ACTIVE INGREDIENTS

IFF | LUCASMEYER COSMETICS

TECHNICAL FILE

GREYVERSE™

ZERO SHADES OF GREY IS ALSO SEXY!

Patented α-MSH biomimetic peptide

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Stimulates melanogenesis and reduces oxidative stress in hair bulb

-

Reverses hair greying process to progressively recover natural hair color for a younger and more confident look!

PRESERVATIVE FREE

ECO FRIENDLY

CLINICALLY PROVEN

SUMMARY

INCI NAME **CAS** **EINECS**	Water (1) (and) Glycerin (2) (and) Palmitoyl Tetrapeptide-20 (3) (1) 56-81-5 (2) 7732-18-5 (3) - (1) 200-289-5 (2) 231-791-2 (3) -
ORIGIN	Biomimetic peptide of the α-Melanocyte Stimulating Hormone (α-MSH)
COSMETIC PROPERTIES	• Binds and stimulates the melanocortin 1 receptor (MC1-R) • Stimulates the melanogenesis process • Stimulates melanin synthesis (↑MITF, ↑TRP-1) • Improves melanosome maturation (↑Melan-A) and their transfer to keratinocytes • Decreases oxidative stress (↑catalase, ↑TRP-2) • Decreases intracellular quantity of H_2O_2
HAIR BENEFITS/ POTENTIAL CLAIMS	• Prevents, delays or reverses the hair greying process • Decreases grey hair density • Provides a younger look • Improves self-confidence
APPLICATIONS	• Anti-aging hair care • Premature grey hair coverage • Post-coloration treatment • Natural color fortifier • Scalp-friendly alternative to chemical dye • Beard & mustache care
RECOMMENDED DOSAGE	0.5-1%: preventive hair care 1-2%: intensive hair care
USAGE PH RANGE	4.0 - 8.0
INCORPORATION	At the end of the formulation (<40°C) Can be heated if needed (tested at 90°C for 2 hours)
INCOMPATIBILITIES	Unknown

INTRODUCTION

Hair color contributes significantly to human social communications as it provides much information regarding an individual's race, ethnicity, age and health status, as well as physical and sexual attractiveness. It is not surprising, then, that many people desire full, shiny, lustrous hair that is still their natural color!

The greying of hair is a natural biological process associated with aging. Men and women of every ethnicity are affected sooner or later. Recent studies show that up to a quarter of people worldwide have more than 50% grey hair by the time they reach 50 years of age.

While elderly people generally accept this new hair color, the same cannot be said for younger people, particularly those with dark hair who compose over 90% of the world's population. Most people want to avoid, delay or hide this inevitable sign of aging as it makes them look older and affects their self-confidence and self-esteem, and this is especially true in the case of young people suffering from premature grey hair.

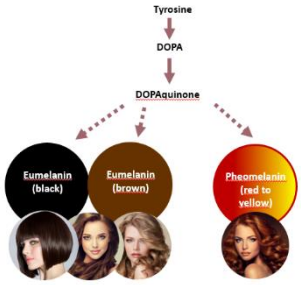
The anti-grey hair market is thus very promising. Most people who want to cover their grey hair choose to color it artificially, and there are several solutions available on the market:
-Hair dye (chemical or vegetable), used by most people as an immediate 100% covering solution (63% of >45-year-old women have dyed their hair within the last 6 months).
-Compositions of reactive substances that reveal their color as a result of an oxidoreduction reaction once they are set on hair, instant root concealers to use in between two shampoos, and coloring vegetable extracts are some of the other types of products proposed.

However, all these products color only the visible part of the hair shaft and the root inevitably grows grey or white, leading to an unsightly color contrast and obliging people to constantly renew the covering action. Until now, there has been no solution targeting the biological causes of the hair greying process in order to stop it or, even better, reverse it by natural repigmentation of the hair shaft.

A breakthrough innovation from Lucas Meyer Cosmetics, Greyverse™ is the first anti-grey hair biomimetic peptide. Clinically effective, it helps men and women suffering from greying hair look naturally younger by reversing the hair aging process and increase their self-esteem.

HAIR GREYING PROCESS

Role of melanin



Hair color varies according to each individual's genetic heritage. The wide range of natural hair colors (different shades of blond, red, brown or black) is the result of the variable amounts and distribution of two types of melanin in the cortex, or middle layer of the hair shaft: eumelanin (black to brown pigments) and pheomelanin (red to yellow pigments). The composition of this blend is genetically determined based on the need for hair protection one's ancestors had as a result of their geographic location.

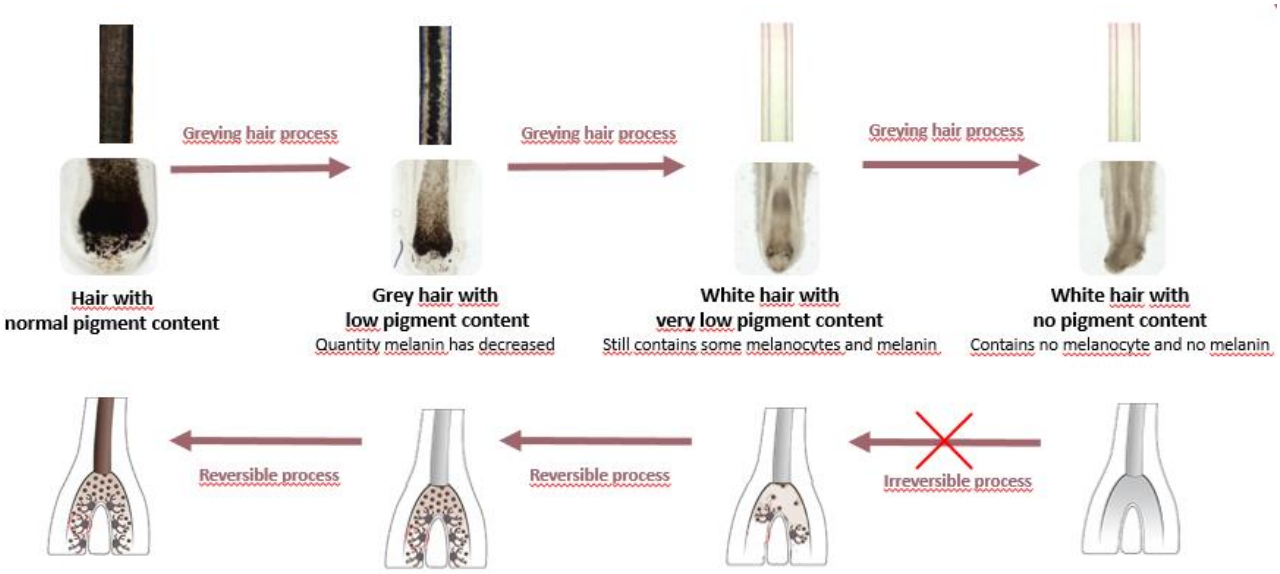
Like in the skin, the role of melanin in hair is mainly to protect it from sun damage - e.g., dry and brittle strands, broken or split ends, thinning and frizz - as the pigment is able to absorb UV rays.
But melanin is also able to neutralize toxins, heavy metals, and other potentially toxic substances that can become highly accumulated in the protein structure of hair. Indeed, the selective and avid binding of these compounds to the melanin pigment limits their access (and thus potential damage) to the living tissue of the highly vascularized scalp.

Synthesis of melanin

Melanin is only produced by melanocytes during the anagen (growth phase) of the hair cycle. Melanocytes are located in the bulb of each hair follicle on the basement membrane surrounding the dermal papilla. Pigments are transferred through melanocyte dendrites into the surrounding keratinocytes in a continuous flow while they proliferate, building the new part of a pigmented hair shaft.

Genetically programmed, the hair greying process, also called canities, usually starts in the 30s for Caucasians, later for Asians (late 30s) and even later for Africans (mid 40s). This unwanted event can also affect some younger people suffering from premature grey hair, sometimes starting before they enter their 20s. Greying usually first appears at the temples, then spreads to the vertex before spreading to the remainder of the scalp. Beard and body hair are usually affected later.

Whatever the case, the appearance of grey color in a hair shaft is due to a gradual loss of melanin over time caused by several factors. As the pigment level gets lower, the color of the hair becomes increasingly light and the hair shaft appears grey. When the pigment level is even lower, it becomes undetectable to the eye (due to changes in light reflection caused by the low pigment level) and the transparent hair shaft appears white. This condition can be reversed as long as some melanocytes are still present in the bulb. The irreversible final step of the hair greying process (a totally white hair shaft) occurs when hair contains no more pigment due to a complete loss of all melanocytes.



Two factors have been identified as the main causes of the hair greying process: the **decrease in melanogenesis** and the **increase in oxidative stress** in the bulb.

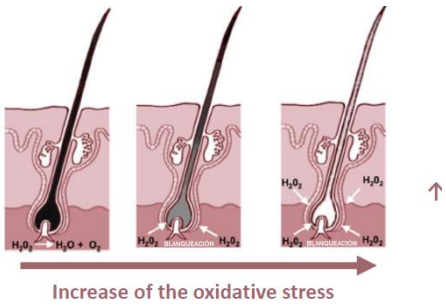
- Over time, the number of melanocytes in the hair bulb and their activity decrease, leading to a decline in the melanogenesis process, which proceeds according to the "melanogenetic clock".

The synthesis of melanin in melanocytes is decreased due a lower stimulation of the process and a lower activity of the enzymes involved (tyrosinase and TRP-1). Also, the transfer of melanosome (melanin-containing vesicle) into the surrounding keratinocytes is reduced due to some defect in melanocyte/keratinocyte interaction. Therefore, the final quantity of pigments in hair shafts dims until hair appears grey, then white.

- Oxidative stress is increased by the decrease of two major enzyme activities:
  1) Catalase. Hair follicle cells produce small amounts of hydrogen peroxide (H_2O_2) as part of the oxygen cycle. This oxidative substance is naturally degraded by catalase into its harmless elements of water and oxygen.

$$H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

With aging, catalase enzyme activity decreases, leading to an accumulation of H_2O_2 that bleaches the melanin pigments and the hair from the inside out, progressively leading to grey hair color.



The buildup of H_2O_2 also inhibits the activity of tyrosinase, the enzyme involved in the synthesis of melanin, thus reducing the capacity of melanocytes to produce pigments.

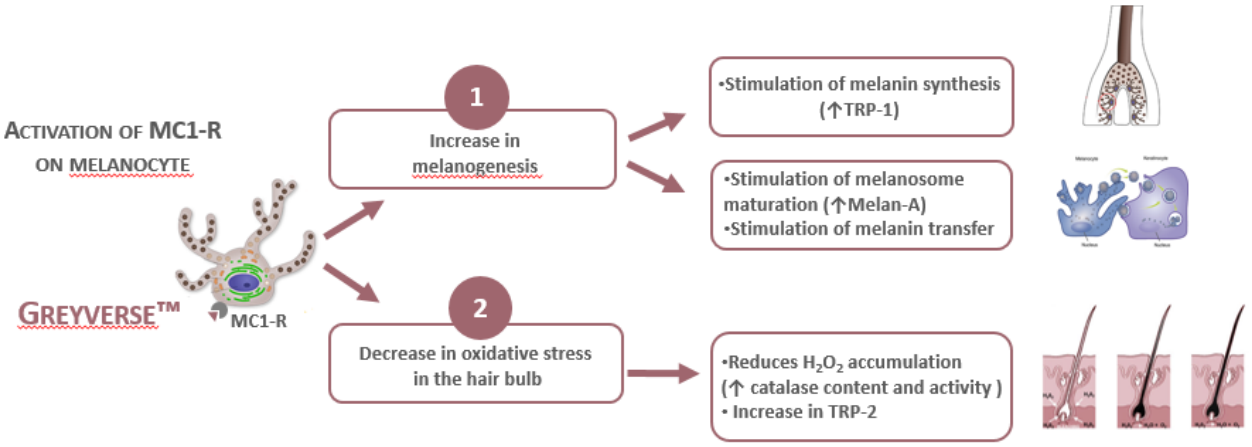
2) TRP-2 (Tyrosine Related Protein-2) initially known as an enzyme involved in the synthesis melanin, is also an antioxidant enzyme that protects and defends melanocytes against reactive oxygen species generation. The decrease in TRP-2 intensifies the loss of melanocytes as they become more sensitive to aggressions from free radicals.

PATENTED PEPTIDE FOR RECOVERING NATURAL HAIR COLOR

Greyverse™ is the result of a wide screening of several peptides tested on different biological targets involved in the hair greying process.

Greyverse™ is a patented Palmitoyl Tetrapeptide (4 amino acids grafted on palmitic acid). The active sequence is derived from the α-MSH structure, known to be the main molecule able to stimulate melanogenesis.

As an analogue of α-MSH, Greyverse™ is able to bind to MC1-R (Melanocortin-1 Receptor), a transmembrane receptor located at the surface of melanocytes. The activation of MC1-R stimulates the melanogenesis process, as well as the catalase expression and activity.



Due to its complete mechanism of action, Greyverse™ is able to act simultaneously and efficiently on the two main factors causing hair greying process:
- It stimulates melanogenesis to increase melanin production in the bulb and favor a better transfer leading to hair repigmentation.
-It lowers oxidative stress by increasing the catalase expression and activity, thus reducing the accumulation of hydrogen peroxide to maintain the full pigment color.

