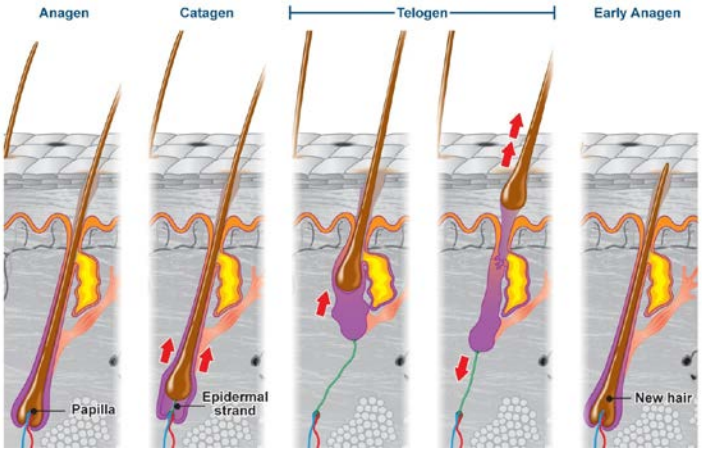


Hair appears much more complex that they look and they play a vital role in our appearance. They are composed of strong structural protein called keratin. Below the surface of the skin is the hair root, which is enclosed within a hair follicle. At the base of the follicle is the dermal papilla. The dermal papilla is feed by the bloodstream which carries nourishment to produce new hair. The dermal papilla plays a crucial role in the dermal-epidermal interactions and is of great importance for the hair formation and growth cycle[4].

Each human head carries roughly 100,000 hair follicles. Each follicle can grow many hairs over a lifetime: on average, each follicle grows a new hair around twenty times. Hair grows about 3-4 mm/day. This hair growth and loss is completely random. At any given time, a random number of hairs will be in various stages of growth and shedding. Over the years, the number of follicles capable of growing hair declines naturally. The decline is especially noticeable on the top of the head.

Between starting to grow and falling out years later, each hair passes through three distinct growth stages.

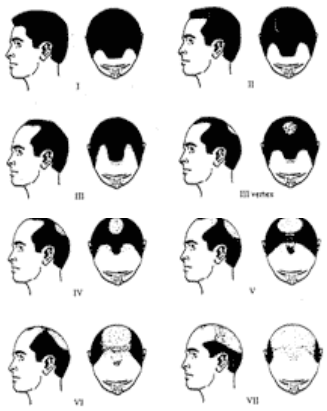
- **Anagen:** the **Growth** phase. Hair is actively growing; this phase can last from 2 to 6 years.
- **Catagen:** the **Transition** phase. The dermal papilla breaks away and the hair stops growing and remains in this phase for only two to three weeks.
- **Telogen:** the **Resting** phase. The hair stays attached to the follicle and falls out only to be replaced by the next building hair in the anagen phase. This phase lasts approximately 100 days.



At the end of the telogen phase the hair follicle re-enters the anagen phase. The dermal papilla and the base of the follicle join together again and a new hair begins to form. If the old hair has not already been shed the new hair pushes the old one out and the growth cycle starts all over again.

In an adult scalp, 70-85% of hairs are in anagen phase and 10% in telogen phase. As we age, the percentage of hair in the telogen phase increases for a slower hair growth rate and the replacement hair gets finer and thinner.

WHAT CAUSES HAIR LOSS?

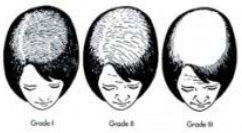


Although causes of hair loss are still not yet fully understood, it can be the results of several factors. The causes of hair loss can be genetic, due to hormonal changes or imbalances (childbirth, menopause), linked to nutrition (deficiency in certain vitamins and minerals), stress, diseases like diabetes or lupus or medications, hair treatment (overstyling and excessive brushing, dye, bleaching) and of course aging.

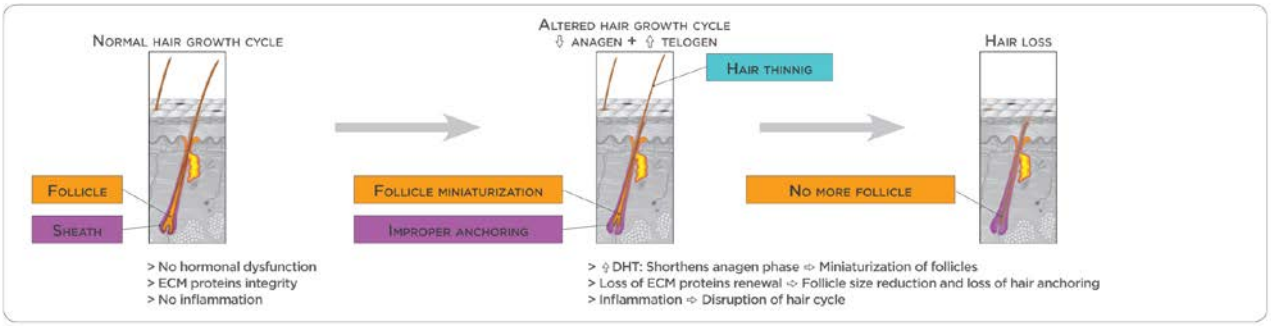
Hair loss can be permanent or temporary.

Most cases of hair loss are due to AGA. Approximately 50% of men by the age of 50 years and 15% of women before the time they reach menopause have some degree of AGA. In men, it begins at the crown, temples, or both. In women, the hair becomes thinner all over the head, and the hairline does not recede. AGA in women rarely leads to total baldness.

A normal hair growth cycle is not affected by any hormonal dysfunction. The hair follicle is surrounded by good quality of connective tissue and dermal papilla and has minimal or absence of neither inflammation nor oxidative stress.



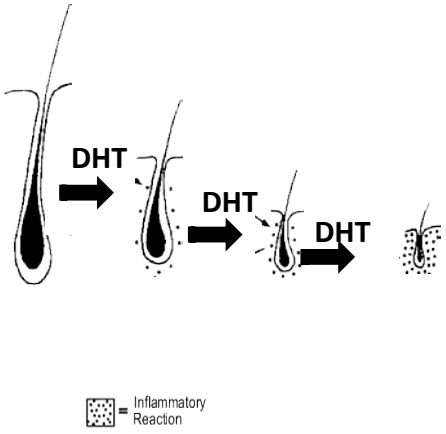
Hair loss sufferers will most of the time have the hair growth cycle disrupted by an increase of dihydrotestosterone (DHT) hormone, loss of connective tissue integrity surrounding the hair follicle, an increase in the inflammatory process in the scalp and is submitted to higher oxidative stress. Of course with aging, the decrease number of hair follicle and size of follicle in the scalp will also lead to thinner hair shaft and an increase in hair loss.

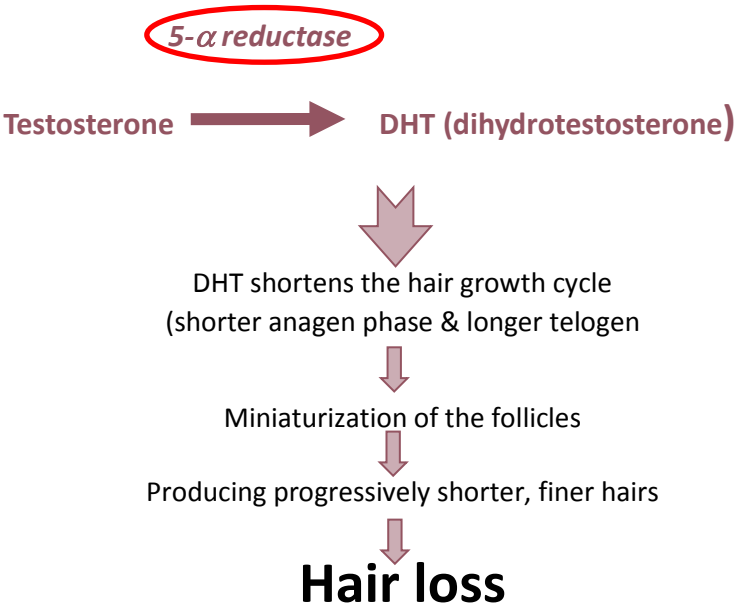


EFFECT OF DHT AND 5-ALPHA-REDUCTASE

Researchers have determined that AGA is related to hormones called androgens, particularly testosterone and its related hormone dihydrotestosterone (DHT)[5].

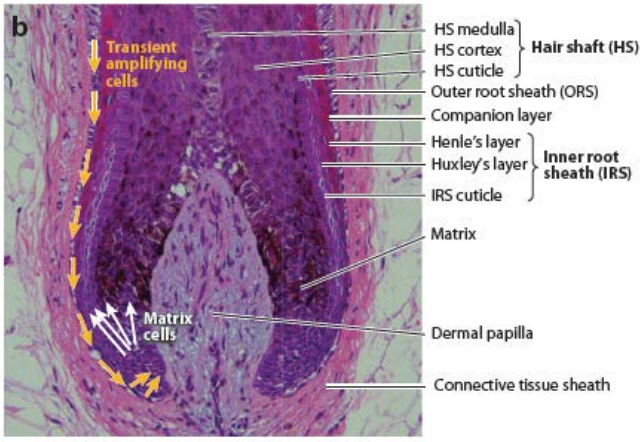
DHT is formed by the action of the enzyme 5α-reductase on testosterone. DHT causes hair loss by shortening the growth phase of the hair cycle (decreasing the anagen phase (growth) and increasing the telogen phase (resting)), causing miniaturization (decreased size) of the follicles, and producing progressively shorter, finer hairs. Eventually these hairs totally disappear. It has been found that in spite of their normal testosterone levels people suffering from alopecia have higher levels of 5α-reductase, DHT and androgen receptors. In men, the 5α-reductase activity is higher in the balding area.





