
Care Creations™
DN-Age™
Hair youthfulness from the inside
BASF
We create chemistry

Anti-aging beauty products and treatments are often focused on skin

However, healthy, beautiful hair is equally important for looking and feeling young!

Take the next step to stay young and defy time: focus on your hair!

We're living longer, and we're torturing our hair like never before. Combined with increased environmental insult and basic chronological aging, and you've got aging hair: brittle, gray, weak,…

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1 | Introduction: hair aging

2 | DN-Age™: product characteristics

3 | Scalp & Hair Care

- *In vitro* data
- *In vivo* data

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« Aging is a basic biological process characteristic of all living organisms… and widely acknowledged to be the consequences of both a genetic program and cumulative environmental wear and tear »

Yaar & Gilchrest, 2001

Hair Aging

2 Hair Loss

1 Hair graying

3 Hair Fragility

Hair graying
or canities

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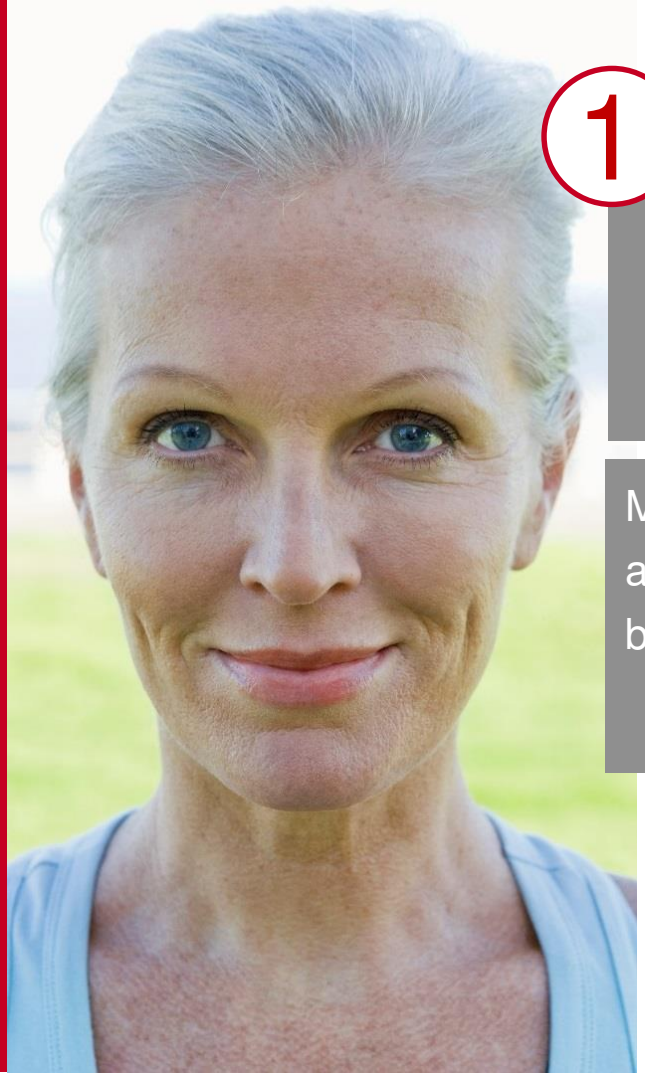
is a progressive **loss of pigment** over time and over several cycles from growing hair shafts:

- Gradual loss of pigment **along the same hair shaft**
- Hair fiber grows in **already depigmented**



1

Gray hair is the most visible sign of aging

More than **70%** of people have at least **25% gray hair** between **ages 45 and 65**

(Panhard *et al.* 2012)

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Hair loss / hair fall

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2

Hair loss, a stressful experience for everyone, but substantially more distressing for women

16% of women under 50 are affected, increasing to a proportion of **30-40%** of women aged 70 years and over.

Affects at least **50%** of men by the age of 50, and up to **70%** of all males in later life.

(Norwood 1975)

Daily hair loss is commonly around **50 to 100** hair/day in **normal scalp** conditions

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Hair fragility

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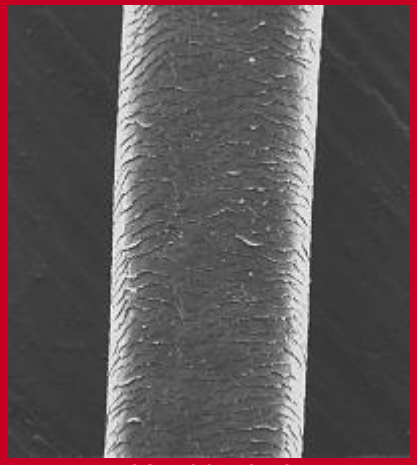
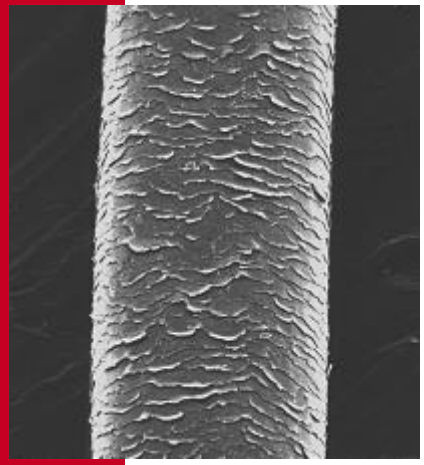
Signs of aged hair:

- ✓ **Thin** (pigmented hair)
- ✓ **Damaged & brittle**
- ✓ **Lack of volume**
- ✓ Lifeless
- ✓ Dull



Healthy hair



Damaged hair

3

Hair is more difficult to manage with aging

Aged hair is more prone to weathering:
flyaway hair, hair tangling, rough hair, hair damage and breakage

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Take care of your hair follicle
to keep youthful hair

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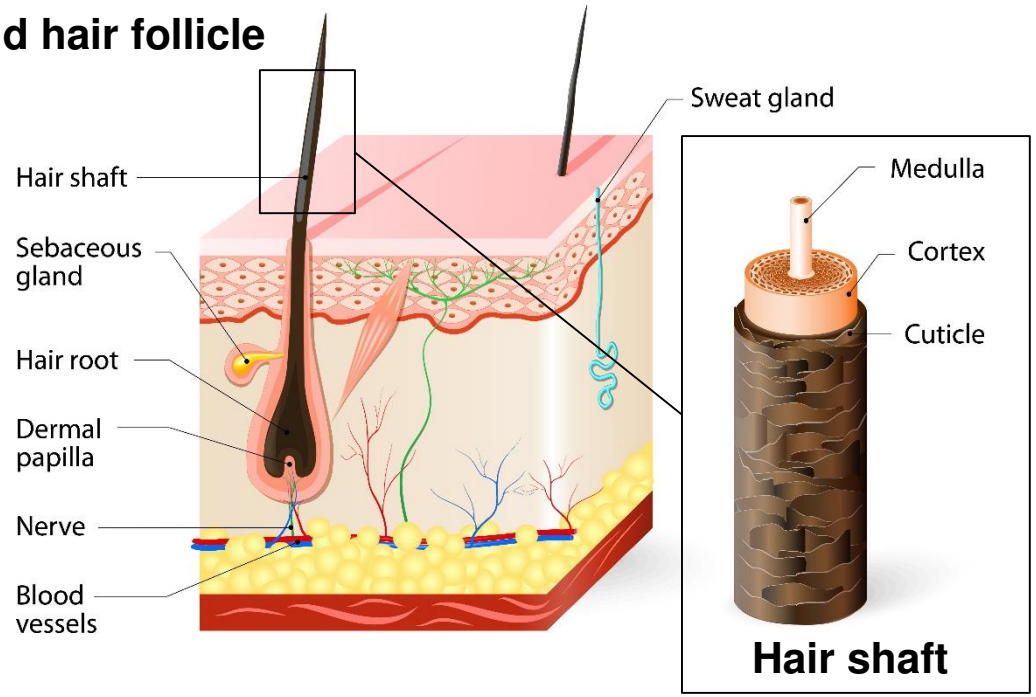
Scalp skin and hair follicle



- Healthy hair follicle gives healthy hair fibers
- Taking care of the hair follicle is taking care of the hair quality

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Take care of your scalp
to keep youthful hair

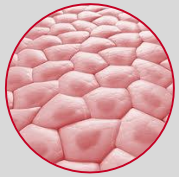
- Scalp directly provides nutrition to the hair follicles and consequently to the hair fibers
- As the skin ages, the scalp ages as well
- Taking care of the scalp is taking care of the hair quality

47% of consumers believe a healthy scalp is a major reason for healthy hair

Source: Mintel - Haircare - China – Jan 16

How does hair age?

HAIR AGING - IMPACTS



SCALP

- Oxidative stress
- Inflammatory stress
- Cellular & molecular damage
- Atrophy of skin scalp (epidermis, dermis and hypodermis)

FOLLICLE

- Loss of hair follicles' melanocytes
- Slow-down of hair growth cycle
- Slow-down and impairment of hair fiber production and quality

HAIR FIBER

- Loss of pigmentation
- Hair loss
- Hair thinning
- Loss of hair quality

Impact of the environment on emerging hair

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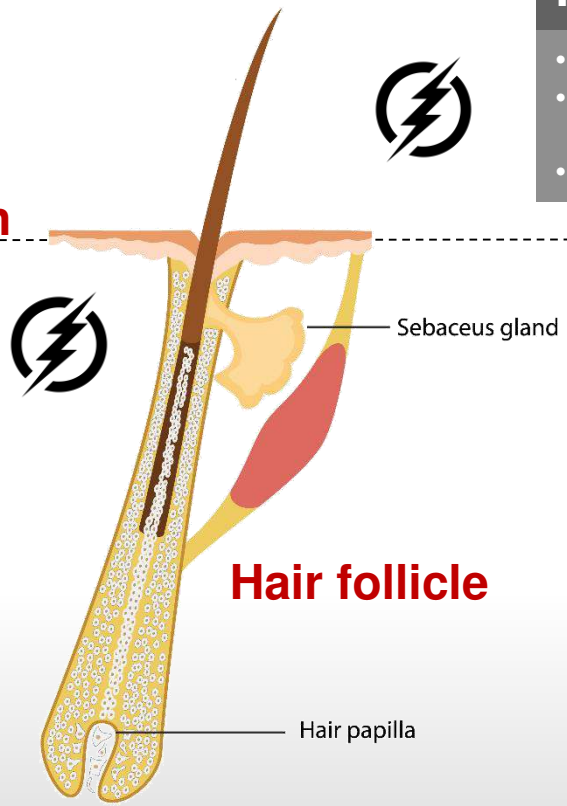
Post-emerging fiber

- UVR
- Chemical agents (hair dyes, pollutants)
- Oxidized scalp lipids

Pre-emerging fiber

- Oxidative metabolism
- UVR
- Smoking
- Inflammation (germs, pollutants, irritants)



(Trüeb 2015)

