

Upcycled active for hair greying prevention

Katharina Kappler – Mibelle Biochemistry

Based on a sustainable upcycling process of a unique locally sourced mandarin from the Greek island of Chios, MelanoGray™ is an anti-hair greying essence that stimulates melanin synthesis and protects melanocytes from stress-induced malfunction. This unique active ingredient was shown to markedly reduce the quantity of grey hair in both men and women, even during times of psychoemotional stress when hair greying can be accelerated.

Hair greying

Hair has a wide-ranging impact on our lives, far beyond its natural role of protection and thermoregulation. Hair and grey hair in particular can greatly influence one's self-esteem and mood. The '50' rule of thumb says that 50% of the population will have at least 50% grey hair by the age of 50 years.

However, studies have shown that this is more likely to be between 6% and 23% of the population, depending on ethnic/geographic origin and natural hair color, with men tending to grey significantly earlier than women.¹ Hair greying, which also called canities, is defined as the progressive loss of natural hair pigmentation.

The mixture of darker, normally pigmented hair and increasingly lighter, less pigmented hair is perceived as progressive greying. While genetic factors play the most predominant role in hair greying, psychoemotional stress, and various diseases, as well as malnutrition and the use of alcohol and certain drugs have been linked to an increased likelihood of premature



greying. In addition, environmental factors – such as smoking, UV radiation, and pesticides – trigger the occurrence of grey hair.

Melanogenesis – the production of hair pigments

The pigmentation of hair is the result of a complex interaction that takes place at the hair follicle between melanocytes, matrix keratinocytes, and dermal papilla fibroblasts. Hair pigmentation occurs only during the anagen phase, which is the growing phase, and

this can last from three to seven years.

The hair pigments, called melanins, are produced in a process which is called melanogenesis in organelles within melanocytes (melanosomes) before they are transferred to keratinocytes, resulting in pigmented hair shafts. From the two different melanin types, the black-brown eumelanin and the reddish-yellow pheomelanin, eumelanin determines how dark the hair is.

The process of eumelanin synthesis can be initiated by activation of the melanocortin 1 receptor (MC1R), e.g. through the hormone α -MSH. The amino acid tyrosine serves as substrate for the synthesis of eumelanin, which is tightly regulated by a range of regulatory proteins and enzymes, including tyrosinase (TYR) and dopachrome tautomerase (DCT).

Anti-greying cosmetic active upcycled from aromatic waste

To counteract hair greying, Mibelle Biochemistry has developed a new cosmetic active ingredient, which is based on an extract of organic mandarins that grow exclusively on the Greek island of Chios.

Mandarins are orange fruits from the largest group of citrus plants and are particularly rich in essential oils and antioxidants such as phenolic acids, flavonoids, limonoids and carotenoids. On Chios, mandarins were introduced in the 13th century after colonization by the Genoese.

