



**Ti-UNic**  
Biotech

**TUN-828<sup>®</sup>**

*For HEALTHY SKIN, HAIR AND SCALP*

## HAIR AND SKIN DAMAGES AND AGING

Every day our Hair and Skin are exposed to external stress factors. UVs, urban pollution, other factors, and stress life main cause free radicals which attack cells causing damage to hair (shaft, bulb, follicle), and skin.  $O_2^-$  and  $H_2O_2$  are called reactive oxygen species that can activate too NF- $\kappa$ B pathway responsible for some stress related-function. They are also released during melanin synthesis. When their level is too high in our body, they initiate chain reactions, such as lipid peroxidation, oxidizing DNA and proteins, or oxidative stress.

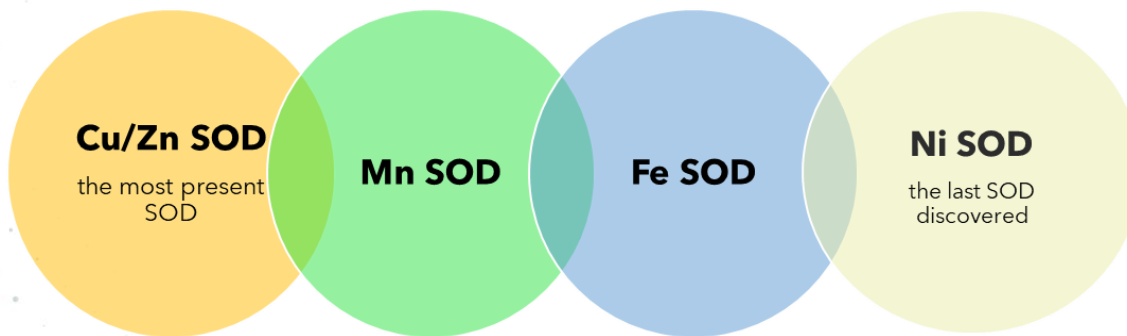
Our Hair and Skin respond to all external and internal attacks; one of them is inflammation. Those compounds activate signaling pathways in keratinocytes that result in overproductions of various inflammatory mediators, such as interleukins (IL 1 $\beta$ , IL-6, IL-8), tumor necrosis factor (TNF- $\alpha$ ), nuclear factor- $\kappa$ B (NF $\kappa$ B), inducible type cyclooxygenase-2 (COX2), and inducible nitric oxide synthase (iNOS). Furthermore, UVR exposure and pollution can further enhance hair aging and scalp and face inflammation by binding to their specific receptors on adjacent cells in hair and skin. UV-A penetrates cuticles layers and cortex, which causes protein oxidation, decreases melanin amount, and launches the aging process.



Pollution (Particle matters) binds to the hair surface and infiltrates the hair follicle something which could affect hair growth and texture. That contributes to scalp irritation, itching hair roots and hair loss, wrinkles, collagen, and elastin breakdown or dryness. To protect our cells, we need antioxidant can seize free radical and can protect our cells. These antioxidants delay or inhibit cellular damage mainly through the free radical scavenging property.

# SUPEROXIDE DISMUTASE

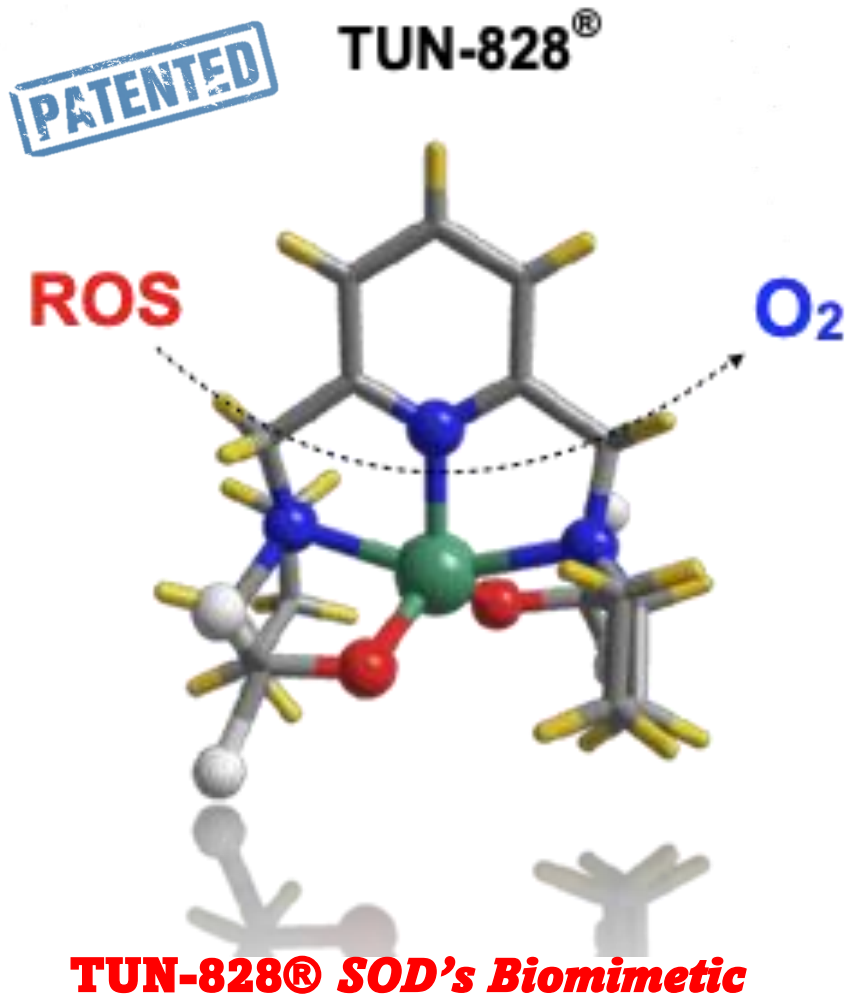
## Metalloenzyme form



Superoxide dismutases are the first antioxidant barrier in the body. Different forms of SOD are found and distinguished based on the metal cofactors present at the active site. It can classify into four groups: copper-zinc SOD (Cu/ZnSOD), nickel SOD (NiSOD), manganese SOD (MnSOD), iron SOD (FeSOD)

### Action

SOD is present in the dermis and epidermis of the skin. They are enzymes that catalyze the dismutation of superoxide anion radical  $O_2^{\cdot-}$  and thus play important roles in defending against the toxicity of reactive oxygen species (ROS).



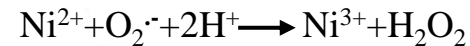
**Protect the Health of your Hair and Scalp**

TUN-828® is an original and unique biomimetic antioxidant able to mimic the active site of the superoxide dismutase (SOD) that is at the top of the biological antioxidant pyramid system. It has the function of converting superoxide anion radicals to oxygen gas.

