ACTIVE INGREDIENTS

TECHNICAL FILE

CAPIXYL™

Multifunctional hair fertilizer

Biomimetic peptide combined with a red clover extract

Stimulates hair follicle regeneration and decreases its miniaturization

Provides fuller, longer hair and lashes while supporting a healthy scalp environment in both leave-on and rinse-off applications

LUCASMEYER COSMETICS

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SUMMARY

INCI NAME	Butylene Glycol (1) (and) Water (2) (and) Dextran (3) (and) Acetyl Tetrapeptide-3 (4) (and) Trifolium Pratense (Clover) Flower Extract (5)
CAS	107-88-0 (1), 7732-18-5 (2), 9004-54-0 (3), 827306-88-7 (4), 85085-25-2 (5)
EINECS	203-529-7 (1), 231-791-2 (2), 232-677-5 (3), - (4). 285-356-7 (5)
ORIGIN	Synergistic combination of a biomimetic peptide with a red clover flower extract rich in biochanin A
COSMETIC PROPERTIES	• Preserves hair follicle stem cell (HFSC) number and activity • Modulates DHT via 5-α reductase activity inhibition • Stimulates ECM renewal and anchoring protein synthesis • Decreases pro-inflammatory cytokines • Rebalances scalp microbiota
HAIR BENEFITS / CLAIMS	• Provides fuller, longer, and healthier hair and lashes • Provides a healthier scalp environment to support hair growth
APPLICATIONS	• Anti-hair loss care • Hair growth care • Scalp care • Lash & brow serums • Makeup
RECOMMENDED DOSAGE	Preventive care: 0.5 – 1.5% Intensive care: 1.5 - 5.0%
PH RANGE USE	4.0 – 8.0
INCORPORATION	Should be incorporated at the end of the formulation at a temperature below 40ºC.
INCOMPATIBILITIES	• No known incompatibility observed

INTRODUCTION

Hair is an essential aspect of one's appearance and well-being. Losing hair can negatively affect both men and women, lowering their self-esteem and self-confidence. Hair loss, also known as alopecia, is a common condition with various causes and types.

Around **85% of men and 33% of women** will at some time face hair loss. The most common type of hair loss, **androgenetic or androgenic alopecia**, is responsible for **95% of all hair loss cases.**

Androgenetic alopecia (AGA), also known as male pattern baldness (MPB), affects millions of people worldwide. **It is estimated to affect about 50% of men and women over 40.** Its prevalence varies by region, gender, and age. In the US, about two-thirds of men experience some hair loss by age 35, and 25% start before age 21. AGA affects 35 million men and 21 million women in the United States. In France, hair loss affects 10 million people, representing 2 out of 3 men and 1 out of 5 women. In Japan, 30% of men experience balding, usually later in life, after age 45. In China, 21% of men and 6% of women have AGA. Furthermore, about **13% of premenopausal women** show some signs of AGA[1]. However, AGA worsens in **women after menopause**, affecting **75% of those over 65 years old**[2].

While AGA is the most prevalent type of hair loss, other types include alopecia areata, alopecia totalis and universalis, trichotillomania, and scarring alopecia.

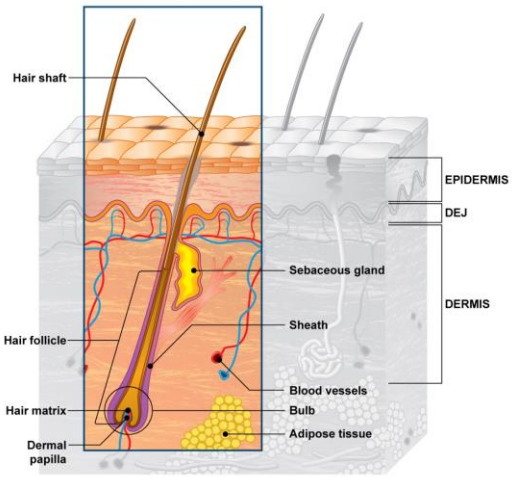
HAIR LOSS MARKET

People increasingly value their appearance and seek a cost-effective and reliable way to restore their hair. This demands high-quality and scientifically validated products that prevent and limit hair loss. In **2022**, the **global hair loss market** was worth **USD 52.37 billion,** based on the sales of products and treatments for hair loss. The market has expanded by an annual average of **6.72% in the past five years.**

The most common products on the market addressing hair loss are Minoxidil (Regain®), a non-prescription vasodilator drug that can reduce or prevent hair loss and stimulate hair growth; finasteride (Propecia®), another drug that works by blocking the enzyme that turns testosterone into dihydrotestosterone (DHT) in AGA and Aminexil®, a patented compound by l'Oréal[3].

HAIR SCIENCE

The biology of hair is more complex than it appears. The hair shaft results from accumulating a solid structural protein called keratin, produced by keratinocytes of the hair follicle (hair matrix).



The mature hair follicle contains keratinocytes that extend into the dermis. The base of the follicle is made of a bulb of specialized dermal cells, the dermal papilla (DP), which play a crucial part in the regulation of successive cycles of hair growth[4]. The DP is also a blood-rich part that supplies nutrients and oxygen to the growing hair attached to it. At the onset of hair growth phases, DP signals the epithelial stem cells residing in the bulge region of the follicle to divide transiently. Stem cell progeny migrate to the follicle's base, surrounding the DP, forming the hair matrix. In response to further signals from the DP, matrix cells proliferate and begin the process of terminal differentiation, moving upward in the follicle and forming the hair shaft and inner root sheath[5].

The hair follicle’s healthy condition is a major criterion for optimal hair growth.

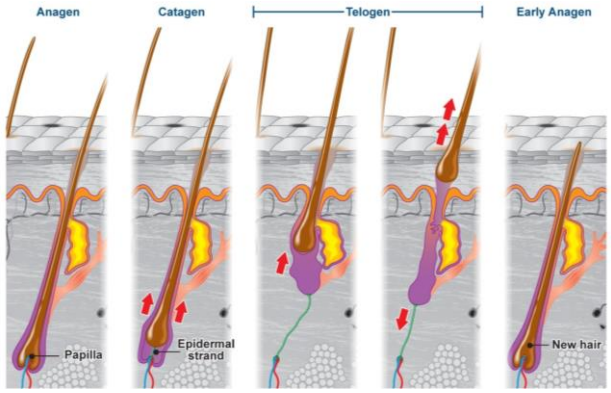
Most organs attain homeostasis upon reaching maturity. However, hair follicles do not exhibit this characteristic. They persist in regenerating new hairs in cycles. This occurs approximately twenty times throughout a person’s life. Each hair follicle and strand undergo three distinct stages, from the growth initiation to the hair's shedding.

- **Anagen (active growth phase):** This phase typically lasts 2 to 6 years, with hair actively growing (3-4mm/day). It’s estimated that about 85-90% of hairs are in the anagen phase at any given time.

- **Catagen (regression phase):** This phase usually lasts 2 to 3 weeks. During this phase, the follicle shrinks toward the surface, active hair growth stops, and the hair progressively detaches from the bottom of the hair follicle. About 1-3% of hairs are in the catagen phase at any time.

- **Telogen (resting phase):** The hair stays attached to the scalp for more or less 3 to 4 months and then falls out. The degenerated hair follicle regenerates and migrates downward to the bottom of the epidermis. Generally, 10 to 15% of hairs are in this phase.

When the telogen phase ends, the hair follicle regenerates and returns to the anagen phase. A new hair growth cycle begins when the dermal papilla and regenerated follicle join each other. New hair starts to form again.

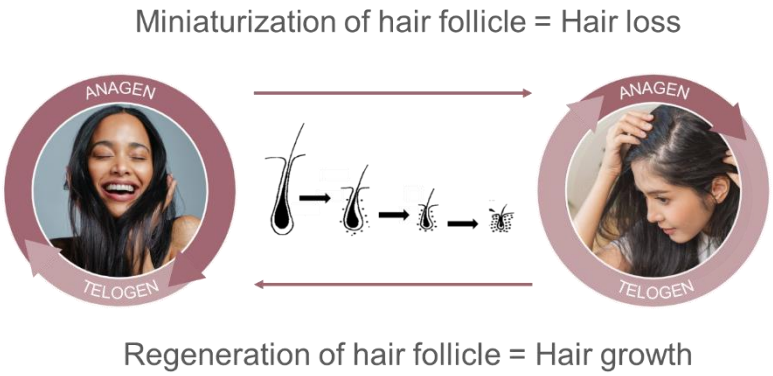


At any given moment, hairs are randomly distributed across the different stages of growth and shedding. Over the years, the number of follicles that can grow hair gradually decreases, a phenomenon that is particularly evident on the scalp's crown. As we age, a more significant proportion of hair in the telogen phase increases, resulting in a reduced rate of hair growth. The new hair that emerges tends also to be finer and less dense.

WHAT CAUSES HAIR LOSS?

Humans have more or less 100,000 hairs on their scalp. A daily loss of 40 to 100 hairs is considered normal, but this number can temporarily reach 175 during seasonal changes (autumn and spring). Alopecia is a loss of more than 100 hairs over a long period. Hair loss can be triggered by several factors: genetic, hormonal changes or imbalances (childbirth, menopause), linked to diet (deficiency in specific vitamins and minerals), stress, diseases like diabetes or lupus, medication, hair treatment (over styling, dye, bleaching) and of course aging.

In all those cases, hair thinning, and loss are due to the same causes: the miniaturization of hair follicles. In this process, hair follicles shrink in size over time due to a shorter anagen phase and a longer telogen phase. Being smaller, the hair follicles produce hair strands that are finer, weaker, and shorter until they can no longer produce any hair (baldness is the final stage of hair loss).



Optimizing the hair follicle's size, environment, and activity is essential to limit hair loss and promote growth.

MAJOR FACTORS CONTRIBUTING TO HAIR MINIATURIZATION

Hair miniaturization is a process that is influenced by various factors, such as:

- **Decrease in HFSC activity:** A reduction in the number and activity of hair follicle stem cells (HFSC) impairs hair follicle size and activity, as these cells are responsible for the continual regeneration of the hair follicle and hair shaft during the hair cycle.

- **Altered extracellular matrix:** The extracellular matrix (ECM) is crucial in directing hair growth and maintaining cell function. If the ECM is not adequately renewed over successive hair cycles, the hair follicle may shrink progressively and lose its functionality. Therefore, maintaining the integrity of the ECM is essential for preventing hair follicle miniaturization and promoting healthy hair growth.

- **High DHT level:** An elevated dihydrotestosterone (DHT) level, associated with high 5α-reductase activity, leads to hair miniaturization. DHT binds to androgen receptors on the hair follicles. This triggers a series of changes that reduce the growth phase and lengthen the resting phase of the hair cycle. Over time, this leads to thinner, shorter, and weaker hairs that eventually fall out.

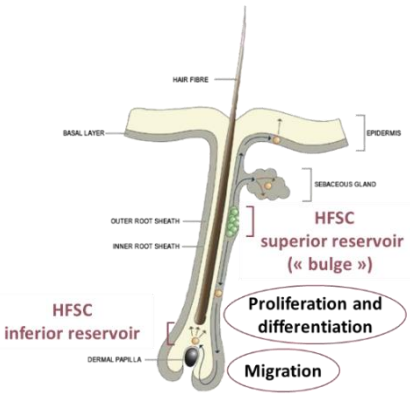
- **Imbalanced scalp microbiota:** An imbalance in the scalp's microbiota leads to increased micro-inflammation and may negatively impact hair follicles' environment and health, contributing to their miniaturization.

- **Micro-inflammation:** Localized micro-inflammation due to excess DHT, chemicals, climate changes, or various external and internal stresses can damage hair follicles and alter their function.

HAIR FOLLICLE STEM CELLS (HFSC) ACTIVITY

Hair follicle regeneration is essential for the initiation of a new hair cycle. This process depends on the activity and the number of hair follicle stem cells (HFSC).

HFSC are located in a specialized microenvironment called the niche, which provides signals and cues to regulate their activity. There are two niches for HFSC: the outer bulge niche (superior reservoir) under sebaceous glands and the hair germ niche (inferior reservoir) in the bulb. The superior reservoir contains the quiescent HFSC, which are dormant and only activated when needed. The inferior reservoir has the primed HFSC, the progeny cells derived from HFSC after dividing and differentiating.

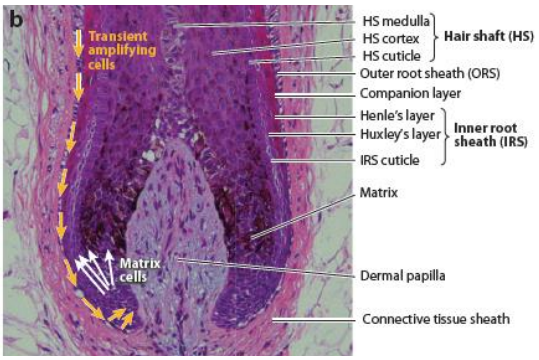


The difference between HFSC and their progeny cells (primed HFSC) is that HFSC are quiescent, meaning they do not divide or differentiate unless signals from the niche or the body activate them. HFSC can also self-renew, producing more HFSC to maintain the stem cell pool. The primed HFSC, on the other hand, migrates to the base of the follicle and surrounds the dermal papilla. These progeny cells are proliferative, dividing and differentiating rapidly and continuously to generate the hair follicle and form the hair shaft[6].

Collagen XVII is highly expressed in the bulge and represents a specific stem-cell anchoring protein required to maintain HFSC quiescence[7]. Furthermore, it was also reported that collagen XVII is also necessary for the self-renewal of HFSC[8].

EXTRACELLULAR MATRIX (ECM) RENEWAL AND INTEGRITY

The dermal papilla (DP) is a fibroblast-containing structure near the base of the hair follicle that plays a vital role in hair growth and follicle health. It initiates and sustains the growth and differentiation of stem cells in the follicle and significantly influences the hair's size, shape, color, and regeneration rate[9].



A direct relationship has been observed between the DP's size and the hair follicle and shaft size[10]. This relationship persists in progressive hair loss in which the size of the follicle and hair it produces is reduced in successive hair cycles until a miniaturized hair follicle results[11]. The volume of the DP depends on the number of cells, which increases either through proliferation or through the migration of cells from the follicular dermal sheath and on the amount of extracellular matrix per cell[12]. Indeed, DP cells are surrounded by a thick ECM rich in collagen III, collagen I, and anchoring proteins such as collagen IV, fibronectin, and laminin, all of which are synthesized actively by the DP cells. This ECM plays a crucial role in maintaining the structure and function of the DP[13].

The highly specialized ECM basement membrane that separates the epithelial compartment (which includes the cells that make up the hair shaft and inner root sheath) and mesenchymal compartment (which consists of the dermal papilla and connective tissue sheath) of the hair follicle also has a prominent expression of these anchoring proteins, along with collagen III, collagen I, collagen VII and glycosaminoglycans. Laminin is crucial in anchoring the hair germ to the dermal papilla, ensuring proper hair anchoring[14]. This membrane is a "boundary" between the two compartments, helping maintain the distinct environments needed to function correctly[15].

The ECM has thus a key role in maintaining cell function, directing hair growth, and ensuring proper hair anchoring. If the ECM is not adequately renewed over successive hair cycles, the hair follicle may shrink progressively and lose its functionality. Therefore, maintaining the integrity of the ECM is essential for preventing hair follicle miniaturization and promoting healthy hair growth.

DIHYDROTESTOSTERONE (DHT) LEVEL

Dihydrotestosterone (DHT), a potent androgen derived from testosterone, plays a significant role in hair miniaturization, particularly in Androgenetic Alopecia (AGA)[16]. The enzyme 5α-reductase catalyzes the conversion of testosterone to DHT. Elevated levels of DHT are detrimental to hair follicles, as they lead to a shortened anagen (growth) phase and an extended telogen (resting) phase within the hair cycle. This hormonal imbalance results in progressively smaller hair follicles and the production of finer, shorter hair strands until they cannot produce any hair and disappear. Individuals with AGA typically exhibit increased 5α-reductase activity, heightened DHT concentrations, and a higher density of androgen receptors in the scalp despite having normal testosterone levels.

SCALP MICROBIOTA

The scalp microbiota refers to the community of microorganisms on the scalp, including bacteria and fungi. When in balance, these microorganisms assist in controlling the body's immune responses by interacting with immune cells. This interaction helps manage inflammation within the body to maintain homeostasis. However, if this microbial balance is disturbed, it can result in persistent micro-inflammation, which may damage the surrounding environment[17].

Maintaining a healthy balance of bacteria on the scalp is especially crucial to avoid issues like dandruff, seborrheic dermatitis, and hair loss. Indeed, any microbiota imbalance, or dysbiosis, can cause micro-inflammation and oxidative stress, potentially contributing to hair loss. Recent studies have shown a microbial shift in patients with alopecia conditions, where the ratios of *C. acnes* to *S. epidermidis* and C*. acnes* to *S. aureus* are significantly higher, while no significant differences in the *S. epidermidis* to *S. aureus* ratio. A notable increase in the abundance of *C. acnes* also accompanies this shift[18].