The island of Chios, which is also known

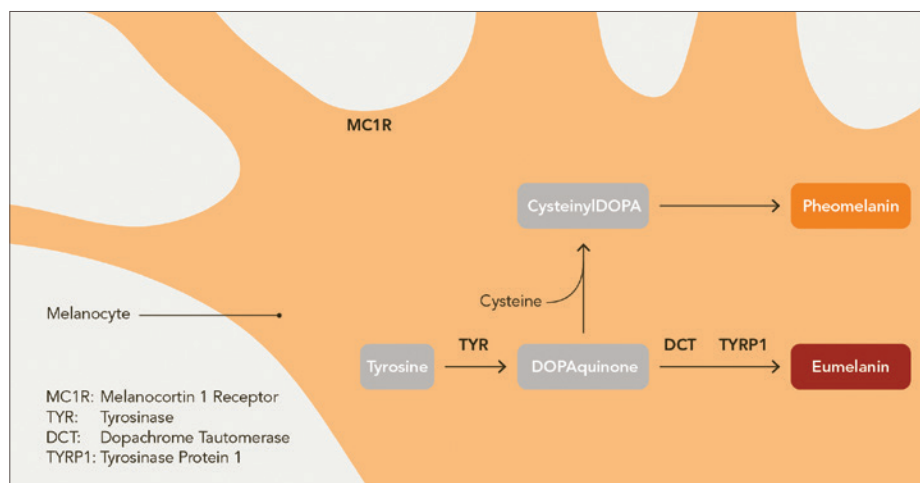


Figure 1: Melanin synthesis

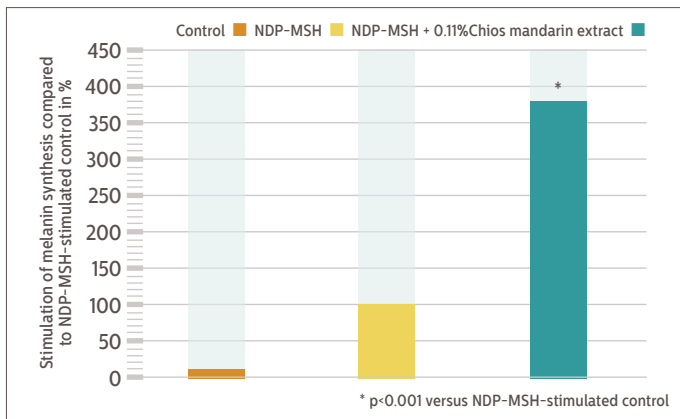


Figure 2: Synergistic stimulation of melanin synthesis

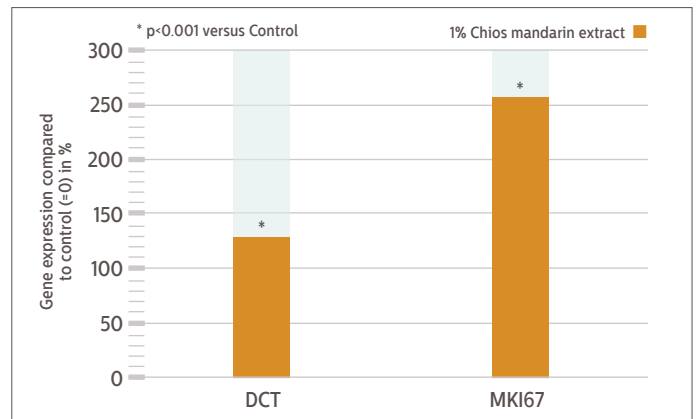


Figure 3: Upregulation of melanogenesis-promoting genes

for the mastic tree, has calcium-rich soil that is based on limestone. The stable temperatures and the Meltemi winds give a mild microclimate that protects the fruits from frost during the winter months. Chios mandarins grow exclusively in very mild climatic conditions in traditional organic cultivation.

The unique mandarin variety from the Greek island of Chios, *Citrus deliciosa* (*Citrus reticulata*) var. Xio, has been registered in the EU since 2012 as a product with Protected Geographical Indication (PGI). Unlike the modern industrially produced fruits, this ancient variety of mandarins contains many seeds, which does not meet today's market standards.

The mandarins of Chios are still highly appreciated for their excellent and intense aromatic properties and delicate fragrance as exquisite perfume essences. To produce perfume essences, both the juice and the peel of the fruit undergo a gentle distillation process in a traditional small distillery.

The remaining rich and thick peel pulp (pomace) is the starting material that is used to produce a Chios mandarin extract in an upcycling-inspired sustainable process. Mibelle Biochemistry's mission was to develop an active ingredient from this very special waste material through an upcycling process to extract the valuable molecules from these special mandarins.

For this active ingredient this unique mandarin peel extract is supplemented with acetyl tyrosine, which is the amino acid that

acts as a substrate for the melanin synthesis in the melanocytes and thus also supports the product efficacy.

Stimulation of melanogenesis

The efficacy of the Chios mandarin extract was demonstrated in various *in vitro* and clinical studies. As shown in B16 melanoma cells after 72 hours of treatment, Chios mandarin extract can stimulate melanin synthesis in a concentration-dependent manner.

In addition, 0.1% Chios mandarin extract synergistically enhanced melanogenesis induced by 0.1 μM NDP-MSH, an α -MSH analogue, in the same cell model, leading to an additional increase of more than threefold in melanin production. Thus, Chios mandarin extract, either alone or in combination with another melanogenesis activator, clearly stimulates melanin synthesis.

Gene expression analysis of human melanocytes treated with 1% Chios mandarin extract for 24 hours revealed the underlying mechanisms. Chios mandarin extract significantly stimulated the expression of DCT (+132%), the gene that encodes the enzyme dopachrome tautomerase needed conversion of dopachrome into 5,6-dihydroxyindole-2-carboxylate, which is a basic step for the production of eumelanin.

In addition, the treatment strongly enhanced the expression of MKI-67 (+257%), encoding a marker of cell proliferation and thus indicating increased melanocyte proliferation.

Furthermore, the increased expression of two genes (HMOX1, TXN) encoding enzymes that are involved in the defense against oxidative stress revealed antioxidant properties of Chios mandarin extract.

This means the Chios mandarin extract achieves its stimulatory effect on melanogenesis through several mechanisms, including the increased production of eumelanin, enhanced melanocyte proliferation, and the protection of melanocytes due to antioxidant properties.

Protection against stress-induced malfunction of melanocytes

While genetic factors play the major role in hair greying, other factors, including stress, have been linked with premature hair greying. Scientific studies confirmed that the hormone noradrenaline, which is released upon stress, is involved in stress-induced hair greying.²

Interestingly, stress-induced hair greying can be reversed.³ Melanocytes treated with 100 μM noradrenaline for 24 hours showed changed gene expression profiles, which indicate a stress-induced malfunction of these cells.