Oxidative stress can impact new pre-emerging hair fibers

UVR: ultraviolet radiation

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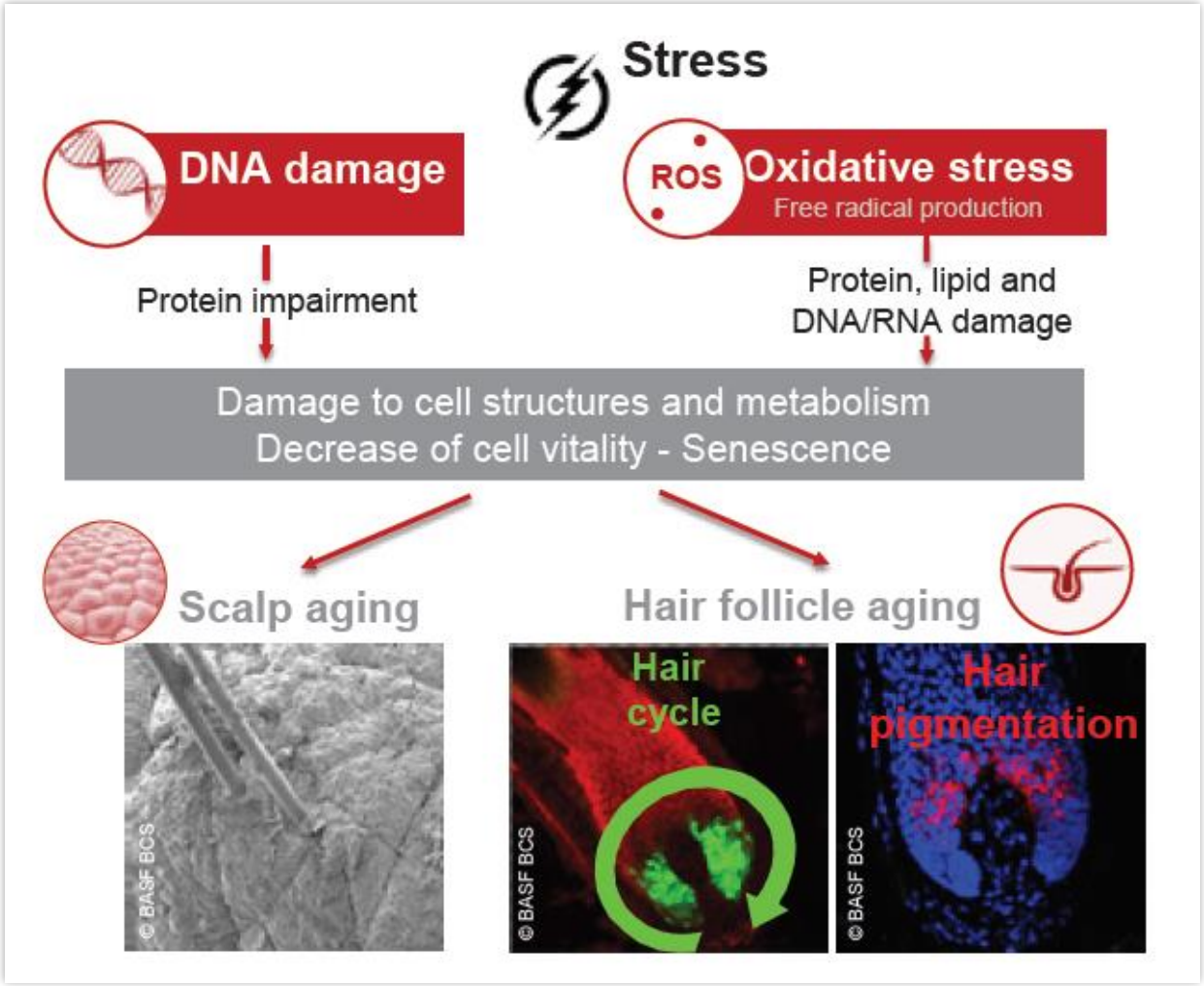
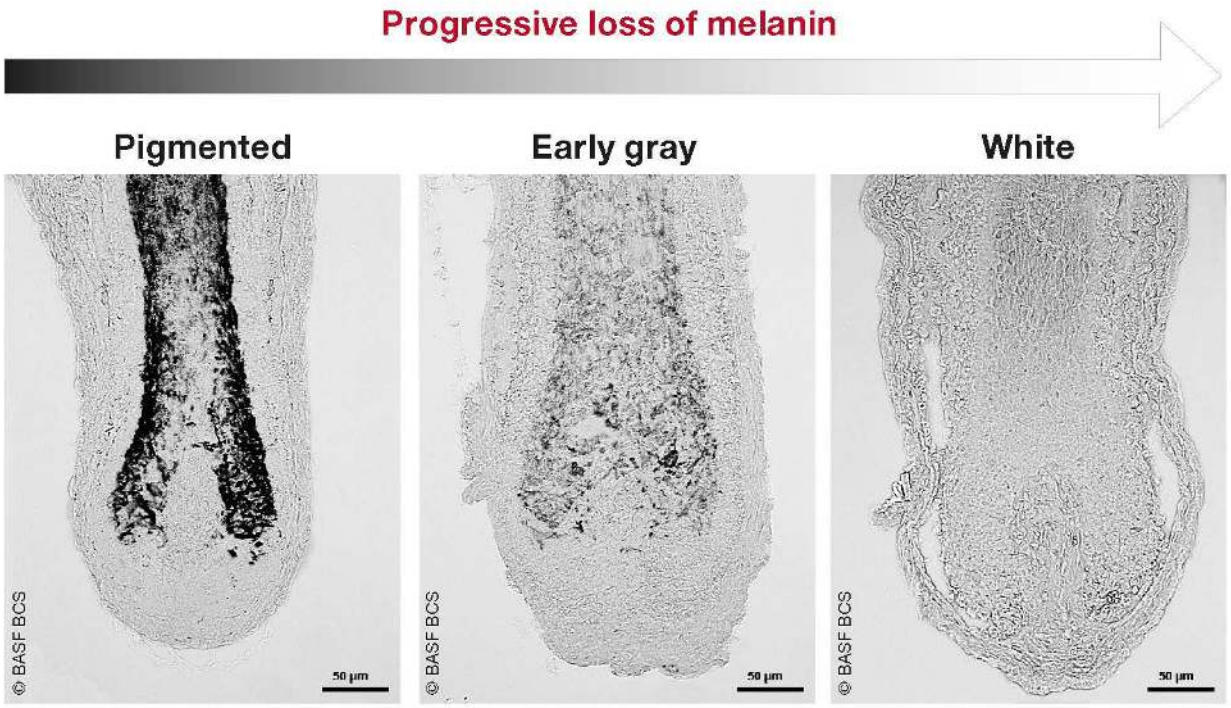
Impact of the environment
on scalp and hair follicle aging



Hair graying
How does hair become white?



Ability of mature melanocytes to produce and transfer pigment is impaired

Hair graying
How does hair become white?

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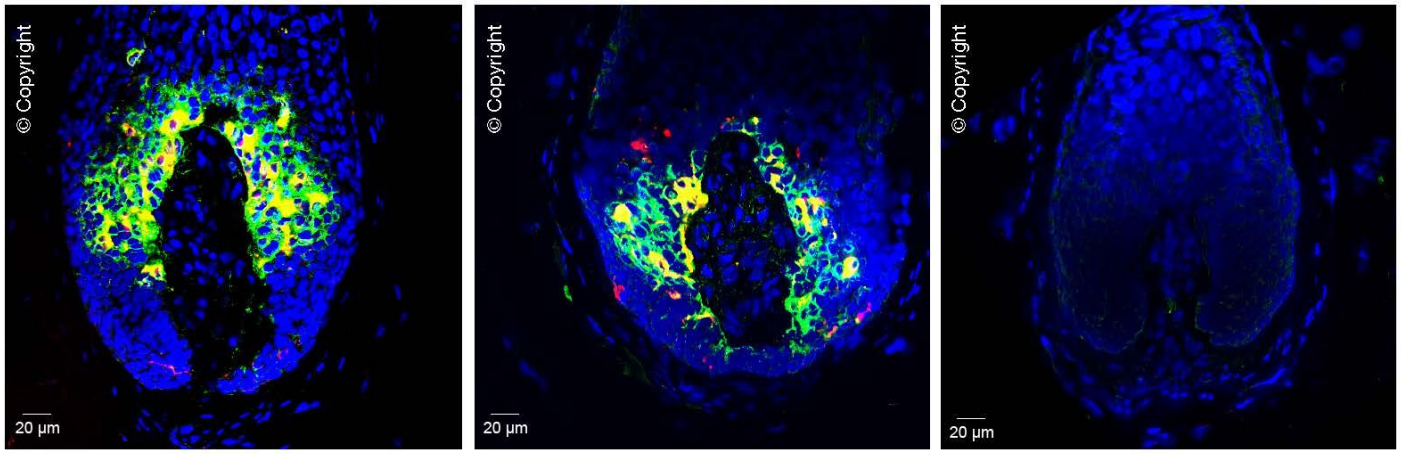
Progressive loss of melanocytes



Pigmented **Early gray** **White**

Mature hair follicle melanocytes are depleted in human hair graying

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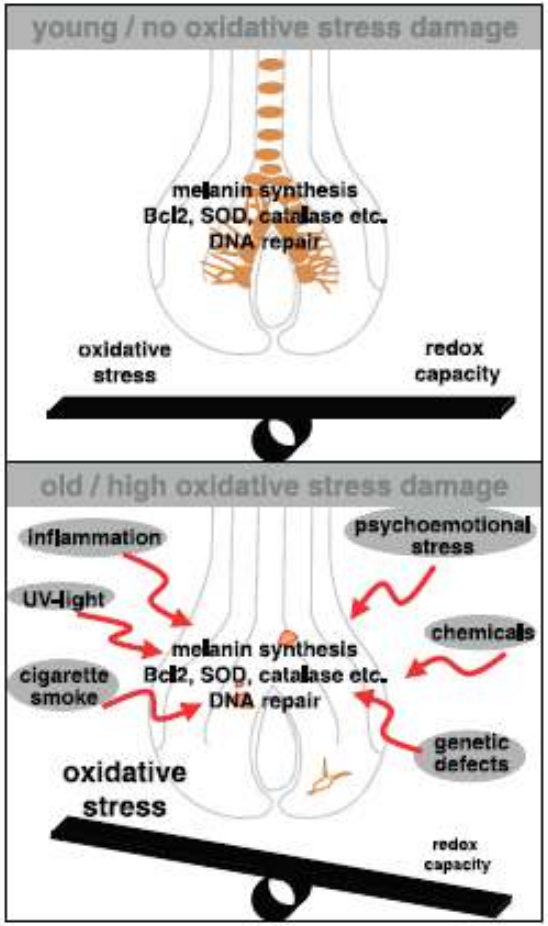
Hair graying
Key role of oxidative stress & DNA damage

In white hair follicle:

- ✓ Accumulation of H_2O_2
- ✓ Higher level of apoptosis in melanocytes
- ✓ Impaired antioxidant mechanisms
- ✓ Impaired DNA repair



(Arck *et al.*, 2006)

Young subjects

Melanocytes in pigmented growing hair follicles can deal with endogenous oxidative stress caused by melanin production

Misbalance of redox status

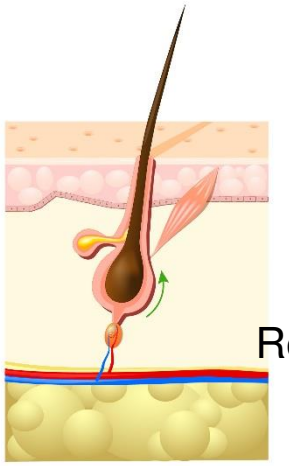
Old subjects

- alteration in pigment production
- anti-oxidant and repair enzymes decrease

This results in break-down of the hair follicle melanocyte redox capacity and subsequent deleterious oxidative stress damage leading to hair graying

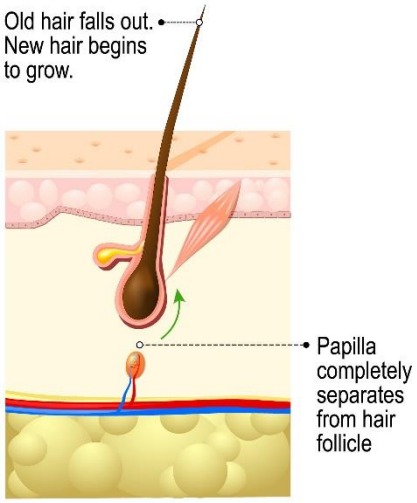
Hair loss / hair fall

Hair growth cycle is impaired

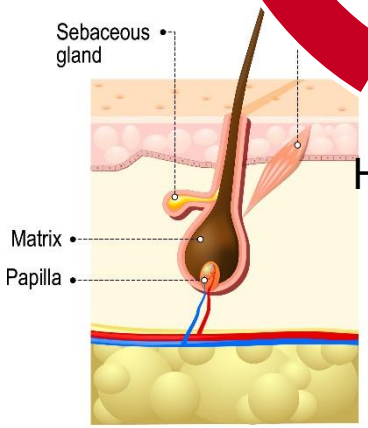


Catagen
Regression phase
(1-2 weeks)

Telogen
Resting phase
(5-6 weeks)



Exogen
Club hair loss



Anagen
Hair growing phase
(2-6 years)

Hair loss:
A : decrease
T : increase
Ratio A/T : decrease

How to keep young and beautiful HAIR?

By acting on both the SCALP and the HAIR FOLLICLE

Care Creations™

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- *In vitro* data
- *In vivo* data

Care Creations™

DN-Age™
Cassia alata

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Botanical information

Family: Fabaceae (*subfamily Caesalpinioideae*)

Species: *Cassia alata* (*Senna alata* (L.) Roxb)

Common name: Golden candle, Candle Bush, Ringworm plant (UK), Dartier, Cassier (Fr)

Distribution: Exotic plant found in diverse habitats especially in **sunny and moist areas** in Caribbean, South America, India, Africa – Native to Central America

Description: Ornamental flowering plants - **Easy to grow** with little invasive potential : forms thickets through natural propagation - Perennial – **Persistent leaves** : give aspect to be **evergreen**

Evoke the natural care for health & youthfulness

Sourcing

Origin: Burkina Faso

Plant part: leaves

Sustainability

- ✓ Respect of the plants ecosystem and biodiversity, no fertilizer or chemicals
- ✓ Monitored harvesting according good practices
- ✓ UICN: non-threatened species, considered invasive in some countries
- ✓ 100% renewable
- ✓ Non genetically modified

Medicinal uses

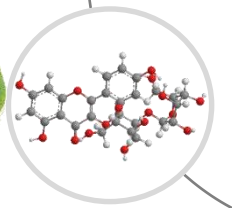
- ✓ Skin diseases in general (dermatitis, eczema, mycosis), anti-inflammation, infectious diseases, anti-diabetic, vitiligo
- ✓ Listed in French pharmacopoeia and Ayurveda

Give your scalp & hair the plant power to fight hair aging

Cassia alata leaves

1 Focused extraction

2 Concentration



Kaempferol-3-O-sophoroside (K3OS)

DN-Age™

Nature identical

Kaempferol-3-O-sophoroside (K3OS) from the candle bush *Cassia alata* is a particularly efficient antioxidant system to fight against UV-induced DNA damage.

BASF Beauty Creations has taken inspiration from plant defense strategies to supply human scalp and hair follicle with a complementary protection to slow down hair aging.

Our targeted extraction of *Cassia alata* leaves provides DN-Age™ titrated in K3OS (flavonoids), which has demonstrated *in vivo* its benefits to preserve hair from graying and keeping with its strength properties.

DN-Age™
A K3OS-titrated extract from leaves

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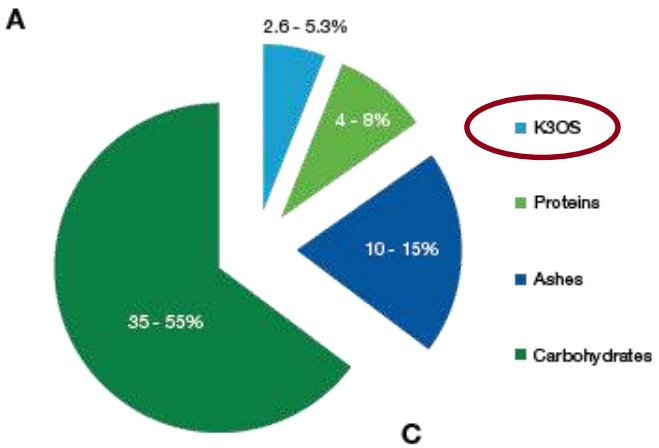
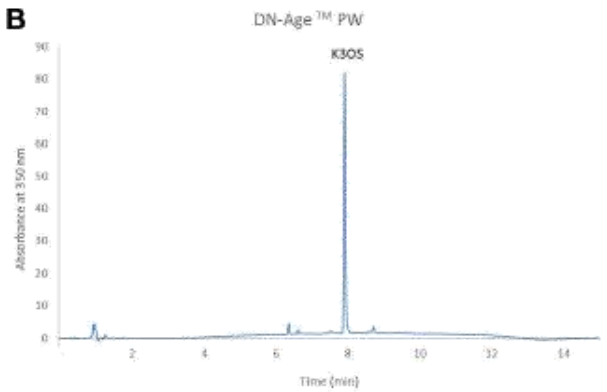
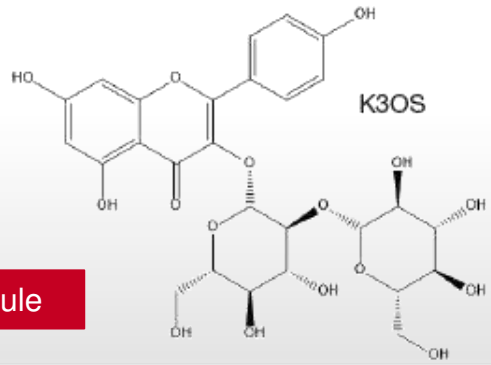
Typical phytochemical composition of the plant extract matter (based on 3 industrial batches).