MICRO-INFLAMMATION

Micro-inflammation within the hair follicle is increasingly recognized as a significant factor in hair miniaturization and the progression of AGA[19,20]. This subtle form of inflammation, termed "micro-inflammation" by Mahe et al., is characterized by its gradual and almost imperceptible nature, differing markedly from the acute, painful, and destructive inflammation observed in classical scarring alopecia[21]. Factors such as thermal stress, mechanical friction, ultraviolet (UV) exposure, abnormal DHT level, and changes in scalp microbiota are thought to initiate this type of inflammatory response, ultimately impacting the hair growth cycle and leading to the characteristic hair follicle shrinkage seen in AGA.

CAPIXYL™: THE MULTIFUNCTIONAL SOLUTION TO ADDRESS HAIR LOSS

Capixyl™ is an innovative active complex combining a **biomimetic peptide** and a **red clover extract**, which work synergistically to limit hair loss and enhance hair growth.

Acetyl tetrapeptide-3 biomimetic peptide

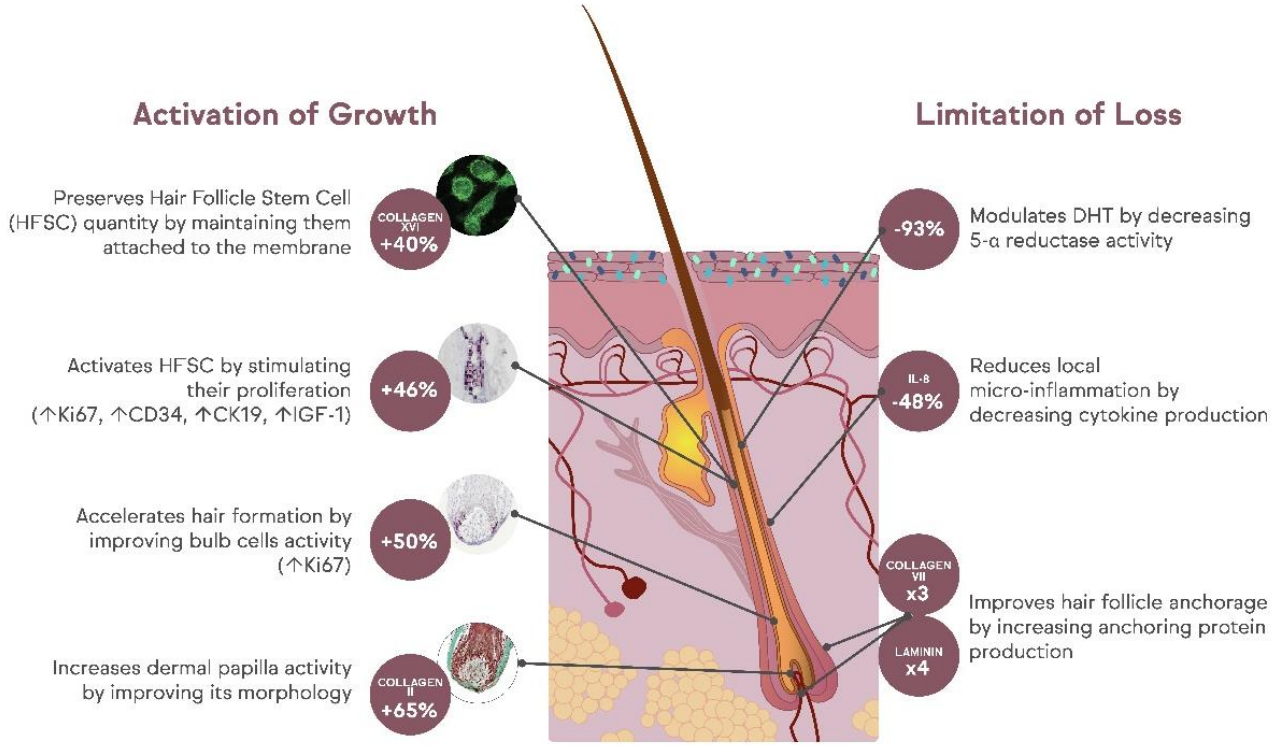
This special peptide, comprised of four amino acids, mimics the SPARC protein function and promotes hair growth by stimulating the production of extracellular matrix proteins. As a result, the hair follicles experience improvements in their dimensions, structure, and anchoring, leading to healthier and stronger hair.

Red clover extract

Red clover *(Trifolium pratense)* 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. These flowers are rich in **biochanin A**, a potent flavonoid with anti-inflammatory properties and an effective inhibitor of 5-α-reductase type I & II activity, allowing the modulation of DHT level; it also has antioxidant properties, limiting free radical damage to the scalp.

MODE OF ACTION

Capixyl™ is a powerful ingredient that effectively promotes hair follicle cell activity, enhances the structural integrity and anchoring of hair follicles, and encourages the synthesis of ECM proteins. Additionally, Capixyl regulates DHT levels by inhibiting 5α-reductase activity and reduces micro-inflammation. All of these actions work together to promote optimal hair regeneration and growth.



Capixyl™ is an excellent solution for promoting optimal hair growth. Its unique blend is specifically designed to rebalance the scalp microbiota, creating a healthy scalp environment that supports healthy hair growth. Whether used in leave-on or rinse-off application, Capixyl™ has been clinically proven to deliver exceptional results in just 4 months.

The effectiveness of Capixyl™ in treating alopecia is undeniable. The clinical data is clear - it increases the hair growth phase and reduces the hair shedding phase to regulate the hair growth cycle, providing remarkable results for both men and women experiencing hair loss. Capixyl™ requires minimal dosage and delivers a rapid response, making it a confident choice for hair loss treatment. It is a superior alternative to Minoxidil, providing faster and better results while remaining safe and reliable.

Capixyl™ is the ultimate multifunctional hair fertilizer for fuller, longer, and healthier hair and lashes.

CAPIXYL™

Multifunctional hair fertilizer

MODE OF ACTION

Stimulates hair follicle regeneration & decreases its miniaturization as it:

- Preserves HFSC activity and number (↑CD34, CK19, IGF-1, Ki-67, Collagen XVII)
- Modulates DHT level (↓ 5-α reductase)
- Stimulates ECM and anchoring protein synthesis (↑ Collagen III, Collagen VII and Laminin)
- Decreases micro-inflammation (↓IL8)

SYNERGISTIC COMPLEX

- Biomimetic peptide combined with a natural red clover flower extract rich in biochanin A, thus meeting consumer needs.

CLINICAL RESULTS

- **Hair:** Proven efficacy in rinse-off and leave-on
  - Increases hair growth phase (anagen)
  - Reduces hair loss phase (telogen)
  - Visibly improves hair density
  - Rebalances scalp microbiota (rinse-off)
- **Lashes:** Increases lash density and length

CONSUMER BENEFITS

- Limits hair loss and promotes hair growth
- Restores microbiota for a healthier scalp
- Provide fuller, longer, and healthier hair and lashes at any stage of life
- Visible results with no side effects in only 90 days
- Suitable for both androgenetic alopecia and general hair loss

MANUFACTURER BENEFITS

- Suitable for hair and lashes
- High efficacy at low dosage
- Tested in rinse-off and leave-on formulas
- Efficient cosmetic alternative to Minoxidil
- Complements the Minoxidil mechanism of action for more results

IN VITRO & EX VIVO STUDIES

EVALUATION OF CAPIXYL™ EFFECT ON HAIR FOLLICLE STEM CELLS (HFSC) ACTIVITY

INTRODUCTION

The hair follicle undergoes repeated cycles of periods of growth (anagen), regression (catagen), and rest (telogen) throughout the life of mammals. Hair follicle regeneration involves stem cell populations that are located in an inferior area and in a superior area (usually called bulge). “Bulge” HFSC are more quiescent than other cells within the follicle[22,23]. HFSC have the potential of generating at least three different cell lineages including hair matrix cells, sebaceous gland cells, and epidermal keratinocytes[24].

Several markers associated with HFSC, including cytokeratin 15, CK19, Nestin, CD200, CD34, CD117, LHx2, and MCSP, have been identified today. Particularly:

- **CD34:** marker used to identify undifferentiated stem cells,
- **CK19 (CytoKeratin 19):** marker of HFSC. CK19 expression is higher in the bulge area of the hair follicle[25].
- **IGF-1 (Insulin-like Growth Factor-1):** growth factor implicated in the early stage of the follicle morphogenesis. IGF-1 can induce stem cell maturation/differentiation and is known to accelerate hair follicle formation and cycling[26]. It was also demonstrated that IGF1 maintains hair follicles in a growth phase (anagen), and removal of IGF-1 leads to a catagen-like regression.

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™ on HFSC activity through the analysis of different markers associated with stem cells (CD34, CK19, and IGF-1).

PROTOCOL

Tested products

Capixyl™ was tested at a concentration of 1%.

Biological materials

Starting with a plastic surgery operation on the scalp of a Caucasian woman aged 49 years, 56 hairs and their bulbs were isolated by micro-dissection. These isolated hairs were placed individually in the wells of two 48 well plates and kept alive in classic cell culture conditions (at 37°C, in an atmosphere enriched to 5% CO_2) with improved Williams medium (Philpott) for 7 days.

Method

- ***Selection of hairs in anagen phase:***

Three days before the beginning of the study (D-3), all the hairs were cut approximately 1 mm from the infundibulum; then, they were photographed with the help of a microscope and a CCD camera joined to software for acquisition and archiving.

On D0, all the hairs were again measured, and only 8 hairs of each batch in the anagen phase were chosen for the rest of the study. The selected hairs grew at an average rate of 50 µm per day for three days before the experiment (between D-3 and D0).

- ***Treatment***

A control batch was left untreated, while Capixyl™ was added to the culture medium at a concentration of 1% from D0 onwards. The culture media was replaced every day.

- ***Sampling***

At D0 and D7, half of the hairs were set by a formaldehyde fixer.

- ***Histological Treatments***

After 24 hours of setting in the swabbed formaldehyde, the samples were dehydrated and coated with paraffin with the help of a Leica TP 1010 dehydration system according to the partner operating procedure. They were placed in a block according to the partner operating procedure with the help of a Leica EG 1160 coating station. Cuts of 5 µm were made according to partner operating procedure with the help of a Minot-type microtome, Leica RM 2125, and mounted on Superfrost® histological glass slides. These cuts were made in view of later immunolabelling.

- ***Immunolabelling of CD34***

Marking of CD34 was performed on frozen cuts with a mouse antibody anti-CD34 human (Santa Cruz, monoclonal TUK3), at 1/100 for 1h at RT with a biotin/streptavidin amplifier system, revealed in fluorescence (FITC).

- ***Immunolabelling of CK19***

Marking of CK19 was performed on frozen cuts with a mouse antibody of anti-CK19 human (Leica, monoclonal), at 1/50 for 1h at RT with a biotin/streptavidin amplifier system, revealed in fluorescence (FITC).

- ***Immunolabelling of IGF-1***

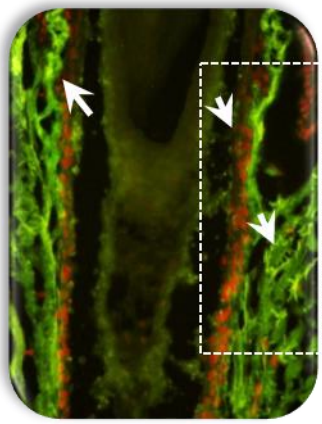
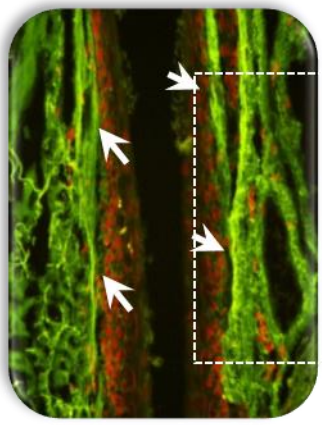
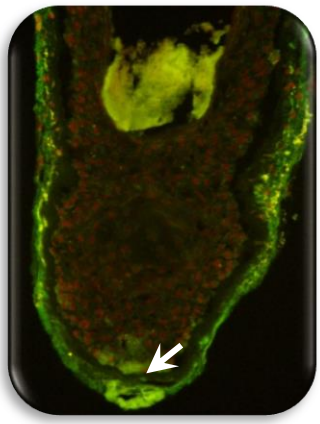
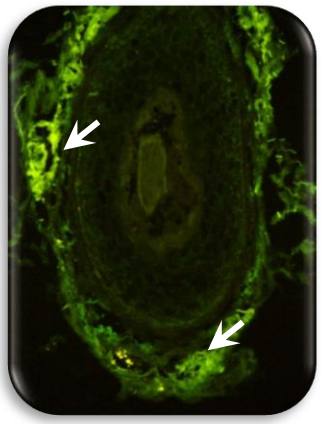
Marking of IGF-1 was performed on paraffin formaldehyde cuts with a rabbit antibody anti-IGF-1 human (Abcam, polyclonal), at 1/100 for 1h at RT with a biotin/streptavidin amplifier system, revealed by peroxidase (RTU Biotinylated PK7200 Vector Kit) and the VIP (Vector SK 4600). The nuclei were counter-colored with Masson hemalum.

Evaluation

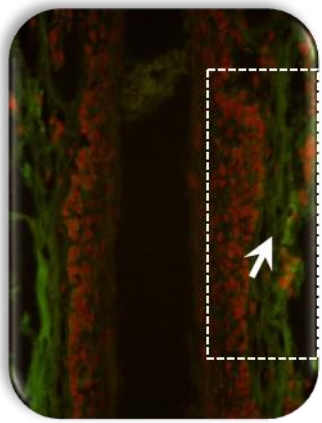
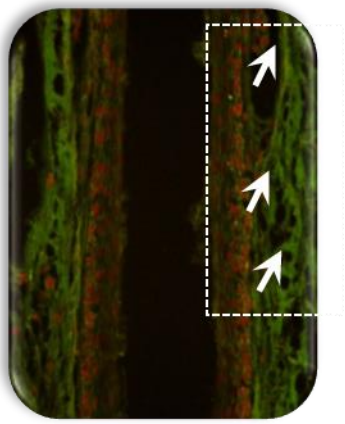
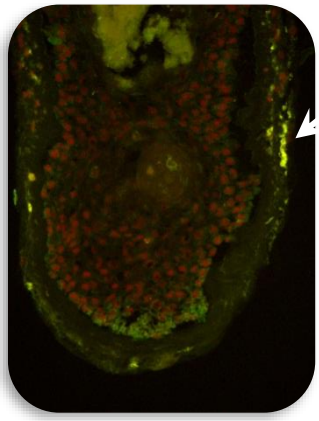
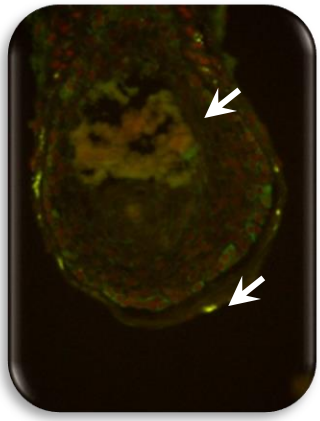
The marking of the 3 markers (CD34, CK19, and IGF-1) was evaluated by microscopic examination of the entire hair length. The observations were performed in the 3 main zones of the hair: the superior stem cells reservoir zone (“bulge”), the median zone (sheath), and the bulb.

