Active Beauty
Redensyl™
The hair growth galvaniser
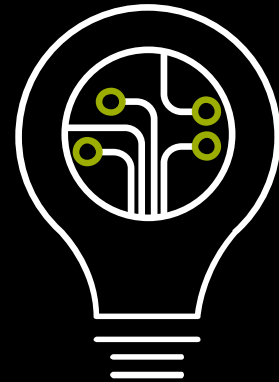
Crafted by green & white technologies

Silver
Redensyl™
in-cosmetics®
Innovation Zone
Best Ingredient
Award 2014

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engage your senses

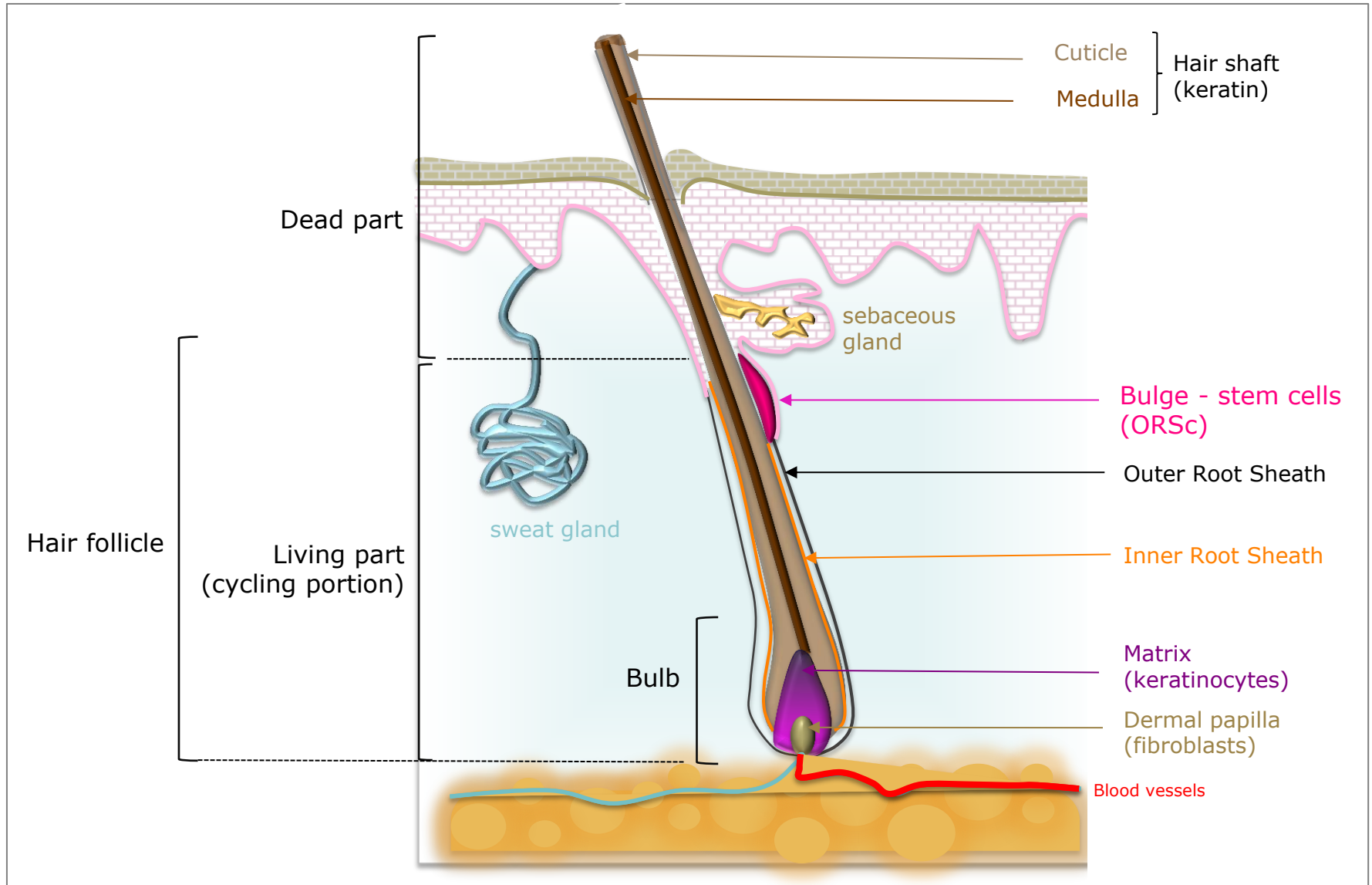# Concept of the product

Hair: continuously self-regenerating

► The full head of hair consists of 110,000 - 150,000 hairs

► The average of scalp surface is 600 cm^2 [1,2]

► Each hair is produced by a single hair follicle

► Each hair follicle produces an average of 30 hairs during our life

► Hair grows at 1 cm per month which corresponds to:
  ► 0.3 to 0.5 mm per day
  ► about 10 cm per year

► We lose naturally from 50 to 100 hair each day.

The hair structure is divided in 2 sections: the hair follicle and the hair shaft

[1,2,3] http://www.hair-science.com

Zoom on the hair structure

Cuticle
Medulla
Hair shaft (keratin)

Dead part

sebaceous gland

Bulge - stem cells (ORSc)

Outer Root Sheath

sweat gland

Inner Root Sheath

Hair follicle

Living part (cycling portion)

Bulb

Matrix (keratinocytes)

Dermal papilla (fibroblasts)

Blood vessels

What are Stem Cells

Stem cells are non differentiated cells

They have two key features:

- Self renewal: they can give birth to other stem cells
- Potency: they can give birth to specialized cell types

Stem cells are divided in two broad categories:

- Embryonic stem cells, which are totipotent: they can create a complete human being
- Adult stem cells, which are multipotent: they can generate an organ

Stem cells are at the origin of our body self regeneration faculties.
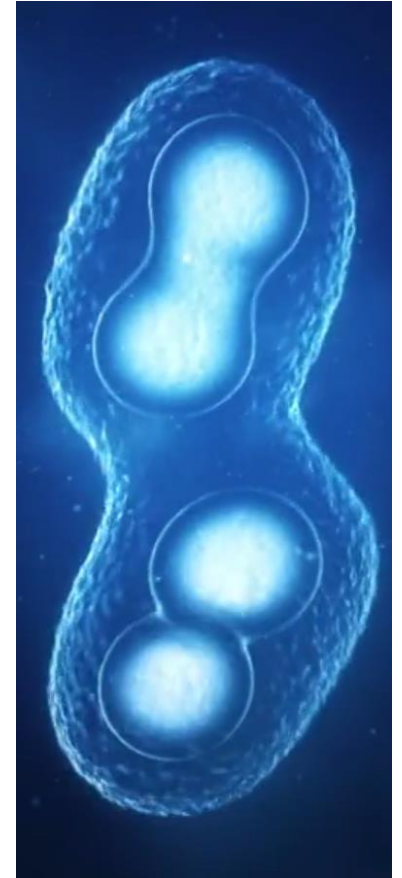
More information on: http://stemcells.nih.gov

Stem cells in regenerative medicine

Stem cells therapy has been a key fundamental research area for more than 30 years.

Concept: introduce new adult stem cells into damaged tissues and organs to regenerate them

Targeted diseases:
- Leukemia (bone marrow)
- Parkinson's and Alzheimer's diseases (neurons)
- Type I diabetes (pancreas)
- Cancers (brain)
- Heart failure (heart)
- Muscles atrophy (muscles)
- Wound healing (skin)
- Baldness (hair) [1]

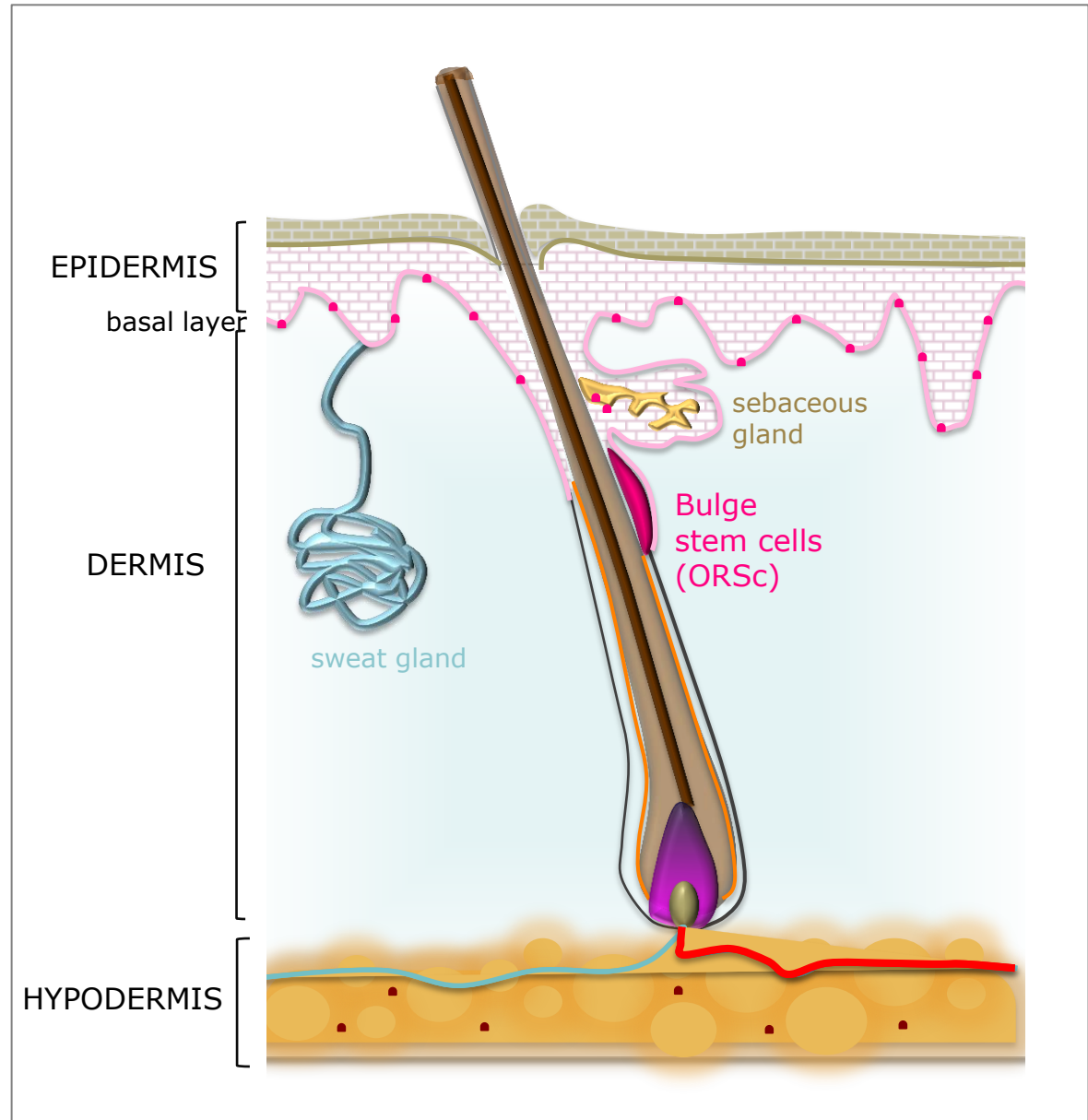**In 2013, 500 clinical trials based on stem cells therapy have been initiated [2]**