UHPLC-PDA typical chromatogram profile (350 nm) of DN-Age™ PW.





K3OS molecule

Titration in K3OS
(replace titration in total flavonoids)

DN-Age™ (PW LS 9827) contains 1.2 - 2.4% of K3OS
(40-50% of active matter)

DN-Age™ (LS 9547) contains 0.1 - 0.2% of K3OS
(~4.15% of active matter)

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DN-Age™
Stability & Formulation

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Storage conditions

Shelf life: **2 years**

Storage temperature: 10-30 °C

Store protected from moisture and light

Formulation method

Add during the final process **below 60°C**, room temperature or cold processing.
pH: 4-8

Compatible with hair care standard formulations

Recommendation: for clear foaming products, to avoid cationic surfactants (opalescence)

Products in formula

1. 2. 3. 4. 5.

2 forms:

1. DN-Age™ PW LS 9827 (powder)
2. DN-Age™ LS 9547 (liquid)

DN-Age™ PW LS 9827 at 0,25% in:

3. hydro-alcoholic lotion
4. cationic hydrogel
5. cationic emulsion

* Updated formula data sheet already available in EMPI for DN-Age™ PW LS 9827

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- *In vitro* data
- *In vivo* data

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Hair aging impacts
Test methods

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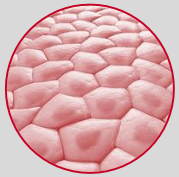
HAIR AGING - IMPACTS



SCALP

1. **Protection of sebum** (from squalene degradation by oxidation)
2. **Protection of DNA** (UV damage)
3. **Inflammation reduction**

FOLLICLE

1. **Protection of hair follicle melanocyte** (against oxidative stress)
2. **Prevention of hair follicle from senescence**

HAIR FIBER

- **Protection of natural hair color** (pigmentation)
- **Prevention of hair fall**
- **Maintenance of hair quality**

Care Creations™

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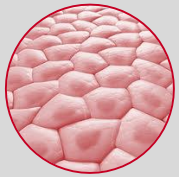
HAIR AGING - IMPACTS



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DN-Age™
Anti-scalp aging – *in tubo* data

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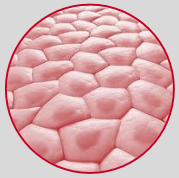
HAIR AGING - IMPACTS



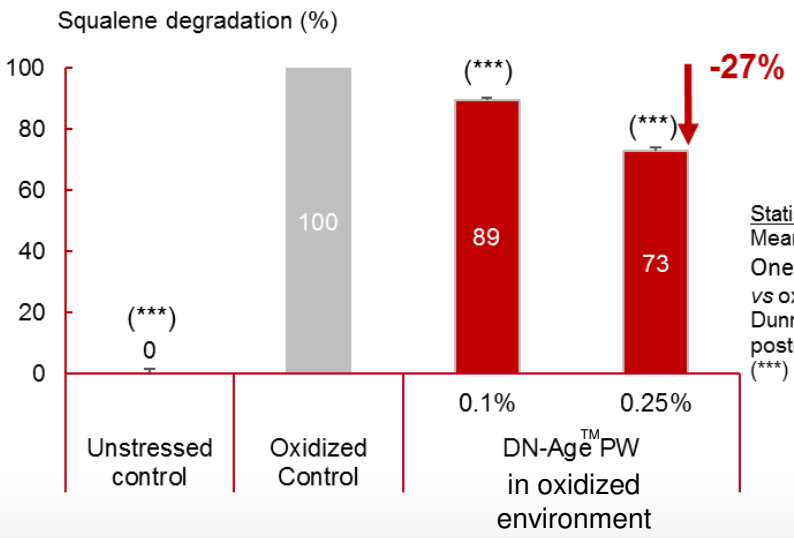
SCALP

- Squalene = key component of the sebum
- Oxidized squalene = marker of oxidative stress on scalp

1. **Protection of sebum**
   (from squalene degradation by oxidation)

- **Sebum protection against oxidation**

In tubo



Statistics:
Mean ± SD, n=3
One way ANOVA *vs* oxidized control
Dunnett's a posteriori test,
(***) p<0.001

Method
Squalene
$^1O_2$*
DN-Age™
Oxidized squalene
GC-MS
Gas chromatography–mass spectrometry

DN-Age™ helps to reduce sebum oxidation to preserve the health and youth of the scalp

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DN-Age™
Anti-scalp aging – *in vitro* data

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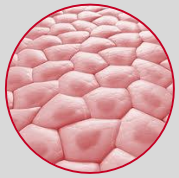
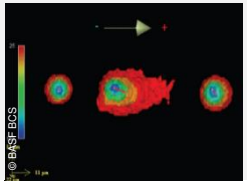
HAIR AGING - IMPACTS



SCALP

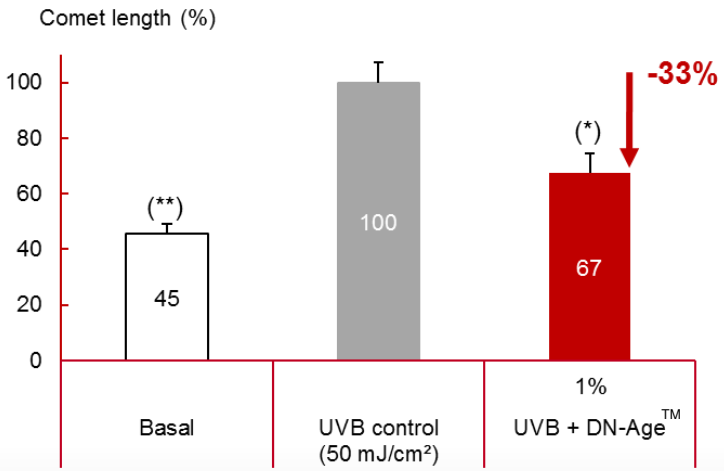
- UV induces DNA damage in cells (*incl.* fragmentation)
- DNA fragmentation is measured using **comet assay**: the intensity of the comet tail relative to the head reflects the number of DNA breaks



2. Protection of DNA
(UV damage)

- Fragmentation (Comet assay)
- Thymine dimers (ICC)

- **DNA damages in human keratinocytes**



In Vitro

Method

Human Keratinocytes
↓ 3 days
DN-Age™
UVB 50 mJ/cm²
↓ 24h
Comet assay
DNA fragmentation

Statistics:
mean +/-SEM
n=3
Student t test vs UVB control
(*) $p<0.05$
(**) $p<0.01$

DN-Age™ helps protect cells from UV-induced DNA damage: 33% less of DNA fragmentation

DN-Age™ LS9547 (liquid form)

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DN-Age™
Anti-scalp aging – *in vitro* data

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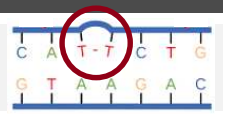
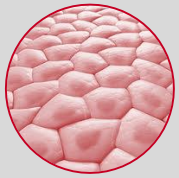
HAIR AGING - IMPACTS

- UV exposure forms direct dimers of thymine (bonds) in the DNA
- Thymine dimers in DNA disturb cell function



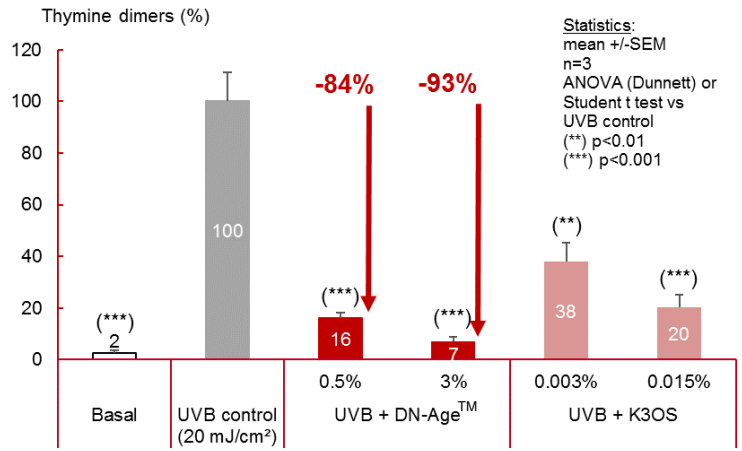


SCALP

2. Protection of DNA
(UV damage)

- Fragmentation (Comet assay)
- Thymine dimers (ICC)

- **DNA damages in human keratinocytes**



Method

Human Keratinocytes

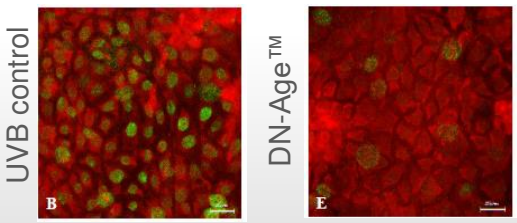
↓ 4 days

DN-Age™

UVB 20 mJ/cm²

↓ 4h

ICC
Thymine dimers



DN-Age™ helps to protect cells from UV-induced DNA damage: 84% less of thymine dimers

DN-Age™ LS9547 (liquid form)
ICC: immunohistochemistry

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DN-Age™
Anti-scalp aging – *in vitro* data

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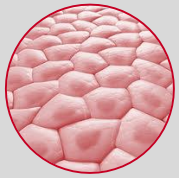
HAIR AGING - IMPACTS

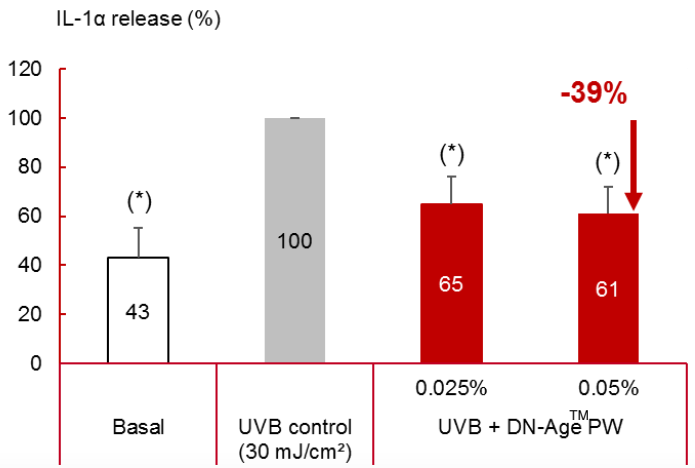


SCALP

- Interleukine 1 alpha (IL-1α) is a mediator of inflammation
- Scalp inflammation plays a role in hair loss

3. Inflammation reduction

▪ **Inflammation in human keratinocytes**



Method

Human keratinocytes
↓ 3-5 days
DN-Age™ PW
↓ 1 hour
UVB 30 mJ/cm²
↓ 1 day
ELISA

Statistics:
Mean ± SEM
n=4
ANOVA *vs* UVB control
Dunnett's test
(*) p<0.05

DN-Age™ helps to decrease inflammation in cells: 39% less of IL-1α

Care Creations™

Hair aging impacts
Test methods

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HAIR AGING - IMPACTS

SCALP

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FOLLICLE

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2. **Prevention of hair follicle from senescence**

HAIR FIBER

- **Protection of natural hair color** (pigmentation)
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Care Creations™

DN-Age™
Anti-hair graying - background

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HAIR AGING - IMPACTS

Oxidative stress impairs the function of follicle's melanocytes which produce the hair pigment

A "caretaker" protein, the ATM, is linked to pigmentation and can be used to measure the level of oxidative stress in the melanocytes

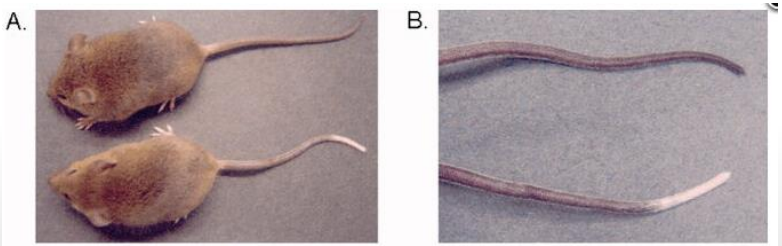
1. **Protection of hair follicle melanocyte**
   (against oxidative stress)
   - ATM as a stress marker
   - ATM protein level (WB or ICC)
   - Activated ATM protein level (WB)

Premature canities associated with **A-T syndrome** (gray hair in some young children) and **vitiligo** (accumulation of H_2O_2)

Mice with ATM$^{-/-}$ mutations have non-pigmented tail regions

WT

ATM -/-



Hibna, J. C., *et al*. The anatomical record. 290:243–250 (2007)

WB: western blot
ICC: immunohistochemistry

A-T: ataxia-telangiectasia syndrome
ATM: ataxia-telangiectasia mutated

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Anti-hair graying - background

