GREYVERSE™

Zero shades of grey is also sexy!

IFF | LUCASMEYER COSMETICS

Grey hair: everybody is affected soon or later!

- Hair color reveals much information regarding an individual's ethnicity, age and health status, as well as physical attractiveness.

  =>Then, many people desire full, shiny, lustrous and colorful hair!
- The greying of hair is a natural biological process associating with aging, but can be amplified by sun exposure or pollution.
- Grey hair process affects the global population:

Men & women

Young & mature

All ethnicities

Caucasian > Asian > African

30s mid 30s 45s

GREY HAIR COVERAGE SOLUTIONS ON THE MARKET

		ADVANTAGES	DISADVANTAGES
Chemical dye		• 100% grey/white hair covering • Fast results	• Repeat every 6 weeks to avoid grey/white roots  • Chemical compounds => scalp irritation
Vegetable dye		• No irritant chemical molecules (ammonia, resorcinol and PPD free)	• Longer application time • Result doesn't last as long as for chemical dye
Instant root concealer		• Immediate covering • Convenient • To be used in between 2 dyes	• Short term effect: lasts until next shampoo • Temporary use • Aerosol
Coloring reaction		• "Non chemical" coloring pigments: bismuth citrate, plant extracts • Coloration by oxidoreduction reaction	• Progressive coloring (several applications needed) • No precise shade

These products color artificially hair shafts
Hair roots inevitably grow grey

NEW MARKET OPPORTUNITY

Progressively recover the **natural hair color**

by stimulating the **natural hair pigmentation process**

With a **clinically proven** action!

Before

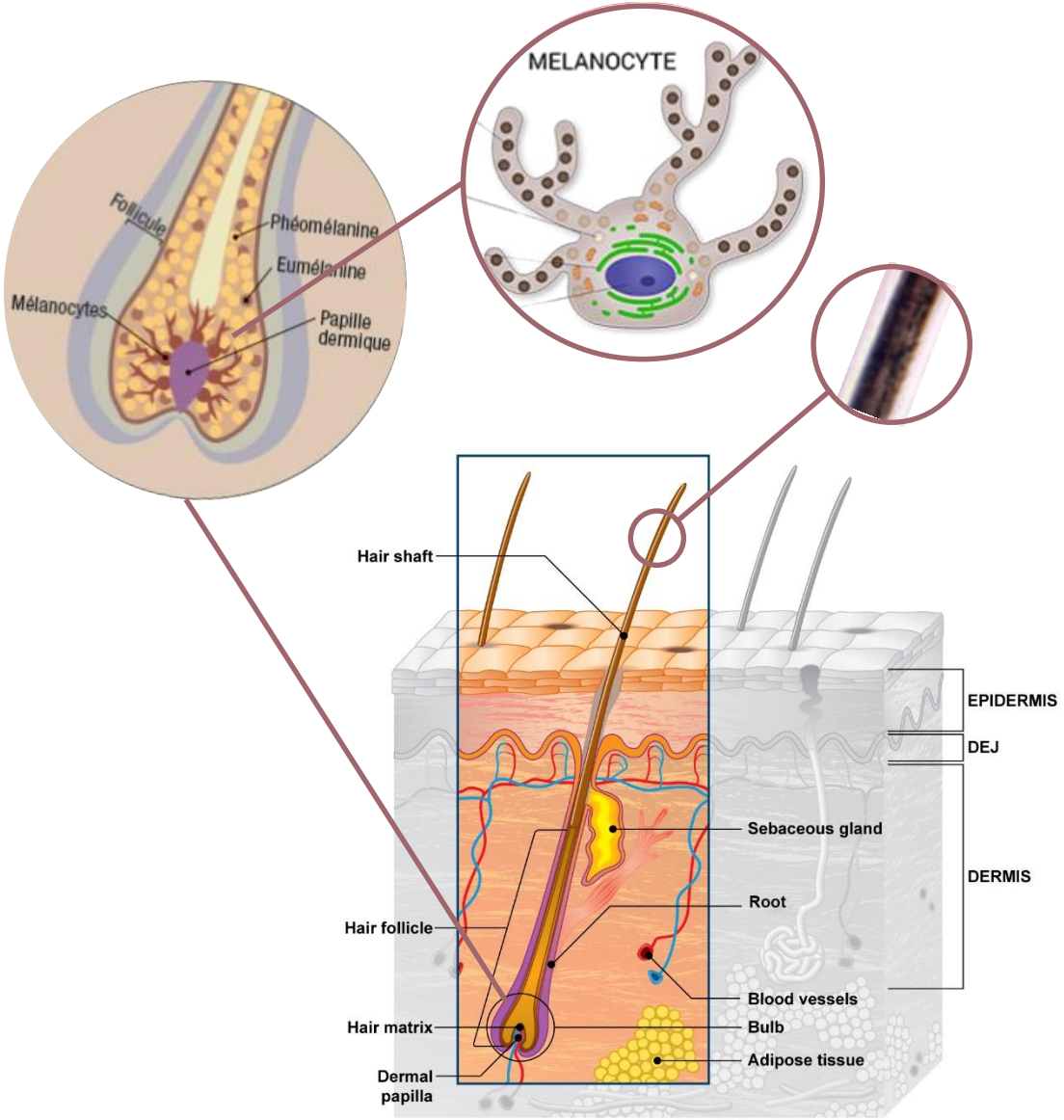
After

HAIR SCIENCE

Hair structure consists of:

- **Hair follicle**: small cavity made up of keratinocytes and melanocytes that extend in dermis.
  From this organ, the hair root develops with a progressive accumulation of **keratinocytes** continuously dividing in the hair matrix of the bulb.
  Melanocytes located in the bulb produce melanin and transfer it to the surrounding keratinocytes. It will provide the constitutive hair color.

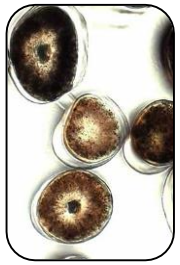
- **Hair shaft**: visible part above skin consist of an accumulation of keratinized pigmented keratinocytes of hair follicle (cells gradually lose their nucleus and become filled with keratin, a hard protein). **The melanin is in the cortex.**



PIGMENTATION STARTS IN THE BULB AT THE SAME TIME AS HAIR GROWS

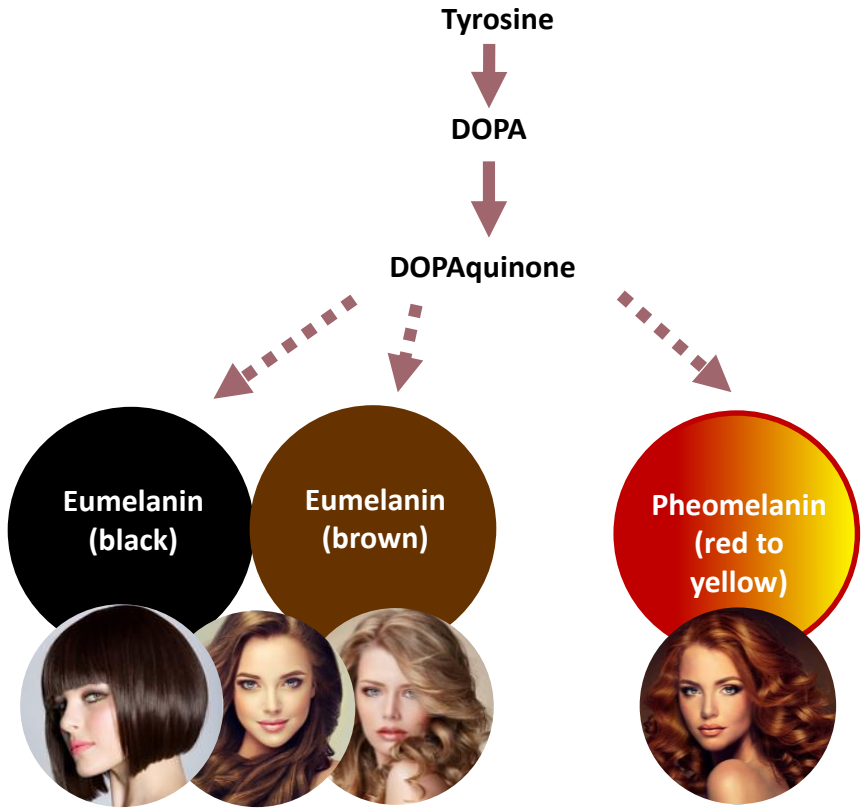
Why hair varies from Blonde to Black?

Hair shaft



transverse section of hair shaft

- The natural color of hair is due the presence of melanin all along the shaft in the middle layer (cortex)
- Color shade depends on the variable amounts and distribution of 2 types of melanin (genetically programmed): **eumelanin** (black-brown pigments) vs. **pheomelanin** (red-yellow pigments)



Variation of eumelanin content

>90% global population is dark hair

WHY DOES HAIR TURN GREY?

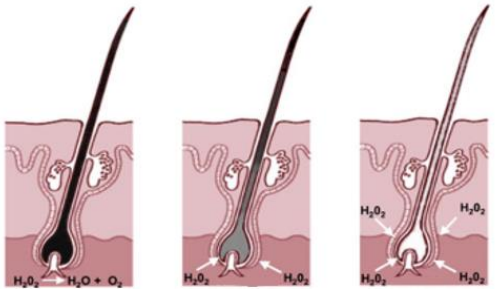
Loss of melanin

1

Decrease in the melanogenesis process

2

Increase in the oxidative stress in the hair bulb



Excess H_2O_2

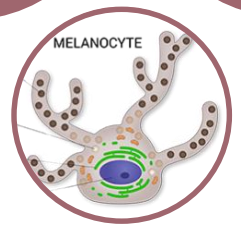
Decrease in melanocyte number

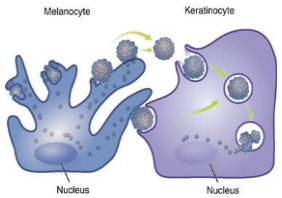
Decrease in melanin production in each melanocyte

Decrease in melanin transfer (melanocytes to keratinocytes)