[1] Yang et al. Nature Communications, 2014
[2] www.clinicaltrials.gov

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Stem cells in skin

Stem cells are mainly found in

- The hypodermis
- The basal layer
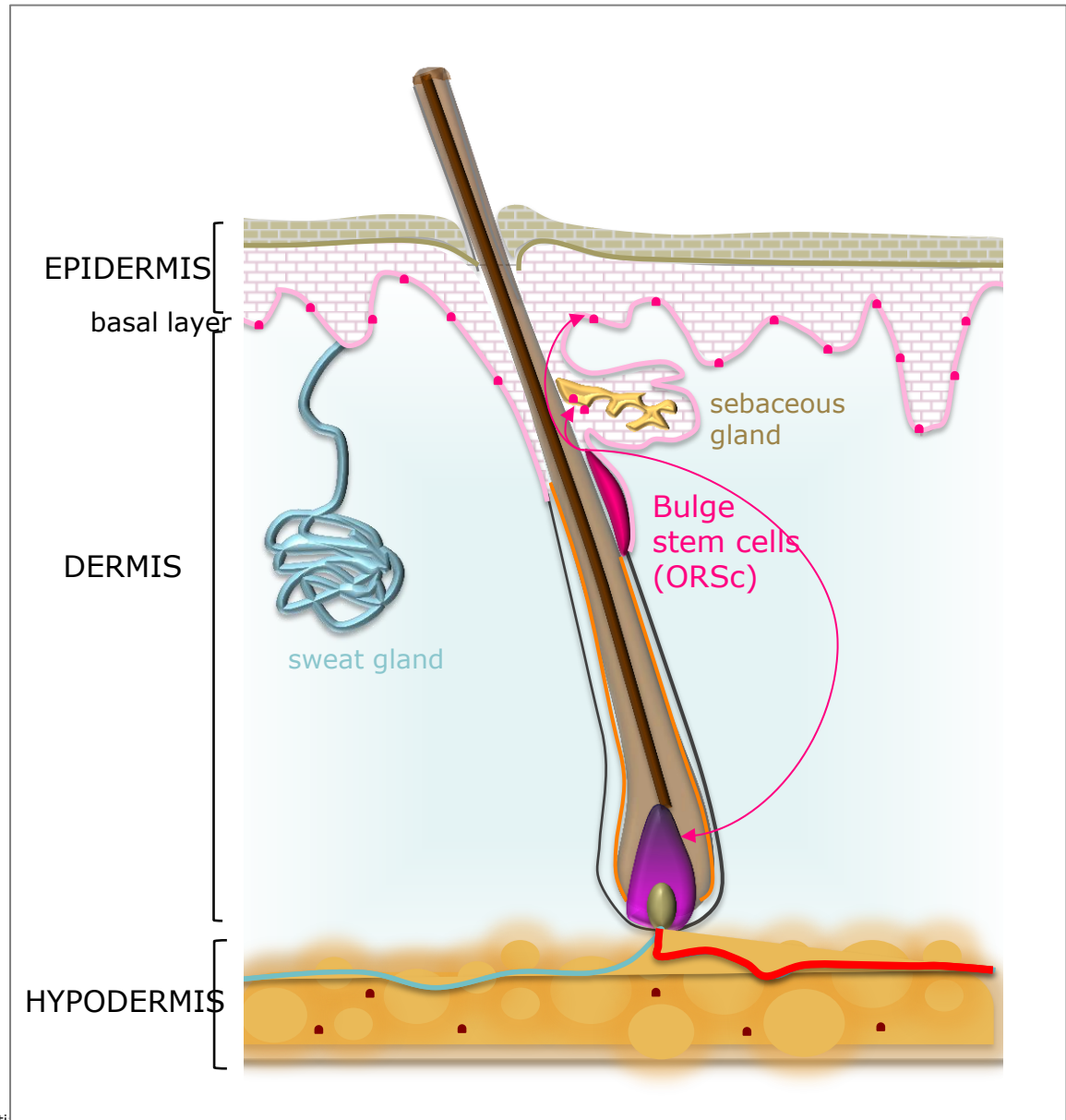- The sebaceous gland
- The bulge (ORSc stem cells)

Diaz-Flores, 2006, Histol Histopathol.

EPIDERMIS

basal layer

sebaceous gland

Bulge stem cells (ORSc)

DERMIS

sweat gland

HYPODERMIS

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Bulge stem cells (ORSc)

Bulge stem cells are mother cells, generating:

- the epidermis cells,
- the hair follicle matrix,
- the sebaceous gland stem cells.

Diaz-Flores, 2006, Histol Histopathol.

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EPIDERMIS

basal layer

sebaceous gland

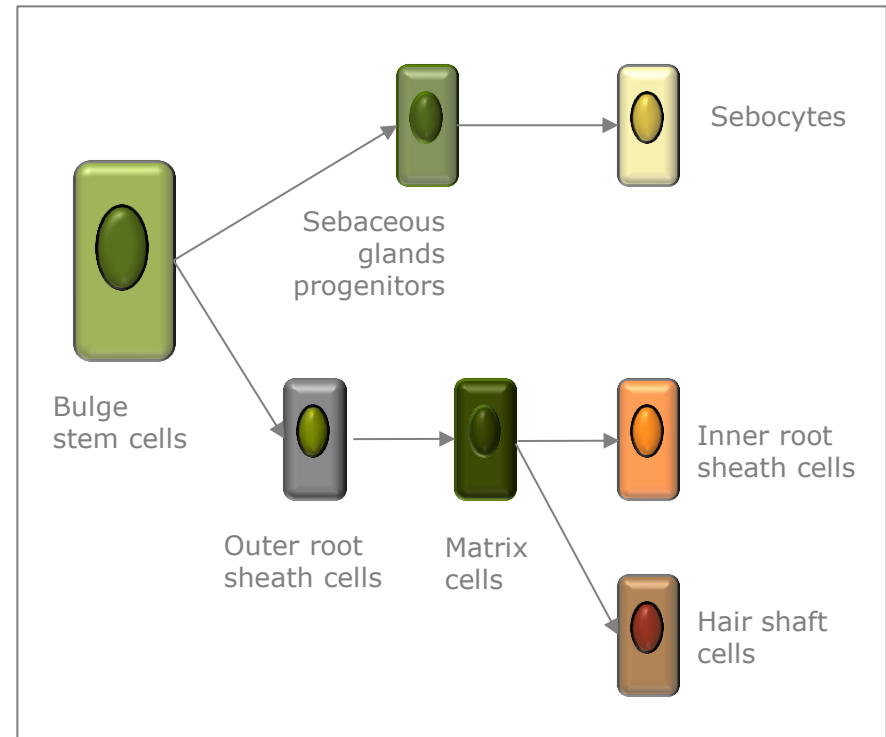Bulge stem cells (ORSc)

DERMIS

sweat gland

HYPODERMIS

Bulge stem cells (ORSc)

Bulge stem cells are expressing the keratin 15 marker.

They have a key role in the regeneration of the hair follicle.

Bulge stem cells are initiating the hair cycle.

THE HAIR CYCLE IS DIVIDED IN 3 STEPS

Sebocytes

Sebaceous glands progenitors

Bulge stem cells

Outer root sheath cells

Matrix cells

Inner root sheath cells

Hair shaft cells

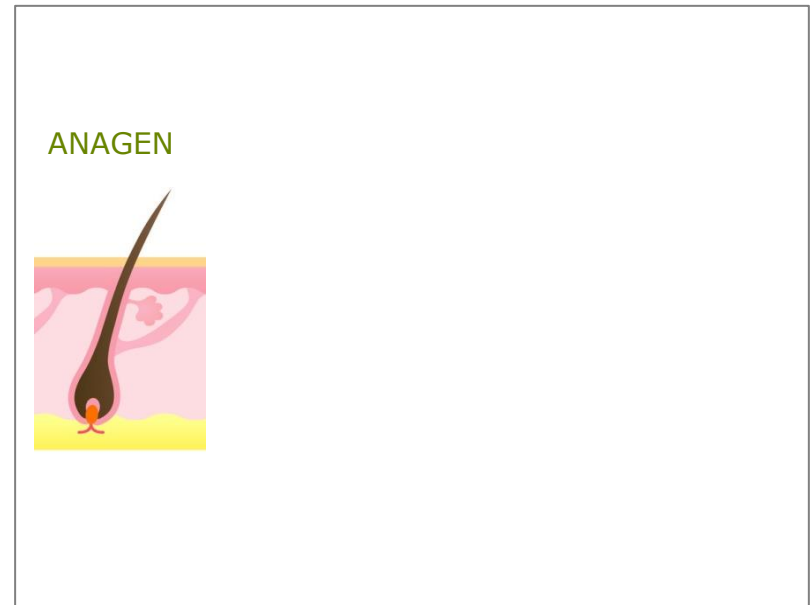Fuchs et al., 2008, Journal of Cell Biology

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Step 1: ANAGEN - Growing phase

Anagen phase is the active phase of the hair (80% to 90% of all hair).

Keratinocytes in the matrix at the root of the hair are dividing rapidly.

During this phase the hair grow about 1 cm every month.

Scalp hair stays in this active phase of growth for 2-6 years.

ANAGEN

Randall 2009. The biology of hair growth, Gurpreet S. Ahluwalia (ed.), Cosmetic Applications of Laser and Light-Based Systems, 3-35, William Andrew Inc.
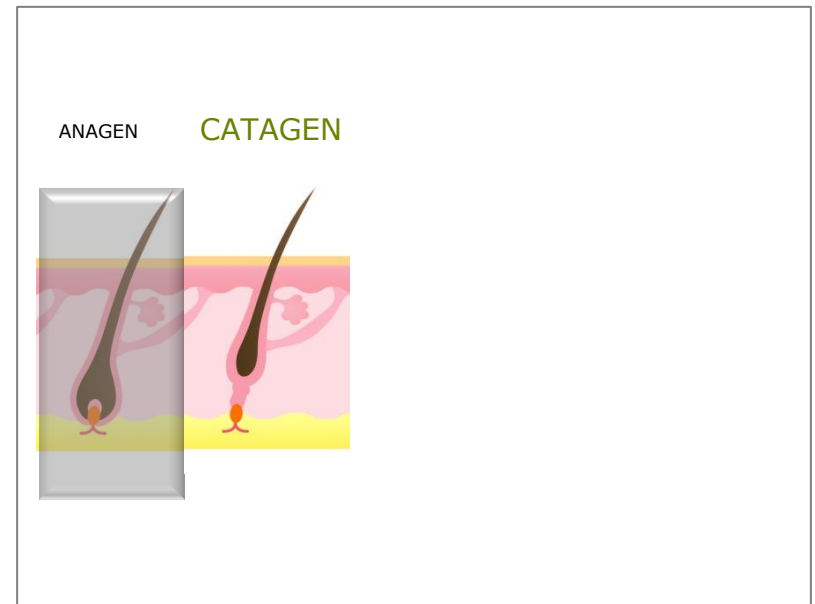
Step 2: CATAGEN - Transition phase

Catagen phase is a transitional stage and 2% of all hairs are in this phase.

This phase lasts for about 2-3 weeks.

During this phase, hair growth stops.
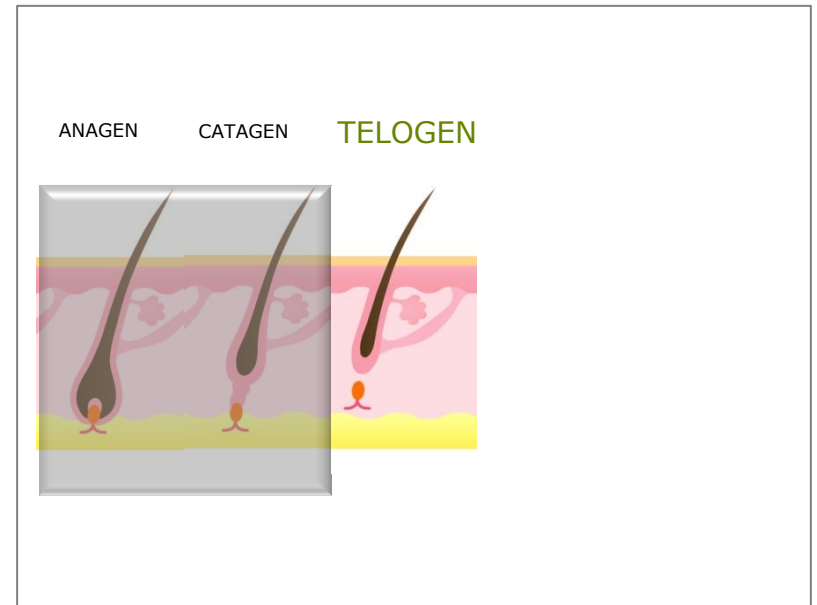
ANAGEN   CATAGEN

Step 3: TELOGEN - Falling phase

Telogen phase is the resting phase and accounts for 10-15% of all hairs.