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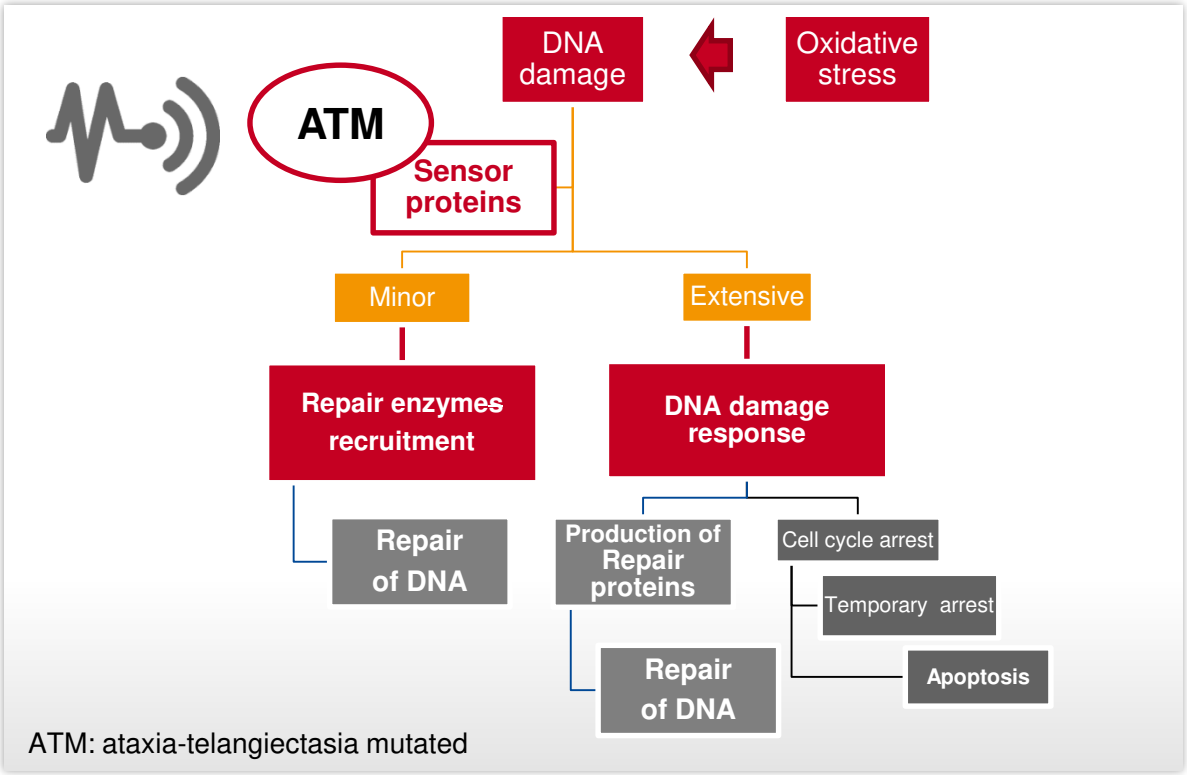
HAIR AGING - IMPACTS

FOLLICLE

1. **Protection of hair follicle melanocyte**
   (against oxidative stress)

- ATM as a stress marker
- ATM protein level (WB or ICC)
- Activated ATM protein level (WB)



ATM: ataxia-telangiectasia mutated

ATM is a key regulator of the cellular response to DNA double strand breaks and oxidative stress

Care Creations™

DN-Age™
Anti-hair graying - collaboration

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Hair follicle melanocytes are susceptible to oxidative stress

- ✓ Recent advances in the biology of pigmentation suggest a possible role of the **ATM protein** (Ataxia-Telangiectasia Mutated) in the graying process, particularly in the management of **redox status** in human hair melanocytes.

- ✓ *In collaboration with the **Centre of skin Sciences** at the University of Bradford and the team of **Prof. Tobin**, we found that **ATM is closely associated with the level of pigmentation in hair follicle** and the **viability of human hair follicle melanocytes***

Stephen K. Sikkink
Desmond J. Tobin

- ✓ **ATM** could act as a **sensor of oxidative stress** in human hair follicle melanocytes *in vivo* and *in vitro* and could be used as a **biomarker of oxidative stress** in human hair follicle melanocytes

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DN-Age™
Anti-hair graying – *in vitro* data

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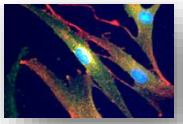
HAIR AGING - IMPACTS



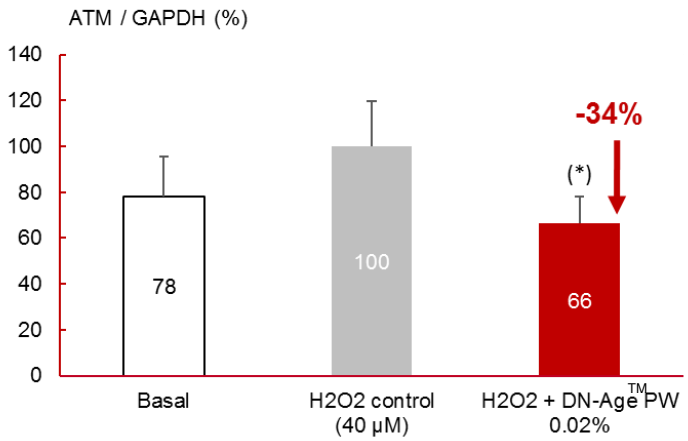
- ATM increases in cell proportionally to the oxidative stress
- Reduction of oxidative stress account for the decrease in ATM induction

FOLLICLE

1. **Protection of hair follicle melanocyte**
   (against oxidative stress)

- ATM as a stress marker
- ATM protein level (WB)
- Activated ATM protein level (WB)

- **ATM level in hair follicle melanocytes**



Method

Hair Follicle Melanocytes
↓ 48h
Oxidative stress By H_2O_2 40 µM
↓ 48h
DN-Age™
↓ 48h
Western Blot

Statistics:
Mean ± SD; n=3
Student's t-test *vs* H_2O_2 control
(*) $p<0.05$

ATM level is decreased with DN-Age™ : DN-Age™ protects hair follicle melanocytes from oxidative stress to preserve from hair graying

Results issued from the collaboration with Bradford University – Prof. DJ Tobin

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DN-Age™
Anti-hair graying – *in vitro* data

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HAIR AGING - IMPACTS

FOLLICLE

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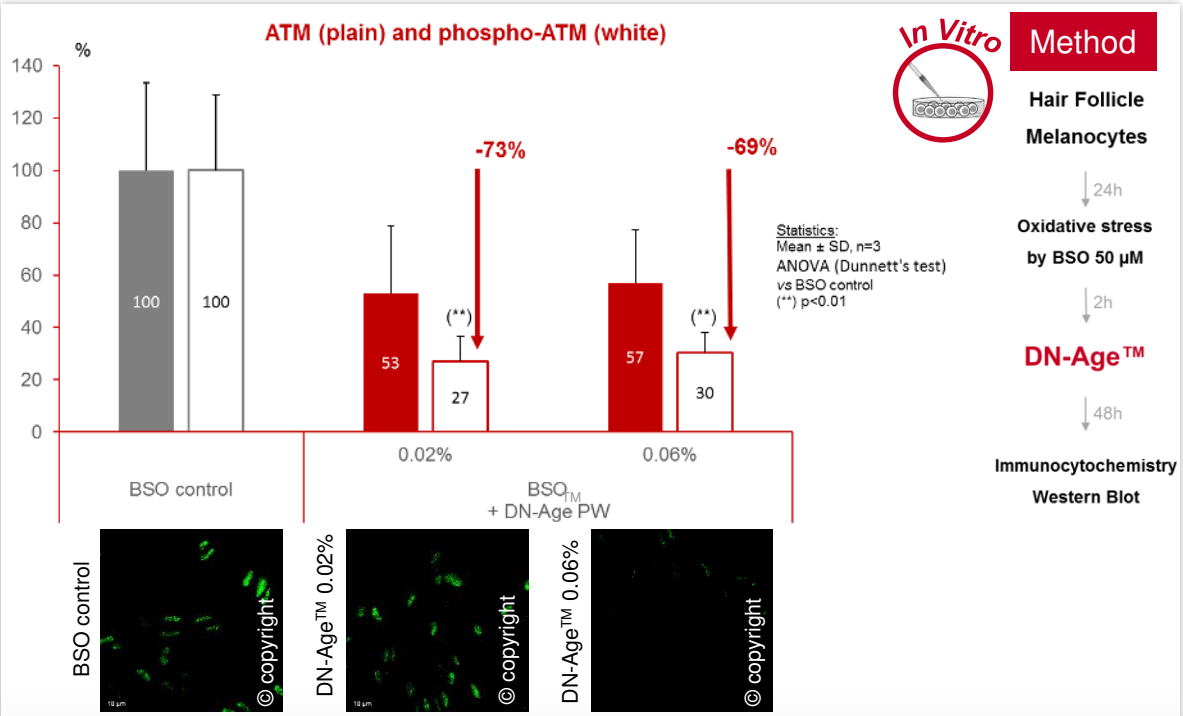

DN-Age™ helps hair follicle melanocytes to cope with oxidative stress

Results issued from the collaboration with Bradford University – Prof. DJ Tobin

BSO : buthionine sulfoximine (H_2O_2 inducer in cells)

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DN-Age™
Anti-hair aging – *in vitro* data

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HAIR AGING - IMPACTS

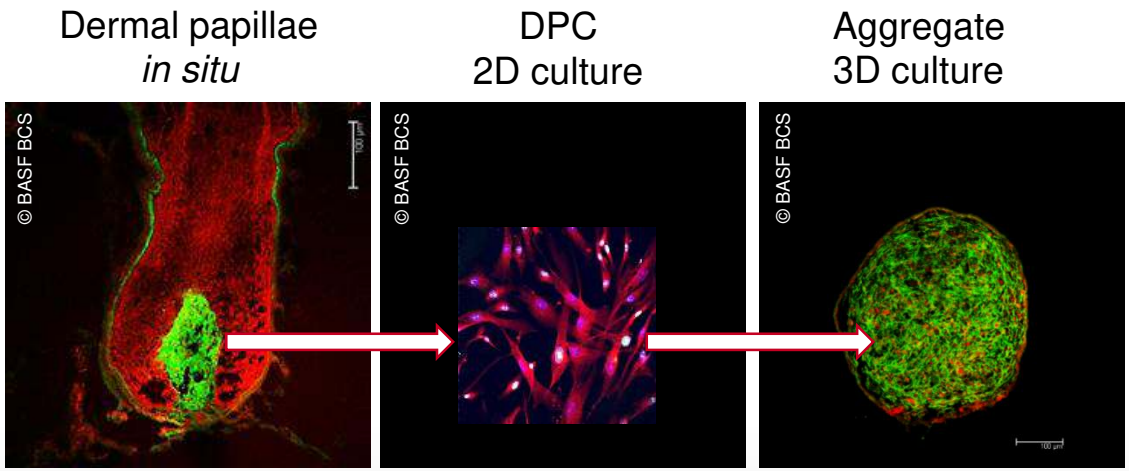
FOLLICLE

2. Prevention of hair follicle from senescence

- 3D cellular models
- Cell number in aggregates
- Autofluorescence in aggregates

New BASF 3D model

A 3D model of pseudo-papillae:
Dermal papilla fibroblasts (DPC) in aggregates



The 3D model of pseudo-papillae preserves specific properties of dermal papillae

Higgins *et al* 2010

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DN-Age™
Anti-hair aging – *in vitro* data

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HAIR AGING - IMPACTS

FOLLICLE

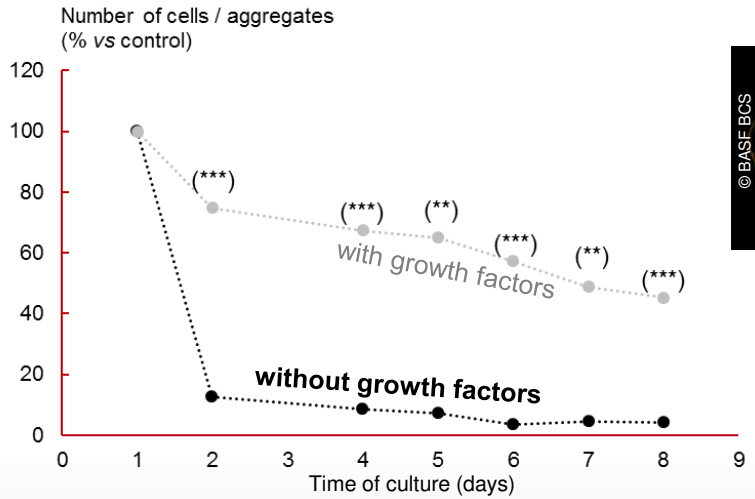
- Dermal papilla fibroblasts cultured in aggregates form a pseudo-papillae
- Deprivation of growth factors is a cellular model of aging process
- Number of dermal papilla fibroblasts is associated with hair growth potential

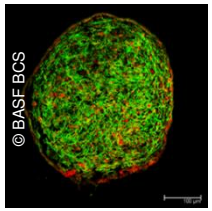
New BASF 3D model

2. Prevention of hair follicle from senescence

- 3D cellular models
- Cell number in aggregates
- Autofluorescence in aggregates

- **Dermal papilla fibroblasts in aggregates**





In Vitro

Method

Dermal papilla fibroblasts aggregation by centrifugation

1-10 days

Number of cells by Flow cytometry

Statistics:
Mean ± SD, n=4-6
Student's t-test or Mann-whitney test
vs untreated control
(**) p<0.01
(***) p<0.001

Number of dermal papilla cells decreases with time in the 3D model of premature aged pseudo-papillae

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DN-Age™
Anti-hair aging – *in vitro* data