Decrease in catalase expression & activity

Decrease in TRP-2

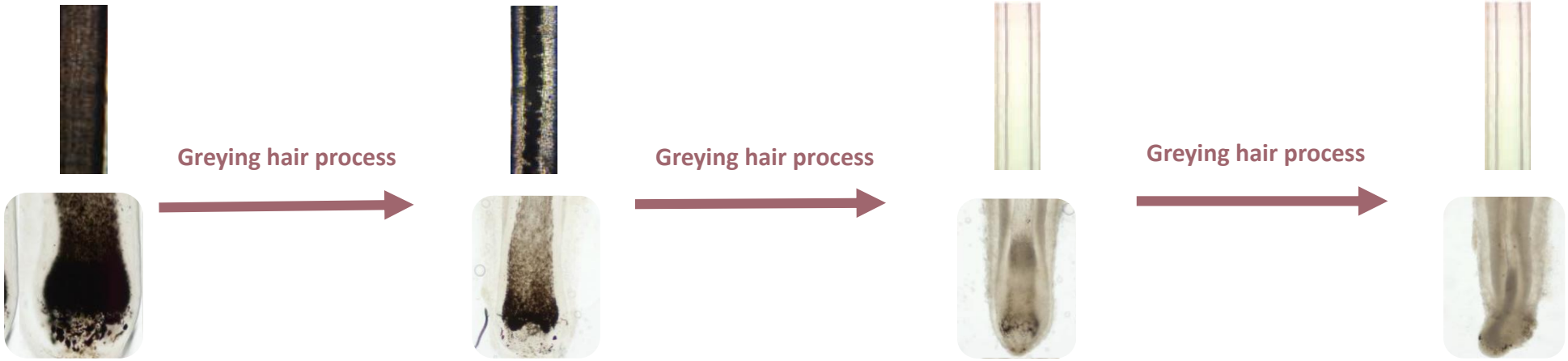




$$H_2O_2 \xrightarrow[\text{Catalase}]{} H_2O + O_2$$

Greying hair process = Gradual loss of melanin

1+2



Greying hair process → Greying hair process → Greying hair process →

Hair with normal pigment content

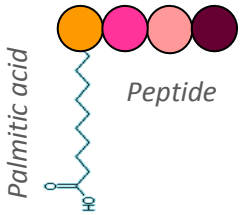
Grey hair with low pigment content
Quantity melanin has decreased

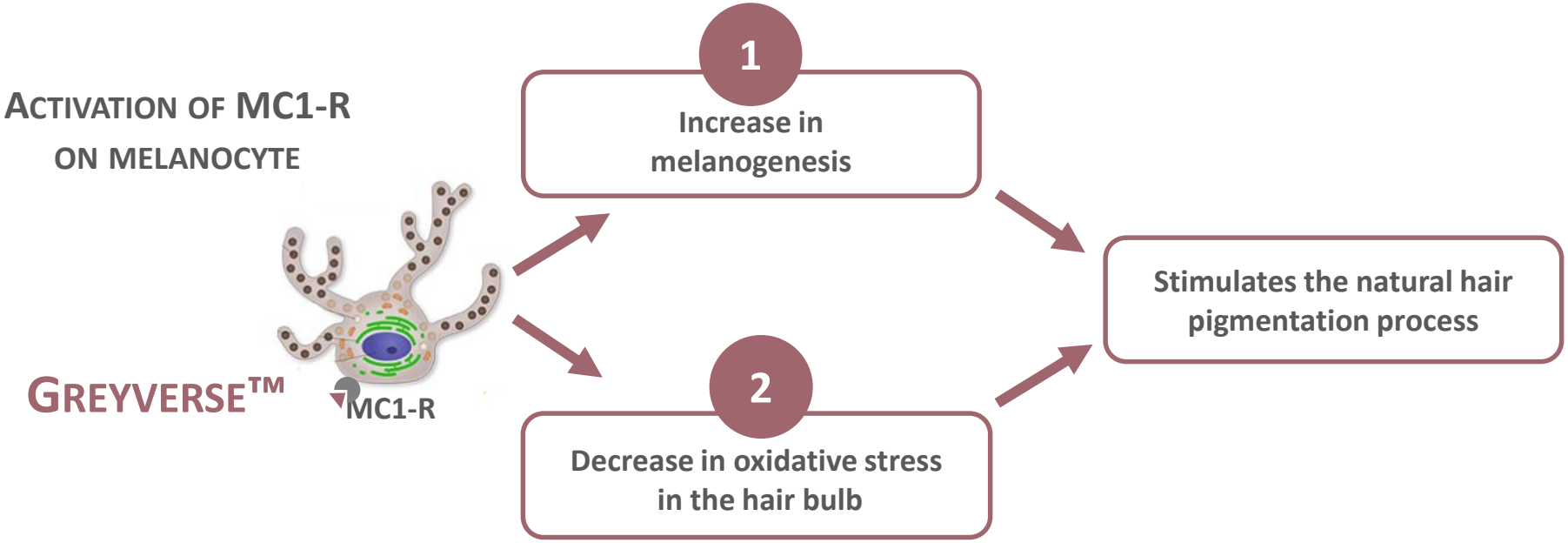
White hair with very low pigment content
Still contains some melanocytes and melanin

White hair with no pigment content
Contains no melanocyte and no melanin

Patented Biomimetic Peptide

- Active sequence of α-MSH (α-Melanocyte Stimulating Hormone) => binding to MC1-R (MelanoCortin 1 Receptor)
- Palmitoyl Tetrapeptide: 4 amino acids grafted on palmitic acid (RSPO certified)





Hair pimentation vs. Skin pigmentation

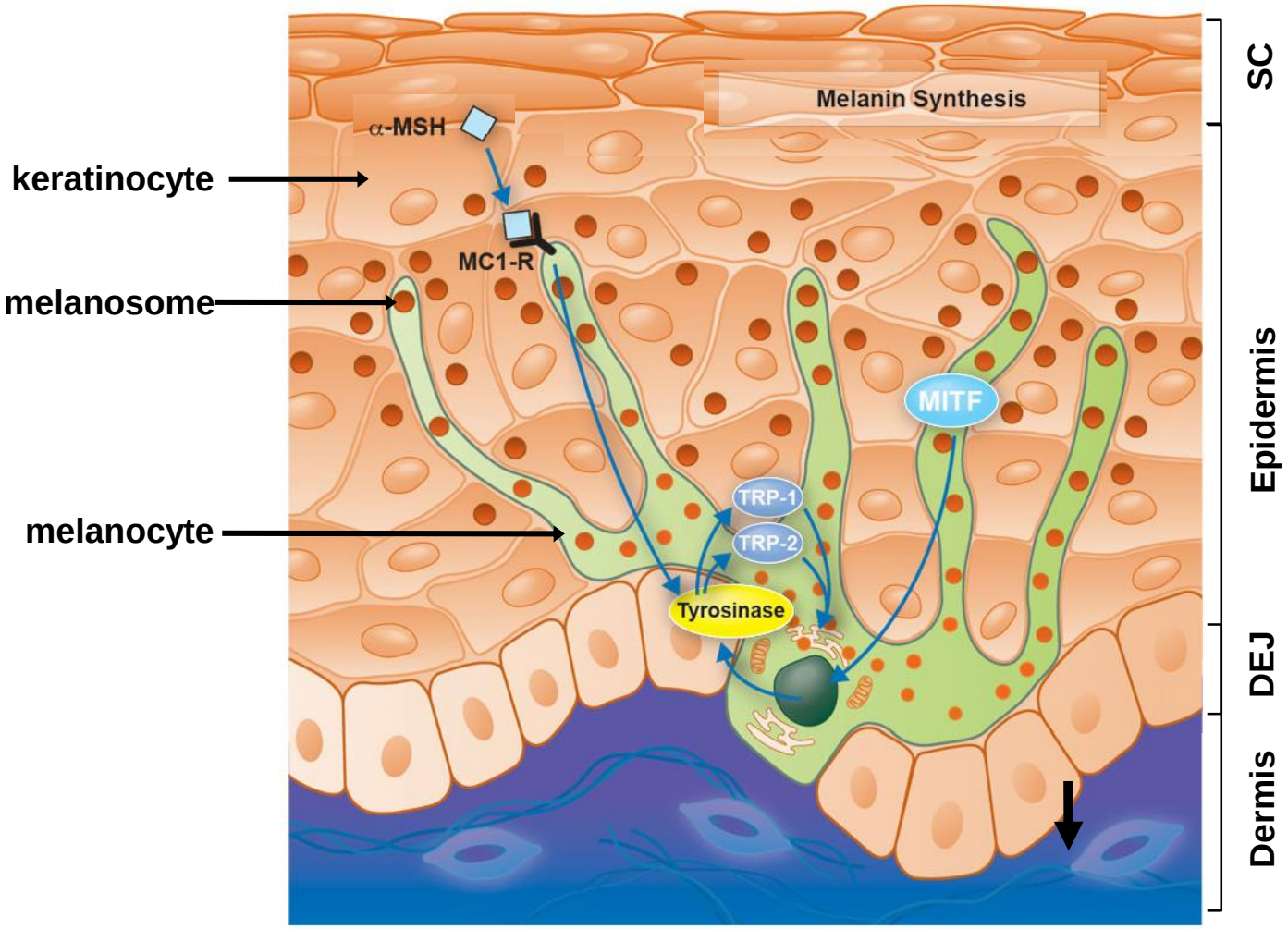
- As in skin, hair melanin protects the fiber against sun-induced free radical damages.

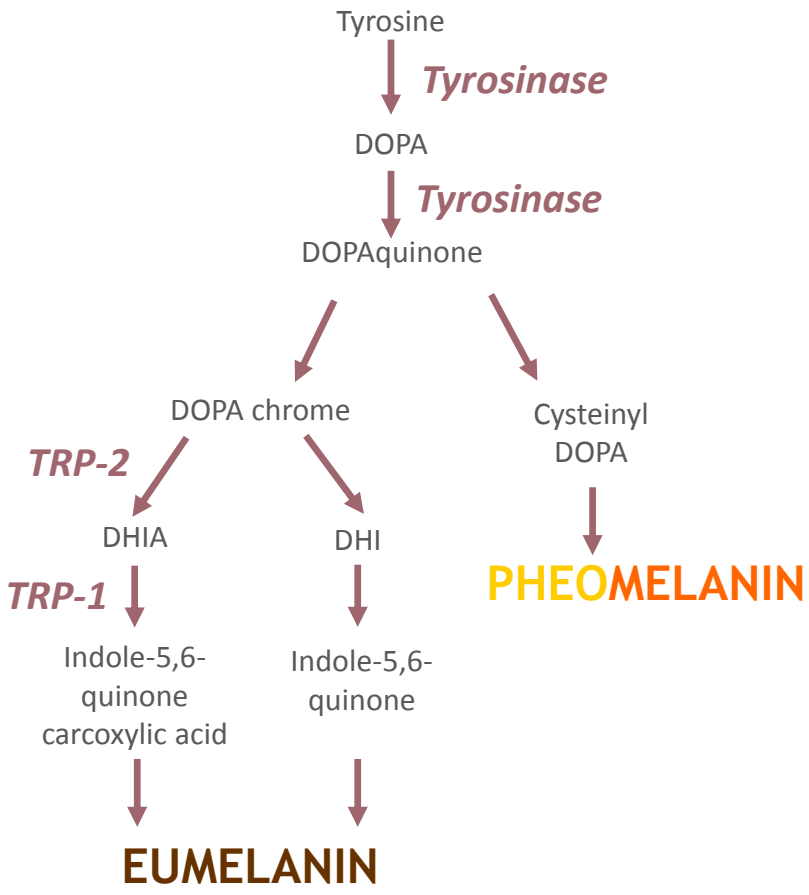
-

	Constitutive pigmentation (genetic)	Facultative pigmentation (sun exposure)	Loss of color with aging
Skin	✓	✓	✗
Hair	✓	✗	✓

Greyverse™ can't darken the natural hair color. It's going to act only on grey hair to recover the genetically programmed hair color.

