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SAHAJ NATURALS

Procapil™

English

sederma

PROCAPIL™

SEDERMA Patents: FR 2 791 684/ WO 00/58347

For enhanced anchorage of the hair: Matrikine and Phytoregulators

PROCAPIL™

SYNOPSIS

Description: Combination of 3 complementary active substances in solution.

INCI name: Butylene Glycol (and) Water (Aqua) (and) PPG-26-Buteth-26 (and) PEG-40 Hydrogenated Castor Oil (and) Apigenin (and) Oleanolic Acid (and) Biotinoyl Tripeptide-1

Objectively-demonstrated cosmetic activity:

- *In vitro* studies:
  - Study of the substantiveness of peptide biotinyl-GHK on the hair follicle - (BIOALTERNATIVES study)
  - Anti-aging study on cultured hair follicles (BIO-EC study):
    In the presence of 2 ppm biotinyl-GHK (i.e. 1% **PROCAPIL™**), superior growth to that of the control (+58%) was obtained, in a similar manner to that in the presence of Minoxidil® 2 ppm (10 µM). With 5 ppm biotinyl-GHK (i.e. 2.5% **PROCAPIL™**), the growth was 121% greater than that of the control.
  - Gene activation by **PROCAPIL™** (DNA array) (BIOALTERNATIVES study)

- *In vivo* studies:
  Placebo-controlled clinical trial over 4 months (Laboratoires DERMSCAN).
  The results of the 4-month clinical trial, covering one cycle of the telogen phase, showed a significant increase in the anagen/telogen ratio comparable to oral Finastéride® treatment in the **PROCAPIL™**-treated group.

Recommended dose for use : 3%

Safety: certified in the context of the UNITIS Charter

Reports available on request:

Expert report
HET CAM test
Patch test on humans
RIPT
Ames' test

CONTENTS

03/2004/V1

1. INTRODUCTION

Hair thinning, morning after morning, until, finally, the hair line has markedly receded: this is the everyday experience of a substantial percentage of the male population. After the worry and disappointment, ultimately, resignation enables the individual to adapt to an unflattering self-image with baldness affecting all of the skull with the exception of the occipital zone.

Alopecia affects 20% of men as of age 20 years and increases by 10% per decade. This means that over half of men aged 50 suffer from baldness.
The strength of male demand in that cosmetic segment will be readily understood.
Alopecia, moderate in the beginning, may occur in young adults and have an androgenic etiology in 95% of cases.

Four centuries before Christ, Hippocrates observed that eunuchs never became bald, thus discovering that baldness was dependent on a specifically male factor.

This androgen-dependent phenomenon also explains why women suffer less from baldness. The handicap only occurs under particular circumstances, such as disease, stress or sometimes the peri-menopausal period, when estrogens fall abruptly and no longer offset the circulating testosterone.

This inequality of men and women spares the latter, with less than 1% complaining of excessive hair loss or established baldness.
There is another difference in favor of women's hair: its life expectancy is markedly greater, up to 7 years, while, on average, it is only half that for men's hair. This explains the great length that a woman's hair may reach.

However, the hair growth cycle is the same in both genders and consists in 3 successive phases:

Each hair is formed at the level of a dermal papilla, which yields a hair bulb, then a hair proper, through the cell division of keratinocytes.
Obeying an 'internal clock', each papilla, located at the base of the hair follicle receives a growth message necessary to trigger the cycle of natural renewal of the hair.

- The first phase, or growth phase, is known as the **anagen** and lasts on average 3 to 4 years.
- The second phase consists of discontinuation of growth over 2 to 3 weeks. This is the **catagen**.
- The third phase is the **telogen**, when the hair falls out. This occurs fairly slowly since it requires regression of the bulbar zone and detachment of the hair shaft (situated in the hypodermis at a depth of about 1.5 mm) prior to expulsion towards the surface. The duration is about 3 to 4 months.

The cycle is repeated about 25 times in a lifetime.

- **MORPHOGENESIS OF THE HAIR**

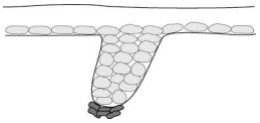
Hair or body hair are engendered by the interaction between the dermis and epidermis.
Induced by an epidermal message, the fibroblasts organize and forward a signal to keratinocytes inducing formation of an epidermal plate (1) which invaginates in the dermis to form a primary bud (2).

In turn, the primary bud emits messages which stabilize the surrounding fibroblasts to form the future dermal papilla (3).
Lastly, the bud gradually differentiates (4 and 5) into a hair follicle under the influence of the messages sent by the dermal papilla.

The different stages in the morphogenesis of a hair after PAUS, 1999

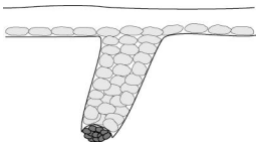
Stage 1

Organization of the epidermal plate following a signal from the keratinocytes



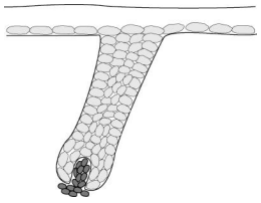
Stage 2

Invagination of the placode in the dermis with formation of the primary bud

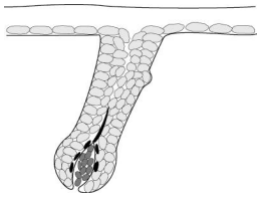


Stage 3

Incipient formation of the dermal papilla following a signal from the fibroblasts



Stage 4



Stage 5

Differentiation: formation of the hair follicle in response to a signal from the dermal papilla

Numerous messengers interact in this dermal-epidermal dialog and their precise roles have yet to be elucidated.

- **HAIR AND THE EXTRACELLULAR MATRIX**

An essential component of hair growth is the physical interaction between the dermis and epidermis, within the dermal papilla where keratinocytes and fibroblasts are condensed.
The dermal papilla is a zone that is particularly rich in collagens and glycosaminoglycans which maintain the close contact between the two cell populations and promote the chemical communication necessary for hair shaft growth. The importance of collagen IV and laminin needs to be stressed, since those matrix components also constitute the basement membrane of the dermoepidermal junction and much of the dermal papilla (with fibronectin), which may be considered a matrix motor for hair growth (JAHODA *et al.*, 1992), (ALMOND-ROESLER B. *et al.*, 1997).

The central role played by interface matrix molecules in the growth and differentiation of the skin and its appendages is clearly illustrated by the work of TAMIOLAKIS (2001) on the embryogenesis of the human skin.
Immunofluorescent labeling between week 12 and 21 shows the strong concentration of laminin, collagen IV and fibronectin in the root sheath of the hair shaft.

The compounds initially only present in the epithelial germinal cells of the hair bulb (week 12) gradually invade the root sheath, then migrate to the area where the hair emerges and to the dermoepidermal junction (DEJ at week 21).

At the initial stage, prior to week 12, at dermoepidermal basal lamina level, vimentin is present and the first hemidesmosomes form (week 8-9).

The importance of the matrix components in the survival and growth of cultured human hair follicles was also demonstrated by WARREN R. *et al.*, 1992.

The role of the interface matrix proteins is particularly clearly illustrated in the sequence of events leading to reconstitution of a new hair. When the bulb has been artificially sectioned and removed, the keratinocytes of the outer root sheath migrate below the compromised zone. Fibroblasts deploy opposite the keratinocytes. In this new interface zone, a matrix consisting of collagen IV, laminin 5 and fibronectin is formed. A new dermal papilla is constituted and becomes operational (COLIN *et al.*, 1992).

It should be noted that collagen IV and laminin 5 are mainly synthesized by keratinocytes and that laminin 5 plays a crucial and irreplaceable role in dermoepidermal cohesion and in the migration or keratinocytes during cicatrization (ROUSSELLE P., 2003).

- **HAIR AND DEFICIENCIES**

Biotin or vitamin H is an essential vitamin made available to the body through the diet.

Biotin deficiency gives rise to anomalies of the skin and appendages: fine, 'uncombable' hair (SHELLEY *et al.*, 1985), alopecia, scaling, pruritus and dermatitis (FRIGG *et al.*, 1989; FRITSCHE *et al.*, 1991).
The cells most sensitive to biotin deficiency include neurons and keratinocytes (SUORMALA *et al.*, 2002). Physiological deficiencies in man give rise to mental retardation and skin anomalies. This is not wholly unexpected, given the shared embryological origin of the skin and brain.

In the epidermis, biotin regulates, in particular, the formation of the late cytokeratins of differentiation (FRITSCHE *et al.*, 1991).

From a biochemical viewpoint, biotin is an enzymatic cofactor indispensable for the correct operation of mitochondrial carboxylases, for which it constitutes the prosthetic group.

Bound covalently to lysine residues of mitochondrial enzymes (pyruvate, propionyl-CoA, 3-methyl crotonyl-CoA and acetyl-CoA carboxylases), biotin, converted to its active form, carboxybiotin, enables transfer of CO_2 groups to acceptors such as pyruvate (Krebs' cycle) and oxaloacetate (lipogenesis): biotin is thus a crucial cofactor in mitochondrial metabolism.

- **STRATEGIC TARGETS USED TO SLOW HAIR LOSS**

First target

The first target is obviously androgenic: the aim is to slow production of dihydrotestosterone (DHT) by 5α-reductase. This metabolite is more active than testosterone (supplied by the blood) since it has a greater affinity for the androgen receptors located, in particular, on the dermal papilla (ANDERSSON S., 2001).
DHT acts by atrophying the hair follicle and, according to a recently advanced hypothesis (SAWAYA *et al.*, 2001), through a pro-apoptotic mechanism *via* caspase 3.

Two isoforms of 5a-reductase are present in the skin but the α1 form seems to be more active at facial level (cf. acne) and in the hair follicle at dermal papilla level, while the α2 form is reported to be more present at inner and outer root sheath level (BAYNE *et al.*, 1999).