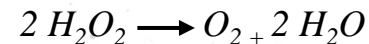
We design a stable SOD mimics **stable at room temperature**, with the Nickel (Ni) **active transition metal center**.

#### TUN-828®'s Reaction - NiSOD:

*Simultaneous oxidation of Ni reduction and reduction of O<sub>2</sub><sup>•-</sup> :*



*Reduction reaction catalyzes by the complex Ni :*





**TUN-828<sup>®</sup>'s  
EFFECTIVENESS**

# ANTIOXIDANT PROPERTIES

Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and Superoxide anion (O<sub>2</sub><sup>·-</sup>) are called reactive oxygen species that can activate the NF-κB pathway responsible for some stress related-functions.

TUN-828®'s capacity tests to scavenge O<sub>2</sub><sup>·-</sup> and H<sub>2</sub>O<sub>2</sub> are determined.

**TUN-828® has the lowest IC50** to inhibit 50% of H<sub>2</sub>O<sub>2</sub> compared to other Antioxidants.

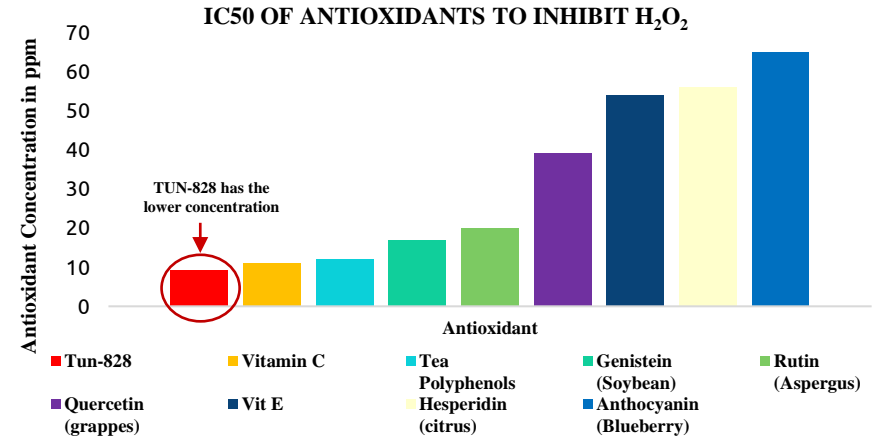
**TUN-828® suppresses hydrogen peroxide and superoxide anion activity.**

Evaluation of **TUN-828®** on HaCaT (Human keratinocyte cell) to scavenge ROS.

Cells are pre-treated with **TUN-828®** for 16 hours. The ROS reaction launched with 1.2mM H<sub>2</sub>O<sub>2</sub> and it was removed after 2 hours.

**TUN-828® reduces 57%** of free radicals present in keratinocyte cells.

**TUN-828® has Antioxidant property of TUN-828®.**  
**TUN-828® decreases oxidative stress in keratinocytes cells.**



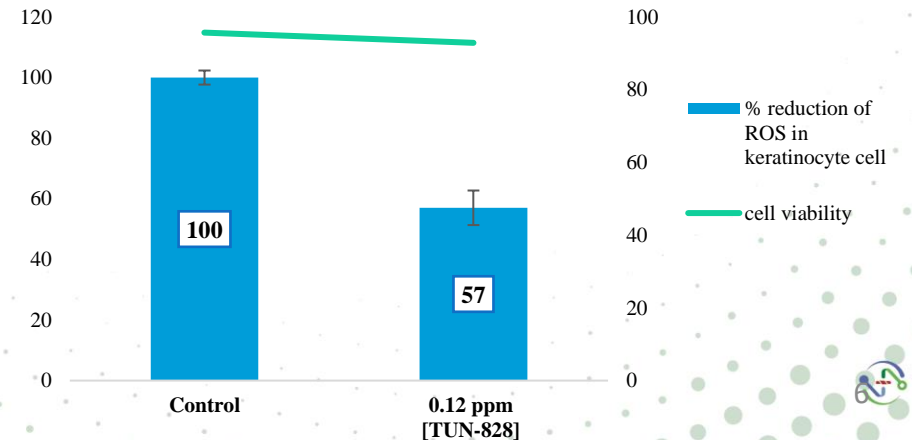
Inhibition of hydrogen peroxide H<sub>2</sub>O<sub>2</sub> TUN-828 (25 ppm)



Inhibition of superoxide anion with TUN-828 (25 ppm)



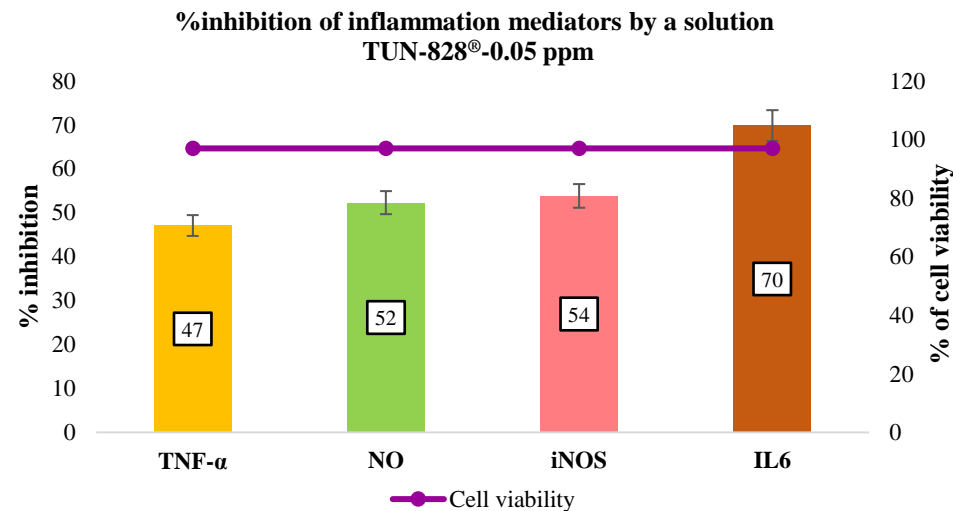
REDUCTION OF ROS IN KERATINOCYTE CELL



# ANTI-INFLAMMATION PROPERTIES

Macrophage is used to establish an in vitro model and lipopolysaccharide (LPS) stimulated the macrophage cell line to secrete the cytokines TNF- $\alpha$  and IL-6 that promote inflammation.

In addition, we also measured the inhibition of NO production, which is critical in multiple cellular signaling pathways, we focus on nitric oxide synthases iNOS, highly expressed in macrophages inflammatory response.



TUN-828® shows good anti-inflammatory properties by inhibiting some inflammation mediators in 2 different inflammation ways:

- NF- $\kappa$ B- pathways with TNF- $\alpha$  and IL-6 inhibition
- and NO production with the inhibition of iNOS and NO production.

