KorneoBioLift – the corneobiology-based anti-wrinkle treatment

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Stem cells are non-differentiated somatic cells which can differentiate into specialized cells and tissue. Whether the particular stem cell develops into an ear, eye or a blood cell is determined by the particular biological milieu with the different growth factors and so-called epigenetic factors.

A distinction is made between embryonic and post-embryonic stem cells. In contrast to the embryonic stem cells that can differentiate into all kinds of different cells and tissues of the organism, the differentiating potential of post-embryonic stem cells is limited to the respective differentiated tissue. Embryonic stem cells only occur in embryonic tissue. The organism regenerates itself from somatic or adult stem cells throughout the entire lifetime. While embryonic stem cells are pluripotent, post-embryonic stem cells are only multipotent. Skin stem cells regenerate all the different tissue layers of the skin and hence have become research topic of today's anti-aging medicine.

Skin stem cells

In the skin, the epidermal stem cells are responsible for the formation and regeneration of all the different kinds of cells in the epidermis. Since the skin progressively differentiates and regenerates, the epidermal stem cells are the most important skin cells in charge of the epidermal homeostasis and the regeneration of the skin barrier. With increasing age, the number of epidermal stem cells and hence the regenerative capacity of the skin decreases, a fact which consequently leads to barrier damages, the loss of skin hydration and elasticity and the formation of wrinkles. The epidermal stem cells occur in the basal layer of the skin. In order to be able to actively influence the regenerative capacity of the stem cells, the active agents need to pass through the protective barrier of the skin and then finally reach the stratum basale in a functionally adequate concentration.

As far as the transport of encapsulated active agents is concerned, a distinction has to be made between penetration and permeation. Penetration means the transport into the horny layer and permeation involves that the active agents pass through the entire skin. The skin barrier is formed by a lipid layer which is almost insuperable for hydrophilic substances. In order to pass the lipid layer, the active agents have to be packed into adequate transport vehicles such as liposomes or nanoparticles. The liposome shell consists of phosphatidylcholine bilayers which also naturally occur as a base material for the formation of cell membranes. Besides its function as a transport vehicle, the liposome shell itself additionally contains skin recovering active agents as for instance polyunsaturated fatty acids and choline which has cell protective functions. Thus, even so-called empty liposomes, i.e. liposomes without encapsulated active agents, already show excellent effects against skin impurities and minor forms of acne.

Epigenetics

Genetics deals with the genetic substance DNA, epigenetics (literally translated: in addition to genetics) provides further coded information on the activity status of genes. Due to epigenetic marks through chemical molecules, particular sequences on the DNA are no longer read. Epigenetic marks can be modified by chemical and physical environmental factors (benzene, bisphenol A, ionizing radiation, laser with particular wave lengths, UV-light), hormonal active chemicals (pesticides, herbicides, medical drugs), but also by nutritional factors (nutritional supplements) and secondary plant components. Also biological, mental and social factors can modulate the epigenome. Thus, epigenetic patterns may modify for instance as a reaction to experienced emotions or a changed nutrition but also in the course of life as a result of the normal aging process. Modifications of the epigenetic marks may cause a multitude of negative effects. They participate in the carcinogenesis and in the formation of congenital maldevelopment [13,14,15].

As already known from twin studies, both the epigenetic information as well as the genetic substance itself are transmitted to succeeding generations. Various environmental influences

but also the personal lifestyle can modify the activity state of genes and these particular modifications can be transmitted to the succeeding generations. In order that the particular organs and tissues can develop, a variety of different genes needs to be read. With a few exceptions only, this process is generally controlled by epigenetic marks and transcription functions without modifying the DNA. Skin cells and nerve cells, for instance, are equipped with the identical genetic material although they fulfill different functions within the organism. Particular sections of the human genome are disconnected by epigenetic mechanisms. Environmental influences such as UV light, medical drugs, hormones or viruses however can "reactivate" these sequences by modifying the DNA methylation pattern. Epigenetic marks are easier to change than the DNA sequence itself. If the mechanisms are known, which will cause health damages, preventive measures can be taken by avoiding unfavorable influences on the one hand, whereas on the other hand pharmacological and dietetic regulatory measures could be realized in order to repair the damages occurred [16]. In the cosmetic field, attempts have been made with vegetable epigenetic factors to protect the skin from premature aging processes and achieve a rejuvenation of the skin.