RESULT

Evaluation of CD34-positive cells

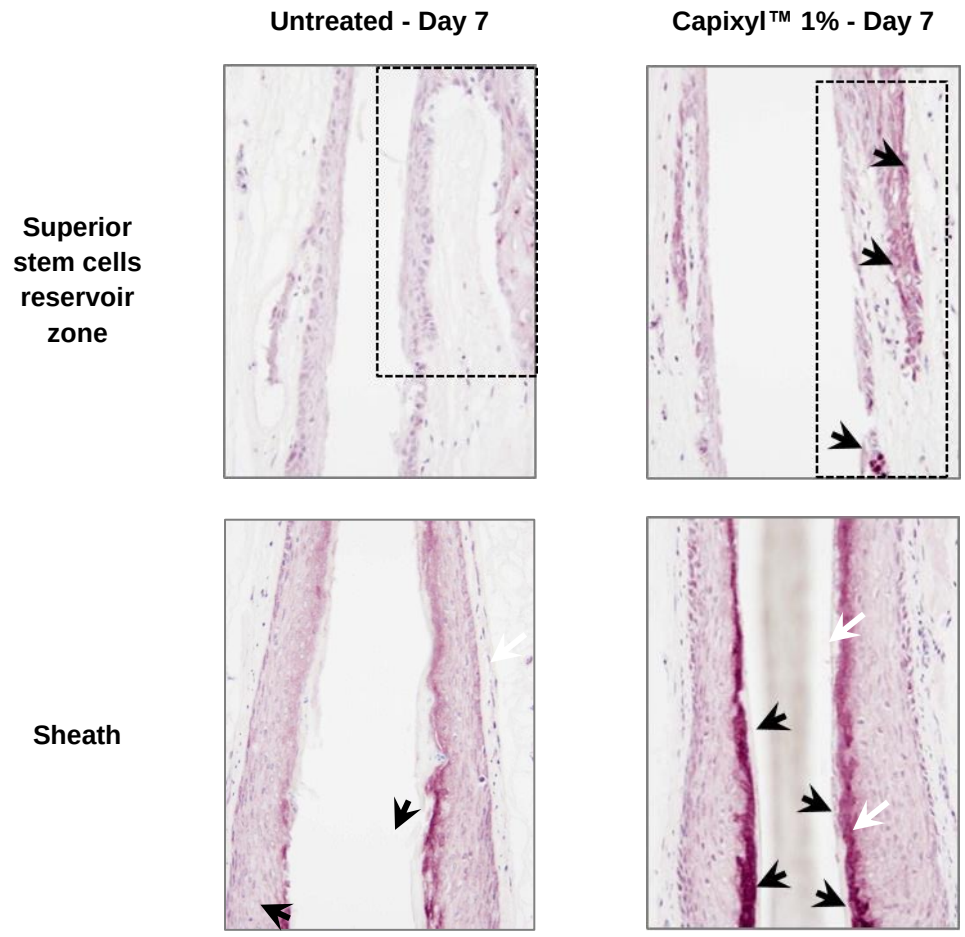
	Untreated - Day 7	Capixyl™ 1% - Day 7	
Superior stem cells reservoir zone			Apparent increase in the CD34 immunostaining (in green) in the superior reservoir zone after 7 days of treatment with Capixyl™
Bulb zone			Moderate increase in the CD34 immunostaining in the bulb zone after 7 days of treatment with Capixyl™

Evaluation of CK19-positive cells

	Untreated - Day 7	Capixyl™ 1% - Day 7	
Superior stem cells reservoir zone			Moderate increase in the CK19 immunostaining in the superior reservoir zone after 7 days of treatment with Capixyl™
Bulb zone			Slight increase in the CK19 immunostaining in the bulb zone after 7 days of treatment with Capixyl™

After 7 days of treatment with Capixyl™, an increase in CD34 and CK19 immunostaining was observed compared to the untreated control, attesting to a rise in HFSC activity and quantity.

Evaluation of IGF-1-positive cells



After treatment with Capixyl™, there was a notable increase in the amount of IGF1 (indicated by pink and violet) in the superior reservoir and root sheath. This increase could be linked to the labeling of CD34 and CK19 and may indicate an activation of stem cell activity and migration along the outer root sheath towards the base of the hair follicle.

CONCLUSION

Capixyl™ increases HFSC quantity and migration for better hair follicle regeneration

EVALUATION OF CAPIXYL™ EFFECT ON COLLAGEN XVII SYNTHESIS

INTRODUCTION

Type XVII Collagen is highly expressed in the bulge and represents a specific stem-cell anchoring protein required to maintain epithelial stem cell quiescence[27]. Furthermore, Tanimuru et al.[28] reported that collagen XVII is also necessary for the self-renewal of hair follicle stem cells (HFSC).

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™ on type XVII collagen synthesis.

PROTOCOL

Tested products

Capixyl™ was tested at the following concentrations: 0.05, 0.1 and 1%. TGF-β was used as a positive control at 10 ng/mL (Sigma).

Biological materials

The keratinocytes NCTC 2544 were isolated from a normal human epithelial cell line. The culture was realized with DMEM complete (Eurobio) containing 10% fetal calf serum (Eurobio), 1% antibiotics (penicillin/streptomycin, Eurobio) and 1% L-glutamine (Eurobio) at 37°C under 5% CO_2 and 95% moisture.

Method

- ***Incubation protocol***

For the test, $5x10^4$ cells were seeded in microplates in DMEM complete for 24h at 37°C under 5% CO_2 and 95% humidity. The culture medium was then replaced with DMEM containing 1% fetal calf serum, 1% antibiotics (penicillin/streptomycin), and 1% L-glutamine at 37°C under 5% CO_2 and 95% moisture for another 24 hours. After the incubation period, TGF-β or Capixyl™ was added for 24 hours before immunolabelling.

- ***Collagen XVII immunolabelling***

Collagen XVII was specifically labeled with a type XVII collagen antibody (Santa CRUZ) diluted at 1% and revealed with a second antibody (anti-goat IgG-FITC, Santa CRUZ).

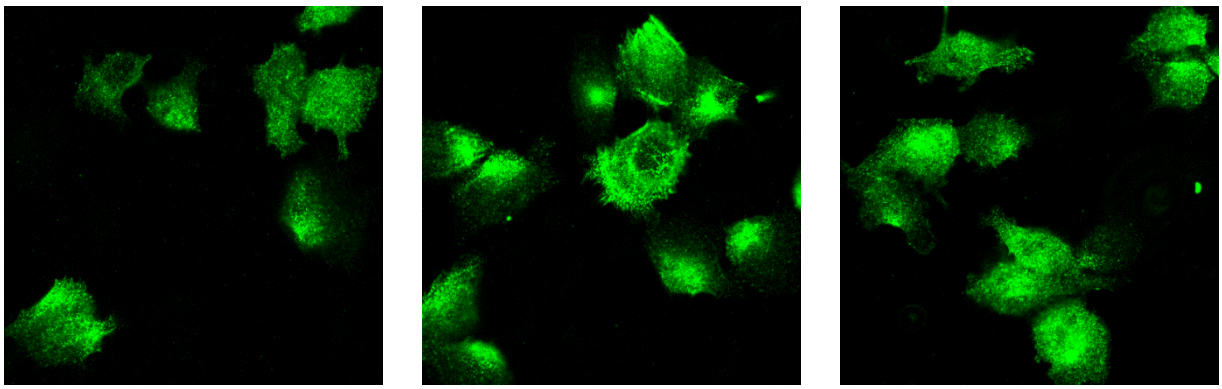
Evaluation

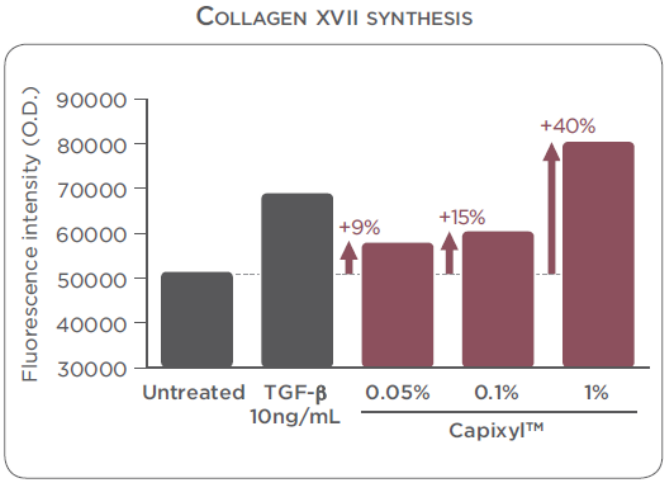
The effect of Capixyl™ on the synthesis of type XVII collagen was evaluated by comparing the fluorescence intensity observed on cells incubated with or without acetyl tetrapeptide-3. The images show the staining of type XVII collagen (green staining) on control (untreated condition),

TGF-β, acetyl tetrapeptide-3-treated fibroblasts. The graph represent a semi-quantitative evaluation of type XVII collagen synthesis.

RESULT

Control (untreated) | **TGF-β 10 ng/mL** | **Capixyl™ 1%**





Capixyl™ stimulated collagen XVII synthesis by up to +40% compared to the untreated condition.

CONCLUSION

Capixyl™ stimulates type XVII collagen synthesis for improved HFSC anchoring and renewal

EVALUATION OF CAPIXYL™ EFFECT ON HAIR FOLLICLE STEM CELLS ACTIVITY

INTRODUCTION

Ki-67 is a protein that is associated with cell proliferation, making it an excellent marker for determining the growth fraction of a given cell population. In the context of hair follicles, Ki-67 is a valuable marker for assessing cell proliferation in hair follicle keratinocytes matrix, which are located below the widest part of the dermal papilla and in HFSC[29]. HFSC in the bulge area can also self-renew, producing more HFSC to maintain the stem cell pool. By using this marker, it is possible to gain insights into the rate at which these cells are dividing and, thus, the activity level of the hair follicles. Furthermore, a direct relationship has been observed between the DP's size and the hair follicle and shaft size[30]. A prominent and well-structured dermal papilla is essential for optimal hair follicle regeneration and growth.

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™ on hair follicle cells activity and the size of the dermal papilla.

PROTOCOL

Tested products

Capixyl™ was tested at a concentration of 1%.

Biological materials

Starting with a plastic surgery operation on the scalp of a Caucasian woman aged 49 years, 56 hairs and their bulbs were isolated by microdissection. These isolated hairs were placed individually in the wells of two 48 well plates and kept alive in classic cell culture conditions (at 37°C, in an atmosphere enriched to 5% CO_2) with improved Williams medium (Philpott) for 7 days.

Method

- ***Selection of hairs in anagen phase:***

Three days before the beginning of the study (D-3), all the hairs were cut approximately 1 mm from the infundibulum; then, they were photographed with the help of a microscope and a CCD camera joined to software for acquisition and archiving.

On D0, all the hairs were again measured, and only 8 hairs of each batch in the anagen phase were chosen for the rest of the study. The selected hairs grew at an average rate of 50 µm per day for three days before the experiment (between D-3 and D0).

- ***Treatment***

A control batch was left untreated, while Capixyl™ was added to the culture medium at a concentration of 1% from D0 onwards. The culture media with or without Capixyl™ was replaced every day.

- ***Sampling***

At D0, D7 and D11, half of the hairs were set by a formaldehyde fixer.

- ***Histological Treatments***

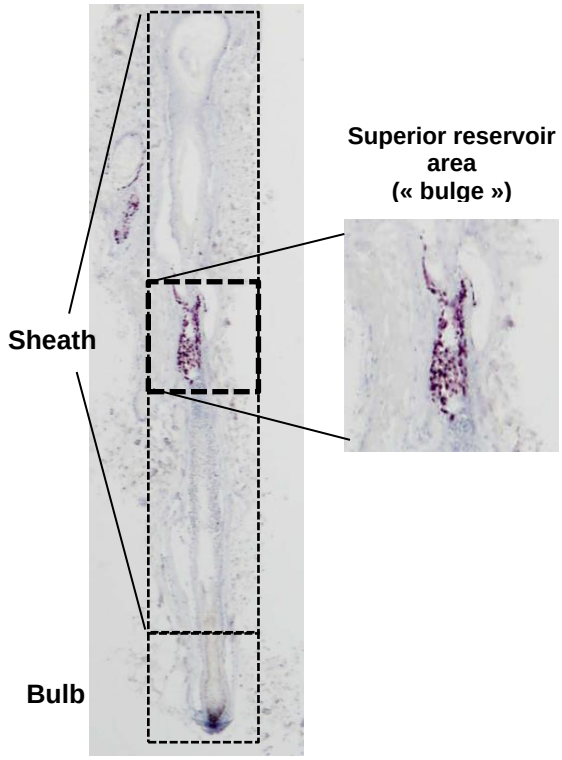
After 24 hours of setting in the swabbed formaldehyde, the samples were dehydrated and coated with paraffin with the help of a Leica TP 1010 dehydration system according to the partner operating procedure. They were placed in a block according to the partner operating procedure with the help of a Leica EG 1160 coating station. Cuts of 5 µm were made according to partner operating procedure with the help of a Minot-type microtome, Leica RM 2125, and mounted on Superfrost® histological glass slides. These cuts were made in view of later immunolabelling.

- ***Immunolabelling of Ki67***

Cells in mitosis were marked on cuts in paraffin with an anti-Ki67, monoclonal 7b11 clone (from Zymed) done on a mouse at 1/100 for 1h at RT with a biotin/streptavidin amplifier system revealed in VIP. The nuclei were counter-colored with Masson hemalum.

Evaluation

The number of Ki67-positive cells was counted over the entire epithelial sheaths/hair follicle length.

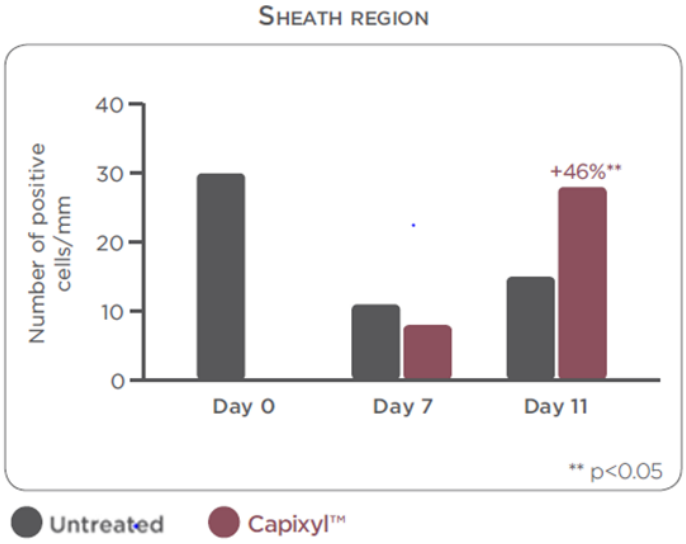


STATISTICAL METHODS

According to normality result, obtained data were submitted to non-parametric Mann-whitney Test. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

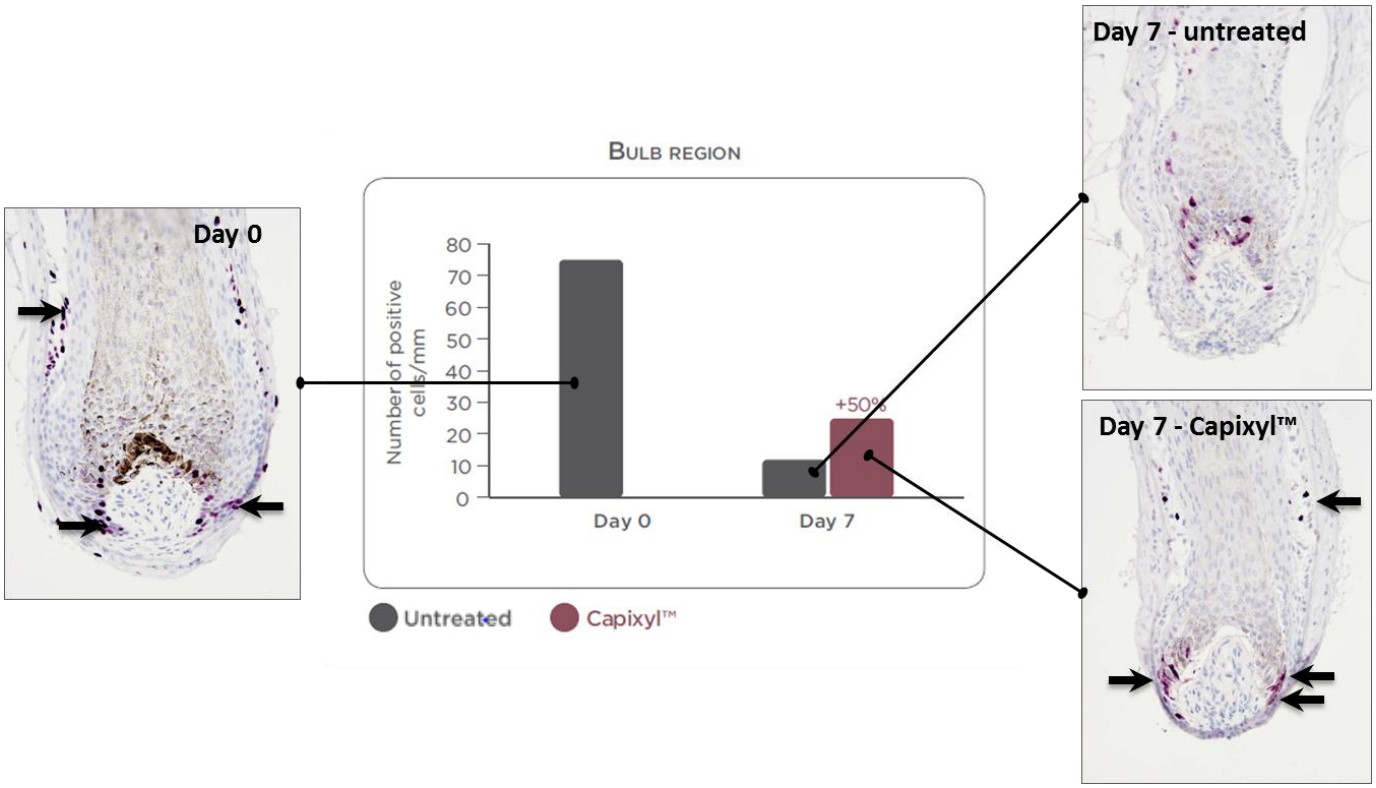
RESULT

Evaluation of the number of Ki67-positive cells in the hair sheaths



Compared to untreated hair on day 7, Capixyl™ did not modify the number of cells in mitosis. However, after 11 days, there was a significant increase in the number of cells in mitosis. The sheaths zone showed intense cell division and activity stimulation, especially in the "bulge" zone, indicating high proliferative activity.