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HAIR AGING - IMPACTS

FOLLICLE

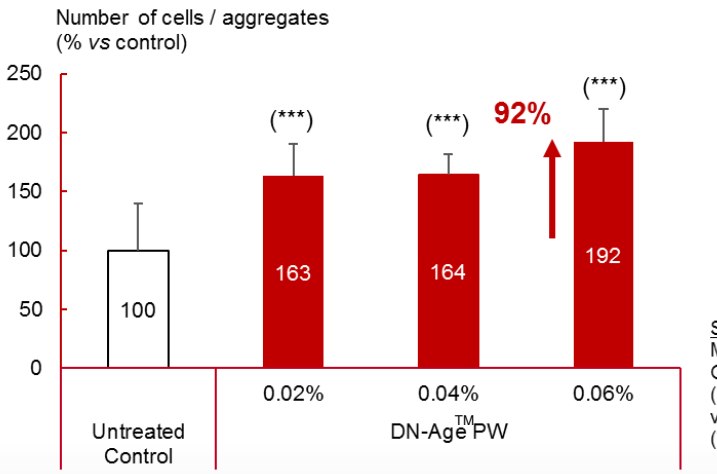
- Dermal papilla fibroblasts cultured in aggregates form a pseudo-papillae
- Number of dermal papilla fibroblasts is associated with hair growth potential

New BASF 3D model

2. Prevention of hair follicle from senescence

- 3D cellular models
- Cell number in aggregates
- Autofluorescence in aggregates

- **Dermal papilla fibroblasts in aggregates**



Statistics:
Mean ± SD, n=3
One way ANOVA
(Fisher LSD test)
vs untreated control
(***) p<0.001

In Vitro

Method

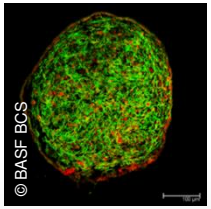
Dermal papilla fibroblasts aggregation by centrifugation

↓

DN-Age™

↓ 5 days

Number of cells, Autofluorescence by Flow cytometry



DN-Age™ improves the number of dermal papilla fibroblasts to prevent the hair from aging

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DN-Age™
Anti-hair aging – *in vitro* data

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HAIR AGING - IMPACTS

FOLLICLE

- Dermal papilla fibroblasts cultured in aggregates form a pseudo-papillae
- Autofluorescence is a marker of senescence

New BASF 3D model

2. Prevention of hair follicle from senescence

- 3D cellular models
- Cell number in aggregates
- Autofluorescence in aggregates

- **Dermal papilla fibroblasts in aggregates**

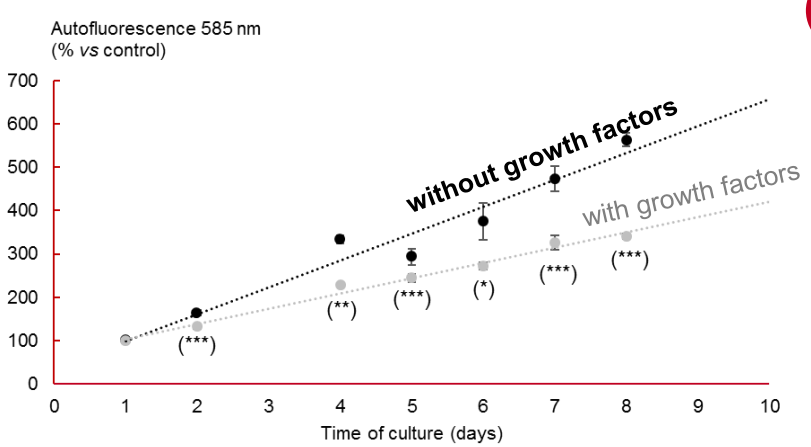
In Vitro

Method

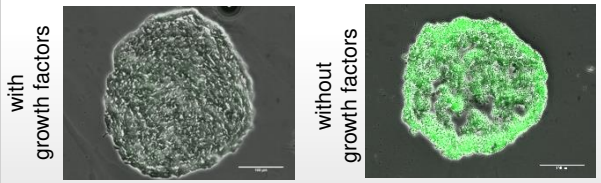
Dermal papilla fibroblasts aggregation by centrifugation

↓ **1-10 days**

Autofluorescence (488/584 nm) by flow cytometry



Statistics:
Mean ± SD, n=4-6
Student t test or
vs untreated control
(*) p<0.05
(**) p<0.01
(***) p<0.001



Autofluorescence increases with time in the 3D model of premature aged pseudo-papillae

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DN-Age™
Anti-hair aging – *in vitro* data

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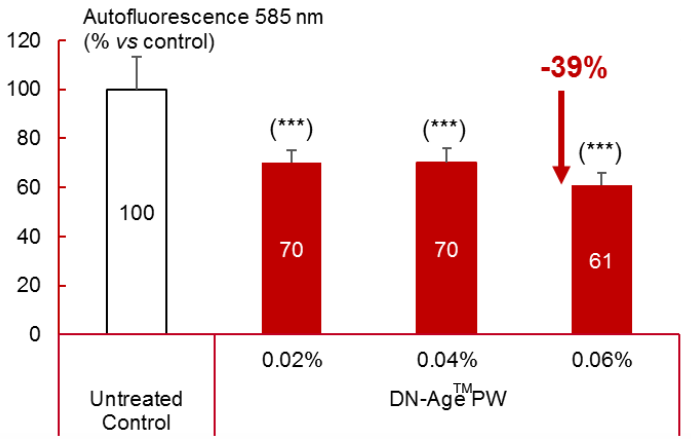
HAIR AGING - IMPACTS

FOLLICLE

- Dermal papilla fibroblasts cultured in aggregates form a pseudo-papillae
- Autofluorescence is a marker of senescence

New BASF 3D model

- Dermal papilla fibroblasts in aggregates



Statistics:
Mean ± SD, n=6-12
One way ANOVA
(Fisher LSD test)
(***) p<0.001

Method

Dermal papilla fibroblasts aggregation by centrifugation

DN-Age™

5 days

Number of cells, Autofluorescence by Flow cytometry

2. Prevention of hair follicle from senescence

- 3D cellular models
- Cell number in aggregates
- Autofluorescence in aggregates

Autofluorescence is decreased with DN-Age™ : follicles are preserved from senescence

Care Creations™

Hair aging impacts
In vitro tests – Summary

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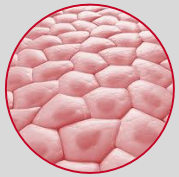
HAIR AGING - IMPACTS



SCALP

- **36% less** of squalene oxidation
- **33% less** of DNA fragmentation
- **93% less** of Thymine dimers
- **39% less** of IL-1α release

Protection of scalp against aging

FOLLICLE

- **73% less** activated ATM in hair follicle melanocytes (less oxidative stress)
- **92% more** cells in pseudo-papilla
- **39% less** autofluorescence in pseudo-papilla
- **48% more** hair fiber elongation

Protection of hair follicle against aging

HAIR FIBER

- **Protection of natural hair color** (pigmentation)
- **Prevention of hair fall**
- **Maintenance of hair quality**

Care Creations™

1 | Introduction: hair aging

2 | DN-Age™: product characteristics

3 | Scalp & Hair Care

- *In vitro* data
- ***In vivo*** **data**

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Hair aging impacts
Test methods

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HAIR AGING - IMPACTS

SCALP

1. **Protection of sebum** (from squalene degradation by oxidation)
2. **Protection of DNA** (UV damage)
3. **Inflammation reduction**

FOLLICLE

1. **Protection of hair follicle melanocyte** (against oxidative stress)
2. **Prevention of hair follicle from senescence**

HAIR FIBER

- **Protection of natural hair color** (pigmentation)
- **Prevention of hair fall**
- **Maintenance of hair quality**

Care Creations™

DN-Age™
Clinical test design

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HAIR AGING - IMPACTS

HAIR FIBER

Clinical test – design

1. Anti-hair fiber graying
2. Hair growth maintenance
3. Hair structure maintenance

Effect of a **scalp treatment** with **DN-Age™** in **emerging hair fiber**

Study 1

Salt & pepper women with **25%-50%** of white hair

√ **Gray hair color** (root)
√ **Hair growth**

Study 2

Women with **perceived brittle** and **fragile hair**

√ **Hair strength** (root)
√ **Consumer perception**

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Clinical study 1
Anti-hair fiber graying

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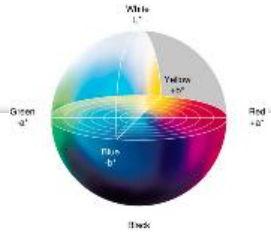
Application period

Day 0

Month 3 Evaluation

Month 6 Evaluation



L* = luminance = relative brightness from total darkness

Volunteer panel

2 groups
- **28** females for Placebo
- **27** females for **DN-Age™ PW**

Characteristics
- Salt & pepper with **25%-50%** of white hair (expert grading)
- 30-70 years old
- **No hair dyes during the study**

Test products

- Hydro-alcoholic lotion (placebo)
- Hydro-alcoholic lotion + **0,25% DN-Age™ PW**

Application

Double blind, randomized study
- Full head on the whole scalp without rinsing (leave-on formula)
- Daily application (once a day)
- **6 months of treatment**

Instrumental evaluation

- **Gray hair color** (base)
  Colorimetry (Chromameter™)
  Time points: baseline, 3 months, 6 months
- **Hair growth**
  Phototrichogram (Trichoscan)
  Time points: baseline, 6 months

Clinical study 1
Chromametry - Method Description

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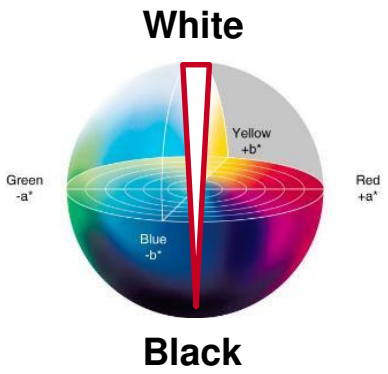
Hair color measurement by chromametry – Minolta

Principle

- The International Commission on Illumination defines chromametry color parameters, the L*a*b color space
- L* = Luminance ; a* = green-red axis ; b* = blue-yellow axis
- Individual Typological Angle (ITA°)= [Arc Tangent ((L*-50)/b*)] 180 / π

Evaluated parameters:

- **L* = Luminance**
- White hair color is directly related to the **value of L*** (increase of L* means increase of white hair)
- Intensity of hair color measurements on hair at the base (close to the scalp) from 3 different scalp areas



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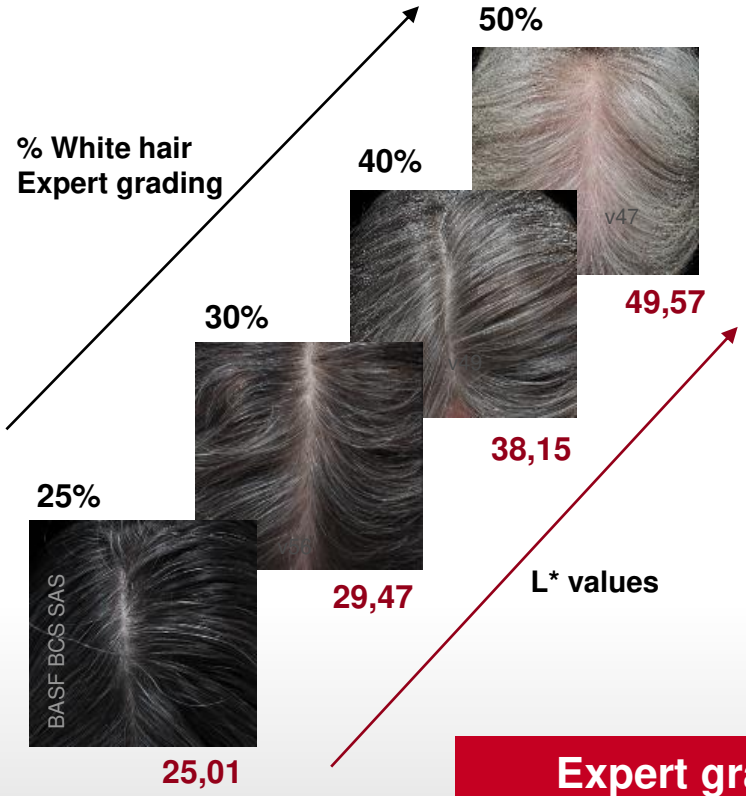
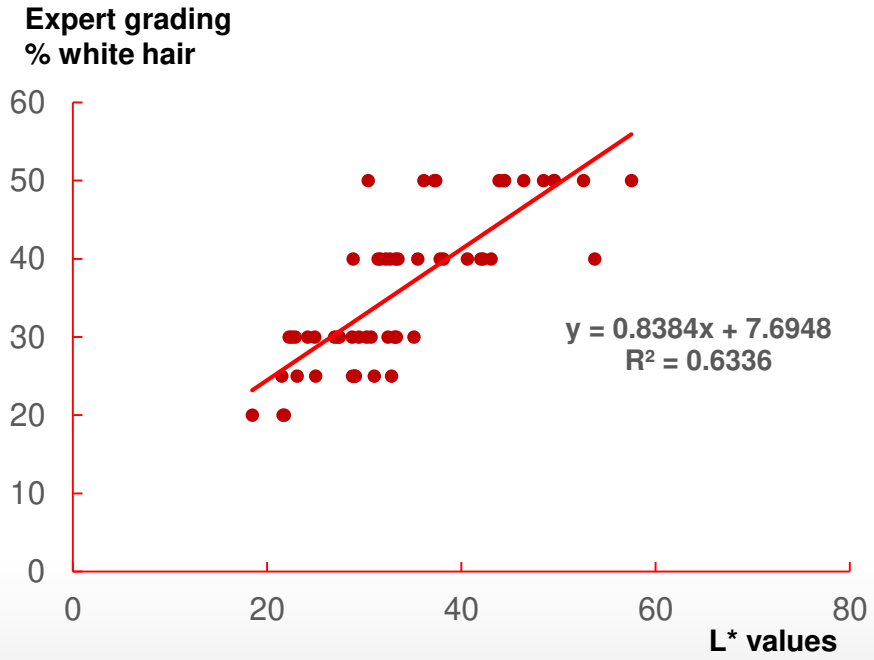
Clinical study 1
Chromameter - Method validation

- **Correlation between expert grading and L* values**





Expert grading and L* values were correlated (R^2=0.63)