L'Oréal's team (GERST, 2002), in a structure/activity relationship study, showed that the specific inhibitors of α2-reductase were not active on cultured hair follicles, unlike specific α1-reductases or mixed α1- and α2-reductases.

The use of a mixed 5α1- and 5α2-reductase inhibitor such as finasteride (a drug originally developed for prostatic hypertrophy because of its action on 5α2-reductase) enabled a considerable reduction in hair loss in patients presenting with a markedly receded hair line.
A 47% increase in hair in the anagen phase was thus obtained after 1 year through simple inhibition of 5α-reductase (VAN NESTE *et al.*, 2000).

This effect is considered due to a local decrease in DHT levels (50%). DHT is thus present at the concentration found in normal scalp (DALLOB *et al.*, 1994).

Second target

The second target is the blood: good capillary perfusion is the mechanism advanced to explain the unexpected success of a peripheral vasodilator, Minoxidil®, originally used as an antihypertensive. Its interesting side effect, fresh growth of hair, was discovered through clinical use of the drug to treat hypertension.

While the effect related to enhanced capillary perfusion should not be minimized, it is now known that Minoxidil® also acts by maintaining active proliferation of the already differentiated keratinocytes in the follicle (BOYERA N, 1997).

Third target

In addition to the hyper-proliferative effect of Minoxidil® (concentration less than 100 µM), a pro-differentiating effect at a higher dose (of the order of a millimole) has been reported. This effect may be obtained in long-term treatment with local accumulation in the follicles. As a result, hair loss is retarded. The hyper-proliferative and pro-differentiating effect thus constitute the 3rd target.

2. THE SEDERMA CONCEPT FOR DELAYING HAIR LOSS

It is clear, on the basis of current understanding of the morphogenesis of hair and the progressive discovery of the potential causes triggering or exacerbating alopecia, that a highly complex and multifactorial mechanism is involved. Attempting to control the genesis of the follicle and the progression of the hair growth cycle is thus tantamount to a wager.

Moreover, very recent genetic studies have shown a substantial number of genes (at least 5) whose mutations have consequences with respect to alopecia (SEDGWICK John, GQ Magazine, 1999).

It would therefore appear important not to neglect the advances already made, particularly with respect to the anti-androgenic and vasodilatory components:
SEDERMA therefore selected two active substances of plant origin acting on those targets: oleanolic acid (extracted from olive tree leaves) for the inhibition of 5α1- and 5α2-reductases and apigenin (flavonoid extracted from citrus) for vasodilation.

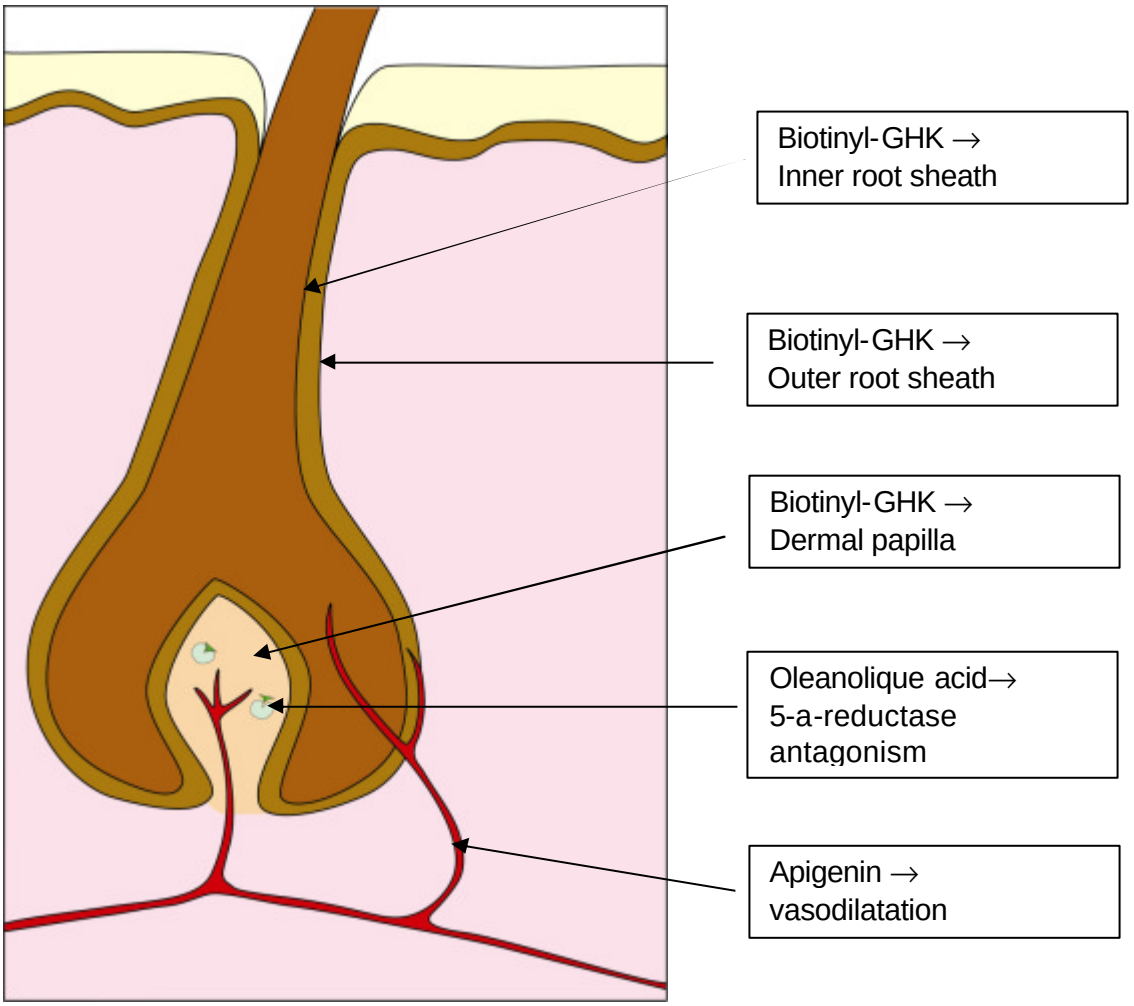
SEDERMA then strengthened those two approaches with an action targeted on the concept of hair anchorage:
If we are able to ensure better 'rooting' of the hair in the skin, enhanced adhesion will be obtained (dermal papilla - hair follicle) with an improvement in the exchanges of chemical messengers at interface level. This enhanced interfacing will have a positive impact on the quality and duration of the anagen phase. Similarly, enhanced anchorage of the hair sheath and dermis should delay the onset of the telogen phase.

With that aim, we selected a peptide sequence endowed with pro-matricial activities: the peptide Glycyl-Hystidyl-Lysine, a member of the Matrikines series (MAQUART *et al.*, 1999), and bound it to vitamin H (biotin). Deficiency in that vitamin gives rise to fine, alopecic hair, sagging skin and dermatitis.
A new entity was thus created: **Biotinyl-GHK, a vitamin-bearing peptide**, with the expectation of a dual matricial and metabolic action.

Thus, three active substances, oleanolic acid to inhibit 5a-reductase, apigenin to enhance blood perfusion and biotinyl-GHK for enhanced anchoring of the hair with strengthened growth, were combined in the new concept:

PROCAPIL™

PROCAPIL™ compound targets



The action mechanism, confirmed by the activation of certain genes (DNA array), matrix strengthening effects, growth of human hair follicle explants in cultures and results of a 4-month placebo-controlled clinical trial, constitute the subject of this dossier.

3. EFFICACY TESTS

3.1. *In vitro* studies

3.1.1. Study on cultured hair follicle explants (Substantiveness of peptide Biotinyl-GHK on the hair follicle - BIOALTERNATIVES study)

Principle

The study was conducted on human skin explants (abdominal plasty) cultured in PBS medium in a moist chamber at 21°C.

Following incubation of the explants with the peptide, immunohistochemical study of sections was conducted to investigate for selective localization of the product around the pilial zone.

Protocol

Skin explants (with hair follicles) were incubated in the presence of 60 ppm peptide for 18 hours and compared to control explants exposed to the peptide-free excipient.

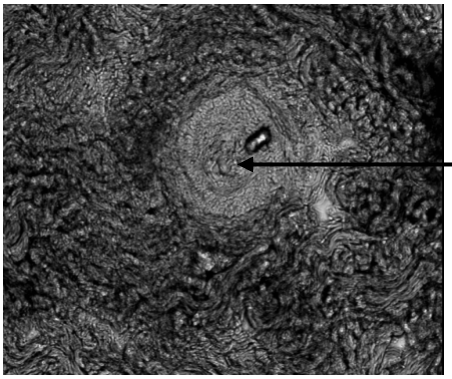
The determinations were conducted in triplicate.

After 18 hours, an 8-mm biopsy was removed from the center of each well and immediately frozen in liquid nitrogen.

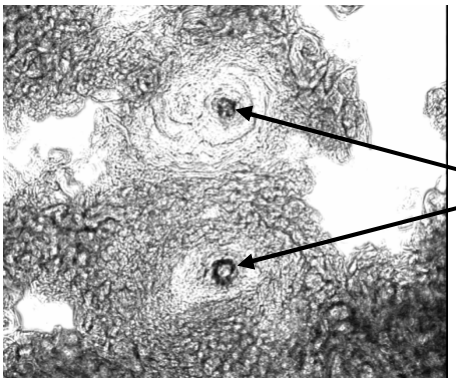
The 15 µm thick sections were made using a freezing microtome (cryostat), then dried and fixed. Biotinyl-GHK was detected by immunolabeling coupled with streptavidine peroxidase.