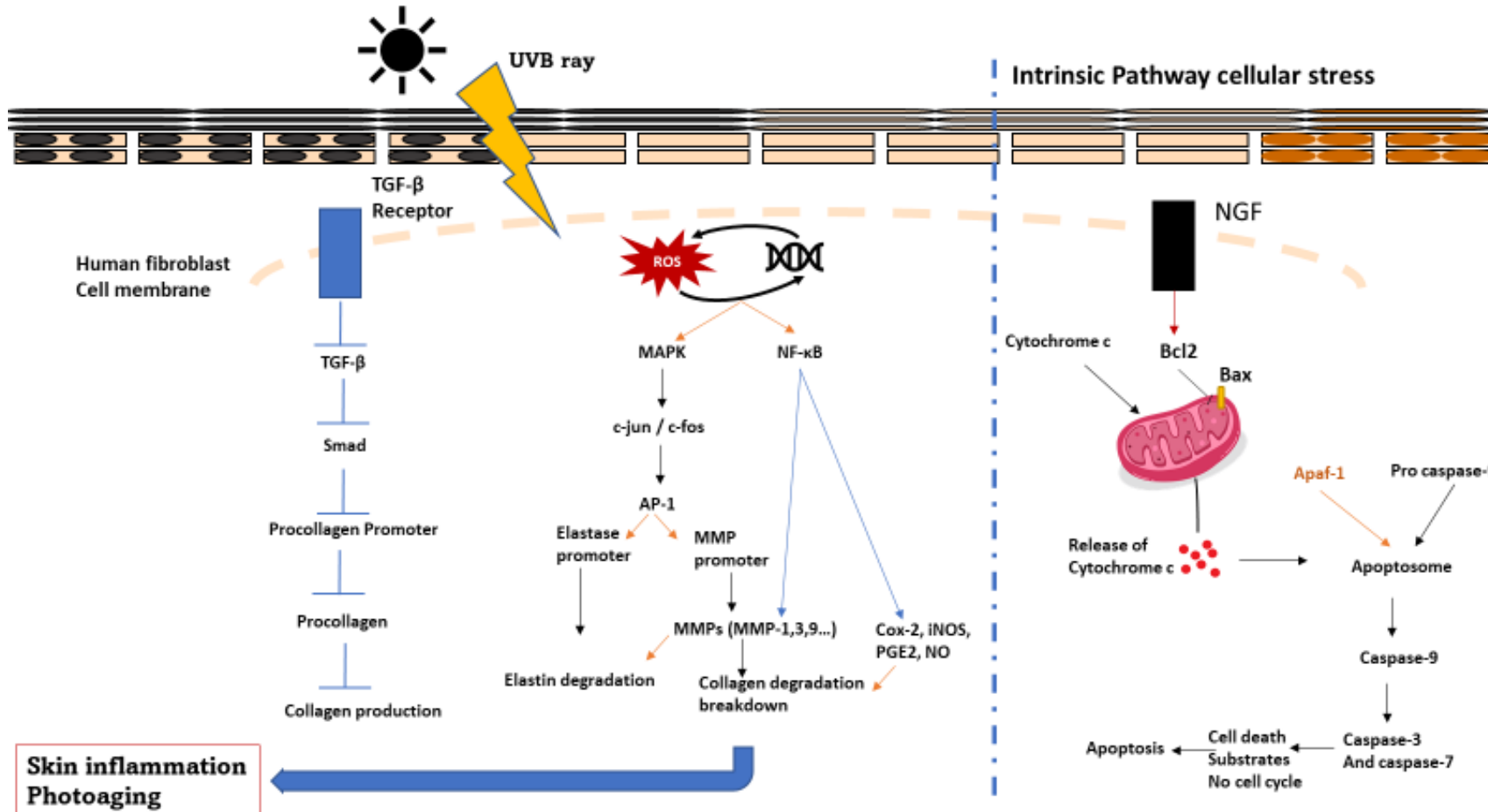
TUN-828® can **inhibit 39% of mast cell degranulation.**

**TUN-828® MODULATES THE EXPRESSION AND DISTRIBUTION OF ANGIOGENIC FACTORS AND INFLAMMATORY MEDIATORS.**

# ANTI-AGING PROPERTIES –UV-B PROTECTION – PHOTOAGING

UVs radiations lead to a lot of damage to the skin, acting mainly on keratinocytes:

- Mediate through the intrinsic effect of the aging process.
- Increase elastase enzyme will induce elastin degradation.
- Induce matrix metalloproteinases such as MMP1, MMP-9, etc...





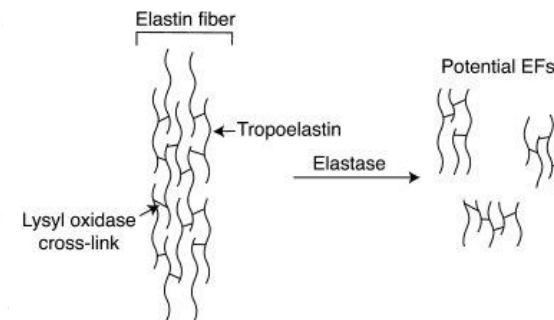
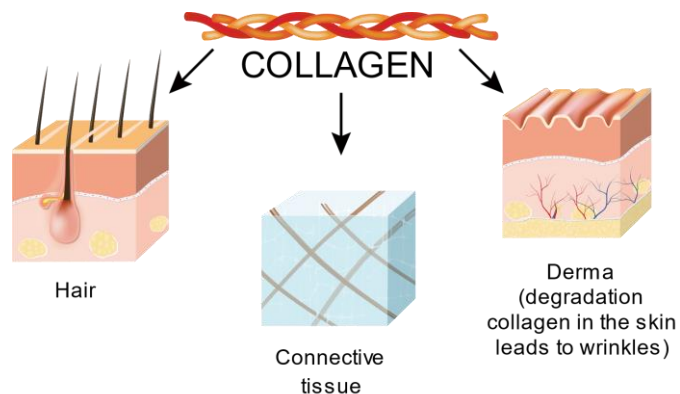
# ANTI-AGING PROPERTIES –UV-B PROTECTION – PHOTOAGING

**PROCOLLAGEN Type I** is a precursor of type collagen I (most abundant human body collagen). Collagens are the major structural proteins of the extracellular matrix that play a crucial role in providing connective tissue structural integrity.

**ELASTIN** is the main component of the elastic fibers, which allow the skin to resume its shape after stretching or contracting. Elastic fibers are gradually degraded with age, especially the thin fibrils in the papillary dermis.

**ELASTASE** is an enzyme that breaks down elastin, an elastic fiber

**MMP-9 or gelatinase  $\beta$ :** can degrade both elastin and partially hydrolyzed collagen. It can degrade collagen type IV an important component of the basement membrane in skin and hair bulbs.