This phase lasts for about 3 months.

During this phase the hair follicle is at rest and the club hair is completely formed.
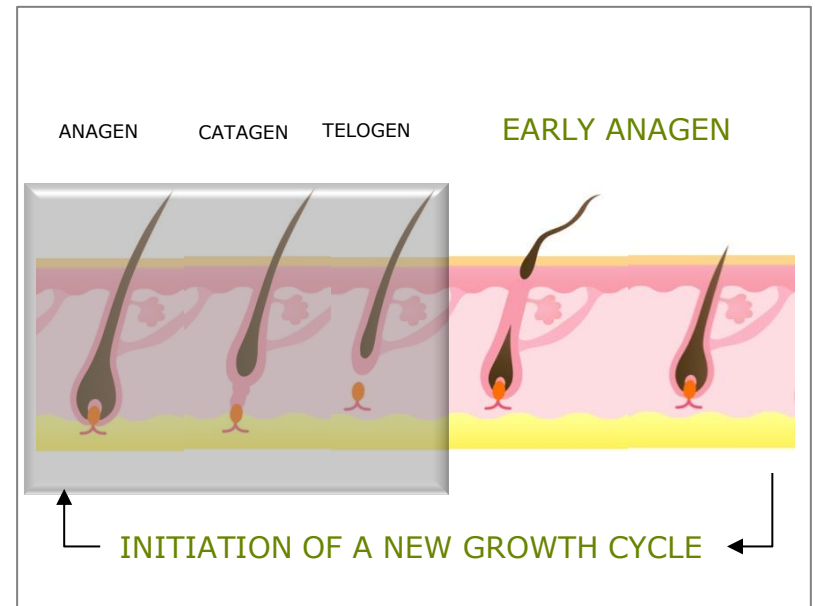
During a period of intensive hair loss, up to 30% of the hairs can be in the telogen phase.

ANAGEN   CATAGEN   TELOGEN

Transition to a new cycle

Early Anagen phase is the activation of a new hair cycle growth.

A new hair is formed and pushes the club hair up the follicle and eventually out.

ANAGEN   CATAGEN   TELOGEN   EARLY ANAGEN

INITIATION OF A NEW GROWTH CYCLE

Early anagen: chronology of the communication

Cells from the dermal papilla activate bulge stem cells (transient activation of the ß-catenin pathway initiates the anagen phase to induce new hair follicle [1,2]).

Bulge stem cells self replicate and migrate into the matrix to create new active keratinocytes.
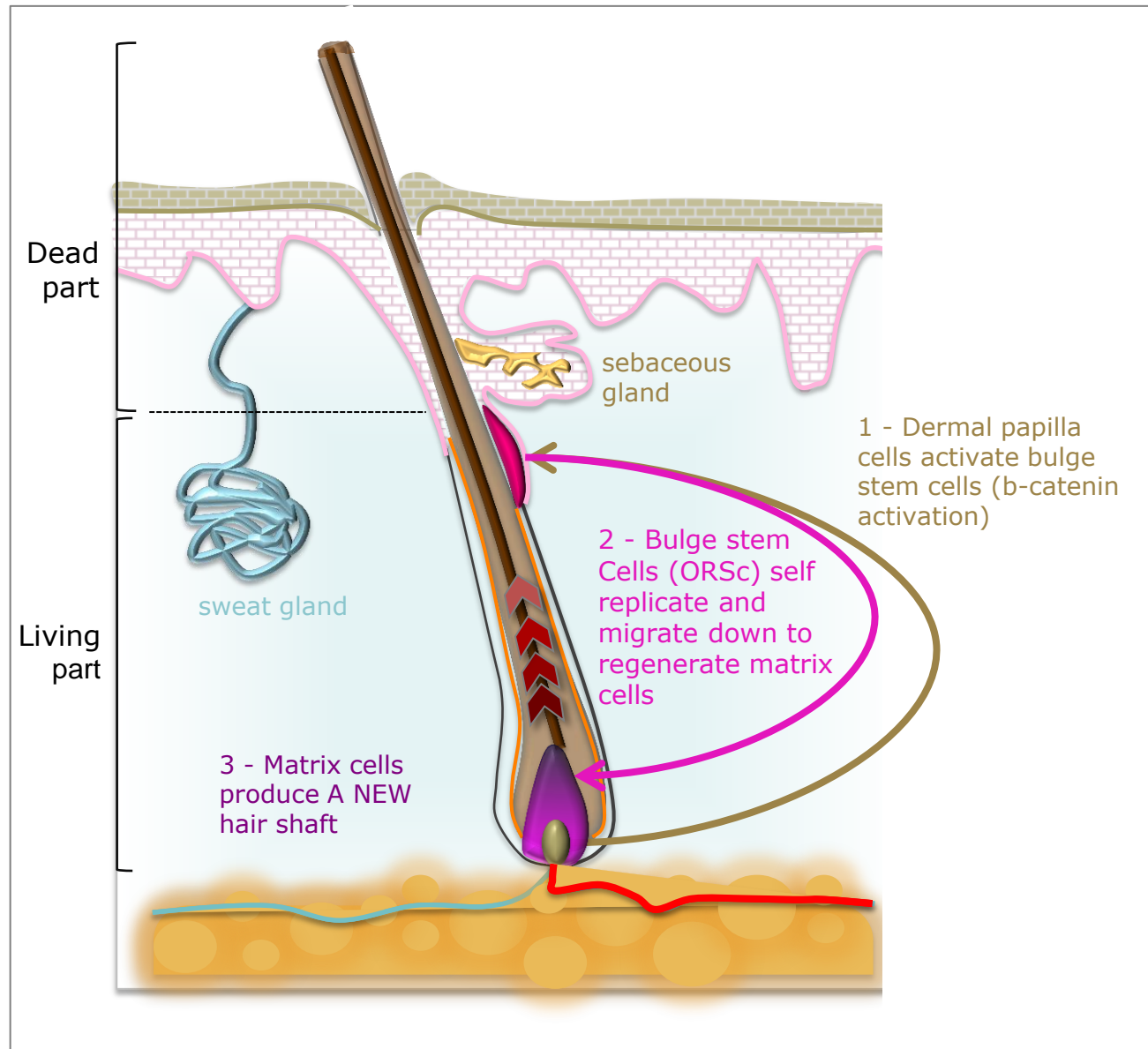
[1] Lo Celso et al, 2004
[2] Shimizu and Morgan, 2004

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Dead part

Living part

sweat gland

sebaceous gland

1 - Dermal papilla cells activate bulge stem cells (b-catenin activation)

2 - Bulge stem Cells (ORSc) self replicate and migrate down to regenerate matrix cells

3 - Matrix cells produce A NEW hair shaft

What happens during hair loss?

Hair loss is a biological problem.

It happens when the number of hairs in anagen phase is lower than those in the telogen phase.

40% of men will have noticeable hair loss (alopecia) by age 35. It reaches 65% by 60 years of age.
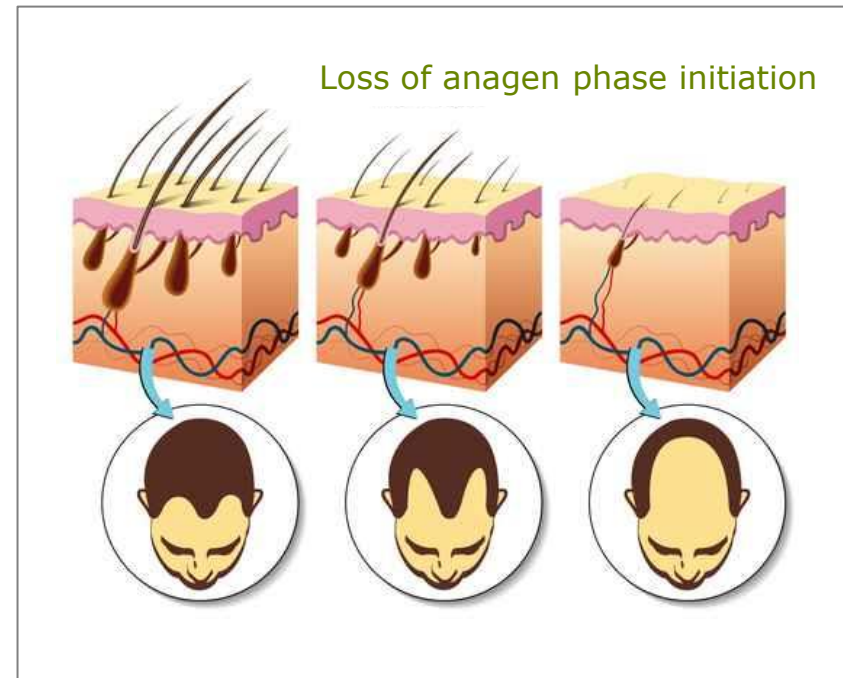
50 to 75% of women suffer noticeable hair loss by age 65.

Hair loss has several origins[1]:
- Hormones (androgenic alopecia)
- Stress
- Aging
- Infections

No matter the causes, hair loss happens when the initiation of the new anagen phase (activation of ORS stem cells) is not activated.

Loss of anagen phase initiation
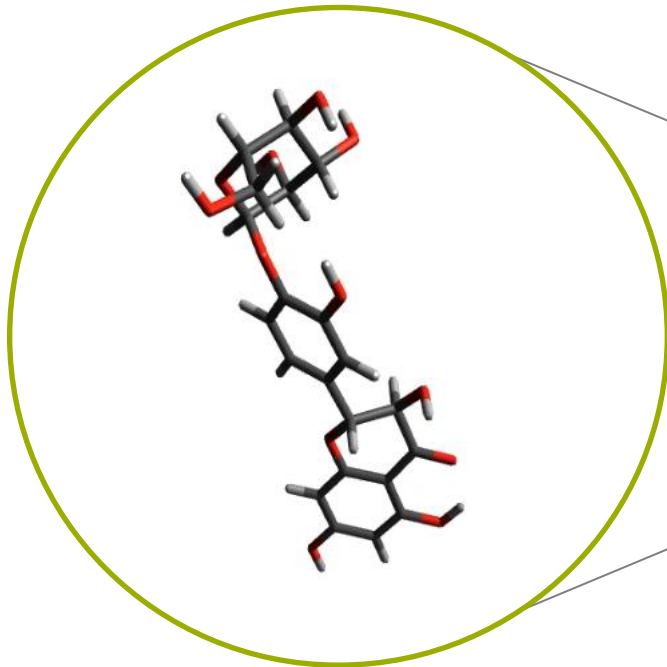
[1] Chen et al., 2012, J Dermatol Sci

In vitro evaluations

Identifying molecules reactivating the bulge (ORS) stem cells to initiate a new anagen phase.