Results

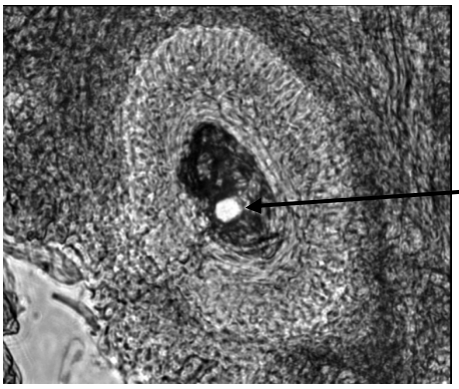
The sections showed the clear peri-pilial localization of peptide biotinyl-GHK.



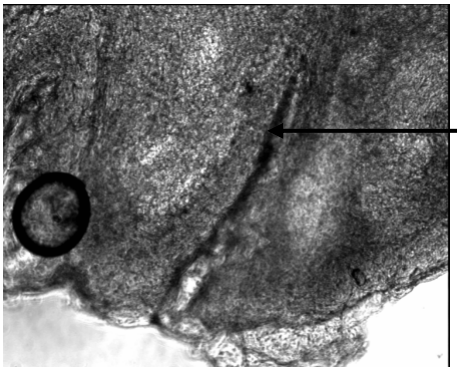
A: image of the control explant: no labeling around the hair shaft (magnification 20X)



B: clear localization of biotinyl-GHK around 2 adjacent hair shafts (magnification 20X)



C: with 40X magnification, the peripheral concentric zone of the hair is strongly labeled by biotinyl-GHK



D: longitudinal section, specific localization of biotinyl-GHK with good distribution along the length of the hair and no labeling of the surrounding tissue (magnification 20X)

Conclusion

Biotinyl-GHK is a substantive peptide that exhibits specific localization around its target: the hair follicle.

3.1.2. Anti-aging study on cultured hair follicles (BIO-EC study)

Principle

The excess hair follicles prepared in the context of a micrograft transplantation session were collected for culturing in a medium similar to that reported by PHILPOTT *et al.*, 1996.

Protocol

The hair follicles were individually incubated at 37°C under an air plus CO_2 (5%) atmosphere for 14 days.

The explants were divided into various groups: control group in the culture medium alone, positive control group (positive reference product) and test group exposed to peptide biotinyl-GHK.

The culture media were changed every 2 days.

General morphology was observed on D0 and D14.

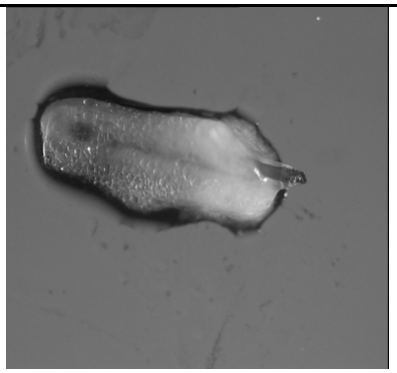
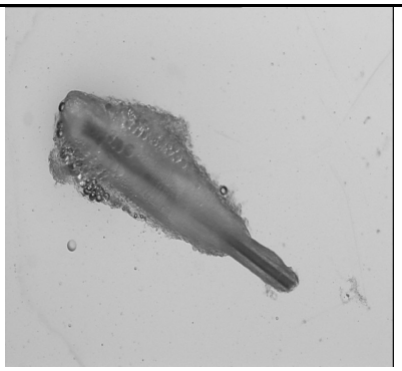
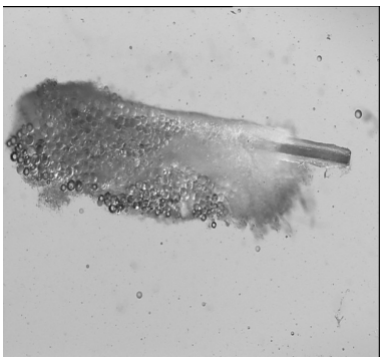
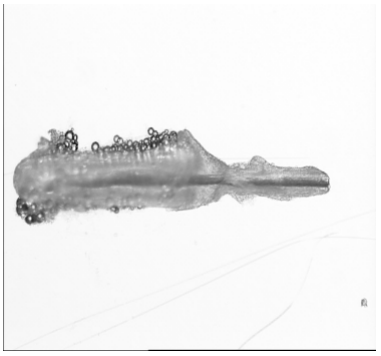
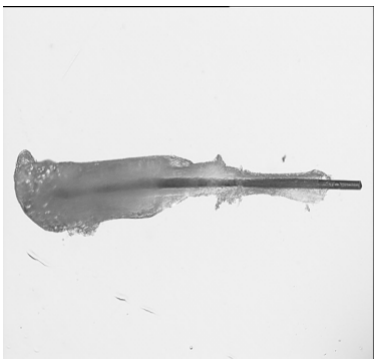
Concomitantly, a fraction of the follicles was frozen with a view to conducting more advanced immunohistochemical studies.

Growth was monitored using a digital camera with images taken on D0, D3, D5, D7, D11 and D14.

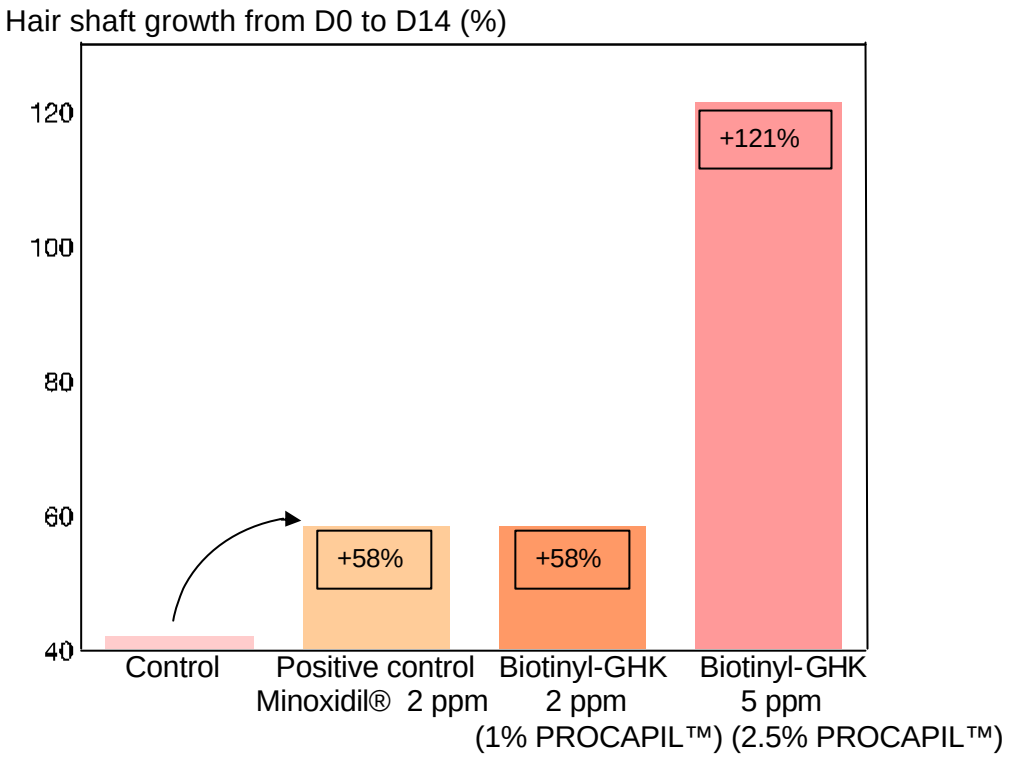
General morphology results

1- Hair shaft growth

The growth determinations were conducted on the free part of the hair shaft (excluding the lower part of the hair bulb).

Growth of the control follicle, T0 to T14 days	Growth of the follicle exposed to Biotinyl-GHK, T0 to T14 days	
		T0
		T7
		T14

The results obtained are reported in the following graph:



Conclusion

Under exposure to 2 ppm peptide (i.e. 1% **PROCAPIL™**), 58% more growth than that of the control was obtained and the growth was similar to that observed in the presence of 2 ppm Minoxidil® (10 µM). With 5 ppm Biotinyl-GHK (i.e. 2.5% **PROCAPIL™**), the growth was 121% greater than that of the control.

2. Anti-aging activity on the root sheath

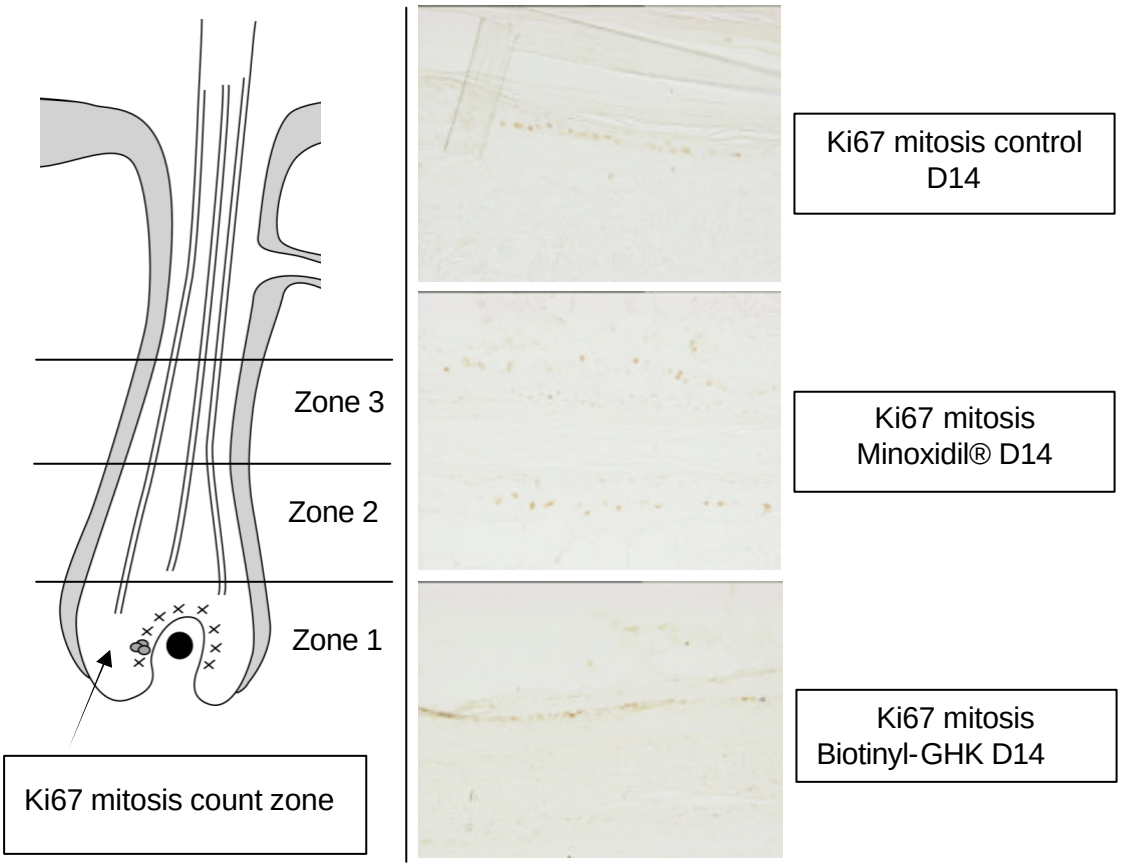
Principle

Mitotic marker Ki67 was used to evidence cell growth activity.

