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Neuron ageing and its effect on skin homeostasis

ABSTRACT

Initiated in the 2000s, 'neurocosmetics' refers to topical ingredients that work on the cutaneous nervous system to restore the mediator-receptor balance in the epidermis. It has led to new ways of inducing a positive action on the skin's nervous system which results in increased skin health.

In 2014 scientists discovered that our touch experiences are processed by neurons in the skin before they reach the brain for further processing.

Meanwhile it has been demonstrated that human skin loses its cutaneous sensitivity with age due to an ageing of sensory fibres in the skin. We know that in the dermis, fibroblasts and neurons are continuously communicating.

Can ageing of sensory fibres impact fibroblasts and therefore skin youth? Working on specific nerves culture models, Codif R&N has highlighted that neuroageing leads to the release of toxic molecules that directly affect fibroblast activity.

By treating this issue Neuroguard opens the door to new neurocosmetic strategies, not only focused on neuromediators, but on the impact of neurons ageing on skin homeostasis and ageing.

Nerves are at the core of how skin functions. The network of nerve endings extends throughout the cutaneous layers, from the hypodermis to the deep layers of the epidermis. They play an important role in the way we perceive our environment through the sense of touch. Nerves also convey our emotions to the skin surface. Thus, tension from stress or annoyance can be manifested on the skin by redness or itching. Nerve endings also play a physiological role inside the skin. Nerves communicate with other cutaneous cells through neurotransmitters that can impact skin pigmentation, tone or the hydration rate. This latter property has been widely studied and put into use during the past few years through a discipline commonly known as neurocosmetics.

Until now, nerve endings were considered pathways. It was thought that the information delivered by touch was first of all transmitted to the central nervous system and then decoded and analysed by the brain. However, in 2014, research scientists showed that information was directly decoded and analysed by the nerve fibres before being sent to the brain.[1] This discovery gives new importance to the key function and place of nerve endings in skin, but also gives rise to a number of questions on the role of nerve endings in the physiological evolution of skin. We know that ageing is accompanied by a loss of sensitivity in touch. Medical research has demonstrated that this loss in sensitivity is directly related to ageing, in particular to the degeneration of nerve endings.[2] What about the impact of neuroageing on the skin itself? Could the ageing of these fibres, which are located inside the skin and are known to communicate with other types of cells, affect skin ageing? If so, how?

The study of the neurodegeneration process, especially in medical applications of the Alzheimer's disease type, have enabled establishing the major role of a neurotoxic peptide called amyloid beta (Aβ), which forms so-called senile plaques on the surface of nerve endings (Fig. 1).[3]

This neurotoxic peptide comes from a membrane protein known as Amyloid Precursor Protein (APP), which is located on the neuron surface. As its name implies, this protein is a precursor that can be cleaved in two different ways. To simplify, we can describe a first type of cleavage that is triggered by a β secretase enzyme and gives rise to the liberation of neurotoxic Aβ. A second type of cleavage is triggered by the liberation of an sAPPα peptide, which, contrary to Aβ has neuroprotective properties (Fig. 2).[4]

These two cleavages exist in a balanced manner within a neuronal population that could be qualified as young. Ageing, and, in a general and repetitive manner, the accumulation of oxidising stress leads to a gradual imbalance, where the cleavage

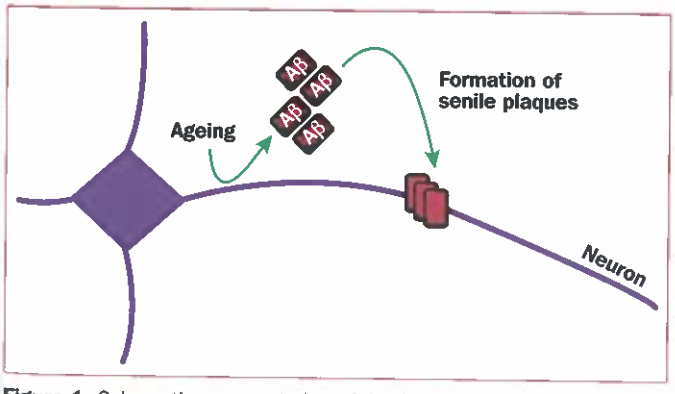

Figure 1: *Schematic representation of the formation of senile plaques on neurons ending.*