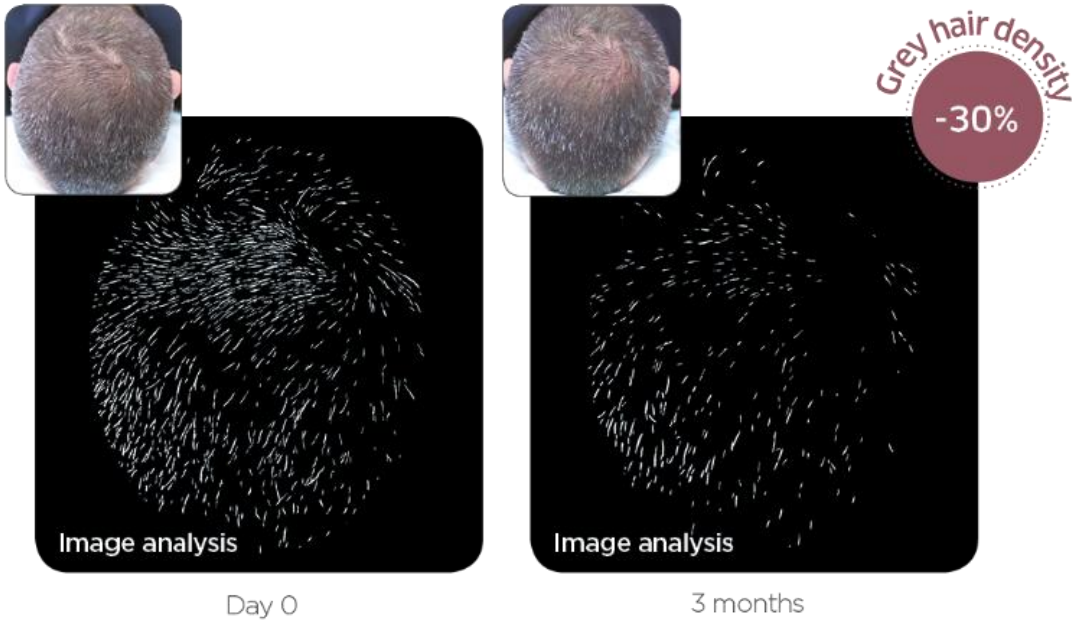
Contrary to skin melanocytes, which are able to produce a higher quantity of melanin than the constitutive skin color if they are stimulated (facultative pigmentation), hair melanocytes cannot produce more than they are genetically programmed (constitutive pigmentation only). Thus, Greyverse™ can only help hair recover its natural color, it cannot darken it.

CLINICALLY PROVEN HAIR REPIGMENTATION

Clinical evidence of the active ingredient's effect is the most important argument, especially for applications such as reversing grey hair.

Tested on selected volunteers with early canities (35 years old, > 20% grey hair), Greyverse™ has been proven to decrease the overall whiteness of hair and to darken grey hair after 3 months of treatment, with outstanding visible results: a 30% reduction in grey hair density.

As a biological process, the hair greying process involves several genes. Evaluating the gene expression and synthesis of related proteins is an innovative way to demonstrate product efficacy.
Based on an analysis of hairs plucked from volunteers, Greyverse™ has demonstrated its ability to modulate some of the main genes involved in pigmentation:
- the increase in MC1-R gene expression increases the number of receptors responsible for inducing melanogenesis
- the decrease in ASIP gene expression (a competitor of α-MSH for the binding to MC1-R) favors the activation of MC1-R by α-MSH to better induce melanogenesis
- the increase in MITF gene expression stimulates melanin synthesis



GREYVERSE™ - 1ST ANTI-GREY HAIR BIOMIMETIC PEPTIDE

Innovative & clinically efficacious ingredient that helps men & women suffering from greying hair to look naturally younger by reversing hair aging and increasing their self-esteem.

EFFICACY STUDIES

IN VITRO EFFECT OF GREYVERSE™ ON MELANIN SYNTHESIS IN HUMAN MELANOCYTES

BACKGROUND

Hair pigmentation is due to the presence of a pigment, melanin, secreted by the melanocytes located in the hair bulb and then transferred into the surrounding keratinocytes [1]. The appearance of greying hair is a phenomenon directly linked to a lack of melanin in the hair bulb.

OBJECTIVE

This study investigated Greyverse™ activity by measuring its effects on the production of melanin in a human melanocyte culture.

PROTOCOL

Biological materials

The melanocytes used were a cell line of primary normal human epidermal melanocytes from adult tissue (HEMas). HEMas cells were grown in an adherent cell culture realized with CnT-40 CellnTec medium at 37°C, 5% CO_2 and 95% humidity.

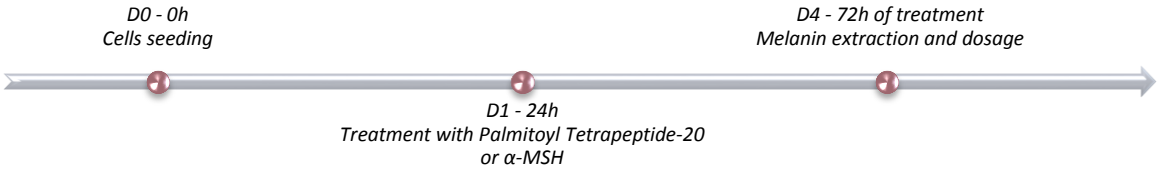
Tested products

- The Palmitoyl Tetrapeptide-20 (pure peptide contained in Greyverse™) was tested at the following concentration: 10^{-7} M (equivalent to 0.5% Greyverse™).
- The positive control, α-MSH, was tested at a concentration of 10^{-6} M.

Evaluation of the activity

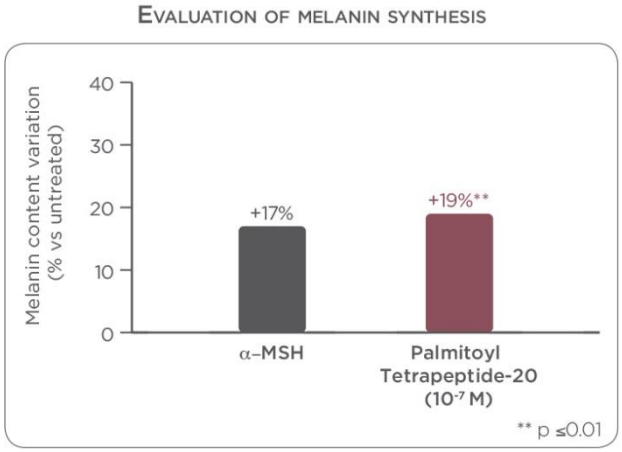
HEMas cells were seeded in 6-well plates at the concentration of 3.10^5 cells/mL in a CnT-40 culture medium and incubated at 37°C, 5% CO_2. After 24 hours, the medium was removed and the cells were treated with Palmitoyl Tetrapeptide-20 (10^{-7} M) or α-MSH (10^{-6} M) for 72 hours.

After 72 hours of treatment, cells were detached for counting, then incubated at 100°C for 10 minutes with NaOH at 1M to extract melanin.



Then, melanin was detected by absorbance at 405 nm. Melanin content was expressed as the percentage of melanin production, compared to cell numbers.

RESULTS



After 72h of treatment, Greyverse™ significantly increased the melanin synthesis by 19% at the concentration of 10^{-7} M.

The results indicate that Greyverse™ has an activity stronger than the positive control α-MSH .

CONCLUSION

A biomimetic peptide of α-MSH, Greyverse™ stimulates the melanin synthesis to improve grey hair repigmentation.

EX VIVO EFFECT OF GREYVERSE™ ON PIGMENTATION IN HUMAN HAIR FOLLICLES

BACKGROUND

Melanosomes are typically divided into four maturation stages (I–IV) determined by their structure and the quantity, quality, and arrangement of the melanin produced.

- Stage I melanosomes have an early matrix organization, are spherical, do not contain tyrosinase activity, and are localized at the periphery of the nucleus.
- Stage II melanosomes are tyrosinase-containing elongated organelles with an organized filamentous matrix. In addition, Mart-1 protein, also known as Melan-A, localized in stage I and/or II, contributes to the melanosome formation.
- Stage III melanosomes, where melanin is uniformly deposited.
- Stage IV melanosomes are melanosomes that are electron-opaque, fully melanized and have a low tyrosinase activity; they are the melanosomes supplied from the dendrites to the neighbouring keratinocytes.

Within melanosomes, at least three key enzymes, tyrosinase, Tyrosinase-related protein 1 (TRP-1), and Tyrosinase-related protein 2 (TRP-2), are absolutely required for the synthesis of different types of melanin.

Moreover, the quality of the pigment depends of the balance of the Agouti signaling protein (ASIP, also known as PARD3) and alpha-MSH expression. The binding of ASIP to MC1R precludes alpha-MSH initiated signaling and thus blocks production of cAMP, leading to a down regulation of eumelanogenesis (brown/black pigment) and increasing synthesis of pheomelanin (yellow/red pigment).

OBJECTIVE

The aim of this study was to determine the effects of Greyverse™ on the pigmentation of isolated human grey hair follicles.
To this end, we checked the hair melanosome biogenesis by melanosomal protein immunohistochemistry (Melan-A and TRP-1) and by the nature of the pigment expressed by the immunostaining of ASIP/PARD3.

PROTOCOL

Tested products

Palmitoyl Tetrapeptide-20 (pure peptide containing in Greyverse™) was tested at the concentrations of 10^{-9} M and 10^{-7} M, equivalent to 0.005% and 0.5% Greyverse™, respectively.

Biological materials

Isolated human grey hair follicle

On a scalp plasty coming from a 71-year-old Caucasian woman who originally had dark hair, grey hair follicles (from the infundibulum to the bulb) were isolated by micro dissection on day 0 (D0). These hair follicles were placed separately in a 48-well plate and kept in survival for seven days in William's medium (Sigma-Aldrich, W1878) supplemented with L-glutamine, insulin, hydrocortisone, serum and antibiotics, at 37°C in a 5% CO_2 atmosphere. Eleven hair follicles were used per condition (untreated or treated). The Palmitoyl Tetrapeptide-20 was diluted in the culture medium on day 0, day 1, day 4 and day 7 whereas the untreated medium was renewed.

Method

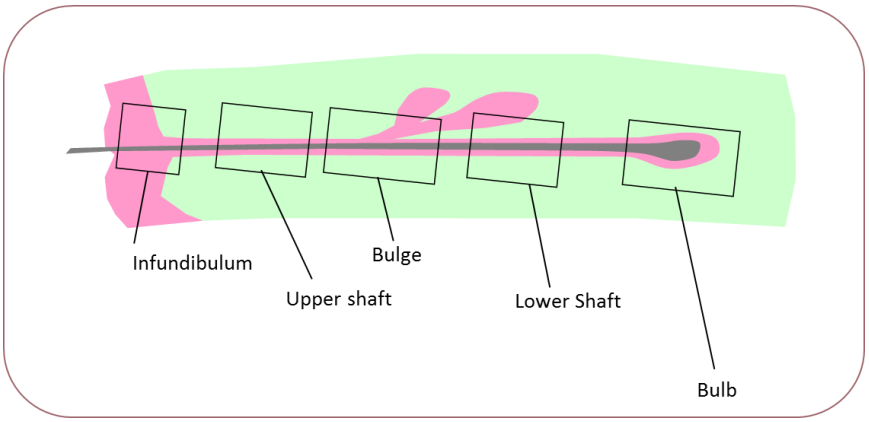
At the end of survival (day 7), the hair shafts were fixed in buffered formalin solution, dehydrated, and paraffin impregnated. 7-µm-thick paraffin sections were mounted on glass slides for immunostainings and stainings.

- For fluorescent immunostainings, the primary antibodies used were anti-PARD3/ASIP (Abcam, ref. ab64646). The sections were then incubated for 30 minutes at room temperature with the Alexa Fluor 488-labeled antibody (Lifetechnologies, ref. A11078). The nuclei were counter-stained using propidium iodide at 0.2µg/mL. The slides were finally mounted using Vectashield® mounting medium (Vector, ref. H-1400).

- For Immunohistochemistry, the hair sections were pre-incubated with hydrogen peroxide (VWR, ref. 23619.264) to inactivate endogenous peroxidase activity. The primary antibodies used were anti-TRP1 (Eurogentec, ref. SIG-38150-1000) and anti-Melan-A (Santa Cruz biotechnologies, ref. sc-20032). Then, the sections were pre-incubated with a biotinylated secondary antibody and incubated with a streptavidin-labeled peroxidase (Vector, Vectastain® Universal ABC kit, ref. PK7200). The staining was revealed by a substrate of peroxidase, VIP (Vector, ref. SK-4600). The nuclei were counter-stained using Mayer hemalun (RAL diagnostics, ref. 320550). The slides were finally mounted using Eukitt mounting medium (VWR, ref. KIND01250).