# ANTI-AGING PROPERTIES

## PROCOLLAGEN SYNTHESIS

**Method:** Human foreskin fibroblast (Hs68) were incubated with TGF- $\beta$  (transforming growth factor) that stimulates proliferation of cells and TUN-828®. Procollagen type I, carboxy-terminal peptide (PIP) released in the culture supernatant as an expression of the collagen synthesis. The cell viability was measured using MTT.

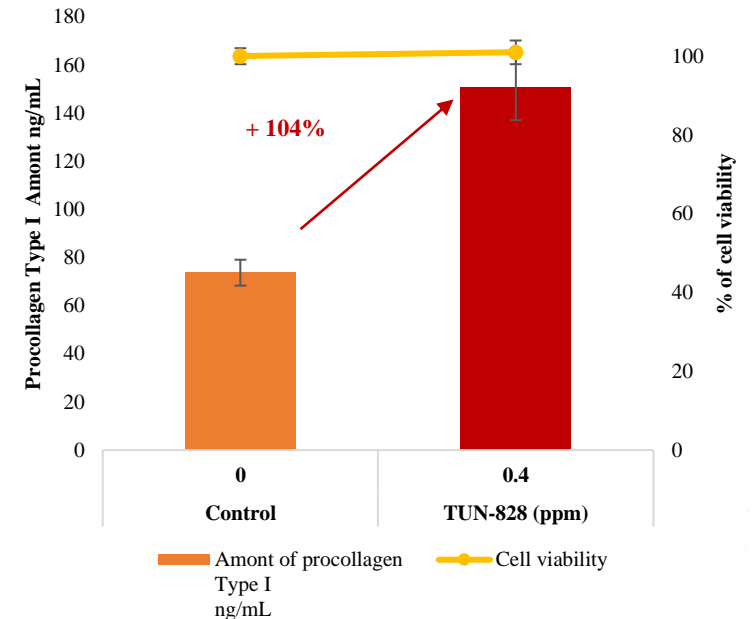
**TUN-828® (0.4 ppm) can increase the procollagen type I level by 104%.**

## INHIBITION OF ELASTASE

**- 90%**

TUN-828® (0.019%) can inhibit 90% of elastase would break elastin and elastic fibers.

Synthesis of procollagen Type I in fibroblast

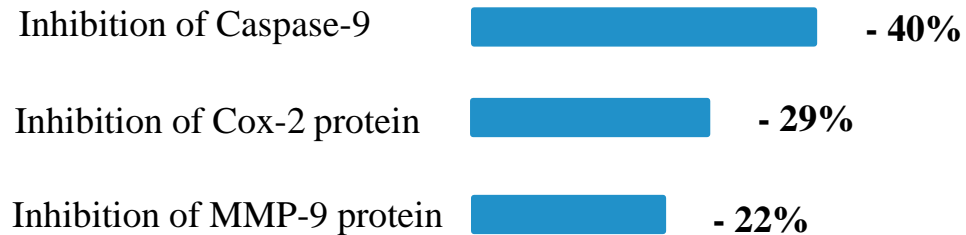


**TUN-828® IN TERMS OF ANTI-AGING CAN BE USEFUL TO PREVENT LOSS OF SKIN ELASTICITY AND THUS SKIN SAGGING.**

# ANTI-AGING PROPERTIES

## UVB PROTECTION – PHOTOAGING

Human epidermal keratinocytes (caspase-9 and Bcl2) and fibroblast (MMP-9 and Cox-2) were pre-treated for 2 hours with a solution containing TUN-828® (0.048 ppm) and after being exposed to UV-B (40 mJ/cm<sup>2</sup>). Inhibition in human fibroblast and keratinocyte of a protein activated by UV-B (40 mJ/cm<sup>2</sup>) with TUN-828®.



TUN-828® (0.048 ppm) can increase the expression of Bcl2 (B-cell lymphoma-2, inhibit apoptosis), can stop the expression of Bax (promote cell death).

TUN-828® has a critical wavelength of 321 nm. It helps you to reduce damages induce after UVB exposition. It can inhibit the expression of MMP-9 protein, Cox-2 protein, and Caspase-9 protein. At the same time, he will increase the expression of the Bcl2 protein that inhibits apoptosis.

**TUN-828® PREVENTS THE DAMAGE INDUCED BY UV-B.**



# **HAIR PROPERTIES**

## HAIR COLOR- MELANOGENESIS IN HAIR

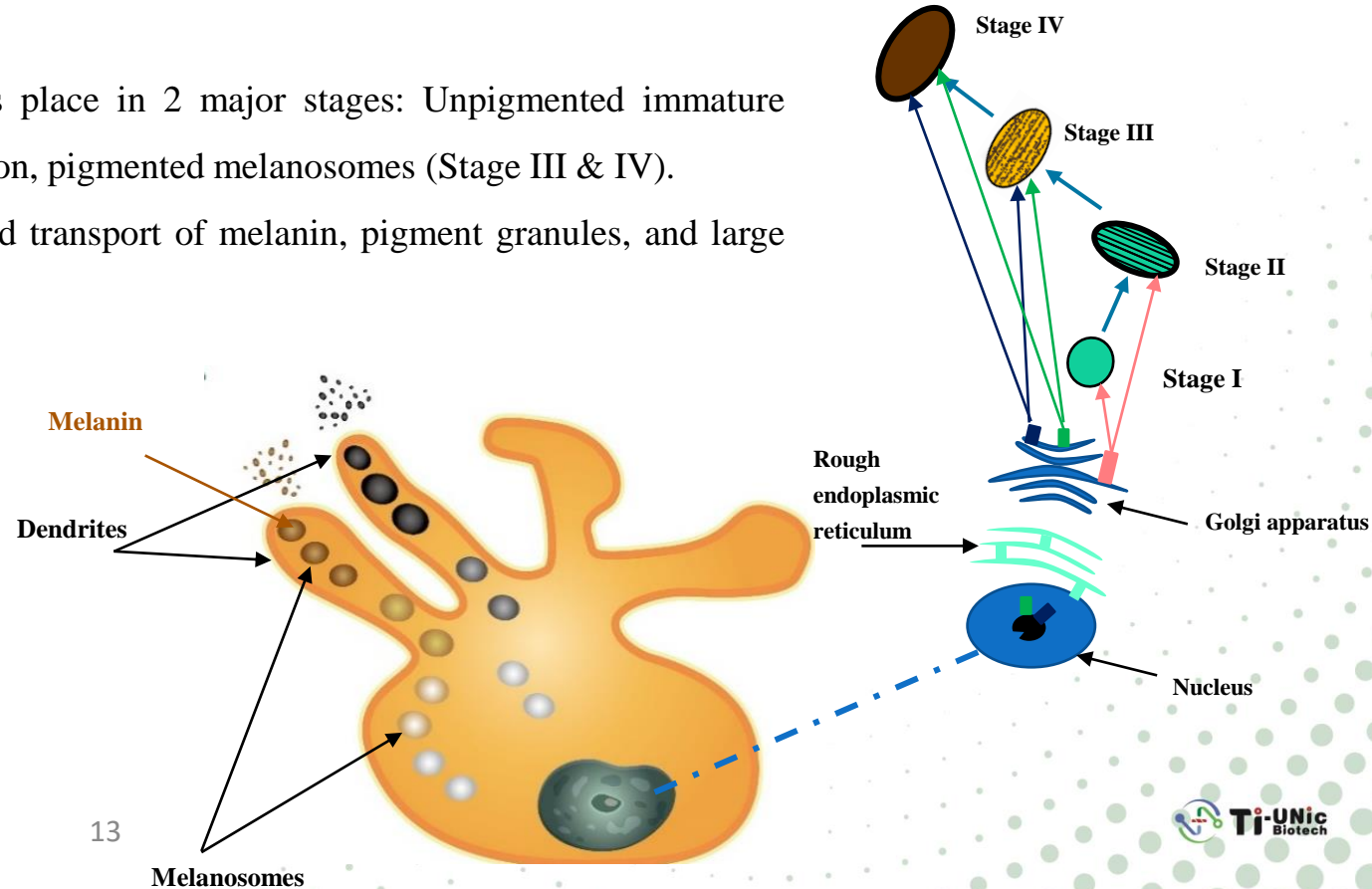
It exists different natural hair colors *Black, Blond, Chestnut, Red hair, etc.* The Hair color comes from **Melanin**, present in hair, skin, or eye, and give them their color. It exists two types of Melanin: *Eumelanin* (**Black** and **Brown** hair) and *Pheomelanin* (**Blond** and **Red** hair).

