

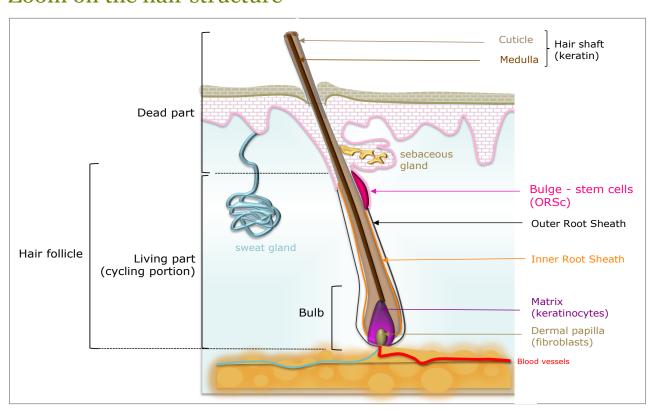
Hair: continuously self-regenerating

- The full head of hair consists of 110,000 - 150,000 hairs
- The average of scalp surface is 600 cm² ^{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



Zoom on the hair structure



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.



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

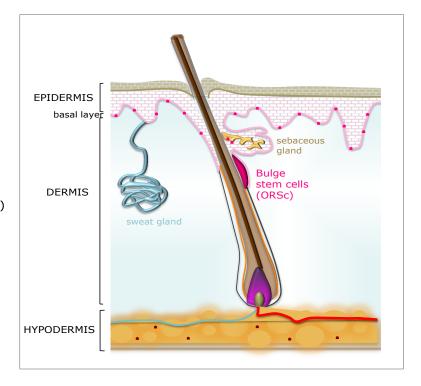
² www.clinicaltrials.gov



Stem cells in skin

Stem cells are mainly found in

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

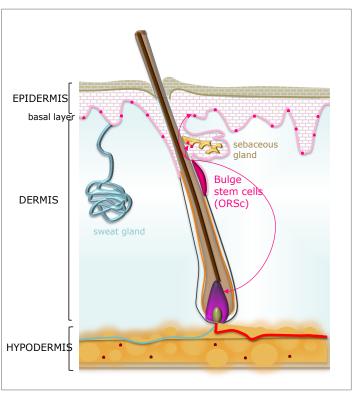


Diaz-Flores, 2006, Histol Histopathol.

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.

Bulge stem cells (ORSc)

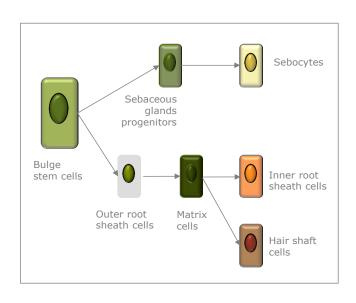


Bulge stem cells are expressing the keratin 15 marker.

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



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.





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.



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.

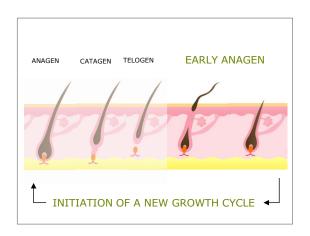




Transition to a new cycle

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

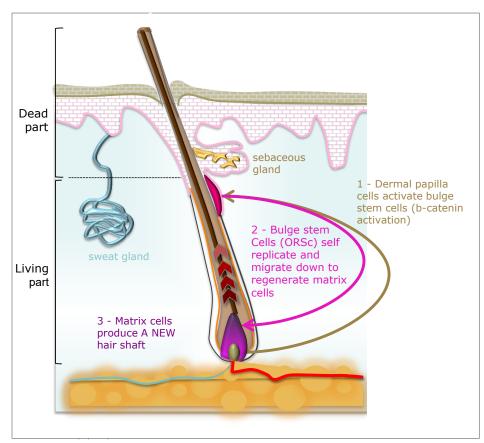
A new hair is formed and pushes the club hair up the follicle and eventually out.



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



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.

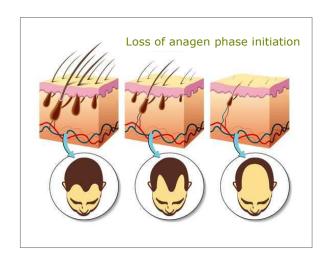
40% of men will have noticeable hair loss (alopecia) by age 35. It reaches 65% by 60 years of age.

50 to 75% of women suffer noticeable hair loss by age 65.