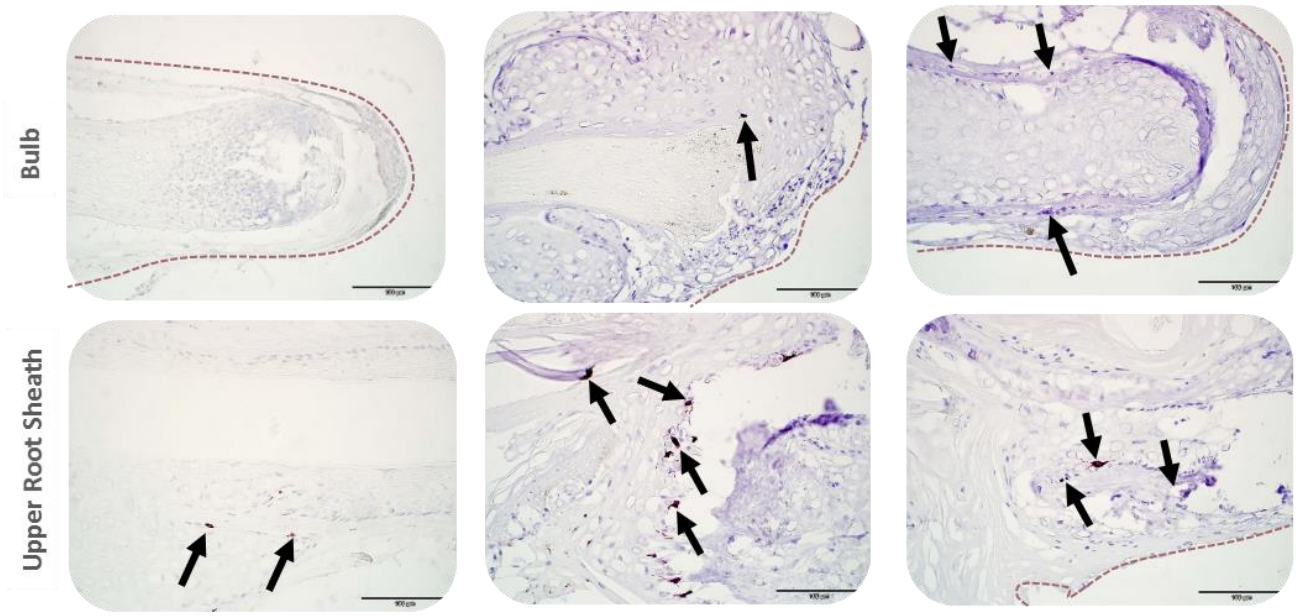
The microscopic observations were performed using a Leica DMLB or Olympus BX43 microscope. Pictures were digitized with a numeric DP72 Olympus camera with CellD storing software. Different hair parts were analyzed: the infundibulum, upper shaft, bulge, lower shaft and bulb.

Hair follicle areas



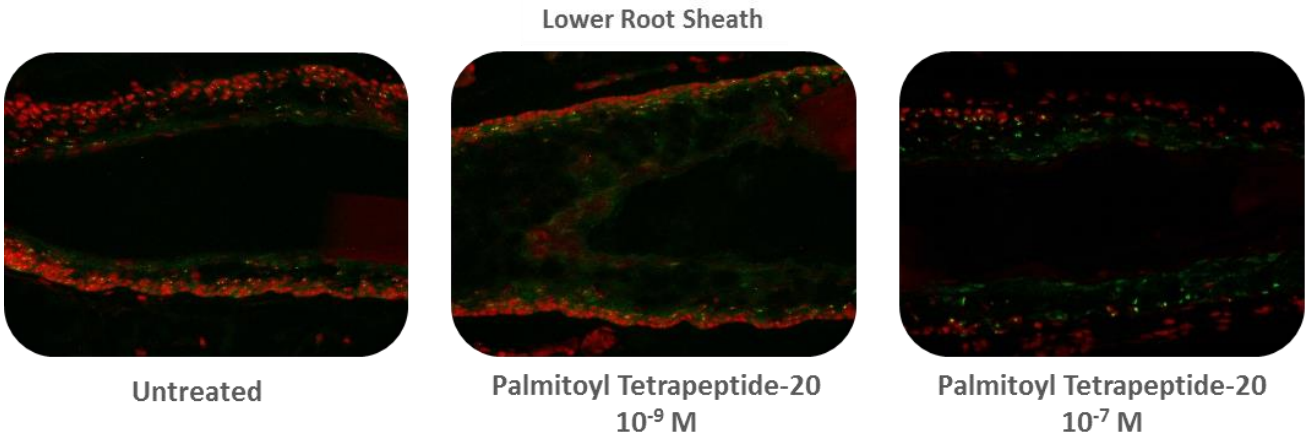
RESULTS

Observation of Melan-A expression in hair follicles



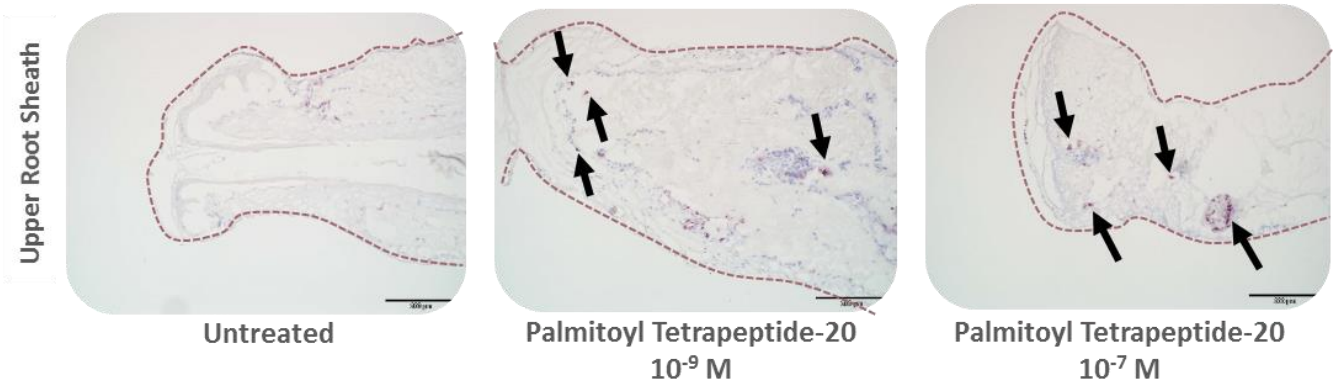
After 7 days of treatments with 10^{-9} M and 10^{-7} M Palmitoyl Tetrapeptide-20, the expression of the melanosome protein, Melan-A, was increased especially in the upper sheath and in the bulb area, a site of melanocyte stem cells, attesting an improvement in the melanosome maturation, necessary for an optimal transfer.

Observation of ASIP/PARD3 expression in hair follicles



Moreover, the expression of the Agouti signaling protein, ASIP/PARD3, was decreased in the lower part of the hair follicle (lower root sheath and bulb). This result indicates that Greyverse™ directs the nature of pigment synthesis: it preferentially increases eumelanin synthesis over pheomelanin synthesis, allowing the natural dark hair color of the donor to be recovered.

Observation of TRP-1 expression in hair follicles



The key enzyme of melanin synthesis, tyrosinase-related proteins 1 (TRP1), was induced in the upper root sheaths, attesting the greater capacity to produce pigments.

Conclusion

Greyverse™ promotes hair pigmentation by increasing melanosome transfer and melanin synthesis, preferably eumelanin.

IN VITRO EFFECT OF GREYVERSE™ ON MELANOSOME TRANSFER IN A CO-CULTURE OF KERATINOCYTES AND MELANOCYTES

BACKGROUND

Hair pigmentation can be influenced by the concentration of melanin as well as by the number, size and distribution of melanosomes around the nucleus of keratinocytes. Melanin is synthesized, stored and transported within a specialized organelle called a melanosome in melanocyte. The transfer of melanin from melanocytes to keratinocytes (melanosome transfer + phagocytosis) is a critical step in skin pigmentation, one that depends on various parameters such as melanocyte dendricity.

OBJECTIVE

In this study, the potential pro-pigmenting effect of Greyverse™ was evaluated in a co-culture of normal human epidermal keratinocytes (NHEK) and normal epidermal melanocytes (NHEM).
In brief, the effect of Greyverse™ on melanosome transfer was evaluated using flow cytometry to quantify a melanosome specific marker (Pmel17) inside keratinocytes identified by the expression of the cell surface marker CD49f.

PROTOCOL

Tested products

- Palmitoyl tetrapeptide-20 (pure peptide contained in Greyverse™) was tested at the concentration of 10^{-9} M (equivalent to 0.005% Greyverse™).
- Positive reference: stimulation mix containing pro-pigmenting factors prepared in accordance with the internal protocol of Bioalternatives (Gençay, France).

Biological materials

The human keratinocytes and human melanocytes were seeded (10:1 ratio) in 24-well plates in a co-culture medium and incubated for 48 hours, with a medium renewal after 24 hours. The medium was then removed and replaced by a co-culture medium containing or not containing the active compound or the positive reference. The cells were then incubated for 48 hours. Half of the medium was then discarded, the treatments were renewed and the cells were incubated for 72 hours. All experimental conditions were performed in n=4.

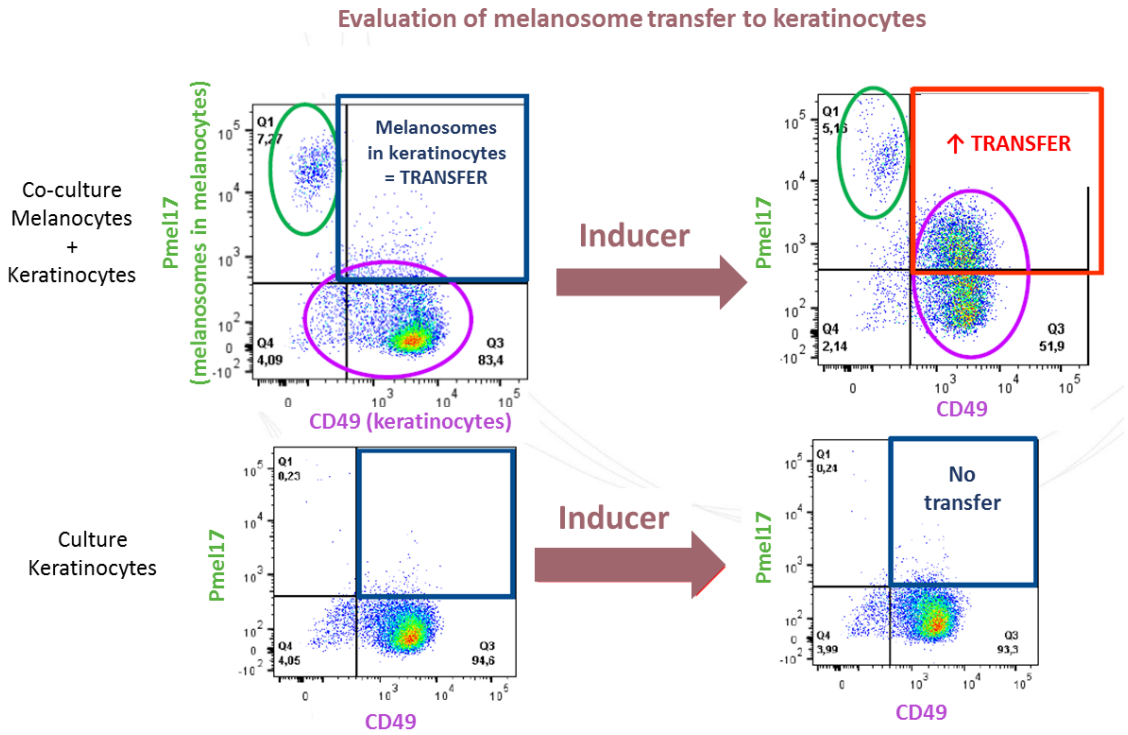
Labelling and cytometry analysis

At the end of the incubation period, the medium was removed and cells were rinsed with a PBS solution. Cells were then detached using a trypsin treatment and transferred to tubes. After several washes in PBS/BSA 0.2% cells were fixed, permeabilized and labeled with the antibodies below:

- Anti-CD49f-FITC: CD49f corresponding to integrin α6 specific to keratinocytes;
- Anti-Pmel17 (specific to melanocytes and melanosomes). Anti-Pmel17 labeling was revealed using a conjugated allophycocyanin (GAM-APC) secondary antibody;
- Or both.

Acquisitions were performed on 10,000 cells for each replicate, after selection of a homogeneous population analyzed for size (FSC) and granularity (SSC) parameters by flow cytometry using a BD FACSVerse™ flow cytometer. Data analysis was carried out using FlowJo vX software.

The melanosome transfer evaluation was performed by quantifying the double-labeled keratinocyte population for CD49f and Pmel17 and calculating the percentage of variation compared to the untreated control.

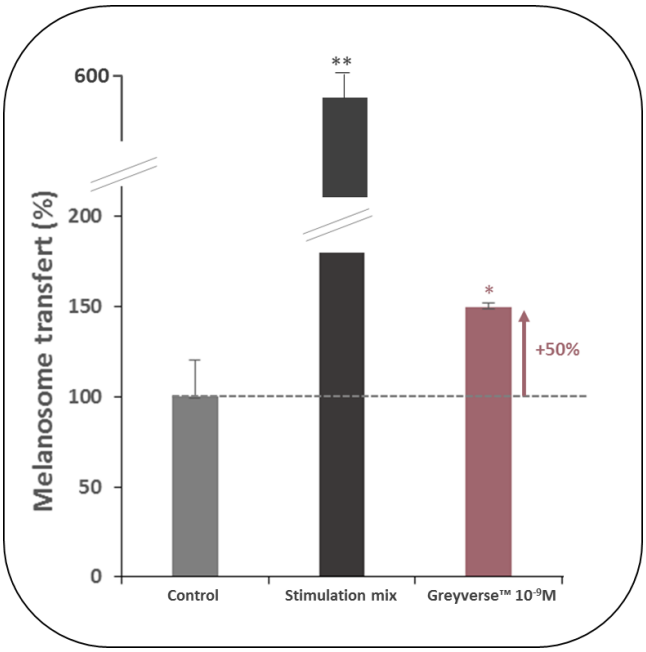


Statistical Analysis

The inter-group comparisons were performed using an Unpaired Student's t-Test: *p<0.05, **p<0.01 and ***p<0.001.