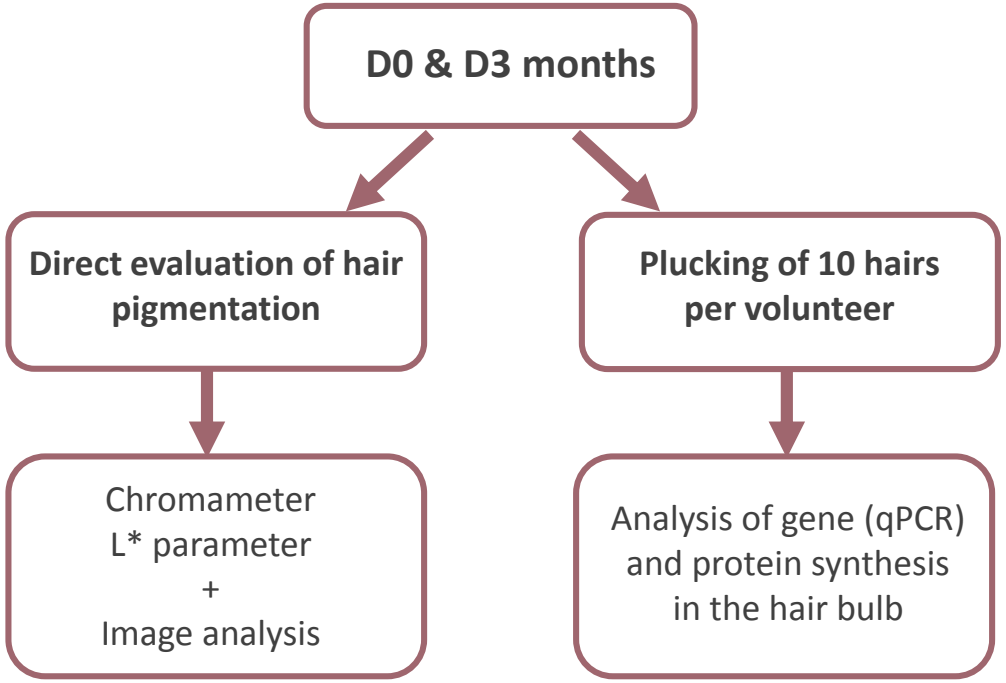
MELANIN SYNTHESIS





MITF = Microphtalmia-associated transcriptor factor
TRP = Tyrosine Related Protein

CLINICAL STUDY



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**

Evolution of Hair Pigmentation

***In vivo* protocol**

- 15 Caucasian men volunteers suffering from early canities (18-35 years old, grey hair > 20%)
- Application 1X/D for 3 months of a lotion containing 2% of **Greyverse™** on the total surface of the scalp by a gently massage
- Evaluation of hair pigmentation by **chromametry** (L* parameter = Luminescence)

Day 0

3 months

Hair whiteness

-5.3% L*

Up to -32% L*

Greyverse™ visibly reverses greying process and improves hair pigmentation

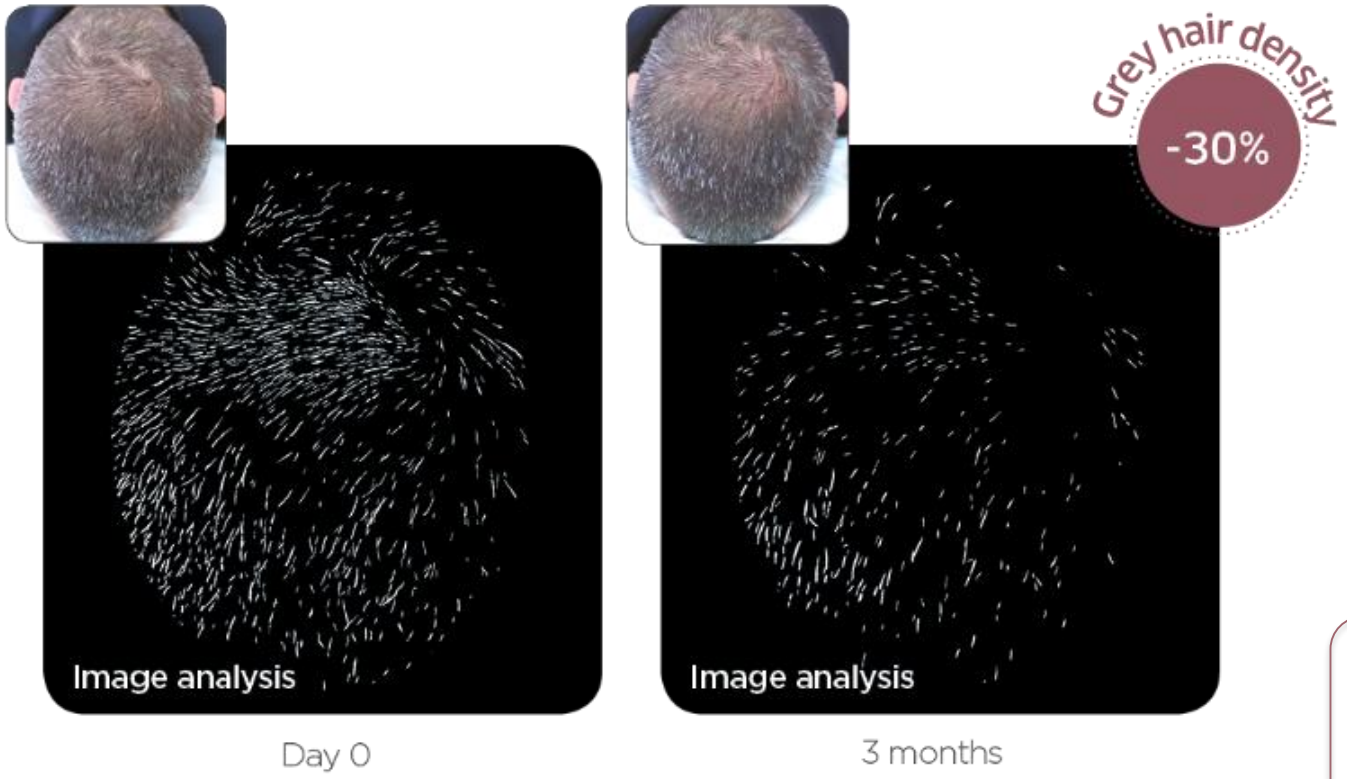
100% positive response

($p \leq 0.1$)

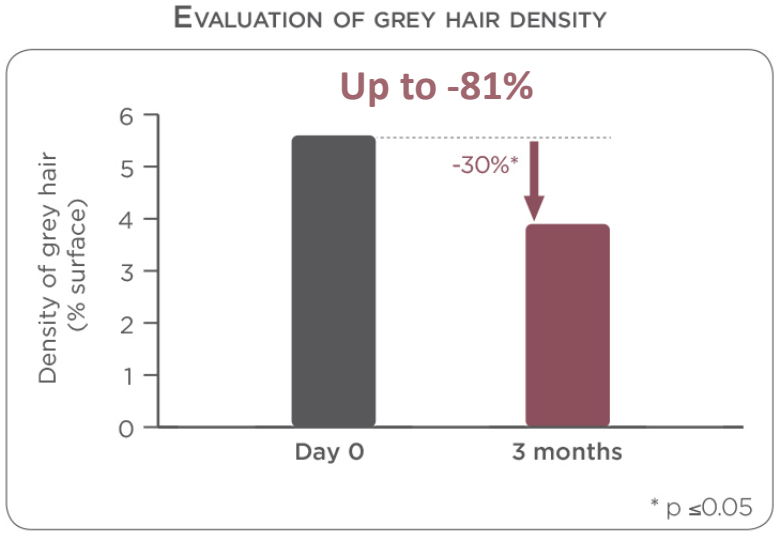
Evolution of Hair Pigmentation

***In vivo* protocol**

- 15 Caucasian men volunteers suffering from early canities (18-35 years old, grey hair > 20%)
- Application 1X/D for 3 months of a lotion containing 2% of **Greyverse™** on the total surface of the scalp by a gently massage
- Evaluation of hair pigmentation by **image analysis**



100% positive response



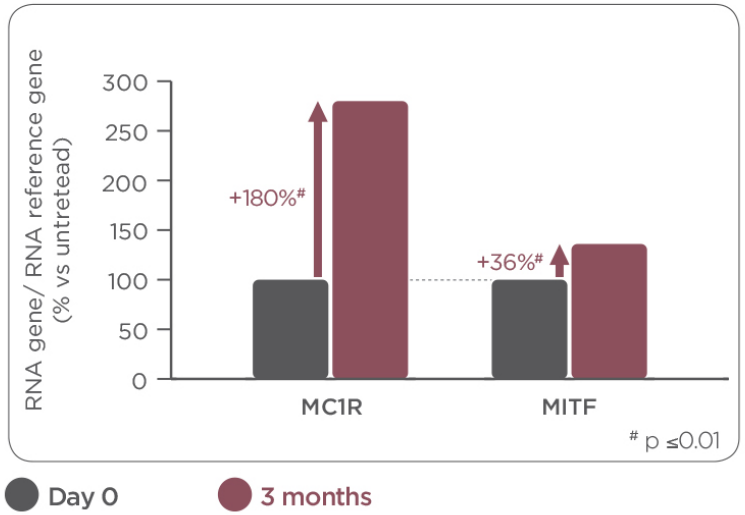
Greyverse™ visibly reverses greying process and improves hair pigmentation

