

From Hydration to Cell Turnover: An Integral Approach to Antiaging

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ABSTRACT: *As the mechanisms of skin aging become better understood, their complexity commands a different approach for antiaging benefits—i.e., integrating multiple complementary actives into a single formulation. In the present article, the authors describe a comprehensive formula designed to effectively address sixteen different mechanisms of skin aging.*

The speed at which information now travels has favored the advancement of science and technology like never before. This is true for all aspects of life, including personal care. The industry's understanding of skin physiological processes has progressed in recent years, and with deeper knowledge more sophisticated cosmetic products have emerged.

Over the past 50 years, cosmetics have evolved from camouflage makeup to the combined health and beauty products that currently predominate the market. In addition, cosmetic products now contain actives that modulate defined physiological processes. The frontier between cosmetic actives and drugs is thinning. In fact, the industry has been flirting so much with phar-

maceutical science that their union has been celebrated with a new word, *cosmeceutical*. What can be learned from this association?

The recent evolution of pharmacological care has initiated a trend toward the development of combination therapies that simultaneously use a variety of drugs to eliminate or control the biochemical causes of a disease. Examples include combinations of antibiotics for tuberculosis, antiretroviral drugs for HIV, or chemotherapeutic drugs for cancer. With the recognition that the body has redundant mechanisms to control any given function, it seems logical to correct a health problem by simultaneously targeting several pertinent mechanisms. This approach was the basis for fantastic improvement

in the success rate of childhood acute lymphoblastic leukemia (ALL), which is now successfully treated in about 80% of patients by the application of intensive combination chemotherapy regimens.¹

The same rationale could be applied to cosmetic care, particularly to antiaging cosmetic care. Throughout the human body, skin aging is a complex process that involves multiple mechanisms that clearly influence each other. A comprehensive integral antiaging approach is thus needed for sustained improvement of the skin. However, this comes with important challenges. An integral approach to skin care requires deep knowledge of the physiological basis for skin aging. It also involves the combination of multiple actives at sufficient concentrations and in a stable formula, a real formulating challenge. The final product also should be clinically efficient and safe—and despite all of this, affordable.

In the present paper, the authors review the changes that occur in skin with age. In addition, the development and testing of an antiwrinkle and firming formulation is described, which combines a long list of actives to fight the signs of skin aging on multiple fronts. A list of the actives found in this serum^a and their respective

Table 1. Actions on skin physiology of the selected actives in the test blend

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Hydration			•		•		•	•			•	•		•			•	•		
Barrier	•	•	•						•									•		
Oxidation					•	•				•					•			•		
DNA						•													•	
Energy				•															•	
Oxygenation						•											•		•	
Immunity	•										•				•					
Pigmentation									•							•				
Keratinization				•									•	•	•					
Skin cohesion													•							
Cell anchorage					•															
Glycation										•										
ECM turnover					•			•					•							
ECM integrity					•			•	•	•			•		•				•	
Inflammation		•	•		•	•		•	•	•								•	•	
Microcirculation								•	•	•										
Ingredients																				
1.	Alteromonas ferment extract										10.	Hesperitin								
2.	Bisabolol										11.	Hyaluronic acid								
3.	Botanical oils (caprylic/capric/succinic triglyceride, sesame seed oil and wheat germ oil)										12.	Imperata cylindrica extract								
4.	Creatine										13.	Palmitoyl oligopeptide (and) palmitoyl tetrapeptide 7								
5.	Dipalmitoyl hydroxyproline										14.	Horsetail, myrrha, wheat germ and hops extracts								
6.	Ethylbisiminomethylguaiacol manganese chloride										15.	Retinol								
7.	Glycerine										16.	Rumex occidentalis extract								
8.	Glycosaminoglycans (GAGs)										17.	Squalane								
9.	Glycyrrhizate (licorice extract)										18.	Tocopheryl acetate								
											19.	Ubiquinone								

effects on physiology are presented in **Table 1**; for the complete INCI listing, see **Integrated Ingredient Concentrate**.

Skin Aging: The Epidermis

Hydration: Skin hydration, which depends on water diffusion and retention, is considerably reduced with aging. Since water binds to protein and is essential to support proper enzymatic reactions, a loss of hydration leads to altered structural and mechanical properties of the skin. The stratum corneum (SC) dries out, causing skin flaking, reducing skin flexibility and precipitating wrinkle formation.

Both lipids and proteins are involved in cutaneous water retention. The SC is rich in long chain lipids that provide a semi-permeable barrier to the passage of water. These lipids represent one of the main mechanisms allowing the SC to hold water.² A decrease in skin lipid content occurs early with aging. Water retention in the epidermis also depends

on charged hygroscopic compounds, among which is hyaluronic acid (HA), a glucosaminoglycan (GAG). HA is synthesized by fibroblasts within the dermis but migrates to the epidermis where it binds and retains water molecules.³ Aging affects the compartmentalization of HA in the skin, favoring dermal—at the detriment of epidermal—localization.⁴ Lipid depletion and the loss of hygroscopic material prevent water diffusion and retention, contributing to dehydration in the upper layers of the skin.