EFFECT OF CONNECTIVE TISSUE INTEGRITY AND RENEWAL

The dermal papilla consists of a highly active group of cells (mainly fibroblasts) shown to be capable of inducing follicle development from the epidermis and production of hair fibers[6,7]. Dermal papilla directs and dictates the generation of a hair follicle and retains this ability throughout the hair cycle. A big and healthy dermal papilla will produces more cells, and will lead to a production of thicker hair fiber by the hair follicle.



Furthermore, the size of hair follicles is determined by the volume of its dermal papilla which depends on the number of cells it contains and on the volume of the extracellular matrix[8]. Dermal papilla cells revealed the typical morphology and growth pattern which is similar to cutaneous fibroblasts. Dermal papilla cells are found to produce considerable amounts of matrix proteins (similar to Extracellular Matrix (ECM)) such as fibronectin, collagen type I and type III[9,10]. A reduction in size of the matrix cells of the hair bulb and its dermal papilla has been associated with AGA condition[11].

It has been demonstrated that molecules which have an activity on the ECM proteins by stimulating the skin remodeling, also increase hair follicle size[12]. Furthermore, skin repair and hair growth enhancement effects are closely linked[13]. Moreover, the quality of the ECM proteins in the dermal papilla is of major importance for the cyclical growth of hair. Healthy dermal papilla will produce good ECM proteins such as collagen type III and anchoring fibers such as laminin and collagen VII which will favor a good hair anchoring in the bulb surrounding tissue. It has also been observed that ECM proteins thickness decreases in alopecia.

If there is improper ECM renewal, hair will eventually lack vigor and will thin. Cycle after cycle, the follicle becomes smaller and finally, miniaturized and fall[14].

EFFECT OF INFLAMMATION

Chronic inflammation of the hair follicle is considered a contributing factor in the pathogenesis of AGA[15]. The follicular microinflammation also plays an integral role in the pathogenesis of AGA in early cases[16]. The term «microinflammation» was proposed by Mahe and colleagues because the process of inflammation in pattern baldness adopts a slow, subtle, painless and lethargic course, in contrast to the inflammatory and destructive process that has been seen in the classical inflammatory scarring alopecia[17]. Among the possible causes cited are heat, frictions, UV radiation and microflora.

LUCAS MEYER COSMETICS UNIQUE SOLUTION TO HAIR LOSS

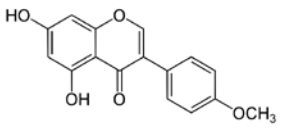
Capixyl™ is an innovative and unique active complex designed to prevent and stop the hair loss process and stimulate hair growth.

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 activity[18]. 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.

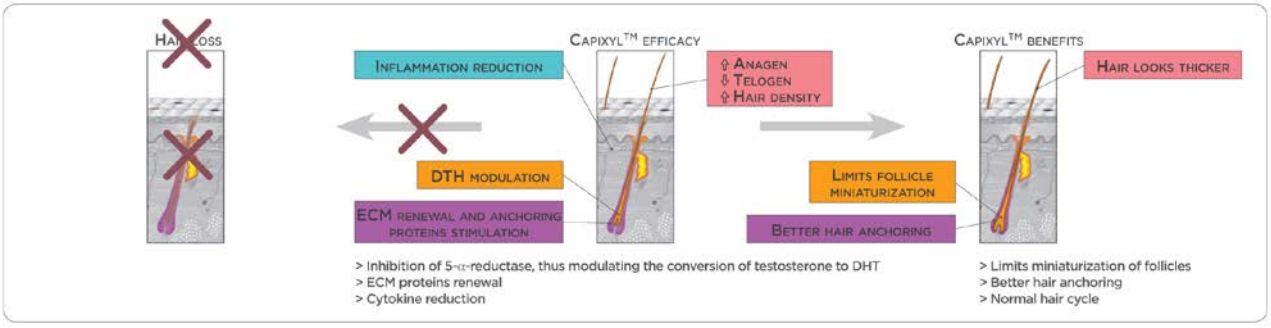


Acetyl Tetrapeptide-3 Biomimetic Peptide

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.

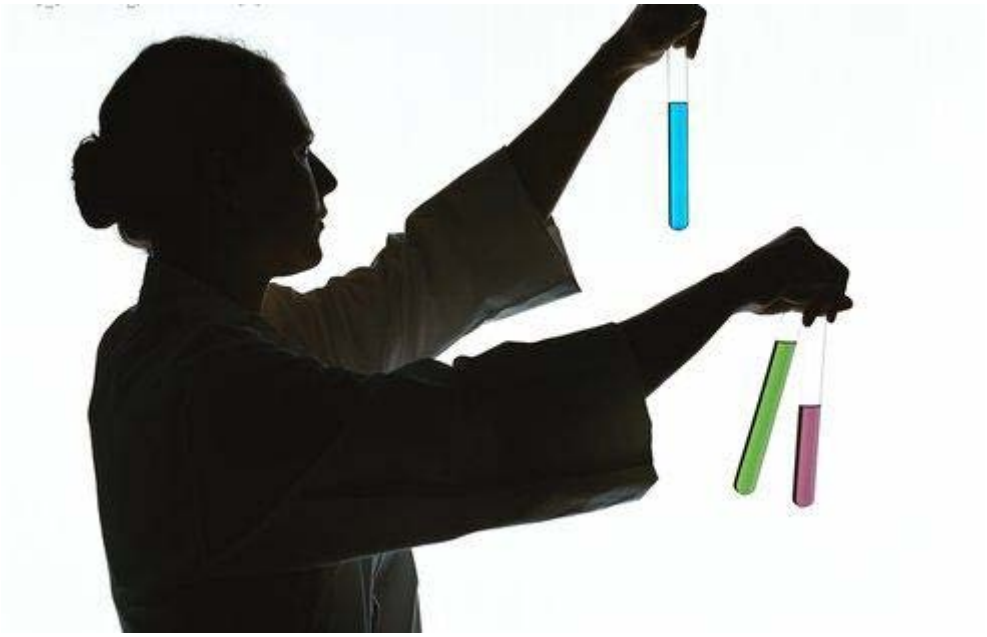
CAPIXYL™ MECHANISM OF ACTION



- Inhibition of 5α-reductase, thereby modulating the conversion of testosterone to DHT → No miniaturization of follicles
- ECM proteins renewal → Better hair anchoring
- Inflammation reduction → Normal hair cycle

Capixyl™ prevents and stops the hair loss process and stimulates hair growth. *Ex vivo* experiments have demonstrated better results in hair growth stimulation than Minoxidil. Clinical data shows that it improves alopecia by increasing the Anagen/Telogen ratio. Capixyl™ is a safe alternative to Minoxidil and provides fast results.

EFFICACY STUDIES



EFFECT OF ACETYL TETRAPEPTIDE-3 ON COLLAGEN SYNTHESIS BY FIBROBLASTS IN VIVO

BACKGROUND

The anatomy of the hair follicle, by contrast with the surface epidermis, is not static or rigid. Human hair grows in a continuous cyclic pattern and the bulbar portion of the follicle undergoes almost total disintegration during the catagen phase. The renewal of the proteins implicated in the formation of new hair follicle through the dermal papilla is thus of importance.

The hair follicle can be recognized as a separate entity within the skin. The dermal papilla consists of a highly active group of cells, especially fibroblasts, shown to be capable of inducing follicle development from the epidermis and production of hair fiber[19,20].

The major biosynthetic product of dermal fibroblasts is collagen[21]. Collagen as a major protein component of the dermis plays an important role in the skin cohesion and renewal.

PROTOCOL

Principle

The dermis is constituted of cells like fibroblasts which produce collagen. Collagen is composed of three chains wound together in a tight triple helix. Collagen is characterized by the presence of 2 specific amino-acids: hydroxylysine and overall hydroxyproline (OH-proline). Through the dosage of hydroxyproline, it is possible to correlate with the quantity of collagen produced by cells.

Thus hydroxyproline estimation may be used to give an indication, unaffected by the presence of other proteins, of the **mature collagen content of biological tissue**. It may also be used to give an indication of the degree of collagen breakdown in physiological or disease processes.

Biological material

It has been established and demonstrated that proteins and collagen synthesis of the dermal-hair papilla cells is very similar to skin fibroblasts[22]. We thus used human fibroblasts (MRC5 cell line) for this assay.

Evaluation of activity

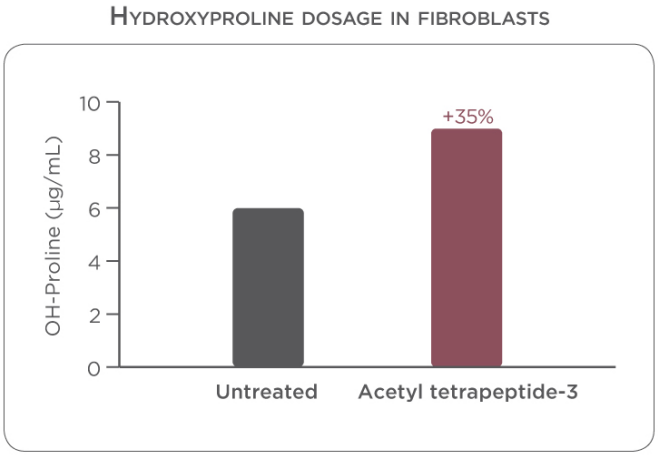
Cells are incubated or not with the acetyl-tetrapeptide-3 at 10^{-7}M for 7 days. After incubation period, Hydroxyproline (OH-proline) dosage is assessed with the Chloramine T reaction and measured by optical density (OD) at 540 nm compared with a range standard[23].

The procedure is based on alkaline hydrolysis of the tissue homogenate and subsequent determination of the free Hydroxyproline in hydrolyzates. Chloramine-T was used to oxidize the free Hydroxyproline for the production of a pyrrole. The addition of Ehrlich's reagent resulted in the formation of a chromophore that can be measured at 540 nm.