DHQG: Dihydroquercetin-glucoside

Origin: Larch tree

MW: 466 g/mol

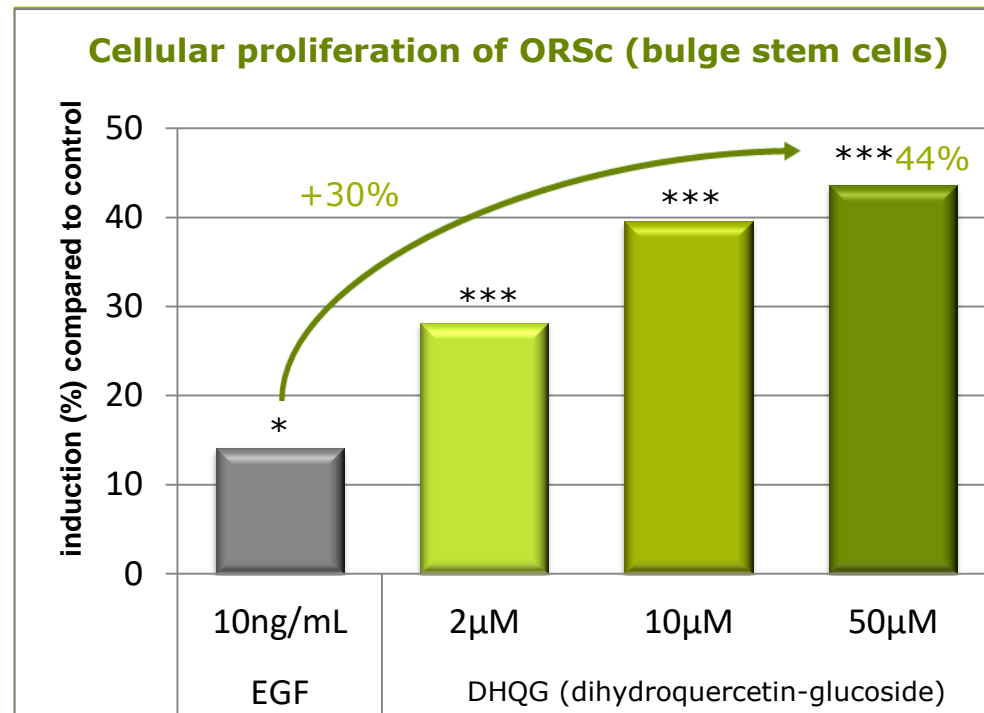**Biotechnology optimisation** (glycosylation)

DHQG: activation of bulge stem cells (*in vitro*)

PROTOCOL:

Incubation of human ORS stem cells (hair follicle bulge stem cells) with increasing concentrations of dihydroquercetin-glucoside (DHQG).

→ DHQG enhances the division of the hair follicle ORS stem cells

(Nota: 50µM DHQG = 1/3 of the amount tested in the clinical assessment)

Cellular proliferation of ORSc (bulge stem cells)

induction (%) compared to control

+30%

***44%

***

***

*

| 10ng/mL | 2µM | 10µM | 50µM |
| EGF | DHQG (dihydroquercetin-glucoside) | | |

*p<0.01, ***p<0.001 compared to control, Student's t Test

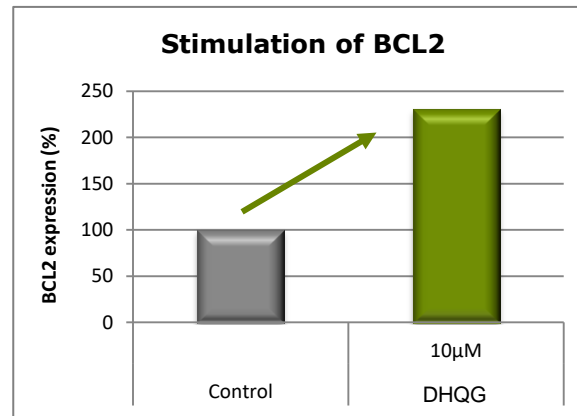# DHQG: effects on hair follicle stem cells genes (*in vitro*)

PROTOCOL:

Incubation of human ORS stem cells (hair follicle bulge stem cells) with different concentrations of dihydroquercetin-glucoside (DHQG). Measure of mRNA expression using qRT-PCR of markers of stem cells' phenotype (K15), anti-apoptosis (BCL2) and differentiation (β-catenin).
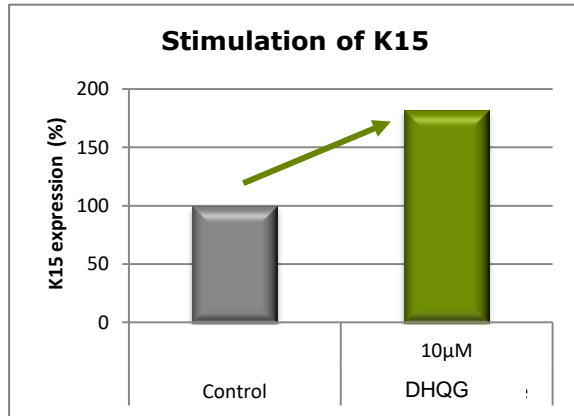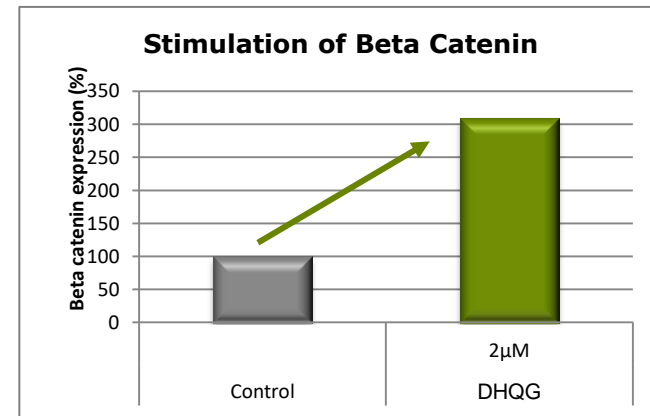

Stimulation of the expression of cytokeratin 15

→ DHQG maintains the hair follicle stem cells' phenotype

Stimulation of the expression of BCL2 marker

→ DHQG protects the hair follicle stem cells from apoptosis

Stimulation of the expression of the beta-catenin marker

→ DHQG stimulates the hair follicle stem cells to initiate the anagen phase

DHQG: stimulation of dermal papilla cells metabolism (*in vitro*)

PROTOCOL:

Incubation of human fibroblasts dermal papilla cells (HFDPc) with increasing concentrations of dihydroquercetin-glucoside (DHQG).
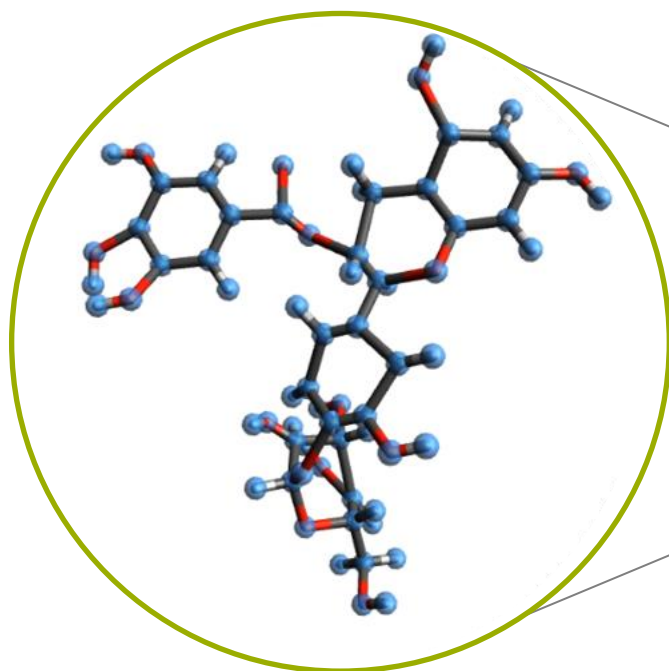
→ DHQG activates the metabolic activity of HFDPc, for a better nourishment of the hair follicle

(Nota: 50µM DHQG = 1/3 of the amount tested in the clinical assessment)

Dermal papilla fibroblasts metabolism activation
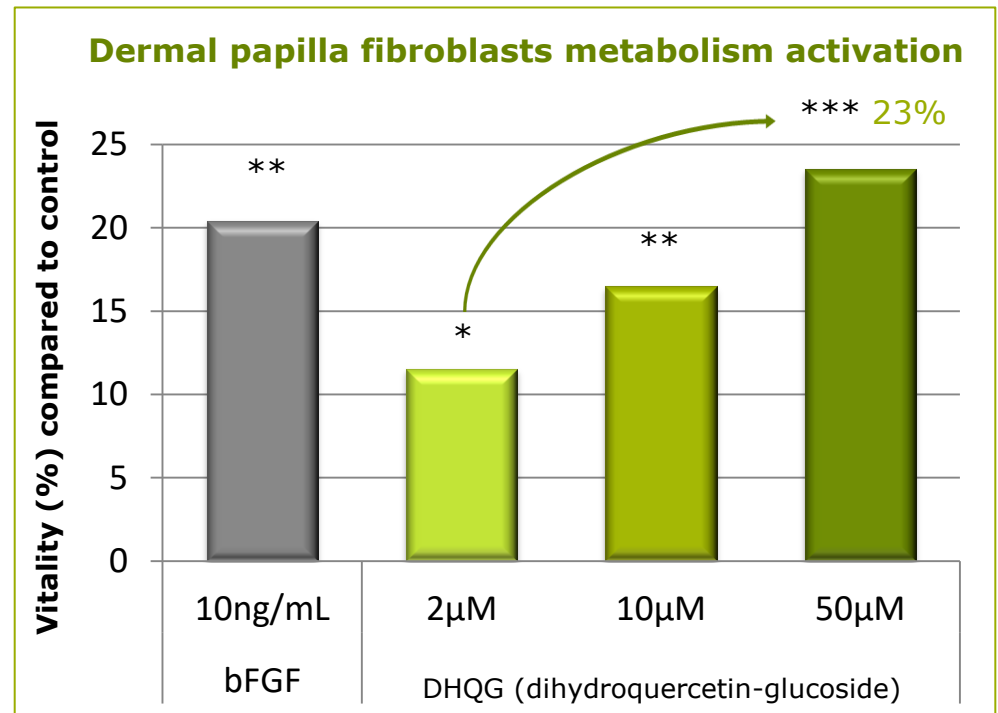
*p<0.05, **p<0.01, ***p<0.001 compared to control, Student's t Test

EGCG2: Epigallocatechin-gallate-glucoside

Origin: Green tea leaves

MW: 604 g/mol

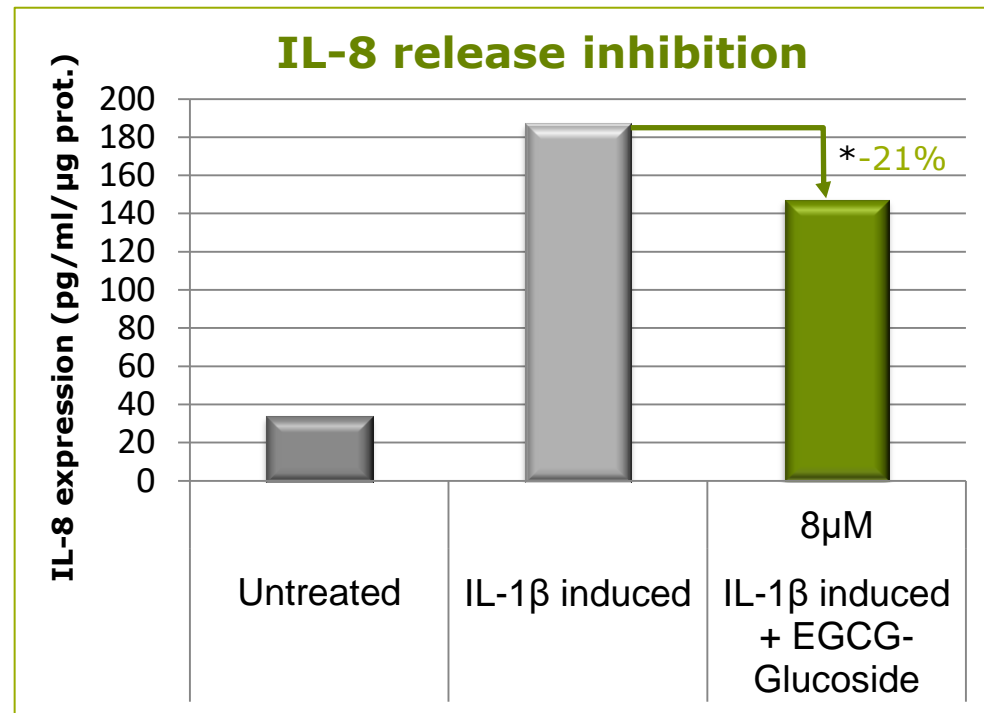**Biotechnology optimisation** (glycosylation)

EGCG2: inhibition of interleukin 8 release (*in vitro*)

PROTOCOL:

Incubation of human normal keratinocytes with epigallocatechin gallate-glucoside (EGCG2). Measure of interleukin 8 release after induction by IL-1b.