Protocol

Freezing microtome sections were made on D0 and D14 and exposed to peroxidase-bound anti-Ki67 antibody.

On the sections, the dividing cells were stained dark brown. A count was conducted on the lower section of the root sheath of the hair shaft under the microscope. All the cells showing Ki67 marker were counted (zone 1).



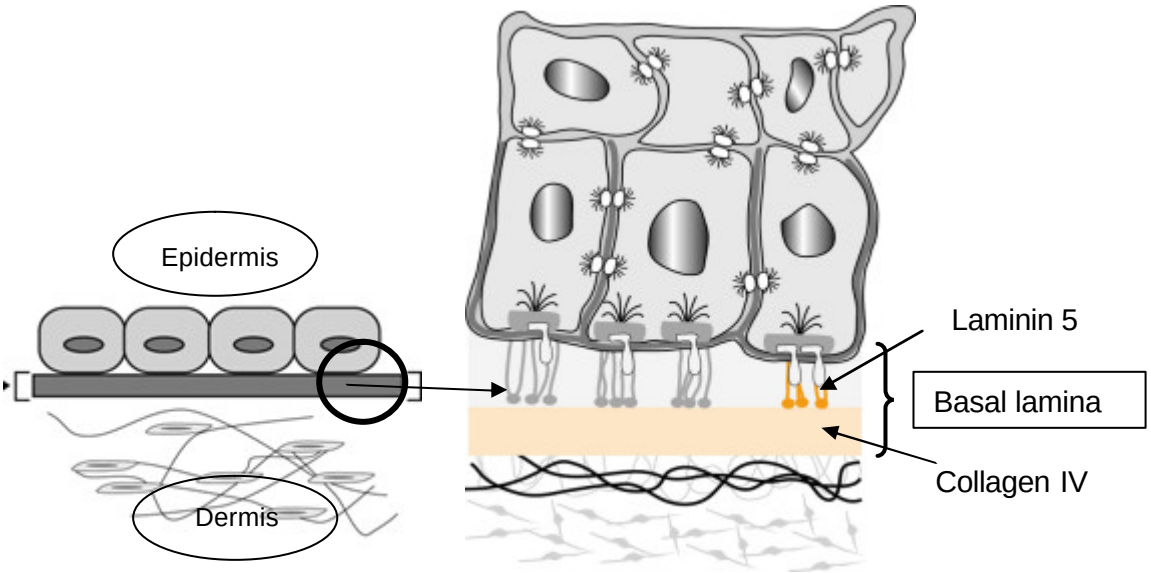
Results

For the control bulb, the results showed a decrease in mitotic keratinocytes on day 14 of culture, reflecting cell aging.
Minoxidil® maintained proliferative activity (as reported by BOYERA *et al.*, 1997), as did 0.3 µM biotinyl-GHK (2 ppm) and approximately 1 µM (5 ppm) biotinyl-GHK. The effect obtained with biotinyl-GHK was superior in that it was obtained at concentrations 10- to 30-fold lower than the Minoxidil® concentration, 10 µM (2 ppm).

3. Stimulation of the adhesion proteins of the root sheath and dermal papilla

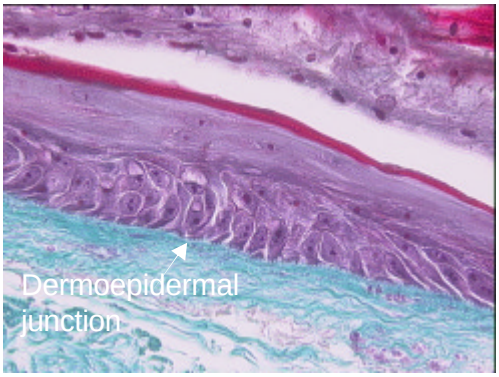
Principle

The quality of the dermoepidermal junction depends on the formation of a very dense basal lamina rich in laminin 5 and collagen IV, on which the keratinocytes of the first basement layer rest and to which they adhere.

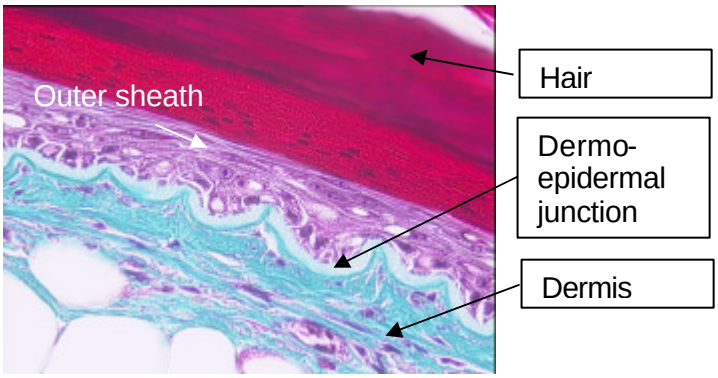


Dermoepidermal junction

a) Morphological observation after 14 days of culturing showed, in the control, a dermoepidermal junction, on the outer sheath side, that was flattened and had lost its basal lamina.
In contrast, when the hair follicle was incubated with biotinyl-GHK for 14 days, the basal lamina persisted and was clearly drawn showing its sinusoidal character. These two findings reflect a strongly adherent and living dermoepidermal junction.



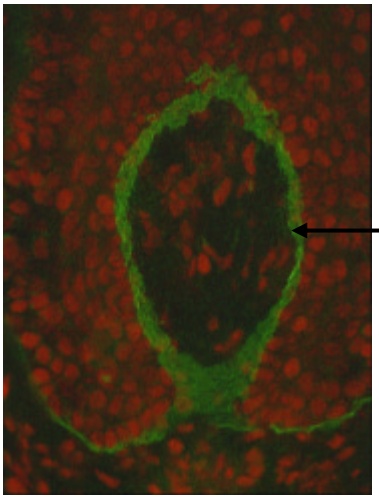
Control: 14 days



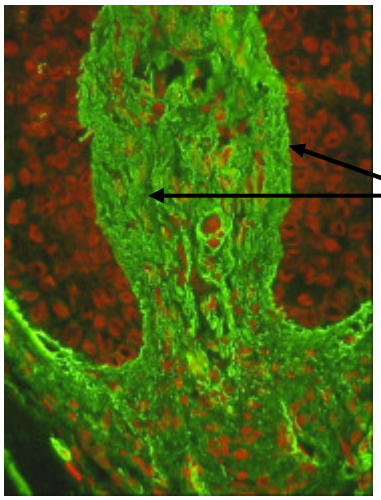
Treated: 14 days

b) Laminin 5 and collagen IV are two proteoglycans of capital importance in the constitution of the basement membrane, the attachment zone for the epidermis and dermis, and, in the case of hairs, between the root sheath and dermis. The matrix components can be detected on histological sections by immunolabeling.

Laminin 5 and collagen IV are also strongly present in the dermal papilla (JAHODA *et al.*, 1992) as shown by the control sections made on D0 using cultured hair follicles.



Laminin 5 in the inner root sheath, interface with the dermal papilla



Collagen IV in the inner root sheath and in the dermal papilla

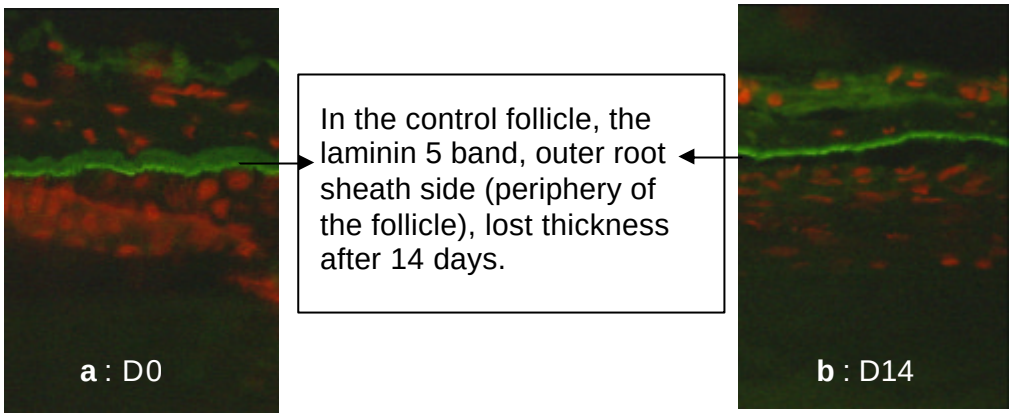
<u>Protocol</u>

The freezing microtome sections of the D0 and D14 samples were exposed to fluorescent antibodies specific to laminin 5 (Tebu) and collagen IV (Cliniscience). The staining obtained consists in green fluorescence. Counter-staining of the nuclei was conducted using propidium iodide, yielding red staining.