ANALYSIS OF GENE EXPRESSION AND RELATED PROTEIN SYNTHESIS

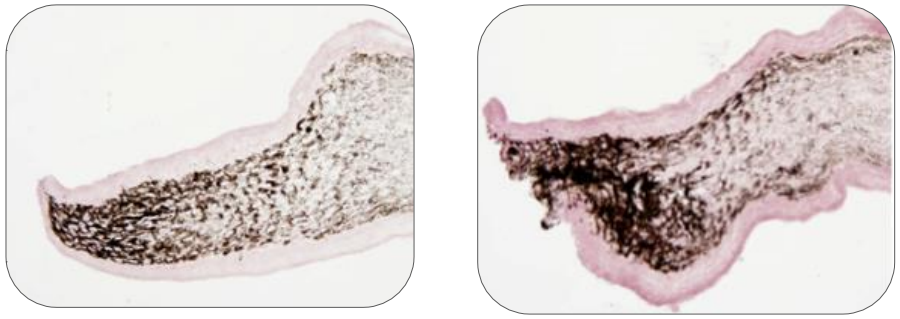
In vivo protocol

- 15 Caucasian men volunteers suffering from early canities (18-35 years old - grey hair > 20%)
- Application 1X/D for 3 months of a lotion containing 2% of **Greyverse™** on the total surface of the scalp by gently massage
- Plucking of 10 hairs per volunteer to extract ARN
- Evaluation of **gene expression** (qPCR) and observation of corresponding **synthetized proteins**

EVALUATION OF GENE EXPRESSION OF PIGMENTATION MARKERS



MC1-R PROTEIN SYNTHESIS IN THE HAIR BULB



Day0 3 months

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

In vitro & *ex vivo* studies

Melanogenesis increase

1

Increase in melanogenesis

Stimulation of melanin synthesis

- Melanin synthesis
- TRP-1

Stimulation of melanin transfer

- Melanosome maturation (Melan-A)
- Melanosome transfer

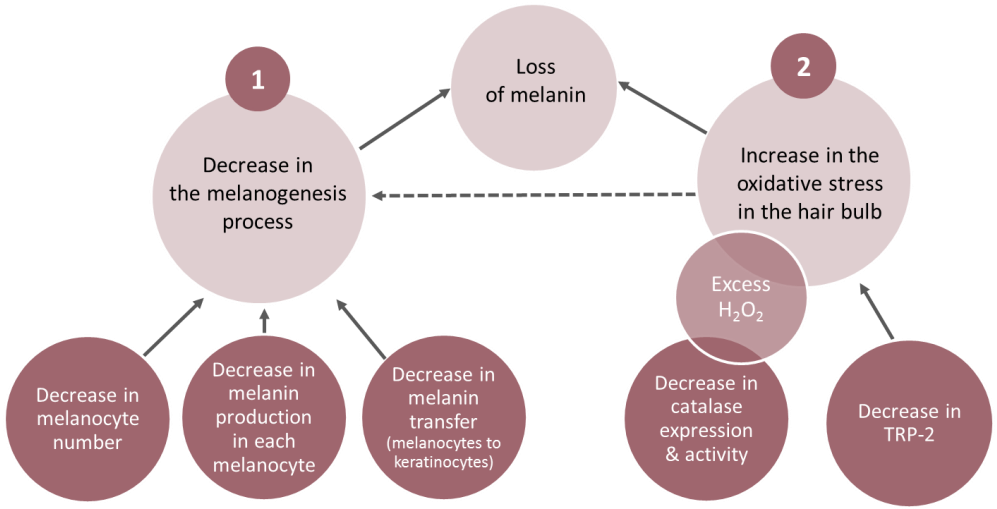
Evaluation of macroscopic hair pigmentation

Oxidative stress

2

Decrease in oxidative stress

- Catalase expression & activity
- H_2O_2 accumulation
- TRP-2



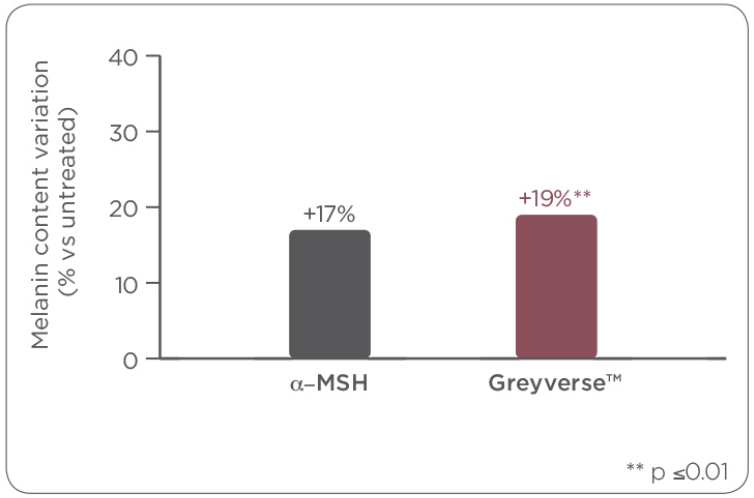
EVALUATION OF MELANIN SYNTHESIS

Melanogenesis increase 1

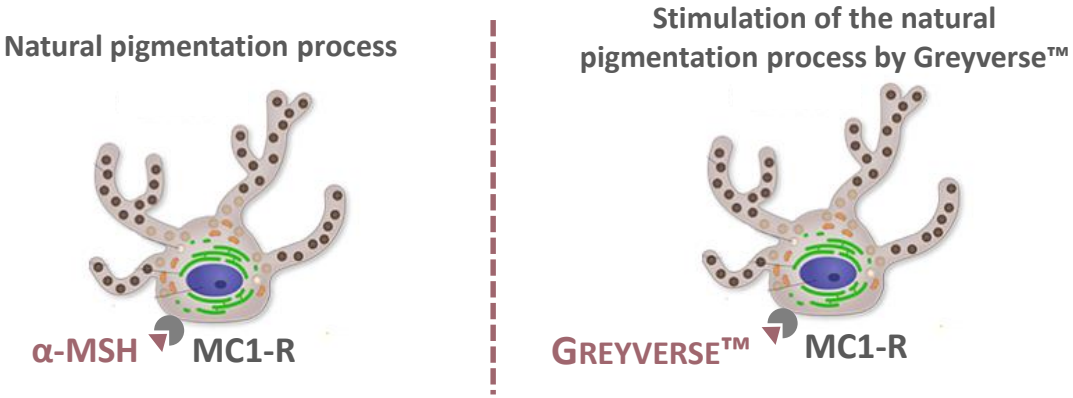
***In vitro* protocol**

- Human melanocyte culture
- Application of pure peptide 10^{-7} M (eq. **Greyverse™**0.5%) or 10^{-6} M α-MSH (positive control) during 72 hours
- Quantification of melanin by absorbance (405 nm)

EVALUATION OF MELANIN SYNTHESIS



ACTIVATION OF MC1-R ON MELANOCYTE



Greyverse™ stimulates the synthesis of melanin to improve grey hair repigmentation

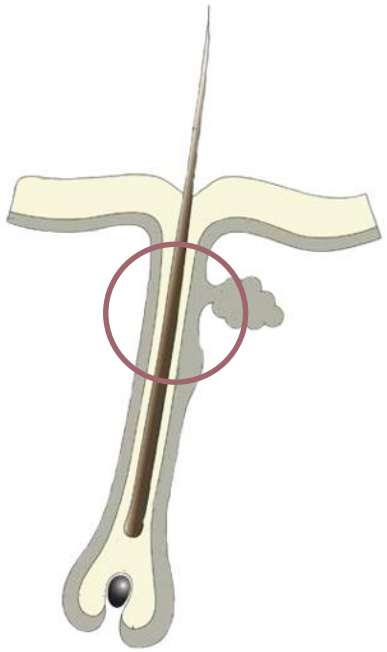
EFFECT ON TRP-1

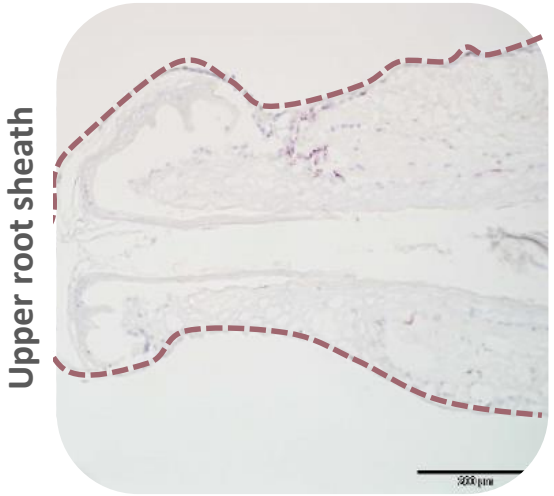
Melanogenesis increase 1

***Ex vivo* protocol**

- Isolated human white hair follicles kept alive during 7 days (Philpott method)
- Application of pure peptide 10^{-9} M and 10^{-7} M (eq. **Greyverse™** 0.005% and 0.5%) at D0
- Immunolabelling of **TRP-1** at D7 after hair microdissection

Tyrosine → (*Tyrosinase*) DOPA → (*Tyrosinase*) DOPAquinone ⇢ (*TRP-1*) Eumelanin





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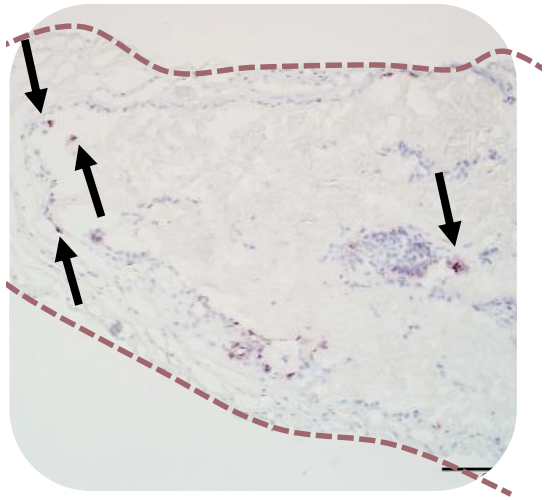
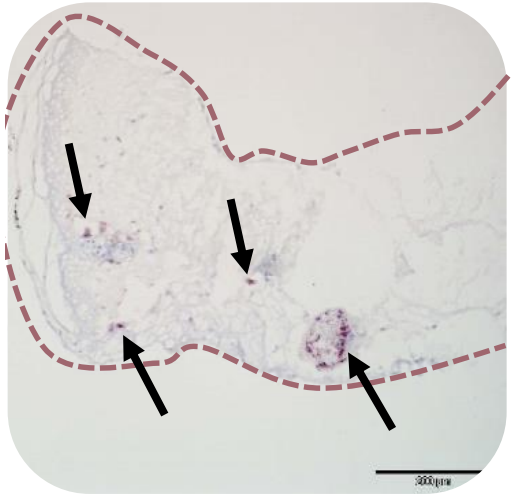


