The quest for a whiter shade of pale in skin care

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Introduction

The quest for a whiter shade of pale dates back to ancient civilizations, when a pale complexion was associated with aristocratic lineage. During the Tang dynasty (618-907), Chinese women used ground pearl powder as a whitening make-up. In ancient Persia, field workers bleached their skin with pure natural hydroquinone to keep it from darkening under the sun. In Europe, during the Renaissance, lead and mercury compounds applied to the face, neck, and chest, became popular as peel-off skin lighteners. Unknown at the time, many of the earlier lightening ingredients were in fact toxic and this led to their ban for cosmetic use.

However, even nowadays, skin lightening remains a popular goal in skin care. A fair skin tone is increasingly associated with a healthy skin and youthful appearance. The field of potential applications for lightening products has grown accordingly to include more specific pigmentation concerns, such as lentigines (dark spots appearing, with older age, on sun-exposed skin), freckles (irregular grouping of pigment-containing cells or melanocytes), melasma (hormonal hyperpigmentation), inflammation-related hyperpigmentation, and even hypopigmentation. Skin lighteners are now perceived as multi-usage cosmetic products although with some geographical and cultural differences. Westerners seek them essentially for their anti-aging benefits, while Asians people rather use them for general lightening of their skin.

With the advancement of science over recent decades, our knowledge of the physiology of skin pigmentation has improved drastically. As we better understand the complex regulation of this physiological process, it is becoming clear that the future of lightening skin care lies in the combination of several actives addressing simultaneously and complementarily the various facets of skin pigmentation. In fact, the trend worldwide is now to offer skin cares that combine multiple modulators of pigmentation with anti-aging actives. This makes sense. After all, factors contributing to skin aging such as UV exposure, hormones, oxidation, and inflammatory reactions also affect skin pigmentation, and irregular skin pigmentation is seen increasingly with age.
This article will review our current knowledge of the mechanisms involved in skin pigmentation, highlight effective ways of modulating these mechanisms with a combination of selected commercially available actives (see Table I), and comment the efficacy and safety of a serum (“Image Blanc™”) integrating this knowledge. The serum described here additionally contains a core anti-aging technology (“REGEN 16™”) which has already been described elsewhere.

Insert table I here

The cellular and molecular physiology of skin pigmentation

The life of melanin, the main pigment responsible for skin pigmentation, is a fascinating journey. Starting within melanocyte cells with the amino acid tyrosine as the raw material, melanin pigments are assembled in specialized incubators called melanosomes, conveyed to finger-like structures at the tip of melanocytes and then gently transferred to neighboring keratinocyte cells that rearrange them as protective umbrellas around their DNA. As these pigmented keratinocytes mature, they progress to the edge of the stratum corneum from which they eventually shed through desquamation, leading to pigment loss. Each step of the melanin journey mobilizes various enzymes and signaling factors that may turn out to be interesting targets for the modulation of skin pigmentation.

- **Melanosome maturation**
  The production and maturation of melanosomes are under the responsibility of melanocytes at the basal layer of the epidermis. Within melanocytes, melanosomes start as spherical vesicles. Their maturation takes place in several steps. In UV-induced tanning, binding of α-melanocyte stimulating hormone (α-MSH) to its receptor (MC1R) at the surface of melanocytes is an early event in melanosome formation. Activation of the receptor triggers a cascade of events within melanocytes that culminates in the induction of the microphthalmia transcription factor (MITF). Signals emerging from non UV-induced pigmentation (intrinsic, hormonal and inflammatory) also converge at MITF level making it a key master of skin pigmentation. Among other things, MITF commands the expression of melanogenic proteins, including that of PMEL-1 whose task is to form a fibrous scaffold on which the biosynthesis and deposition of melanin will eventually take place.

  Melanosome maturation can be prevented early, using antagonists of α-MSH such as Lepidium sativum (cress) sprout extract or the small molecule undecylenoyl phenylalanine. Acting a step further, α-bisabolol extracted from chamomile is able to interrupt the cAMP signaling cascade normally turned on by α-MSH. α-Bisabolol additionally reduces MITF expression, as also do thioctic acid (α-lipoic acid), and oligopeptide-68, the latter being a peptide derived from TGF-beta. Working at a more distal point, a Pisum sativum (pea) extract has been shown to act directly on PMEL-1, the scaffolding protein, to inhibit melanosome maturation.

- **Pigment production**
  In the later steps of melanosome maturation, three important proteins for melanogenesis, namely tyrosinase (TYR), tyrosinase-related protein-1 (TRP1), and dopachrome oxidase (DCT), are processed and transported into the organelle. The three enzymes work in concert to produce two distinct types of pigments. One is a red/yellow pigment (pheomelanin) while the second is a
black/brown pigment (eumelanin). The balance between these two pigments determines the color of a person skin and hair. The MC1R receptor, at the surface of melanocytes, partly controls the switch between eumelanin and pheomelanin. Eumelanin is the preferred pigment as it confers better sun protection. As just mentioned above, activation of MC1R leads to induction of MITF which, besides being involved in melanosome maturation, also controls the expression of TYR, TRP1, and DCT, thus pigment production.