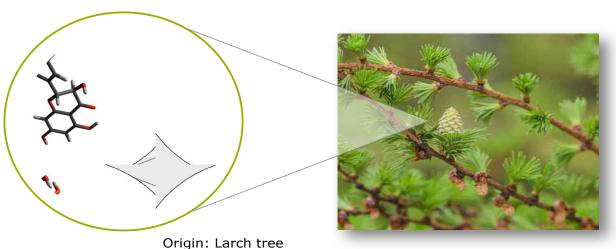
Hair loss has several origins1:

- 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.



DHQG: Dihydroquercetin-glucoside



MW: 466 g/mol

Biotechnology optimisation (glycosylation)

Morgan's

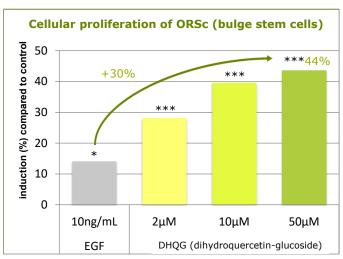
DHQG: activation of bulge stem cells (in vitro)

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: $50\mu M$ DHQG = 1/3 of the amount tested in the clinical assessment)

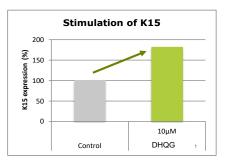


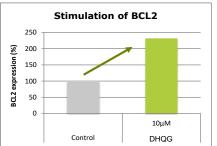
*p<0.01, ***p<0.001 compared to control, Student's t Test

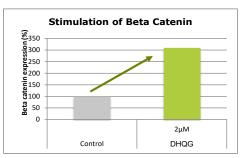
DHQG: effects on hair follicle stem cells genes (in vitro)

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



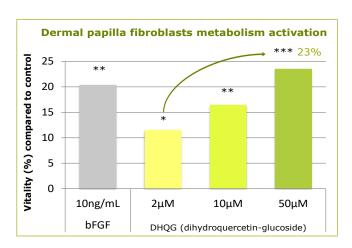
DHQG: stimulation of dermal papilla cells metabolism (in vitro)

PROTOCOL:

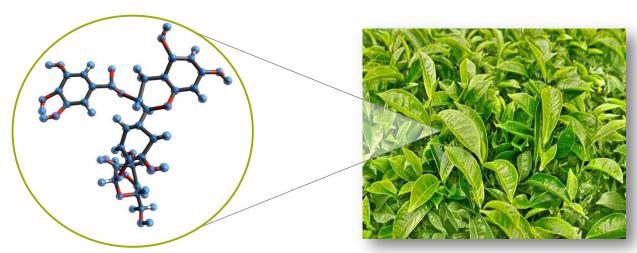
Incubation of human fibroblasts dermal papilla cells (HFDPc) with increasing concentrations of dihydroquercetinglucoside (DHQG).

 DHQG activates the metabolic activity of HFDPc, for a better nourishment of the hair follicle

(Nota: $50\mu M$ DHQG = 1/3 of the amount tested in the clinical assessment)



EGCG2: Epigallocatechin-gallate-glucoside



Origin: Green tea leaves

MW: 604 g/mol

Biotechnology optimisation (glycosylation)



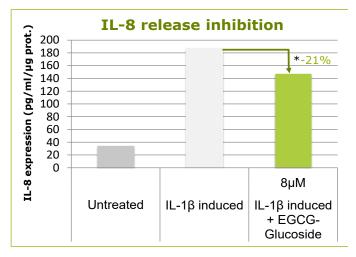
EGCG2: inhibition of interleukin 8 release (in vitro)

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: $8\mu M EGCG2 = 1/2$ of the amount tested in the clinical assessment)



*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

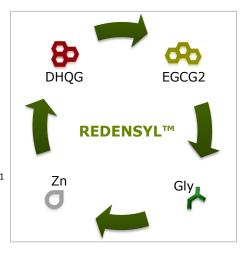
- Highly potent antioxidant
- Inhibitor of interleukin 8 release

Zinc, because

- Zn increases incorporation of cystine in hair proteins¹
- 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,
- The KAPs possess either high cysteine or high glycine-tyrosine content 2



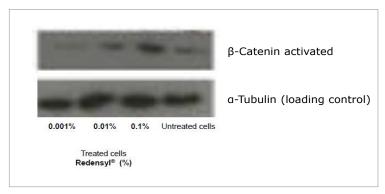
¹ Hsu et al., 1971, J. Nutr. 101. ² Rogers et al., 2002, JBC Papers in Press.