An integral approach to this effect of aging should therefore facilitate both water diffusion and retention in the skin. Botanical oils such as caprylic/capric/succinic triglyceride, sesame seed oil, wheat germ oil, squalane, glycerin and tocopheryl acetate are rich in fatty acids that diffuse into the skin to efficiently replenish skin lipids, thus easing water diffusion through successive layers in the epidermis.⁵

Hyaluronic acid, an anionic non-sulphated GAG, and vegetal-derived dipalmitoyl hydroxyproline, an amino acid naturally found in collagen, can further support skin hydration by fix-

ing water molecules. As a hygroscopic material, GAG possesses the capacity to bind water roughly 1000 times its own weight⁶ while hydroxyproline has been linked to collagen structural stabilization through the formation of water bridges.⁷

In addition, a well-characterized marine GAG extract has been reported to deliver additional GAGs for improved hydration, and the blend of horsetail, myrrh, wheat germ and hops extracts further reinforces hydration potential. Also, an extract of *Imperata cylindrical* root, a plant found in desert environments, is rich in potassium and 3-dimethyl-sulfopropionate (DMSP) and acts as an osmoregulator to trap moisture within the skin cell wall.^{8,9} Taken together, these materials would be expected to restore skin flexibility and slow wrinkle formation, as will be examined.

Barrier function: Poor hydration alters the barrier function of the skin, leaving it more vulnerable to internal and external stress. Permeability barrier disruption is associated with skin irritation and inflammation, in part through the recruitment of inflammatory cells that generate reactive free radicals as

^a Deep Wrinkles Integral Correction Concentrate—IDC (INCI: see **Integrated Ingredient Concentrate sidebar**) is a product of Immanence Integral Dermo Correction Inc.

well as inflammatory lipids (PGE2) and cytokines.¹⁰ Therefore, an approach to aging would be the inclusion of anti-irritant actives.

Exopolysaccharides (EPS), notably those synthesized by *Alteromonas macleodii*, a microorganism that survives harsh conditions of deep sea hydrothermal vents, could impart such a benefit. These EPS form a protective shield at the surface of the skin and work at the cellular level to reduce the expression of ICAM-1, an adhesion molecule involved in the recruitment of inflammatory cells.¹¹ Additional anti-irritants could be delivered through incorporation of bisabolol, botanical oils (caprylic/capric/succinic triglyceride, sesame seed oil, and wheat germ oil), tocopheryl acetate, and/or dipotassium glycyrrhizate (licorice extract), the latter having been shown to slow the production of PGE2, a major inflammatory lipid in the skin.¹² Through reduced irritation and inflammation, this combined formulation could help to restore and maintain skin barrier function.

Oxidation: Reactive oxygen species (ROS) are important contributors to skin aging. Derived from molecular oxygen, ROS can form from interaction of ionizing radiations with biological materials, as a by-product of cellular metabolism, or can be synthesized by neutrophils as a defense mechanism against bacteria. ROS are molecules that have lost or gained an electron and electrons are not stable in odd numbers; thus, in their quest for missing electrons, ROS take electrons from cellular and extracellular components in their surroundings. Importantly, ROS are responsible for alterations affecting the structure and function of lipids at the membrane of cells and of proteins within the extracellular matrix (ECM).¹³ Exposure to UV is an important trigger of ROS formation and is associated with cutaneous photoaging.

The skin possesses natural antioxidant defenses under the form of superoxide dismutase (SOD) and catalase activities that can transform ROS into inoffensive molecules. Unfortunately, both activities decrease with

age and sun exposure, leaving more free radicals responsible for cellular damage.^{14,15} To address these concerns, antioxidants, such as stabilized citrus flavonoid (hesperitin), dipalmitoyl hydroxyproline, tocopheryl acetate, retinol (a carotenoid), ubiquinone (CoQ10), and ethylbisiminomethylguaiacol manganese chloride (a trademarked salen-manganese complex^b), may be used. The flavonoid, hydroxyproline, tocopheryl acetate, retinol and ubiquinone are known ROS scavengers. The salen-manganese complex is an SOD and catalase mimetic with self-regenerating potential due to the presence of a manganese atom at the active catalytic site that has the ability to bounce back and forth from a redox state of III to II to III upon reactions with ROS.^{16,17} A formulation including these materials

Like health care, a combined treatment regimen could be applied to personal care, particularly for antiaging.

could support natural skin defenses and replenish the deficiencies that may occur with aging.

DNA damage and repair: ROS may also affect DNA, generating damage that prevents its exact replication during cell division or introducing mutations that precipitate the process of aging. ROS-induced DNA damage occurs as various types. DNA base oxidation, thymidine dimer (T-T) formation, and DNA strand breaks are commonly seen following UV exposure and are associated with skin photoaging.¹⁸ It is estimated that thousands of DNA alterations rise in each cell daily.¹⁹ Fortunately, the skin has developed mechanisms to repair this damage, such as mismatch repair, nucleotide excision repair and double-strand break repair that operate through various enzymes. However, not surprisingly, the efficiency of these repair systems diminishes with time, a phenomenon associated with accelerated aging.²⁰

The salen-manganese complex and ubiquinone previously mentioned have

shown to be effective in protecting skin cells from oxidative DNA damage induced by UV light.²¹ Going a step further, the complex can stimulate cellular DNA repair in keratinocytes following UV exposure, thus supporting healthy cell viability in the presence of ROS.

Energy production: Importantly, oxidative stress affects the functioning of mitochondria, which are in every cell including epidermal keratinocytes and dermal fibroblasts. Mitochondria couple cellular respiration with energy production to form adenosine triphosphate (ATP), which is stored as ATP-creatine with the help of creatine kinase enzymes. The energy banked in these links is released upon ATP consumption and serves to support cellular metabolism. However, the accumulation of ROS damage with aging alters mitochondrial integrity and reduces creatine kinase activity, thus affecting cell metabolic activity and leading to cellular senescence.²²⁻²⁵ Additional ROS also are produced as by-products of an impaired mitochondrial respiration. Ultimately, old mitochondria produce less ATP, causing an energy crisis that, at the skin level, translates into a dull complexion.