Natural pigmentation produces in cells named Melanocytes. They have a dendric form and are present in the hair follicle and basal epidermis. Melanocytes transfer melanin produces to keratinocytes. So, like this, the hair is pigmented from the root to the tip.

**Melanogenesis** is the production of melanin. It takes place in 2 major stages: Unpigmented immature organelle, pre-melanosomes (stage I & II), and Maturation, pigmented melanosomes (Stage III & IV).

**Melanosome** is a cellular site of synthesis, storage, and transport of melanin, pigment granules, and large organelles. Exists 4 stages of melanosomes maturation:

- **Stage I and II:** White, unpigmented, and organized.
- **Stage II and III:** Melanin deposit.
- **Stage IV:** Melanosomes transfer to keratinocytes.



# HAIR COLOR- MELANOGENESIS IN HAIR

We focus on 3 precursors of melanin :

**PMEL**, Transmembrane protein, is a main constituent of fibers.

Those protein fibers are physical support of Melanin in Melanosome and are specific to pre-melanosome (stage I & II).

**TYRPI** (Tyrosinase-related proteins 1) and **Tyrosinase**, those enzymes engaged in melanin synthesis. They are transported to pre-melanosome and play a role in the stage where the pigment is synthesized and accumulated (stages III & IV).

## Experimental Procedures

Cell type: Human Melanocytes

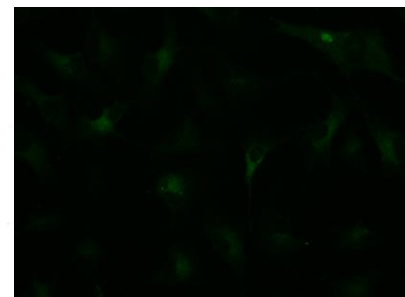
PMEL and TRP1 (*in situ* immunofluorescence detection – epifluorescence microscopy and image analysis) and Tyrosinase Activity on cell protein extracts (colorimetric enzymatic assay).

## EFFECT OF TUN-828® ON PMEL EXPRESSION

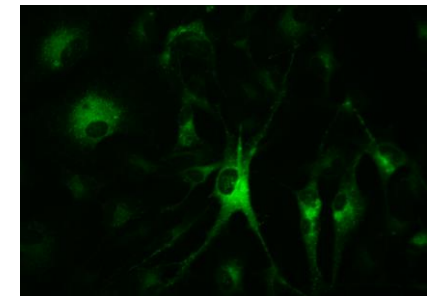
Pre-Melanosome

- Stage I: formation of PMEL fibrils (small fibers)
- Stage II: PMEL is completely organized such as parallel sheets in ellipsoidal shape.

**TUN-828® INCREASES PMEL LEVELS BY 41%**



Control



TUN-828® 0.0001%

The visualization of the *in-situ* PMEL level is presented as a range of specific signal intensity (in green).

*In-vitro* test with Human Melanocyte Growth Suppl

# HAIR COLOR- MELANOGENESIS IN HAIR

## EFFECT OF TUN-828® ON TYRP1 ACTIVITY

**TUN-828® INCREASES THE TYRP1 LEVEL BY 79%.**

## EFFECT OF TUN-828® ON TYROSINASE ACTIVITY

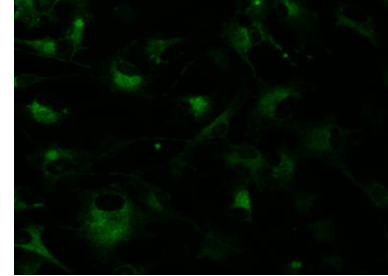
Tyrosinase is an enzyme that activates melanin biosynthesis. Melanogenesis is initiated with the first step of tyrosine oxidation to dopaquinone catalyzed by tyrosinase.

**TUN-828® EXPANDS TYROSINASE ACTIVITY BY 33%.**

## EFFECT OF TUN-828® ON MELANIN SYNTHESIS

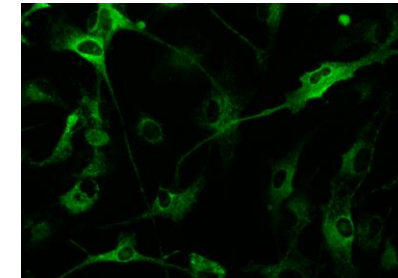
**TUN-828® IMPROVES MELANIN SYNTHESIS OF 28 % IN MELANOCYTES.**

**TUN-828® INCREASES MELANOGENESIS**

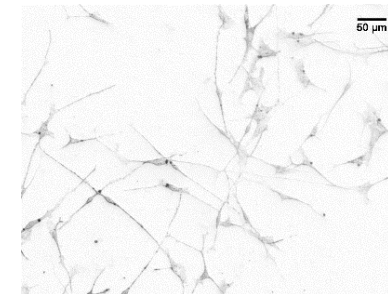


Control

*In situ visualization of TRP1 (green). The signal for TRP1 was obtained by specific immunofluorescence detection that absorbs and emits light at different wavelengths*