Evaluation of the number of Ki67-positive cells in the bulb area



On day 7, the hair treated with Capixyl™ showed an increase in the number of cells undergoing mitosis compared to untreated hair. Additionally, Capixyl™ increased the dermal papilla's size, resulting in an ideal hair bulb structure for optimal hair growth.

CONCLUSION

Capixyl™ stimulates cell activity, ensuring optimal hair follicle activity and hair formation

EVALUATION OF ACETYL TETRAPEPTIDE-3 EFFECT ON ECM PROTEIN SYNTHESIS

INTRODUCTION

The dermal papilla (DP) supports HFSC activity, hair follicle regeneration, and hair anchoring. The dermal papilla cells, mostly fibroblasts, are surrounded by a thick extracellular matrix (ECM). This ECM is rich in various proteins, such as fibronectin, type I, and type III collagen, all of which are synthesized actively by the DP cells[31]. This ECM plays a crucial role in maintaining the structure and function of the DP.

Furthermore, the basement membrane is responsible for the interface between epithelial and fibroblast cells, particularly between hair germ and dermal papilla cells. Among its components, laminin is crucial in anchoring the hair germ to the dermal papilla and regulating the hair cycle[32].

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™'s peptide, acetyl tetrapeptide-3, on the synthesis of different extracellular matrix proteins (collagen III & laminin) by immunofluorescence.

PROTOCOL

Tested products

Acetyl tetrapeptide-3, Capixyl™'s peptide, was tested at a concentration of 10^{-7}M.

Biological materials

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

Method

Cells were incubated or not with acetyl tetrapeptide-3 for 3 days. Cells were then fixed on the slide, and proteins (type III collagen 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.

- ***Incubation protocol***

For the test, $3X10^4$ cells were seeded in microplates in DMEM complete for 24h at 37°C under 5% CO_2 and 95% humidity. The culture medium was then replaced with DMEM containing 1% fetal calf serum, 1% antibiotics (penicillin/streptomycin), and 1% L-glutamine at 37°C under 5% CO_2

and 95% moisture for another 24 hours. After the incubation period, acetyl tetrapeptide-3 was added for 24 hours before immunolabelling.

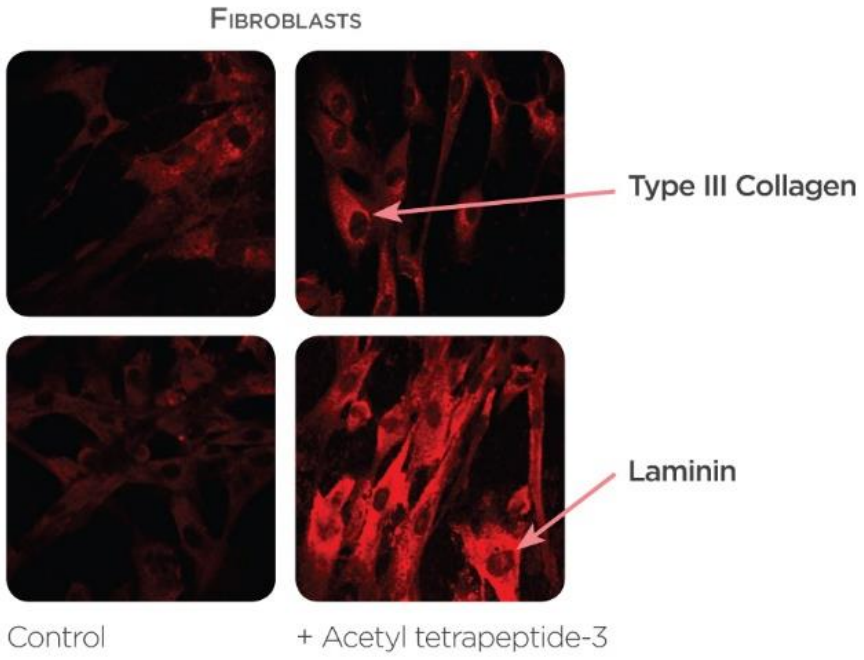
- ***Collagen III and laminin immunolabelling***

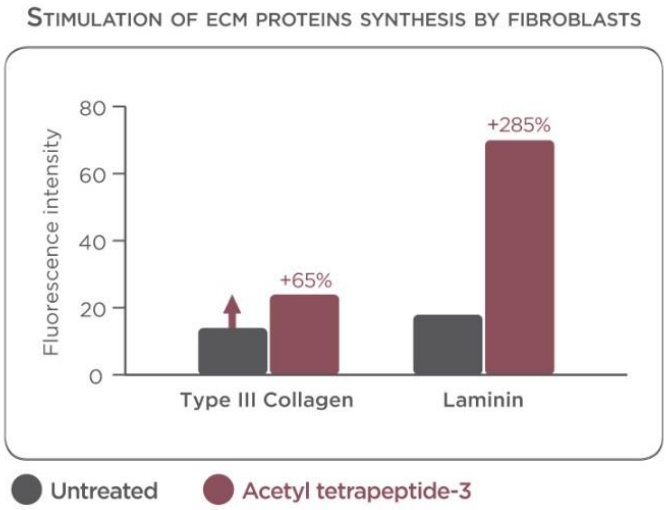
Collagen III and laminin were specifically labeled with a type III collagen and laminin antibodies and diluted at 1% and revealed with a second antibody.

Evaluation

The effect of acetyl tetrapeptide-3 on the synthesis of type III collagen and laminin by fibroblasts was evaluated by comparing the fluorescence intensity observed on cells incubated with or without acetyl tetrapeptide-3. The images show the staining of type III collagen and laminin (red staining) on control (untreated condition) and acetyl tetrapeptide-3-treated fibroblasts. The graph represent a semi-quantitative evaluation of type III collagen and laminin synthesis.

RESULT





After treatment with acetyl tetrapeptide-3, fibroblasts increased the production of extracellular matrix proteins such as type III collagen and laminin.

CONCLUSION

Acetyl tetrapeptide-3 strongly induces the synthesis of ECM proteins, such as type III collagen and laminin, by fibroblasts.

Capixyl™'s peptide stimulates the production of ECM proteins, favoring a better hair follicle structure and hair anchoring

EVALUATION OF ACETYL TETRAPEPTIDE-3 ON COLLAGEN VII SYNTHESIS

INTRODUCTION

The highly specialized ECM basement membrane that separates the epithelial compartment (which includes the cells that make up the hair shaft and inner root sheath) and mesenchymal compartment (which consists of the dermal papilla and connective tissue sheath) of the hair follicle also has a prominent expression of collagen IV, fibronectin, and laminin, along with collagen III, collagen I, collagen VII, and glycosaminoglycans. This membrane is a “boundary” between the two compartments, helping maintain the distinct environments needed to function correctly[33].

The connective tissue-epithelial junction (CEJ) resembles the dermo-epidermal junction (DEJ). The application of corticoids experimentally decreases skin metabolism. The repairing effect of acetyl tetrapeptide-3 on the dermal-epidermal junction following corticoid application is thus evaluated by immunohistological staining of collagen VII proteins.

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™’s peptide, acetyl tetrapeptide-3, on type VII collagen synthesis.

PROTOCOL

Tested products

Acetyl tetrapeptide-3, Capixyl™’s peptide, was tested at a concentration of 10^{-5}M.

Biological materials

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

Method

On day 0, a dermocorticoid (Diproson®) was applied to the skin explants surface to disrupt the DEJ. Acetyl tetrapeptide-3 was added to the cell culture on day 0 and day 1. The treatment was stopped on day 3, and skin explants were prepared for immunohistochemical labeling of collagen VII using an ABC peroxidase kit. The labeling was revealed by AEC substrate (brown color).

Evaluation

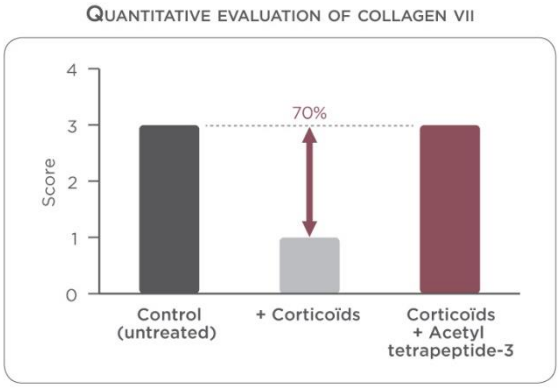
The repair of the DEJ with acetyl tetrapeptide-3 was evaluated through the analysis of immunohistological staining of collagen VII proteins.

Scores ranging from 0 (no staining) to 4 (strong staining) 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

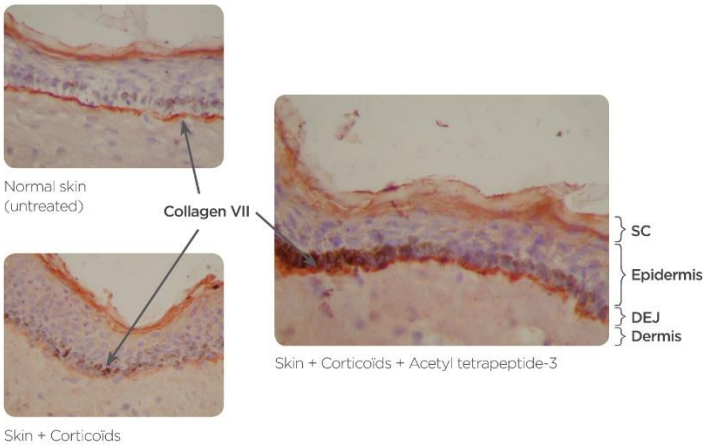
RESULT

Semi-quantitative evaluation of collagen VII staining



Microscopic observations of the collagen VII immunohistological labeling

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



The microscopic observations of untreated skin showed intense labeling of collagen VII along the dermal-epidermal junction. This label corresponds to a score of 3. As expected, application of dermocorticoids on the skin explant reduced collagen VII staining by 70% (score 0.9) demonstrating a altered dermal-epidermal junction properties.

However, the application of acetyl tetrapeptide-3 on the skin, in addition to dermocorticoids, restored the usual amount of collagen VII along the DEJ. The score calculated after the labeling was the same as that obtained for the untreated skin (score 3).

CONCLUSION

The addition of acetyl tetrapeptide-3 on the skin surface restored the DEJ by stimulating the production of collagen VII. These results can be correlated with interfollicular collagen VII since the DEJ is very similar to the CEJ in structure and collagen VII distribution.

Capixyl™'s peptide stimulates collagen VII synthesis for improved hair follicle structure and anchoring

EVALUATION OF ACETYL TETRAPEPTIDE-3 EFFECT ON GENERAL COLLAGEN SYNTHESIS

INTRODUCTION

A direct relationship has been observed between the DP's size and the hair follicle and shaft size[34]. The volume of the DP depends on the number of cells and the amount of extracellular matrix per cell[35]. Indeed, the DP cells are surrounded by a thick extracellular matrix (ECM). This ECM is rich in various proteins, such as fibronectin and collagen, which are synthesized actively by the DP cells[36]. This ECM undergoes extensive changes in concert with the hair cycle. For instance, in the telogen phase, the volume of the DP extracellular matrix is much reduced, ultimately leading to a decrease in the size of the hair follicle[37]. If the ECM renewal is inadequate over successive cycles, the follicle may progressively shrink, leading to hair miniaturization and loss[38].

Collagen is composed of three chains bound together in a tight triple helix. Collagen is characterized by the presence of 2 specific amino acids: hydroxylysine and overall hydroxyproline (OH-proline). Hydroxyproline dosage can be used to give an indication, unaffected by the presence of other proteins, of the mature collagen content of biological tissue.

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™'s peptide, acetyl tetrapeptide-3, on general collagen synthesis.

PROTOCOL

Tested products

Acetyl tetrapeptide-3, Capixyl™'s peptide, was tested at a concentration of 10^{-7}M.

Biological materials

It has been demonstrated that collagen synthesized by dermal papilla cells is similar to that of skin fibroblasts. Therefore, human fibroblasts (MRC5 cell line) were used in this assay.

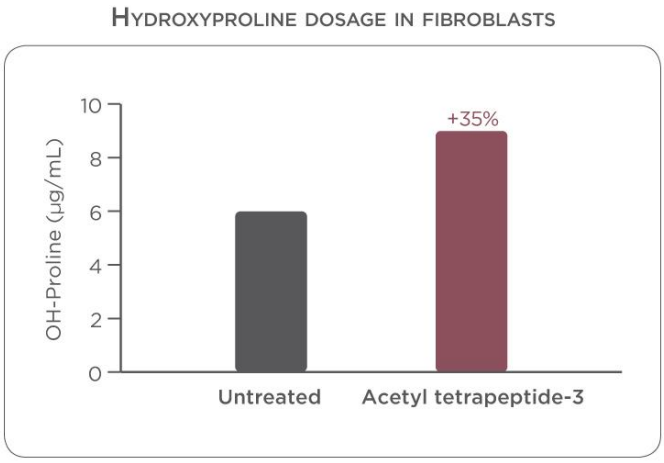
Method

Cells were incubated or not with acetyl tetrapeptide-3 for 6 days. After the incubation period, hydroxyproline (OH-proline) dosage was assessed with the chloramine-T reaction and measured by optical density (OD) at 540 nm compared to a range standard. The procedure was based on alkaline hydrolysis of the tissue homogenate and subsequent determination of the free hydroxyproline in hydrolysates. Chloramine-T was used to oxidize free hydroxyproline to produce a pyrrole. The addition of Ehrlich's reagent resulted in the forming of a chromophore that can be measured at 540 nm.

Evaluation

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

RESULT



Cells treated with acetyl tetrapeptide-3 showed increased levels of hydroxyproline, which is directly correlated with mature collagen content.

CONCLUSION

Capixyl's peptide increases collagen synthesis, favoring ECM renewal.

EVALUATION OF CAPIXYL™ EFFECT ON HAIR FOLLICLE MORPHOLOGY

INTRODUCTION

The highly specialized ECM basement membrane that separates the epithelial compartment (which includes the cells that make up the hair shaft and inner root sheath) and mesenchymal compartment (which consists of the dermal papilla and connective tissue sheath) serves as a "boundary" between the two compartments, helping to maintain the distinct environments needed for each to function correctly[39]. The basement membrane plays an essential role in maintaining the health and cohesion of the hair follicle.

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™ on the hair follicle morphology.

PROTOCOL

Tested products

Capixyl™ was tested at a concentration of 1%.

Biological materials

Starting with a plastic surgery operation on the scalp of a Caucasian woman aged 49 years, 56 hairs and their bulbs were isolated by microdissection. These isolated hairs were placed individually in the wells of two 48 well plates and kept alive in classic cell culture conditions (at 37°C, in an atmosphere enriched to 5% CO_2) with improved Williams medium (Philpott) for 7 days.

Method

- ***Selection of hairs in anagen phase:***

Three days before the beginning of the study (D-3), all the hairs were cut approximately 1 mm from the infundibulum; then, they were photographed with the help of a microscope and a CCD camera joined to software for acquisition and archiving.

On D0, all the hairs were again measured, and only 8 hairs of each batch in the anagen phase were chosen for the rest of the study. The selected hairs grew at an average rate of 50 µm per day for three days before the experiment (between D-3 and D0).

- ***Treatment***

A control batch was left untreated, while Capixyl™ was added to the culture medium at a concentration of 1% from D0 onwards. The culture media was replaced every day.