→ EGCG2 inhibits the release of interleukin 8, a cytokine involved in hair loss (Kuwano 2007. Br J Dermatol)

(Nota: 8µM EGCG2 = 1/2 of the amount tested in the clinical assessment)

IL-8 release inhibition

IL-8 expression (pg/ml/ µg prot.)

*-21%

Untreated | IL-1β induced | 8µM IL-1β induced + EGCG-Glucoside

*p<0.05 compared to control, Student's t Test

Creation of Redensyl™

Based on these research results we combined:

DHQG
- Activator of stem cell division
- Maintenance of their stem cell properties
- Protection against apoptosis
- Stimulation of dermal fibroblasts metabolism

EGCG2
- Highly potent antioxidant
- Inhibitor of interleukin 8 release

Zinc, because
- Zn increases incorporation of cystine in hair proteins[1]
- Deficiency in zinc is associated with hair loss

Glycine, one of the top 10 amino acid in hair
- The main structural proteins in the hair fiber are the hair keratins and the hair keratin-associated proteins, KAPs
- The KAPs possess either high cysteine or high glycine-tyrosine content [2]
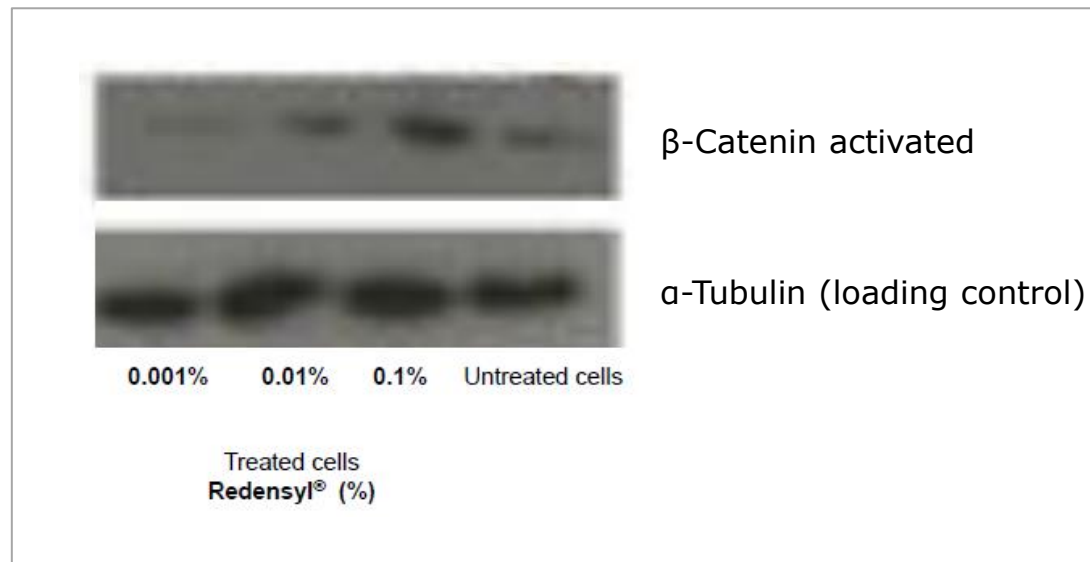
[1] Hsu et al., 1971, J. Nutr. 101.
[2] Rogers et al., 2002, JBC Papers in Press.
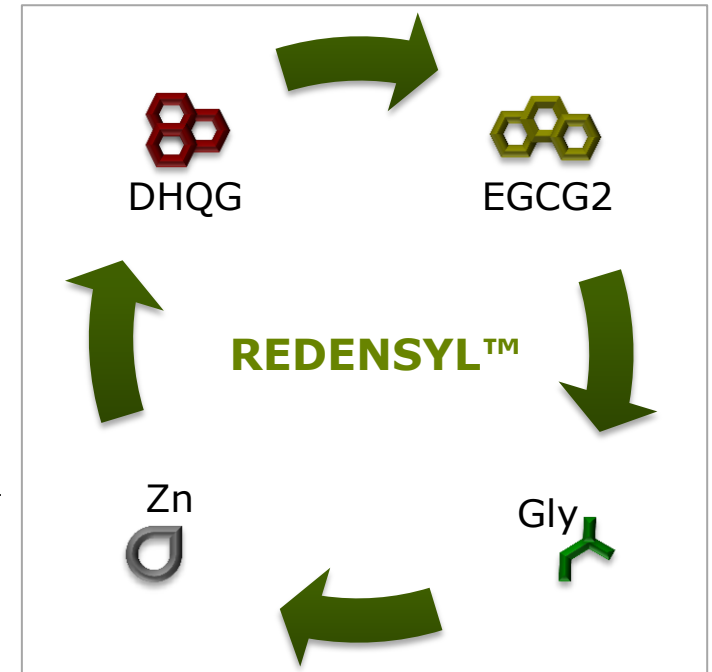
Redensyl™: Activation of β-Catenin (*in vitro*)

PROTOCOL:

Western blot analysis run on human androgenic alopecic ORSc (3 donors) treated with Redensyl™. Measurement of β-Catenin activation.

β-Catenin activated

α-Tubulin (loading control)

0.001%   0.01%   0.1%   Untreated cells
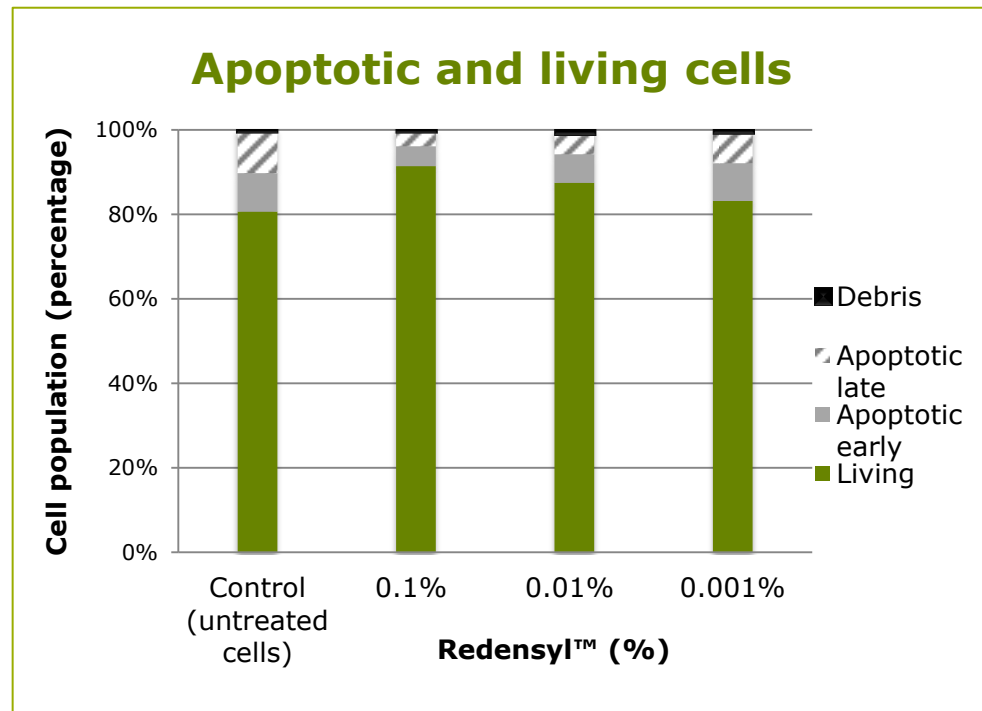
Treated cells
Redensyl® (%)

→ Redensyl™ activates β-Catenin in androgenic alopecia ORSc which confirms the very good results observed during the q-RT PCR with DHQG.

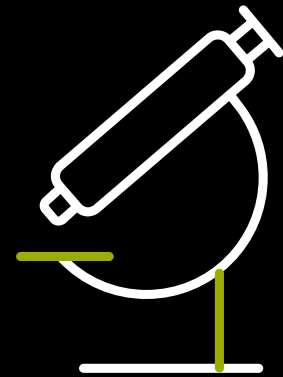# EGCG2: inhibition of interleukin 8 release (*in vitro*)

PROTOCOL:

Apoptosis Annexin V assay run on human androgenic alopecic ORSc (3 donors) treated with Redensyl™. Measurement of living cells situation proportions.

→ Redensyl™ protects androgenic alopecia ORSc from apoptosis which confirms the very good results observed during the q-RT PCR with DHQG.

Apoptotic and living cells

Chart: Cell population (percentage) vs Redensyl™ (%). Categories: Control (untreated cells), 0.1%, 0.01%, 0.001%. Legend: Debris, Apoptotic late, Apoptotic early, Living.

Ex vivo evaluations

Hair follicle growth test (Philpott test)

PROTOCOL:

24 hair follicles from men suffering from alopecia were maintained alive with either 1% of Minoxidil or 1% of Redensyl™ during 10 days. Hair growth was measured at D7 and D10.
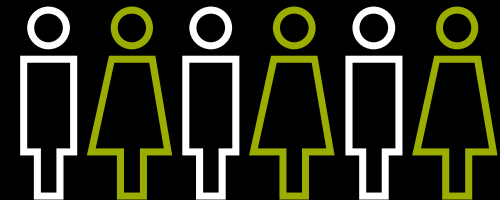

Hair growth after 7 and 10 days
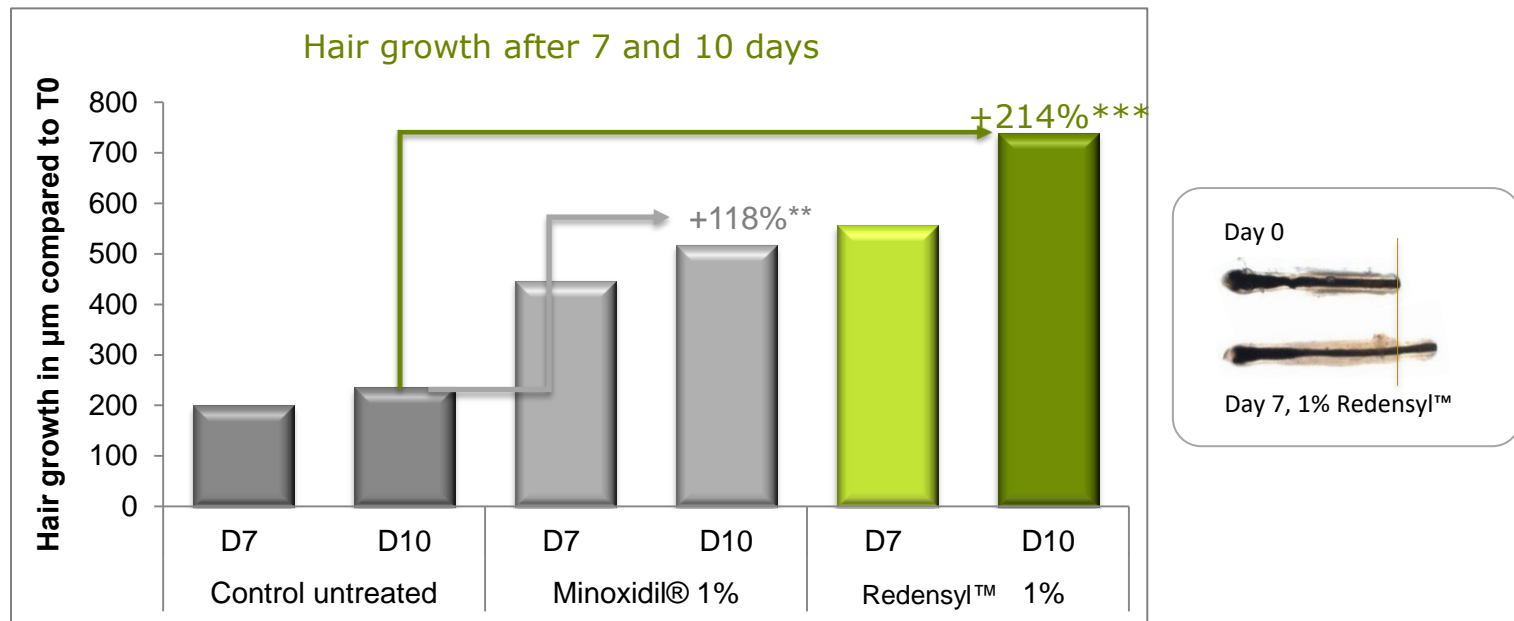
**p<0.1, ***p<0.001 compared to untreated, Student's t Test