To address this problem, ubiquinone, a natural component of the respiratory chain in mitochondria, can be used for energy production. The topical supplementation of ubiquinone compensates for its reduced presence in aged skin, thus supporting cellular energy production.²⁶ In addition, creatine, a natural energizer, is essential to regenerate the pool of ATP. When applied topically, creatine is taken up by keratinocytes, thus increasing creatine kinase (CK) activity for improved mitochondrial function and reduced ROS production.²⁵ Studies of creatine have shown increased cellular energy in its presence and synergistic effects when used in combination with ubiquinone, for improved skin vitality.²⁶ This synergy most likely works at the mitochondrial level, since ubiquinone is known to stabilize endogenous mitochondrial activity.

Oxygenation: Skin oxygenation also declines with aging, often as a result of urban pollution exposure. Oxygen supplementation to the skin is assured

^b EUK134 (INCI: Ethylbisiminomethylguaiacol Manganese Chloride) is a product of Cayman Chemical.

in two complementary ways, internally and externally. Within the body, blood carries oxygen that is delivered to cells via diffusion following its release from the hemoglobin content of red cells. There are no blood vessels present within the epidermis. As a consequence, oxygen must diffuse from the small vessels that irrigate the dermis to the upper layers. Unfortunately, dermal capillaries become more fragile with age and oxygen diffuses less efficiently from the deeper layers to the upper layers of the skin.²⁷

From external sources, oxygen is absorbed through cellular respiration, which is influenced by cellular metabolism and the quality of ambient air. Over time, urban living can contribute to poor oxygenation of the skin cells, thus negatively affecting the complexion of the skin.²⁸

An integral solution to aging should therefore improve skin oxygenation. In the course of ROS neutralization by the salen-manganese active previously mentioned, a water molecule is produced and molecular oxygen released in situ. Squalane and ubiquinone further assist by carrying ambient oxygen to cells, in turn supporting ATP production. In theory, combining these ingredients should bring more oxygen to the skin for a radiant complexion.

Immunity: The skin, being exposed to the external world, has developed immune functions assumed primarily by Langerhans cells in the epidermis. Langerhans cells are dendritic cells originally recruited from the bone marrow from where they migrate to the epidermis. Langerhans cells act as sentinels to alert the immune system to the presence of pathogens and other foreign materials. Not surprisingly, the immune functions of the skin are altered with aging and sun exposure; Langerhans cells are depleted from the epidermis and have fewer dendrites and reduced antigen-trapping capacity, leaving the skin immunosuppressed.²⁹ The consequences are increased skin susceptibility to viral infections and skin cancer.

Therefore, maintaining proper immunosurveillance appears to be a viable strategy for healthy skin. Retinol, for one, is known to help protect the skin from bacterial invasion, and

hyaluronic acid was recently found to facilitate Langerhans cells maturation and migration in the skin.³⁰ EPS extract protects the integrity of Langerhans cells when subjected to UV exposure;¹¹ in fact, beta-glucan-containing polysaccharides from plants, mushrooms and microorganisms are often found to have nonspecific immunopotentiating activity. These ingredients may preserve the immune function in skin.

Pigmentation: Skin aging and photoaging also affect melanocytes. In response to various stimuli—i.e., UV, hormones, cytokines, etc.—melanocytes synthesize photoprotective melanin pigments and transfer them to keratinocytes where they accumulate, affecting skin pigmentation. Aged skin often exhibits irregular pigmentation, particularly in chronically sun-exposed areas. This heterogeneity results from the presence of hypopigmented areas that coexist with zones of hyperpigmentation known as *lentigines* or age spots. Hypopigmentation is caused by a decrease in the number of active melanocytes, whereas anarchical melanin production and accumulation is associated with the formation of lentigo.^{31, 32}

Microarray analysis evaluation of solar lentigo demonstrates the up-regulation of genes related to inflammation, fatty-acid metabolism, and melanin production but down-regulation of cornified envelope-related genes.^{31, 32} Accordingly, it seems that increased pigment production in lentigo is associated with keratinization impairment, on a chronic inflammation background. The incidence of lentigo augments with age, affecting more than 90% of fair-skinned individuals over age 50 and making it a major cosmetic concern in an aging society.³³

One sure way to address pigmentation is to inhibit tyrosinase, an enzyme involved in the formation of melanin in the skin. Tyrosinase inhibitors include, among others, an extract derived from *Rumex occidentalis*, a plant native to the northern Canadian prairies region; dipotassium glycyrrhizate, a licorice extract; and retinol. *Rumex* and licorice extracts appear to inhibit tyrosinase by binding or deforming the enzyme molecule around the active site, according to manufacturer data, while retinol inhibits pigment maturation and transfer.³⁴

Keratinization: Keratinization, i.e., the process by which the epidermis forms its outer protective layer, the SC, is also modified with aging. Normal keratinization relies on a delicate balance between cell proliferation, differentiation and desquamation.³⁵ Skin renewal begins with the generation of new keratinocytes from stem cells residing in the stratum basale, the inner layer of the epidermis. As new cells continue to form, keratinocytes migrate upward and differentiate into corneocytes. Keratin, a highly fibrous protein, is produced during the differentiation process and causes cell walls to harden, thus forming the SC. The terminal differentiation of keratinocytes ultimately results in cell death. Old corneocytes are shed from the SC through desquamation. Aging is associated with reduced epidermal proliferation, rapid differentiation and slower desquamation of the SC.³⁶ Keratinocytes that remain in the skin longer tend to become more keratinized, therefore the aged cell is more rigid and the aged skin is more fragile.

Keratinization concerns can be addressed with creatine for energy support as well as materials such as myrrh, wheat germ, horsetail and hops extracts, which are reported to harbor regenerating potential, according to manufacturer data. An antiaging regimen may also include the combination of palmitoyl oligopeptide and palmitoyl tetrapeptide 7, which are peptides that mimic fragments of ECM proteins and are capable of activating genes involved in cellular turnover.³⁷ Other peptides have also presented regenerating potential.³⁸ Finally, retinol could be employed to boost cell renewal at the basal layer within the epidermis and in the differentiation of cells as they migrate upward to the SC.³⁹ However, the irritancy potential of retinol should be neutralized through the inclusion of anti-irritants in the same formulation. With such interventions, the epidermis may simultaneously find increased volume and softness.