Greyverse™
0.005%



Greyverse™
0.5%

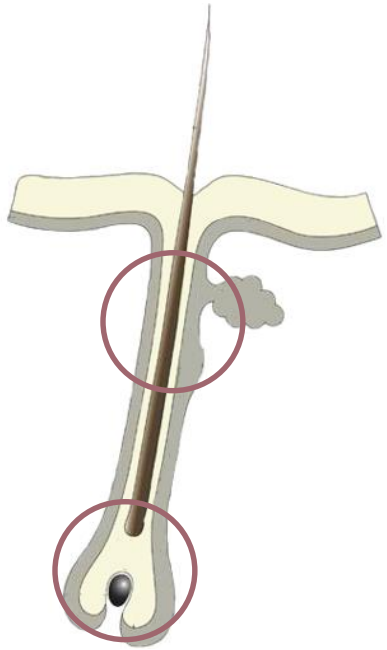
Greyverse™ improves TRP-1 synthesis to stimulate melanin synthesis

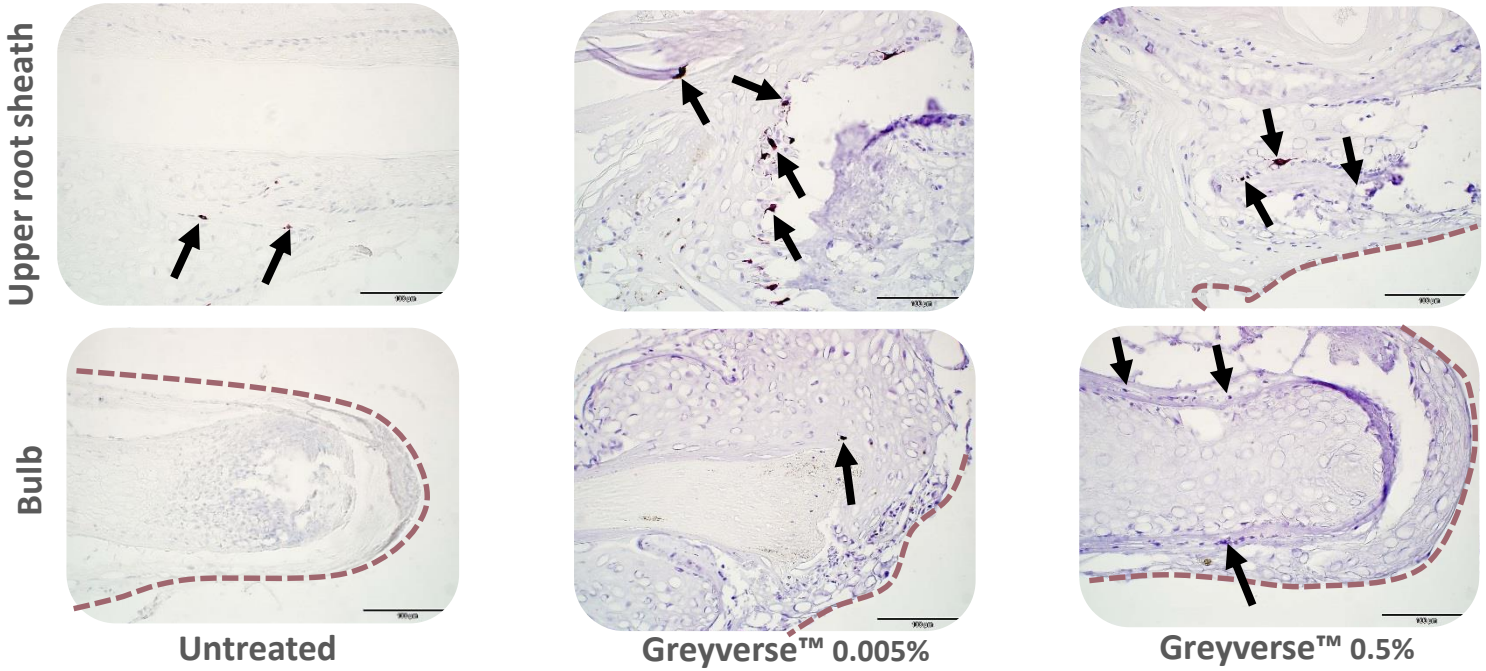
EVALUATION OF MELANOSOME MATURATION

Melanogenesis increase 1

***Ex vivo* protocol**

- Isolated human white hair follicles kept alive during 7 days (Philpott method)
- Application of pure peptide 10^{-9} M and 10^{-7} M (eq. **Greyverse™** 0.005% and 0.5%) at D0
- Immunolabelling of **Melan-A** (pink/violet) at D7 after hair microdissection





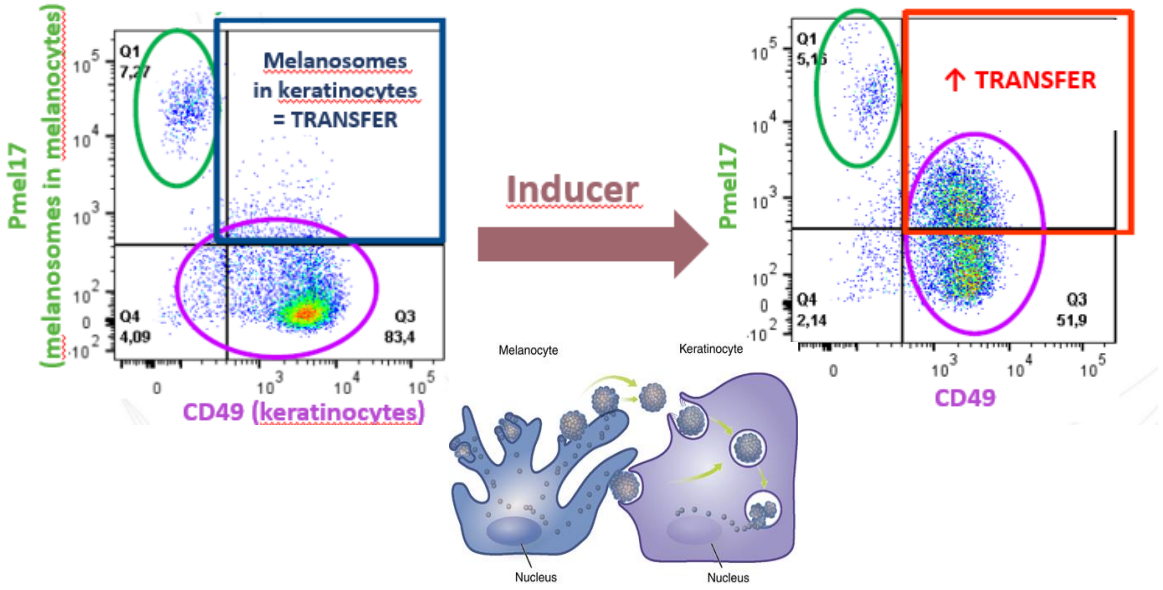
Greyverse™ increases Melan-A markers attesting to a better maturation of melanosome necessary for an optimal transfer

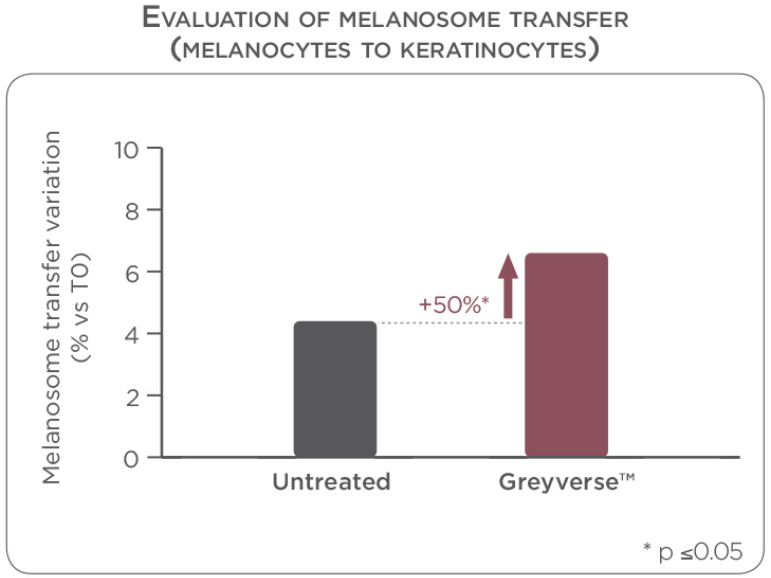
EVALUATION OF MELANOSOME TRANSFER

Melanogenesis increase 1

In vitro protocol

- Co-culture human melanocytes + keratinocytes with pure peptide 10^{-9} M (eq. **Greyverse™** 0.005%)
- Labelling of melanosome marker (**Pmel17**) & keratinocyte marker (**CD49**)
- Quantification of **melanosome transfer** (= quantity of Pmel17 in keratinocytes) by flow cytometry





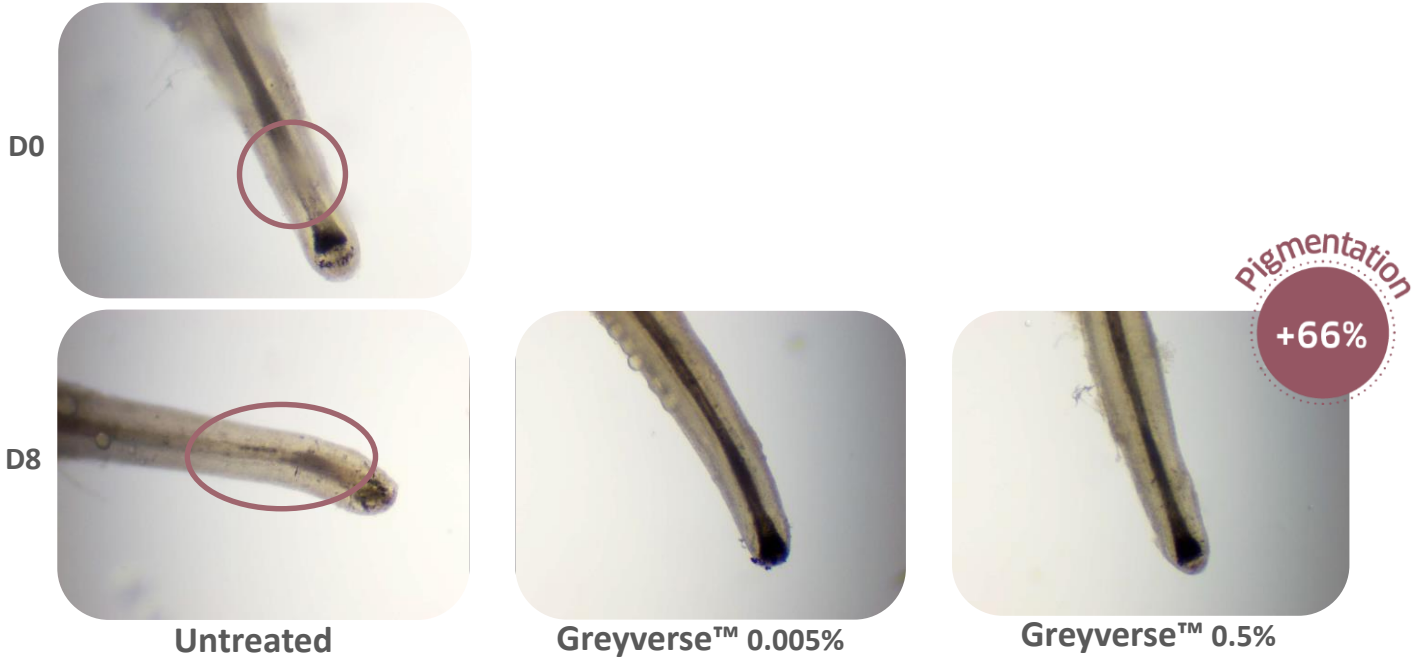
Greyverse™ stimulates melanosome transfer thus increasing melanin content in hair shaft and leading to natural hair pigmentation recovery.