The observations were conducted on the inferior zone of the follicle above and below the bulb (zones 1 and 2, cf. diagram page 15).

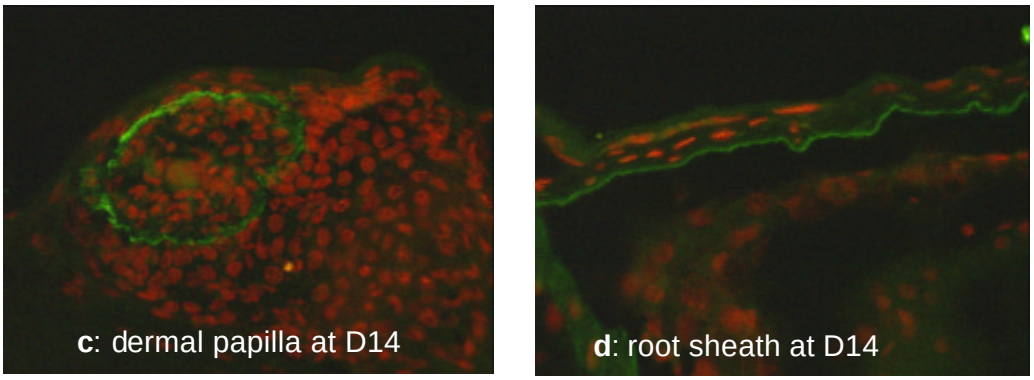
Results

a) Laminin 5

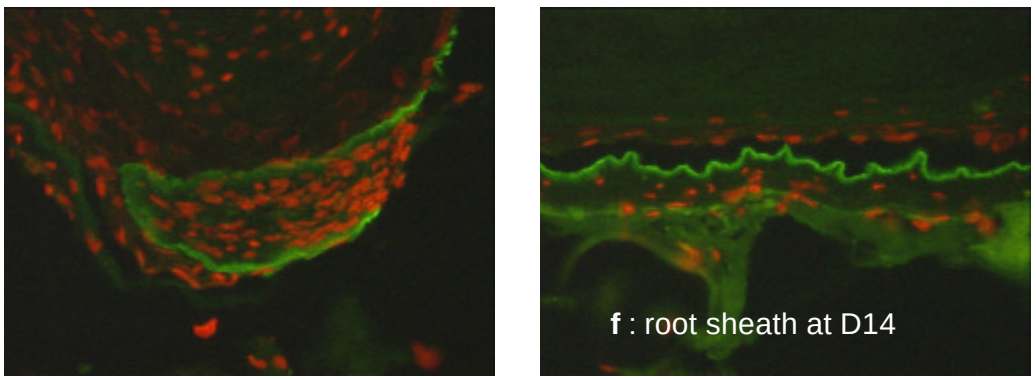


In the control follicle, the laminin 5 band, outer root sheath side (periphery of the follicle), lost thickness after 14 days.

This led us to investigate for laminin 5 loss in the presence of the various products at D14.

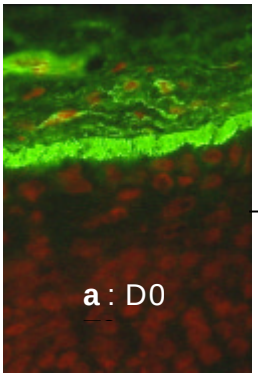


Following exposure to 2 ppm (10 µM) Minoxidil®, the follicle showed a laminin 5 band that remained thick and strip-like after 14 days

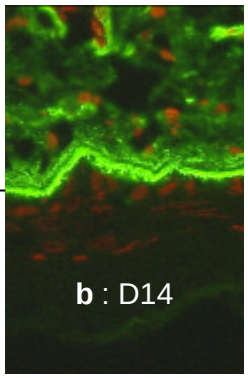


Following exposure to 2 ppm (0.3 µM) Biotinyl-GHK, laminin 5 remained strongly present at papilla level and in the outer root sheath after 14 days

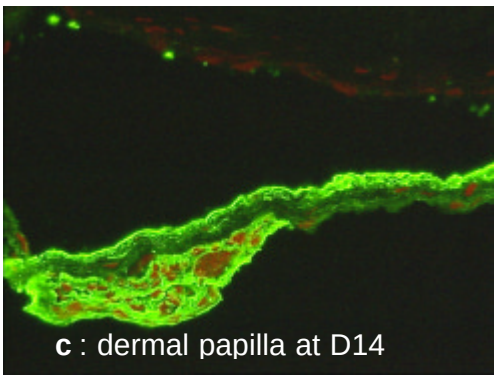
b) Collagen IV



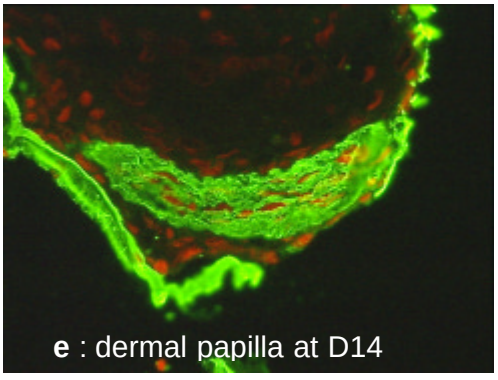
In the control, the collagen IV band, very thick at T0, has destructured and lost density at T14 days. The papilla lost its labeling (photos not shown)



This led us to investigate for collagen IV loss at D14 in the presence of the various products.



Exposure to 2 ppm (10 µM) Minoxidil® induced a loss of collagen IV density in the dermal papilla and in the root sheath after 14 days.



In the presence of Biotinyl-GHK, after 14 days, collagen IV remained strongly present in the dermal papilla (e) and was very thick and structured at root sheath level (f). The structure observed is almost the same as that of the control at D0 (a).

Conclusion

The protective and reparative effects of peptide Biotinyl-GHK on the constituents of the root sheath and dermal papilla, collagen IV and laminin 5, were clearly demonstrated. On hair follicle explants cultured over 14 days, the effects observed were more marked than those induced by Minoxidil® at Biotinyl-GHK concentrations that were 30-fold lower.

In terms of general morphology, Biotinyl-GHK is endowed with very marked anti-aging activity on hair follicle keratinocytes (14-day culture) with maintenance of a viable root sheath (mitoses, Ki67) and enhanced structuring by adhesion proteins (collagen IV and laminin 5) responsible for anchorage in the dermis.

3.1.3. Gene activations by PROCAPIL™ (BIOALTERNATIVES study)

Principle

The DNA array study employed a panel of 600 genes selected for their interest with respect to cell function. The study showed the marker genes up-regulated and down-regulated, thus enabling definition of a profile of the mechanisms responsible for the action of the cosmetic active substance on keratinocyte and fibroblast populations.

Protocol

The DNA array study was conducted on SkinEthic® reconstituted human epidermis samples incubated in the presence of **PROCAPIL™** (complex consisting of 3 active substances: peptide biotinyl-GHK, oleanolic acid and apigenin).

Incubation was conducted for 18 hours. The mRNA present in the cells was reverse transcribed to yield DNA and amplified (RT-PCR method) to obtain a legible signal vs. the control cultures.

The resulting image is a snapshot, at time point 18 hours, of the genes up-regulated or down-regulated by **PROCAPIL™**.

Results

The tables on the following pages show the results considered significant vs. the control: at least a 30% positive or negative change.

When smaller changes (20 to 30%) were observed in several related genes, those changes were nonetheless considered to have a degree of mechanistic significance.

Genes up-regulated vs. the control (100%) and coding for proteins:

Change in gene expression under exposure to PROCAPIL™	%
Adhesion complex proteins	
Desmosomal proteins 1&3 (Desmogleins)	135% / 138%
Desmocollin 1	146%
Fibronectin receptor β-subunit	134%
Vimentin	138%
Laminin binding protein	146%
Integrin β1 & β2	134% / 144%
Antioxidant enzymes	
Thioredoxins peroxidases (TDPX2 & AO372)	152 and 174%
SOD (mitochondrial & cytosolic)	150 and 169%
Metallothioneins MTH & HMT	188 and 190%
CYP b-reductase	160%
Stress proteins	
HSP 27	164%
HSP 90	139%
Anti-inflammatory proteins	
Interferon γ antagonist	135%
Cell metabolism enzymes	
Mitochondrial trifunctional protein & Acyl CoA precursor	123 and 128%
Ornithine decarboxylase	132%
Glutamine synthetase	136%
Acetyl CoA transferase	137%
Isocitrate dehydrogenase	189%
iNOS	143%
NADP isocitrate dehydrogenase	189%
Proliferation / differentiation markers	
Proliferating cell nuclear antigen (PCNA)	191%
Cytokeratins 10, 14 and 16	154 / 150 / 144%
Steroid receptor co-activator	160%

Genes down-regulated vs. the control (100%) and coding for proteins:

Change in gene expression under exposure to PROCAPIL™	%
Pro-inflammatory proteins	
Interferon γ receptor	-57%
Angiogenic and matrix-remodeling factors	
Vitronectin	-52%
TIMP1/TIMP2	-43% / -24%
Antichymotrypsin α1	-43%
Lysyl hydroxylases 1&2	-50% / -29%
Heparan sulfate proteoglycan	-40%
Collagen 1 subunit	-46%
Cell proliferation regulation	
Retinoic binding proteins CRABP1/CRABP2	-34% / -63%
Vit. D3 receptor	-40%

Interpretation

The up-regulated genes reflect a cell profile oriented towards high growth activity with very strongly expressed cell metabolism enzymes (123 to 189%, depending on the enzyme). Antioxidant protective enzymes were also associated since it is necessary to protect the cell against the oxygen free radicals systematically generated by the high level metabolic activity.