RedensylTM: Activation of β -Catenin (in vitro)

PROTOCOL:

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



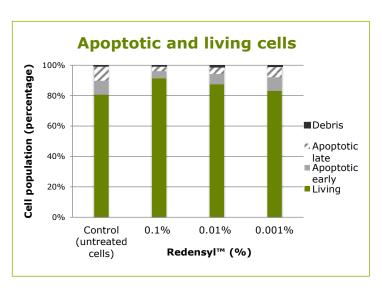
• RedensylTM activates β -Catenin in androgenic alopecia ORSc which confirms the very good results observed during the q-RT PCR with DHQG.

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.

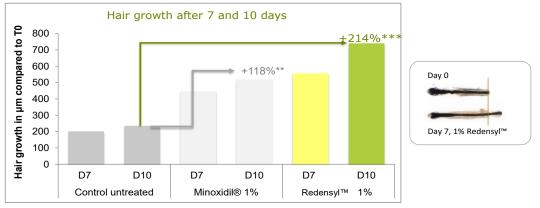


Hair follicle growth test (Philpott test)



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.



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.

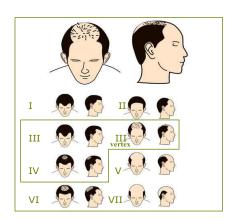
Protocol of clinical trial

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

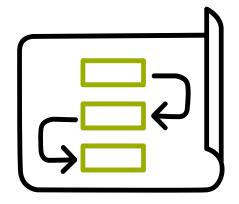




Evaluated parameters

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:

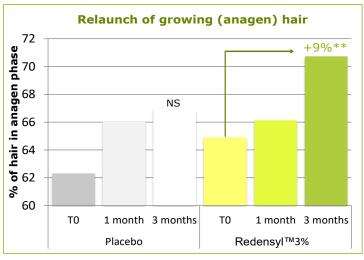
- Shaving of a 1.5 cm² area
- Analysis on 0,7 cm²

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 microcirculation)
- RedensylTM stimulates up to +9% the number of hair in anagen phase.



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

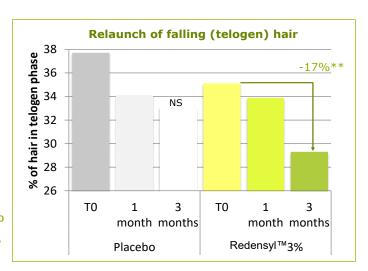


Counting of telogen hair (=falling)

PROTOCOL:

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

- Slight non significant placebo effect up to D84 (activation of microcirculation)
- Redensyl™ reduces down to -17% the number of hair in telogen phase.

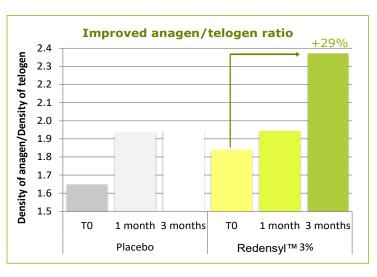


Follow up of the anagen/telogen ratio

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



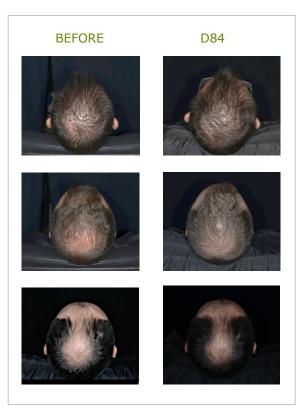


Pictures of volunteers

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





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	



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 / cm²		
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		



• 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 / cm²	
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	

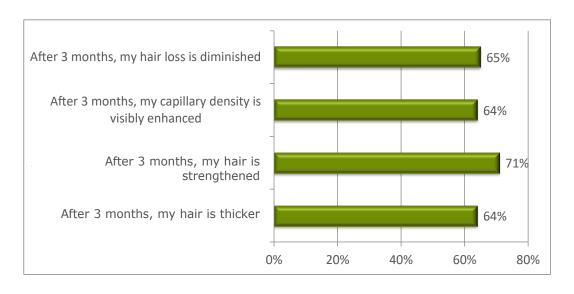


Hair looks thicker with a visible improvement of the density

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.