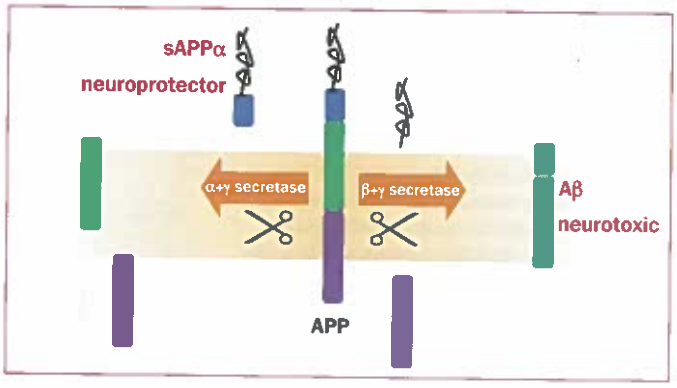

Figure 2: *Schematic representation of APP processing.*

liberating the neurotoxin becomes dominant,[5] triggering the neuronal degeneration process that we call neuroageing.

The goal of the study we describe here is to understand the impact of this phenomenon on the neuroageing of cutaneous cells, in particular the fibroblasts. Does neuroageing affect fibroblasts? Can neuroageing lead to fibroageing?

After answering these questions, we will have a look at how and why the treatment of neuroageing represents a new anti-ageing possibility for lessening wrinkles in individuals over sixty.

How neuroageing can affect fibroageing

The hypothesis we are testing here is whether neurons placed under neuroageing conditions secrete messages that are potentially toxic for fibroblasts.

Neuroageing was simulated by the addition of the A factor (1 µM) to embryo cortical neurons. After 24 hours of culture, we observed a decline in viability in the order of 45%, thus illustrating the impact of neuroageing (Fig. 3). This neuron culture was then inoculated into human dermal fibroblast cultures, where we observed a 48% drop in fibroblast viability, as compared to fibroblast cultures receiving neuron cultures which were not exposed to neuroageing. This first result confirms the hypothesis that neurons exposed to neuroageing secrete messengers that direct impact fibroblast viability. Communication between nerves and fibroblasts becomes toxic under neuroageing conditions. The drop in viability is also accompanied by a decrease in the synthesis of collagen and elastin, respectively 86% and 81%. Therefore, neuroageing directly affects fibroageing, suggesting repercussions on global skin ageing.

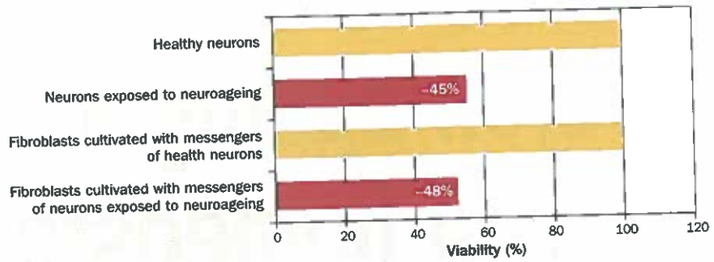

Figure 3: *Impact of neuroageing on neurons and fibroblasts viability. Measure of the viability of neurons cultures non exposed to Ab (Healthy neurons) and neurons exposed to 1 µm Ab for 24 hours (neurons exposed to neuroageing). The culture medium of healthy neurons is then inoculated to culture of fibroblasts (fibroblast cultivated with messengers of healthy neurons) while the culture medium of neurons exposed to Ab is inoculated to a second culture of fibroblasts (fibroblasts cultivated with messengers of neurons exposed to neuroageing).*