- ***Sampling***

At D0 and D7, half of the hairs were set by a formaldehyde fixer

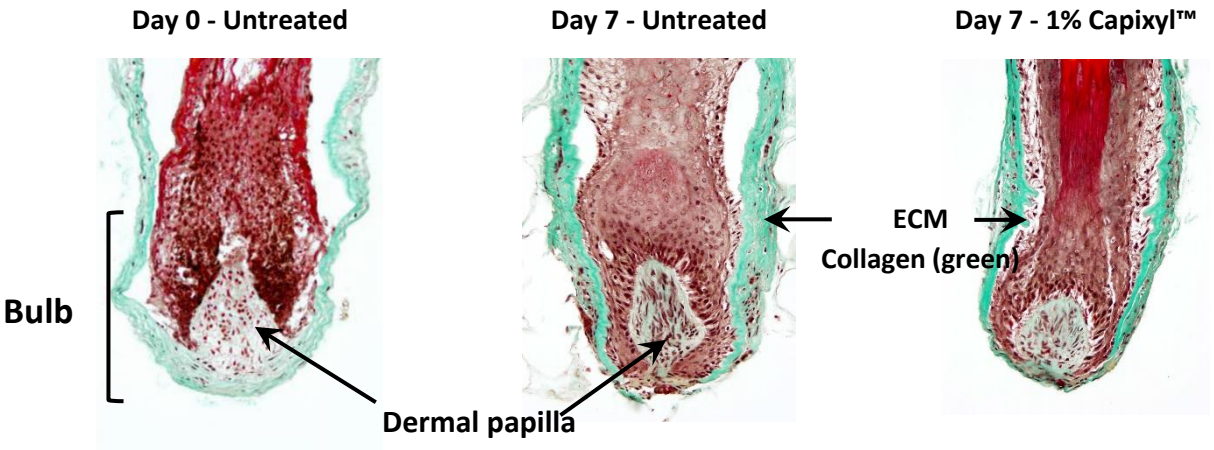
- ***Histological Treatments***

After 24 hours of setting in the swabbed formaldehyde, the samples were dehydrated and coated with paraffin with the help of a Leica TP 1010 dehydration system according to the partner operating procedure. They were placed in a block according to the partner operating procedure with the help of a Leica EG 1160 coating station. Cuts of 5 µm were made according to the partner operating procedure with the help of a Minot-type microtome, Leica RM 2125, and mounted on Superfrost® histological glass slides. These cuts were made in view of later immunolabelling. The cuts in paraffin were colored with Masson trichrome, the Goldner variant, according to the operating procedure.

Evaluation

General morphology was evaluated by microscopic examination along the entire hair length. Photos were taken of the bulb zone, the middle of the hair (sheath), and the “bulge” zone.

RESULT



After 7 days of treatment with Capixyl™, the morphology of hair follicles at the bulb level was improved compared to the untreated control. The epithelial sheaths adhered well to the underlying connective tissue and the dermal papilla showed an excellent structure.

CONCLUSION

Capixyl™ improves the structure and cohesion of hair follicles, creating an optimal environment for increased activity.

EVALUATION OF BIOCHANIN A EFFECT ON 5A-REDUCTASE ACTIVITY

INTRODUCTION

The enzyme 5α-reductase catalyzes the conversion of testosterone to DHT. Elevated levels of DHT are detrimental to hair follicles, as they lead to a shortened anagen (growth) phase and an extended telogen (resting) phase within the hair cycle. This hormonal imbalance results in progressively smaller hair follicles and the production of finer, shorter hair strands.

OBJECTIVE

The aim of the study was to evaluate the capacity of biochanin A, a primary component of red clover extract, to modulate the activity of both type I and type II 5α-reductase.

PROTOCOL

Tested products

Biochanin A was tested at a concentration of 100 µM. EGCG (epigallocatechin gallate) isolated from green tea was used as a positive control and tested at 100 µM.

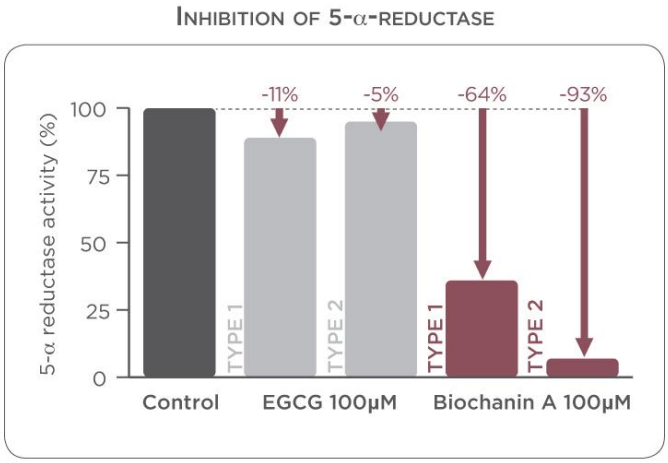
Method

Cells were plated and incubated with test compounds (biochanin A or positive control) for 1 hour at 37°C before adding ^{14}C-testosterone, at a final concentration of 1.5 µM. Cells were incubated for an additional 3 hours, and radioactive steroids were extracted.

Evaluation

The amount of labeled testosterone and DHT were determined by TLC (Thin Layer Chromatography) to quantify the 5α-reductase activity. Results are expressed in percent inhibition of 5-α reductase activity in the presence of biochanin A or ECGC.

RESULT



These results showed that 5α-reductase (type I and II) activity decreases in the presence of biochanin A. The inhibition observed with biochanin A is higher than the effect observed with ECGC, a well-known 5α-reductase activity inhibitor.

CONCLUSION

Biochanin A effectively decreases 5-α reductase activity, thereby down-modulating the conversion of testosterone into DHT

EVALUATION OF CAPIXYL™ EFFECT ON IL-8 PRODUCTION

INTRODUCTION

Androgenetic alopecia is a common hair disorder resulting from the interplay of genetic, endocrine, and aging factors leading to a patterned hair follicular miniaturization. Micro-inflammation seems to be a potential active player in this process. In AGA, clusters of abnormal inflamed streamers or fibrous tracts surrounding the hair follicle are observed. Interleukine-8 (IL-8) is a cytokine secreted by several cell types and is one of the primary mediators of the inflammatory response. Decreasing the level of inflammation on the scalp and hair follicle area could positively affect hair loss prevention.

OBJECTIVE

The aim of the study was to evaluate the capacity of a red clover extract and Capixyl™ to modulate the production of IL-8.

PROTOCOL

Tested products

Capixyl™ and the red clover extract were tested at the following concentrations: 0.5 and 1%. Dexamethasone (DMS) at 1µM was used as a positive control.

Biological materials

Experiments were done using monolayers of cells derived from normal human fibroblasts (NHDF).

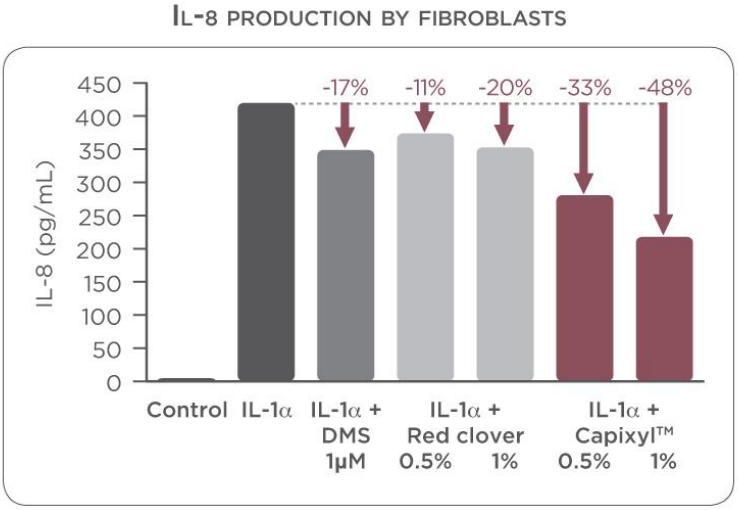
Method

After seeding, confluent NHDF cells were cultured for 24 hours in complete medium containing 10% fetal calf serum (Eurobio), 1% antibiotics (penicillin/streptomycin, Eurobio) and 1% L-glutamine (Eurobio) at 37°C under 5% CO_2 and 95% moisture. At the end of this period, fibroblasts were treated with IL-1α as an inducer of IL-8 production, in the presence/absence of Capixyl™, red clover, or DMS, for 24 hours in a medium without serum.

Evaluation

The content of IL-8 was quantified in the medium using a highly sensitive and specific enzyme immunoassay (ELISA) kit. The results are expressed as pg/mL of IL-8 synthesis reported to fibroblast viability.

RESULT



As expected, IL-1a activated IL-8 production, which was decreased with DSM treatment. When cells were treated with the red clover extract or Capixyl™, the synthesis of IL-8 decreased.

The effect of Capixyl™ was dose-dependent and higher than the effect observed with DMS, an anti-inflammatory reference. Moreover, Capixyl™ showed greater efficacy than the red clover extract alone, demonstrating a synergistic effect between the peptide and the red clover extract.

CONCLUSION

Capixyl™ decreases the production of pro-inflammatory cytokines

EVALUATION OF CAPIXYL™ EFFECT ON HAIR FOLLICLE GROWTH

INTRODUCTION

In 1990, Philpott et al. reported the successful maintenance and growth of human hair follicles in vitro for the first time. The importance of this model to hair follicle biology has been demonstrated[40].

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™ on the growth speed of hair shafts.

PROTOCOL

Tested products

Capixyl™ was tested at a concentration of 1%.

Biological materials

Starting with a plastic operation on the scalp of a white woman aged 49 years, 56 hairs and bulbs were isolated by microdissection. These isolated hairs were placed individually in wells of two 48 well plates and kept alive in classic cell culture conditions (at 37°C, in an atmosphere enriched to 5% CO_2) with improved Williams medium (Philpott) for 11 days. 15 hairs per batch were kept alive to preserve about 10 hairs in the anagen phase per batch.

Method

- ***Selection of hairs in anagen phase:***

Three days before the beginning of the study (D-3), all the hairs were cut approximately 1 mm from the infundibulum; then, they were photographed with the help of a microscope and a CCD camera joined to software for acquisition and archiving.

On D0, all the hairs were again measured, and only 8 hairs of each batch in the anagen phase were chosen for the rest of the study. The selected hairs grew at an average rate of 50 µm per day for three days before the experiment (between D-3 and D0). For each batch, 8 hairs were chosen with an average daily growth of 160 µm.

The numbers of follicles per treatment are:
- n = 22 follicles treated with Capixyl™
- n = 34 untreated follicles

- ***Treatment***

A control batch was left untreated, while Capixyl™ was added to the culture medium at a concentration of 1% from D0 onwards. The culture media was replaced every day.

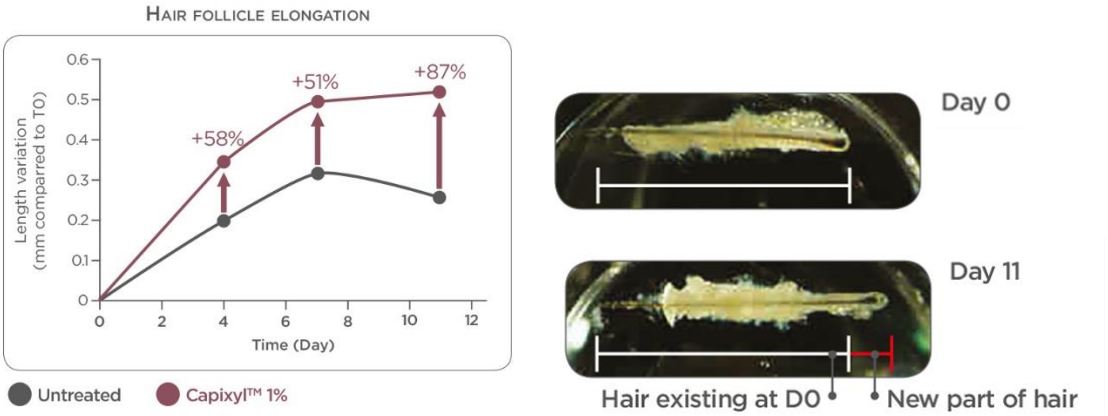
- ***Measuring Hairs***

On days D4, D7, and D10, the hairs chosen on D0 were photographed with the help of a microscope and a CCD camera joined to software for acquisition and archiving.

Evaluation

On D4, D7, and D11, the variation in size was measured in relation to D0.

RESULT



Compared to the untreated condition, Capixyl™ resulted in a 58% increase in hair growth on day 4, 51% on day 7, and 87% on day 11.

CONCLUSION

Capixyl™ stimulates hair growth

EVALUATION OF ACETYL TETRAPEPTIDE-3 EFFECT ON HAIR FOLLICLE GROWTH VS MINOXIDIL

INTRODUCTION

In 1990, Philpott et al. reported the successful maintenance and growth of human hair follicles in vitro for the first time. The importance of this model to hair follicle biology has been demonstrated.

OBJECTIVE

The aim of the study was to evaluate the effect of Capixyl™ peptide, acetyl tetrapeptide-3, on the growth speed of hair shafts compared to Minoxidil.

PROTOCOL

Tested products

Acetyl tetrapeptide-3, Capixyl™'s peptide, and Minoxidil were tested at a concentration of 10^{-7}M and $120x10^{-7}$M, respectively.

Biological materials

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 a scalpel bald.

Method

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

The follicles were cultured in a specific medium with or without the test substances: acetyl tetrapeptide-3 at 10^{-7}M or the reference Minoxidil at $120x10^{-7}$M for 7 days. Untreated follicles cultured in the same conditions were used as 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

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:

$$\text{Percentage of growth} = \frac{\text{lenght at Tx} - \text{lengh at T0}}{\text{length at T0}} \times 100$$

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

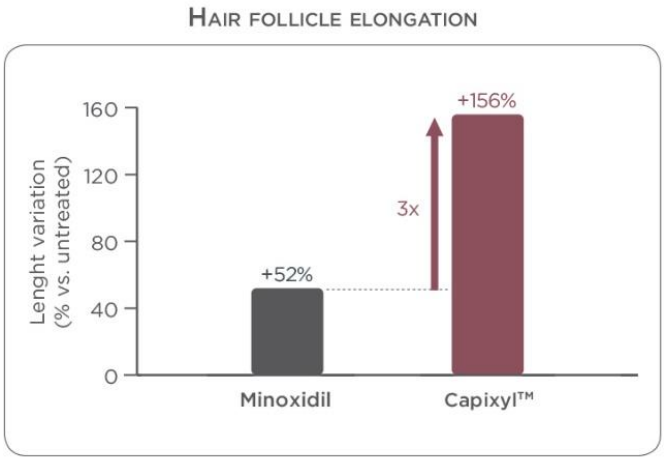
In parallel, the **normalized activity** after treatment ("hair growth induced by the treatment") was calculated following the equation:

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

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

The growth percentages and activity were determined after 7 days of treatment with acetyl tetrapeptide-3 or Minoxidil compared to untreated follicles.

RESULT



Stimulation of hair growth in these experimental conditions was observed. The activity was better with acetyl tetrapeptide-3 and demonstrated a 47% growth, higher than with Minoxidil.

CONCLUSION

Acetyl tetrapeptide-3 strongly stimulates hair follicle growth, improving hair growth. Results obtained with acetyl tetrapeptide-3 are higher than those obtained with Minoxidil, a reference in hair growth treatment.

Capixyl™' peptide better stimulates hair follicle growth than the market reference Minoxidil.

CLINICAL STUDIES

EVALUATION OF CAPIXYL™ EFFECT ON THE HAIR GROWTH CYCLE AND HAIR DENSITY – LEAVE-ON

BACKGROUND

The hair growth cycle consists of four stages. The first three (anagen, catagen, and telogen) involve the growth and development of the hair follicles that produce each strand. The final stage (exogen) is when the hair falls out, and new hair emerges to replace it. Each stage has a different duration that can be influenced by age, diet, stress, hormonal changes, or medication side effects. These factors can also cause hair loss by shortening the anagen stage, triggering an immune response against the hair follicles, or increasing the number of hairs in the telogen stage. Hair loss can harm one's social and emotional well-being, leading to low self-esteem, anxiety, depression, and more.

OBJECTIVE

The aim of the study was to evaluate the efficacy of Capixyl™ in limiting hair loss and promoting hair growth in leave-on conditions.

PROTOCOL

Panel

The assay was conducted on 29 volunteers suffering from AGA (mean age of 46), who had been chosen using the following criteria: less than 70% hairs in the anagen phase. Patients were clinically evaluated, and individual case histories were recorded to rule out possible pathologies such as iron deficiency anemia, thyroid-related conditions, or other possible pathologies.

Tested product

Ingredient	Active formula %	Placebo formula %
Water	75.0	80.0
Alcohol	20.0	20.0
Capixyl™	5.0	0.0

Test conditions

Twenty (20) drops of the tested products were applied in the evening and gently distributed with the fingertips on the experimental area for 4 consecutive months. Every week, patients were given a plastic bag, where they had to collect all the hairs on their pillows, combs, and clothes daily; they had to bring the bag to the laboratory for hairs to be counted. Efficacy was objectively evaluated by using instrumental measurements (digital trichogram with TrichoScan™). Measurements from

14 Capixyl™-treated volunteers were compared with those of 15 Placebo-treated ones. Every volunteer assessed the product's acceptability daily.

