Active Beauty
Synchronight™
The microbiome-activated
digital stress defender

Crafted by Green Fractionation
Skin melatonin: the key for a happier and younger life

Melatonin is a well-known sleep-related hormone naturally secreted by our organism (brain and skin), on a daily rhythm cycle. Its production peak happens at night, and plays a crucial role in our capacity to fall asleep and in our sleep quality, as described thoroughly by the literature. However, melatonin has other interesting properties: it also acts as a powerful anti-ageing agent, thanks to its strong antioxidant properties, and the triggering of a biological defences cascade through its binding to the melatonin specific receptor (MT1R).

When exposed to digital stress (digital devices, blue light...), melatonin production rhythm is disturbed, ending up in skin ageing (loss of antioxidant defences and damages to the mitochondria) and a perturbation of the sleep-related functions (difficulties to fall asleep, waking-up several times at night, fatigue in the morning). This results in a vicious circle of premature ageing of the skin, more exposed to external aggressions and unable to restore itself during resting phases of the human body.

In a world where 80% of people are not totally satisfied with their sleep quality, and where consumers are also convinced of the strong link between sleep quality and skin health, it becomes clear that melatonin protection is one of the key to investigate for both well-ageing and well-being.

A natural shield against digital stress, activated by the microbiome in a melatonin-like molecule

Our researchers have investigated specific tetraterpenoids, generally known for their antioxidant and anti-inflammatory benefits, such as crocin and crocetin, which are identified for their additional benefits against blue light and UV radiations, as well as for their ability to improve sleep quality (as food supplements).

Crocin can be found abundantly in the fruits of Gardenia, a beautiful and inspiring Asian plant, with pure white flowers and vivid red fruits. Relying in the NADES technology (NATural Deep Eutectic Solvent), our experts for Green Fractionation crafted a stabilised Gardenia Fruit extract enriched in crocin, called Synchronight™.

By further collaborating with our experts in Skin Physiology and Skin Microbiomics, we demonstrated that crocin not only protects the natural skin melatonin cycle, but can also be converted into crocetin by the skin microflora, triggering the same receptors as melatonin (MT1R). Synchronight™ therefore behaves as a vegetal melatonin-like molecule, activating biological mechanisms of skin repair and defences, connected to the circadian rhythm, ultimately linked to the well-being.

Synchronight™ - for younger skin and better nights!

Synchronight™ protects the cutaneous production of melatonin when skin is exposed to a digital stress thanks to its blue light absorbing properties. Melatonin can therefore achieve its natural role of fighting against the deleterious effects of digital pollution on premature skin ageing. But moreover, thanks to its activation by the skin microbiome into a vegetal melatonin-like molecule, Synchronight™ can take an active part into skin defences and well-being mechanisms.

Clinical tests have indeed highlighted that Synchronight™ reverses visible signs of ageing (~21% wrinkles number versus placebo) while contributing to a general improvement of volunteers sleep quality (easiness to fall asleep, number of wake-ups during night).

Synchronight™ is the perfect natural ingredient to fight against the damages of digital stress, both for premature ageing prevention and well-being improvement. It can also be combined with the Givaudan patent-pending technology DreamScentz™, a breakthrough sensory innovation to enhance sleep experience.

Focus on the product

Synchronight™ actions
Protecting the skin from digital stress: anti-ageing efficacy

1. Protection of mitochondria network and cells spreading \textit{(in vitro)}

Human dermal primary fibroblasts (57 years old donor) were treated with an equivalent of Synchronight™ at 2% or left untreated. Cells were then loaded with Mitotracker Green dye and seeded into a CYTOOplate™ with extra-large Y-micropatterns. After 2 hours of treatment, cells were irradiated with blue light for 1 hour at 20 J/cm².

Live imaging analysis was performed to qualify the mitochondrial network, by measuring its characteristic metrics, and F-actin was stained to analyse the cells spreading.

**Results:** After exposure to blue light, the mitochondrial network appears to be damaged. The network is less fragmented in presence of Synchronight™, demonstrating its protective effect.

While blue light significantly decreases the characteristic dimensions of the mitochondrial network, Synchronight™ at 2% enables to significantly protect it, by increasing its length by +51% and reducing the number of branches by –19%.