As a key enzyme in melanin production, tyrosinase has traditionally been a major target for skin whitening products. Inhibitors of tyrosinase activity include Glycyrrhiza glabra (licorice) root extract, phenylethyl resorcinol, Rumex occidentalis (willow) extract, the arbutin-containing Uva-Ursi (bearberry) leaves extract, and magnesium ascorbyl phosphate. It is also possible to stabilize tyrosinase in its inactive conformation using diacetyl boldine, an active derived from the bark of a Chilean tree. Other inhibitors of melanin production act through down-regulation of TYR, TRP-1, or DCT gene and protein expression. Ingredients from this category include Humulus lupulus (hop) strobile, oligopeptide-68, and octadecenedioic acid. Not surprisingly, ingredients acting upstream of tyrosinase, will also inhibit melanin formation. These include the previously discussed Lepidium sativum (cress) sprout extract, undecylenoyl phenylalanine, α-bisabolol, thioctic acid (α-lipoic acid), oligopeptide-68, and Pisum sativum (pea) extract. Vitamin E, for its part, has been shown to favor production of the lighter pheomelanin pigment, leading to lighter skin pigmentation.

- **Melanocyte dendricity**

As melanosomes are maturing, filling up with dark pigments, they also develop finger-like (dendrite) projections in preparation for pigment transfer. This is a remarkable ability requiring a considerable reorganization of the cell cytoskeleton. Very few cell types in the body have such potential. Melanocyte dendrite formation is regulated through multiple signaling pathways in response to UV rays, hormonal stimulation, and other incentives, including pro-inflammatory stimuli such as prostaglandin E2 (PGE2). All these signals converge at the level of MITF who contributes largely to melanosome dendricity. The formation of dendrites opens highways on which fully matured melanosomes can now be transported.

Actives with the potential to inhibit dendrite formation include α-bisabolol, thioctic acid (α-lipoic acid), and oligopeptide-68, for their action on MITF, and dipotassium glycyrrhizate, through inhibition of PGE2.

- **Melanosome transport**

Upon melanogenic stimulation, melanosome, otherwise packed in the center of melanocytes, are mobilized toward the tip of the dendrites. The process involves special structures (microtubules) acting as rails for the cargo of melanosomes, the driving force being assured by motor proteins. Pretty much as sushi trains, melanosomes move up and down dendrites until they are picked up at the tip of a dendrite and hold in place by a complex of proteins, in the wait for their transfer to keratinocyte cells. Again, MITF plays a role in melanosome transport by controlling the expression of proteins essential for the capture of melanosomes at the tip of the dendrites.

Useful actives in inhibiting melanosome transport include those acting on MITF, like α-bisabolol, thioctic acid (α-lipoic acid), and oligopeptide-68.
• Melanosome transfer & uptake

How exactly melanosomes are transferred from the dendrites of melanocytes to keratinocytes is still a matter of debate. So far, four mechanisms have been proposed: cytophagocytosis, exocytosis, membrane fusion, and vesicles trafficking\(^{25,4}\). Cytophagocytosis implies that the tip of a melanocytic dendrite is pinched off and engulfed by a neighboring keratinocyte. According to exocytosis, melanosomes and melanocytes fuse through their membranes to release melanin pigments in the extracellular space, where they are taken up by keratinocytes through phagocytosis. In the membrane fusion model, a melanocytic dendrite fuses with a keratinocyte creating a channel for melanosomes transfer. The vesicles trafficking model implies that melanin containing vesicles are shed in the extracellular space before being absorbed by keratinocytes. Chances are that more than one process is used at any given time.

Actives that inhibit dendrite formation on melanocytes will also inhibit the transfer of melanosomes to surrounding keratinocytes. As discussed above, \(\alpha\)-bisabolol, thiocytic acid (\(\alpha\)-lipoic acid), oligopeptide-68, and dipotassium glycyrrhizate fall in this category. To more directly inhibit melanosome phagocytosis by keratinocytes, it is possible to use Artocarpus heterophyllus (Jack fruit) seed extract. A plausible explanation for this activity is that Jack fruit extract contains a lectin and lectins have the potential to inhibit close interactions between melanocytes and keratinocytes as required for pigment exchange\(^{25}\). Another way to interfere with pigment transfer is to modulate calcium flow regulation in melanocytes\(^{26}\) and diacetyl boldine has been shown to do that. Retinol also inhibits pigment transfer, but the precise mechanism remains to be fully elucidated\(^{27}\).