METHOD

Evaluation of hair growth cycle phases and hair density

- ***Principle***

TrichoScan is suitable for the analysis of human scalp hair in AGA. TrichoScan is a non-invasive method that combines standard chemiluminescence microscopy with automatic digital image analysis to measure 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***

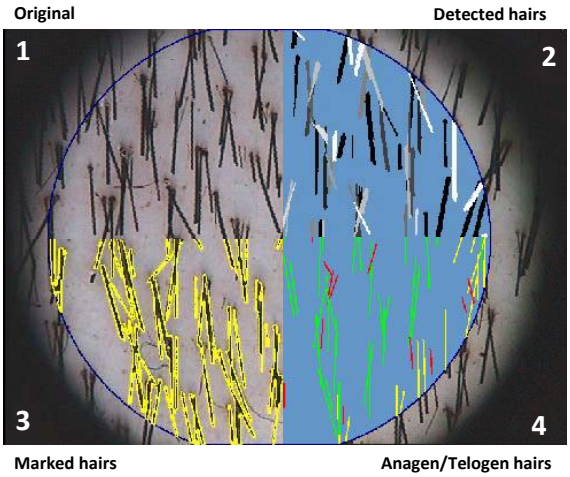
1. A shaving mask is positioned on the volunteer's head to shave a 1.8 cm^2 area on the zone or zones to be studied.
2. Three days later, as hairs do not typically contrast well enough with the scalp (due to gray or fair hairs) skin for digital photography, hairs must be dyed and subsequently cleaned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

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

- ***Recording the images***

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

1. Original image
2. Detection of hair by the software
3. Specifically 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, and grouping follows via a particular statistical procedure.



- ***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 phases. This software is calibrated based on 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 on each volunteer.

Anagen hair density (n/cm^2): In the TrichoScan procedure definition, anagen hair is detectable three days after complete hair shaving. Within this time, only anagen hairs should grow significantly.

Telogen hair density (n/cm^2): 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 lengths. TrichoScan identifies non-growing hairs as telogen hairs and growing hairs as anagen hairs.

Ratio A/T (growth index): Comparison of the numbers of anagen and telogen hair indicates the percentage of active hair follicles.

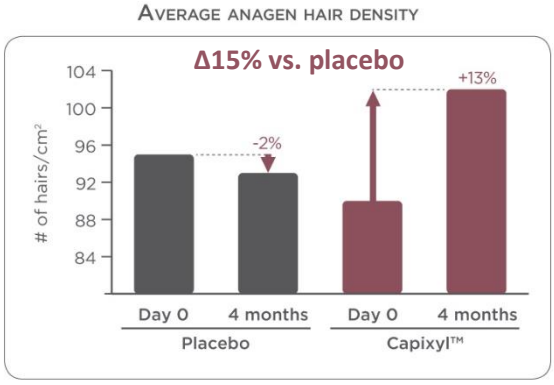
- ↑A/T ratio = activation of hair growth → efficacy of treatment
- ↓A/T ratio = loss of hair growth activity → alopecia continues

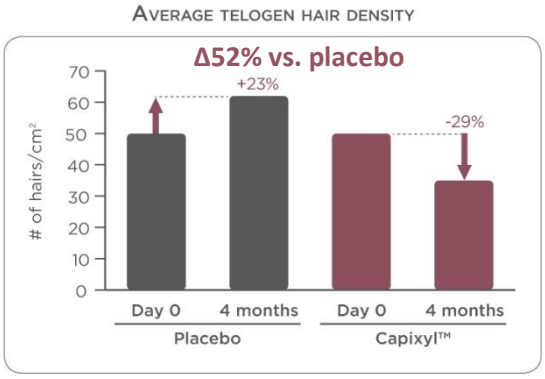
STATISTICAL METHODS

Based on results of normality and variance homogenization tests, the non-parametric unpaired Kolmogorov-Smirnov test or the non-parametric paired Wilcoxon test were used. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

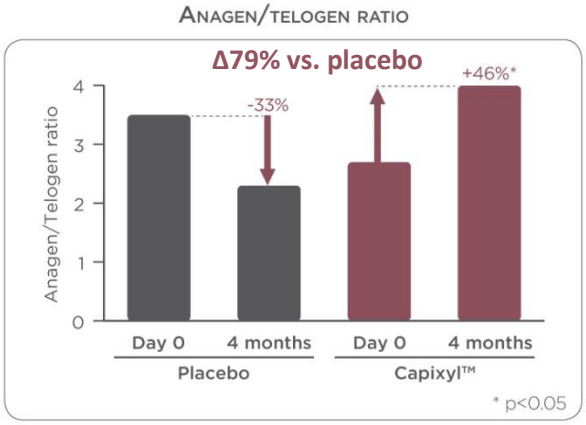
RESULTS

Clinical evaluation of hair growth cycle phases



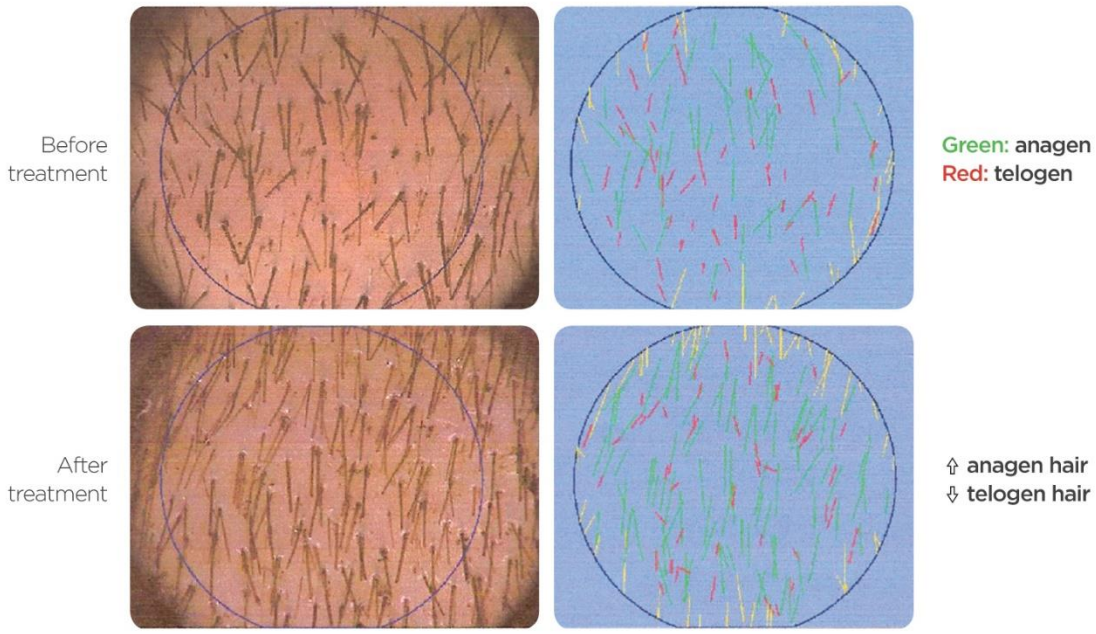


Capixyl™ induced an apparent increase in the anagen hair density compared to placebo, demonstrating the efficacy of the treatment for hair growth after 4 months of application compared to day 0. Capixyl™ also induced a strong reduction in the telogen hair density compared to the placebo, indicating decreased hair loss with Capixyl™ treatment.



Capixyl™ significantly increased the Anagen/Telogen ratio (representing the growth index) by 46% compared to a reduction of -33% with the placebo.

Clinical evaluation of hair density



CONCLUSION

Capixyl™ regulates the hair growth cycle for optimal growth and visually increases hair density

EVALUATION OF CAPIXYL™ EFFECT ON THE HAIR GROWTH CYCLE – RINSE-OFF

OBJECTIVE

The aim of the study was to evaluate the efficacy of Capixyl™ in limiting hair loss and promoting hair growth in rinse-off conditions.

PROTOCOL

Panel

23 healthy volunteers (both genders) aged 18-65 years old with general hair loss due to stress, fatigue, season, and more.

Tested products

The tested cosmetic product complies with regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009.

Shampoo formula

Ingredient	INCI name	Active formula %
Plantacare 2000 UP/MB	Decyl glucoside	8.00
Genencare® OSMS BA	Betaine	3.50
Microcare® NB	Sodium Benzoate	0.30
Dermosoft® 700 B	Levulinic Acid (and) Sodium Levulinate (and) Glycerin (and) Aqua	1.00
Deionized water	water	84.60
Xanthan gum FNCSP-PC	Xanthan gum	1.10
Capixyl™	**Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and)Trifolium Pratense (Clover) Flower Extract**	**1.50**

Conditioner formula

Ingredient	INCI name	Active formula %
Dissolvine Na	Tetrasodium EDTA	0.10
Satiaxane™ VPC 911	Xanthan Gum	0.30
Emulgade® 1000 NI	Cetearyl Alcohol (and) Ceteareth-20	5.00
Lanette® 0-0R	Cetearyl Alcohol	4.00
Saboderm TCC	Capric/Caprylic Triglycerides	9.00
Dekaben C4	Phenoxyethanol, Methylparaben, Ethylparaben, Butylparaben, Propylparaben	0.80
Sun E1000	Tocopherol (and) Helianthus Annuus (Sunflower) Seed Oil	0.20
Dehyton® K COS	Cocamidopropyl betaine	3.00
Deionized water	water	76.02
Citric Acid 50% solution	Water (and) Citric Acid	0.08
Capixyl™	**Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and)Trifolium Pratense (Clover) Flower Extract**	**1.50**

Test conditions

Volunteers used the shampoo and conditioner combination every two days for 90 days. The shampoo was first massaged onto the scalp for at least one minute and rinsed with plenty of water. The conditioner was massaged in for at least two minutes and rinsed with plenty of water.

METHOD

Evaluation of hair growth cycle phases

The hair growth cycle was evaluated by using the TrichoScan method, which can measure the ratio of anagen (growth) phase and telogen (fall) phase hairs. The technique involves shaving a small area of the scalp and taking digital images of the hair with a special camera. The images are then analyzed by software that calculates important hair growth parameters, such as hair density, diameter, and growth rate. The results indicated the percentage of hair in the anagen and telogen phases and the ratio between the two phases relative to day 0.

Self-assessment

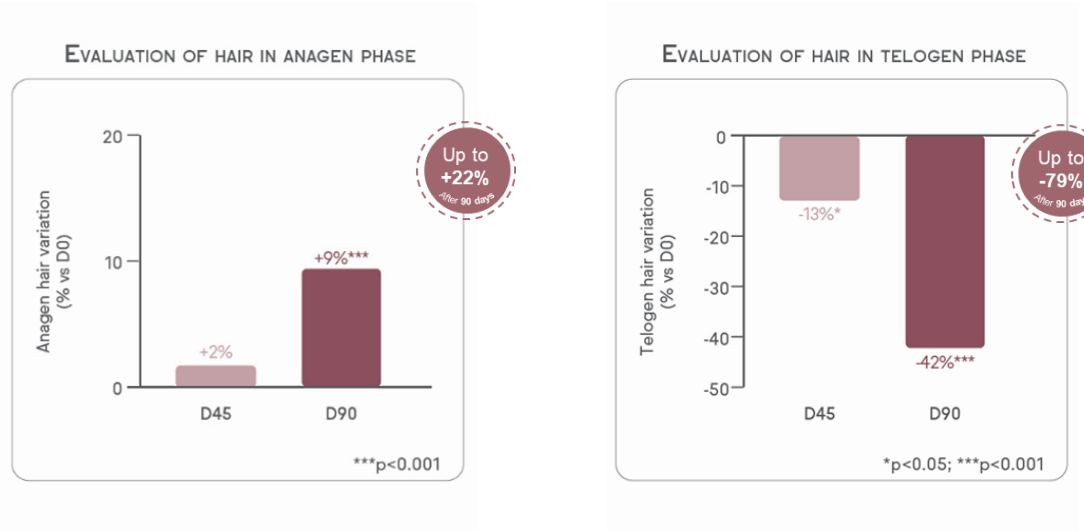
The volunteers completed a subjective evaluation questionnaire after 45 and 90 days of using Capixyl™ to assess the product's efficacy.

STATISTICAL METHODS

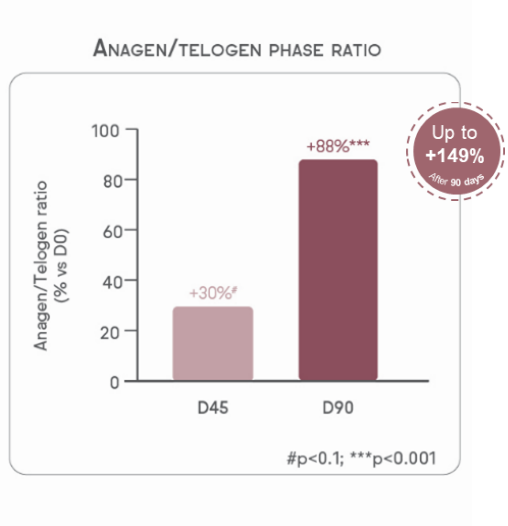
Based on the results of normality and variance homogenization tests, the One-way ANOVA nonparametric test following the Friedman post hoc test was applied. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

RESU LTS

Clinical evaluation of hair growth cycle phases

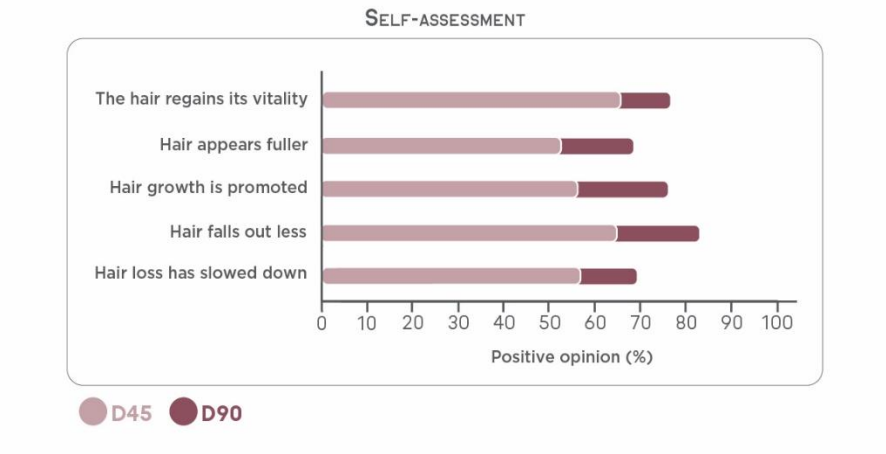


After only 90 days, Capixyl™ significantly increased the percentage of hair in the growth phase (anagen) by 9% (up to +22%) while also significantly decreasing the percentage of hair in the shedding phase (telogen) by 42% (up to -79%).



Capixy™ significantly increased the anagen/telogen ratio, reflecting the growth index, by 30% and 88% (up to +149%) after respectively 45 and 90 days.

Self-assessment



Most users approved Capixyl™ efficacy in rinse-off application after only 45 days.

CONCLUSION

With a short response time and low dosage, Capixyl™ successfully regulates the hair growth cycle of both men and women suffering from general hair loss for improved hair growth and limited hair loss.

Capixyl™ shows incredible efficacy in rinse-off application by successfully regulating the hair growth cycle

EVALUATION OF CAPIXYL™ EFFECT ON SCALP MICROBIOTA

BACKGROUND

Human hair and scalp are host to various micro-organisms, and balance is crucial for hair and scalp health. Hair follicles are home to 25% of the cutaneous microbial population. Like cutaneous microbiotas, the hair microbiota includes commensal microbes and opportunistic pathogens, constantly interacting with the host, eliciting and evading the host's immune system.

Studies have highlighted that bacteria of the phyla *Actinobacteria*, *Firmicutes*, and *Proteobacteria* predominate on the scalp's surface and that the most abundant organisms are *Cutibacterium* species and *Staphylococcus* species. Maintaining a healthy balance of bacteria on the scalp is especially crucial to avoid issues like dandruff, seborrheic dermatitis, and hair loss. Indeed, any microbiota imbalance, or dysbiosis, can cause inflammation and oxidative stress, potentially contributing to hair loss. Recent studies have shown a microbial shift in patients with alopecia, where the ratios of *C. acnes/S. epidermidis* and *C. acnes/S. aureus* are significantly higher, with no significant differences in the *S. epidermidis/S. aureus* ratio (beneficial ratio). A notable increase in the abundance of specific bacteria species*, such as Rothia, Bacteroidetes, Acinetobacter and Streptococcus* bacteria*,* also accompanies this shift[41].

OBJECTIVE

The aim of the study was to evaluate the ability of Capixyl™ to counteract the microbial shift observed in the scalp microbiota of people suffering from hair loss.

PROTOCOL

Panel

21 healthy volunteers (women and men) aged 18-65 with general hair loss due to stress, fatigue, season, and more.

Tested products

The tested cosmetic product complies with regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009.