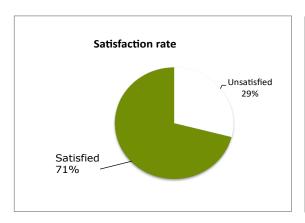


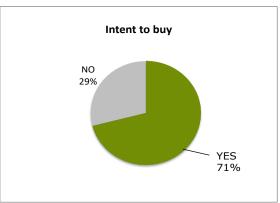


Self assessment

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.





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

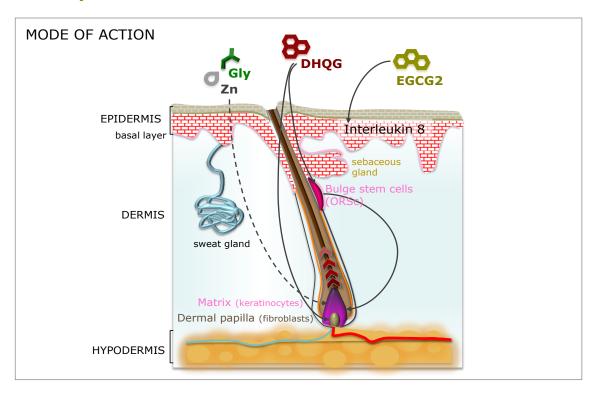
An average +8% increase of hair density, corresponding to,

- **+10,000 new hairs** on a total 600 cm² scalp surface
- Up to +28,200 new hairs





Redensyl™ - the Hair Growth Galvanizer



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



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 grafts²
- 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

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



² Bernstein Medical center www.bernsteinmedical.com/hair-transplant/follicular-unit-transplantation/graft-numbers/



Focus on the product

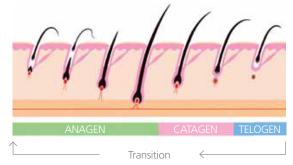
Hair loss in numbers

It is a known fact that 40% of men will have noticeable hair loss (alopecia) by age 35. This number reaches 65% by 60 years of age. Women are also deeply impacted by such process: 50 to 75% suffer noticeable hair loss by age 65. Hair loss can be devastating to one's self image and emotional well being.

The normal cycle for hair

The hair cycle is made of three phases:

- > Anagen phase during which the hair is growing (± 3 years),
- > Catagen phase also called the transition phase (± 3 weeks),
- > Telogen phase during which the hair is dying and falling (± 3 months), which is followed by the anagen phase again.



Hair loss and stem cells

When suffering from hair loss, the telogen phase is prolonged, and the transition to the anagen phase becomes more difficult. Hair become thinner and the percentage of hair transitioning to the telogen phase continues to increase.

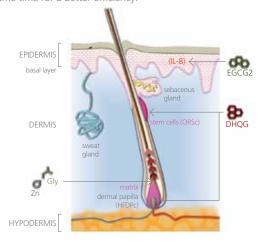
The problem comes from the fact that the hair follicle stem cells (also called ORSc) located in the bulge along the hair, are less productive, and less adapted to improve the quality of the matrix (made of keratinocytes) supporting the hair follicle growth. Furthermore, the fibroblasts located in the dermal papilla (also called HFDPc) are less efficient in communicating with the stem cells, meaning that the matrix will not be renewed as it used to. Initiating the anagen phase becomes more sluggish, and hair loss becomes a part of daily life.

Redensyl®: acting on stem cells and HFDPc to re-activate hair growth

Redensyl® is made of patented molecules targeting the ORSc and the HFDPc at the same time for a better efficiency:

- Dihydroquercetin-glucoside (DHQG): a stabilized polyphenol which activates the division of hair follicle stem cells, while maintaining their differentiation properties. It protects stem cells from apoptosis (BCL2 activation), and drives them towards the anagen cycle (β-catenin activation), while boosting the metabolism of dermal papilla fibroblasts.
- ➤ EGCG-glucoside (EGCG2): a stabilized EGCG derivative used to reduce the typical inflammatory state of alopecic scalp (reduction of IL-8), and capture free radicals¹.
- ➤ Glycine: a major constituent of hair proteins, mainly keratin associated proteins (KAP), which favors hair growth².
- > Zinc: a very important co-factor for numerous enzymes, favoring the incorporation of cystin in keratin for a stronger hair shaft³.

Redensyl ® shows outstanding results after 3 months at the clinical level.





Biological activity

Four actions on ORSc stem cells (in vitro tests)

1. Stimulating ORSc proliferation:

ORSc proliferation was tested with increasing concentration of dihydroquercetin-glucoside (DHQG, the major component of Redensyl®) by following the BrdU cell proliferation assay, using EGF as a reference. Measurement of cell proliferation is proportional to the amount of incorporated BrdU.