Skin Aging: The Dermal-Epidermal Level

Skin cohesion: One of the most striking manifestations of skin aging, as revealed by histological techniques, is



Figure 1. Effect of the test serum on skin parameters

the flattening at the dermal-epidermal junction (DEJ) and loss of dermal papillae. The latter forms villousities that facilitate nutritional exchanges and metabolic by-product evacuation between the dermis and the epidermis. Their disappearance with aging contributes to the slowing of epidermal cell turnover. The DEJ is a structure of major importance for skin cohesion since it anchors the epidermis to the underlying dermis. This zone is constituted by various types of anchoring fibrils such as fibronectin, laminin and collagen IV. Collagen IV is an exclusive member of the basement membranes, whose structure forms supramolecular networks that influence cell adhesion, migration and differentiation.⁴⁰ Collagen IV is essential for the mechanical stability of the skin. Studies have shown that collagen IV content decreases with age after 35 years, weakening the skin structure and contributing to wrinkle formation.⁴¹

Therefore, an approach to antiaging would be to improve skin cohesion. Palmitoyl oligopeptide and palmitoyl tetrapeptide 7 are lipopeptides derived from a collagen precursor that cells perceive as a sign of excessive ECM degradation.³⁷ These peptides trigger positive feedback within the skin and promote the synthesis of collagen IV and fibronectin at the DEJ, thus favoring better cohesion between the dermis and the epidermis for firmer skin.⁴²

Skin Aging: The Dermis

Cell anchorage: Another feature of aged skin is the progressive disorganization of the ECM within the dermis that

generates wrinkles and sagging. Dermal ECM is made up of collagen, elastin, proteoglycans and GAGs that together establish the structural integrity of the skin.⁴³ In the dermis, ECM components are produced by fibroblasts to form a tridimensional network that, in return, provides support and anchorage for cells. Proper attachment of dermal fibroblasts to the ECM promotes cell functions, including migration, proliferation and differentiation. Thus, not surprisingly, any modification in the dermal ECM tridimensional network potentially contributes to skin aging by affecting the biomechanical properties of the skin.

This aspect of cell aging may be addressed through dipalmitoyl hydroxyproline amino acid, which has been reported to promote interactions between fibroblasts and the ECM. Stimulating the reorganization of collagen fibers into a denser, more supportive matrix would firm the skin.

Protein modification: The ECM is rich in long-lived proteins.⁴⁴ As such, these proteins are vulnerable to various post-translational modifications that tend to accumulate with time and UV exposure, affecting their structure and biological functions. Glycation is a good example of such a modification. Resulting from the non-enzymatic addition of sugars to proteins, glycation generates abnormal cross-linking between adjacent proteins. The process is accelerated in the presence of high glucose levels and free radicals. In the aging body, cross-links affecting glycosylated collagen and elastin fibers contribute to harden-

ing and brittleness of the skin, and interfere with skin renewal.^{45, 46}

Since glycation is linked to ROS production,⁴⁴ antioxidants would limit the formation of abnormal cross-linking of proteins. Specifically, the citrus flavonoid hesperitin has been reported to inhibit hemoglobin glycation *in vivo*⁴⁷ and collagen glycation *in vitro*, thus inhibiting glycation could preserve skin's resilience and suppleness.

ECM turnover and integrity: As noted, the maintenance of the ECM relies on a delicate balance between production and degradation of its components. Both mechanisms are altered with aging, a phenomenon amplified by sun exposure, cigarette smoke and urban pollution. The dermis of aged skin contains a decreased number of fibroblasts with a reduced capacity to produce collagen and elastin fibers.^{48, 49} In addition, with aging, fragmentation of both fibers is increased through the activation of specific enzymes: matrix metalloproteinases (MMPs) for collagen and neutrophil elastase for elastin.^{50, 51}

Fragmentation of dermal ECM has important consequences for skin physiology. Fibroblasts cannot attach to fragmented collagen and are thus deprived of the level of mechanical tension required to maintain efficient collagen synthesis. These collapsed fibroblasts produce high levels of collagen-degrading enzymes, alighting a self-perpetuating, deleterious cycle. Moreover, reduction in mechanical tension has been associated with increased levels of ROS that accelerate the aging process and reduce the expression of antioxidant enzymes in the skin.⁴⁸ ECM turnover in the skin may therefore be supported in two complementary ways: by stimulating the production of ECM fibers—i.e., collagen, elastin and GAGs—and by inhibiting specific proteolytic enzymes to prevent their degradation.

The first task may be achieved through the combined actions of dipalmitoyl hydroxyproline, palmitoyl oligopeptide and palmitoyl tetrapeptide 7 that mimic endogenous signals, and GAGs that modulate cell-matrix interaction, thus favoring the production of fibers.⁵² The second objective may be attained with the same marine GAG extract, which also

has proven anti-MMP activity that preserves collagen fibers.⁵³ Additional anti-MMP activity may be provided by palmitoyl oligopeptide and palmitoyl

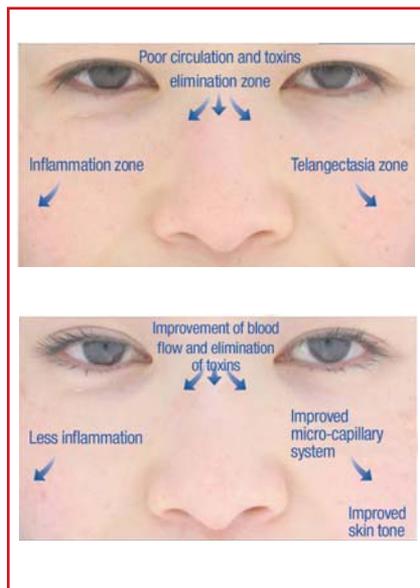


Figure 2. Effect of the test serum on skin irritation



Figure 3. Effect of the test serum on pockets and rings under the eyes

tetrapeptide 7, retinol and ubiquinone, while dipalmitoyl hydroxyproline and hesperitin citrus extract display anti-elastase activities that preserve elastin fibers. GAG components of the ECM could be protected with glycyrrhizate, which has anti-hyaluronidase activity.