Shampoo formula

Ingredient	INCI name	Active formula %
Plantacare 2000 UP/MB	Decyl glucoside	8.00
Genencare® OSMS BA	Betaine	3.50
Microcare® NB	Sodium Benzoate	0.30
Dermosoft® 700 B	Levulinic Acid (and) Sodium Levulinate (and) Glycerin (and) Aqua	1.00
Deionized water	water	84.60
Xanthan gum FNCSP-PC	Xanthan gum	1.10
Capixyl™	**Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and)Trifolium Pratense (Clover) Flower Extract**	**1.50**

Conditioner formula

Ingredient	INCI name	Active formula %
Dissolvine Na	Tetrasodium EDTA	0.10
Satiaxane™ VPC 911	Xanthan Gum	0.30
Emulgade® 1000 NI	Cetearyl Alcohol (and) Ceteareth-20	5.00
Lanette® 0-0R	Cetearyl Alcohol	4.00
Saboderm TCC	Capric/Caprylic Triglycerides	9.00
Dekaben C4	Phenoxyethanol, Methylparaben, Ethylparaben, Butylparaben, Propylparaben	0.80
Sun E1000	Tocopherol (and) Helianthus Annuus (Sunflower) Seed Oil	0.20
Dehyton® K COS	Cocamidopropyl betaine	3.00
Deionized water	water	76.02
Citric Acid 50% solution	Water (and) Citric Acid	0.08
Capixyl™	**Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and)Trifolium Pratense (Clover) Flower Extract**	**1.50**

Test conditions

Volunteers used the shampoo and conditioner combination every two days for 90 days. The shampoo was first massaged onto the scalp for at least one minute and rinsed with plenty of water. The conditioner was massaged in for at least two minutes and rinsed with plenty of water.

METHOD

Evaluation of scalp microbiota

Scalp microbiota samples from volunteers who applied Capixyl™ according to the established protocol were collected using the Sequential Skin Kit, following the manufacturer's instructions (Sequential Skin Ltd., UK). A Sequential Skin adhesive patch was applied to the participant's scalp for 10 seconds. The patch was peeled and stored in a 15 ml tube containing a preservation solution (Sequential Skin Ltd., UK). Subsequently, microbial DNA was extracted according to the Sequential Skin DNA extraction protocol (Sequential Skin Ltd., UK). Bacterial detection was performed using the 16S RNA marker to quantify the bacterial community using qPCR sequencing. Once the total DNA was collected, the purity and quality of the samples were assessed, and high-throughput qPCR was performed. The raw qPCR data was cleaned, and bacterial analysis was performed. Absolute abundance was calculated for significant bacteria on the scalp, including *S. epidermidis*, *S. aureus*, and *C. acnes.*

- ***Evaluation of bacterial ratios***

The ratio of *C. acnes to S. epidermidis*, *C. acnes to S. aureus*, and S. *epidermidis to S. aureus* was determined at day 0 and after 90 days of product application.

- ***Evaluation of the correlation between bacteria modulation and hair cycle phases***

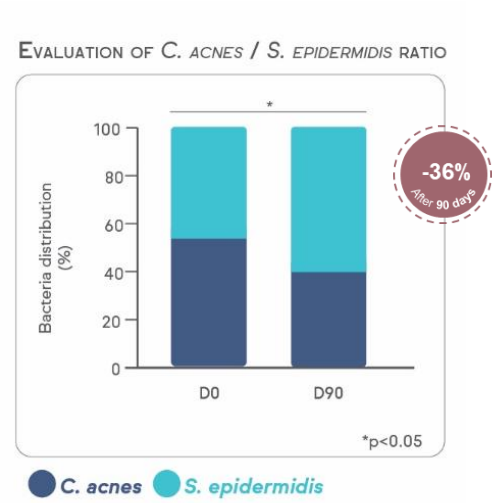
The absolute abundance of bacteria involved in hair loss was evaluated before and after 90 days of shampoo and conditioner combination application. Subsequently, the correlation between the variation at day 0 and day 90 in the absolute abundance of bacteria identified and the variation at day 0 and day 90 in the percentage of hair in anagen and telogen phases obtained in the hair growth assessment study was determined.

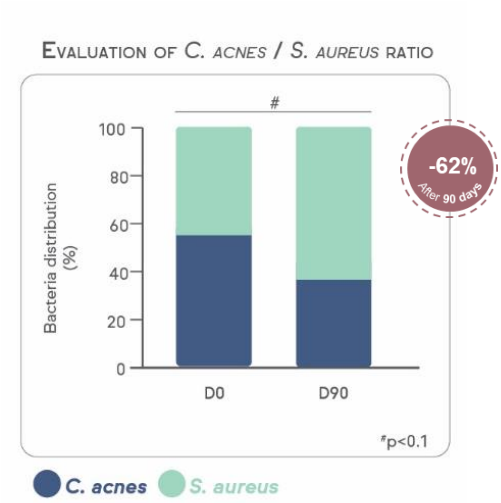
STATISTICAL METHODS

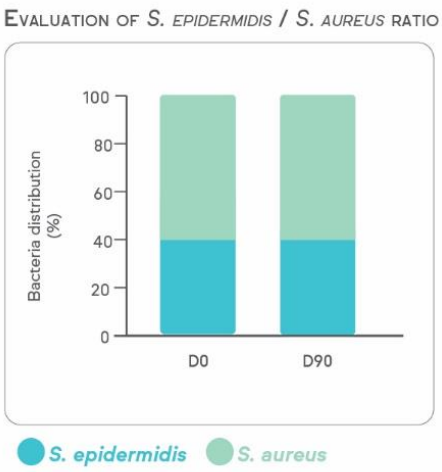
Based on the results of normality and variance homogenization tests, the non-parametric paired Wilcoxon test and the Spearman r test were applied. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

RESULTS

Clinical evaluation of bacterial ratios







After only 90 days, Capixyl™ significantly decreased the ratio of *C. acnes to S. epidermidis* by 36% and reduced the ratio of *C. acnes to S. aureus* by 62%. Capixyl™ did not alter the ratio of *S. epidermidis to S. aureus*.

Clinical evaluation of the correlation between bacteria modulation and hair cycle phases

EVALUATION OF THE CORRELATION BETWEEN BACTERIA MODULATION AND HAIR CYCLE PHASES

Bacteria modulation vs D0	Increase in anagen phase	Decrease in telogen phase
↓ *Rothia sp.* (*)	*	*
↓ *Bacteroidetes sp.* (***)	*	
↓ *Acinetobacter sp.* (**)	#	*
↓ *Streptococcus sp.* (*)		*

*(***p<0.001; **p<0.01; *p<0.05; #p<0.1)*

The significant decrease of *Rothia sp.*, *Bacteroidetes sp*., and *Acinetobacter sp.* was significantly correlated with the increase in the anagen phase observed after 90 days of shampoo and conditioner combination application. Similarly, the significant decrease of *Rothia sp.*, *Acinetobacter sp.*, and *Streptococcus sp*. was significantly correlated with a decrease in the telogen phase observed after 90 days of shampoo and conditioner combination application.

CONCLUSION

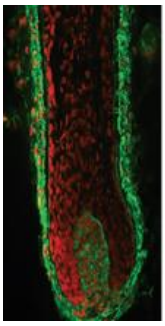
Capixyl™ has the ability to restore the balance of the scalp microbiota without harming the beneficial bacteria nearby.

Capixyl™ rebalances scalp microbiota to support healthy hair growth

EVALUATION OF CAPIXYL™ EFFECT ON LASH DENSITY, NUMBER, AND LENGTH

BACKGROUND

As lash characteristics are similar to scalp hair, Capixyl™ represents the ideal and perfect solution to modulate lash growth to avoid loss and stimulate the biological mechanisms behind their growth. Little is known about lashes compared with human hair. In the embryo, lashes 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[42]. 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 6 to 8 mm in the lower lid[4344].



The terminal lash fiber is characterized by a regular curve shape, more or less marked depending on the ethnic origin[45]. Microscopy of lashes revealed a structure very close to that of hair fiber, with three compartments from the outside to the inside. However, the lash fiber is much shorter than scalp hair due to a shorter hair cycle. The growth rate is approximately 120 µm daily, and the duration of the anagen and telogen phases is estimated to be 1-4 and 4-9 months, respectively[46]. At any given time, 59-85% of lash follicles are in the telogen phase, depending on whether they are in the upper or lower lid[47].

OBJECTIVE

The study aimed to evaluate the efficacy of Capixyl™ in increasing lash density, number, and length.

PROTOCOL

Panel

17 healthy women volunteers aged 25 to 68 years old with damaged lashes.

Tested product

The tested cosmetic product complies with regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009.

Ingredient	INCI name	Active formula %	Placebo formula %
Deionized water	Water	88.10	90.60
Glycerin	Glycerin	3.00	3.00
Chlorophenesin	Chlorophenesin	0.25	0.25
Carbopol Ultrez 10	Cabomer	1.00	1.00
Hydroxyde sodium 20%	Sodium Hydroxide	2.30	2.30
PVP VA S30	VP/Va copolymer	2.00	2.00
Borax decahydryte	Sodium Tetraborate Decahydrate	0.10	0.10
Boric acid	Boric acid	0.66	0.66
Microcare MT	Water (and) methylisothiazolinine	0.09	0.09
Capixyl™	**Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and)Trifolium Pratense (Clover) Flower Extract**	**2.50**	**0.00**

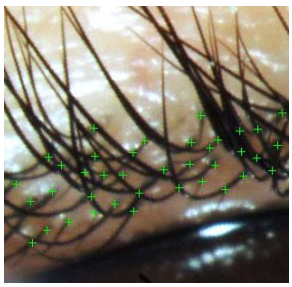
Test conditions

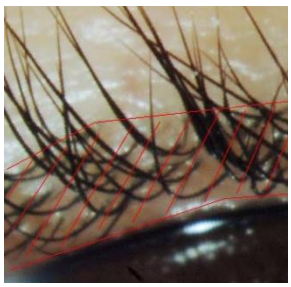
Capixyl™, or a placebo gel, was applied on the upper and lower lashes of the eye in the morning, before the application of the usual mascara, and a second time alone, in the evening, for 8 weeks. Lash density, number, and length were evaluated at D0 and after 4 and 8 weeks of product application. One eye was treated with a placebo while the other eye was treated with Capixyl™.

METHOD

Evaluation of lash density

At D0, 4, and 8 weeks, photographs of the upper lashes were taken with a camera fixed on a biomicroscope. The number of lashes in the specific area was then analyzed and counted. After counting, the surface where the lashes grew was measured, and the density was determined.



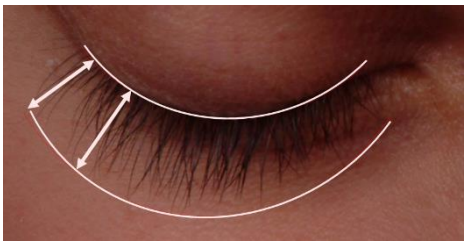


Evaluation of the number of new lashes

Compared to mature lashes, young lashes are less colored, thinner, and 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 to the morphology and characteristics of young lashes, was then counted.

Evaluation of lash length

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 length of the lashes (mm) was measured.

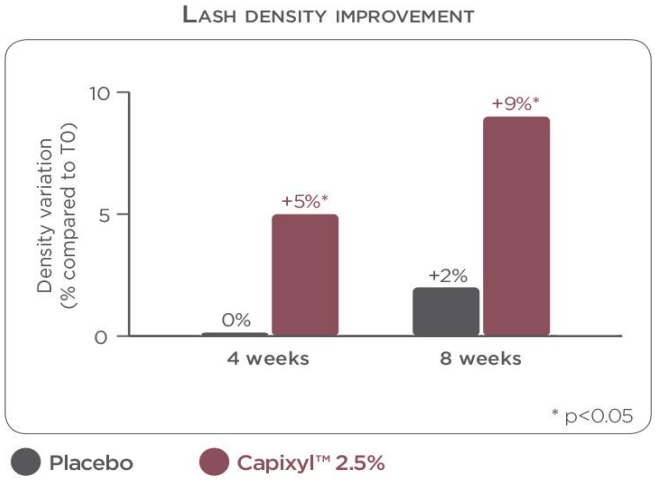


STATISTICAL METHODS

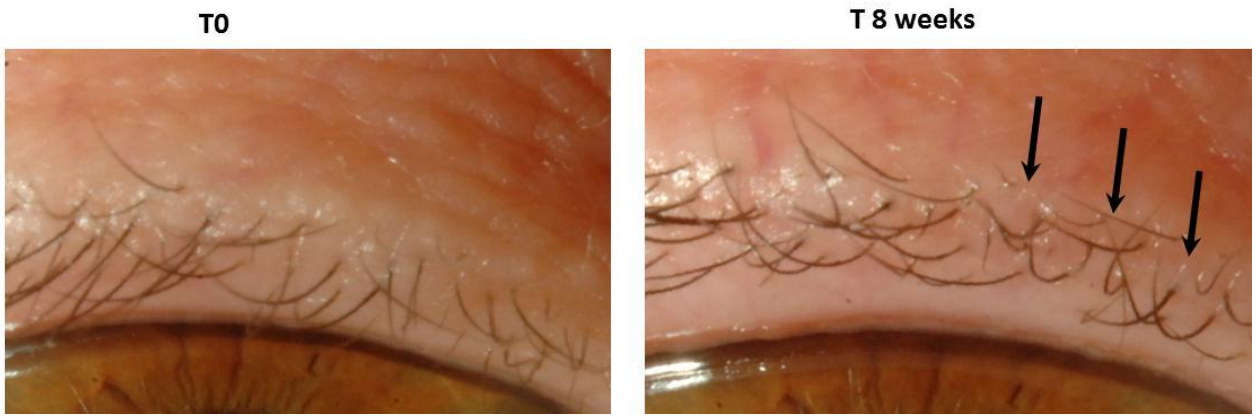
Obtained Data were submitted to two-ways of Student T-test. The statistical significance value is $p<0.01$ (#$p<0.1$, *$p<0.05$, **$p<0.01$, ***$p<0.001$).

RESU LTS

Clinical evaluation of lash density

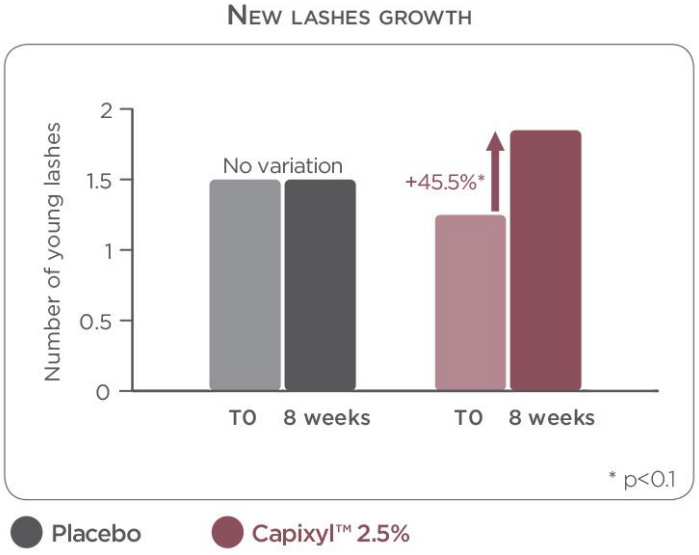


Vol #14

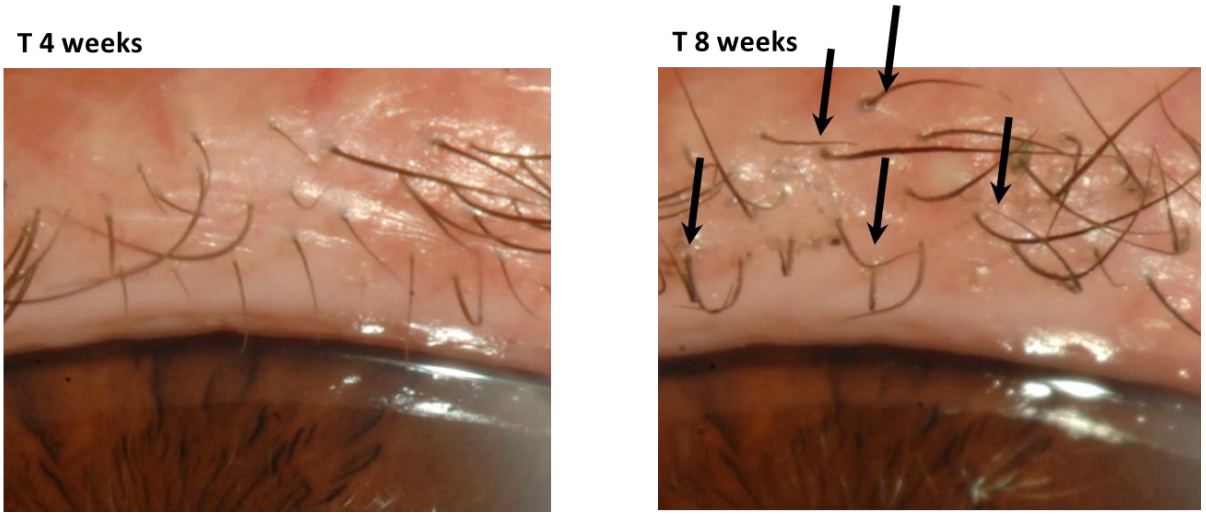


A clear and significant change in lash density was observed when lashes were treated with Capixyl™ compared to placebo. Lash density increased by up to 27% after 8 weeks of Capixyl™ application. More than 60% of subjects saw an improvement in the density of their lashes after 4 weeks and 93% after 8 weeks.