Results analysis

The effect of the Acetyl-tetrapeptide-3 and its potential activation on collagen synthesis on fibroblasts was evaluated by comparing the quantity of Hydroxyproline produced by MRC5 cells incubated with or without Acetyl-tetrapeptide-3.

RESULTS



As represented in the figure above, the cells treated with Acetyl tetrapeptide-3 show highly elevated level of Hydroxyproline direclty correlated with mature collagen cotent in cells.

CONCLUSION

By stimulating the collagen synthesis and collagen content in fibroblasts, Acetyl-tetrapeptide-3 can be used to renew dermal papilla dermis collagen.

Acetyl-tetrapeptide-3 stimulates collagen production for better ECM integrity for a better hair follicle anchoring.

EFFECT OF ACETYL TETRAPEPTIDE-3 ON THE SYNTHESIS OF ECM PROTEINS BY FIBROBLASTS IN VITRO

BACKGROUND

As already described, dermal part of hair follicle, such as dermal papilla cells and dermal sheath cells are thought to play an important role in the induction and maintenance of hair growth[24]. Those cells have the ability to induce hair follicle formation by interaction with the epithelial cells of hair follicle[25].

The major biosynthetic product of dermal fibroblasts is collagens[26]. It is present in more than ten genetically types found in different tissues and synthesized by specialized connective tissues cells. Dermal fibroblasts synthesize mostly type I and type III, and in addition small amounts of types V and VI collagen[27]. Collagen types I and III are not uniformly distributed in the dermal connective tissues showing an accumulation of type III collagen in the papillary dermis[28]. Furthermore, the presence of laminin was also detected in the papilla dermis cells and seems to be important in the first days of growth for proper follicle anchoring[29].

The size of hair follicle is thought to be determined by the volume of its dermal papilla which depends on the number of cells and on the volume of the extracellular matrix[30].

PROTOCOL

Principle

The effect of Acetyl tetrapeptide-3 on the synthesis of different extracellular matrix proteins (collagen III & laminin) was evaluated by selective immunofluorescence in comparison with untreated fibroblasts.

Immunofluorescence is a technique allowing the visualization of a specific protein (collagen III or laminin) in cells or tissue sections by binding a specific antibody chemically conjugated with a fluorescent dye such as rhodamine (red color).

Biological material

As previously described, fibroblasts from dermal papilla and dermis fibroblasts are similar. Thus, human fibroblasts (MRC5 cell line) were used for this assay.

Evaluation of activity

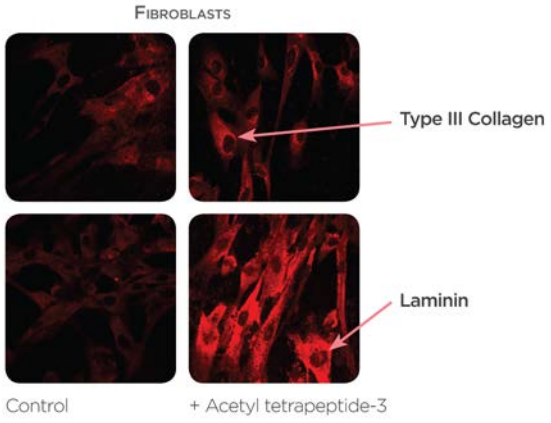
Cells are incubated or not with Acetyl tetrapeptide-3 at 10^{-7}M for 3 days. Cells were then fixed on the slide and proteins (collagen type III or laminin) were detected with specific antibodies coupled to a fluorochrome which can be detected and quantified by a confocal microscope (Axioplan and Zeiss LSM510), which allowed a semi-quantitative evaluation.

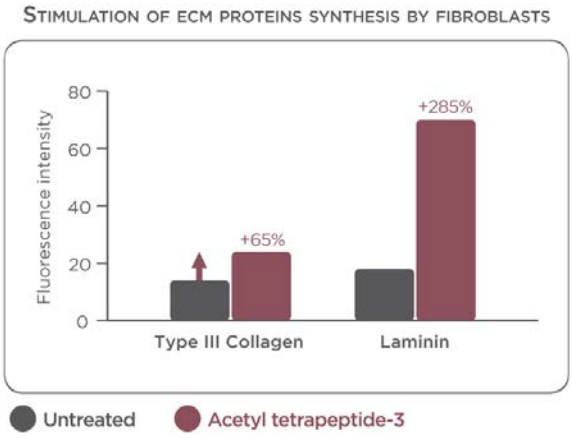
Results analysis

The effect of Acetyl tetrapeptide-3 and its potential activation on collagen types III or laminin synthesis on fibroblasts was evaluated by comparing the fluorescence intensity observed on cells incubated with or without Acetyl tetrapeptide-3.

RESULTS

Represented here, are the microscopic images of the fibroblasts, untreated (left) or treated (right) with Acetyl tetrapeptide-3. The graphs represent the semi quantitative evaluation of the extra cellular matrix protein synthesis (type III collagen and laminin).





These results showed a significant increase of the synthesis of extracellular matrix (type III collagen and laminin) proteins in fibroblasts treated with Acetyl tetrapeptide-3.

CONCLUSION

Acetyl tetrapeptide-3 powerfully induces the synthesis of ECM proteins by fibroblasts such as type III collagen and laminin. It has been demonstrated that molecules which have an activity on the ECM proteins by stimulating the skin remodeling, also increase hair follicle size[31].

Acetyl tetrapeptide-3 stimulates dermal papilla extracellular matrix proteins thus has a direct effect on hair follicle size and better anchoring

EFFECT OF ACETYL TETRAPEPTIDE-3 ON THE SYNTHESIS OF COLLAGEN VII IN HUMAN SKIN EXPLANTS *EX VIVO*

BACKGROUND

Little is known regarding the junction that separates the follicular epithelial and its adjacent connective tissue, the connective tissue-epithelial junction (CEJ). However, this junction presents around the anagen hair follicle is closely similar to that seen at the DEJ[32] and proteins found in the normal interfollicular epidermis were expressed mainly during the anagen phase[33].

Collagen VII (the major constituent of anchoring fibrils), is located in the middle part of the follicular basement membrane zone and around the hair papilla. As the composition and structure of the anagen hair follicle dermal-epidermal junction (DEJ) is similar to the interfollicular skin, the effect of Acetyl-tetrapeptide-3 on type VII collagen synthesis was evaluated on human skin explants.

PROTOCOL

Principle

This biological model uses human normal skin explants. The skin metabolism is experimentally decreased by the application of corticoïds, which modified the skin metabolism like the one observed in natural aging. Furthermore, it has been described that corticoïds decrease the hair papilla cells (fibroblasts) growth[34]. The repair of dermal-epidermal junction with the Acetyl tetrapeptide-3 is then evaluated by the analysis of immunohistological staining of collagen VII proteins.

Biological material

Four Human skin explants were obtained from patients undergoing plastic surgery (Caucasian women 35-45 years old) and maintained in culture.

Evaluation of activity

The first day, a surface skin application of corticoïds was done[35]. Acetyl tetrapeptide-3 at 10^{-3}M was added on the cell culture for 2 days. Skin explants were prepared for specific collagen VII immunohistological labelling using ABC peroxydase Kit and revealed by AEC substrate (brown color).

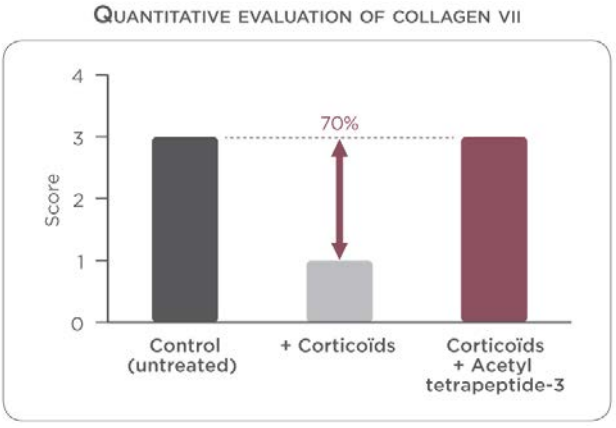
Results Analysis: Semi-quantitative histological evaluation of collagen VII labeling:

Scores ranging from 0 (negative) to 4 (maximum) were defined as the following parameters:

No collagen VII labelling	Score 0
Slight Collagen VII labelling	Score 1
Moderate Collagen VII labelling	Score 2
Normal Collagen VII labelling **(normal skin)**	Score 3
Over-expression of Collagen VII labelling	Score 4

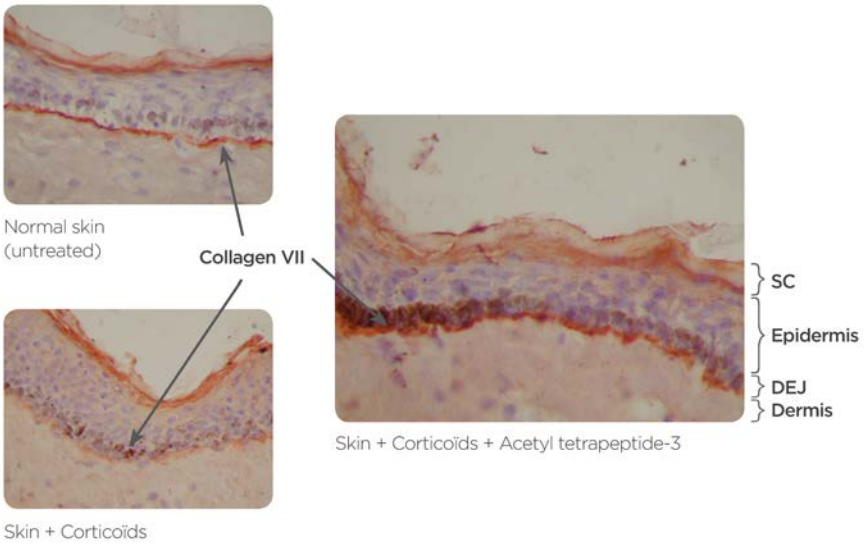
RESULTS

Semi-quantitative evaluation of the Collagen VII staining in the human skin explants



Microscopic observations of the collagen VII immunohistological labelling

The presence of collagen VII in the dermal-epidermal junction is evidenced by its specific labelling using coupled antibodies (brown-red color along the DEJ).