Inflammation: Proteolytic activity itself and the ECM fragments generated are potential triggers of inflammation. MMPs and elastase both facilitate the migration of inflammatory cells through their ability to proteolyze the ECM. Degradation of collagen and elastin also leads to the generation of fragments that further contribute to recruit inflammatory cells that, when activated, induce the release of additional proteolytic enzymes.^{51, 54} In this aspect, skin aging can be viewed as a chronic inflammatory state.

As previously described, protecting the skin from inflammation should be part of an integral approach to skin care. This can be done through numerous mechanisms. Controlling MMP activity on the ECM with the marine GAG extract described limits the recruitment of inflammatory cells.⁵⁵ Additional ingredients may include bisabolol, botanical oils such as caprylic/capric/succinic triglyceride, sesame seed oil and wheat germ oil, tocopheryl acetate and/or dipotassium glycyrrhizate to slow the production of PGE2, a major inflammatory lipid in the skin. Finally, adding antioxidants in the formulation such as hesperitin, dipalmitoyl hydroxyproline, carotenoids, ubiquinone and the salen-manganese complex, would control inflammation in the skin by limiting ROS production. Interestingly, combining carotenoids with ubiquinone has been reported to enhance the skin's protection from inflammation and premature aging caused by sun exposure.⁵⁶ Stopping chronic microinflammation processes is increasingly being recognized as a way of improving skin aging.⁵⁷

Microcirculation: An increase in the release of pro-inflammatory mediators, proteolytic activity, and ROS ultimately affect skin microcirculation with aging. These three factors converge to alter the structure of dermal microcapillaries, causing vasodilatation and increased vessel permeability. As a consequence, plasmatic fluids tend to leak within

the dermis, generating edema and contributing to the formation of dark circles under the eyes. Vasodilatation is also associated with rosacea, a cosmetic concern in progression with aging.⁵⁸ An integral antiaging approach therefore may protect the integrity of skin microvessels, in part through antioxidant activities. The flavonoid content of citrus extract has vasoregulatory activity, which is known to help reduce eye puffiness. This action may be reinforced with a licorice extract that inhibits histamine and PGE2 release, both important mediators of vasodilatation. Protection of the capillary bed could be supported through reduced proteolytic deterioration of the ECM around blood vessels. The latter benefit may come from a marine GAG extract, which inhibits vascular permeability⁵⁹ and has clinically proven activity in improving dark circles, telangiectasia, rosacea and psoriasis.⁶⁰

Integrating the Actives

As one might imagine, when combining such an array of materials, several considerations must be taken into account. For instance, for every INCI name, there are several options available on the market and they are not the same. The quality of the active ingredients is crucial and the selection of actives must not be based merely on their reported activity, in vitro and in vivo claim substantiation literature, and/or their marketing appeal, but also by evaluating the ingredient's purity, source (i.e., manufacturing process), physicochemical properties, compatibilities and limitations, and performance in different systems. Besides choosing the right actives, quality excipients are also important for this integrated approach. And special attention must be paid to the rheological properties and interactions with the selected active molecules.

Beyond the quality of the actives and excipients, the process design itself is another key element the formulator must carefully work. While the specific approach to developing the serum described here is proprietary, it should be noted that the selection and set up of the appropriate equipment, parameters and values are important. As a final

comment—the more complex the system, the less forgiving it is.

Efficacy Testing

The integrated approach to antiaging was tested by formulating all the ingredients described into a serum (see **Integrated Ingredient Concentrate**) and conducting clinical trials under the supervision of highly qualified dermatologists and independent cosmetic experts. It should be noted that the studies described here are preliminary and were designed to obtain a first impression of the potential for this approach. The benefits observed serve as proof of concept and warrant a more extensive clinical study including rigorous controls.

First study: A preliminary study was conducted by an independent testing laboratory to evaluate the antiwrinkle potential of the serum. For this purpose, 20 women aged 35–62 years applied approximately 1–2 g of the serum to the face, neck and the eye contour twice daily for 12 weeks. Wrinkle reduction was measured under normal conditions of use by optical profilometry of silicone imprints of skin, taken on day 0, 7, 28 and 56, respectively, while skin hydration was assessed using corneometry (see **Figure 1**). The results demonstrated that the integral approach improved the appearance of fine lines and wrinkles as well as the tone, texture, consistency and general appearance of the skin. The feedback provided by the corneometry study helped refine the final formulation for maximal support of skin hydration.

Second study: The serum was subsequently tested on Asian skin, since recent studies have indicated Asian consumers may be more sensitive and prone to itching than other ethnicities.⁶¹ A positive outcome would thus suggest the material's safety and efficacy for all types of skin. A pilot study was conducted in collaboration with a renowned clinic and spa in Tokyo. For this study, 25 women and men aged 26–62 years applied between 1–2 g of the serum under normal use conditions twice daily for a period ranging from 1 to 4.6 months, depending in the participant, on the face, neck and the eye contour. The effect of the product was documented through photographs

and dermatologist comments, as well as a self-assessment questionnaire, after 1, 2, 3 and 4.6 months of treatment, respectively, compared to the baseline (see **Figure 2**).