→ Redensyl™ increases hair growth by +214% compared to untreated

→ Redensyl™ acts almost 2x more than Minoxidil, the benchmark reference.
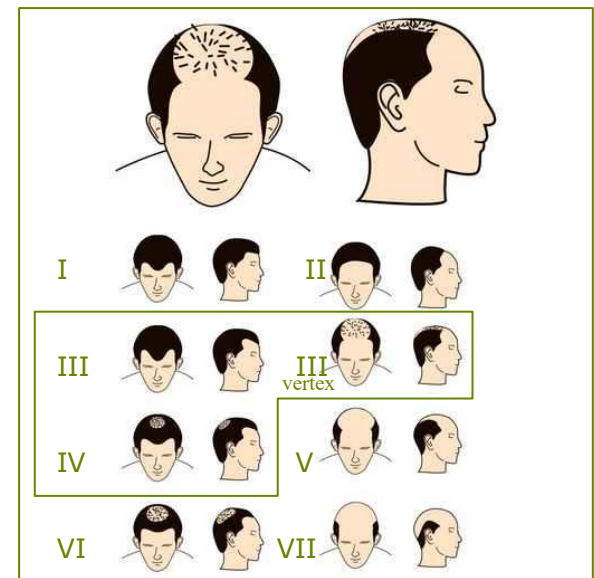
Clinical evaluation

Protocol of clinical trial

VOLUNTEERS

- 26 men aged 18 to 70 years old
- Brown to dark hair
- Qualified for a grade 3 to 4 alopecia (Norwood scale)
- With minimum 150 hair/cm2 and 40 telogen hairs/cm2

PROTOCOL

- Double blind clinical trial versus a placebo
- Applying the formula once a day
- 50% of volunteers received the placebo
- 50% received the formula with 3% Redensyl™
- Clinical study was performed under the control of a dermatologist.
- Period of the test: autumn

Clinical formula: AQUA, ALCOHOL DENAT., BUTYLENE GLYCOL, GLYCERIN, XANTHAN GUM, DISODIUM EDTA, CITRIC ACID, (+/-) REDENSYL™ 3%
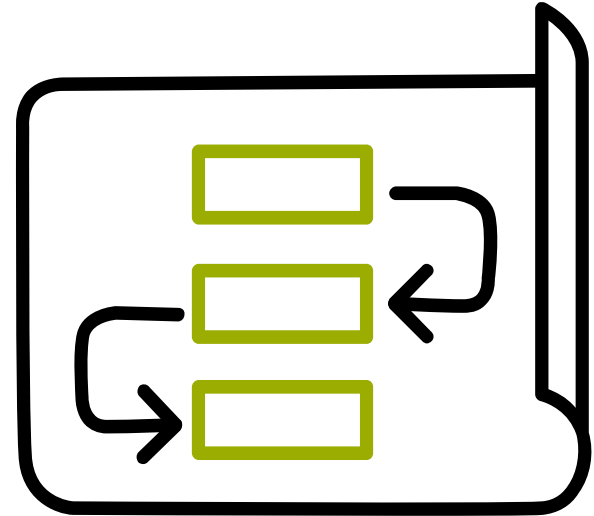
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Evaluated parameters

Clinical measures at D0, D30 and D84

- Macro pictures on scalp
- Density of hair in anagen phase
- Density of hair in telogen phase
- Ratio anagen/telogen
- Pictures of the head
- Self assessment questionnaire at D84

Nota:

- Shaving of a 1.5 cm^2 area
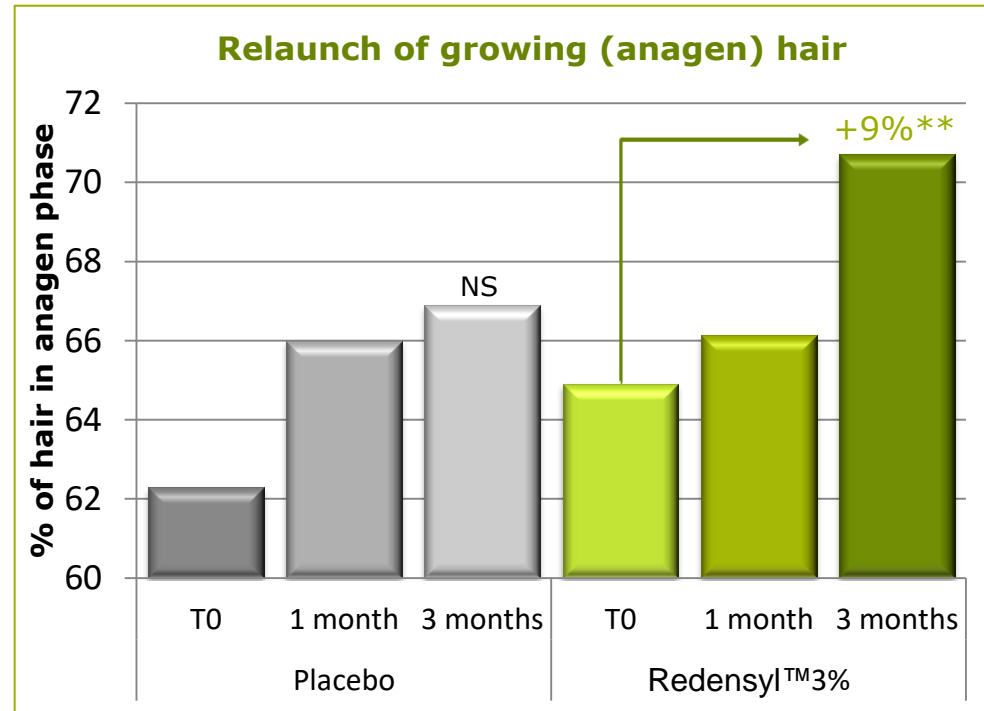- Analysis on 0,7 cm^2

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Counting of anagen hair (=growing)

PROTOCOL:

Analysis of the volunteers' scalp of the number of hair in anagen phase.

→ Slight non significant placebo effect up to D84 (activation of micro-circulation)

→ Redensyl™ stimulates up to +9% the number of hair in anagen phase.

Relaunch of growing (anagen) hair

+9%**

**p<0.01, compared to untreated, Student's t Test
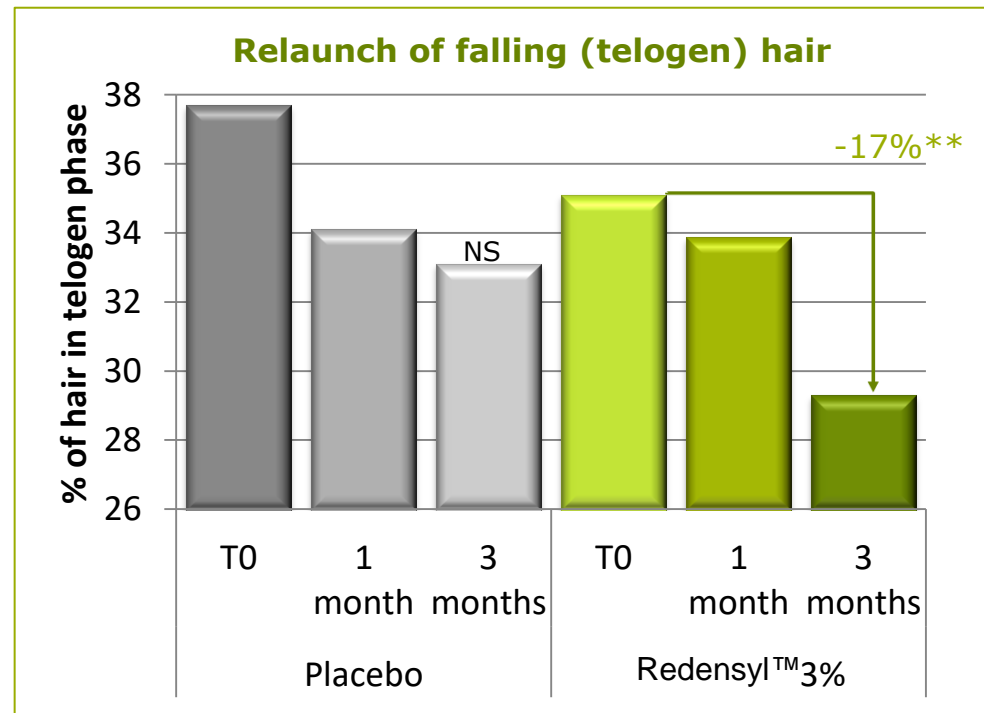
Counting of telogen hair (=falling)

PROTOCOL:

Analysis of the volunteers' scalp of the number of hair in telogen phase.

→ Slight non significant placebo effect up to D84 (activation of micro-circulation)

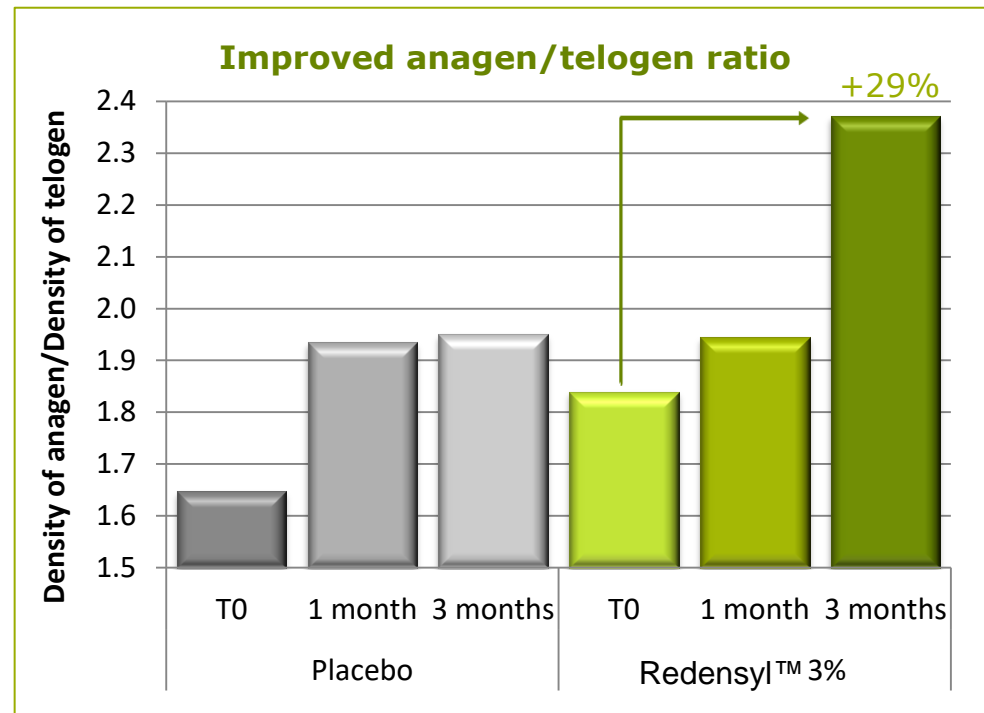→ Redensyl™ reduces down to -17% the number of hair in telogen phase.

Relaunch of falling (telogen) hair

**p<0.01, compared to untreated, Student's t Test

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Follow up of the anagen/telogen ratio

PROTOCOL:

Analysis of the volunteers' scalp of the number of the ratio of anagen versus telogen hair.