The microscopic observations of normal untreated skin show a strong labelling of the collagen VII along the dermal-epidermal junction (**Figure A**). This labelling is quantified using a score ranging evaluation table and corresponds to a score equal to 3.

We clearly observed a dramatically decrease of the collagen VII staining after application of dermocorticoïds on skin (**Figure B**). The semi-quantification evaluation of this labelling represents a decrease of 70% in collagen VII (score 0.9) which demonstrates a strong alteration of the dermal-epidermal junction properties.

The application of Acetyl tetrapeptide-3 on the skin (**Figure C**), in addition to dermocorticoïds restores the normal amount of collagen VII along the DEJ as the score calculated after the labelling is the same than the one obtained for the untreated skin (score 3) (**Figure A**).

CONCLUSION

In this aging human skin experimental model we can observe an alteration of the collagen VII along the dermal-epidermal junction after application of dermocorticoïds.

The addition of Acetyl tetrapeptide-3 on the skin surface restores totally the dermal-epidermal junction by the stimulation and/or the protection of collagen VII.

These results obtained in this skin model can be correlated with the interfollicular skin (in anagen hair follicle) as the expression and the distribution of the collagen VII in the DEJ is similar in both types.

Acetyl tetrapeptide-3 provides a repairing effect at the dermal-epidermal junction level, improving hair anchoring

EFFECT OF ACETYL TETRAPEPTIDE-3 ON HUMAN HAIR FOLLICLES *EX VIVO*

BACKGROUND

In 1990, Philpott and al. reported the first time the successful maintenance and growth of human hair follicles *in vitro*[36]. The importance of this model to hair follicles biology had been demonstrated. This study was assessed in order to measure the growth speed of hair shafts on isolated human hair follicles in cultured with **Acetyl tetrapeptide-3**.

PROTOCOL

Principle

Human anagen hair follicles were isolated by microdissection from human scalp skin. Isolation of the hair follicles was achieved by cutting the follicle at the dermo-subcutaneous fat interface using scalpel blad.

Biological Material

Hair follicles recovered from human scalps are immersed in a specific culture medium. Successive washing is performed and the follicles dissection is made under binocular microscope according to the Philpott technique in order to specifically select follicles which are in the anagen phase.

The follicles are cultured in a specific medium added or not with Acetyl tetrapeptide-3 at $4x10^{-9}$M for 8 days. Untreated follicles but cultured in the same conditions represent our negative control.

The numbers of follicles per treatment are:

- n = 6 follicles treated with Acetyl tetrapeptide-3
- n = 6 untreated follicles

Evaluation of activity

Growth of hair follicles in culture was assessed by measuring increases in follicle length over the 8 days culture period, each 24 hours with a micrometer incorporated in the microscope optical.
The percentage of growth was calculated as follows:

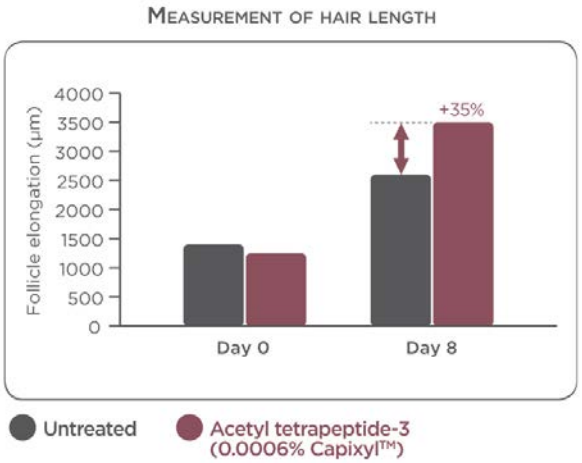
Percentage of growth: $\frac{\text{Length at Tx} - \text{Length at T0}}{\text{Length at T0}} \times 100$

Tx: Time x after treatment *T0: Time 0, at the beginning of the treatment*

Results analysis

The growth percentage was determined after 8 days treatment of follicles with **Acetyl tetrapeptide-3** in comparison with untreated follicles.

RESULTS



In these experimental conditions, we observed a strong stimulation of hair growth by 35% in comparison with untreated follicles.

CONCLUSION

Acetyl tetrapeptide-3 is able to strongly stimulate the human hair growth after 8 days treatment.

Acetyl tetrapeptide-3 stimulates hair growth

EFFECT OF ACETYL TETRAPEPTIDE-3 ON HUMAN HAIR FOLLICLES *EX VIVO* – COMPARATIVE STUDY

BACKGROUND

In 1990, Philpott and al. reported the first time the successful maintenance and growth of human hair follicles *in vitro*[37]. The importance of this model to hair follicles biology had been demonstrated.
This study was assessed in order to measure the growth speed of hair shafts on isolated human hair follicles in cultured with **Acetyl tetrapeptide-3** in comparison with the Minoxidil.

PROTOCOL

Principle

Human anagen hair follicles were isolated by microdissection from human scalp skin. Isolation of the hair follicles was achieved by cutting the follicle at the dermo-subcutaneous fat interface using scalpel bald.

Biological Material

Hair follicles recovered from human scalps are immersed in a specific culture medium. Successive washing is performed and the follicles dissection is made under binocular microscope according to the Philpott technique in order to specifically select follicles which are in the anagen phase.

The follicles are cultured in a specific medium added or not with the test substances: Acetyl tetrapeptide-3 at 10^{-7}M or the reference Minoxidil at $120x10^{-7}$M for 7 days. Untreated follicles but cultured in the same conditions represent our negative control.

The numbers of follicles per treatment are:

- n = 15 follicles treated with Acetyl tetrapeptide-3
- n = 27 follicles treated with Minoxidil
- n = 27 untreated follicles (control)

Evaluation of activity

Growth of hair follicles in culture was assessed by measuring increases in follicle length over the 7 days culture period, each 24 hours with a micrometer incorporated in the microscope optical.
The percentage of growth was calculated as follows:

$$\textbf{Percentage of growth:} \quad \frac{\textbf{Acetyl tetrapeptide-3 stimulates hair growth}}{} \times 100$$

Tx: Time x after treatment *T0: Time 0, at the beginning of the treatment*

In parallel, the **normalized activity** after treatment (“hair growth induced by the treatment”) was calculated following the equation:

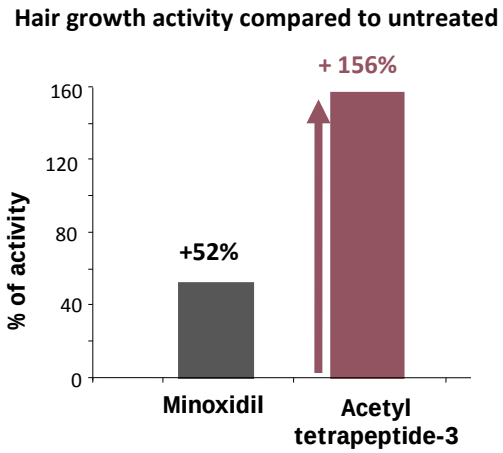
$$\textbf{Activity} = \frac{(\text{length } \textbf{P} \text{ D7} - \text{length } \textbf{P} \text{ D0}) - (\text{length } \textbf{C} \text{ D7} - \text{length } \textbf{C} \text{ D0})}{(\text{length } \textbf{C} \text{ D7} - \text{length } \textbf{C} \text{ D0})} \times 100$$

C = control (untreated follicles) ; *P = Acetyl tetrapeptide-3*

Results analysis

The growth percentages and activity were determined after a 7 days treatment of follicles with **Acetyl tetrapeptide-3** or Minoxidil in comparison with untreated follicles.

RESULTS



We observed a strong stimulation of hair growth in these experimental conditions. The activity is better with **Acetyl tetrapeptide-3** and has demonstrated a 47% growth which is higher than the growth obtained with Minoxidil.

CONCLUSION

Acetyl tetrapeptide-3 is able to strongly stimulate the human hair growth. Results obtained with Acetyl tetrapeptide-3 are higher than with Minoxidil, a reference in hair growth treatment.

Acetyl tetrapeptide-3 stimulates hair growth with higher activity than the reference hair growth product, Minoxidil

EFFECT OF BIOCHANIN A ON 5α-REDUCATSE ACTIVITY IN VIVO

BACKGROUND

The enzyme 5α-reductase catalyses the conversion of testosterone to dihydrotestosterone (DHT), a process thought to amplify the androgenic response.

DHT causes hair loss by shortening the growth phase of the hair cycle (decreasing the anagen phase (growth) and increasing the telogen phase (resting)), causing miniaturization (decreased size) of the follicles, and producing progressively shorter, finer hairs.

PROTOCOL

Principle

In this experiment, the capacity of Biochanin A to modulate the activity of both type I and type II 5α-reductase activity was measured in intact cells.

Biological Material

EGCG (epigallocathechin gallate) isolated from green tea served as a positive control for the inhibition of 5α-reductase activity[38].
ECGC and Biochanin A were used at 100 µM.