In photographs, one can see wrinkle reduction as well as a more even and radiant complexion following treatment. Upon visual assessment, the disappearance of bags under the eyes was noted, supporting a possible improvement of the skin microcirculation. Moreover, skin initially showing signs of irritation, acne or rosacea improved under treatment with the serum.

Third study: Four additional case studies were carried out in collaboration with an eminent member of the Taiwanese Association of Dermatologists. For these case studies, 3 women aged 41, 43 and 72 years, and a man aged

Besides choosing the right actives, quality excipients are important for this integrated approach.

77 years, were recruited. Participants applied 1–2 g of the serum twice daily to the face, neck and the eye contour for a period ranging 2–3 months. The effect of the product was documented through photographs and dermatologist comments (see **Figure 3**).

The results from this study corroborated those of the Japanese study. Again, the serum appeared to have improved the skin microcirculation since visual assessment revealed a reduction of pockets and rings under the eyes. The complexion was revived and more even. The skin looked firmer and the sebum production was apparently normalized.

Fourth study: The last study consisted of a consumer test realized by an independent European quality control laboratory for the cosmetic industry. For this consumer test, 114 respondents were recruited. All were women with ages ranging from 45 to 65 years. Participants applied 1–2 g of the serum twice daily to the face, neck and the eye contour for a period of 4 weeks.

The results highlighted several quali-

ties relating to antiaging effects. Up to 95% of the users reported a moisturizing effect; 90% considered the product to be comfortable; 84% said it made the skin smoother and provided a soothing effect; and 82% described a firming effect. Seventy-eight percent noted a more even tone while nearly 68% saw a decrease in fine lines and wrinkles.

When compared to results obtained by the testing company in the past for similar products, the serum statistically distinguished itself for moisturizing effects ($p \leq 0.0001$), comfort ($p = 0.007$), and antiwrinkle properties ($p = 0.007$).

Safety Testing

Especially considering the many actives used in this single formulation, it was crucial to test the safety of the material. Preliminary safety assessments were based on ingredient safety reviews and information on the final formulation, including the intended and reasonably foreseeable uses, the physicochemical and microbiological specifications of the raw materials and the finished product, its stability, and a history and record of any reported undesirable effects linked to the use of the product. In reviewing the safety and toxicity profile of all the ingredients used and their history of safe use, researchers concluded that safety hazards were not probable from the use of this integral serum when used as directed or from foreseeable conditions of misuse.⁶²

Ocular irritation: The Irritation Assay System provides an in vitro alternative to traditional animal tests for ocular irritancy. To perform this standardized assay, the test sample is applied to a synthetic biobarrier composed of a semipermeable membrane. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a solution containing proteins, glycoproteins, lipids and low molecular weight components associated in a complex macromolecular matrix. Reaction of the test sample with these macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the solution. Comparison of these optical density measurements to those produced by

standard chemical irritants permits the calculation of an irritancy score that has been shown to be directly related to the potential corneal irritancy of the test material. The results of the study indicated that the serum could be classified as a minimal ocular irritant and should be well-tolerated on the skin and in the eye area (data not shown).

48-hr single patch test: The irritation potential of the serum was clinically evaluated on 30 healthy volunteers, following one close contact application under a semi-occlusive patch that was maintained in close contact with the skin for 48 hr. The results obtained under these experimental conditions indicated that the blend, applied in a pure form, could be considered as only slightly irritating and well-tolerated by the skin (data not shown).

HRIPT: Human repeat insult patch testing (HRIPT) was also conducted to document the cutaneous irritating and sensitizing potentials of the product, following repeated applications on the skin, under occlusive patch, in 50 healthy adult volunteers. The nondiluted product 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 product (data not shown).

Challenge test: Finally, 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 test showed that the serum met the Personal Care Products Council's requirements and guidelines for antimicrobial preservative effectiveness (data not shown).

Conclusion

With the recognition that skin aging involves alterations in various molecular and physiological processes, it now seems desirable to simultaneously address them all, for a more complete skin response. The trend is definitely there. However, formulating many actives in a finished product that is efficient, stable and appealing to the consumer remains a challenge.

The integral approach described is a proof of concept and as noted, the benefits observed warrant a more extensive clinical study including rigorous controls. The test serum was found to successfully combine various potent and well-known actives to address the major mechanisms of skin aging with striking anti-irritation and anti-puffiness properties. This integral approach was

well received by a panel of consumers that highlighted its moisturizing effect, the comfort it provides to the skin, and its antiwrinkle properties. Importantly, with the varied and numerous actives used in this integral cosmeceutical approach, the blend proved to be safe although it should be noted that the use of a complimentary product with SPF is recommended under sun exposure. Overall, the results validate that it is indeed possible to address skin aging with a more integrative approach.



Lab Practical: Integrating Actives

- The quality of the active ingredients and excipients is crucial.
- Special attention must be paid to rheological properties and ingredient interactions.
- The process design itself is another key element; the appropriate equipment, parameters and values are necessary.
- The more complex the system, the less forgiving it is.