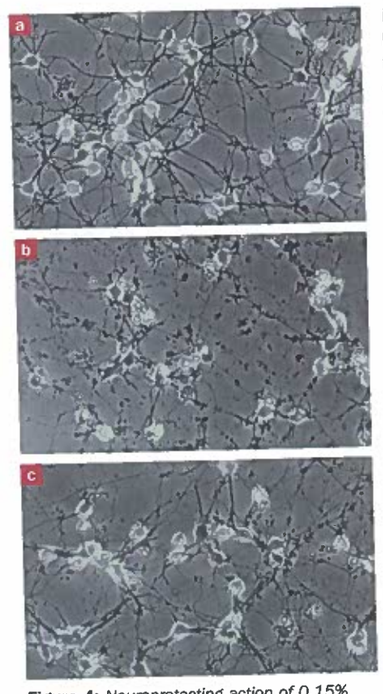

Figure 4: *Neuroprotecting action of 0.15% Neuroguard.* **a)** *neurons not exposed to neuroageing presents healthy nuclei, visible synapses and a dendrite network which is well developed.* **b)** *neurons exposed to neuroageing with farctionated nuclei (apoptotic), thinner, shorter and fractionated dendrites with a de-structured network and synapses which are no more visible.* **c)** *neurons exposed to neuroageing + 0.15% Neuroguard recover same characteristics than healthy neurons.*

Protecting neurons from neuroageing

To block the effects of neuroageing, Neuroguard uses a two-step strategy. First, it protects neurons from the causes of neuroageing, primarily free radicals. Used at 0.15%, Neuroguard (now referred to as 'the new hydrolysed algin') provides protection in the order of 42% against hydrogen peroxide toxicity on neuron viability in cultures. Next, it stimulates the neuronal synthesis of sAPPa by 87%. The neuroprotective action of sAPPa is probably due to binding of the BACE 1 receptor, which is directly involved in the inhibition of β-secretase.[6] Figure 4 shows the neuroprotective effect of the new hydrolysed algin on the neuron network. Whereas neurons which have not been exposed to neuroageing (Fig. 4) show active synapses, an extensive network and integral cell nuclei, the effect of neuroageing (Fig. 4b) is characterised by fragmented nerve endings, 'burnt-out' nuclei and a limited network. By stimulating the sAPPa neuroprotector, the new hydrolysed algin enables recovering the characteristics of neurons that have not been exposed to neuroageing.

A complementary experiment enabled demonstrating that the protective action of the new hydrolysed algin (0.15%) on neuron viability is effective when it is used before neuroageing starts (45% protection), as well as when neuroageing is already underway (34% protection). An optimal protection of 52% is obtained when the new hydrolysed algin is used both before and after the start of neuroageing. The new hydrolysed algin is thus able to prevent and repair damage from neuroageing on nerve fibres.

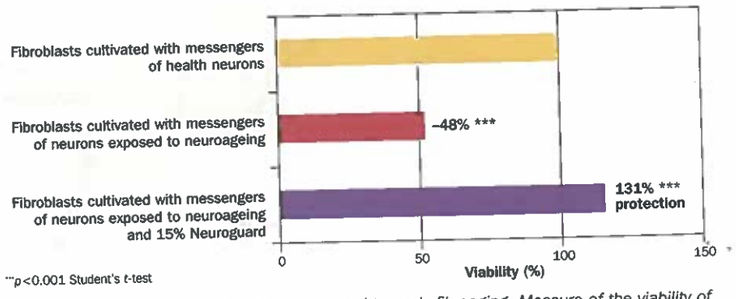

Figure 5: *Protective action of 0.15% Neuroguard towards fibroaging. Measure of the viability of fibroblasts exposed to messengers released by neurons not exposed to neuroageing (healthy neurons), exposed to neuroaging (1 µm Ab for 24 hours), and exposed simultaneously to neuroageing and Neuroguard.*

Impact of neuroprotective action on fibroageing

In spite of an observed drop in the viability of fibroblasts exposed to neuroaeging messengers, through its neuroprotective action, the new hydrolysed algin provides 131% protection against decreased fibroblast viability that had been induced by neuroageing (Fig. 5). By protecting neurons against neuroageing, the new hydrolysed algin re-establishes a healthy communication between nerve endings and fibroblasts, thereby protecting dermal cells from fibroageing.

Under these conditions, collagen and elastin synthesis are once again optimal, with respective variations of +405% and +832% as compared to synthesis obtained under conditions of neuroageing (Fig. 6).

Neuroprotection: a new key to treating the deep wrinkles of people over sixty

The benefits of this neuroprotective approach were evaluated on a panel of 20 volunteers, ages 62 to 74. The depth and extent of crow's foot wrinkles, as well as skin roughness, were evaluated before and after 28 and 56 days of twice-daily application of a cream containing 1.5% new hydrolysed algin. The study showed a visible decrease in the volume and extent of wrinkles from 28 days and significant variations after 56 days of treatment (Figs. 7 & 8). In a similar manner, skin roughness was less after 28 days, with a significant variation after 56 days of treatment.