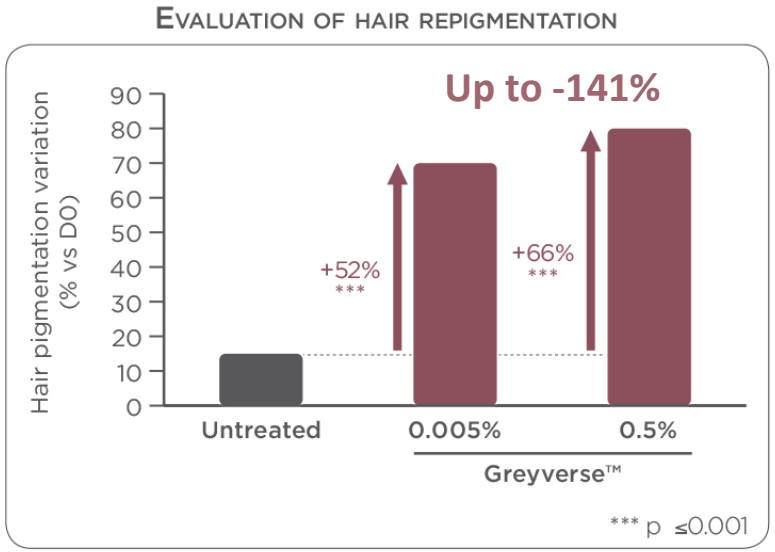
EVALUATION OF MACROSCOPIC HAIR PIGMENTATION

Melanogenesis increase 1

***Ex vivo* protocol**

- Isolated human white hair follicles kept alive during 7 days (Philpott method)
- Application of pure peptide 10^{-9} M and 10^{-7} M (eq. **Greyverse™** 0.005% and 0.5%) at D0
- Evaluation of the hair follicle **pigmentation** by microscopic analysis at D8





Greyverse™ visibly improves hair pigmentation

Greying hair process - Oxidative Stress

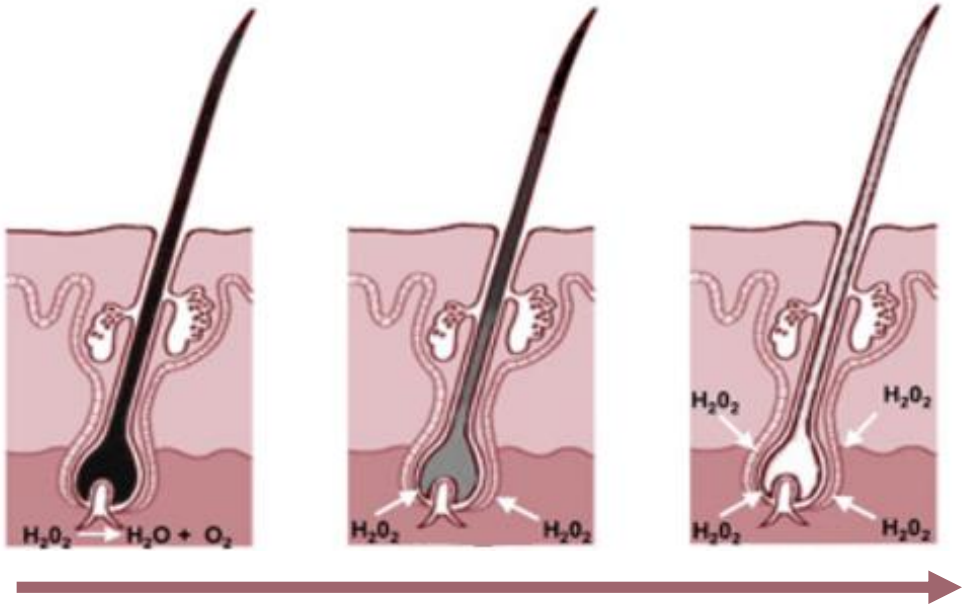
Oxidative stress 2

- Bulb 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 harmless elements of water and oxygen
- With aging, catalase content and activity decrease leading to an accumulation of H_2O_2 which bleaches melanin => grey hair

Hair with normal pigmentation

Normal catalase activity

$$H_2O_2 \xrightarrow[\text{Catalase}]{} H_2O + O_2$$



Increase in H_2O_2 = Increase in oxidative stress

Grey hair

Low catalase activity

$$\uparrow H_2O_2 \xrightarrow[\downarrow\text{Catalase}]{} \downarrow H_2O + O_2$$

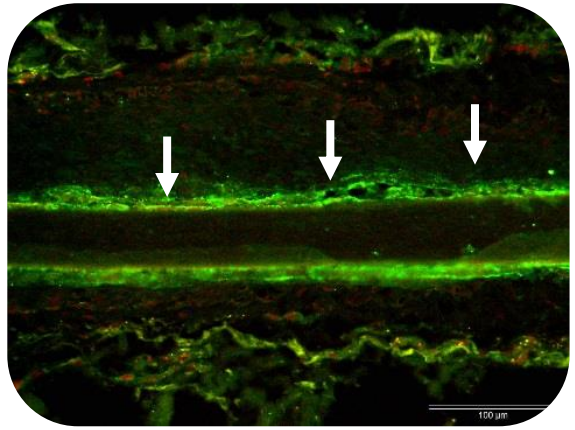
EFFECT ON CATALASE

Oxidative stress 2

Ex vivo protocol (catalase production)

- Isolated human white hair follicles kept alive during 7 days (Philpott method)
- Application of pure peptide 10^{-7} M (eq. **Greyverse™** 0.5%) at D0
- Immunolabelling of **catalase** at D7 (yellow/green) after hair microdissection

Lower root sheath



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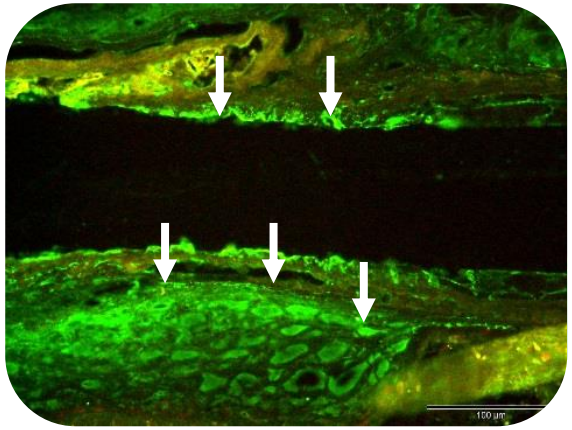
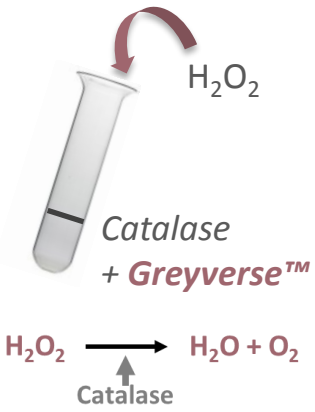


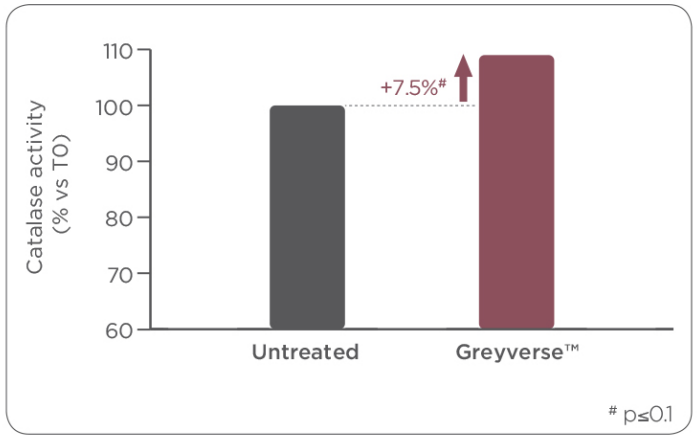
Greyverse™

In tubo protocol (catalase activity)

- **Greyverse™** 10^{-5} M was mixed with catalase
- Addition of hydrogen peroxide (H_2O_2)
- Evaluation of the enzymatic activity of **catalase** by measuring the quantity of H_2O_2 which has not been degraded by the enzyme.