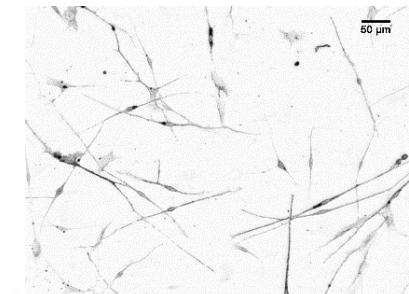


TUN-828® 0.0001%



Control

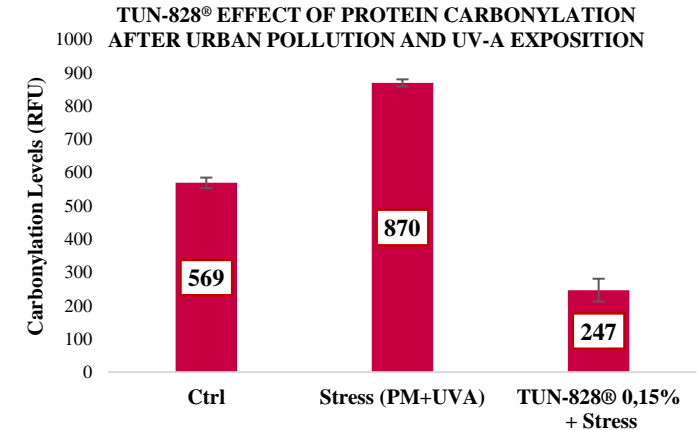
*Melanin is visualized by bright-field microscopy as a black signal on white background.. \*In-vitro test with Human Melanocyte Growth Suppl*



TUN-828® 0.0001%

## EFFECT OF TUN-828® ON POLLUTION AND UV-A STRESS DAMAGES

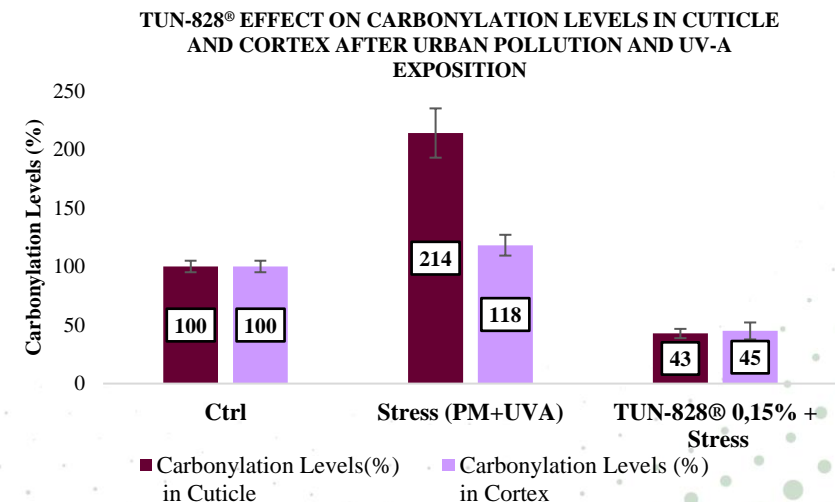
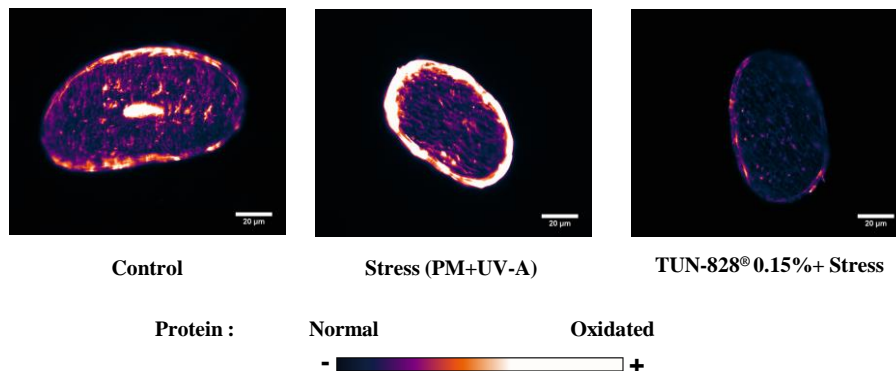
- Biological model: Human Hair Shafts (Caucasian).
- Incubation time: Overnight (16 hours) (prior to stress).
- Hair shaft was dipped in a water solution containing 0,15% of TUN-828®, shafts dry naturally at room temperature.
- Stress conditions: Urban Pollution: Particulate Matter (PM 2,5 + PM 10) (150 µg/cm<sup>2</sup>, applied as powder + UV-A irradiation (LED source, 84 J/cm<sup>2</sup>; 6 hours of irradiation).



TUN-828® (0.15%) shows **100% protection** on hair shafts against urban pollution-induced and UV-A damages.

## Visualization of carbonylated proteins (on hair cross-section)

TUN-828® shows a protection of internal (cortex) and external (cuticle) protection on hair shaft after an exposition of urban pollution and UV-A.

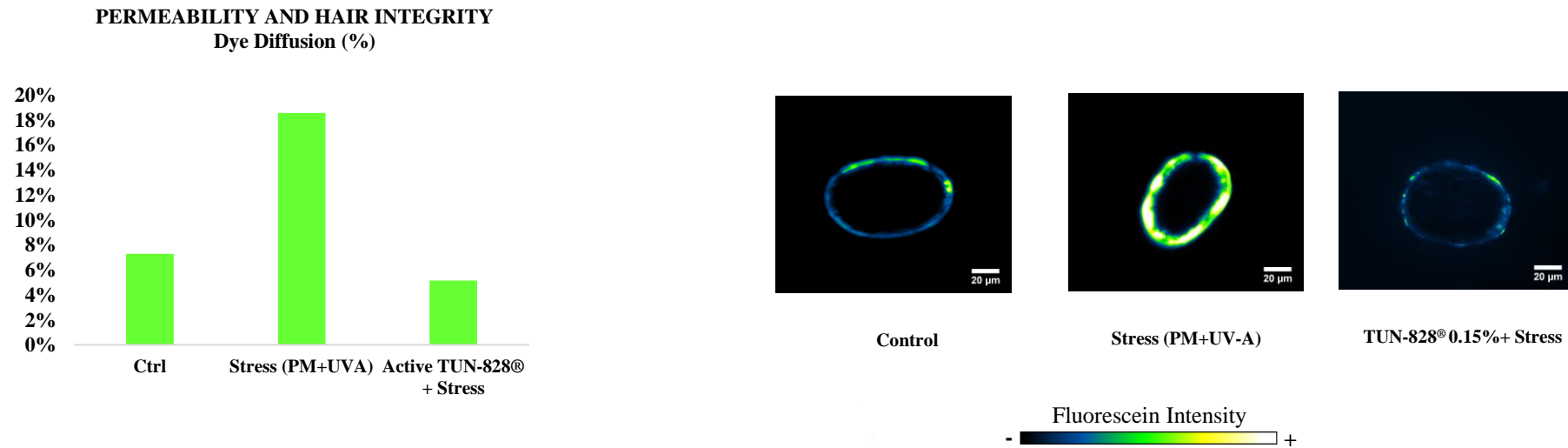




# HAIR PROTECTION AGAINST POLLUTION AND UV-A

## INTEGRITY OF HAIR SHAFT

The stress (urban pollution) increases fluorophore penetration on hair structure. The presence of the TUN-828® (0.15%) protects from hair impairment by the preservation of structural hair integrity. It considers the condition, strength, and overall healthiness of the hair.