• Keratinocyte maturation and skin desquamation

Following their uptake by keratinocytes, melanosomes fuse with lysosomal structures and are transported close to the cell nucleus. There, melanin pigments rearrange themselves forming a cap that protects the nuclear keratinocyte DNA from the deleterious effects of UV radiation\(^{28}\). As part of their maturation process, pigmented keratinocytes move upward to the stratum corneum from which they eventually shed. Any intervention that increases epidermal renewal and desquamation will thus help getting rid of acquired pigmentation\(^{18}\).

Adenosine monophosphate can be used to stimulate epidermal turnover. Adenosine is the building block of adenosine 5'-triphosphate (ATP), the main intracellular source of energy. Since energy is essential for cell proliferation and maturation, supporting ATP levels with topical adenosine safely accelerates epidermal turnover\(^{29}\). Topical adenosine also proved to be effective for treating hyperpigmentary disorders, such as melasma\(^{30}\). To favour proper epidermal desquamation, treatment with a mixture of Centella asiatica (gotu kola) extract, Carica papaya (papaya) extract, and Iris florentina (iris) extract has been reported to be helpful. Other actives such as retinol\(^{18}\), Salix nigra (willow) bark extract, and Vibrio exopolysaccharide extract are also effective for desquamation and additionally stimulate keratinocyte renewal.

Oxidative and inflammatory incentives for skin pigmentation
Oxidative stress and inflammation can result from both normal and pathological reactions. Whatever the cause, both processes have a big influence on skin pigmentation and on skin aging as a matter of fact. Recognizing that has provided new tools to address pigmentation concerns.

**Oxidation**

Skin pigmentation is greatly influenced by ultraviolet (UV) radiation from sun exposure. UV rays trigger the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that may dangerously alter the DNA of skin cells. Since melanin pigments can act as ROS scavengers, the skin response to UV exposure and ROS challenge naturally involves the proliferation of melanocytes and the release of hormones and factors that stimulate melanogenesis. Given their low level of natural antioxidant (catalase) expression, melanocytes themselves are especially sensitive to ROS and prolonged excessive exposure quite often results in irregular pigmentation and may even cause the appearance of white patches (vitiligo).

Numerous cosmetic ingredients have proven to be useful in supporting natural skin antioxidant defenses for better control of skin pigmentation. Following is a short list of interesting actives, on that aspect. For instance, dipalmitoyl hydroxyproline, *Glycyrrhiza glabra* (licorice) root extract, *Lepidium sativum* (cress) sprout extract, phenylethyl resorcinol, retinol, squalane, thioctic acid (α-lipoic acid), vitamin E, ubiquinone, and a mixture of *Helianthus annuus* (sunflower) seed oil, ethyl ferulate, *Rosmarinus officinalis* (rosemary) leaf extract, and disodium uridine phosphate have documented ROS scavenging abilities. Dimethylmethoxy chromanol can neutralize RNS oxidative molecules. Most interestingly, a small salen-manganese complex (EUK-134™) can be used to mimic the antioxidant activity of two important endogenous antioxidant enzymes (SOD and catalase). EUK-134™ has the unusual ability of regenerating its antioxidant potential which makes it a long lasting molecule.

**Inflammation**

Pigmentary changes are also common features of post-inflammatory events, including sunburns of course, but also mechanical injuries or cutaneous disorders such as eczema or acne for example. These changes involve the release of cytokines, growth factors, and pro-inflammatory lipids such as leukotrienes and PGE2. These mediators can increase melanin production by melanocytes and/or stimulate melanin transfer to keratinocytes. This leads to hyperpigmentation, a problem more common in people with darker skin. In older individuals, chronic inflammation associated with aging favors the formation of age-spots (lentigines). This cosmetic concern affects more than 90% of white people older than 50 years and even appears at a much younger age in Asian skin.

Many actives have anti-inflammatory activity that may help reduce such pigmentation problems. For instance, α-bisabolol is a good inhibitor of leukotrienes synthesis and glycyrrhizate inhibits the production of PGE2. *Cynara scolymus* (artichoke) leaf extract and thioctic acid (α-lipoic acid) both interfere with the activation of NF-kB, a major intracellular signaling molecule involved in inflammation reactions. Palmitoyl tripeptide-8 reduces the production of an inflammatory cytokine (IL-8). *Humulus lupulus* (hop) strobile inhibits the release, by keratinocytes, of an inflammatory factor (GM-CSF) involved in lentigo formation. Other actives also have non-specific anti-inflammatory actions, such as sesame seed oil & wheat germ oil, *Glycyrrhiza glabra* (licorice) root extract, *Salix nigra* (willow) bark extract, a mixture of hesperidin methyl chalcone &
dipeptide-2 & palmitoyl tetrapeptide-7, and also a mixture composed of ethyl ferulate, *Rosmarinus officinalis* (rosemary) leaf extract, and disodium uridine phosphate.