EVALUATION OF CATALASE ENZYMATIC ACTIVITY



Greyverse™ improves catalase production and activity to reduce the greying hair process due to oxidative stress caused by excess H_20_2

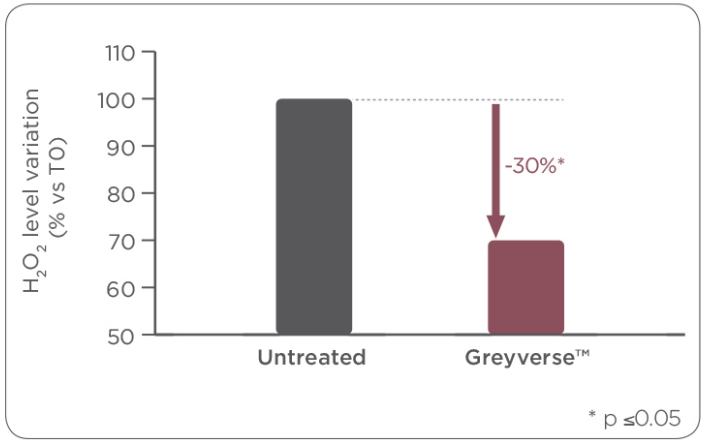
EFFECT ON H_2O_2 ACCUMULATION

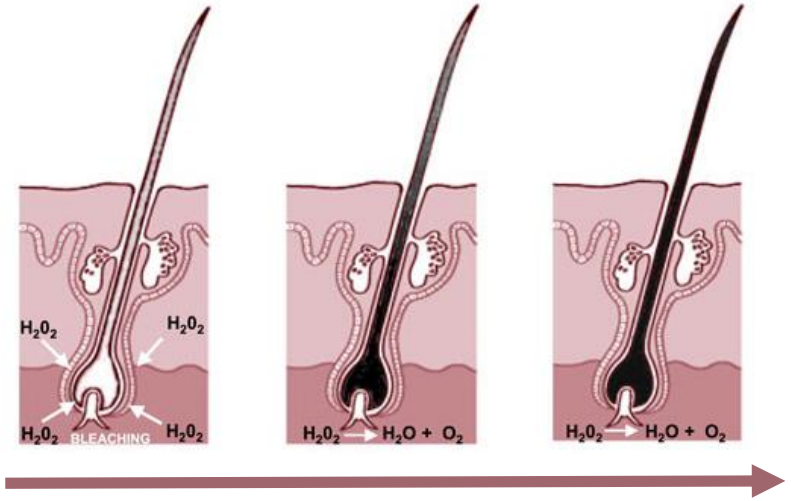
Oxidative stress 2

***In vitro* protocol**

- Human culture of fibroblasts from dermal papilla
- Addition of **Greyverse™** 10^{-5}M
- Evaluation of intracellular of H_2O_2 level using fluorescence

EVALUATION OF H_2O_2 PRODUCTION IN HAIR BULB





Decrease in H_2O_2 = Decrease in oxidative stress

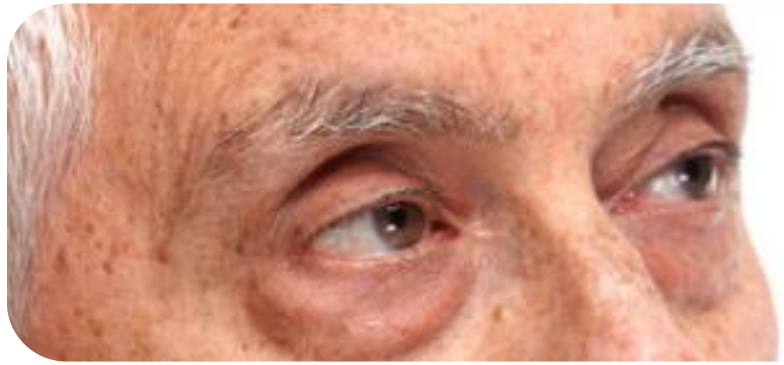
Greyverse™ decreases H_2O_2 accumulation thus decreasing oxidative stress involved in the greying hair process

Greying hair process - Oxidative Stress

Oxidative stress 2

- TRP-2 (Tyrosine Related Protein-2) initially known as an enzyme involved in the synthesis of melanin is also an important antioxidant enzyme which protects and defends melanocytes against reactive oxygen species.

- The decrease in TRP-2 intensifies the loss of melanocytes as they become more sensitive to free radicals aggressions



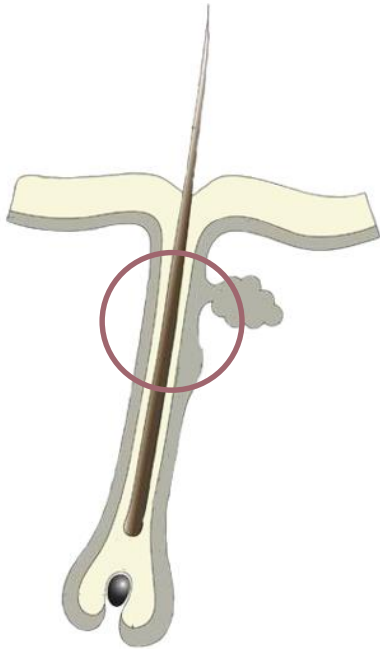
Eyelashes don't turn grey because TRP-2 don't decrease over time

EFFECT ON TRP-2

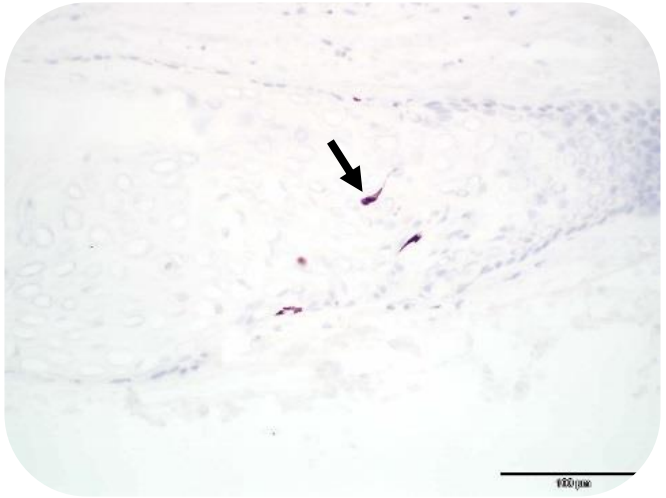
Oxidative stress 2

***Ex vivo* protocol**

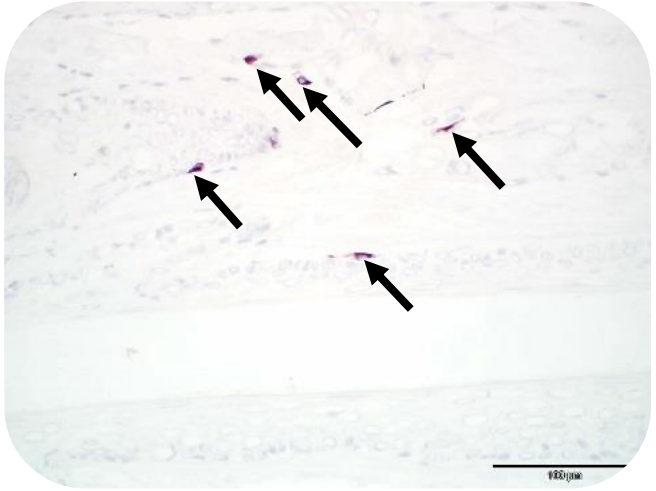
- Isolated human white hair follicles kept alive during 7 days (Philpott method)
- Application of pure peptide 10^{-9} M and 10^{-7} M (eq. **Greyverse™** 0.005% and 0.5%) at D0
- Immunolabeling of **TRP-2** at D7 after hair microdissection



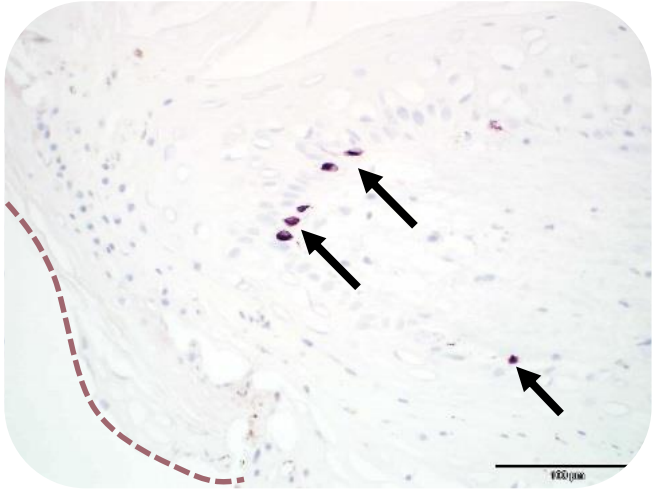
Upper root sheath



Untreated



Greyverse™
0.005%

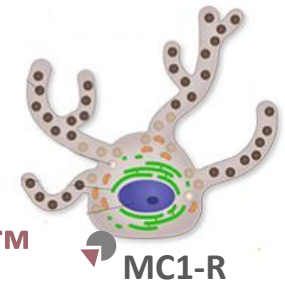


Greyverse™
0.5%

Greyverse™ increases TRP-2 thus reducing oxidative stress in the bulb and protecting melanin synthesis

GREYVERSE™ - MECHANISM OF ACTION

ACTIVATION OF MC1-R ON MELANOCYTE



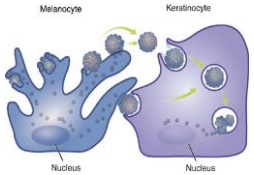
GREYVERSE™

1

Increase in melanogenesis

- Stimulation of melanin synthesis (↑TRP-1)

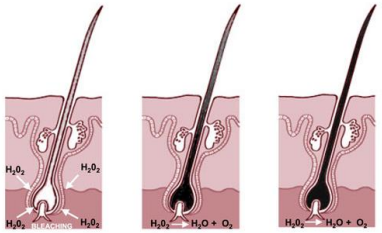
- Stimulation of melanosome maturation (↑Melan-A)
- Stimulation of melanin transfer



2

Decrease in oxidative stress in the hair bulb

- Reduces H_2O_2 accumulation (↑ catalase content and activity)
- Increase in TRP-2



Greyverse™ restores natural hair color

GREYVERSE™ PROJECT HISTORY - STARTED IN 2011!

IFF | LUCASMEYER COSMETICS

800,000€ investment

COSMETIC VALLEY FRANCE

Centre de Santé Sabouraud www.centresabouraud.fr

Medical center specialist in hair (research and treatment)
Biopsies on volunteers

Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

Public lab with a hair study department
Genic expression

ICSN CNRS

Public lab with a hair study department
Biologic activity screening

ALES GROUPE

French brand
Formulation for the clinical study

Laboratoire BIO-EC

Testing lab
Evaluation of the *ex vivo* and clinical efficacies

Steps

1- Identification of the genes involved in canities process (analysis of the genomic expression)

2- screening of several peptides for their capacity to modulate melanogenesis and oxidative stress

3- Development of active ingredients to treat and prevent greying hair

=> Greyverse™!

GREYVERSE™ - 1ST ANTI-GREY HAIR BIOMIMETIC PEPTIDE

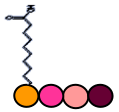
Innovative & clinically efficacious ingredient that helps men & women suffering from greying hair to look naturally younger by reversing hair aging and increasing their self-esteem.