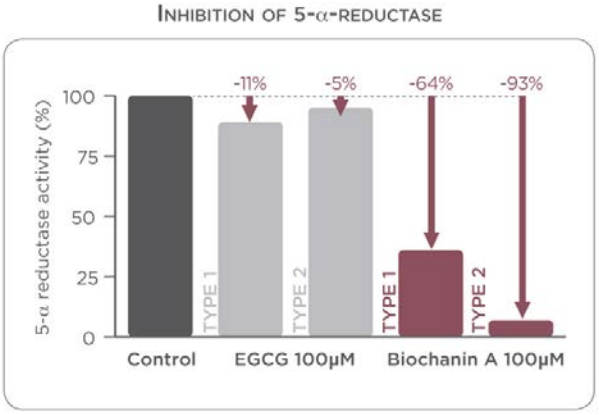
Evaluation of activity

Cells were plated and incubated with test compounds (Biochanin A or positive control) for 1 hour at 37°C before the addition of ^{14}C-testosterone, at a final concentration of 1.5 µM. Cells were incubated for an additional 3 hours and radioactive steroids were extracted. The amount of labelled testosterone and DHT were determined by TLC (Thin Layer Chromatography) in order to quantify the 5α-reducatse activity.

Results analysis

Results are expressed in percent inhibition of 5α–reductase activity in the presence of 100 µM concentration of Biochanin A or ECGC.

RESULTS



These results showed that 5α–reductase (type I and II) activity is significantly decrease in presence of Biochanin A at 100 µM.

The inhibition observed with Biochanin A is higher than the effect observed with ECGC, a 5α–reductase activity inhibitors reference.

Biochanin A decreases decrease 5-α-reductase activity and thus reduce the conversion of testosterone into DHT

EFFECT OF CAPIXYL™ AND RED CLOVER ON IL-8 PRODUCTION BY HUMAN FIBROBLASTS

BACKGROUND

Androgenetic alopecia is a common cosmetic hair disorder, resulting from interplay of genetic, endocrine, and aging factors leading to a patterned follicular miniaturization. Micro-inflammation seems to be a potential active player in this process[39.] In AGA we observed clusters of abnormal inflamed streamers or fibrous tracts surrounding the hair follicle[40].

Interleukine-8 (IL-8) is a cytokine secreted by several cell types and is one of the major mediators of the inflammatory response.

Decreasing the level of inflammation on the scalp and hair bulb area can have a positive effect on hair loss prevention.

PROTOCOL

Principle

In this experiment, the capacity of red clover extract and Capixyl™ to modulate the production of IL-8 was measured in normal human fibroblasts, in order to evaluate its anti-inflammatory potential.
Inflammation is chemically induced in keratinocytes using IL-1α a physiological pro-inflammatory agent.

Biological Material

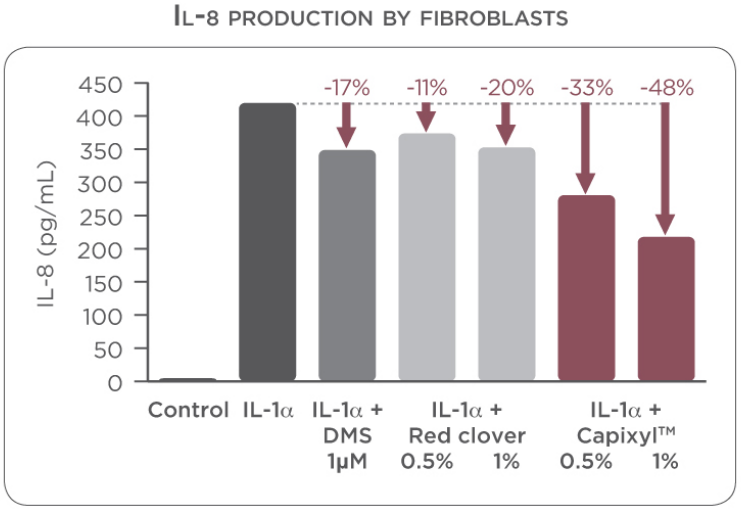
Experiments were done using monolayers of cells derived from normal human fibroblasts (NHDF). IL-1α is used at 0.0075 ng/mL to induce inflammation in fibroblasts.
Dexamethasone (DMS) at 1µM served as a positive control and anti-inflammatory agent[41]. Dexamethasone is a potent synthetic member of the glucocorticoïd class of steroid hormones and acts as an anti-inflammatory with potency about 20-30 times that of hydrocortisone[42].

Evaluation of activity

After seeding, confluent NHDF cells were cultured for 24 hours. At the end of this period, the fibroblasts were incubated in the presence or absence of Capixyl™, red clover or DMS and IL-1α was added concomitantly to the medium in order to mimic an inflammatory reaction during 24 hours. IL-8 content was quantified in the medium, using a highly sensitive and specific enzyme immunoassay (EIA) kit.

Results analysis

Results are expressed as pg/ml of IL-8 produced by fibroblasts.



RESULTS

These results showed that fibroblasts secreted IL-8 after stimulation by IL-1α, acytokine that is triggering the inflammation process in cells. When cells are treated with DMS, red clover extract or Capixyl™, we observed a significant decrease in the synthesis of IL-8 attesting a decrease in the inflammation process.

The effect of Capixyl™ has a dose dependant effect and is higher than the effect observed with the DMS, an anti-inflammatory reference. We also observed a better activity of the Capixyl™ in comparison with the red clover extract alone, which demonstrates a synergistic effect of the peptide and the red clover extract.

Capixyl™ decreases pro-inflammatory cytokines with a synergistic action compared to red clover extract alone

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 cm^2 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.

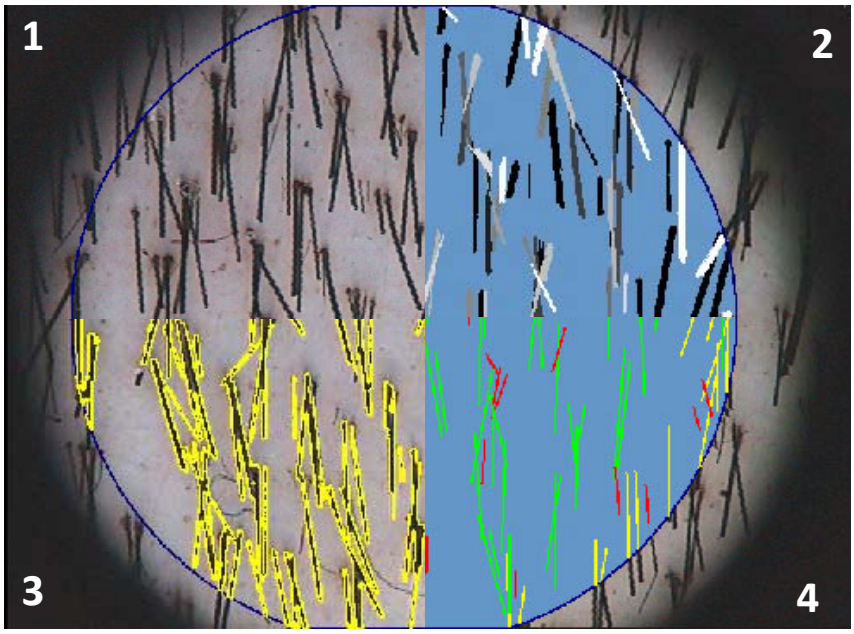
Patients were asked not to wash their hair for two days prior to the evaluation with TrichoScan.

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.

Original **Detected hairs**



Marked hairs **Anagen/Telogen hairs**

PROTOCOL

Formulations used in the clinical study

- **Capixyl™ lotion**: 5% Capixyl™, 20% alcohol, 75% water (pH = 5.4)
- **Placebo lotion**: 20% alcohol, 80% water

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/cm^2): 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/cm^2): 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.

↑ A/T ratio = activation of hair growth ➔**efficacy of treatment**

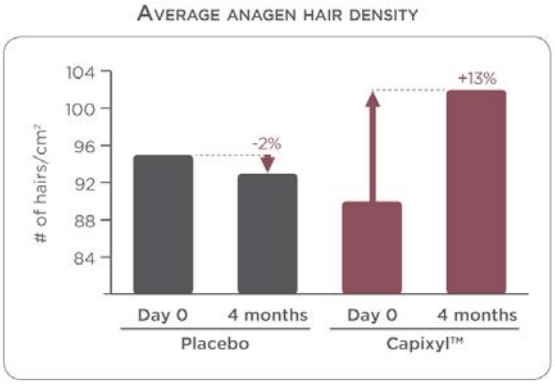
↓ A/T ratio = loss of hair growth activity ➔**alopecia continues**

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.

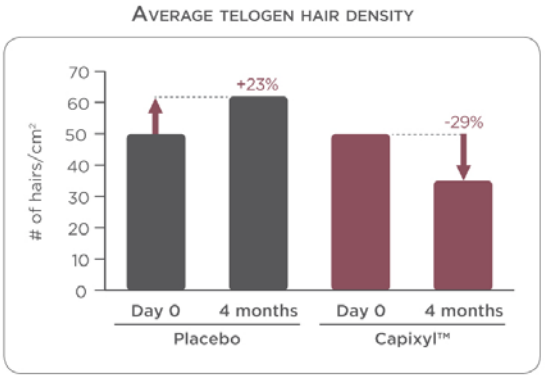
During successful treatment, the anagen hair count should increase and therefore this approach can be used to monitor a treatment response.



Determination of telogen hair count

In the software sense a telogen hair is a non-growing 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).



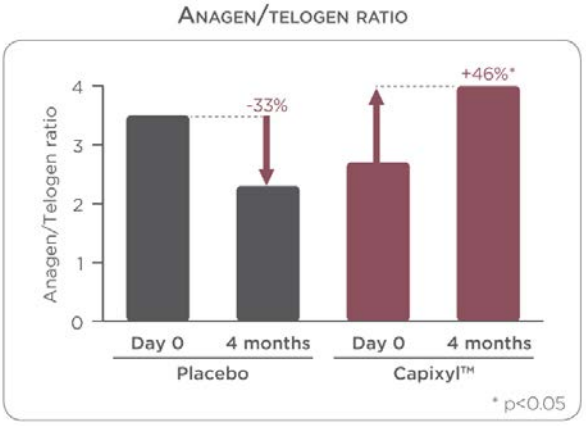
Capixyl™ induces a clear increase in the anagen hair density in comparison with placebo, demonstrating an efficacy of the treatment for hair growth after 4 months application.