DN-Age™
Improvement of hair color at the root

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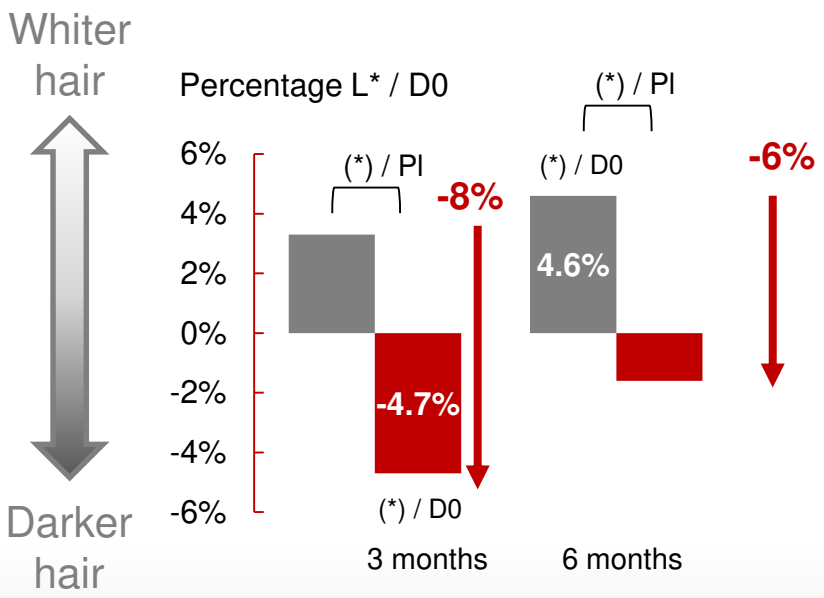
HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. **Anti-hair fiber graying**
2. Hair growth maintenance
3. Hair structure maintenance



DN-Age™ improves the appearance of gray hair

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DN-Age™
Improvement of hair color at the root

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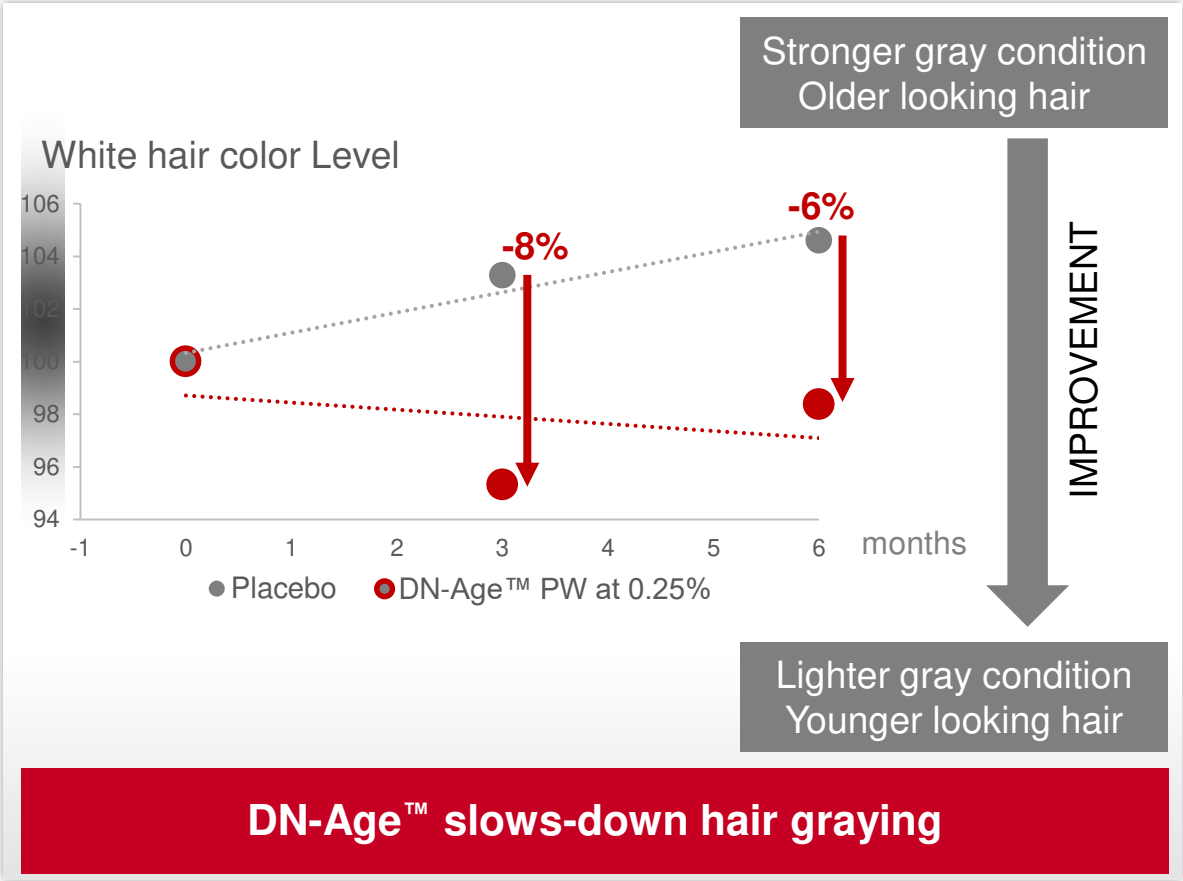
HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. **Anti-hair fiber graying**
2. Hair growth maintenance
3. Hair structure maintenance



DN-Age™ slows-down hair graying

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DN-Age™
Improvement of hair color at the root

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HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. **Anti-hair fiber graying**
2. Hair growth maintenance
3. Hair structure maintenance

A difference of 8% in hair color between 2 different persons is visible

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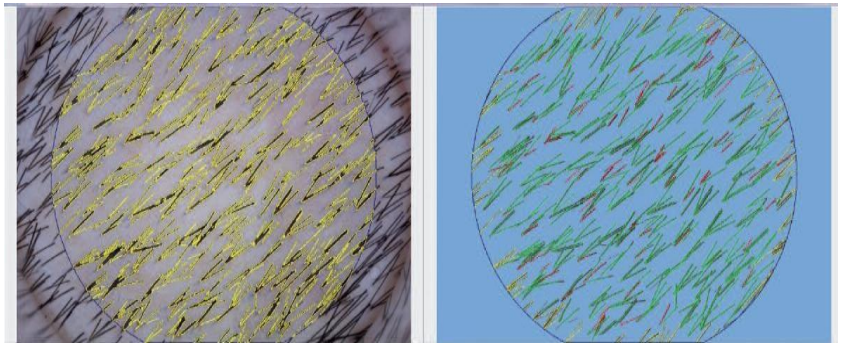
Clinical study 1
Phototrichogram – Method

- **Hair growth parameters – Trichoscan**



- **Considered parameters:**
  - ✓ Anagen hair density: number of growing hair fibers/cm²
  - ✓ Telogen hair density: number of ingrowing hair fibers/cm²
  - ✓ A/T ratio

Measurements

Day 0
1st measurement + shaving

Month 6
Evaluation

- ✓ Shaving of 1cm² area (on the right side of the scalp)
- ✓ Three pictures are taken with the Trichoscan (Dermoscan).
- ✓ 48 hours later (T0+48h and T6+48h) another three pictures are taken of the same area.

DN-Age™
Reduces hair fall

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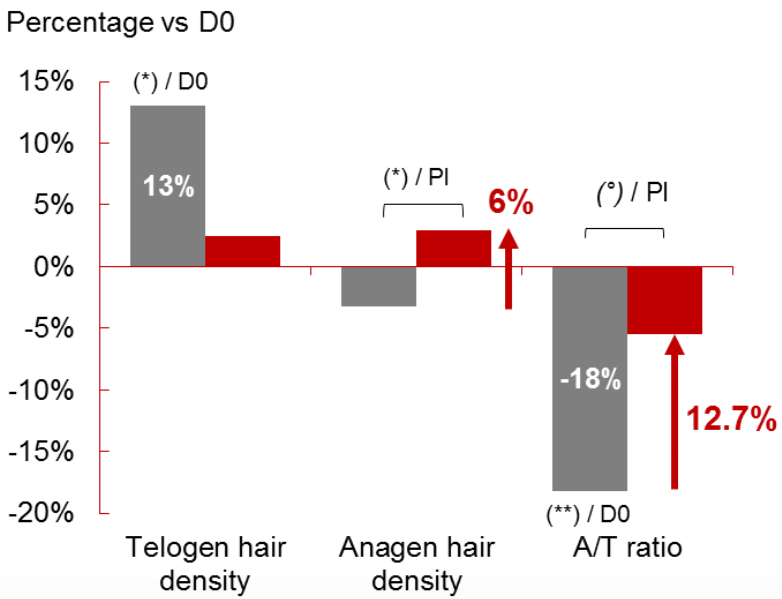
HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. Anti-hair fiber graying
2. **Hair growth maintenance**
3. Hair structure maintenance



DN-Age™ counteracts hair fall

Care Creations™

DN-Age™
Reduces hair fall

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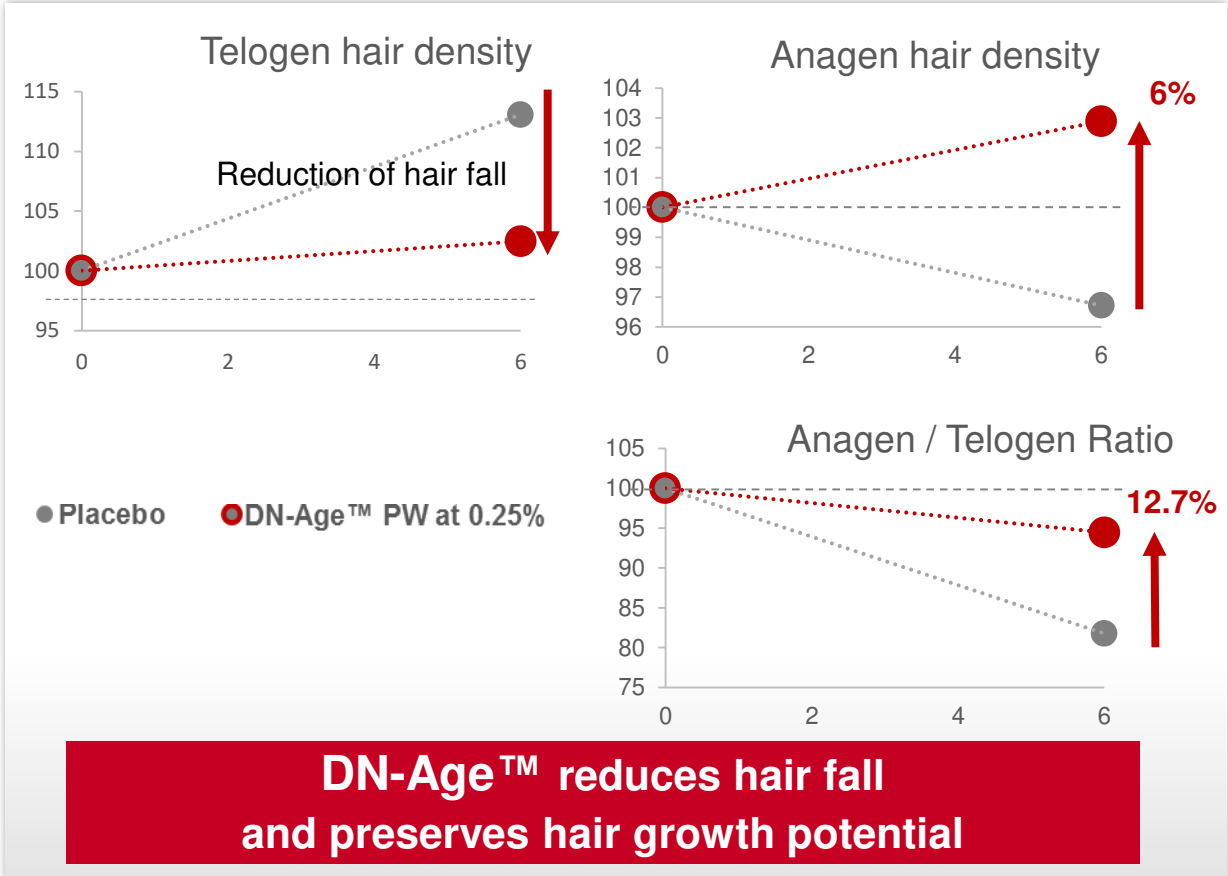
HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. Anti-hair fiber graying
2. **Hair growth maintenance**
3. Hair structure maintenance



DN-Age™ reduces hair fall and preserves hair growth potential

Care Creations™

Clinical study 2:
Tensile behavior

Day 0 → **Application period** → Month 6 Evaluation

Single hair strength measurement

Volunteer panel

2 groups

- **17** females for Placebo
- **20** females for **DN-Age™ PW**

Characteristics

- 34-65 years old
- Fragile & brittle hair (self-perception)
- Fine/medium hair (expert evaluation)
- **Be willing to use hair colorant**

Test products

- Hydro-alcoholic lotion (placebo)
- Hydro-alcoholic lotion + **0.25% DN-Age™PW**

Application

Double blind, randomized study

- Full head on the whole scalp without rinsing (leave-on formula)
- Daily application (once a day)
- **6 months of treatment**

Instrumental evaluation

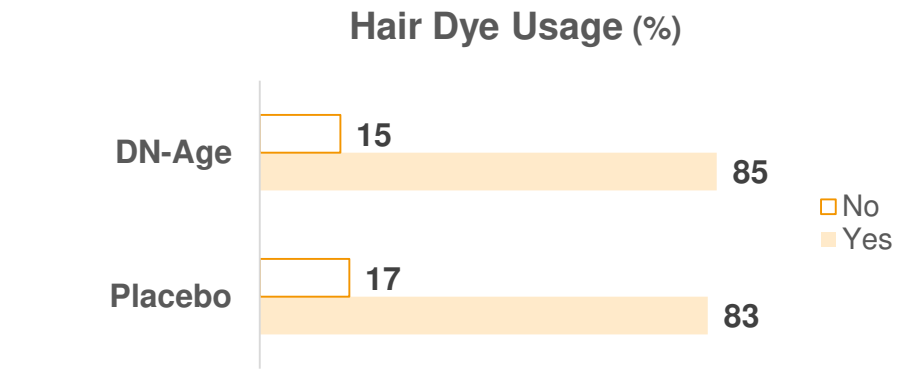
- **Tensile strength**
  DIA-STRON measurements (close to the scalp): single fiber diameter and tensile property
  Time points: baseline, 6 months
- **Subjective questionnaire**
  Time points: at 6 months
- **Images of hair fiber by electron microscopy (illustration only)**
  Time points: baseline, 6 months