Genes that are important for melanocyte function and melanogenesis were significantly downregulated. These genes included HMOX1 encoding heme oxygenase 1, which protects the cells from oxidative stress, MC1R encoding melanocortin 1 receptor which is directly involved in melanogenesis and CCN3 encoding cellular communication network factor 3.

The downregulation of CCN3 can lead to

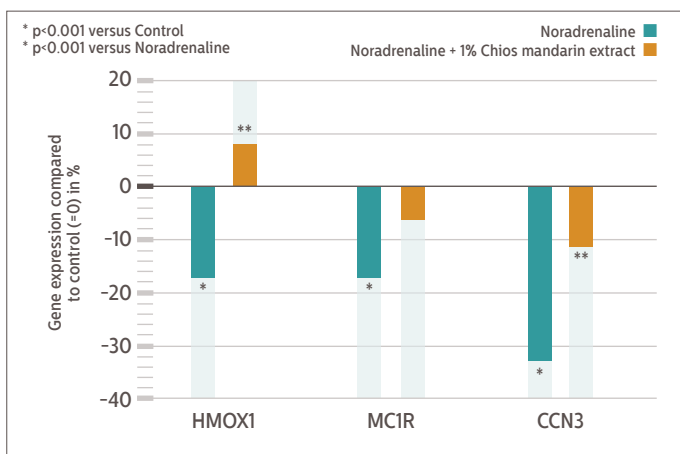


Figure 4: Restored gene expression in stressed cells

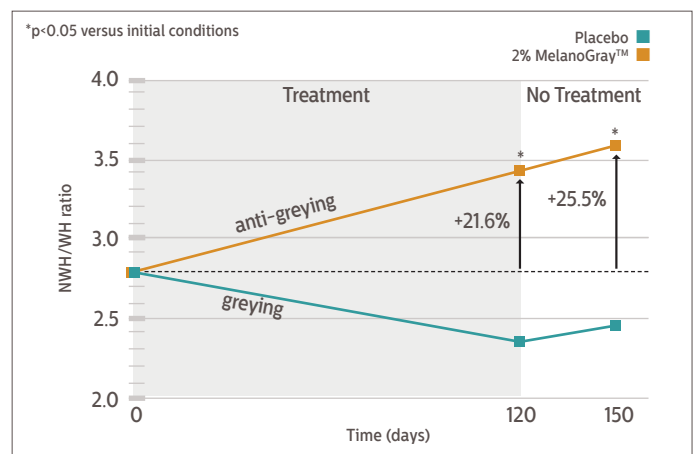


Figure 5: Long-lasting anti-greying effect

reduced cell adhesion and detachment of melanocytes from the basal membrane, which in turn can lead to a decreased melanin production.

Gene expression could be restored in all cases by adding 1% Chios mandarin extract to the cells during the treatment with noradrenaline. Thus, by reducing the negative effects of noradrenaline-induced stress, this Chios mandarin extract could help to prevent or reverse the hair greying process.

Anti-greying effect

The *in vitro* results suggested that Chios mandarin extract can reduce hair greying. Thus, the efficacy of the Chios mandarin extract-based active ingredient was analyzed in a randomized, placebo-controlled clinical study including 55 female and male volunteers with grey hair.

The study was performed in Spain during the first wave of the COVID-19 pandemic in Europe in spring 2020. The timing of this study may have involved psychological stress for the volunteers due to the lockdown situation.

A hair serum with 2% Chios mandarin active was used daily for 120 days and the numbers of white (WH) and non-white (NWH) hair were calculated based on pictures taken with a TrichoScan microcamera.

Whereas the proportion of grey hair further increased in the placebo group, the treatment with the active ingredient led to a reduction of grey hair by 5% compared to the placebo group after 120 days of treatment. The ratio of non-white and white hair (NWH/WH), which indicates an anti-greying effect, was significantly increased by 21.6%.

This effect was long-lasting, as the NWH/WH ratio was still significantly increased in 77% of volunteers 30 days after the last application (day 150). Thus, treatment with 2% Chios mandarin extract-based active ingredient leads to a long-lasting significant reduction of grey hair, even during times of enormous stress, where hair greying could be accelerated.

Conclusion

Derived from a unique locally sourced mandarin, MelanoGray is an attractive upcycling ingredient that stimulates melanin synthesis by different mechanisms, including increased production of eumelanin, enhanced melanocyte proliferation, and protection of melanocytes from stress-induced malfunction.

The combination of these effects leads to a long-lasting reduction of grey hair by prevention of hair greying and reversal of stress-induced hair greying. **PC**

References

1. Panhard S *et al.* Greying on the human hair: a world-wide survey revisiting the '50' rule of thumb. *British Journal of Dermatology*. 167: 856 – 873 (2012)
2. Zhang B *et al.* Hyperactivation of sympathetic nerves drives depletion of melanocyte stem cells. *Nature*. 577: 676 – 681 (2020)
3. Rosenberg A *et al.* Quantitative mapping of human hair greying and reversal in relation to life stress. *eLife*. 10: e67437 (2021)