RESULTS

EVALUATION OF MELANOSOME TRANSFER
(melanocytes to keratinocytes)



After 72 hours of treatment, Greyverse™ significantly increased the melanosome transfer between melanocyte and keratinocyte cells by 50%.

CONCLUSION

Greyverse™ stimulates melanosome transfer, thus increasing melanin content in the hair shaft and leading to natural hair pigmentation recovery.

EX VIVO EFFECT OF GREYVERSE™ ON MACROSCOPIC PIGMENTATION IN ISOLATED HUMAN HAIR FOLLICLES

OBJECTIVE

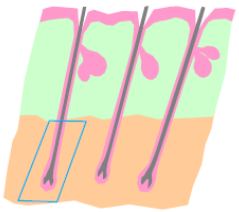
To complete the previous data, the aim of this study was to evaluate the activity of Greyverse™ on the macroscopic hair pigmentation of isolated hair follicles maintained in survival.

PROTOCOL

Tested products

Palmitoyl Tetrapeptide-20 (pure peptide contained in Greyverse™) was tested at the concentrations of 10^{-9} M and 10^{-7} M, equivalent to 0.005% and 0.5% Greyverse™, respectively.

Biological materials



On a scalp plasty coming from a 76-year-old woman, 74 inferior hair follicles (lower root sheath and bulb) were isolated by microdissection.

These hair follicles were placed in 48-well plates and kept in survival for 8 days at 37°C in a 5% CO_2 atmosphere, in a Philpott medium composed of William's medium (ref. W1878, Sigma-Aldrich) supplemented with L-Glutamine, insulin-transferrin-selenium, hydrocortisone, penicillin/streptomycin antibiotics and serum (300 µl per well).

Method

The entire culture medium was renewed (300 µl per well) on day 0, day 1, day 4 and day 6 and the products to be tested were added to it.

The untreated batch did not receive any treatment other than the renewal of the medium.

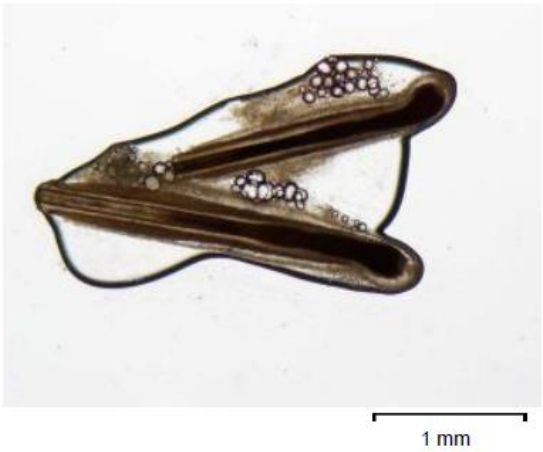
Hair follicle pigmentation analyses

Microscopic observation of grey hair follicle pigmentation

The method consisted in mounting selected hairs on clear glass slides for microscopic observation (hairs were then fixed)

- Hair pigmentation on mounted hairs

Selected hair follicles were fixed and mounted on clear glass slides using a Clarine-Eukitt mounting medium to visualize the hair pigmentation by microscopic observation.
The microscopic observations were performed using a Leica DMLB or Olympus BX43 microscope. Pictures were digitized with a numeric DP72 Olympus camera with CellD storing software.

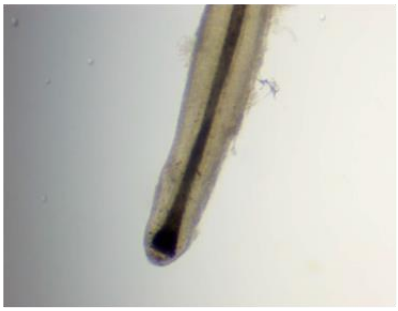


Microscopic picture (objective x2.5) of inferior follicles mounted on clear glass slides

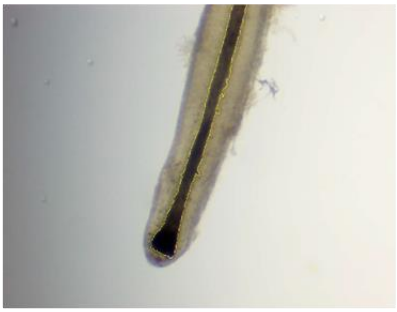
Picture analysis

All image processing was performed with Fiji software [2]. The first part of the processing allowed the hair follicle to be extracted from the rest of the image and its surface measured (in mm^2). The "subtract background" function was used to remove smooth continuous backgrounds from the image. Then, the image intensities were enhanced using the *Enhanced Local Contrast (CLAHE)* plugin. This filter allows the local contrast in an image to be equalized (it reduces inhomogeneity in terms of intensity), while preserving the selected regions of interest and limiting the amplification of noise. This method helped increase hair detection. Finally, an automatic threshold level was set to create the corresponding binary image of the hair, and calculate area statistics (hair surface in mm^2).

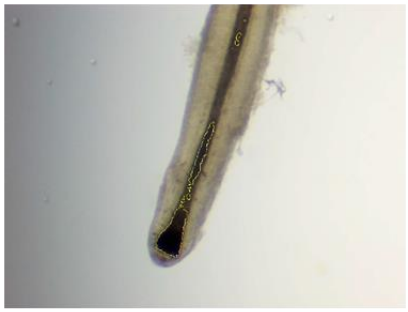
In the second part of the processing, an automatic thresholding was used on each raw image in order to select pixels corresponding to the pigmented area only. Using binary images of the selected hair (first part of the pipe) as an inclusive mask, the surface of these pigments (in mm^2) was computed in the hair area only.



Microscopical hair follicle image



Binary hair follicle image to calculate area statistic



Pigmented area fraction of the hair follicle

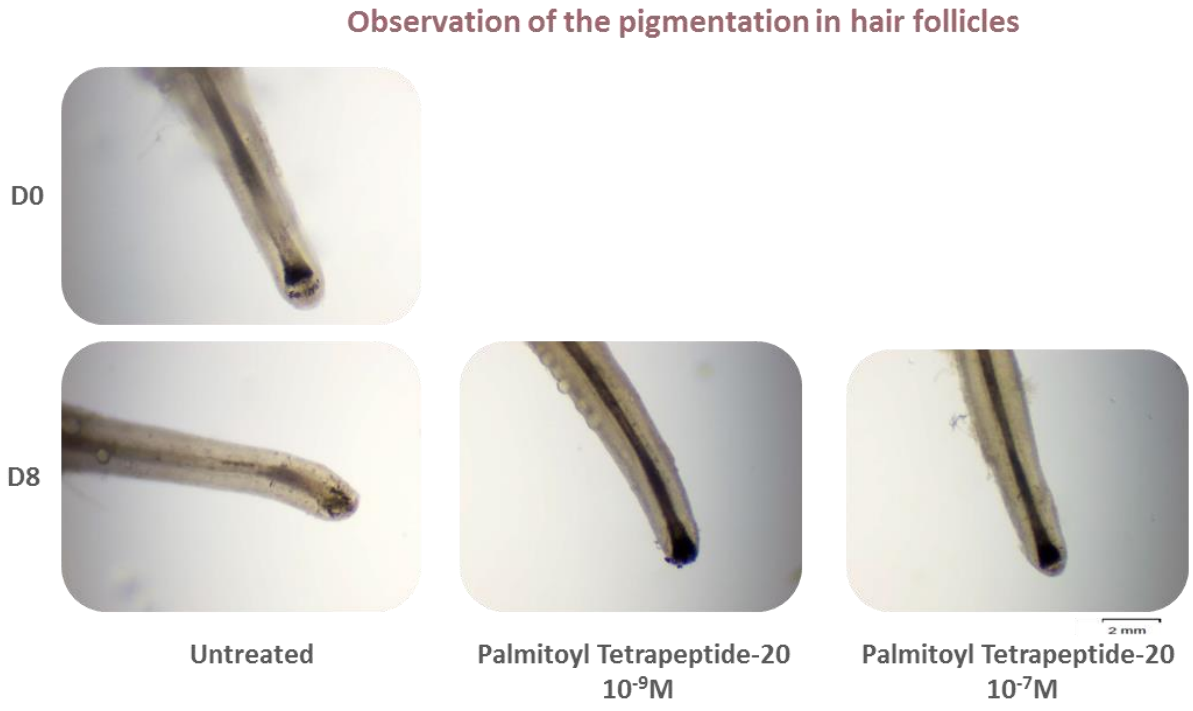
Statistical Analysis

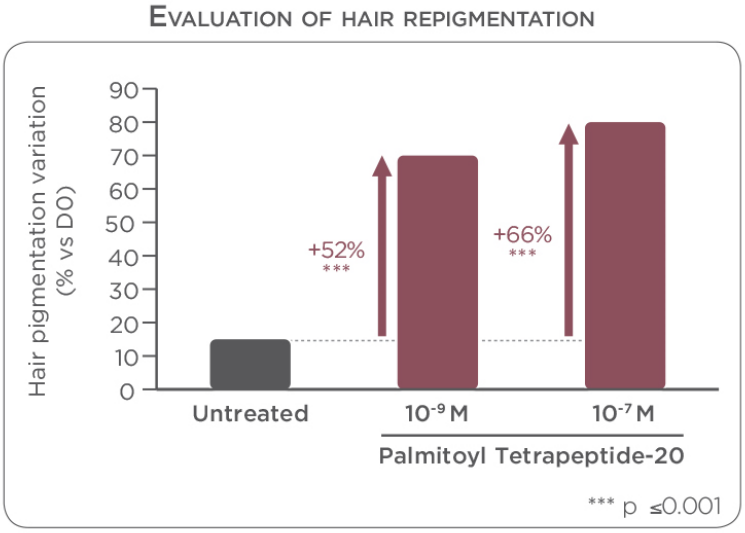
The inter-group comparisons were performed by an Unpaired Student's t-Test: *p<0.05, **p<0.01 and ***p<0.001.

RESULTS

The micro-dissected hair follicles were put in survival and photographed alive (20 hairs per batch) on day 0 (before treatment) and day 8 (after treatment).
The evolution of the hair bulb pigmentation was estimated by image quantification on day 8 compared to day 0, for each isolated hair follicle. The results were expressed as a percentage of variation of area hairs exhibiting an increase in hair pigmentation.

According to the experimental conditions, after 8 days of survival and compared to day 0, the Palmitoyl Tetrapeptide-20 Fgrey increased hair pigmentation by 52% and 66% at concentrations of 10^{-9} M and 10^{-7}M, respectively.





The color of the treated hair follicles had increased, attesting a higher melanin content.

CONCLUSION

Greyverse™ visibly darkens hair by increasing the melanin content in the hair shaft.

IN TUBO EFFECT OF GREYVERSE™ ON CATALASE ENZYMATIC ACTIVITY

BACKGROUND

Recently, Shi and *al.* [3] have shown that a deficiency of catalase, an enzyme that allows the transformation of hydrogen peroxide into oxygen and water ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$) in human hair follicles, largely contributes to the greying of hair. Moreover, it was shown that it induces a change in the conformation of the tyrosinase structure synthesized by hair follicle melanocytes, especially by the oxidation of the methionine residues of this key enzyme in melanin production. This structural modification of the tyrosinase leads to a loss of its enzymatic activity and stops melanin synthesis in the melanocytes of the hair follicle.

OBJECTIVE

The aim of this study was to determine the direct effect of Greyverse™ on catalase activity by measuring the quantity of H_20_2 not degraded by the enzyme.

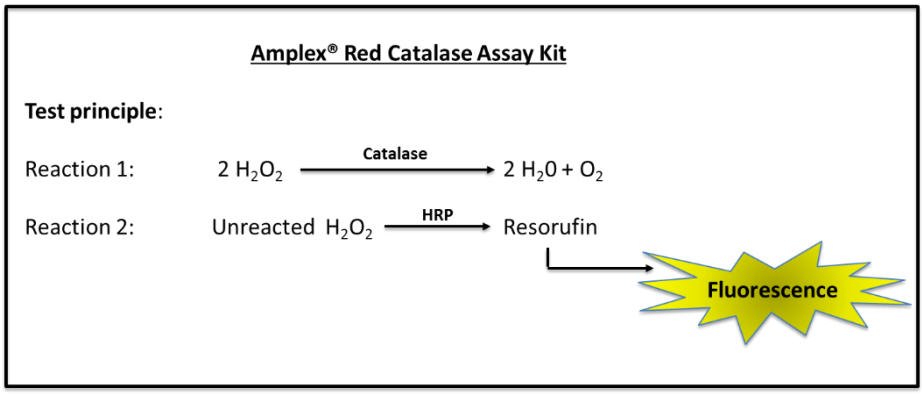
PROTOCOL

Tested products

- Palmitoyl Tetrapeptide-20 was tested at the concentration of 10^{-5} M.