Cell spreading is also significantly affected by blue light, with a decrease down to -26% of correctly spread cells. Synchronight™ at 2% is protecting significantly cells spreading (+20% cell area, +57% spread cells).

\[*** p<0.001 \text{ one way ANOVA test}\]

<table>
<thead>
<tr>
<th>Biological activity</th>
<th>Untreated</th>
<th>Blue light</th>
<th>Blue light + Synchronight™ 2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Network length (µm)</td>
<td>454.1</td>
<td>259.7 (-43%*** )</td>
<td>391.9 (+51%*** )</td>
</tr>
<tr>
<td>Nb of branches / Total length</td>
<td>0.464</td>
<td>0.678 (+46%*** )</td>
<td>0.549 (-19%*** )</td>
</tr>
</tbody>
</table>

2. Reduction of oxidised proteins content \textit{(ex vivo)}

Human skin explants (35 years old donor) were cultured for 5 days in day/night cycles (12 hours of day / 12 hours of dark), with a 3 hours exposition to blue light for some of them (63.75 J/cm²). A dose equivalent to Synchronight™ at 2% was topically applied on some of the explants exposed to blue light. On the last day, immuno-staining of the oxidised proteins was performed and image analysis was used to quantify oxidised proteins content.

**Results:** After exposure to blue light, oxidised proteins content significantly increases up to +93%. Synchronight™ at 2% enables to significantly reduce proteins oxidation, by decreasing their content by -81%, close to basal level.

\[** p<0.01, * p<0.05 \text{ Mann Whitney test}\]
Protecting the skin from digital stress: melatonin cycle preservation *(in vitro)*

Sensory neurons (derived from human induced Pluripotent Stem cells) and keratinocytes (derived from a 30 years old donor) were co-cultured under a cyclisation protocol (a drop in temperature to mimick the “night” phase, together with a pause in glutamate use).

At the end of D-3, a nutriment shock was performed to synchronise the cells. At the end of D-1, a new shock was performed to initiate a new cycle on the synchronised co-culture. From this day, a dose equivalent to Synchronight™ at 4% was also added into the medium to one third of the culture samples.

On D0 and D1, 30 minutes before the “night” phase, two third of the co-cultured cells samples (including the ones treated with Synchronight™) were exposed to blue light (20 mJ/cm²). The culture supernatants were taken 30 minutes before the “night” phase, then 2 hours, 5 hours and 8 hours after the shift to the “night” phase and an ELISA assay was performed to dose the amount of released melatonin.

**Results:** The synchronisation of the cells induces a cyclisation of the release of melatonin after 24 hours (D1). The quantity of cutaneous melatonin is significantly increased after 2 hours, 5 hours or 8 hours in comparison to the level 30 minutes before the “night” phase.

Blue light stress disturbs or delays the cycle of melatonin release, as there is no significant increase in melatonin release under these conditions.

The use of Synchronight™ at 4% enables a full protection of the natural melatonin release cycle, avoiding any perturbation caused by the blue light, as the level of melatonin and its rhythmed cycle is preserved at cutaneous level.

\* p<0.05 Mann Whitney test
Microbiome activation into a melatonin-like molecule

1. Conversion of crocin into crocetin by the skin microbiome (in vitro)

A sampling of skin’s microbiome was performed on 7 volunteers with a sterile gauze impregnated with NaCl on 5 areas: forehead, cheek, nose, neck, forearm. Microbiome was cultured in liquid medium (buffered HT medium) at 30°C in presence of crocin. Supernatants were collected upon time and analysed by HPLC-MS to detect crocin and crocetin.

Results: The Stratum Microbium™ totally converts crocin into crocetin, by removing the glucoside moieties of crocin over time.

2. Melatonin-like behaviour of crocetin (bioinformatics)

Docking studies were performed with models derived from crystallographic structures to evaluate the possibility of crocin and crocetin to interact with a melatonin specific receptor (MT1R).

The best model reproducing experimental data was selected, and affinities were calculated for crocin, crocetin and melatonin.

Bioinformatics simulation of melatonin, crocin and crocetin binding to MT1R (in orange)

Results: While crocin has a negative score of affinity (highly disfavoured interaction), crocetin has an affinity with MT1R close to the one of melatonin itself, meaning it may be able to bind to this receptor, and trigger the biological cascade of mechanisms which would be triggered by the binding of melatonin: activation of the antioxidant defences and positive activity on the circadian rhythm.
Anti-ageing and well-being efficacy (clinical test)

To evaluate the well-being & anti-ageing benefits of Synchronight™ in face care applications, thanks to its action against digital stress, a clinical test was conducted in double blind versus placebo. This study was carried out on 40 women (average age 39 years old, from 18 to 50 years old), selected for being in front of a digital screen more than 4 hours per day, of which at least 2 consecutive hours in the evening, with their devices set at 100% luminosity. 2 groups of volunteers were formed, and panellists applied a cream with or without the equivalent of Synchronight™ at 2% twice a day (morning and evening) for 56 days.