Clinical evaluation of the number of new lashes

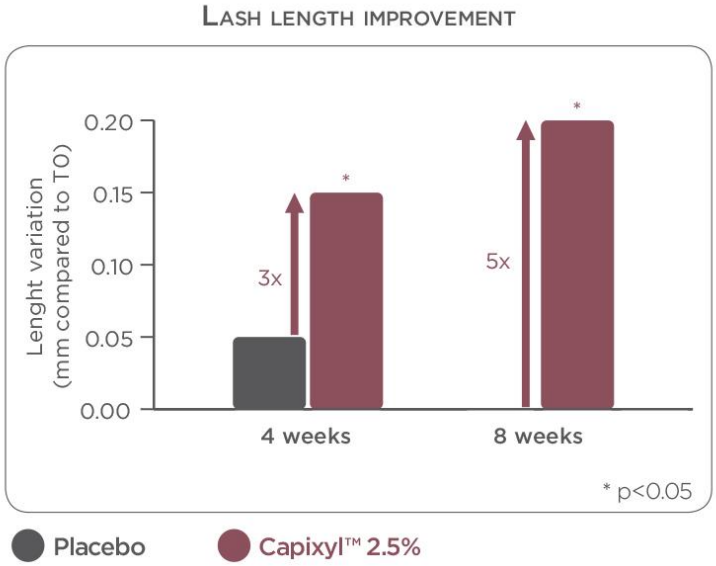


Vol #10



A significant increase of 45.5% in the number of new lashes was observed after the application of Capixyl™ for 8 weeks compared to the placebo treatment, where no new lashes were counted. These new lashes were visible after 8 weeks on the upper eyelid area.

Clinical evaluation of lash length



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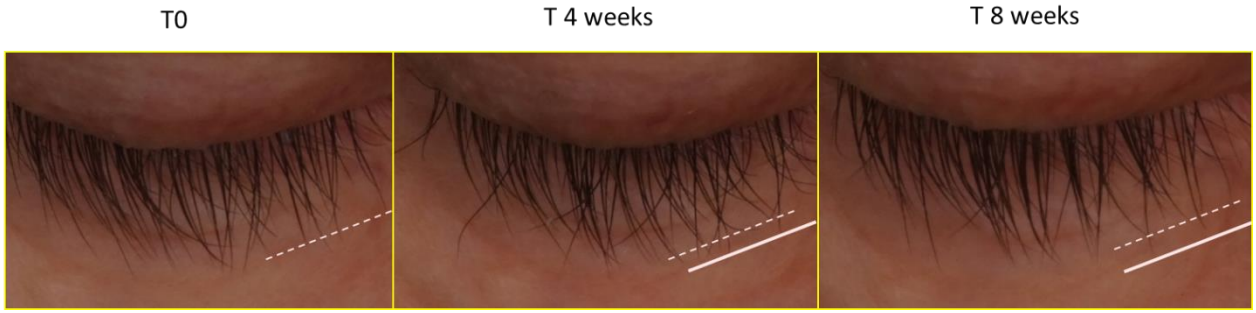
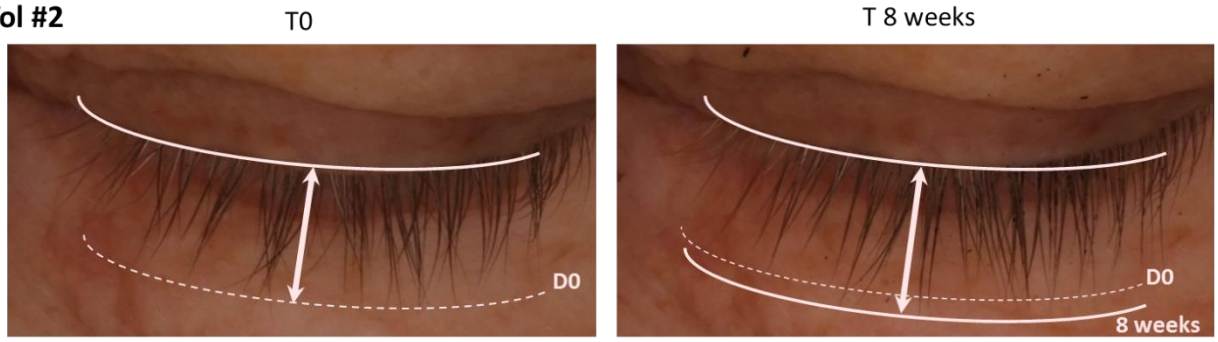


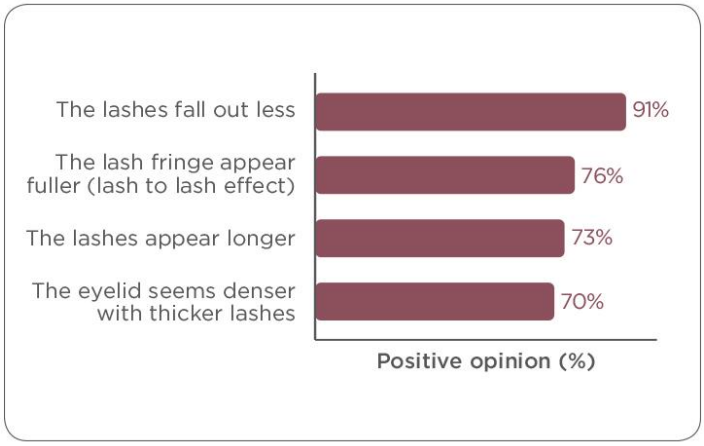
Vol #3

Vol #2



A significant increase in lash length was observed after 4 and 8 weeks of treatment with Capixyl™. Growth up to 0.70 mm was measured after only 4 weeks of treatment. The lashes were 3 and 5 times longer after, respectively, 4 and 8 weeks of application of Capixyl™ compared to the placebo.

Self-assessment



Volunteers noticed a real improvement when they used Capixyl™ as a lash treatment.

CONCLUSION

Capixyl™ induces a clear and significant increase in lash density and length

CONCLUSION

Capixyl™ is an exceptional active complex that combines a biomimetic peptide and a red clover extract rich in biochanin A. Working in perfect synergy, Capixyl™ targets the root causes of hair loss, hair miniaturization and promotes speedy hair growth while simultaneously limiting hair loss. In addition, Capixyl™ rebalances the scalp microbiota, which is crucial for maintaining a healthy scalp environment, essential for optimal hair growth.

Capixyl™ multifunctional efficacy on the hair follicle and scalp microbiota is clinically proven in leave-on and rinse-off applications. Providing rapid and low-dosage efficacy, Capixyl™ successfully regulates the hair growth cycle in both men and women experiencing hair loss.

It is the ultimate multifunctional hair fertilizer for fuller, longer, healthier hair and lashes.

COSMETIC APPLICATIONS

- Anti-hair loss care
- Hair growth care
- Scalp care
- Lash & brow serums
- Makeup

RECOMMENDATIONS FOR USE

1. Recommended dosage

The recommended dosage of Capixyl™ is up to 5%.

2. Addition in formula

Capixyl™ must be preferably introduced at the end of the formulation process, below 40°C.

3. Recommended pH

pH value must be adjusted between 4.0 and 8.0 to ensure proper organoleptic characteristics of the formulated product. Outside the recommended pH range, the stability and aspects of the formula should be evaluated case by case by the formulator.

REFERENCES

[1] Raine-Fenning NJ, Brincat MP, Muscat-Bron Y. Skin aging and menopause : implications for treatment. Am J Clin Dermatol. 2003;4(6):371-8.
[2] Androgenetic Alopecia. Robert P Feinstein, MD, Associate Clinical Professor, Department of Dermatology, Columbia University College of Physicians and Surgeons
[3] Suzuki M. Hair Loss mechanisms and treatment materials : a review. Cosmetics and toileterries. 2008. 9, 123; 51-56.
[4] Bernard BA. Hair biology: an update. *Int J Cosmet Sci.* 2002. 24(1):13-6.
[5] Millar S. E. (2002). Molecular mechanisms regulating hair follicle development. *The Journal of investigative dermatology*, *118*(2), 216–225.
[6] Ji, S., Zhu, Z., Sun, X., & Fu, X. (2021). Functional hair follicle regeneration: an updated review. *Signal transduction and targeted therapy*, *6*(1), 66.
[7] Abreu, C. M., & Marques, A. P. (2021). Recreation of a hair follicle regenerative microenvironment: Successes and pitfalls. Bioengineering & translational medicine, 7(1), e10235.
[8] Shintaro Tanimura,Yuko Tadokoro, Ken Inomata, Nguyen Thanh Binh, Wataru Nishie, Satoshi Yamazaki, Hiromitsu Nakauchi, Yoshio Tanaka, James R. McMillan, Daisuke Sawamura,6 Kim Yancey, Hiroshi Shimizu, and Emi K. Nishimura.Hair Follicle Stem Cells Provide a Functional Niche for Melanocyte Stem Cells. *Cell Stem Cell* 2011. 8, 177–187.
[9] Morgan B. A. (2014). The dermal papilla: an instructive niche for epithelial stem and progenitor cells in development and regeneration of the hair follicle. Cold Spring Harbor perspectives in medicine, 4(7), a015180.
[10] Scott EV, Eckel T 1958. Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. J Invest Dermatol 31: 281–289
[11] Miranda BH, Tobin DJ, Sharpe DT, Randall VA 2010. Intermediate hair follicles: A new more clinically relevant model for hair growth investigations. Br J Dermatol 163: 287–295
[12] Elliott K, Stephenson TJ, Messenger AG 1999. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: Implications for the control of hair follicle size and androgen responses. J Invest Dermatol 113: 873–877
[13] Katsuoka K., Mauch C., Schell H., Hornstein O.P., Krieg T. Collagen-type synthesis in human-hair papilla cells in culture. *Arch. Dermatol. Res.* 1988; 280(3): 140-144.
[14] Tsutsui, K., Machida, H., Nakagawa, A. *et al.* Mapping the molecular and structural specialization of the skin basement membrane for inter-tissue interactions. *Nat Commun* **12**, 2577 (2021).
[15] Katsuoka K., Mauch C., Schell H., Hornstein O.P., Krieg T. Collagen-type synthesis in human-hair papilla cells in culture. *Arch. Dermatol. Res.* 1988; 280(3): 140-144.
[16] Randall VA. Androgens and human hair growth. *Clin. Endocrinol.* 1994. 40(4):439-57.
[17] Constantinou, A., Kanti, V., Polak-Witka, K., Blume-Peytavi, U., Spyrou, G. M., & Vogt, A. (2021). The Potential Relevance of the Microbiota to Hair Physiology and Regeneration: The Emerging Role of Metagenomics. Biomedicines, 9(3), 236.
[18] Pinto, D., Sorbellini, E., Marzani, B., Rucco, M., Giuliani, G., & Rinaldi, F. (2019). Scalp bacterial shift in Alopecia areata. PloS one, 14(4), e0215206.
[19] El-Domyati M, Attia S, Saleh F, Abdel-Wahab H. Androgenetic alopecia in males: a histopathological and ultrastructural study. *J Cosmet Dermatol*. 2009. 8(2): 83-91.

[20] Chittur S, Parr B, Marcovici G. Inhibition of Inflammatory Gene Expression in Keratinocytes Using a Composition Containing Carnitine, Thioctic Acid and Saw Palmetto Extract. Evid Based Complement Alternat Med. 2009 Aug 19. [in printing]

[21] Mahé YF, Michelet JF, Billoni N, Jarrousse F, Buan B, Commo S, Saint-Léger D, Bernard BA. Androgenetic alopecia and microinflammation. *Int J Dermatol.* 2000. 39(8): 576-584.

[22] Jahoda CA, Whitehouse J, Reynolds AJ, Hole N. Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. Exp Dermatol. 2003 12: 849-859.

[23] Blanpain C, Fuchs E. Epidermal stem cells of the skin. Annual Rev Cell Dev Biol. 2006. 22: 339-373.

[24] Alonso L1, Fuchs E. Stem cells of the skin epithelium. Proc Natl Acad Sci USA. 2003 Sep 30;100

[25] Hu, X. M., Li, Z. X., Zhang, D. Y., Yang, Y. C., Fu, S. A., Zhang, Z. Q., Yang, R. H., & Xiong, K. (2021). A systematic summary of survival and death signalling during the life of hair follicle stem cells. *Stem cell research & therapy*, *12*(1), 453.

[27] Abreu, C. M., & Marques, A. P. (2021). Recreation of a hair follicle regenerative microenvironment: Successes and pitfalls. Bioengineering & translational medicine, 7(1), e10235.

[28] Shintaro Tanimura,Yuko Tadokoro, Ken Inomata, Nguyen Thanh Binh, Wataru Nishie, Satoshi Yamazaki, Hiromitsu Nakauchi, Yoshio Tanaka, James R. McMillan, Daisuke Sawamura,6 Kim Yancey, Hiroshi Shimizu, and Emi K. Nishimura.Hair Follicle Stem Cells Provide a Functional Niche for Melanocyte Stem Cells. *Cell Stem Cell* 2011. 8, 177–187.

[29] Lee Byung-Woo, Oh Jin-Woo, Seo Minchul, Hwang Jae-Sam, Yoon Byungil. Expression of p63 and its association with cell proliferation at different stages of murine hair follicle cycle. J Biomed Transl Res 2018;19(1):10-15.

[30] Scott EV, Eckel T 1958. Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. J Invest Dermatol 31: 281–289

[31] Katsuoka K., Mauch C., Schell H., Hornstein O.P., Krieg T. Collagen-type synthesis in human-hair papilla cells in culture. *Arch. Dermatol. Res.* 1988; 280(3): 140-144.

[32] Tsutsui, K., Machida, H., Nakagawa, A. *et al.* Mapping the molecular and structural specialization of the skin basement membrane for inter-tissue interactions. *Nat Commun* **12**, 2577 (2021).

[33] Katsuoka K., Mauch C., Schell H., Hornstein O.P., Krieg T. Collagen-type synthesis in human-hair papilla cells in culture. *Arch. Dermatol. Res.* 1988; 280(3): 140-144.

[34] Scott EV, Eckel T 1958. Geometric relationships between the matrix of the hair bulb and its dermal papilla in normal and alopecic scalp. J Invest Dermatol 31: 281–289

[35] Elliott K, Stephenson TJ, Messenger AG 1999. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: Implications for the control of hair follicle size and androgen responses. J Invest Dermatol 113: 873–877

[36] Katsuoka K., Mauch C., Schell H., Hornstein O.P., Krieg T. Collagen-type synthesis in human-hair papilla cells in culture. *Arch. Dermatol. Res.* 1988; 280(3): 140-144.

[37] Messenger, A. G., Elliott, K., Temple, A., & Randall, V. A. (1991). Expression of basement membrane proteins and interstitial collagens in dermal papillae of human hair follicles. The Journal of investigative dermatology, 96(1), 93–97.

[38] El-Domyati M, Attia S, Saleh F, Abdel-Wahab H. Androgenetic alopecia in males: a histopathological and ultrastructural study. *J Cosmet Dermatol.* 2009. 8(2): 83-91.

[39] Katsuoka K., Mauch C., Schell H., Hornstein O.P., Krieg T. Collagen-type synthesis in human-hair papilla cells in culture. *Arch. Dermatol. Res.* 1988; 280(3): 140-144.

[40] Philpott M.P., Green M.R., Kealey T. Human hair growth in vitro. J. Cell. Sci. 1990; 97 : 463-471.

[41] Pinto, D., Sorbellini, E., Marzani, B., Rucco, M., Giuliani, G., & Rinaldi, F. (2019). Scalp bacterial shift in Alopecia areata. PloS one, 14(4), e0215206.
[42] Montagna W, Ford DM. Histology and cytochemistry of human skin. 3. The eyelid. Arch Dermatol. 1969. 100(3):328-335.
[43] Na JI, Kwon OS, Kim BJ, Park WS, Oh JK, Kim KH, Cho KH, Eun HC. Ethnic characteristics of eyelashes: a comparative analysis in Asian and Caucasian females. Br J Dermatol. 2006. 155(6): 1170-1176.
[44] Liotet S, Riera M, Nguyen H. The lashes. Physiology, structure, pathology (author's transl). Arch Ophtalmol (Paris). 1977. 37(11): 697-708.
[45] Na JI, Kwon OS, Kim BJ, Park WS, Oh JK, Kim KH, Cho KH, Eun HC. Ethnic characteristics of eyelashes: a comparative analysis in Asian and Caucasian females. Br J Dermatol. 2006. 155(6): 1170-1176.
[46] Thibaut S, De Becker E, Caisey L, Baras D, Karatas S, Jammayrac O, Pisella PJ, Bernard BA. Human eyelash characterization. Br J Dermatol. 2010 Feb 1;162(2):304-10.
[47] Elder MJ. Anatomy and physiology of eyelash follicles: relevance to lash ablation procedures. Ophthal Plast Reconstr Surg. 1997 Mar;13(1):21-25.