Stem cells from the old apple variety "Uttwiler Spätlauber"

Plant stem cells have been studied in terms of their regenerating potential for the dermal stem cells. An extract has been isolated from stem cells of the old apple variety "Uttwiler Spätlauber" and encapsulated into liposomes for cosmetic formulations. The regenerative and cell recovering effects however are not attributed to the stem cells but to the epigenetic factors due to the fact that vegetable stem cells only can regenerate vegetable tissue but not the human skin. Epigenetic factors support stem cells in their differentiation. These so-called phytofactors are supposed to protect the dermal stem cells and prevent the skin aging process [1]. Vegetable phytohormones for instance also show hormonally stimulating effects on the human skin. The effects of the apple stem cell extract on human skin cells however have only been studied in cell cultures. Whether liposomally encapsulated epigenetic factors, mixed into a cream and applied on the skin ever reach the stratum basale in adequate concentration, hence the area where the dermal stem cells reside, has not yet been proved. It can rather be assumed that other vegetable actives as for instance the phytohormones gained from the stem cell extract improve the skin hydration and elasticity and in this way smooth out the wrinkles.

3S-Therapy (Stimulated-Self-Serum Skin Therapy or Dracula-Therapy)

In the United States, platelet rich plasma (PRP) has been applied for years to accelerate the wound healing process of sports injuries. Dr. Daniel Sister, the French anti-aging specialist, was the first to introduce this therapy, called 3 S or Dracula Therapy, in cosmetic applications for the revitalization of the skin. Platelet rich plasma is gained from a blood sample and then used for mesotherapy or for the wrinkle injection treatment. Growth factors are released from the blood platelets, mesenchymal stem cells activated and DNA repair mechanisms stimulated, wound healings are accelerated and the collagen synthesis by fibroblasts is stimulated. The skin is tightened, the skin texture improved and the wrinkles are smoothed out. As we are dealing with natural substances of the skin, they will not cause the side effects known of synthetic fillers such as allergies, intolerances or granulomas.

NO production in the skin

In the course of evolution, organisms have learned to develop effective mechanisms against the negative influence of free radicals in order to protect the natural structures of the body. In the so-called power plants of the cells, the mitochondria, the body systematically applies free radicals in order to generate energy. Other physiological processes utilize nitrogen radicals, as for instance nitrogen monoxide (NO), as a mediator in the signal transfer in nerve cells and for the triggering of the planned cell death (apoptosis) of epidermal cells. A number of natural enzymes of the skin generate specifically defined radicals, whereas others provide for their destruction. This leads to the conclusion that specific steady-state levels of natural radicals of the body are not only present in the different skin layers but rather seem to essentially contribute to the epidermal homeostasis. Upward deviations from this natural homeostasis over a certain period of time are called "chronic oxidative stress", downward deviations are designated as "reductive stress". Such deviations are observed in connection with pathological processes such as chronic inflammatory processes, degenerative processes such as arteriosclerosis, Alzheimer's disease, diabetes but also with chronic skin diseases and barrier disorders. Hence, radical scavengers in appropriate concentration are important factors in the skin care and should be adequately integrated into the treatment. It should be men-

tioned though that there are rather few data regarding the concentration of free radicals in the epidermis and dermis.

In this context, a short discussion of the apoptosis (planned cell death) of the skin cells is indicated. On their way from the reducing milieu of the basal layer into the oxidizing atmosphere in the upper section of the stratum corneum, the keratinocytes (from their formation from stem cells up to the akaryote corneocytes) pass a transition area where the adequate formulation of the "steady state redox condition" is essential. In the upper part of the stratum corneum, oxidizing attacks from the outside need to be successfully warded off. In this very critical zone, a multitude of different nitrogen containing substances is found which, among others, result from the degradation of membrane forming phospholipids, sphingomyelins and proteins. In higher concentrations, the nitrogen containing substances are rather effective radical and ROS scavengers. On the one hand, they can modify reactive radicals into inert radicals and on the other hand, they can directly react with peroxides and atmospheric nitric oxides.