STOPS AND REVERSES THE GREYING HAIR PROCESS

- Promotes pigmentation process
  - ↑ melanin synthesis
  - ↑ melanosome transfer
- Reduces oxidative stress

ORIGIN

- Patented α-MSH biomimetic tetrapeptide linked to a palmitic chain



CLINICALLY PROVEN

- Outstanding and visible decrease in grey hair density
- Modulates gene expression and related proteins synthesis

CONSUMER BENEFITS

- Fights hair aging
- Prevents the appearance of 1st grey hair
- Helps recover natural hair color

MANUFACTURER BENEFITS

- Easy to formulate
- Opportunity to have a new claim
- Complements dye or hair care product line
- Alternative to chemical dye

PRODUCT INFORMATION

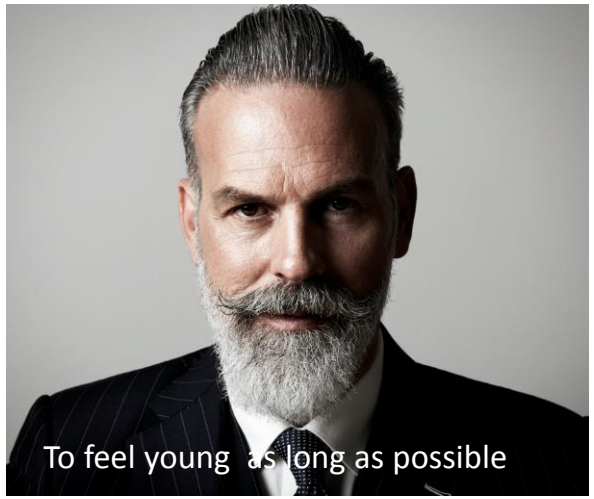
INCI NAME	Glycerin (and) Water (and) Palmitoyl Tetrapeptide-20
ADDITIVE	None
APPEARANCE	Colorless aqueous transparent solution
FORMULATION	Should be incorporated at the end of the formulation at a temperature below 40°C Could be heated if needed (tested at 90°C for 2 hours)
DOSAGE	0.5-1%: preventive hair care 1-2 %: intensive hair care
OPTIMUM PH	4.0-8.0
APPLICATIONS	• Anti-aging hair care • Premature grey hair coverage • Post-coloration treatment • Natural color fortifier • Scalp-friendly alternative to chemical dye • Beard & mustache care

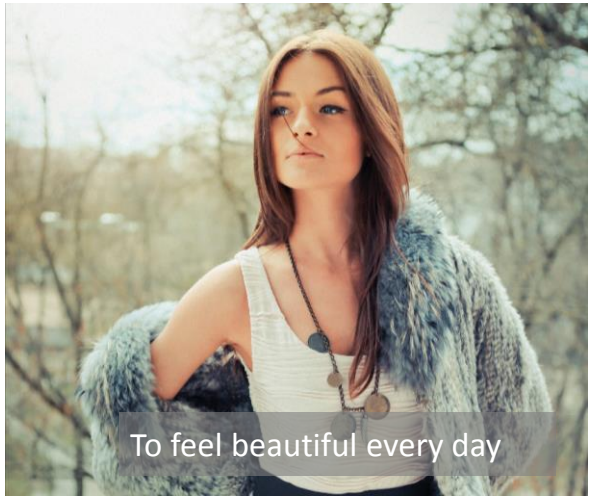
PATENTED

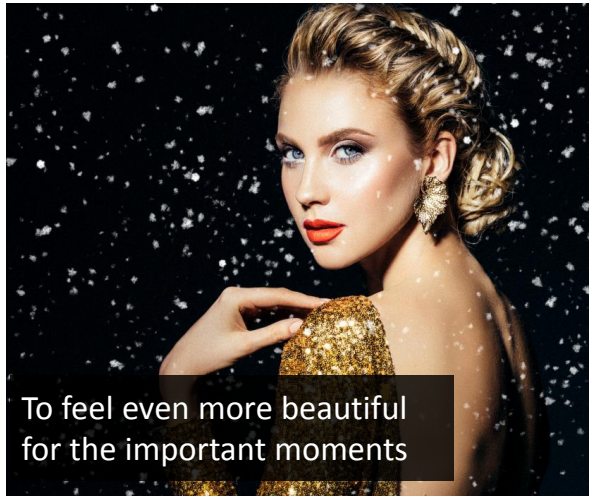
PRESERVATIVE FREE

ECO-FRIENDLY

APPLICATIONS - CLAIMS







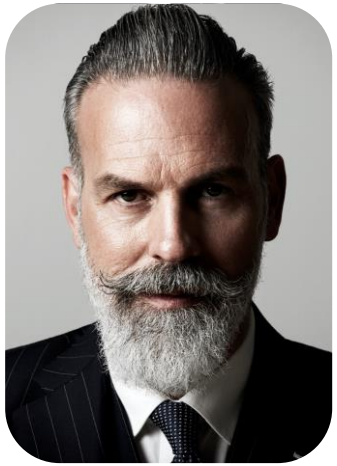
Dosage:
- Intensive treatment: 2%
- Preventive action: 0.5 - 1%

Applications:
- Shampoo
- Conditionners
- Leave-on
- Post-coloration Traitement
- Natural color fortifier
- Anti-aging hair care
- Early grey hair coverage
- Darker hair look
- Scalp-friendly alternative to coloration
- Beard & mustache care

HAIRESISTIBLE SERUM

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



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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

Toxicological Studies

- Eye Irritation (HET-CAM) 10% Greyverse™ tested
- Skin irritation (48-hours single patch-test) 10% Greyverse™ tested
- Acute toxicity (OCDE 423)
- Irritation and Sensitization (HRIPT) 10% Greyverse™ tested
- Genotoxicity (AMES according to OECD 471) 10% Greyverse™ tested
- Phototoxicity
  - -*in vitro* test 3T3 NRU (according to OECD 432) 10% Greyverse™ tested
  - -*in vivo* test (Evaluation of the photosensitizing potential in adult volunteers with normal skin) 10% Greyverse™ tested

Ecotoxicity

- Biodegradability (OECD 301D) pure Greyverse™ tested
- Aquatic toxicity on daphnies (OECD 202) pure Greyverse™ tested
- Freshwater Alga and Cyanobacteria, Growth Inhibition Test (according to OECD 201) pure Greyverse™ tested

Excellent safety profile

Consumers' target vs. concerns

Prevention

Men & Women

They want to maintain their full young and attractive look

First grey hair

Men & Women

They want to delay the first dye by slowing down the greying process and recovering a colorful hair

Users of dye

Men & Women

They will extend time between hair coloration = save time & money...

Consumers who don't use chemical/oxidative dye

Men & Women

"natural" alternative to chemical dye

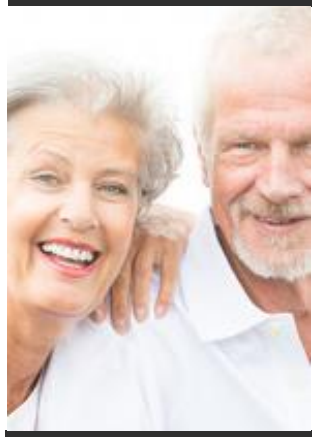
Solution for root coverage

Most of men usually don't use dye because of its non natural result

They are waiting for a product that can recover their natural hair color

Signs of aging

Men & Women



Crossover of skin care into hair care for a anti-aging claim

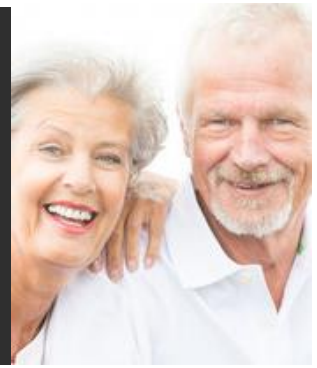
Consumers' target vs. lifestyle profiles

Slow Beauty		No use of chemicals Dyes are very effective to hide grey hair but contains some chemical compounds which can induce some intolerances or side effects
Naturopathy		Stimulate body's natural capacities and defenses "Prevent is better than heal"
#NoMakeup trend		Movement started with Alicia Keys No cover up to show who you really are

Active complex

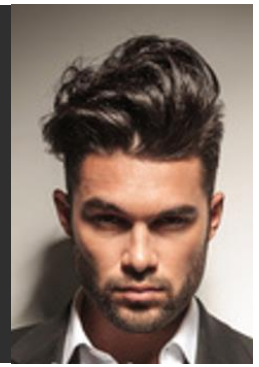
Anti-aging

Greyverse™
+ Capixyl™



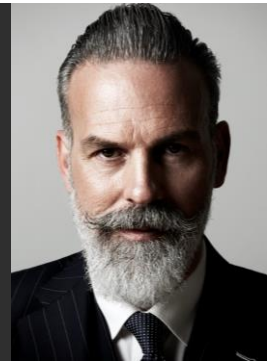
Youth keeper
(grey hair prevention)

Greyverse™ + Riboxyl™



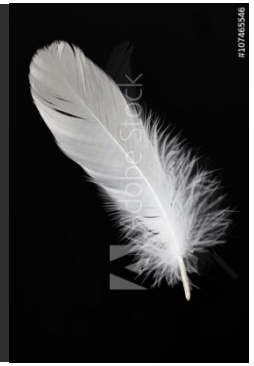
Beard care

Greyverse™ +
Amisol Trio™



Soothing Scalp after dye

Greyverse™
+Tazman Pepper™
+ Defenscalp™

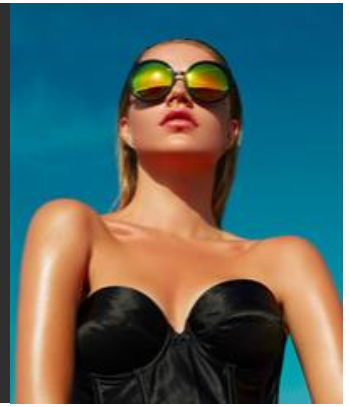


Anti-pollution

Greyverse™
+ Exo-P™

Sun protection

Greyverse™
+ Superox-C™

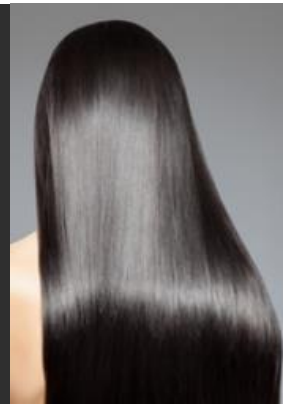


Dye long lasting
Color Intensifier

Greyverse™
+ Sunflohair™

Shiny hair
conditioner

Greyverse™
+ Phytenso™



THANK YOU!