*\*In situ visualization of fluorescein penetration, on hair cross-section*

**TUN-828® PRESERVES THE HAIR INTEGRITY.**  
**TUN-828® PROTECTS PROTEINS OF HAIR FROM OXIDATION.**



**CLINICAL STUDY**



**Subject:** 28 completed the study, target enrollment 31 (Asian adult), 20-75 years old.

**Usage:** every evening 1 hour before going to bed, for 6 months.

**Hair Product test:** subjects used a hair revitalizer/toner containing 1% of TUN-828<sup>®</sup>.

**Ingredient list:** Water, Alcohol, 1,2-Hexanediol, Diglycerin, Caprylhydroxamic Acid, Dipropylene Glycol, PPG-26-Buteth-26, PEG-40 Hydrogenated Castor oil, Nickel Bis (Hydroxy Diphenyl Methylpyrrolidino Methyl) Pyridinediyl t-Butylisocyano Perchlorate, Parfum.

**Methods:** The Rate measured by the DermoPrime Hair analysis System to analyze: Scalp Condition, Keratinization, Hair density (hair/cm<sup>2</sup>), and Hair thickness.

The test was performed on the hair on both sides of the ears (Right and Left) and the top of the head.

The study was supervised by a **dermatologist** and the subject respond to a self-assessment questionnaire.



**RESULTS\*:** TUN-828<sup>®</sup> leads to significant improvement in hair and scalp health.

|                 | MONTH 1 | MONTH 6 |   |
|-----------------|---------|---------|---|
| HAIR DENSITY    | + 23 %  | + 40%   | TUN-828 <sup>®</sup> <b>EXPANDS</b> HAIR DENSITY.                 |
| HAIR THICKNESS  | + 14%   | + 9%    | TUN-828 <sup>®</sup> <b>INCREASES</b> HAIR THICKNESS.             |
| SENSITIVE SCALP | - 44 %  | - 40%   | TUN-828 <sup>®</sup> <b>REDUCES THE</b> SENSITIVITY OF THE SCALP. |
| KERATINIZATION  | + 52%   | + 49%   | TUN-828 <sup>®</sup> <b>IMPROVES</b> KERATINIZATION.              |

\*p >0,01 p >0,05

\*Efficacy test carried out under dermatologist supervision.

## SUBJECT'S SELF-ASSESSMENT QUESTIONNAIRE\*

Subject self-assessments were perceived as statistically significant improvements in overall hair and scalp health.

- **70% Felt their scalp is comfortable.**
- **70% Remarked the reduction of sensitive scalp conditions.**
- **70% Noticed their hair shine Increased.**
- **60% Perceived their hair density increased.**
- **58% Felt their hair become stronger.**
- **58 % Noted their hair becomes healthy.**
- **58.06% of subjects felt that the hair loss was significantly reduced when combing their hair,**  
**Based on the condition of hair loss after daily shampooing.**
- **48.4% Discerned that inflammatory state was improved.**
- **35.48% Observed that their scalp itching had improved.**
- **29% of the subject has grey hair (50% of the total subjects) noticed that the proportion of black hair has increased.**

\*Efficacy test carried out under dermatologist supervision.

## Woman 40s



Before Use  
Gray hair density 74/cm<sup>2</sup>



0 day

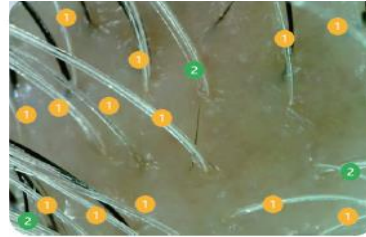


After Use  
Gray hair density 36/cm<sup>2</sup>

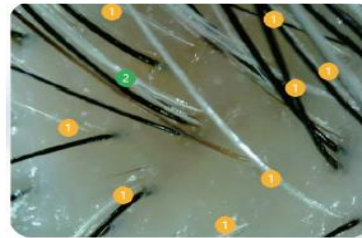


After 30 days

## Woman 55s



Before Use  
Gray hair density 79/cm<sup>2</sup>

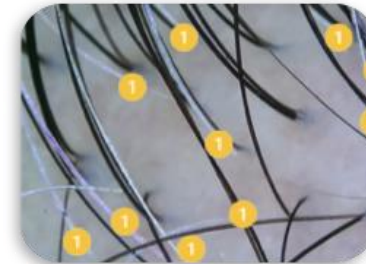


After Use (30 days)  
Gray hair density 41/cm<sup>2</sup>

## Man 61s



Before  
Grey Hair density 157/cm<sup>2</sup>



After Use (30 days)  
Grey Hair density 40/cm<sup>2</sup>

**TUN-828<sup>®</sup> IMPROVES THE HEALTHY APPEARANCE OF HAIR AND SCALP**

# CLINICAL STUDY OF TUN-828® IN SKINCARE

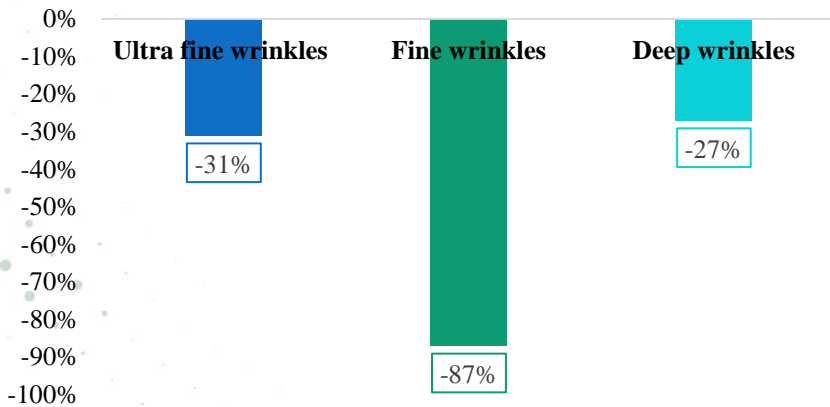


**Subject:** 10 women completed the study, (Asian adults), 30-60 years old.

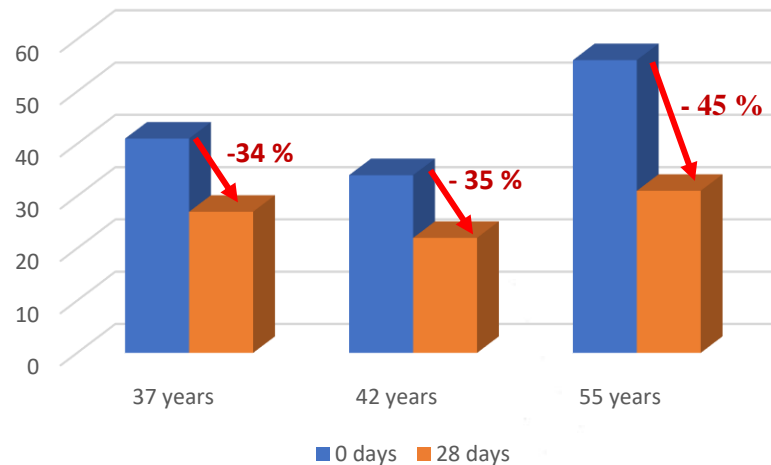
**Usage:** The subjects applied the cream every morning and evening on cleansed skin, for 1 month.