Markers of cell proliferation such as proliferating cell nuclear antigen (PCNA), steroid receptor co-activator and cytokeratins 10, 14 and16 (proliferation and differentiation) were markedly up-regulated, but also associated with protein HSP27 (164%), indicating pro-differentiation activity (JONAK, 2002).
The differentiation was accompanied by an increase in several adhesion proteins: those enabling cohesion between cells and the adhesion and the deployment of keratinocytes in cell layers (desmogleins, desmocollins); those involved in cell attachment to the basal lamina (laminin binding protein, vimentin, integrin α and β) and lastly those that ensure anchoring to the surrounding dermis (desmogleins, desmocollins).

Gene down-regulation was reflected in the decreased expression of the interferon receptor (-57%), associated with an increase in the interferon antagonist (+135%), both making a strong anti-inflammatory contribution.

Thus, the genes involved in matrix remodeling and angiogenesis were temporarily down-regulated, while the cell proliferation pathways were intensified by a decrease in the factors with a negative impact on those pathways: CRABP 1/2 (cytoplasmic retinoic acid binding proteins) and vitamin D3 receptor (transcription factor for cell proliferation and differentiation).

The strengths of the markers:

Desmogleins are adhesion proteins that are indispensable for between-keratinocyte adhesion (GARROD *et al.*, 2002; NUBER *et al.*, 1996) and which contribute to the formation of the outer root sheath of the hair.
They are also involved in anchoring the root sheath to dermal structures: mice in which the desmoglein genes have been knocked out loose their telogen hair prematurely (Hanakawa Y, 2002).

Vimentin is a constituent of the matrix synthesized by keratinocytes at the junction between the epithelial tissue and mesenchyma (dermis) which plays a role in the morphogenesis of hair (TAMIOLAKIS *et al.*, 2001).

Cytokeratins 10 (differentiation), 14 and 16 (morphogenesis of the hair and keratinocyte proliferation) and the metabolic enzymes and markers of cell mitosis (proliferating cell nuclear antigen) characterize keratinocytic hyperactivity oriented towards the morphogenesis of new tissues.
It is interesting to note that the vitamin D3 receptor and the receptors for retinoic acid (CRABP 1/2) are temporarily down-regulated: inhibition of transcription is removed promoting *de novo* synthesis of DNA, cell proliferation (KROHN *et al.*, 2003) and follicular survival (BILLONI, 1997).
Since receptor activity is also dependent on steroids such as the androgens, of which dihydrotestosterone (DHT), the low level of receptor expression also reflects the absence of hormonal activation.

There are subtle interactions between retinoid, steroid and vitamin D3 receptors (and in the presence or absence of their co-effectors). Those receptors are therefore important factors in the morphogenesis of the hair follicle.
The action of **PROCAPIL™** thus involves those essential factors for hair morphogenesis and growth.
Among the various genes up-regulated, the different effects of peptide biotinyl-GHK (adhesion and proliferation gene), biotin (strong mitochondrial activity) and oleanolic acid (deactivation of the CRABP 1 and 2 and vitamin D3 pathways) are patent.

Conclusion on the *in vitro* data

The remarkable consistency of the data generated by the DNA-array study on synthetic epidermis and the morphological study on cultured human hair follicle explants is worthy of note:

- **High anti-aging activity with Ki67, enhanced general morphology (root sheath and papilla), antioxidant cellular enzymes and PCNA markers of proliferation activated.**
- **High *de novo* synthesis of proteins of the adhesion complex (collagen IV, laminin 5, vimentin, desmogleins and desmocollins).**
- **Marked stimulation of cell metabolism (mitochondrial enzymes) and growth activation (hair shaft and cytokeratins 10, 14 and 16).**

The above data are consistent with the profile of a product promoting hair morphogenesis and strengthening the anchorage of the root sheath in the dermis.

The product is substantive and specifically localized on the hair (immuno-localization along the length of the follicle, absence in the surrounding tissue).

3.2. *In vivo* studies

Four-month placebo-controlled clinical trial (Laboratoires DERMSCAN).

Principle

Since men are mainly affected by a receding hair line and incipient baldness, a study in male subjects presenting with that problem was set up. A study duration of 4 months was selected in order to totally cover the telogen.

The videotrichogram method was used to establish and monitor the time course of the ratio of the proportion of hairs in the anagen phase and the proportion in the telogen phase (A/T parameter).

Protocol

- Inclusion criteria

Thirty-five male subjects of Caucasian origin, aged between 18 and 50 years and presenting with more than 20% of their hair in the telogen phase were included.

- Exclusion criteria

Gray hair on the vertex.
Diseases of the scalp.
Intake of corticosteroids, immunosuppressants or retinoids in the 6 months or anti-inflammatories in the week preceding the study.
Local application of Minoxidil® or any local 'anti-hair loss' treatment, applied topically or taken orally, or trophic treatment of the hair in the last 3 months.
Topical or oral treatment of the scalp (anti-seborrheic, anti-dandruff daily friction in the 4 weeks preceding the study).
Change in dietary or exercise habits during the study.
Immoderate use of alcohol or tobacco.

- Product application

The product or placebo was applied twice daily to the scalp using gentle massage.
PROCAPIL™ was formulated as a 3% dilute alcohol lotion with the appearance of a colorless liquid. The placebo was indistinguishable (formulae given in appendix 1).

- Compliance / Safety

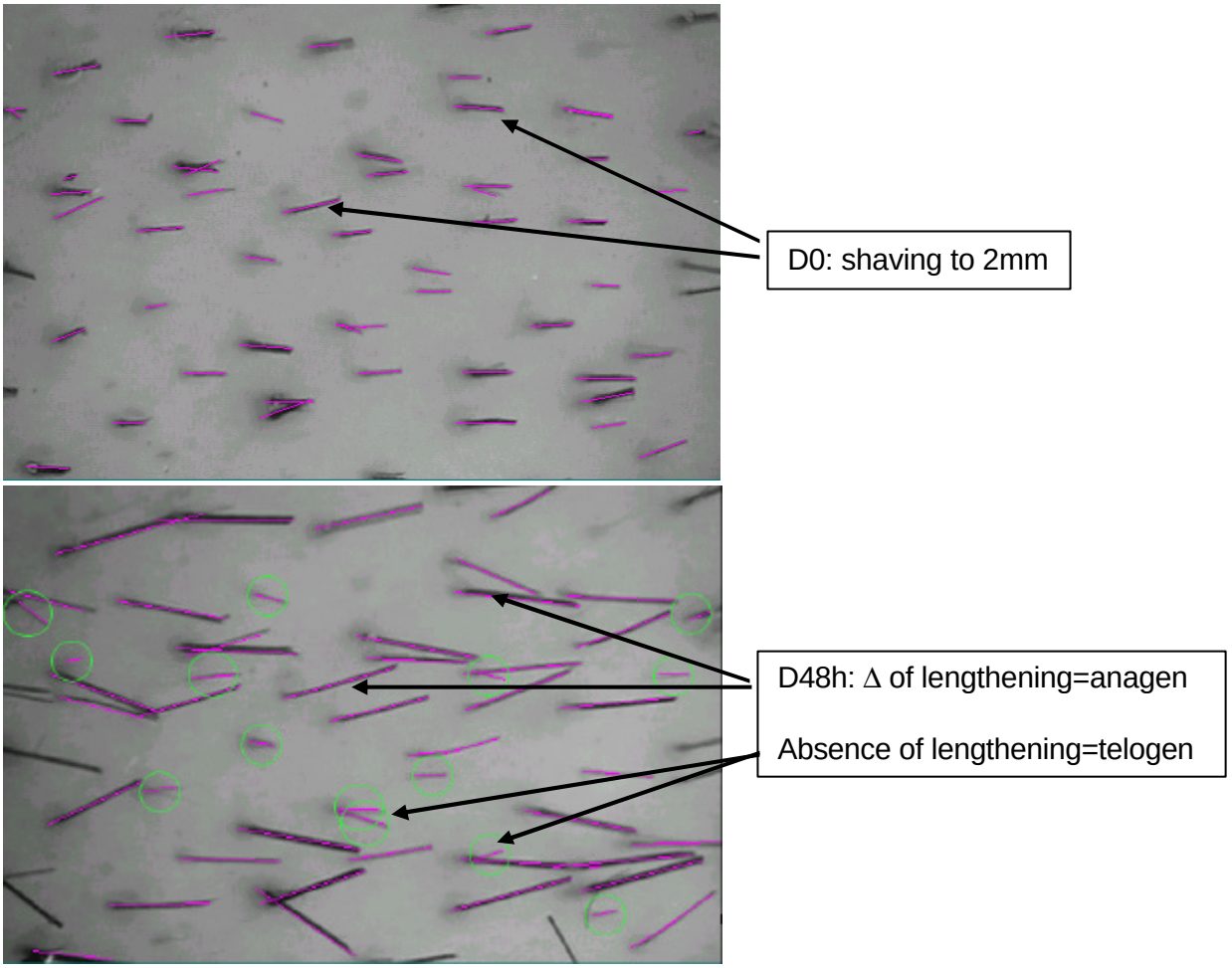
Compliance and safety visits were conducted after 4, 8 and 12 weeks of treatment.

At time points T0 and T4 months, a physical examination of the scalp was conducted by a dermatologist and safety was assessed by subject interview.

- Videotrichogram

The system used consisted in a MORITEX SCOPEMAN® MS-500 videomicroscope fitted with a mobile 25X objective with optical fiber, connected to a digital image acquisition system.
The images were analyzed by the COUNT-HAIR® program developed by Laboratoires DERMSCAN.
Image acquisition at T0 and after 4 months was conducted on the same shaved hair zone (about 1 cm^2/ 200 hairs, on average), after marking.

The parameters monitored were the length and growth rate of the hair and the proportion of hairs in the anagen phase and proportion in the telogen phase.



- Hair samples: morphological analysis and immunolabeling of collagen IV and laminin 5.