Method

The catalase activity was evaluated using the Amplex® Red Catalase Assay Kit purchased by Molecular Probes (Invitrogen, ref: A22180) in compliance with the instructions of the manufacturer. In the assay, catalase first reacts with H_2O_2 to produce water (H_20) and oxygen (O_2). Then, the Amplex Red reagent reacts with a 1:1 stoichiometry with any unreacted H_2O_2 in the presence of horseradish (HRP) to produce the highly fluorescent oxidation product, resorufin. A correlation with catalase activity is then calculated.



A 10^{-5} M Palmitoyl Tetrapeptide-20 solution was incubated with 6.25 mU catalase in a 96-well plate for 10 minutes at room temperature. Then, an extemporaneously prepared solution of H_2O_2 (20 µM final concentration) was added and the mixture was incubated for 30 minutes at room temperature in the dark. A mixture of HRP (0.2U/mL final concentration) and Amplex Red fluorogenic probe (50 µM final concentration) was then added and the plates were incubated in the dark at 37°C for 60 minutes.

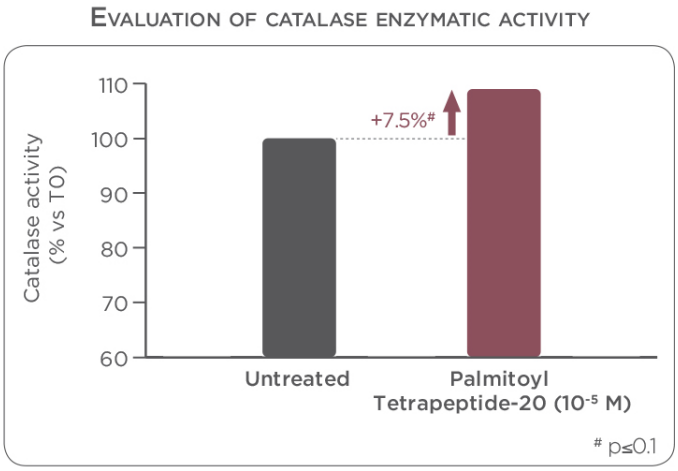
Evaluation of the activity

The generated fluorescence of formed resorufin was immediately recorded (λ_{ex} = 544nm, λ_{em} = 590 nm) using a Polar Star Omega (BMG, Ortenberg, Germany) reader.

Statistical Analysis

The inter-group comparisons were performed by an Unpaired Student's t-Test: #p<0.1, *p<0.05, **p<0.01 and ***p<0.001.

RESULTS



The Palmitoyl Tetrapeptide-20 is able to significantly increase*, in tubo,* catalase activity by 7.5%.

CONCLUSION

Greyverse™ improves catalase activity to reduce the greying hair process due to oxidative stress caused by excess H_2O_2.

IN VITRO EFFECT OF GREYVERSE™ ON THE INTRACELLULAR H_2O_2 LEVEL IN HAIR FOLLICLE DERMAL PAPILLA FIBROBLASTS

BACKGROUND

Recently, Shi and *al.* have shown that a deficiency of catalase, an enzyme that allows the transformation of hydrogen peroxide into oxygen and water ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$) in human hair follicles, largely contributes to the greying of hair [3].

OBJECTIVE

The aim of this study was to determine the effect of Greyverse™ on the intracellular H_2O_2 level due to its action on the increased catalase activity.

PROTOCOL

Tested products

- Palmitoyl Tetrapeptide-20 was tested at the concentration of 10^{-5} M.
- The 2',7'-Dichlorofluorescein diacetate (DCFH-DA) was used at a final concentration of 10 µM.

Biological materials

HFDP cells are fibroblasts isolated from human hair dermal papilla and maintained in a specific follicle dermal papilla cell medium (Promocell, Ref: C-26501) containing fetal serum (0.04 mL/mL), bovine pituitary extract (0.004 mL/mL), basic fibroblast growth factors (recombinant human, 1 ng/mL), insulin (recombinant human, 5µg/mL) and antibiotics (penicillin/streptomycin/Eurobio, CABPES01-OU) at 37 °C under 5% CO_2 and 95% humidity.

Method

The intracellular levels of H_2O_2 were evaluated by using a fluorescent dichlorofluorescein assay adapted to flow cytometry which allowed the detection of picomole levels of hydroperoxides [4]. Using the fluorogenic probe and the 2',7'-Dichlorofluorescein diacetate (DCFH-DA) (Sigma, Ref: D6883), we assessed the levels of intracellular hydrogen peroxide within the untreated control and the treated cells. HFDPC cells were seeded at 2.10^6 cells/well in 12-well plates with 500 µl complete medium and incubated for 24 hours at 37°C. Palmitoyl Tetrapeptide-20 (10^{-5} M) was then added and incubated for 18 hours at 37°C. At the end of incubation, DCFH-DA probe (10 µM final concentration) was added for an additional 15 minutes.

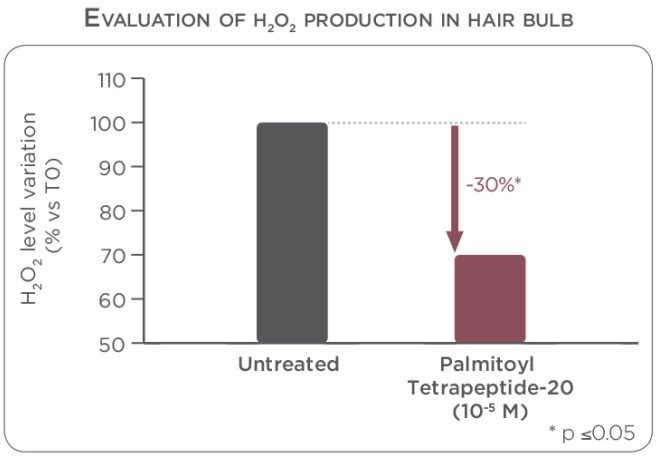
Evaluation of the activity

Cells were then trypsinized, washed and immediately scanned on an FACS (FC500 flow cytometer, Beckman-Coulter) with excitation and emission settings of 485 nm and 529nm, respectively. The emitted fluorescence is assumed to be proportional to the concentration of hydrogen peroxide in the cells [5]. The results are expressed as a percentage of decrease in the H_2O_2 production by HFDPC in comparison with the untreated control cells.

Statistical Analysis

The inter-group comparisons were performed by an Unpaired Student's t-Test: *p<0.05, **p<0.01 and ***p<0.001.

RESULTS



Greyverse™ is able to decrease the internal H_2O_2 production in an HFDPC culture by 30%.

CONCLUSION

Greyverse™ decreases H_2O_2 accumulation thus decreasing oxidative stress involved in the greying hair process.

EX VIVO EFFECT OF GREYVERSE™ ON OXIDATIVE ENZYME EXPRESSION

BACKGROUND

Oxidative stress is one of the main causes of the greying process. The excess of free radicals degrades melanin and lowers the capacity of the melanocytes to synthetize melanin.
Shi and *al.* have shown that a deficiency of catalase, the enzyme that allows the transformation of hydrogen peroxide into oxygen and water ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$) in human hair follicles, largely contributes to the greying of hair [3].
Moreover, TRP-2 expression was shown to be uncoupled to that of tyrosinase and TRP-1 in hair bulb melanocytes, whatever the hair colour and ethnic origin [6], suggesting a specific role of this enzyme apart from pigmentation through melanin synthesis. Michard et al. investigated the TRP-2 effects on oxidative stress-mediated toxicity [7]. They demonstrated that TRP-2 over-expression reduces H_2O_2-induced DNA damage, with a significant improvement of respiration and survival rate without any impact on cell proliferation. Additionally, they demonstrated that TRP-2 silencing partially restores sensitivity to oxidative stress. The importance of TRP-2 as a protector for hair follicle and hair colour preservation is further confirmed by the fact that TRP-2 expression is preserved in eyelash follicle melanocytes [8]: it would explain eyelash protection against greying.

Altogether, we can seriously presume that catalase and TRP-2 contribute to protecting hair follicle melanocytes from oxidation, and that their specific lack leads to hair follicle susceptibility to oxidation [9] and largely contributes to the greying of hair.

OBJECTIVE

The aim of this study was to determine *ex vivo* the effects of Greyverse™ on oxidative enzymes (catalase and TRP-2) implicated in the greying hair process.

PROTOCOL

Tested products

Palmitoyl Tetrapeptide-20 (pure peptide contained in Greyverse™) was tested at concentrations of 10^{-9} M and 10^{-7} M, equivalent to 0.005% and 0.5% Greyverse™, respectively.

Biological materials

Isolated human grey hair follicle

On a scalp plasty coming from a 71-year-old Caucasian woman, grey hair follicles (from the infundibulum to the bulb) were isolated by microdissection on day 0. These hair follicles were placed separately in a 48-well plate and kept in survival for seven days in William's medium (Sigma-Aldrich, W1878) supplemented with L-glutamine, insulin, hydrocortisone, serum and antibiotics, at 37°C in a 5% CO_2 atmosphere. Eleven hair follicles were used per condition (untreated or treated). Palmitoyl Tetrapeptide-20 was diluted at 10^{-9} M and 10^{-7} M in the culture medium on day 0, day 1, day 4 and day 7 whereas the medium of the untreated hair follicles was renewed.

Method

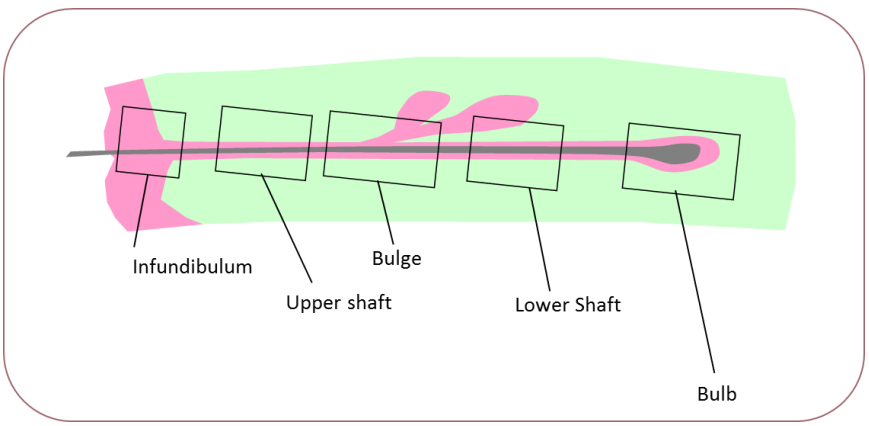
At the end of survival (day 7), hair follicles were fixed in a buffered formalin solution, dehydrated, and paraffin impregnated. 7-µm-thick paraffin sections were mounted on glass slides for immunostainings and stainings.

- For fluorescent immunostainings, the primary antibody used was i-PARD3/ASIP (Abcam, ref. ab64646). The sections were then incubated for 30 minutes at room temperature with the Alexa Fluor 488-labeled antibody (Lifetechnologies, ref. A11078). The nuclei were counter-stained using propidium iodide at 0.2µg/mL. The slides were finally mounted using Vectashield® mounting medium (Vector, ref. H-1400).

- For Immunohistochemistry, the hair sections were pre-incubated with hydrogen peroxide (VWR, ref. 23619.264) to inactivate endogenous peroxidase activity. The primary antibodies used were anti-TRP1 (Eurogentec, ref. SIG-38150-1000) and anti-Melan-A (Santa Cruz biotechnologies, ref. sc-20032). Then, the sections were pre-incubated with a biotinylated secondary antibody and incubated with a streptavidin-labeled peroxidase (Vector, Vectastain® Universal ABC kit, ref. PK7200). The staining was revealed by a substrate of peroxidase, VIP (Vector, ref. SK-4600). The nuclei were counter-stained using Mayer's hemalum (RAL diagnostics, ref. 320550). The slides were finally mounted using Eukitt mounting medium (VWR, ref. KIND01250).

The microscopic observations were performed using a Leica DMLB or Olympus BX43 microscope. Pictures were digitized with a numeric DP72 Olympus camera with CellD storing software. Different hair parts were analyzed: infundibulum, upper shaft, bulge, lower shaft and bulb.