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.

Determination of Anagen/Telogen ratio (A/T)

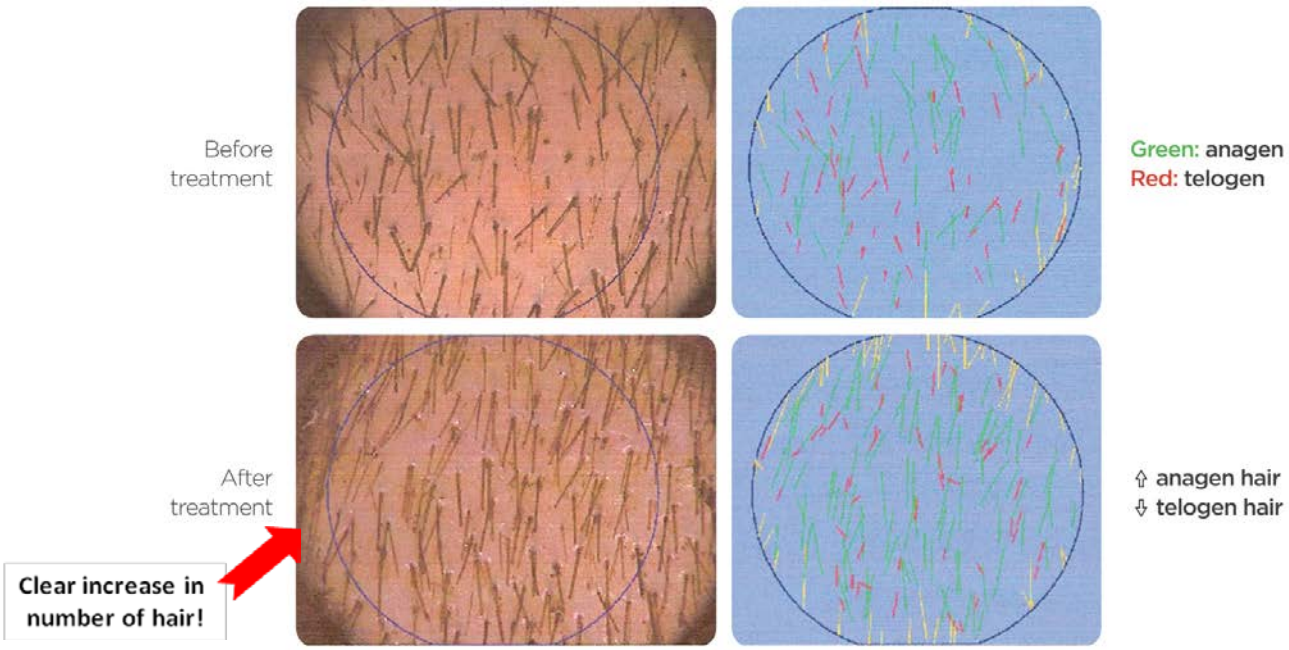
The A/T ratio is a comparison of the numbers of anagen and telogen hair follicles, which is an indication of the percentage of active hair follicles.

The graphic shows the averaged beginning-of-study 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.

Before & After Pictures

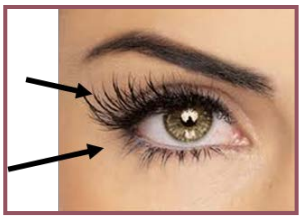


Capixyl™ increases the A/T ratio of 46% compared to a reduction of -33% for the placebo attesting the efficacy for stimulating hair growth and reducing hair loss

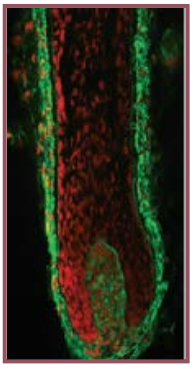
CLINICAL STUDY ON EYELASHES

As eyelash characteristics are very similar to those of scalp hair, Capixyl™ represents the ideal and perfect solution to modulate eyelash growth in order to avoid eyelash loss and stimulate biological mechanisms behing eyelash growth.

Compared with human hair, little is knowm about eyelashes. In the embry, eyelashes develop between the 7th and 8th week. They grow in imperfect rows of five to six in the upper and three to four in the lower lid[43].



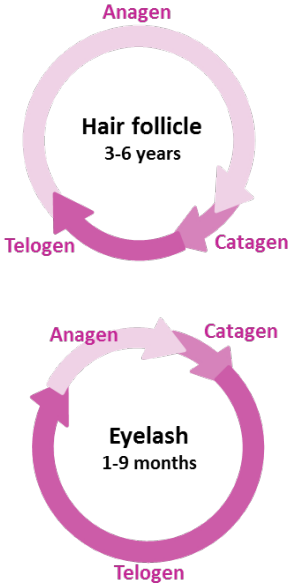
Their mean number is 90-160 in the upper and 75-80 in the lower lid, while their length varies from 8 to 12 mm in the upper and from 6 to 8 mm in the lower lid[44],[45].



The terminal eyelash fibre is characterized by a regular curve shape, more or less marked depending on ethnic origin[46]. Microscopy of eyelash revealed a structure very close to that of hair fibre, with three compartments from the outside to the inside.
However, the eyelash fibre is much shorter than scalp hair, due to a shorter hair cycle. The growth rate is approximately 120 µm daily[47] and the duration of anagen and telogen phases is estimated to be 1-4 and 4-9 months respectively. At any given time, 59-85% of eyelash follicles are in the telogen phase, depending on whether upper or lower lid[48].

Comparison between hair growth cycle and eyelash growth cycle

	Anagen Growth phase	Catagen Transition phase	Telogen Resting phase	Growth rate
Hair follicle	2 - 6 years 70 - 85% hair	100 days 15 - 30% hair		0.3 - 0.6 mm daily
Eyelash	1 - 3 m (ref.1) 22 - 55 d (ref.2) 15 - 40%	4 - 9 m (ref.1) ~2 m (ref.2) 60 - 85% lashes		0.12 mm daily



OBJECTIVES

The aim of the study was to evaluate the efficacy of Capixyl™ versus placebo under normal conditions of use.

Relevancy

Cosmetic products present effects on ciliary characteristics, which are shown through biometrological measurements and subjective evaluations. These observations were performed in normal conditions of use.

Study design

Design: The study was open and randomized. The treated eye on which Capixyl™ gel was applied was randomized.

The average age of the subjects was: 49.4 ± 14.4 years old (extreme values: 25 - 68 years old).

17 subjects were included according to the following criteria:

Conditions of use (application)

Capixyl™ or placebo gel was applied on the upper and lower lashes of the eye designed by the randomization, in the morning, before the application of the usual mascara and a second time, alone, in the evening, during 8 weeks.

2 steps application of 2.5% Capixyl™ eye gel - twice a day (morning and evening) for 8 weeks:

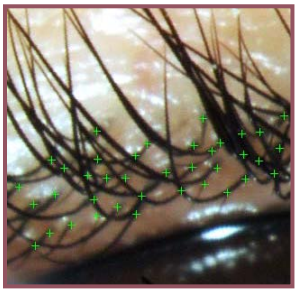
1. Application of the gel to skin using applicator: «draw the applicator carefully along the skin at the base of the upper and lower eyelashes (where the eyelashes meet the skin)".

2. Application of the gel on lashes using the brush as close to the roots of your lashes as possible and wiggle before brushing out to get more product applied to every lash. Lightly brush the tip back and forth across lower lashes.

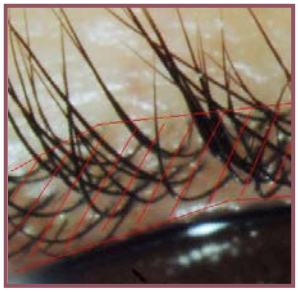
Parametres evaluation

Three biological parameters were evaluated at D0, after 4 and 8 weeks:

1. Lash density:



At D0, 4 8 weeks, photographs of the upper lashes were taken with a camera fixed on a biomicroscope. The number of lashes in the specific area were then analyzed and counted.



After counting, the surface where the lashes growth was measures and the density was determined.

2. Evaluation of the number of young lashes:

Young lashes have the followed characteristics (in comparison with adult lashes):

- less coloring
- thinner
- shorter

At D0, 4 weeks and 8 weeks, photographs of the upper lashes were taken with a camera fixed on a biomicroscope.

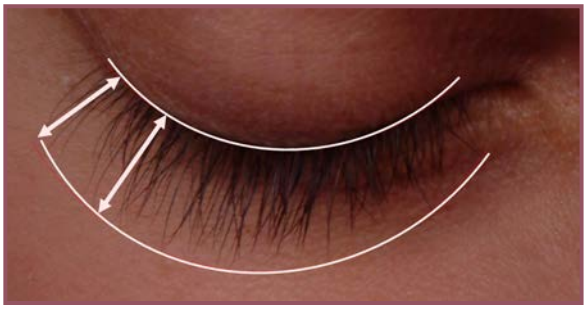
The number of new lashes according the morphology and characteristics of young lashes were then counted..