Globally, the diversity and complementarities of the ingredients discussed above should altogether be addressing all known major mechanisms involved in skin pigmentation. To verify this hypothesis, these ingredients were formulated in a serum and tested under dermatological control for clinical efficacy in the modulation of skin pigmentation.

**Clinical Efficacy**

This integrated approach to skin pigmentation was tested in a case study conducted in Canada, under the supervision of a medical doctor. This clinical study was aimed at evaluating the potential of the serum described above to reduce the appearance of hyperpigmentation (age spots and melasma) of the face and hands of volunteers. For this purpose, 10 Caucasian women aged between 38 and 82 applied the serum locally, twice daily for up to 10 weeks, on hyperpigmented spots present on their face and hands. The effect of the serum was documented through before-and-after photographs and doctor evaluation.

Figure 1, Figure 2, and Figure 3 are representative of results obtained with the serum, when applied to the face. As can readily be seen on photographs, a noticeable improvement in the appearance of dark spots is observed within 1 to 2 months of twice daily local application. According to doctor evaluation, global improvement in skin pigmentation was estimated to reach between 20% and 80%, depending on the volunteer. The serum was very effective in improving the appearance of melasma on younger skin (Figure 1). A noticeable improvement in the appearance of age spots was also seen with older skin (Figure 2 and 3). No adverse effects were reported in the course of the study.

The study just described is preliminary and was designed to gain a first impression of the potential of this approach. The benefits reported here serve as a proof of concept and warrant a more extensive clinical study including rigorous controls.

**Safety Testing**

- **HRIPT**
  Human repeat insult patch testing (HRIPT) was conducted to document the cutaneous irritating and sensitizing potentials of the serum, following repeated applications on the skin, under occlusive patch, in 50 healthy adult volunteers. The non diluted serum was repetitively applied during an induction period of 3 weeks, followed by a rest period, and a challenge period. Under the conditions of the test, no evidence of dermal irritation or sensitization was observed for the tested serum (data not shown).

- **CHALLENGE TESTS**
Challenge tests were conducted that involved the standard protocol of exposing the material to specified types of bacteria and fungi to determine whether it is adequately preserved over its intended shelf-life. Interpretation of the data was based on official protocols. Results from the challenge tests showed that the serum met the Personal Care Products Council (PCPC) requirements and guidelines for antimicrobial preservative effectiveness (data not shown).

Conclusion

Skin lightening products are gaining in popularity on the global market. Early generations of skin whitening agents were effective but safety concerns led to their ban from the cosmetic market, forcing the industry to look at new innovative and safer ways to improve skin pigmentation. This has been a big incentive to better understand the physiology of skin pigmentation and elucidate its complexity.

One clear link that has emerged is the close relationship between aging and skin pigmentation concerns. The recently developed microarray technology has pointed at up-regulation of genes related to inflammation, fatty-acid metabolism, and melanin production, but down-regulation of cornified envelope-related genes. Accordingly, it seems that increase uneven pigmentation in aging is associated with keratinization impairment, on a chronic inflammation background. Thus, simply targeting tyrosinase activity to solve pigmentation problems does not seem adequate anymore. Skin pigmentation needs to be addressed globally, as part of the aging process.

The trend is toward the integration of multiple actives covering all mechanistic aspects of skin pigmentation, as well as addressing the skin aging process. The serum “Image Blanc™” described here does it all and leads the way for better integrated skin cares.
References


# Table 1. Mechanisms of pigmentation modulation by selected actives

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**Legend:**
- MMA: Melanosome maturation
- PPr: Pigment production
- Den: Dendricity
- PTp: Pigment transport
- PUp: Pigment uptake
- Dsq: Desquamation
- Oxd: Oxidation
- Inf: Inflammation
Figure 1. Effect of the serum on pigmented spots of the face:

Subject: Caucasian woman
Age: 38
Type of skin: normal, except for pigmented spots
Application: on pigmented spots
Frequency: 2 x/day
Duration: 35 days

Doctor evaluation:
Improvement = 60%
Figure 2. Effect of the serum on pigmented spots of the face:

Subject: Caucasian woman
Age: 62
Type of skin: normal, except for pigmented spots
Application: on pigmented spots
Frequency: 2 x/day
Duration: 70 days

Doctor evaluation:
Improvement = 50%

"Image Blanc™ and REGEN 16™ are products of Immanence Integral Dermo Correction"
Figure 3. Effect of the serum on pigmented spots of the face:

Subject: Caucasian woman
Age: 82
Type of skin: normal, except for pigmented spots
Application: on pigmented spots
Frequency: 2 x/day
Duration: 70 days

Doctor evaluation:
Improvement = 40%

*Image Blanc™ and REGEN 16™ are products of Immanence Integral Dermo Correction*