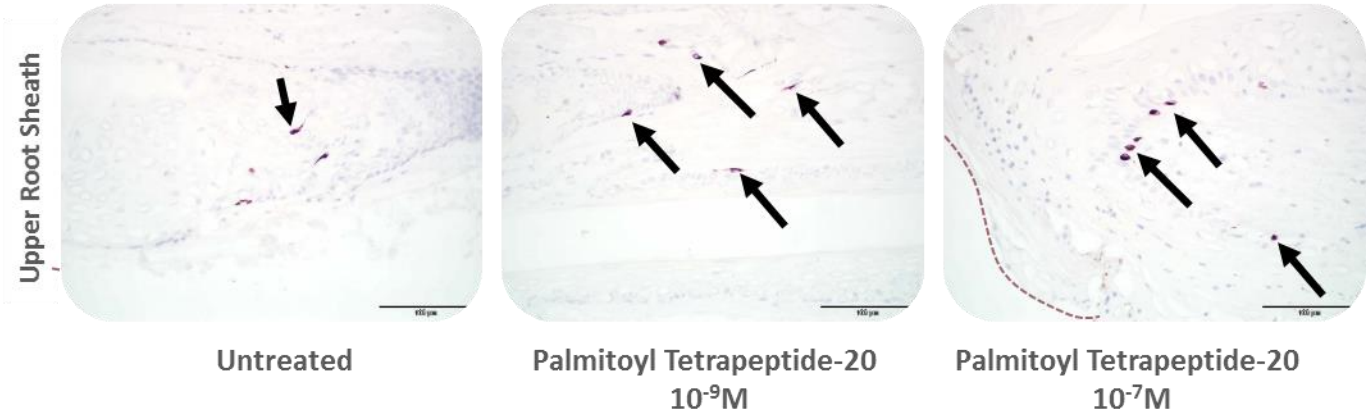
Hair follicle areas



RESULTS

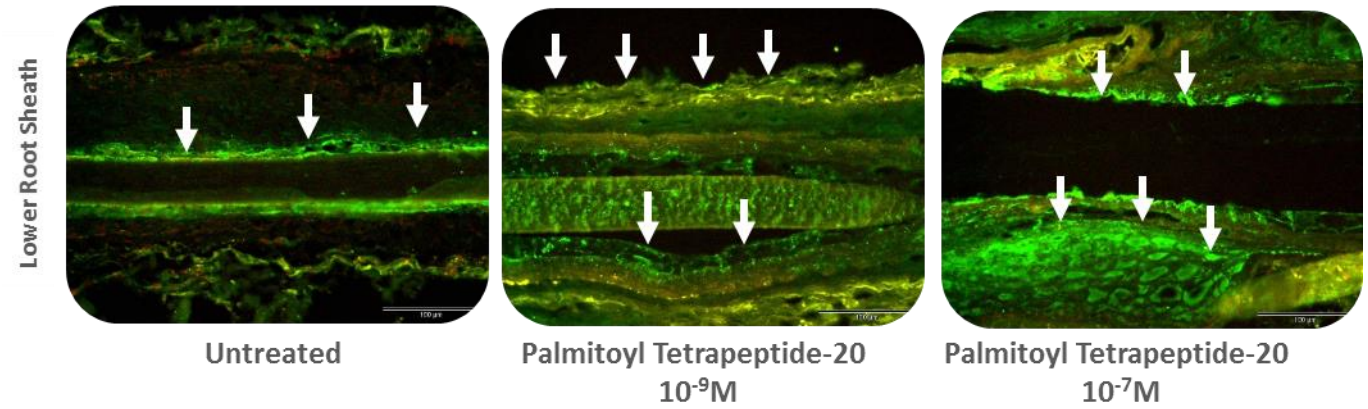
After 7 days of treatments with 10^{-9} M and 10^{-7} M Palmitoyl Tetrapeptide-20, an antioxidant activity was observed by immunostainings on micro-dissected human hair follicles.

Observation of TRP-2 in hair follicles



The tyrosinase-related proteins TRP-2 was induced in the upper root sheaths.

Observation of catalase expression in hair follicles



The antioxidant enzyme catalase, known to block the deleterious whitening effect of hydrogen peroxide in hair, was increased in the lower root sheaths.

CONCLUSION

Greyverse™ decreases the enzymes involved in oxidative stress thus reducing the greying hair process.

CLINICAL STUDIES

CLINICAL EVALUATION OF HAIR PIGMENTATION

OBJECTIVE

The primary aim of the study was to evaluate the direct effect of Greyverse™ on hair pigmentation and its indirect effect on gene expression and related protein synthesis using the plucked hair shafts of volunteers.

PROTOCOL

Subjects

Fifteen healthy Caucasian male volunteers were included in a clinical study after informed consent. They were between the ages of 18 and 35 (mean age 33) and exhibited premature hair greying, with more than 20% white hairs.

Test conditions

A pipette of 3 mL of the tested product was applied each evening topically on dry hair scalp, with massage to facilitate the hair scalp distribution, without rinsing. The application was repeated every night for 3 months. Monitored parameters were instrumentally evaluated by means of non-invasive bio-engineering techniques at the baseline (T0) and after 3 months (T3 months) of product use. In addition, the hair of volunteers was photographed at two different time stamps: T0 and T3 months.

Tested product

A hydro alcoholic lotion containing 2% Greyverse was used for the study.
The formula conformed to Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance) and to its annexes.

INCI Name	%
WATER	76.2
ALCOHOL DENAT.	14.7
PROPYLENE GLYCOL	2.0
PEG-40 HYDROGENATED CASTOR OIL	1.5
LECITHIN	1.0
PHENOXYETHANOL	0.8
GLYCERIN	0.6
PARFUM	0.5
CHLORPHENESIN	0.25
PVP	0.15
PANTHENOL	0.1
PYRIDOXINE HCL	0.1
SODIUM HYDROXIDE	0.075
ETHYL ESTER OF HYDROLYZED SILK	0.0175
TOCOPHEROL	0.01
Greyverse™	**2**

METHODS

Determination of the brightness (or luminance) of the hair with a Chroma Meter

Before treatment (T0), the measurement of hair color intensity was performed by colorimetry using a Chroma Meter (Chromatique CR 200™ MINOLTA, France) on three different hair areas. The vertical dimension L* parameter determining the brightness (or luminance) from zero (black) to 100 (white) was evaluated. After 3 months of treatment (T 3 months), the same areas were measured by Chroma Meter.

Analysis of protein expressions directly on the hair of the volunteers

On day 0, and after 3 months of treatment, several hair shafts were plucked from the scalp of each volunteer. Half of the plucked hairs of each volunteer were fixed in standard formalin-buffered solution for 24 hours, dehydrated and embedded in paraffin, and half were frozen at -80°C for immunostainings.

Seven µm sections of frozen hair shafts were fixed with acetone at -20°C for 10 minutes, while 5 µm sections of paraffin-embedded hairs were deparaffinized and incubated with a pH6-antigen retrieval solution for 20 minutes at 98°C (Dako, ref. K8005) for immunostainings. The hair sections were incubated for 30 minutes with 3% normal rabbit, goat or horse serum (Vector, Vectastain® Universal ABC kit, ref. PK7200) in PBS to block nonspecific binding sites and stained with specific primary antibodies diluted in PBS with 3% BSA.

- For fluorescent immunostainings, the primary antibody used was anti-PARD3/ASIP (Abcam, ref. ab64646) for 1 hour at room temperature. The sections were then incubated for 30 minutes at room temperature with Alexa Fluor 488 antibody (Lifetechnologies, ref. A11008). The nuclei were counter-stained using propidium iodide at 0.2µg/mL. The slides were finally mounted using Vectashield® mounting medium (Vector, ref. H-1400).

- *For Immunohistochemistry,* the hair sections were pre-incubated with hydrogen peroxide (VWR, ref. 23619.264) at 0.3% for 10 minutes to inactivate endogenous peroxidase activity. The primary antibody used was anti-MC1R antibody (Santa Cruz biotechnologies, ref. sc-28990) for 1 hour at room temperature. Then, the sections were incubated for 30 minutes at room temperature with a biotinylated secondary antibody and incubated for 30 minutes at room temperature with a streptavidin-labeled peroxidase (Vector, Vectastain® Universal ABC kit, ref. PK7200). The staining was revealed by a substrate of peroxidase, VIP (Vector, ref. SK-4600). The nuclei were counter-stained using Mayer hemalun (RAL diagnostics, ref. 320550). The slides were finally dehydrated and mounted using Eukitt mounting medium (VWR, ref. KIND01250).

The microscopic observations were realized using a Leica DMLB or Olympus BX43 microscope. Pictures were digitized with a numeric DP72 Olympus camera with CellD storing software.

Analysis of gene expressions directly on the hair of the volunteers

On day 0, and after 3 months of treatment, ten hair shafts were plucked from the scalp of each volunteer and frozen at -80°C for ARNs extraction. The hairs were ground by a potter in the presence of Trizol® solution (Thermo Fischer Scientific, ref: 15596026). The lysate was transferred to a new Eppendorf and heated at 55°C in the presence of proteinase K for 10 minutes. Then chloroform was added before centrifusion at 4°C. The aqueous phase was then precipitated in 100% ethanol.

RNA extraction was followed on a Qiagen mini-column and the standard extraction protocol was then performed according to the manufacturer's instructions (Qiagen extraction kit). The RNA obtained was

controlled on Bioanalyzer Agilent 2100. The analysis of the expression of 3 genes (MC1-R, MITF, ASIP) was performed in microfluidic quantitative PCR on FLEX 12x12 plates, with normalization by B2M household gene.

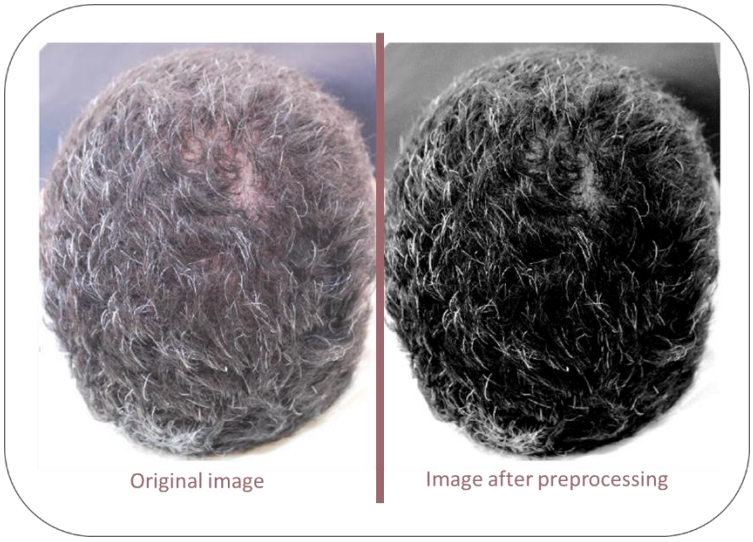
Image analysis

- ***Segmentation of white hair***

Following the clinical study, image analyses were used to assess the effect of Greyverse™ on the amount of white hair over time.

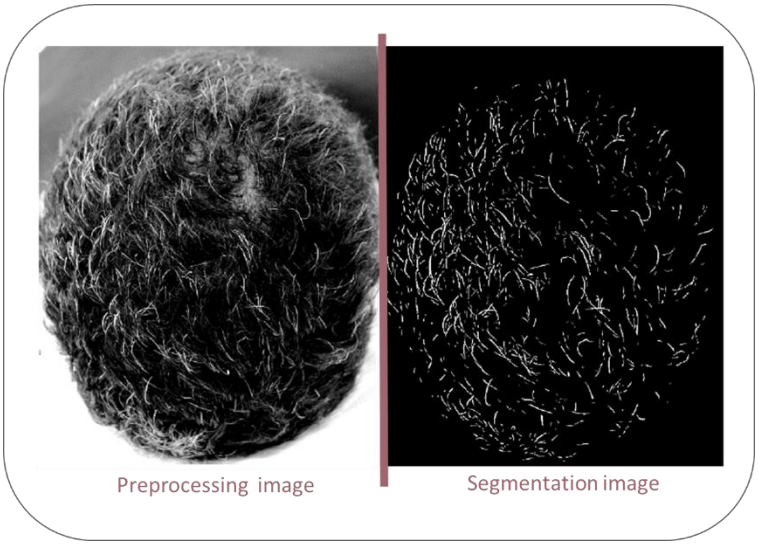
Pre-processing

First, the images were projected on a guiding axis in order the highlight the white hair on the images. The following images illustrate the result of this preprocessing step:



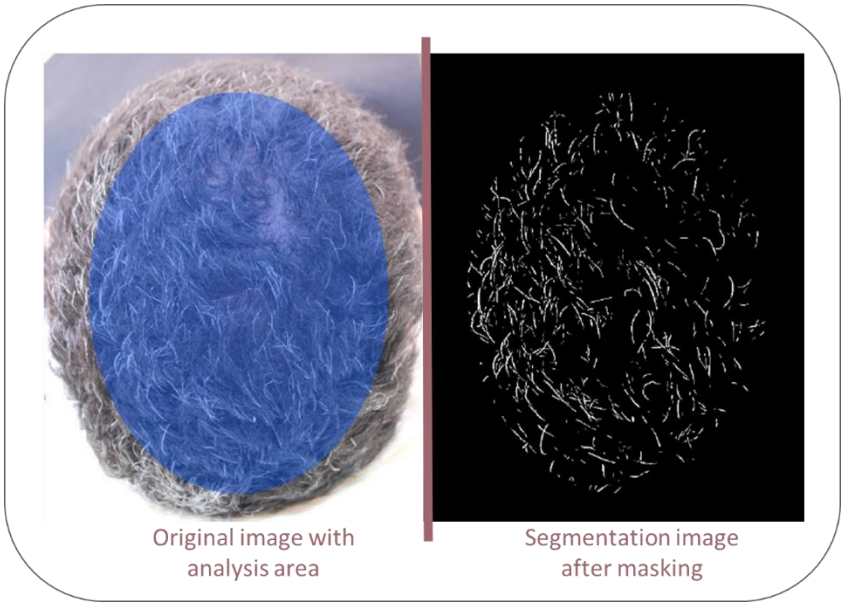
Segmentation

In order to segment the white hair, an algorithm was applied allowing the lines on an image to be extracted. Elements smaller than hair were removed. The following images illustrate the results of the segmentation:



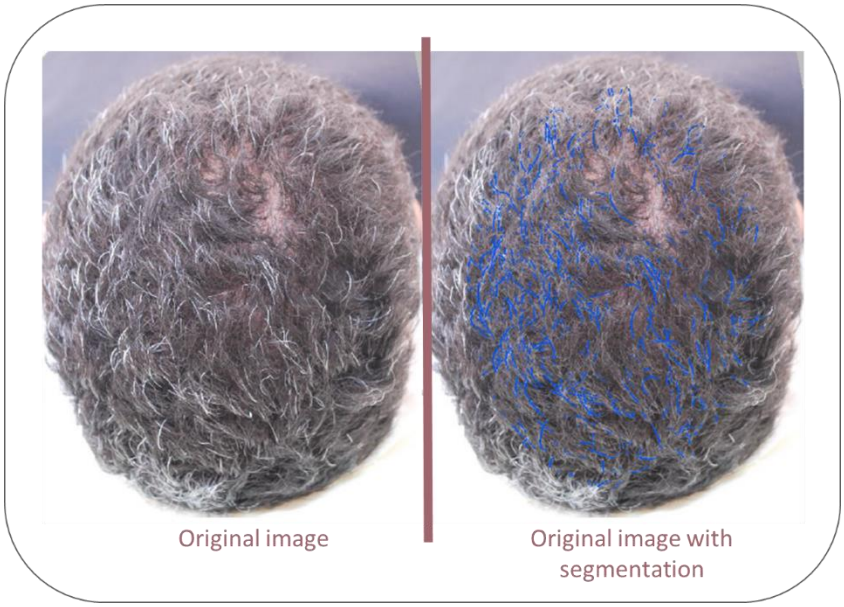
Masking

In order to exclude blurry areas and keep only the white hair located on the center of the head, an analysis area was defined for each subject at T0. Then, this area was replaced automatically at other time points thanks to a specific special registration algorithm. The following images illustrate the defined region of interest and the result of this masking step:



Illustration

Once the segmentation was computed, the resulting mask was applied to the original image. The following images illustrate the result of the segmentation:



- ***Evaluation of white hair density***

The density of the white hair is defined as the surface of the segmentation divided by the surface of the analysis area. A decrease in the number of white hairs results in a decrease in the density.

The resulting data and percentage variations were submitted to the Wilcoxon test. If the test provides a p-value less than 0.05, the variation is significant over the time of the studied parameter.

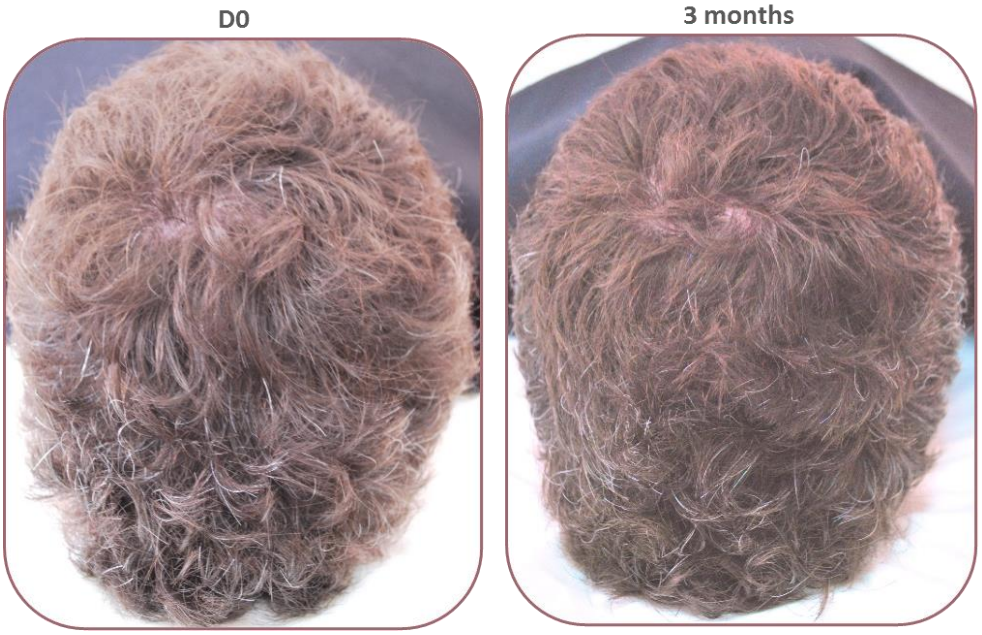
STATISTICAL METHODS

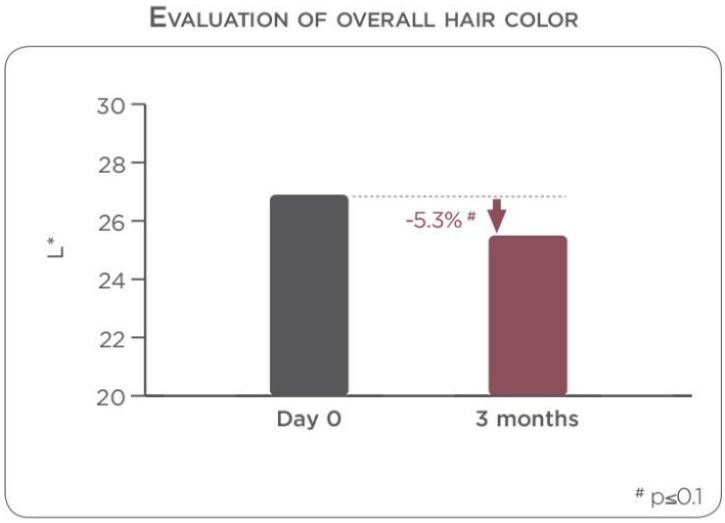
For the clinical study, the resulting data and percentage variations were submitted to the Unpaired Student's t-Test in the case of the Chroma Meter and gene expressions results (#p≤0.1) while the Wilcoxon test was used (*p<0.05) for the data obtained from the image analyses.

RESULTS

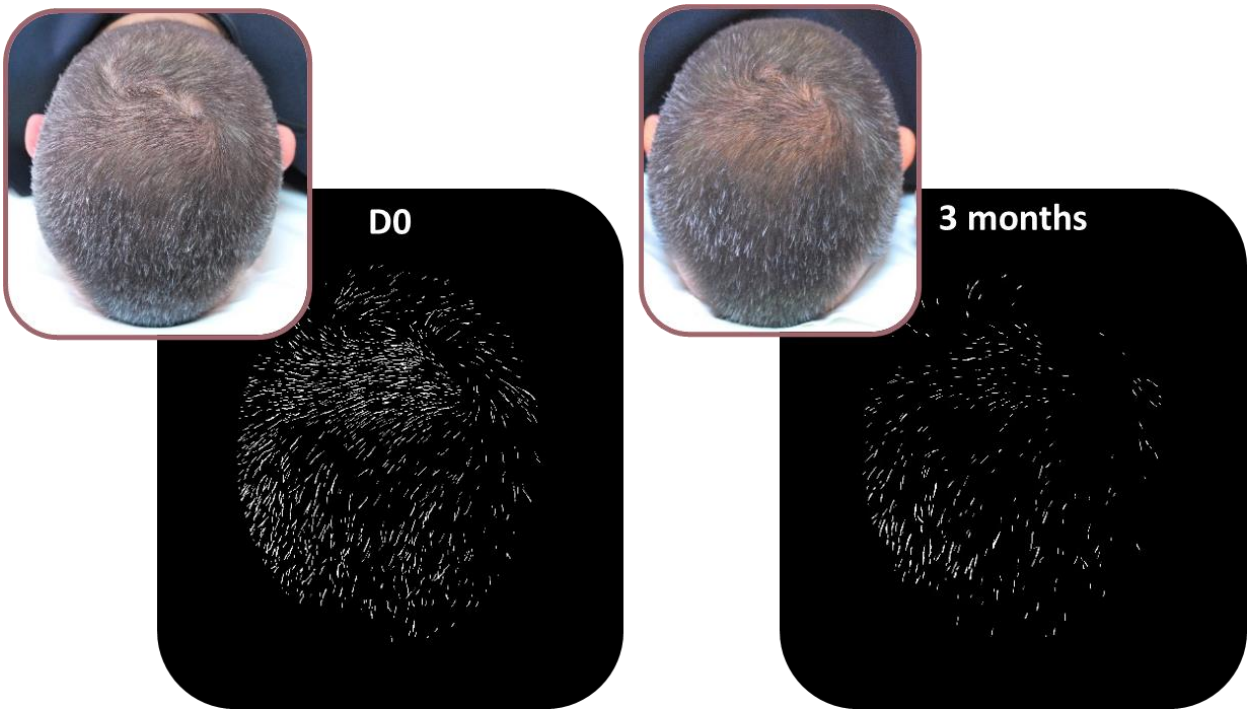
CHROMAMETER RESULTS

After three months of treatment with the lotion containing 2% Greyverse™, the hair became darker for 100% volunteers. Indeed, the brightness parameter L* was significantly decreased by 5.3% # (26.92 a.u ± 6.35 on day 0 vs 25.50 a.u ± 6.33 after 3 months; # p-value=0.0941), up to by 32%.

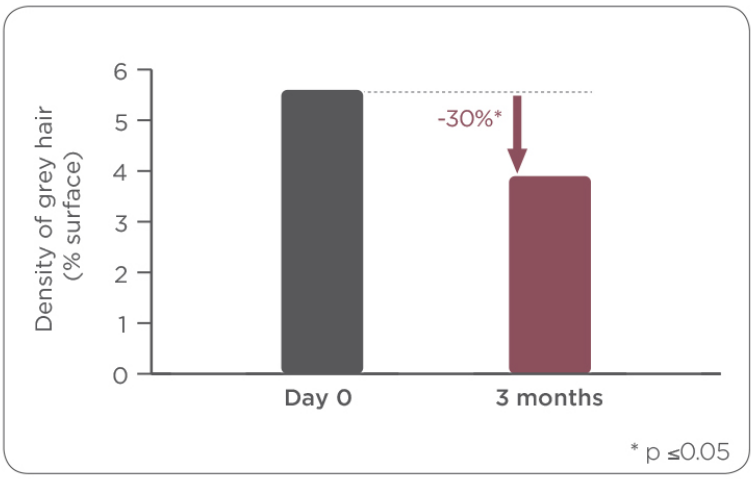




These results were confirmed by image analysis:



EVALUATION OF GREY HAIR DENSITY

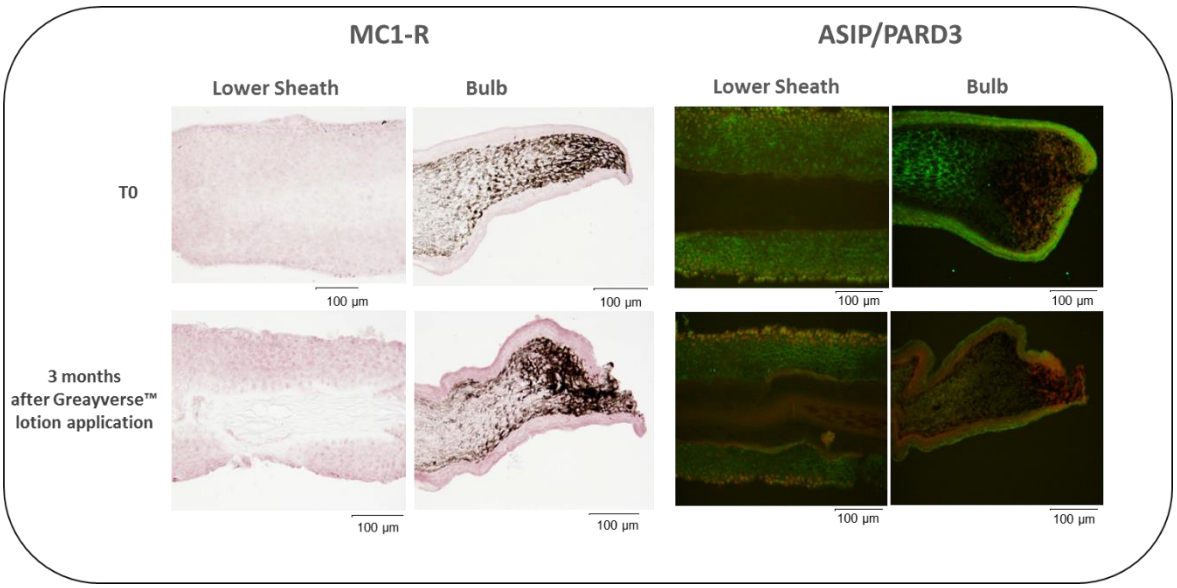


After image analysis, the density of white hair was significantly decreased by 30% after 3 months of Greyverse™ application.