→ After 3 months, Redensyl™ improves the ratio of anagen/telogen by +29%, reaching 2.37 from the initial 1.83

Improved anagen/telogen ratio

+29%

Density of anagen/Density of telogen

Placebo: T0, 1 month, 3 months
Redensyl™ 3%: T0, 1 month, 3 months

Pictures of volunteers

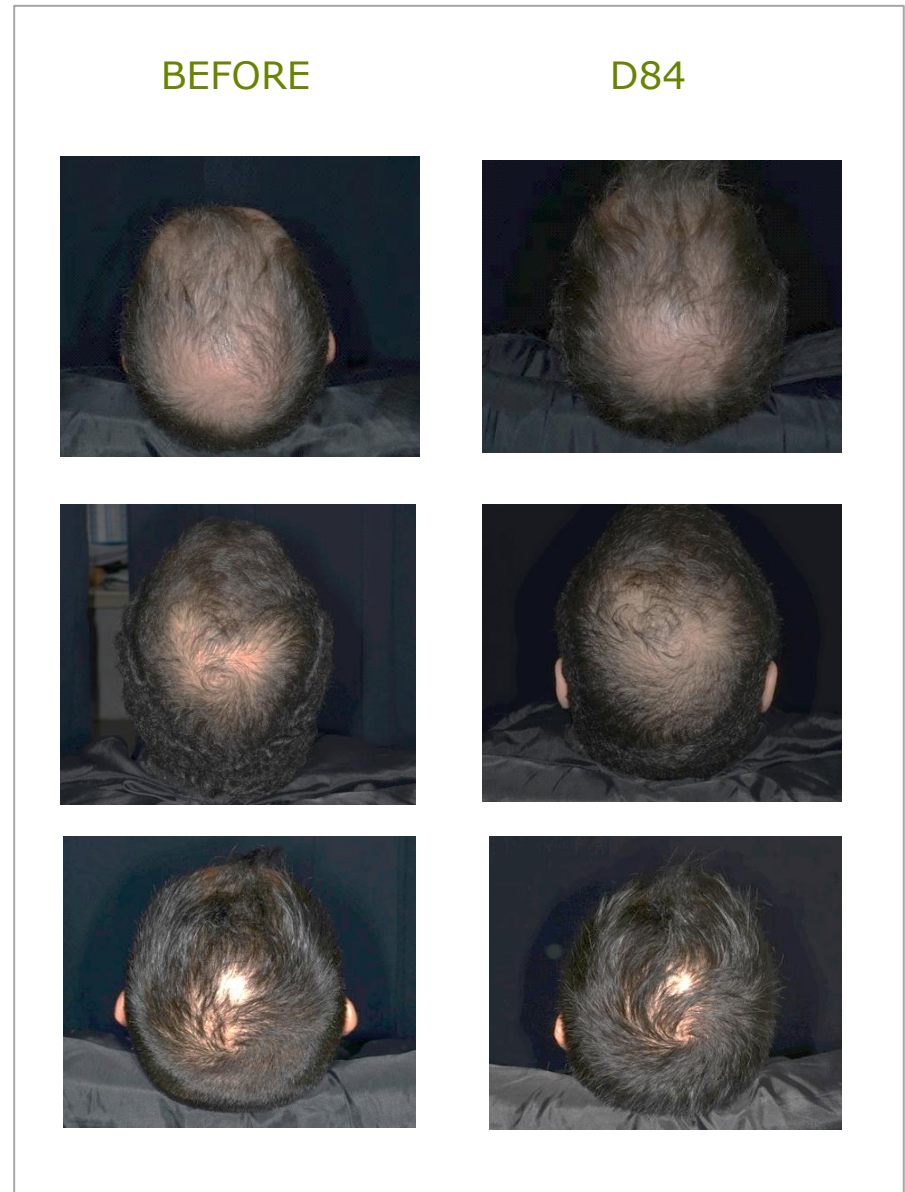|	BEFORE	D84
Increased density 52 years old		
Reduction of the vortex 38 years old		
Reduction of the bald area diameter 42 years old		
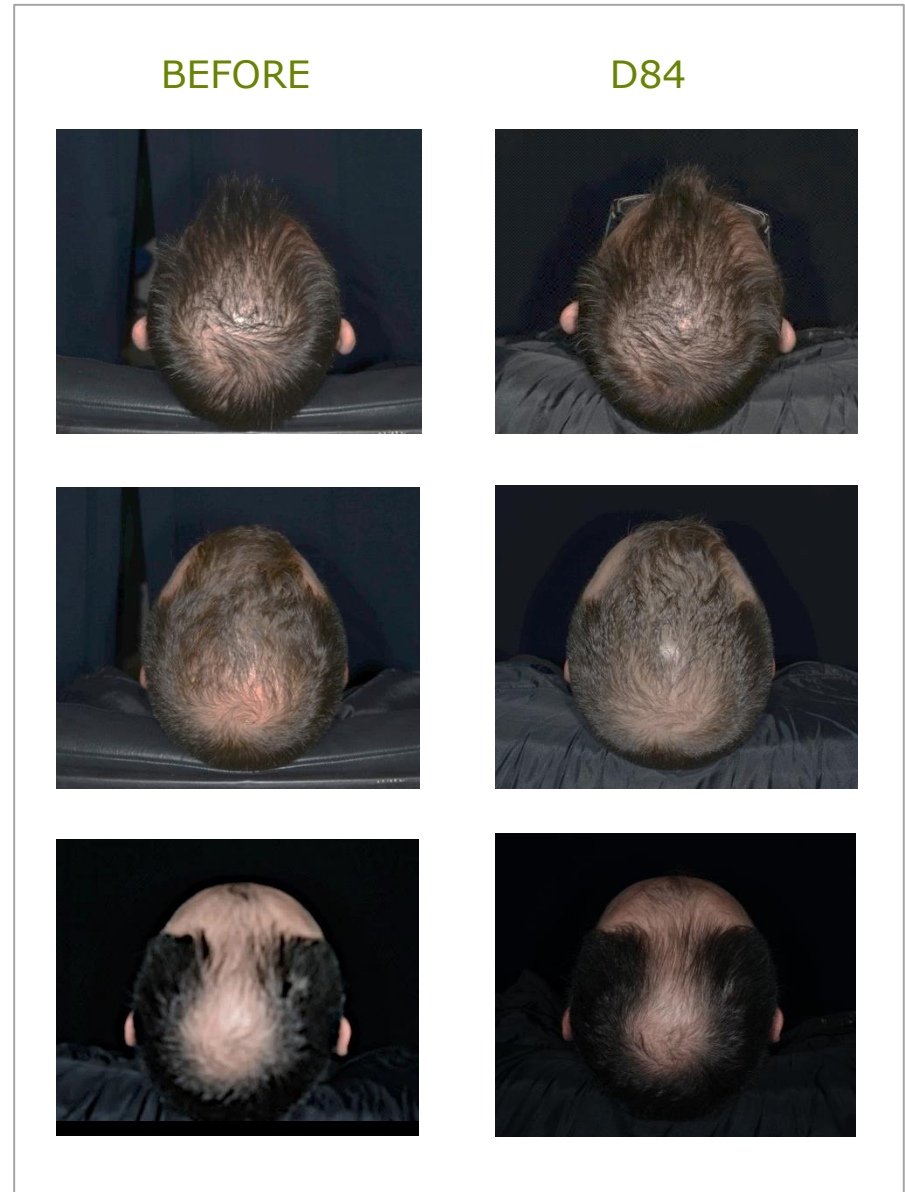
Pictures of volunteers

Increased density
46 years old

Reduction of the vortex
36 years old

Increased density
29 years old

BEFORE   D84

Details – before/after

Criteria	#3
Age	**52 years old**
% of new anagen hair	**+ 10.8%**
% of density of hair increase	**+ 17%**
Number of new hairs / cm^2	**+ 47 hairs/ cm²**
Total number of new hairs on the scalp (600 cm²)	**+ 28,200 hairs**
Number of new hair per month on the scalp	**+ 9,400 hairs**

Analysis area - Day 0
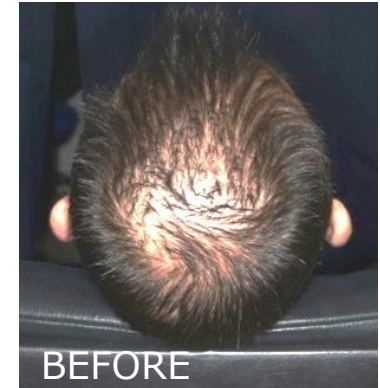
Analysis area - Day 84

BEFORE

AFTER

→ **Visible redensification of the scalp**

Details – before/after

| Criteria	#6
Age	42 years old
% of new anagen hair	+ 19.2%
% of density of hair increase	+ 17%
Number of new hairs / cm^2	+ 43 hairs / cm^2
Total number of new hairs on the scalp (600 cm^2)	+ 25,800 hairs
Number of new hair per month on the scalp	+ 8,600 hairs

Analysis area - Day 0

Analysis area - Day 84

BEFORE

AFTER

→ **Hair loss stopped, a visible increase of hair density**

Details – before/after

Volunteer	#26
Age	29 years old
% of new anagen hair	+ 9.2%
% of density of hair increase	+ 17%
Number of new hairs / cm^2	+ 29 hairs / cm^2
Total number of new hairs on the scalp (600 cm^2)	+ 17,400 hairs
Number of new hairs per month on the scalp	+ 5,800 hairs

Analysis area - Day 0

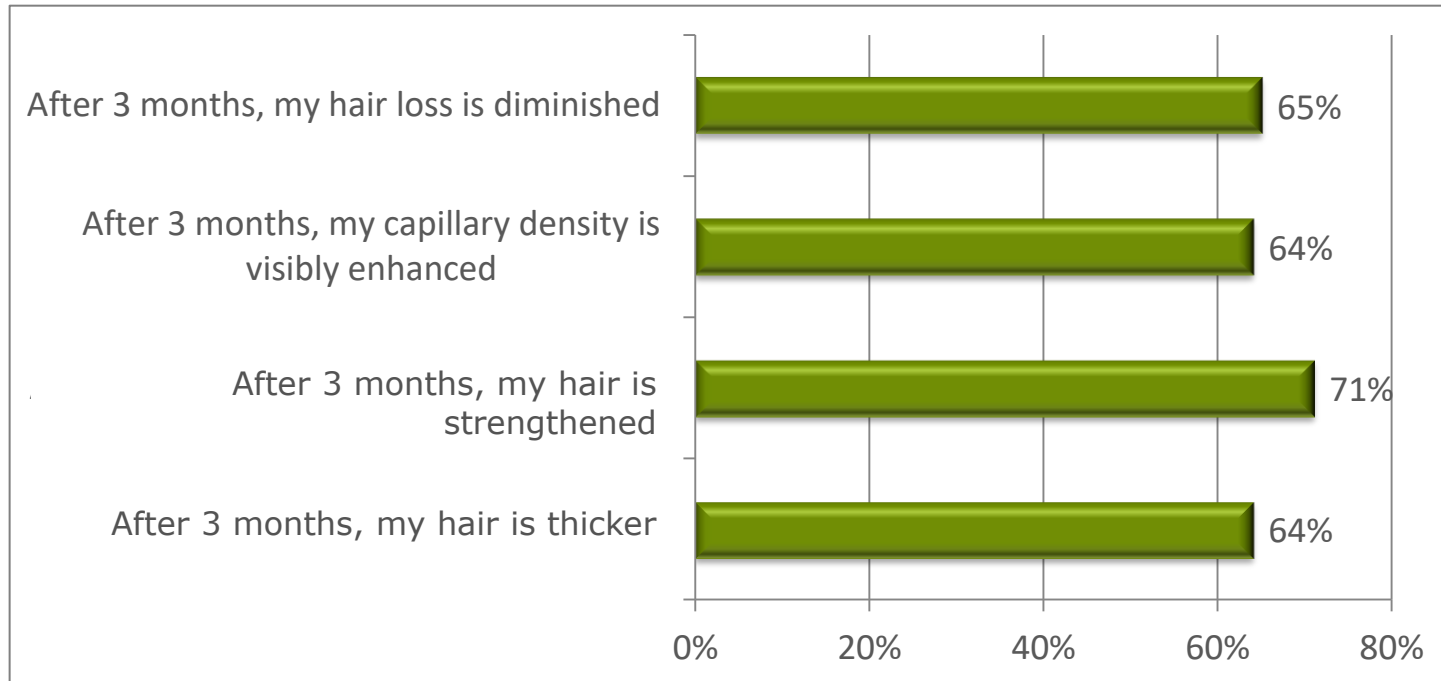Analysis area - Day 84

BEFORE
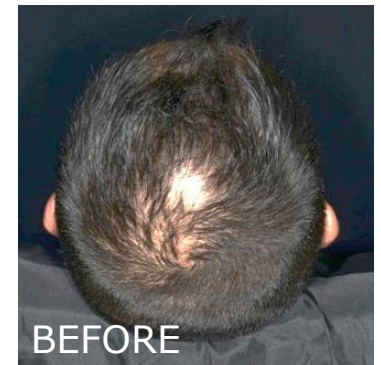
AFTER

→ **Hair looks thicker with a visible improvement of the density**

Self assessment

A self-evaluation run by the volunteers after 84 days.