Results: DHQG increases the cellular proliferation of the ORSc. More stem cells are produced with increasing doses of DHQG. *p<0.01, **p<0.001 compared to control, Student's t-test



ORSc were treated with $10\mu M$ of DHQG to evaluate the potential of this molecule to maintain the ORSc as real stem cells. The mRNA expression of cytokeratin 15, a major stem cell marker, was quantified by qRT-PCR.

Result: DHQG at 10µM multiples by almost 2 times the mRNA synthesis of K15, a qualification marker of stem cell's phenotype.

3. Avoiding apoptosis:

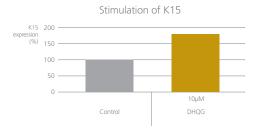
ORSc were treated with $10\mu M$ of DHQG to evaluate the protective potential of this molecule against apoptosis. The mRNA expression of BCL2, a major anti-apoptotic marker, was evaluated by qRT-PCR

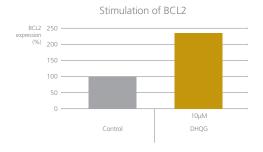
Result: DHQG at 10µM increases by 2 times the mRNA synthesis of BCL2, showing the anti-apoptosis effect of this molecule.

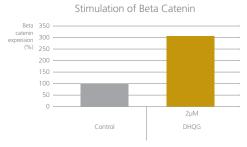
4. Activating differentiation:

ORSc were treated with $2\mu M$ of DHQG to evaluate the potential of this molecule to induce the cells differentiation process. The mRNA expression of β -catenin, a major differentiation marker, was quantified by qRT-PCR.

Result: DHQG at 2μ M multiples by more than 3 times the mRNA synthesis of β -catenin, showing its differentiation inducing activity on stem cells.







Summary: DHQG stimulates hair follicle stem cells division, maintains their stem cells status, protects them from apoptosis, and boosts their differentiation.



Biological activity

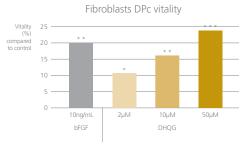
Increase of Fibroblasts DPc vitality (in vitro tests)

Human fibroblasts dermal papilla cells (HFDPc) were incubated for 48 hours in a basal medium and treated with increasing doses of DHQG (the major component of Redensyl®) or bFGF as a reference.

Their metabolic activity was evaluated thanks to a XTT reduction assay.

Results: DHQG helps the HFDPc to improve their metabolic activity, for a better nourishment of the hair follicle.

*p<0.05, **p<0.01, ***p<0.001 compared to control, Student's t-test

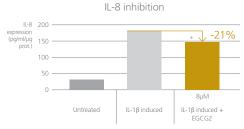


Decrease of skin irritation (in vitro tests)

EGCG2 was tested for its ability to reduce IL-8, a cytokine involved in scalp irritation. An irritated skin is more prone to hair loss. Normal human keratinocytes were put in a culture medium and were stressed using IL-1β and treated for 48h with EGCG2, a major component of Redensyl®. IL-8 in the supernatant was quantified by ELISA test.

Results: EGCG2 confirms its anti-irritation potential by inhibiting IL-8 release by 21%.

*p<0.05 compared to untreated, Student's t-test



Increase of hair follicle length (ex vivo Philpott test)

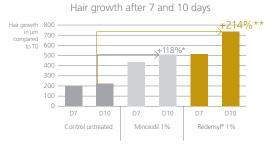
Redensyl® was tested at 1% versus Minoxidil at 1% as a benchmark reference to evaluate its potential on hair follicle growth. Hair of four male donors suffering from alopecia were maintained alive in normal hair culture conditions. After 7 and 10 days hair growth was measured compared to day 0 with pictures analysis.

Results: Redensyl® increases hair growth by +214% compared to untreated, and shows almost two times higher results than Minoxidil, the benchmark reference.

*p<0.1, **p<0.001 compared to untreated, Student's t-test

Visible increase of hair follicle size







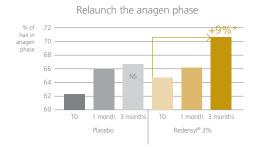


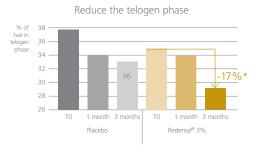
Reactivate the hair growth cycle (clinical evaluation)

The efficiency of Redensyl® at 3% was evaluated in a double-blind test versus a placebo. Twenty six male volunteers were selected by following specific inclusion criteria: between 18 to 70 years old, brown to dark hair, with a minimum density of hair of 150 hair/cm² and 40 telogen hair/cm², with clinically confirmed grade 3 to 4 alopecia.