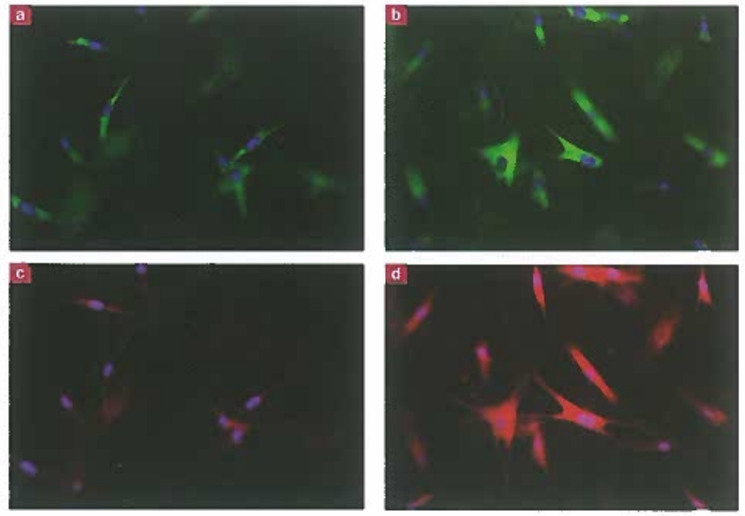

Figure 6: *Impact of neuroprotective action of Neuroguard on the synthesis of extra-cellular matrix. Proteins. Visualisation of collagen synthesis (green fluorescence) in cultures of fibroblasts exposed to messengers of neurons submited to neuroageing* **a)** *without, or* **b)** *with 0.15% Neuroguard. Visualisation of elastin synthesis (red fluorescence) in cultures of fibroblasts exposed to messengers of neurons submited to neuroageing* **c)** *without, or* **d)** *with 0.15% Neuroguard.*

Conclusion

Neuroguard revolutionises neurocosmetics by directly targeting the ageing of nerve endings. Its neuroprotective action blocks neuroageing and re-establishes a healthy communication between nerve endings and fibroblasts, thus protecting dermal cells from premature ageing. Under these conditions, collagen and elastin synthesis are reactivated, deep wrinkles are diminished and skin texture is smoother. With an action focused on neuroageing, Neuroguard provides an appropriate and well-targeted answer for treating wrinkles in individuals aged sixty and older. PC

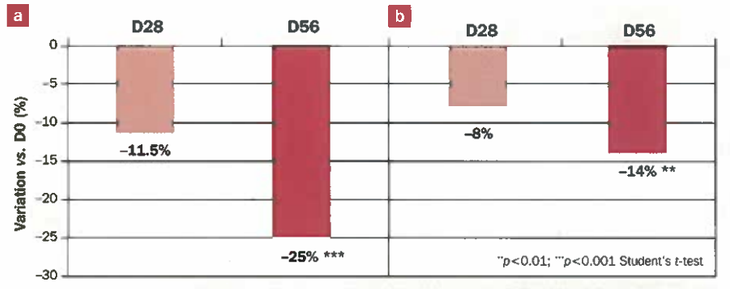

Figure 7: *Effect of 1.5% Neuroguard on both* **a)** *volume, and* **b)** *area of crow's feet wrinkles after 28 and 56 days treatment.*

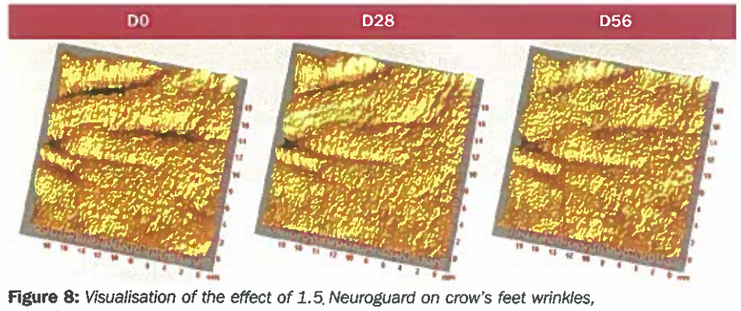

Figure 8: *Visualisation of the effect of 1.5 Neuroguard on crow's feet wrinkles, using fringe projection.*

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