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References

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1. M Stanulla and M Schrappe, Treatment of childhood acute lymphoblastic leukemia, *Semin Hematol* 46(1) 52-63 (2009)
2. PM Elias, Stratum corneum defensive functions: An integrated view, *J Invest Dermatol* 125(2) 183-200 (2005)
3. S Sakai, R Yasuda, T Sayo, O Ishikawa and S Inoue, Hyaluronan exists in the normal stratum corneum, *J Invest Dermatol* 114(6) 1184-7 (2000)
4. LJ Meyer and R Stern, Age-dependent changes of hyaluronan in human skin, *J Invest Dermatol* 102(3) 385-9 (1994)
5. JW Fluhr, R Darlenski and C Surber, Glycerol and the skin: Holistic approach to its origin and functions, *Br J Dermatol* 159(1) 23-34 (2008)
6. JE Silbert, Proteoglycans and glycosaminoglycans, in: *Biochemistry and Physiology of the Skin*, LA Goldsmith, ed, Oxford University: New York, NY 448-461 (1983)
7. A De Simone, L Vitagliano and R Berisio, Role of hydration in collagen triple helix stabilization, *Biochem Biophys Res Commun* 372(1) 121-5 (2008)
8. VR Leite et al, Hydrating effects of moisturizer active compounds incorporated into hydrogels: In vivo assessment and comparison between device, *J Cosmet Dermatol* 8(1) 32-9 (2009)
9. VR Leite e Silva et al, Hydrating effects of moisturizer active compounds incorporated into hydrogels: In vivo assessment and comparison between devices, *J Cosmet Dermatol* 8(1) 32-9 (2009)
10. CR Thornfeldt, Chronic inflammation is etiology of extrinsic aging, *J Cosmet Dermatol* 7(1) 78-82 (2008)
11. A Thibodeau, Protecting the skin from environmental stresses with an exopolysaccharide formulation, *Cosm & Toil* 120(12) 81-89 (2005)
12. J Graf, Herbal anti-inflammatory agents for skin, *Skin Therapy Lett* 5(4) 3-5 (2000)
13. S Briganti, M Picardo, Antioxidant activity, lipid peroxidation and skin diseases. What's new, *J Eur Acad Dermatol Venereol*, 17(6) 663-9 (2003)
14. YH Wei, YS Ma, HC Lee, CF Lee and CY Lu, Mitochondrial theory of aging matures—Roles of mtDNA mutation and oxidative stress in human aging, *Zhonghua Yi Xue Za Zhi* (Taipei) 64(5) 259-70 (2001)
15. L Declercq, I Sente, L Hellemans, H Corstjens and D Maes, Use of the synthetic superoxide dismutase/catalase mimetic EUK-134 to compensate for seasonal antioxidant deficiency by reducing pre-existing lipid peroxides at the human skin surface, *Int J Cosmet Sci* 26(5) 255-63 (2004)
16. A Thibodeau, The crucial role of metalloproteinase inhibitors and regenerating antioxidants in the age-related alterations of the skin, *SOFW*, 131(4) 10-20 (2005)
17. SR Doctrow et al, Salen-manganese complexes as catalytic scavengers of hydrogen peroxide and cytoprotective agents: Structure-activity relationship studies, *J Med Chem*, 45(20) 4549-