3. Length of the lashes:

Assessment of the mean length of the upper ciliary fringe (for each eye)

At D0, 4 weeks and 8 weeks, photographs of the upper ciliary fringe were taken with a digital camera

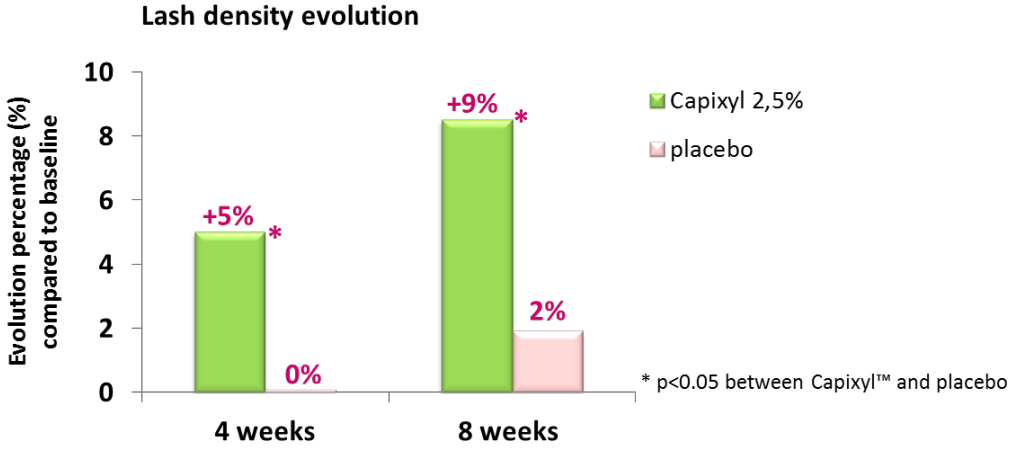
The mean of the length of the lashes (mm) was measured



Results

Evaluation of lash density

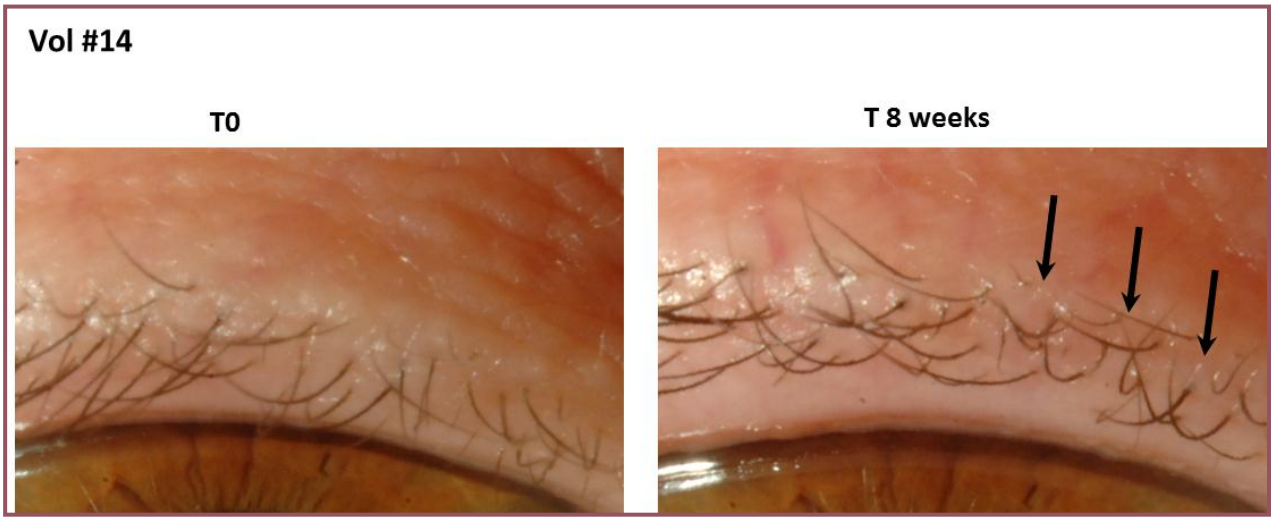
Lash density was measured after 4 and 8 weeks for each volunteers; results showed here after presented the average of lash density evolution of all the volunteers.



We observed a clear and significant evolution of lash density when eyelashes were treated with 2.5% Capixyl™ in comparison with placebo treatment (on the other eye).
The lash density is increased up to 27% after 8 weeks application of Capixyl™ gel.

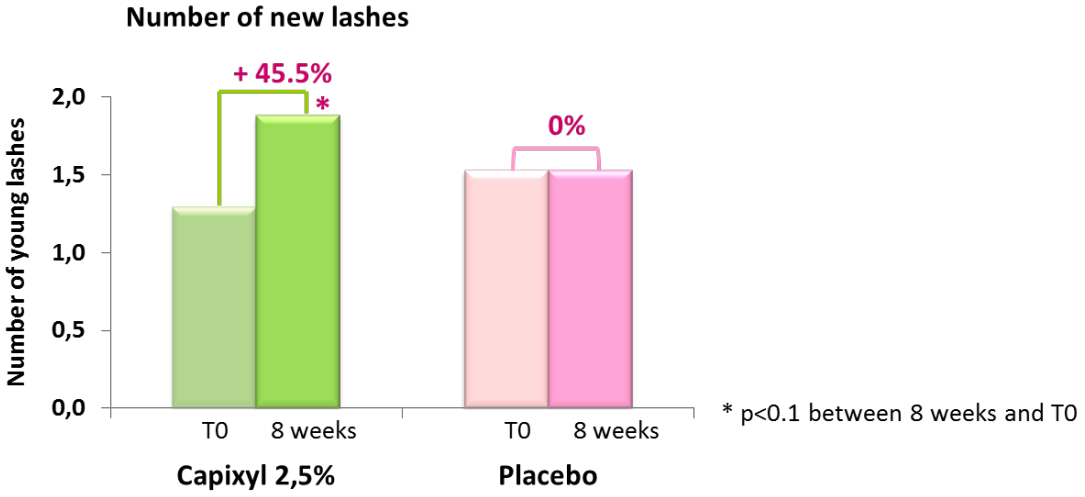
It is important to note that after 4 weeks, 73% of the subjects improved in lash density and 93% had an effect after 8 weeks.

The improvement in lash density also indicated that Capixyl™ treatment induced an increase in new lashes and decrease in falling lashes.



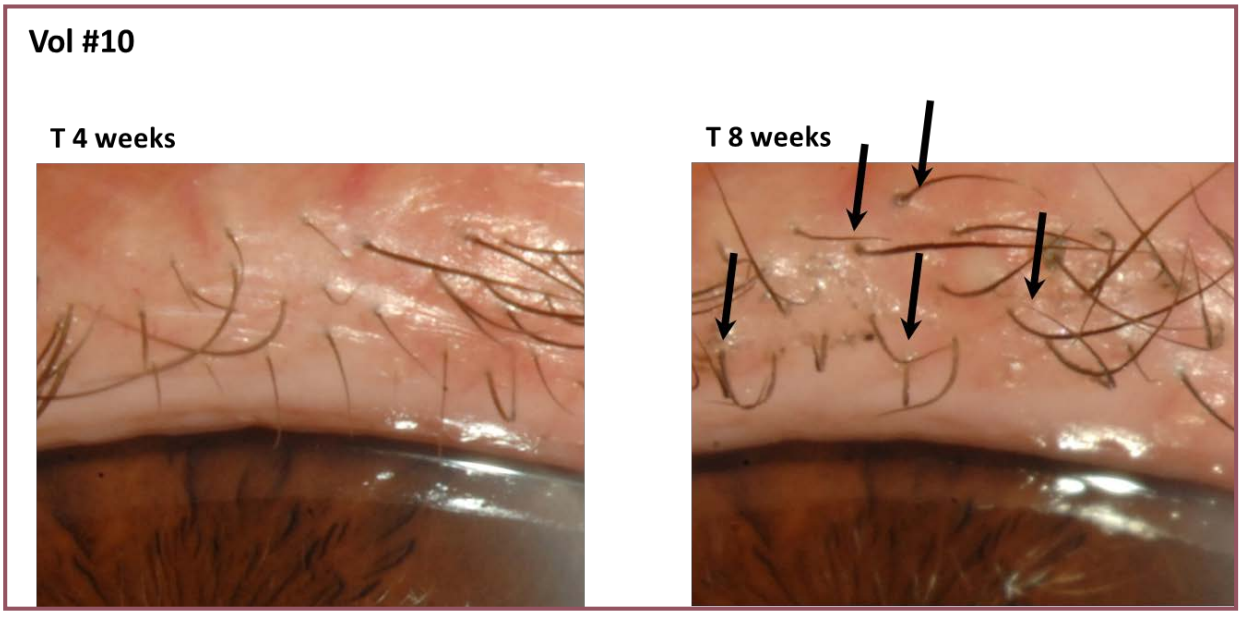
Evaluation of number of new lashes

New lashes were identified and counted after 8 weeks treatment with gel containing 2.5% Capixyl™ or placebo gel.



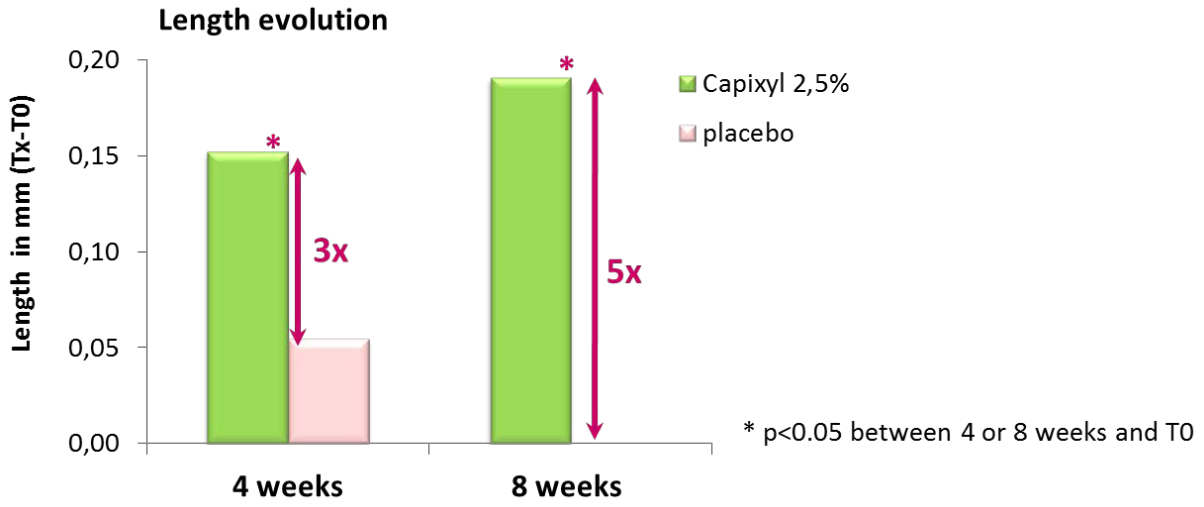
We observed a significant increase in new lashes after application of Capixyl™ gel by 45.5% in comparison with placebo treatment no new lashes were measured.