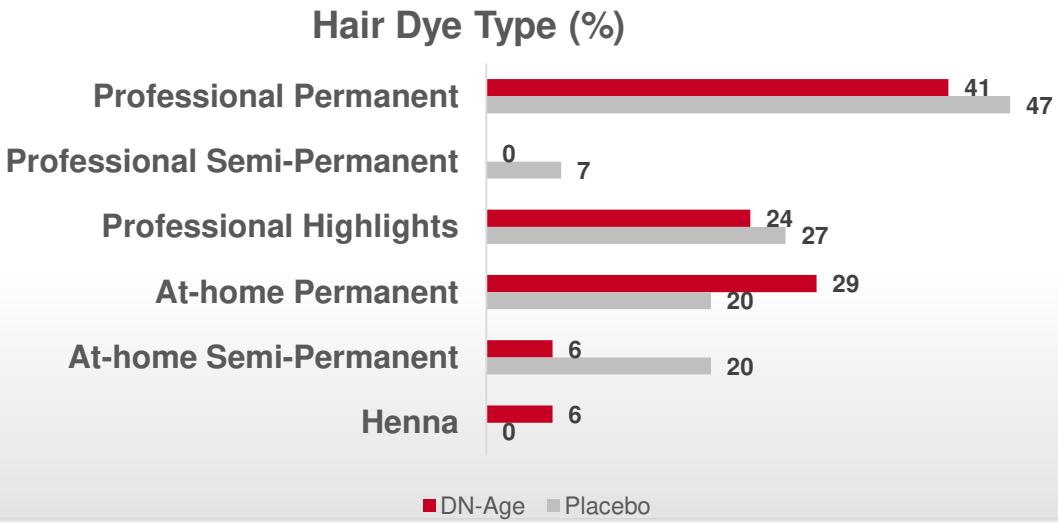
Clinical study 2:
Consumer behavior at baseline

- **Volunteer habits & practices**

✓ More than 80% of volunteers had hair dye usage in the two groups

✓ Hair dye type was mainly professional permanent





Clinical study 2:
Hair treatment description

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Before treatment, at D0

DN-Age™ Treatment

Hair strength and quality measured on hair before and after treatment

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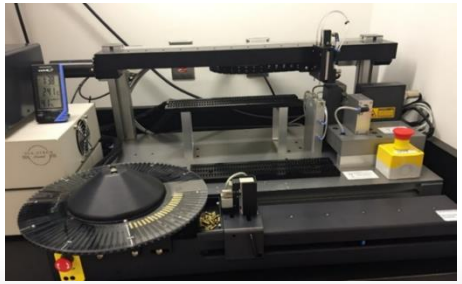
Tensile behavior of hair root
Method description



Single hair strength measurement

Tensile test – Dia-Stron

Mechanical properties of emerging hair fibers can be measured.

At least 50 fibers/volunteer, close to the scalp, were collected by a cosmetologist across the whole head and 40-45 were included for analysis.

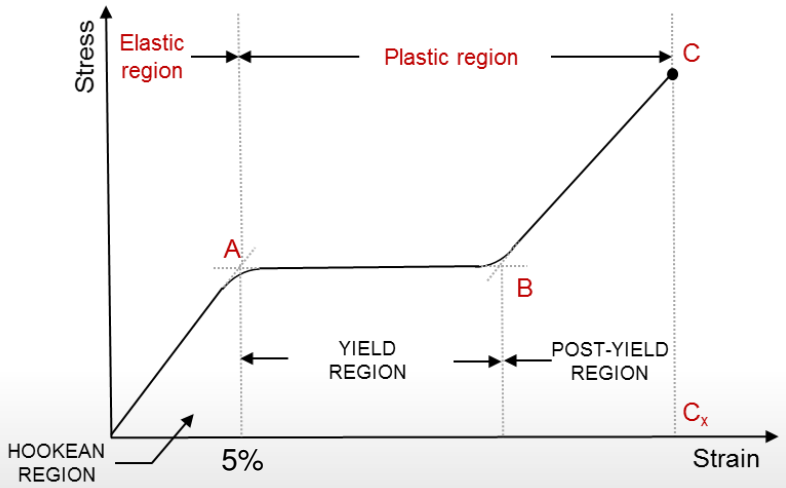
(At least a total of 2'800 single fibers were analyzed)

Tensile stress-strain curve

- ✓ **Post-Yield Gradient (gmf / % Strain):** area under the curve in the plastic segment where the stress suddenly increases before the break. Linked to disulfide cross-links of the fibers and resistance of β-keratin to stretching.
- ✓ **Break Extension (% Strain):** Break extension is the strain [in %] at the fiber break point. Extensibility max. of hair fiber.

A = Hookean limit
B = Turnover point
C = Break point
C_x = % extension to break

Stress-strain curve



DN-Age™
Clinical test design

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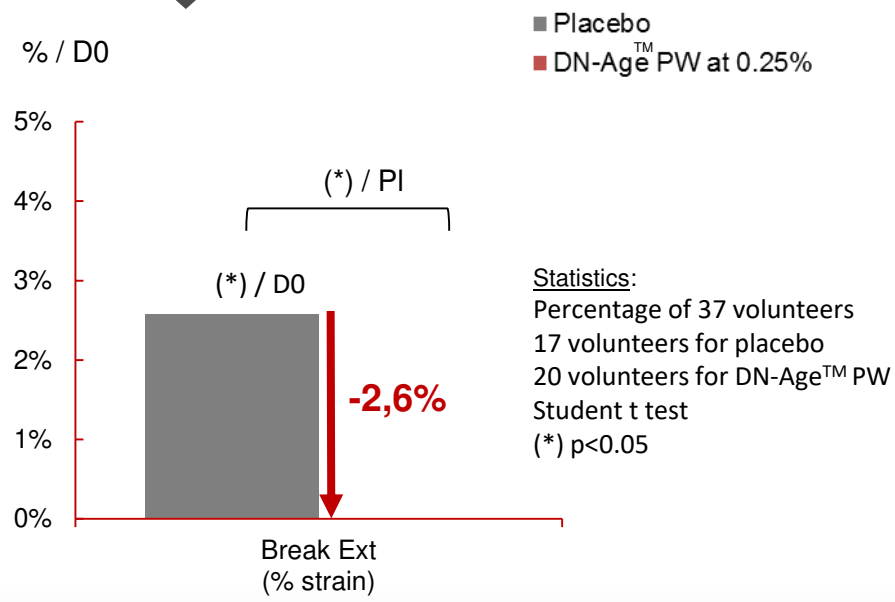
HAIR AGING - IMPACTS



Statistics:
Percentage of 37 volunteers
17 volunteers for placebo
20 volunteers for DN-Age™ PW
Student t test
(*) p<0.05

DN-Age™ counteracts the loss of hair resistance to stretching

Break extension (% Strain)

= Extensibility maximum of hair fiber
Break extension is the strain at the fiber break point

Increase of break extension in hair fiber due to:
- ✓ Bleaching (H_2O_2)
- ✓ Reductant use (ammonia)
- ✓ UV exposure

Increase in extensibility is linked to the reduction of disulfide groups in cortical domains (Rebenfeld 1966)

Care Creations™

DN-Age™
Clinical test design

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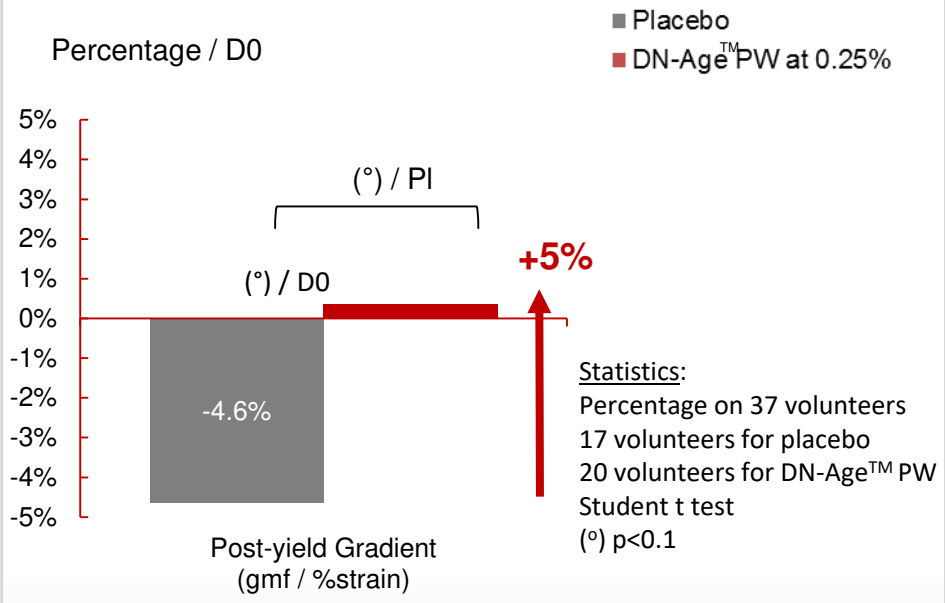
HAIR AGING - IMPACTS



Emerging hair fibers are stronger with DN-Age™

Post-Yield Gradient (gmf / % Strain)

= Ability of hair fiber to deform

Area under the curve in the plastic segment where the stress suddenly increases before breakage

Decrease of post-yield gradient in hair fiber after:

- ✓ Reductant use (ammonia)
- ✓ UV exposure

Dependent on the disulfide cross-links of the cortical domains of the fibers

(Rebenfeld 1966)

Care Creations™

DN-Age™
Clinical test design

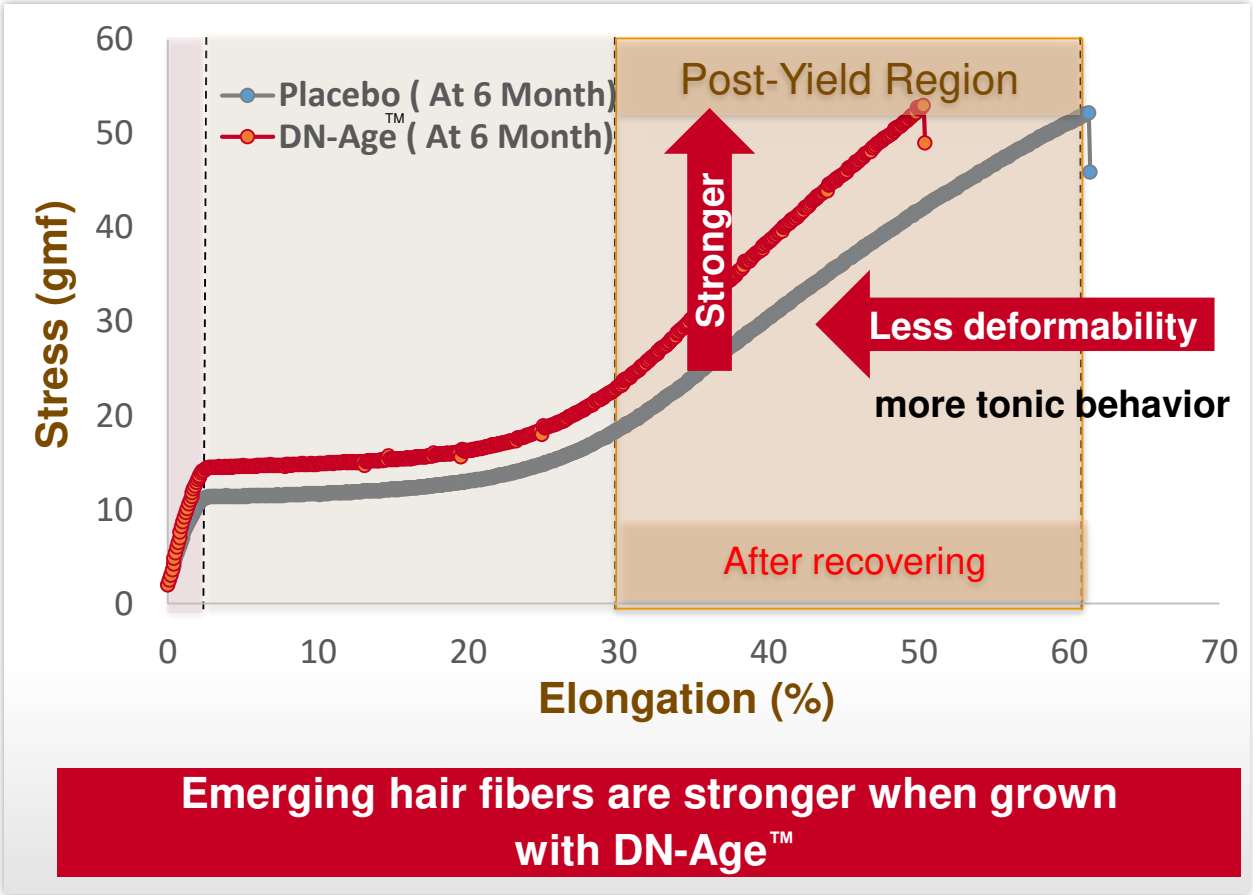
BASF
We create chemistry

HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. Anti-hair fiber graying
2. Hair growth maintenance
3. **Hair structure maintenance**