At T0 and at the end of the study, 24 hairs were sampled from the border of the alopecic zone using tweezers. Six subjects in the treatment group and 6 in the placebo group underwent sampling.
The hairs were fixed in Bouin's fluid (12 hairs) or frozen immediately (12 hairs) prior to shipment to BIO-EC for analysis.

Results

a) Clinical trial

Out of the 35 subjects included in the study, 18 were allocated to the **PROCAPIL™** group (37 ± 2 years) and 17 to the placebo group (38 ± 1 year). Subject allocation to the **PROCAPIL™** and placebo groups was randomized.

- Safety

PROCAPIL™ was very well tolerated by all volunteers over the 4 months of use.

- Videotrichogram

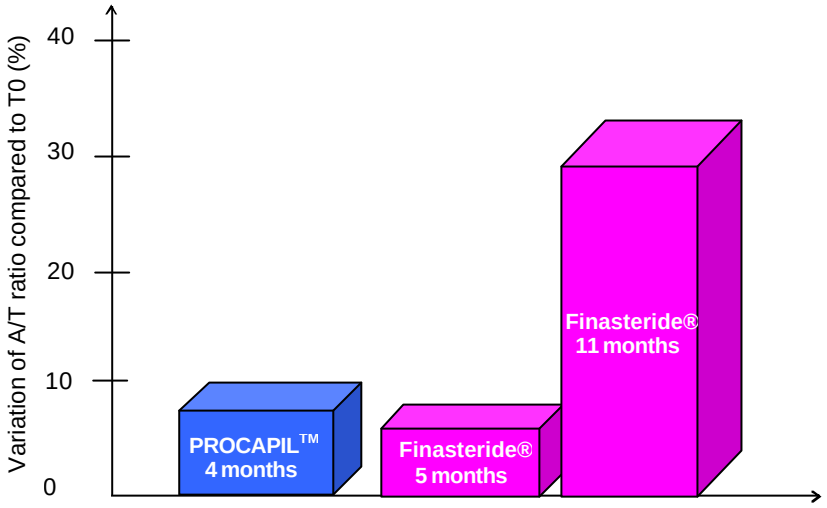
Clinical studies intended to measure the impact of a treatment on hair scalp health use various criteria of evaluation. Hair density (number of hair/cm²) is used for products claiming the growth/regrowth. Percentages of hair in anagen and telogen phases (growth or loss) as well as ratios of these percentages are more adapted to the analysis of the hair anchoring and hair vitality (still) present on the scalp. These latter parameters have thus been chosen for the study.

Anagen / telogen ratio

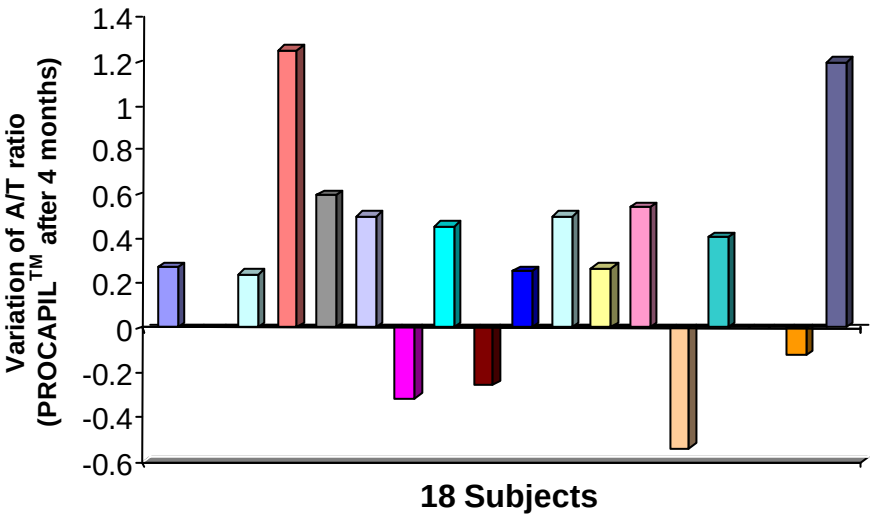
The figure below shows the anagen/telogen ratio at baseline and after 4 months of treatment in comparison with data published for the Finasteride® after using by oral way (Van Neste et al. 2000) for 11 months.

After 4 months of **PROCAPIL™** treatment, the volunteers showed a marked improvement in the proportion of anagen phase hairs, significatively superior compared to T0 (+10%, $p<0.05$). The placebo is inactive. The comparison with the data published for the Finasteride® by oral administration shows that **PROCAPIL™** has also a remarkable activity.

In fact, a moderate 8% variation of A/T ratio (compared to T0) is reported for Finasteride® after 5 months, variation increasing strongly to reach 33% after 11 months.



In the PROCAPIL™ group, 67% of the subjects presented with an improvement in A/T ratio and, for 3 subjects out of the 12 improved, the A/T increase was 31.2, 33.5 and 46.3%, respectively.
In contrast, in the placebo group, there was a trend toward a decrease in anagen hairs.



Growth rate

The mean hair growth rate for the 17 subjects showed no significant difference, baseline vs. end of study. However, a trend toward improvement was shown by 8 volunteers, who presented with an increase in growth rate, in the **PROCAPIL™**-treated group.

In the placebo group, the mean growth rate tended to fall (-3%) and the majority of the subjects did not present with any improvement: 11 out of 16 subjects.

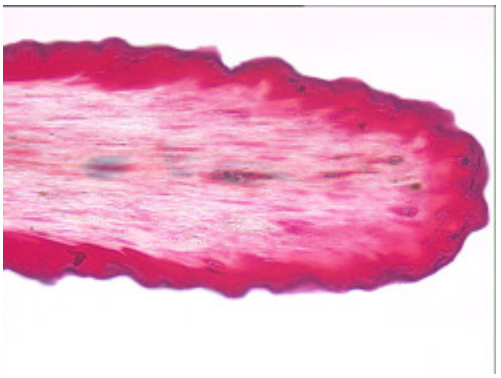
These results let us conclude that PROCAPIL™ is overall a powerful moderator of hair loss thanks to its hair anchoring action in the skin, as proven by the pictures in the next chapiter.

b) Morphological changes in the hair after 4 months

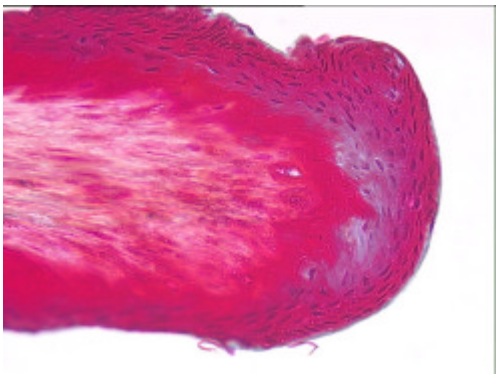
The between-group differences were serially observed using telogen hair (sampled by pulling out) after 4 months.

- Differences at time point 4 months, **PROCAPIL™** vs. placebo

The **PROCAPIL™**-treated group showed much more highly structured hair bulbs:

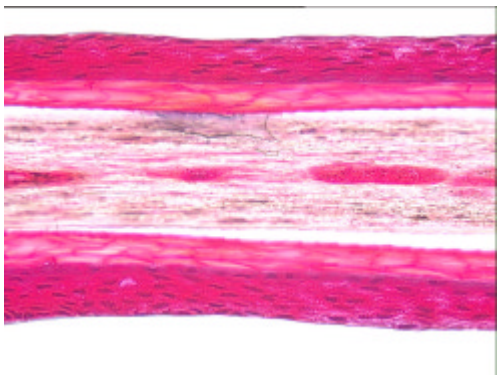


Placebo T4 months (bulb)

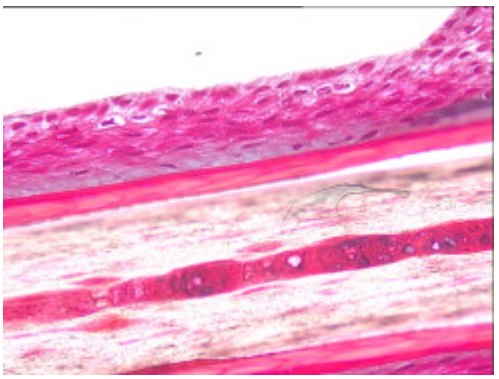


PROCAPIL™ T4 months (bulb)

Moreover, the **PROCAPIL™**-treated hair showed root sheaths with well differentiated cell bases, very clearly anchored with respect to the inner hair shaft, but also with a very good quality outer interface (anchoring in the dermis).