Illustration showed here after attest the evolution between 4 and 8 weeks as we can cleary observed new lashes at the upper lid area.



Evaluation of lashes lenght

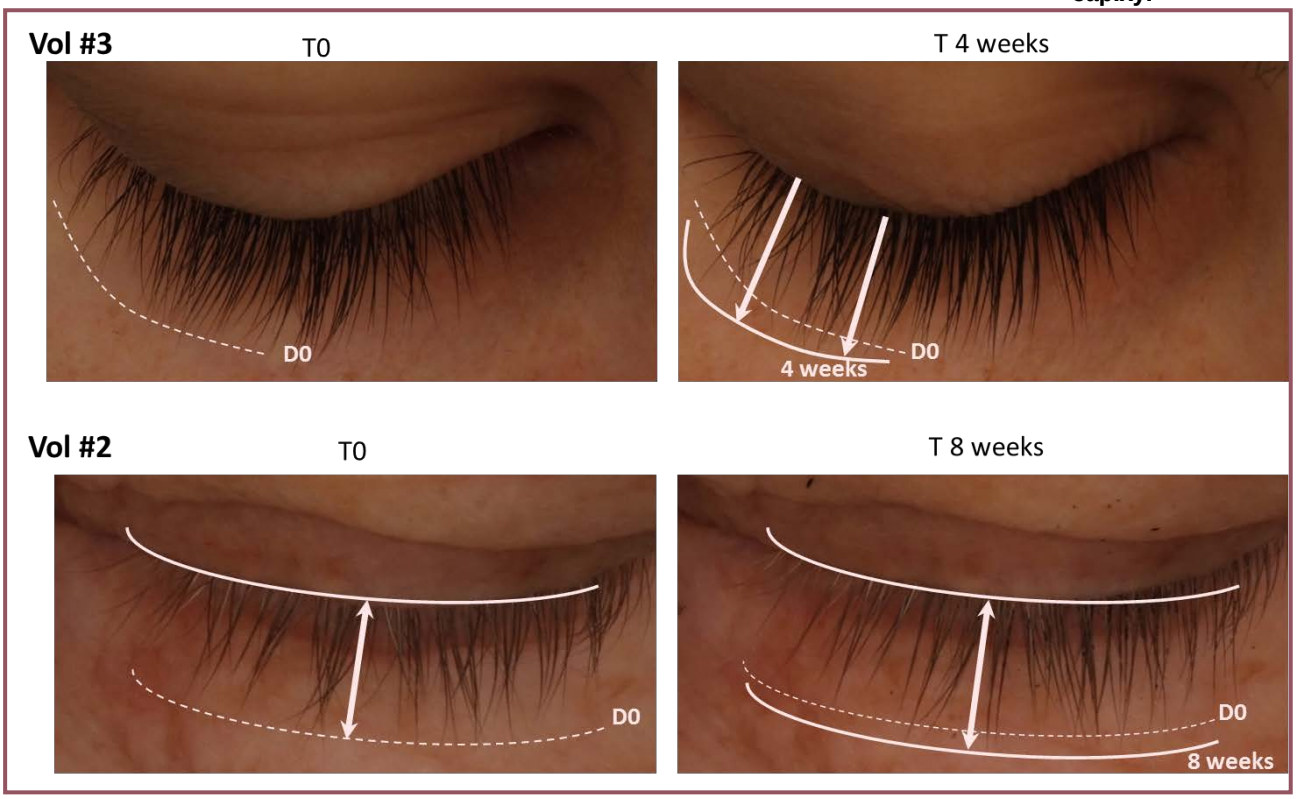
The length of lashes were measured at D0 and after 4 and 8 weeks treatment of gel containing or not Capixyl™ 2.5%.



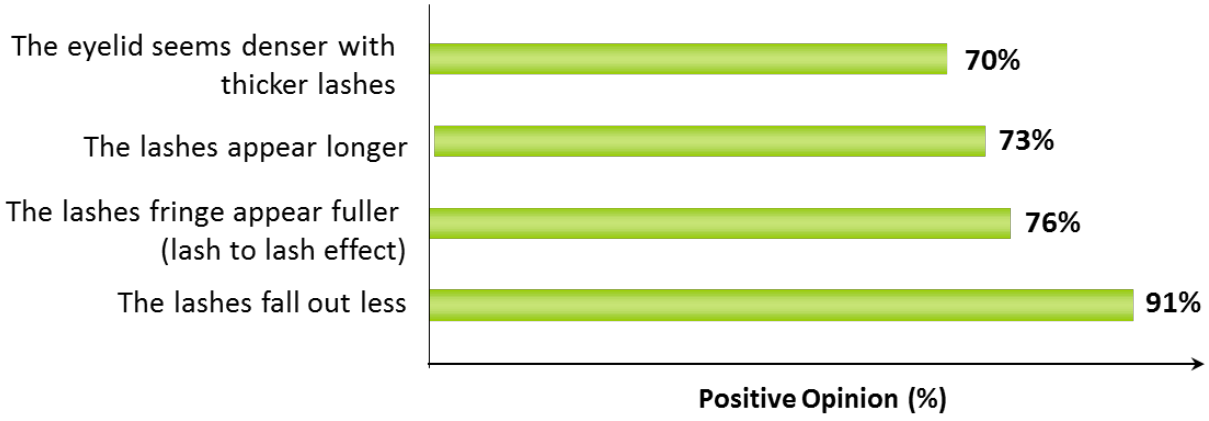
A significant increase of lash length was observed after 4 and 8 weeks treatment with Capixyl™. We measured up to 0.70 mm growth after 4 weeks treatement.

The evolution of lash length is 3 and 5 times better after Capixyl™ application (4 and 8 weeks respectively) in comparison with placebo.

After 8 weeks 73% of the subjects saw an improvement in the length of their lashes.



Consumer perception



CONCLUSION

Capixyl™ is an innovative and powerful cosmetic ingredient providing fuller, thicker and healthier looking hair.
Capixyl™ promotes thicker, fuller and more luscious looking lashes!
It is a complex combination of red clover extract rich in Biochanin A with a patented signal peptide.
It has outstanding results.
It has higher activity than Minoxidil.
It has clinical results to prove its significant anti-hair loss effect and hair regrowth.
Capixyl™ is an innovative & powerful active for eyelashes growth and for decreasing the lash loss process.
Capixyl™ has clinical results to prove its significant anti-lash loss effect and lash regrowth.

COSMETIC APPLICATIONS

- **Hair treatment**
- **Men hair care product**
- **Leave-on**
- **Scalp treatment**
- **Tonics**
- **Anti-aging hair care product**
- **Treatment for seasonal hair loss**
- **Hair treatment for menopaused women**
- **Eyelashes**
- **Eyelash growth mascara**
- **Eyelash serum**
- **Eyelash conditioner**
- **Anti-lash loss**
- **Eyelash treatment**
- **Active makeup**

RECOMMENDATION OF USE

Capixyl™ is easy to use and has excellent stability.

Capixyl™ should be incorporated at the end of the manufacturing process, at a temperature below 40°C.

Recommended dosage:

- Intensive treatment: 5%

- Preventive care: 0.5 – 2.5%

ANTI-HAIR LOSS POTION

	INGREDIENTS	INCI NAME	%
A	Water	Water	qsp
	Sodium Phosphate	Sodium Phosphate	1.117
	Citric Acid	Citric Acid	0.715
B	Tinogard Q	Tris (Tetramethylhydroxypiperidinol) Citrate	0.025
	UCON 50-HB-3520	PPG-28-Buteth-35	0.50
	Mirasil DMCO	PEG/PPG - 22/24 Dimethicone	0.50
	Capixyl™	Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and) Trifolium Pratense (Clover) Flower Extract	5.00
	Ethanol	Alcohol	20.00
C	Eumulgin HPS	Coceth-7 (and) PPG-1-PEG-9 Lauryl Glycol Ether (and) PEG-40 Hydrogenated Castor Oil	0.60
	Parfum Neva	Fragrance	0.10

Mixing Procedure

1. Solubilize Sodium Phosphate in water, after complete solubilization, add citric acid.
2. One phase A is homogenous, introduce Phase B ingredients one at the time (in indicated order) and homogenize after each ingredient addition.
3. Mixed the solubilisant with the fragarance and then slowly add this pre-mix to the mix (phase A & B)..
   (NB : the fragrance pre-mix should be completly transparent before introducing it into the mix).
4. Check pH.

Caractéristiques

Aspect : Clear solution
pH = 5,50 – 5,70
Stabilitiy : 1 month at 45°C / 3 month at 40°C

Formulation Example for Eyelash Treatment

Formulation (gel) developed in partnership with SECOS SAS (Cheops group)

Commercial Name	INCI	%
water	aqua	68.100
glycerine	glycerin	3.000
chlorophenesine	chlorphenesin	0.250
carbopol Ultrez 10	cabomer	1.000
water hydroxyde sodium 20%	aqua sodium hydroxide	2.300
water	aqua	20.000
PVP VA S30	VP/VA copolymer	2.000
Borax decahydryte	sodium tetraborate decahydrate	0.100
Boric acid	Boric acid	0.660
microcare MT	aqua methylisothiazolinine	0.090
Capixyl™	bytylene glycol aqua acetyltetrapeptide-3 red clover exctract	2.500

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