PROTEIN EXPRESSIONS DIRECTLY ON THE HAIR OF THE VOLUNTEERS

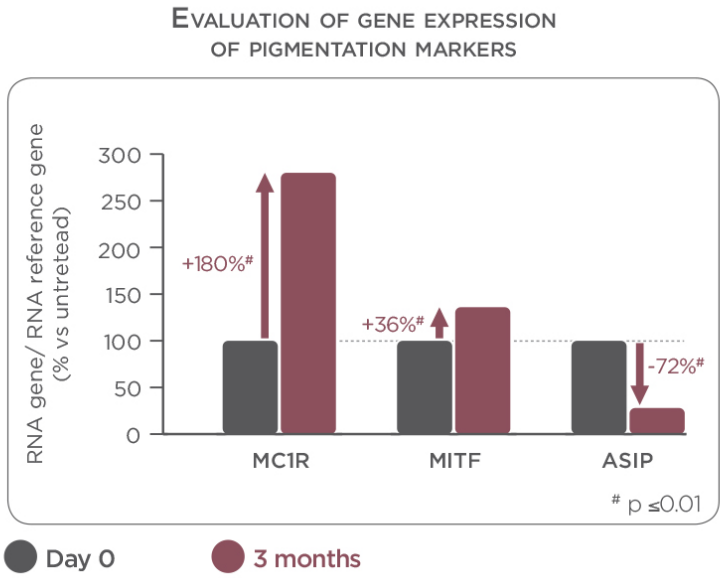
After 3 months of treatment with the lotion containing 2% Greyverse™, a stimulation of melanosome biogenesis and eumelanin synthesis in plucked hairs could be observed by immunostainings. An increase in MC1-R was observed, whereas ASIP/PARD3 was decreased in the lower root sheaths and bulb in more than 50% of the volunteers.

EVALUATION OF EXPRESSIONS OF PIGMENTATION MARKERS IN HUMAN HAIR FOLLICLES



GENE EXPRESSIONS DIRECTLY ON THE HAIR OF THE VOLUNTEERS

The results obtained by protein synthesis analysis were confirmed by gene expression analysis. After 3 months of treatment with the lotion containing Greyverse™, gene expressions of pigmented markers were increased by 180% for MC1-R, 36% for MITF and decreased by 72% for ASIP.



Greyverse™ improves the expression of melanogenesis genes thus progressively stimulating the pigmentation process

ADDITIONAL DATA

IN VITRO EFFECT OF GREYVERSE™ ON THE TRANSACTIVATION OF THE MC1-R RECEPTOR

BACKGROUND

Pigmentation process goes through the activation of some melanocortin receptors like the MC1-R, which has a higher affinity for the α-MSH and is expressed by most cutaneous cells.

The first step was to check the ability of Greyverse™ to bind to and specifically stimulate the MCR-1 receptor.

PROTOCOL

Biological materials

Human Embryonic Kidney (HEK293) cells were co-transfected using calcium phosphate and selected in 200 µg/mL hygromycin, with two vectors, one of the vectors containing the gene that codes for MC1-R (hMC1-R) and the other vector containing the gene that codes for the luciferase (CRE-Luc). The cDNA for the human MC1R (hMC1R) receptor comes from the RNAm of NHEM amplified by PCR. HEK cells were grown in DMEM with 10% fetal bovine serum without sodium pyruvate either without (wild-type cells) or with 200 µg/mL hygromycin (stably hMC1R-transfected cells). The cells were passaged once weekly using trypsin or EDTA to remove the cells from the culture plates. We have previously shown that non-transfected HEK293 cells do not express an endogenous MC1R.

Tested products

The dose-response of Palmitoyl Tetrapeptide-20 (pure peptide contained in Greyverse™) was tested on the transactivation studies in HEK293-hMC1-R.

The α-MSH was chosen as a reference agonist.

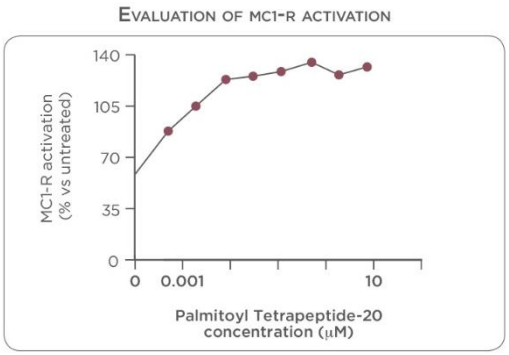
Evaluation of the activity

When the plasmid construct was activated by an agonist (Greyverse™ or α-MSH), there was a luminescent signal measured and quantified by a luminometer. The AC_{50} was determined. This corresponded to the concentration at which 50% of the luminescence was activated compared to the control.

RESULTS

The results below indicate the dose-response of MC1-R receptor activation by the peptide.

MC1-R ACTIVATION BY GREYVERSE™



	AC50 (nM)	Maximum activation at 25 µM (%)
Pure Peptide	0.16	137%

AC_{50} was determined from the results. It corresponds to the concentration of Palmitoyl Tetrapeptide-20 necessary to activate 50% of the maximum activation of the receptor. The lower the AC50, the better the affinity of the molecule for its receptor.

CONCLUSION

Greyverse™ is an agonist of the MC1-R receptor

IN VITRO EFFECT OF GREYVERSE™ ON cAMP PRODUCTION

BACKGROUND

In the previous study, we demonstrated the affinity of the Greyverse™ for the MCR-1 receptor. The goal of this second study was to confirm this affinity and to reveal the effectiveness of the Greyverse™ binding to its receptor.

The agonist binding to the MCR-1 receptor will activate the adenylate cyclase which will produce the cAMP (Cyclic adenosine monophosphate) from the adenine.

With the cAMP quantification, we will evaluate two parameters:

- The affinity of the ligand to its receptor
- If its binding is effective

The assay method used the radio labeling of adenine by tritium, which was introduced in the culture cell medium and transformed in AMPc-^{3}H.

PROTOCOL

Biological materials

Human melanocytes (M4Be cell line) expressing the MC1-R receptor.

Tested products

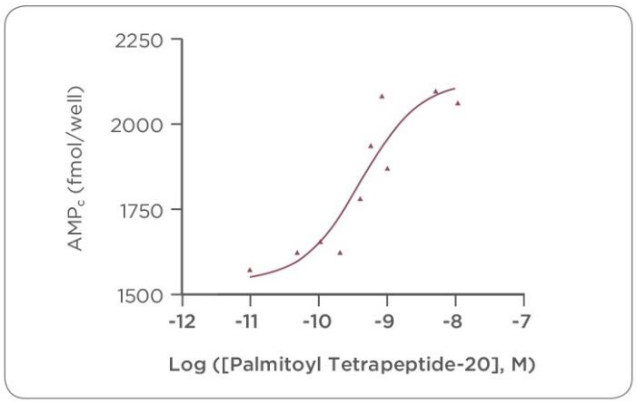
Palmitoyl Tetrapeptide-20 (pure peptide contained in Greyverse™) was tested at the following concentrations: 10^{-8} M, 10^{-9} M, 10^{-10} M and 10^{-11} M.

AMPc assay: to inhibit AMPc degradation, phosphodiesterase inhibitors were added to the culture medium.

RESULTS

The results below indicate the level of cAMP production as evaluated by the radioactivity:

GREYVERSE™ DOSE-EFFECT ON cAMP PRODUCTION



Pure peptide concentration (M)	AMPc (fmoles/wells)
10^{-11}	1576
5.10^{-11}	1629
10^{-10}	1660
2.10^{-10}	1631
4.10^{-10}	1787
6.10^{-10}	1942
8.10^{-10}	2087
10^{-9}	1879
5.10^{-9}	2104
10^{-8}	2071

EC_{50} was determined from the results. It corresponds to the concentration of Palmitoyl Tetrapeptide-20 necessary to activate 50% of the maximum stimulation of AMPc synthesis.

In two independent experiments, EC_{50} was found in very low quantities: 0.42mM and 0.23mM respectively.

CONCLUSION

Greyverse™ has a high affinity for the MC1-R receptor.

IN VITRO EFFECT OF GREYVERSE™ ON THE MELANIN SYNTHESIS IN A B16F1 CULTURE

BACKGROUND

The α-MSH induces the production of melanin after fixation on its specific receptor, the MC1-R. This study investigated the agonist activity of Greyverse™ to the MC1-R receptor by measuring its effects on the production of melanin in a B16F1 culture.

PROTOCOL

Biological materials

B16F1 cell lines were isolated from murine melanoma. These cells expressed only type 1 of the MC-R receptor.

Tested products

Palmitoyl Tetrapeptide-20 (pure peptide contained in Greyverse™) and α-MSH were tested at increasing concentrations.

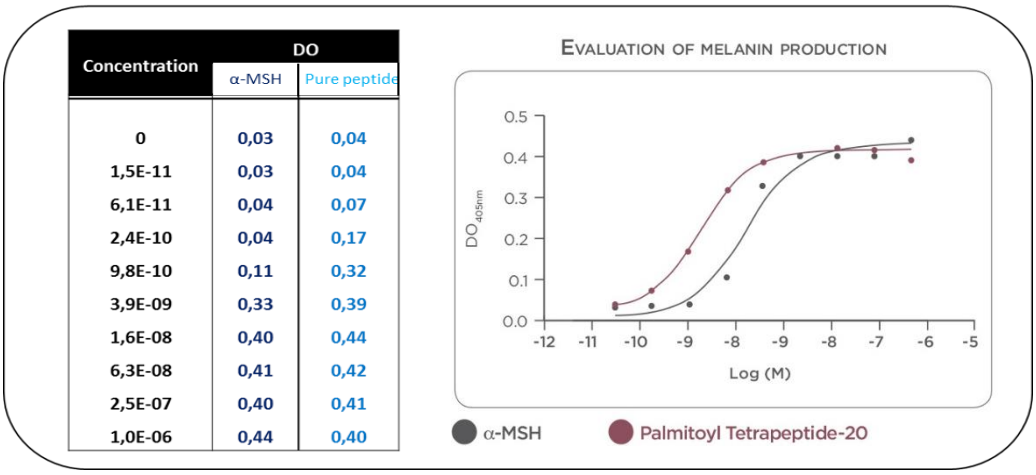
Evaluation of the activity

Cells were treated by α-MSH with or without the peptide. After 72 hours of treatment, the melanin secretion was quantified by spectrophotometer at 405 nm.

RESULTS

EFFECT OF GREYVERSE™ ON MELANIN PRODUCTION

Concentration	DO	
	α-MSH	Pure peptide
0	0,03	0,04
1,5E-11	0,03	0,04
6,1E-11	0,04	0,07
2,4E-10	0,04	0,17
9,8E-10	0,11	0,32
3,9E-09	0,33	0,39
1,6E-08	0,40	0,44
6,3E-08	0,41	0,42
2,5E-07	0,40	0,41
1,0E-06	0,44	0,40



EC_{50} was determined from the results. It corresponds to the concentration of Palmitoyl Tetrapeptide-20 or α-MSH necessary to activate 50% of the maximum stimulation of melanin synthesis.

The results indicate that the peptide had an agonist activity stronger than the α-MSH activity. The EC_{50} obtained for the peptide and the α-MSH were 0.36nM and 2.2nM (10 fold higher) respectively.

Greyverse™ is a biomimetic peptide of α-MSH thus stimulating melanin synthesis through the activation of the MC1-R receptor.

CONCLUSION

Greyverse™ is an innovative and clinically effective ingredient able to act on the different causes of the hair greying process, offering a unique solution to finally recover natural hair color.

A peptide biomimetic of α-MSH, Greyverse™ stimulates melanogenesis and reduces oxidative stress in the hair bulb to reactivate the production of melanin and increase the quantity of pigment in the hair shaft.

Greyverse™ delivers outstanding clinical results when it comes to decreasing grey hair density, helping men and women achieve a younger look and boost self-esteem.

COSMETIC APPLICATIONS

- Oily scalp and hair
- Anti-aging hair care
- Premature grey hair coverage
- Post-coloration treatment
- Natural color fortifier
- Scalp-friendly alternative to chemical dye
- Beard & mustache care

RECOMMENDATIONS FOR USE

Greyverse™ should be used at a temperature of 40°C or below. However, it can be heated if needed.

Recommended dosage:
0.5-1%: preventive care
1–2%: intensive care

FORMULAS

HAIRESISTIBLE SERUM 17.100.01 C195

INGREDIENTS	INCI NAMES	%
A Deionized Water	Water	91.70
Dissolvine® NA	Tetrasodium EDTA	0.10
B Lecigel™	Sodium Acrylates Copolymer (and) Lecithin	1.50
C Vitapherole® E-1000	Tocopherol (and) Helianthus Annuus (Sunflower) Seed Oil	0.20
D Verstatil® PC	Phenoxyethanol (and) Caprylyl Glycol	1.00
E Greyverse™	Glycerin (and) Water (and) Palmitoyl Tetrapeptide-20	2.00
Capixyl™	Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and) Trifolium Pratense (Clover) Flower Extract	2.00
Defenscalp™	Water (and) Epilobium Angustifolium Flower/Leaf/Stem Extract	1.50

MAXIMALE BEARD SERUM 17.100.02 C195

INGREDIENTS	INCI NAMES	%
A Deionized Water	Water	91.70
Dissolvine® NA	Tetrasodium EDTA	0.10
B Lecigel™	Sodium Acrylates Copolymer (and) Lecithin	1.50
C Vitapherole® E-1000	Tocopherol (and) Helianthus Annuus (Sunflower) Seed Oil	0.20
D Verstatil® PC	Phenoxyethanol (and) Caprylyl Glycol	1.00
E Greyverse™	Glycerin (and) Water (and) Palmitoyl Tetrapeptide-20	2.00
Capixyl™	Butylene Glycol (and) Water (and) Dextran (and) Acetyl Tetrapeptide-3 (and) Trifolium Pratense (Clover) Flower Extract	2.00
Canadian Willowherb™	Water (and) Epilobium Angustifolium Flower/Leaf/Stem Extract	1.50

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