**Skin Product test:** subjects used a face cream formulated with 1% of TUN-828®

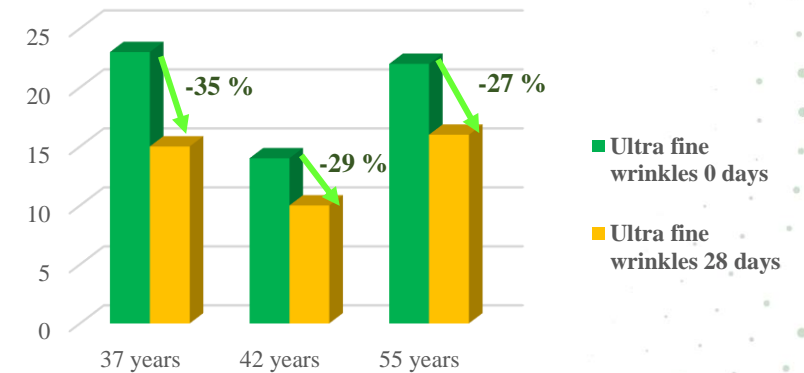
Wrinkles reduction after 28 days using a cream formulate with 1% of TUN-828®



Measure of wrinkles number on crew's feet aerea



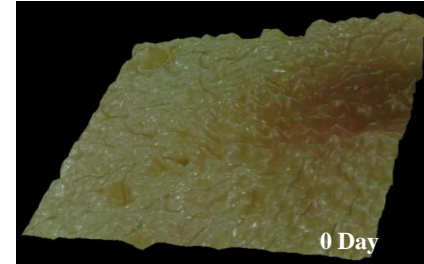
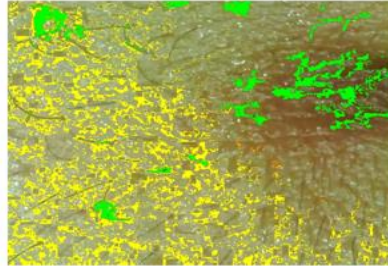
Measure of Fine wrinkles numbers of crew's feet area



# CLINICAL STUDY OF TUN-828® IN SKINCARE

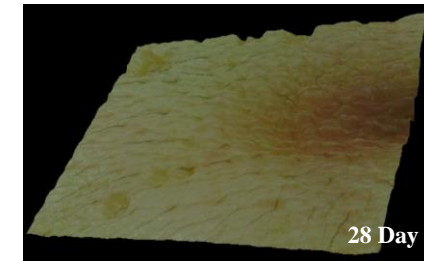
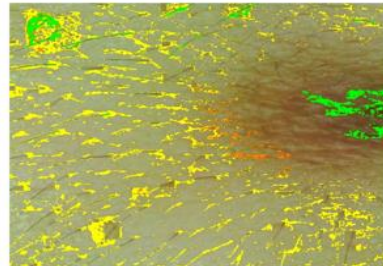
Age: 55 years  
Day :0

- Ultra-fine wrinkles
- Fine wrinkles
- Deep wrinkles
- Very deep wrinkles



Age: 55 years  
Day :28

- Ultra-fine wrinkles
- Fine wrinkles
- Deep wrinkles
- Very deep wrinkles



- 27% ultra fine wrinkles

- 64% of Deep wrinkles



Surface skin representation of Crow's feet area of a woman aged 55 years old, before and 28 days after using a cream formulated with 1% of TUN-828 ®



**INCI Name:** *Nickel Bis (Hydroxy Diphenyl MethylPyrrolidino Methyl) Pyridinediyl t-Butylisocyano Perchlorate / PEG-40 hydrogenated castor oil/ Water/ Glycerin/ Propanediol/ Butylene Glycol.\**

*\* Exist in oil type too.*

## **SPECIFICATION**

**Recommended dosage:** 0,5 ~ 2 %

**Color:** Light yellow

**Appearance (25°C):** liquid

**pH value (25°C):** 6~8

**Expiry date:** 3 years

**Storage:** From -20 ° C to 20 ° C and avoid direct sunlight.

**Formulation:** Regardless of the influence of pH and temperature, it can be directly added to the aqueous solution and stirred evenly.

## **TOLERANCE TEST:**

**Phototoxicity:** Not cause phototoxicity (OECD 432).

**Skin irritation:** Non-irritant (OECD 439).

**Skin sensitization:** Non-sensitizer (OECD 442C).

## **COSMETIC PRODUCTS SUGGESTION:**

**Skincare:** Cream (Day and Night), Essence, Lotion, Toner, etc.

**Eyecare products:** Gel, Cream, Essence, etc.

**Bodycare:** Lotion, etc.

**Haircare:** Shampoo, Conditioner, Lotion, Hair Mask, Toner, etc.

**INCI Name :** *Nickel Bis (Hydroxy Diphenyl MethylPyrrolidino Methyl) Pyridinediyl t-Butylisocyano Perchlorate / PEG-40 hydrogenated castor oil/ Water/ Glycerin/ Propanediol/ Butylene Glycol.*

## ANTIOXIDANT

- ◆ Prevents oxidative stress.
- ◆ Reduces H<sub>2</sub>O<sub>2</sub> amount in cells.
- ◆ Mimics SOD properties.

## ANTI AGING

- ◆ Reduces the look or signs of aging.
- ◆ Collagen Type I booster.
- ◆ Prevents skin elasticity loss.

## ANTI INFLAMMATORY

- ◆ Good for sensitive skin.
- ◆ Relieves redness.
- ◆ Reduces stress reactions.

## HAIRCARE

- ◆ Prevents Hair shaft damage from UVA and pollution.
- ◆ Maintains Hair integrity from urban pollution stress.
- ◆ Preserves internal and external Hair proteins.
- ◆ Increases natural hair pigmentation.
- ◆ Stimulates Hair Melanogenesis.



**TUN-828<sup>®</sup>**  
**SOD BIOMIMETIC**  
**FOR A YOUNGER LOOKING SKIN AND HAIR**

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