- 58 (2002)
18. J Cadet, T Douki, J Pouget and JL Ravanat UVB and UVA induced formation of photoproducts within cellular DNA, in: *From DNA Photolesions to Mutations, Skin Cancer and Cell Death*, E Sage, R Drouin, M Rouabhia, eds, RSC Publishing: London (2005) pp 1-14
 19. DA Goukassian and BA Gilchrist, The interdependence of skin aging, skin cancer and DNA repair capacity: A novel perspective with therapeutic implications, *Rejuvenation Res* 7(3) 175-85 (2004)
 20. F Altieri, C Grillo, M Maceroni and S Chichiarelli, DNA damage and repair: From molecular mechanisms to health implications, *Antioxid Redox Signal* 10(5) 891-937 (2008)
 21. D Decraene et al, A synthetic superoxide dismutase/catalase mimetic (EUK-134) inhibits membrane-damage-induced activation of mitogen-activated protein kinase pathways and reduces p53 accumulation in ultraviolet B-exposed primary human keratinocytes, *J Invest Dermatol* 122(2) 484-91 (2004)
 22. H Corstjens, L Declercq, L Hellemans, I Sente and D Maes, Prevention of oxidative damage that contributes to the loss of bioenergetic capacity in ageing skin, *Exp Gerontol* 42(9) 924-9 (2007)
 23. M Greco, G Villani, F Mazzucchelli, N Bresolin, S Papa and G Attardi, Marked aging-related decline in efficiency of oxidative phosphorylation in human skin fibroblasts, *FASEB J* 17(12) 1706-8 (2003)
 24. A Navarro and A Boveris, The mitochondrial energy transduction system and the aging process, *Am J Physiol Cell Physiol* 292(2) C670-86 (2007)
 25. H Lenz et al, The creatine kinase system in human skin: Protective effects of creatine against oxidative and UV damage in vitro and in vivo, *J Invest Dermatol* 124(2) 443-52 (2005)
 26. T Blatt et al, Stimulation of skin's energy metabolism provides multiple benefits for mature human skin, *Biofactors* 25(1-4) 179-85 (2005)
 27. E Chang, J Yang, U Nagavarapu and GS Herron, Aging and survival of cutaneous microvasculature, *J Invest Dermatol*, 118(5) 752-8 (2002)
 28. E Berardesca, H Maibach, Transcutaneous CO₂ and O₂ diffusion, *Skin Pharmacol*, 6(1) 3-9 (1993)
 29. HC Wulf, J Sandby-Møller, T Kobayasi and R Gniadecki, Skin aging and natural photoprotection, *Micron* 35(3) 185-91 (2004)
 30. ME Mummert, Immunologic roles of hyaluronan, *Immunol Res* 31(3)189-206 (2005)
 31. H Aoki, O Moro, H Tagamia and J Kishimoto, Gene expression profiling analysis of solar lentigo in relation to immunohistochemical characteristics, *Br J Dermatol* 156(6) 1214-23 (2007)
 32. E Goyarts, N Muizzuddin, D Maes and PU Giacomoni, Morphological changes associated with aging: Age spots and the microinflammatory model of skin aging, *Ann NY Acad Sci* 1119 32-9 (2007)
 33. M Brenner and VJ Hearing, Modifying skin pigmentation—Approaches through intrinsic biochemistry and exogenous agents, *Drug Discov Today Dis Mech.* 5(2) e189-e199 (2008)
 34. JP Ortonne, Retinoid therapy of pigmentary disorders, *Dermatol Ther* 19(5) 280-8 (2006)
 35. E Houben, K De Paepe and V Rogiers, A keratinocyte's course of life, *Skin Pharmacol Physiol* 20(3) 122-32 (2007)
 36. H Tagami, Functional characteristics of the stratum corneum in photoaged skin in comparison with those found in intrinsic aging, *Arch Dermatol Res* 300 Suppl 1:S1-6 (2008)
 37. C Mas-Chamberlin, P Mondon, O Peschard and K Lintner, Matrikine technology and barrier repair: The ultimate in anti-age skin care?, *Cosmetic Science Technology* (2004)
 38. L Zhang and TJ Falla, Cosmeceuticals and peptides, *Clin Dermatol* 27(5) 485-94 (2009)
 39. G Bellemère, GN Stamatias, V Bruère, C Bertin, N Issachar and T Oddos, Antiaging action of retinol: from molecular to clinical, *Skin Pharmacol Physiol* 22(4) 200-9 (2009)
 40. J Khoshnoodi, V Pedchenko and BG Hudson, Mammalian collagen IV, *Microsc Res Tech* 71(5) 357-70 (2008)
 41. F Vázquez, S Palacios, N Alemañ and F Guerrero, Changes of the basement membrane and type IV collagen in human skin during aging, *Maturitas* 25(3) 209-15 (1996)
 42. KA Bush, BR Downing, SE Walsh and GD Pins, Conjugation of extracellular matrix proteins to basal lamina analogs enhances keratinocyte attachment, *J Biomed Mater Res A*, 80(2) 444-52 (2007)
 43. H Järveläinen, A Sainio, M Koulu, TN Wight and R Penttinen, Extracellular matrix molecules: Potential targets in pharmacotherapy, *Pharmacol Rev*, 61(2) 198-223 (2009)
 44. D Schmid, R Muggli, F Zülli, Collagen glycation and skin aging, *Cosm & Toil* 117 118-24 (2002)
 45. AJ Bailey, Molecular mechanisms of ageing in connective tissues, *Mech Ageing Dev* 122(7) 735-55 (2001)
 46. NC Avery and AJ Bailey, The effects of the Maillard reaction on the physical properties and cell interactions of collagen, *Pathol Biol (Paris)* 54(7) 387-95 (2006)
 47. B Manuel, Y Keenoy, J Vertommen and I De Leeuw, The effect of flavonoid treatment on the glycation and antioxidant status in Type 1 diabetic patients, *Diabetes Nutr Metab*12(4) 256-63 (1999)
 48. J Varani et al, Decreased collagen production in chronologically aged skin: Roles of age-dependent alteration in fibroblast function and defective mechanical stimulation, *Am J Pathol* 168(6) 1861-8 (2006)
 49. GC Sephel and JM Davidson, Elastin production in human skin fibroblast cultures and its decline with age, *J Invest Dermatol* 86(3) 279-85 (1986)
 50. GJ Fisher, J Varani and JJ Voorhees, Looking older: Fibroblast collapse and therapeutic implications, *Arch Dermatol* 144(5) 666-72 (2008)
 51. F Antonicelli, G Bellon, L Debelles and W Hornebeck, Elastin-elastases and inflamm-aging, *Curr Top Dev Biol* 79 99-155 (2007)
 52. Schaefer, M Roux, HW Stuhlsatz, R Herken, B Coulomb, T Krieg, H Smola, Glycosaminoglycans modulate cell-matrix interactions of human fibroblasts and endothelial cells in vitro, *J Cell Sci*, 109(Pt 2) 479-88.(1996)
 53. A Thibodeau, Metalloproteinase Inhibitors, *Cosm & Toil* 115(11) 75-82 (2000)
 54. TL Adair-Kirk and RM Senior, Fragments of extracellular matrix as mediators of inflammation, *Int J Biochem Cell Biol* 40(6-7) 1101-10 (2008)
 55. E Dupont et al, Modulation of the contact hypersensitivity response by AE-941 (Neovastat), a novel antiangiogenic agent, *J Cutan Med Surg* 7(3) 208-16 (2003)
 56. B Fuller, D Smith, A Howerton and D Kern, Anti-inflammatory effects of CoQ10 and colorless carotenoids, *J Cosmet Dermatol* 5(1) 30-8 (2006)
 57. CR Thornfeldt, Chronic inflammation is etiology of extrinsic aging, *J Cosmet Dermatol*, 7(1) 78-82 (2008)
 58. GH Crawford, MT Pelle and WD James, Rosacea: I. Etiology, pathogenesis, and subtype classification, *J Am Acad Dermatol* 51(3) 327-41 (2004)
 59. R Béliveau et al, The antiangiogenic agent neovastat (AE-941) inhibits vascular endothelial growth factor-mediated biological effects, *Clin Cancer Res* 8(4) 1242-50 (2002)
 60. E Dupont et al, Antiangiogenic properties of a novel shark cartilage extract: Potential role in the treatment of psoriasis, *J Cutan Med Surg* 2(3) 146-52 (1998)
 61. AV Rawlings, Ethnic skin types: Are there differences in skin structure and function? *Int J Cosmet Sci* 28(2) 79-93 (2006)
 62. The Cosmetic Ingredient Review Web site, available at www.cir-safety.org (accessed Jan 25, 2010)

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