Emerging hair fibers are stronger when grown with DN-Age™

Care Creations™

DN-Age™
Clinical test design

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HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. Anti-hair fiber graying
2. Hair growth maintenance
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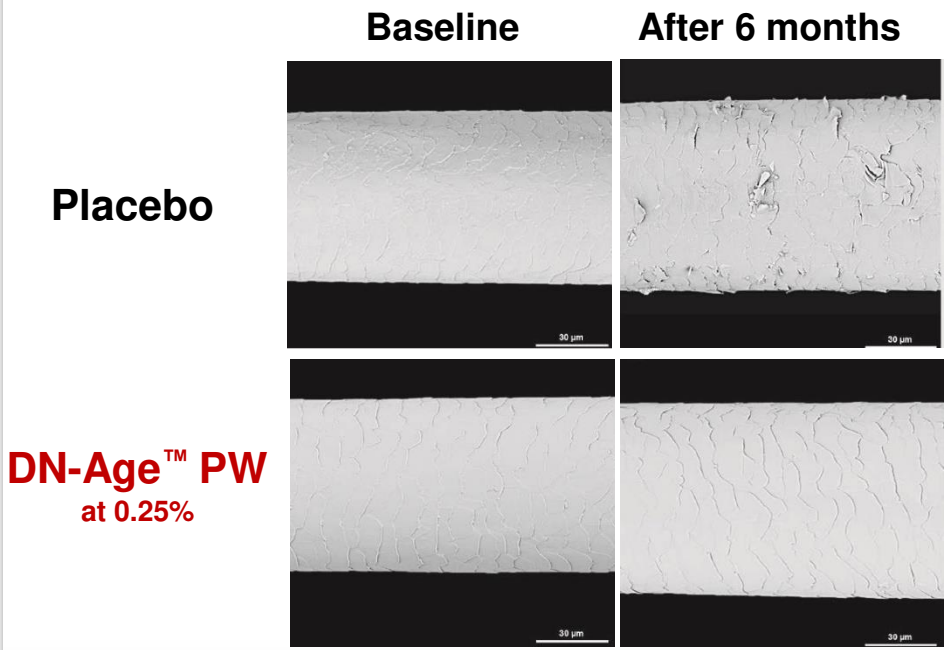

Hair fiber near the scalp treated with DN-Age™ shows a **well-preserved cuticle morphology**

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DN-Age™
Consumer perception

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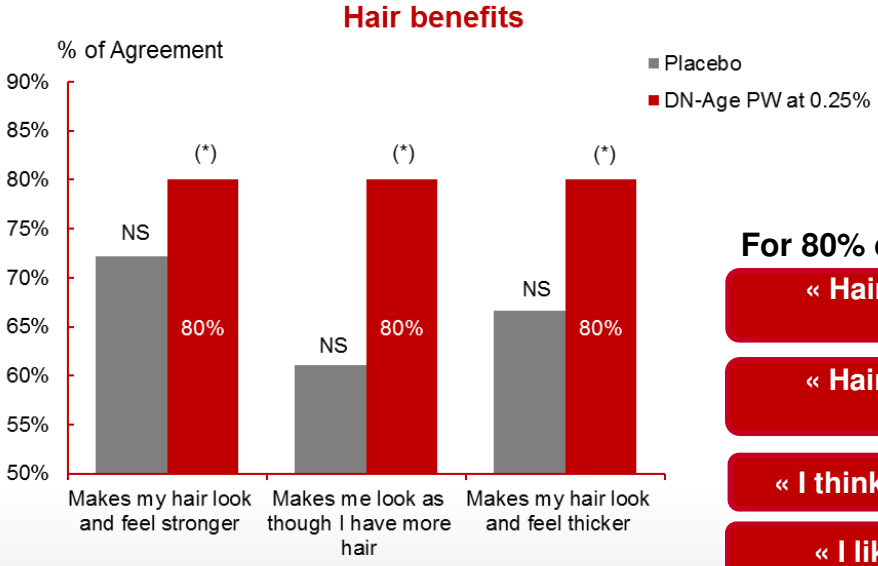
HAIR AGING - IMPACTS

HAIR FIBER

Clinical test

1. Anti-hair fiber graying
2. Hair growth maintenance
3. **Hair structure maintenance**

After scalp treatment (6 months)



For 80% of people:

- **« Hair looks and feels stronger »**
- **« Hair looks and feels thicker»**
- **« I think I have more hair »**
- **« I like the product »**

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DN-Age™
Anti-hair aging – Summary

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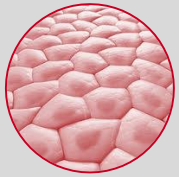
HAIR AGING - IMPACTS

SCALP



- **36% less** of squalene oxidation
- **33% less** of DNA fragmentation
- **93% less** of Thymine dimers
- **39% less** of IL-1α release

Protection of scalp against aging

FOLLICLE

- **73% less** activated ATM in hair follicle melanocytes (less oxidative stress)
- **92% more** cells in pseudo-papilla
- **39% less** autofluorescence in pseudo-papilla
- **48% more** hair fiber elongation

Protection of hair follicle against aging

HAIR FIBER

- **8% less appearance of gray hair**
- **6% less of hair fall**
- **Hair structure and strength maintenance**
- **Perceptible stronger, thicker & more dense hair**

Protection of new created hair fiber against aging

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DN-Age™

Claims
Scalp & hair Care

Defy time!
"Gives your hair a youthful boost"

Natural color extender
"Protects against the appearance of gray hair"

Hair strengthener
"Makes your hair fibers stronger and thicker"

2-in-1: Scalp and Hair care
"Protects your scalp and keeps your hair from aging"

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DN-Age™
Conceivable claims * (1/2)

* Assessment of local legislation and competition law is mandatory for the distributor of the cosmetic product.
[1] *In vitro* results; [2] *Ex vivo* results; [3] Clinical study; [4] Application study

DN-Age™
Conceivable claims * (2/2)

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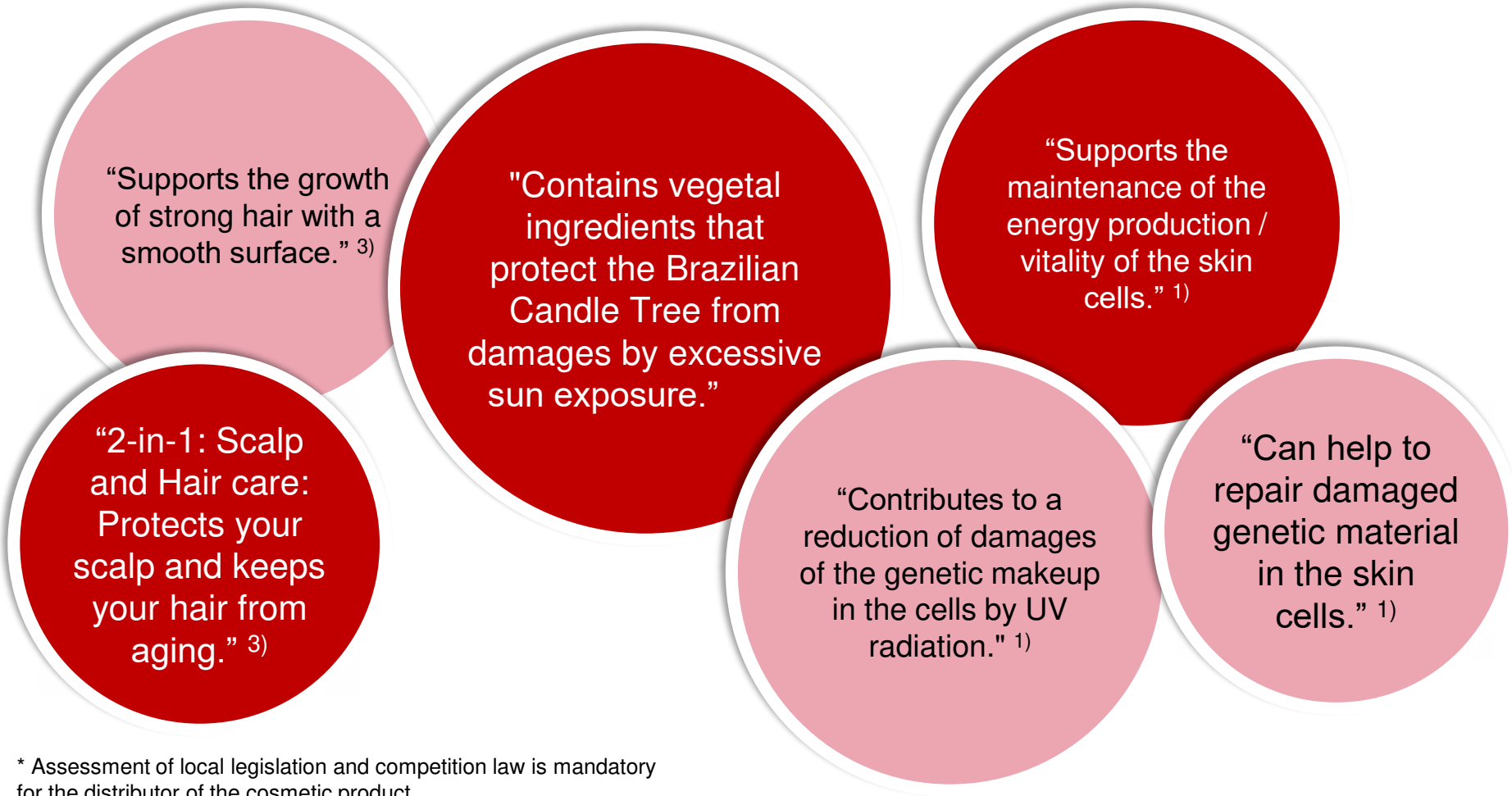

* Assessment of local legislation and competition law is mandatory for the distributor of the cosmetic product.
[1] *In vitro* results; [2] *Ex vivo* results; [3] Clinical study; [4] Application study

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DN-Age™
Example of formula

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1 **Youth-keeper lotion**
For scalp (leave-on)

2 **Gold & Beauty protect hair spray**
Pearly hair gel (leave on)

3 **Rejuvenation hair cream**
Conditioning mask (rinse off)
For scalp & hair

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Youth-keeper lotion

Scalp lotion (leave-on) (no. HB-FR-17-BC-50629/06)

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Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	84.35	
	Elestab™ 50 J	Chlorphenesin, Methylparaben	0.30	Preservative
	Rheocare® XGN	Xanthan Gum	0.10	Stabilizer
B	Ethanol 96%	Alcohol	10.00	Cooling agent
C	Water, demin.	Aqua	5.00	
	DN-Age™ PW LS 9827	Maltodextrin, Cassia Alata Leaf Extract	0.25	Active Ingredient

Specifications

pH value (23°C)	6,2
Appearance -	Liquid hair lotion

Performance

Additional performance has not been evaluated

Manufacturing Process

1- Mix ingredients of A until homogeneous at 50°C. Cool at room temperature.
2- Add B into A while mixing.
3- Add C while mixing until homogeneous.

Stability test

Stable 3 months at 4°C, RT, 40°C, 45°C

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Gold & Beauty protect hair spray

Pearly hair gel (leave-on) (no. HB-FR-17-BC-50822-02)

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Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	92.51	
	Euxyl PE 9010 *(Schülke)*	Phenoxyethanol, Ethylhexylglycerin	1.00	Preservative
B	Dehyquart® A-CA	Cetrimonium Chloride	0.50	Conditioning agent
	DN-Age™ PW LS 9827	Maltodextrin, Cassia Alata Leaf Extract	0.25	Active ingredient
C	Perfume*	Parfum	0.26	Fragrance
	Eumulgin® CO 40	PEG-40 Hydrogenated Castor Oil	0.27	Solubilizer
	Ethanol 96%	Alcohol	4.30	Solvent
D	Cosmedia® Ultragel 300	Polyquaternium-37	0.90	Rheology modifier
E	Reflecks™ Dimensions Brilliant Bronze GB90D	Calcium Sodium Borosilicate, Iron Oxide, Titanium Dioxide, Silica	0.01	Effect pigment

Specifications

pH value (23°C)	4.3
Viscosity (Brookfield; RVT; spindle 5; 50 rpm; 23°C)	3120 mPas
Appearance -	Shiny orange lotion

Performance

Additional performance has not been evaluated

Manufacturing Process

1- Mix the ingredients of phase A at 35°C.
2- When phase A is homogenesous, add ingredienbts of phase B one by one.
3- Mix ingredients of phase C.
4- Add Phase C in phase A+B while stirring
5- Add Phase D and E while stirring.

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Rejuvenation hair cream

Conditioning mask (rinse off) (no. HB-FR-17-BC-50819-01)

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Phase	Ingredients	INCI	% by weight	Function
A	Cosmedia® Triple C	Polyquaternium-37, Dicaprylyl Carbonate, Lauryl Glucoside	2.00	Rheology modifier
	Dehyquart® F 75 T	Distearoylethyl Hydroxyethylmonium Methosulfate, Cetearyl Alcohol	1.00	Emulsifier (O/W)
	Cutina® HVG	Hydrogenated Vegetable Glycerides	2.50	Consistency agent
	Lanette® O	Cetearyl Alcohol	3.00	Consistency agent
	Cetiol® CC	Dicaprylyl Carbonate	0.50	Emollient
B	Water, demin.	Aqua	87.05	
	Sodium Benzoate	Sodium Benzoate	0.40	Preservative
C	DN-Age™ PW LS 9827	Maltodextrin, Cassia Alata Leaf Extract	0.25	Active Ingredient
	Water, demin.	Aqua	3.00	
D	Citric Acid (50% solution)	Citric Acid	q.s.	pH Adjustment
E	Perfume*	Parfum	0.30	Fragrance

Specifications	
pH value (23°C)	4.1
Viscosity (Brookfield; RVT; spindle TD, helipath; 5 rpm; 23°C)	26 000 mPas
Appearance -	Yellow/beige emulsion

Performance

Additional performance has not been evaluated

Manufacturing Process

Heat phase A and B to 80-85 °C
Add phase B to A while stirring.
Cool down while stirring to 30 °C, then add phase C.
Adjust pH using phase D.
Add phase E.

Care Creations™

DN-Age™

Take the next step to stay young and defy time, focus on your hair!

A natural, Cosmos approved, titrated botanical extract with demonstrated *in vitro* antioxidant and DNA protective properties and unprecedented hair rejuvenating benefits observed *in vivo*

Care Creations™

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