Testers claim to see reduced hair loss, improved capillary density, stronger and thicker hair after three months of treatment.

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Self assessment

A self-evaluation run by the volunteers after 84 days.

71% of the testers are satisfied by the product, and 71% of them would like to buy the product.

Satisfaction rate

Unsatisfied
29%

Satisfied
71%

Intent to buy

NO
29%

YES
71%

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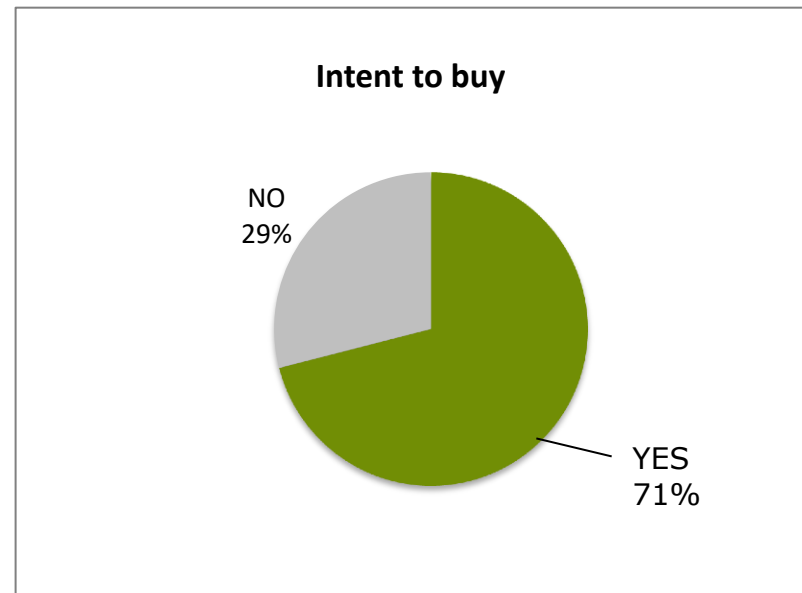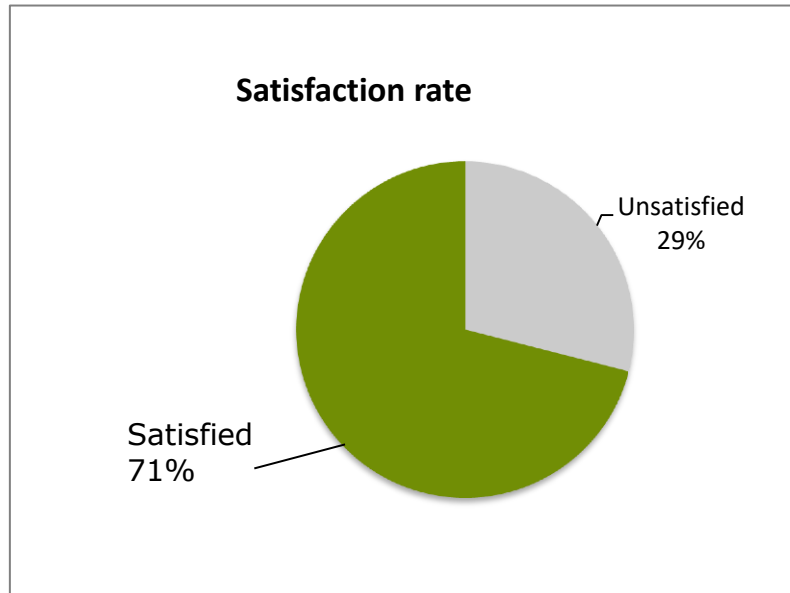
Summary of the clinical assessment

Within 84 days on grade 3 to 4 alopecic volunteers:

85% of volunteers showed clinical improvements:

- ✓ **+9%** of anagen hair
- ✓ **-17%** of telogen hair
- ✓ **+29%** in the anagen/telogen ratio

- ✓ An average **+8%** increase of hair density, corresponding to,
    - ✓ **+10,000 new hairs** on a total 600 cm^2 scalp surface
    - ✓ Up to **+28,200 new hairs**

Redensyl™

Summary

Redensyl™ - the Hair Growth Galvanizer

MODE OF ACTION

Gly
Zn
DHQG
EGCG2

EPIDERMIS
basal layer

Interleukin 8

sebaceous gland

Bulge stem cells (ORSc)

DERMIS

sweat gland

Matrix (keratinocytes)
Dermal papilla (fibroblasts)

HYPODERMIS

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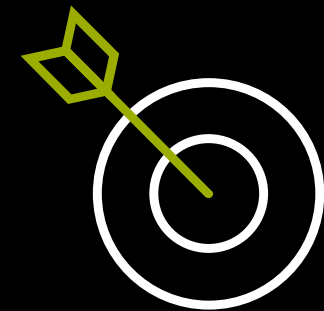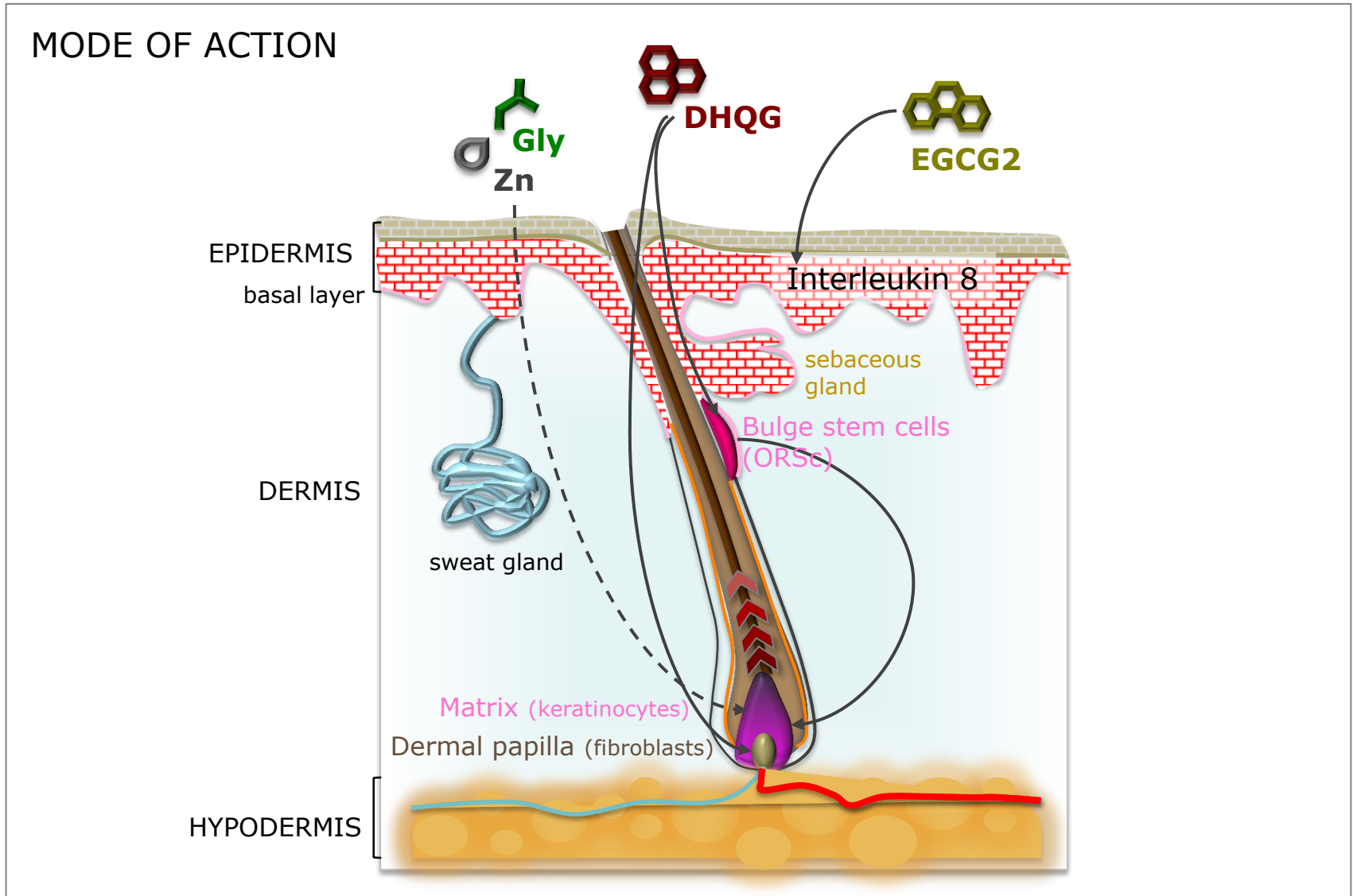
Targeting existing hair follicle stem cells

1. A reactivation of the bulge stem cells

2. A metabolic boost of dermal papilla cells

3. A shut down of inflammatory reactions

4. Excellent results on grade 3 to 4 alopecic volunteers:

   - Hair are **denser**

   - Hair look **thicker**

   - **Increase** in hair **growth**

   - **Decrease** in hair **loss**

   - A **better ratio anagen/telogen**

   - **Visible results** in 84 days

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Comparison with esthetic surgery

Hair transplantation surgery:

- A hair transplantation surgery enables to make an average of 2016 grafts [1]

- Grade 3 to grade 4 alopecic patients need between 1600 to 2200 hair grafts [2]

- Each graft contains 4 hair [2], so each transplantation gives 6400 to 8800 new hairs

- 65% of the patients undergo a single hair transplantation [1]

- Up to 3 hair grafts sessions can be needed to get the appropriate hair density [1]

→ Redensyl™ gives better results than one hair transplantation surgery (+10,000 new hairs in average, up to +28,200)

[1] International Society of Hair Restoration Surgery: 2013 Practice Census Results
[2] Bernstein Medical center www.bernsteinmedical.com/hair-transplant/follicular-unit-transplantation/graft-numbers/

Applications

MEN

- Anti hair loss lotion and shampoo
- Hair growth spray
- Anti aging global hair serum
- Shampoo for thin hair
- Preventive hair care shampoo

WOMEN

- Mask, or leave-on hair care products
- Preventive hair care shampoo
- Post trauma hair treatment
- Anti aging global hair serum

- Eyelash growth mascara
- Eyelash growth primer
- Eyebrows redensifier

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Technical information
Redensyl™

INCI	Glycerin (And) Water (And) Sodium Metabisulfite (And) Glycine (And) Larix Europaea Wood Extract (And) Zinc Chloride (And) Camellia Sinensis Leaf Extract
Origin	White and Green technologies
Preservation	Preservative free
Appearance	Clear yellow solution
Solubility	Water soluble
Dosage	1% to 3%
Processing	Can be added at the end of the formulation process under stirring below 50°C. Can be heated for a short term with the oil phase of formulation
Compliance	INCI CHINA IECIC LISTED · HALAL APPROVED · VEGAN SUITABLE

Thank you

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