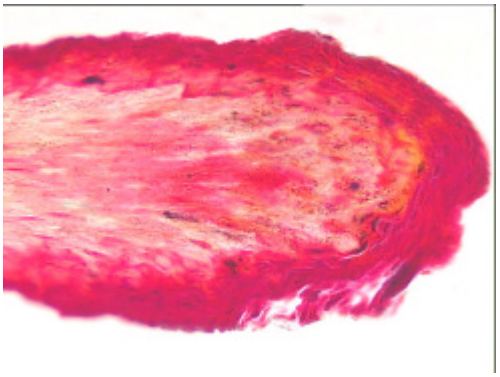
Placebo T4 months (root sheath)



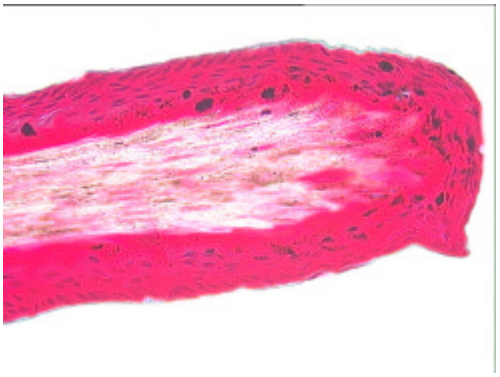
PROCAPIL™ T4 months (root sheath)

- Difference, T0 vs. T4 months, for anagen and telogen hairs

In a given subject, marked differences were observed, T0 vs. T4 months.
As shown below, the bulb zone of telogen hair has been very markedly improved:

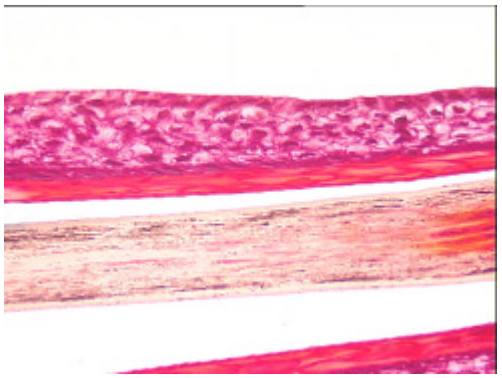


PROCAPIL™ T0 (bulb)

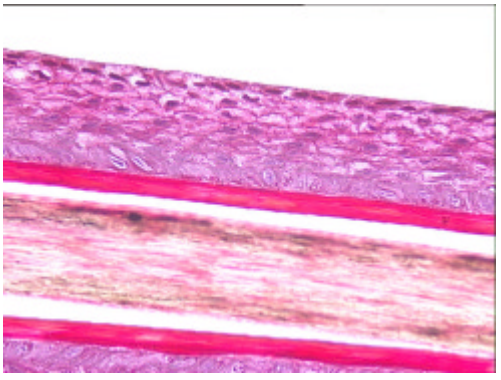


PROCAPIL™ T4 months (bulb)

The root sheath of anagen hairs also improved, with thickening and clearly defined cell bases:

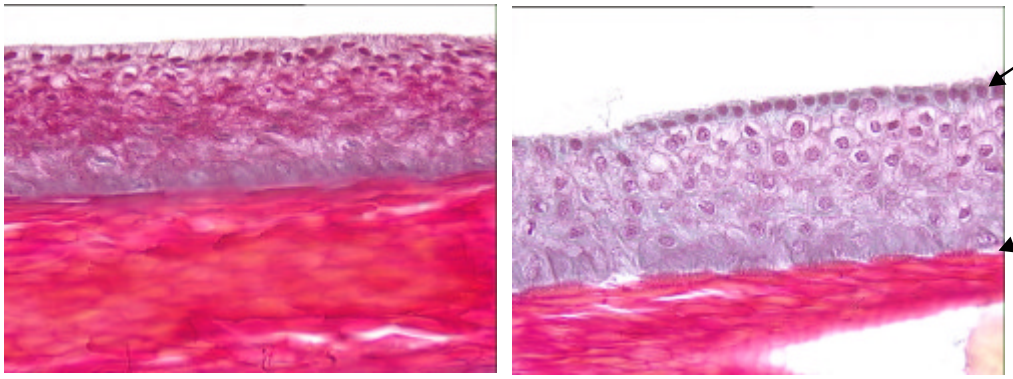


PROCAPIL™ T0 (root sheath)



PROCAPIL™ T4 months (root sheath)

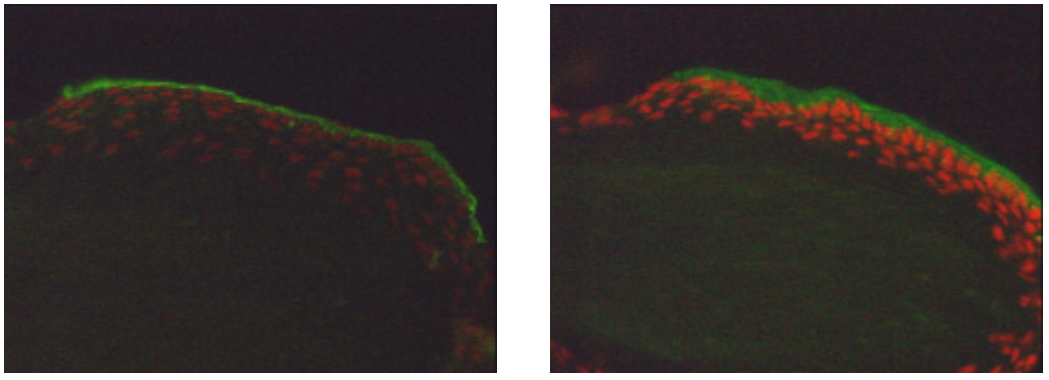
In the **PROCAPIL™** group, the root sheath was observed to be of high quality with a perfectly structured basal lamina ensuring optimum dermal-epidermal adhesion on the outer side of the hair.
On the inner root sheath side, anchoring zones with the hair shaft were observed.
In contrast, in the placebo group, those two zones were not very structured.



Placebo T4 months **PROCAPIL™** T4 months

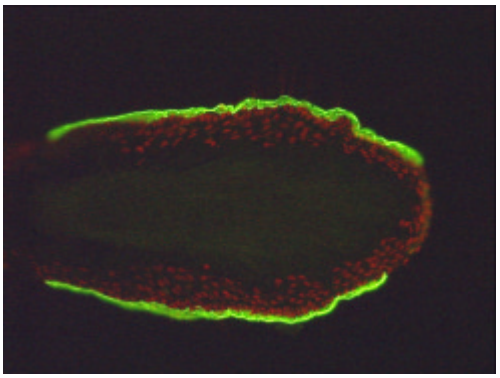
The immunofluorescence findings with respect to the markers collagen IV and laminin 5 further reinforced the previous findings:

Greater laminin 5 fluorescence of the root sheath was observed for the telogen bulbs in the **PROCAPIL™** group**.**

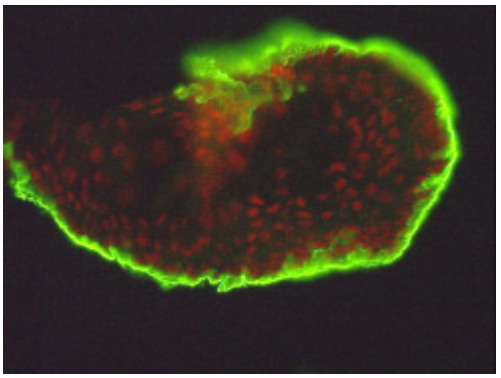


Placebo T4 months **PROCAPIL™** T4 months

Collagen IV labeling of the telogen bulb was also more marked in the **PROCAPIL™** group:



Placebo T4 months



PROCAPIL™ T4 months

Conclusion on the *in vivo* data

The results of the 4month clinical trial covering a complete telogen phase showed a significant increase in the anagen/telogen ratio comparable to oral Finastéride® treatment in the PROCAPIL™-treated group.

This finding was perfectly in line with the morphological findings made on hair samples taken from a few subjects in the PROCAPIL™ and PLACEBO groups:

Reconstitution, on the telogen hair, of a perfectly structured root sheath with a structured and regular basal lamina for good anchoring in the dermis. This was confirmed by the greater presence of adhesion complex proteins: collagen IV and laminin 5.
The inner root sheath showed adhesion motifs between the hair shaft and the sheath.

4. OVERALL CONCLUSION

PROCAPIL™ is a potent Anti-Hair Loss complex that targets the three phenomena responsible for hair loss:

- **5α-reductase, which converts testosterone to DHT**
- **Inadequate blood perfusion**
- **Failing anchorage of the hair in the dermal papilla.**

PROCAPIL™ consists of 3 active substances which act together:

- **peptide Biotinyl-GHK, a Matrikine, which acts on the anchoring of the hair thanks to adhesion proteins**
- **apigenin, a citrus extract flavonoid with a vasodilatory effect**
- **oleanolic acid, extracted from olive tree leaves, which inhibits the production of dihydrotestosterone *via* 5α-reductase.**

The data obtained *in vitro* on human follicles and by analysis of the activated genes have demonstrated:

- **The substantiveness of the product *vis-à-vis* the hair shaft and its selective localization**
- **Improvement in hair morphology with a living root sheath well structured by adhesion proteins, of which vimentin, desmogleins, desmocollins, laminin 5 and collagen IV**
- **Potent activity on keratinocytic multiplication and hair morphogenesis**

These highly positive characteristics were shown to be effective *in vivo*:

The 4-months clinical trial covering the telogen phase compared PROCAPIL™ and placebo and confirmed the marked anti-hair loss activity of the complex:

- **Out of 18 volunteers in the PROCAPIL™ group, 67% showed significant improvement in the mean anagen/telogen ratio ($p<0.05$), in the same range that is reported for Finasteride® after a treatment of 5 months by oral administration. Certain subjects showed after using PROCAPIL™ for 4 months, an improvement greater than 30 or even 46%.**
- **The morphological and immunohistological analyses of the hair samples taken at the start and end of the study showed that the bulb of telogen hair, root sheath and laminin 5 and collagen IV densities were markedly improved in the PROCAPIL™ group, in contrast to what was observed in the placebo group.**

The above set of results enables confirmation that PROCAPIL™ acts by promoting enhanced anchorage of telogen hair in the dermis via regeneration of the root sheath. PROCAPIL™ thus slows hair loss and improves the health of hair follicles.

For optimum effect, we recommend use of PROCAPIL™ at a concentration of 3%.

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APPENDIX

Formulations used for the clinical trial

Starting material	INCI name	Supplier	Placebo %	Product %
Phase 1				
Demineralized water	Water (*Aqua*)		q.s. 100	q.s. 100
Citric acid	Citric Acid		0.26	0.26
Sodium citrate	Sodium Citrate		1.20	1.20
Incroquat CTC 30	Cetrimonium Chloride	Croda	1.00	1.00
Phase 2				
Ethanol	Ethanol		8.00	8.00
Fragrance	Fragrance		q.s.	q.s.
Crillet 1	Polysorbate 20	Croda	0.40	0.40
Phase 3				
PROCAPIL™	(cf. Synopsis)	SEDERMA	-	3%
PROCAPIL™ excipient			3%	-

Cosmetic
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