Screen exposure time was compared between the 2 groups thanks to a daily logbook filled by all the volunteers, making sure that digital stress exposure time was not significantly different during the study, either during the day, or more specifically during the evening. No significant differences were detected with an average of 4.5 hours daily exposure for the placebo group versus 5.1 hours for Synchronight™, and respectively 2.6 hours versus 2.4 hours in the evening, validating the possibility to compare the groups.

1. Anti-ageing: reduction of wrinkles number

The anti-ageing efficacy of Synchronight™ was analysed by the quantification of crow’s feet wrinkles number using VISIA® analysis at D0, D28 and 56.

Results: As confirmed by the visual observation of volunteers pictures, Synchronight™ at 2% induces a significant reduction of crow’s feet wrinkles number, down to -21% versus placebo (-26% versus D0).

Student’s t-test: * p<0.05, ** p<0.01

2. Anti-ageing: self-assessment by volunteers

Panellists were asked to assess both products efficacy on their skin quality after 56 days of use. Significant differences were noticed in terms of skin hydration and smoothness.

My skin is more hydrated. 80% (vs 40% for placebo)°

My skin is smoother. 75% (vs 45% for placebo)°

# p<0.1 χ² test
3. Well-being: improvement of sleep quality

The well-being benefits of Synchronight™ were studied by analysing daily logbooks of the volunteers during 56 days. The logbook was filled by the volunteers every day in order to collect data about different parameters, such as: source of exposure to digital stress, screen exposure duration, easiness to fall asleep, number of nocturnal awakenings...

Results: Synchronight™ at 2% has a significant impact on the sleep quality versus placebo, according to the perception of volunteers.

It makes it easier to fall asleep (it was easy to fall asleep 90.6% of the nights versus 84.8% of the nights only with the placebo), and drastically decreasing the number of awakenings every night (20.0 nights without awakenings over 28 versus only 14.3 for the placebo).

The median number of awakenings during 28 nights was drastically reduced as well, with only 3.0 awakenings in average versus 23.0 for the placebo.

A focus was made more precisely on the youngest part of the panel (18 to 35 years old). It is noticeable that the activity of Synchronight™ on the well-being is even stronger on this younger population, with a drastic improvement of the easiness to fall asleep, for more than 97% of the volunteers, and of the nights without awakenings (close to 22 nights without awakenings over a period of 28 nights).

All of these results are conserved in the same order of magnitude and the same statistical significativity after 2 months of use of both active and placebo (results not shown here).

***p<0.001, **p<0.01 dichotomic analysis with Fisher test
*p<0.05, #p<0.1 non parametric analysis with Mann Whitney test
Technical information

Suggested INCI: Glycerin (and) Betaine (and) Water (and) Gardenia Jasminoides Fruit Extract (and) Maltodextrin

Cosmos: Approval pending

Origin: Green fractionation

Preservation: Preservative free

Appearance: Orange to amber liquid

Solubility: Water soluble

Dosage: 2%

Processing: Can be added to the water phase at the end of the formulation process, at a pH between 5 and 8, and a temperature below 40°C.

Claims: Blue light protection, anti-wrinkle, sleep quality improvement, antioxidant, safeguard melatonin natural production cycle, skin protection.

Applications: Anti-ageing serum or cream, night cream, well-being products, skincare for millennials, cosmetics to preserve from modern lifestyle damages.

Givaudan Active Beauty Sales Offices

Europe

Givaudan France SAS
19-23 rue de la Voie des Bans
FR-95100 Argenteuil (France)

Givaudan UK Ltd
Magna House
76-80 Church Street
Staines, TW18 4XR (United Kingdom)

Naturex SA
250 rue Pierre Bayle - BP 81218
84911 Avignon Cedex 9 (France)

Asia Pacific

Givaudan Singapore Pte Ltd
1 Pioneer Turn
627576 Singapore (Singapore)

Givaudan Shanghai Ltd
298 Li Shi Zhen Road
Pudong Zhang Jiang High Tech Park
201203 Shanghai (China)

Latin America

Givaudan do Brasil Ltda
Av. Engº Billings - 2185, Edificio 31, 1ºAndar - Jaguaré
05321-010 São Paulo - SP (Brazil)

global.cosmetic@givaudan.com

www.givaudan.com