Volunteers applied the placebo or the product with 3% of Redensyl® on their whole scalp daily for 3 months.

A shaved area of 1.5cm² was defined on each volunteer to allow the measurements on a window of 0.7cm² at D0, D28 and D84. Phototrichograms were realized using a NIKON camera associated with Canfield® Epiflash System and a contact plate to press hair on the scalp. Analysis were run with Photoshop CS5 extended® and permitted to define if hair were in anagen, telogen or undetermined phase.





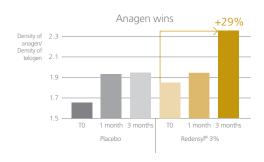
*p<0.01 compared to untreated, Student's t-test

Results: A non significant placebo effect is observed (mechanical activation of micro-circulation), with almost no more evolution after 1 month. Redensyl® increases the percentage of hair in anagen phase by 9% compared to T0 after 3 months, and decreases the percentage of hair in the telogen phase by 17% compared to T0 after 3 months.

Rebalance the anagen/telogen ratio (clinical evaluation)

The ratio Anagen/Telogen was evaluated by comparing the density of hair in anagen phase and in telogen phase.

Results: Redensyl® significantly increases the ratio Density of Anagen / Density of Telogen. After 3 months the ratio reaches 2.37 while the placebo shows almost no evolution after one month.



As a consequence, density of hair was also measured and was increased by an average +8% in three months while using Redensyl® at 3%.





Redensyl®: Visible results after 3 months (clinical evaluation)

85% of volunteers show clinical improvements. More anagen hair, a higher density, more visible hair.

Examples of the clinical results of three volunteers (29 to 52 years old) treated with Redensyl® during 3 months.

Criteria Volunteer	#3 (52 years old)	#6 (42 years old)	#26 (29 years old)
% of new anagen hair	+ 10.8%	+ 19.2%	+ 9.2%
% of density of hair increase	+ 17%	+ 17%	+ 17%
Number of new hair / cm ²	+ 47 hair/cm ²	+ 43 hair/cm ²	+ 29 hair/cm ²
Total number of new hair on their scalp (600 cm²)	+ 28,200 hair	+ 25,800 hair	+ 17,400 hair
Number of new hair per month on their scalp	+ 9,400 hair	+ 8,600 hair	+ 5,800 hair
Macro pictures (Phototrichograms) Results: Hair look thicker, with a visible improvement of the density.	10	10	10
Scalp pictures	3 months	3 months	3 months
Results: Hair loss stopped, a visible increase of hair density is noticeable.	Before	Before	Before
	After	After	After

Summary



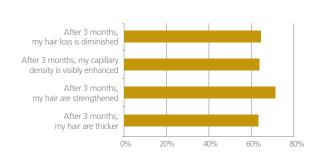
Self-evaluation of Redensyl® (clinical)

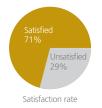
A self-evaluation after 3 months was run by the volunteers.

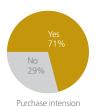
Results: Testers claim Redensyl® at 3% reduced their hair loss, improved the capillary density by strengthening and thickening their hair after three months of treatment.



+10,200 hair in average in 3 months: better results than one hair transplantation procedure 1.







Technical information

Suggested INCI: Water, Glycerin, Sodium Metabisulfite, Glycine, Larix Europaea Wood Extract, Zinc Chloride, Camellia Sinensis Leaf Extract

Origin: Plant extracts and Biotechnology

Preservation: Preservative-free

Appearance: Clear, yellow liquid

Solubility: Water soluble

Dosage: 1-3%

Processing: Can be added at the end of the formulation process under stirring or homogenizing or can be heated for a short time

with the water phase of formulation. Formulate at temperature below 50°C.

Claims

Claims: Anti-hair loss, stimulation of hair growth, re-densification of hair on scalp, stimulation of eyelash growth,

activation of eyebrow growth.

Applications: Anti-hair loss treatment, hair lotion, hair serum, anti-aging hair serum, eyelash growth serum, active mascara,

eyebrow enhancers.