Whereas already smallest amounts of the enzymatic radical scavengers such as SOD (superoxide dismutase), glutathione peroxidase, catalase and thioredoxin that occur in epidermal cells, quickly and very effectively degrade oxygen compounds, nitrogen compounds compensate their inferior reactivity with higher concentrations and rather show unspecific reactions. Since many of the radical scavengers belong to the NMF (Natural Moisturizing Factor) and contribute to the osmotic equilibrium of the skin, it is recommended to adequately adapt the formulation of skin care products to the NMF. Moisturizers play an important role in the skin [10,11]. They increase the elasticity of the skin and have a tightening effect. About 40 percent of the natural NMF consist of amino acids which are not only responsible for the hydration balance of the skin but also influence the osmotic equilibrium. The fact that the amino acids and urea of the skin also represent a natural skin protection against ROS (Reactive Oxygen Species) and hence against radicals still is rather unknown [17,18]. The following examples for the destruction of nitrite which forms of nitric oxide radicals may illustrate the process:

Destruction through glycine

$$H_2N\text{-}CH_2\text{-}COOH + NO_2^- \rightarrow HO\text{-}CH_2\text{-}COO^- + N_2 + H_2O$$

Destruction through urea:

$$H_2N\text{-}CO\text{-}NH_2 + 2\ NO_2^- \rightarrow CO_3^{2-} + 2\ N_2 + 2\ H_2O$$

In contrast to nitric oxide radicals which are harmful for the skin, nitrogen monoxide (NO) is a natural messenger substance of the body that participates in numerous metabolism processes as e.g. the control of the blood pressure. NO is formed from the amino acid L-arginin by specific enzymes in line with the demand. Bioactive nitrogen monoxide can also be formed through non-enzymatic process. This occurs by photolysis of nitrite (NO_2^-) and nitrosated proteins, i.e. S-nitroso-albumin. Blue light (453 nm) releases nitrogen monoxide in the deeper layers of the human skin. The skin is equipped with relatively high concentrations of copper ions and nitrite and has a low pH level of 5.5 to 6. A radiation with blue light leads to the formation of nitrogen monoxide from nitrite. NO reduces inflammatory processes, supports the regeneration of the skin and inhibits fibrosis [2]. NO-induced signaling pathways lead to a reduction of the intracellular calcium concentration in the muscular cells and hence cause a relaxation of the mimic muscles. In this way the wrinkles are reduced and the skin is tightened [3,4]. Blue light with a wave length of 453 nm up to the radiation dose of 250 J/cm^2 has no toxic effects on skin cells and causes a higher proliferation and differentiation rate in human keratinocytes and endothelium cells [5].

The most important advantage of blue light radiation is its natural approach. Exclusively the natural processes of the body are stimulated and endogenic NO is released without any external application of chemical or pharmaceutical substances. The discovery of nitrogen monoxide as a signaling substance for muscle cell relaxation by Robert F. Furchgott, Louis J. Ignarro and Ferid Murad has been rewarded with the Nobel Prize for Medicine as long as 1998 [6]. A number of additional protective characteristics of nitrogen monoxide complement these processes and protect the cells from oxidative stress in a variety of different ways. Already low NO concentrations can intensify essential components of the antioxidative protection as for instance the glutathione metabolism (GSH) by forming S-nitroso-glutathion. Since it is a weak radical, it is able to react with highly reactive radicals and hence prevents DNA damages, damages of the cell membrane through lipid peroxidation as well as the oxidation of proteins which are essential for the cell functions [7,8,12].

KorneoBioLift – an autologous cell therapy for the anti-wrinkle treatment

The 3S therapy has been further refined by the Institute of Applied Corneotherapy (IAK) at Leichlingen, Germany. With the help of a

patented specialized system for molecular diagnostics (CPT™ Cell Preparation Tube), mononuclear cells and adult stem cells have been isolated from 20 ml of blood. By means of centrifugation, a FICOLL™ gradient evolves and separates the erythrocytes from the remaining cells. Growth factors, vital substances and epigenetic factors concentrate with the stem cells in the plasma. Subsequently trace elements such as copper, zinc, manganese and amino acids as for instance L-Arginin are added to the cells via transfer system and the cell suspension is irradiated with blue light in order to stimulate the nitrogen monoxide formation. The cell suspension then is transferred into injection syringes via sterile system and used for mesotherapy or anti-wrinkle injections. In the dermis and lower epidermis, the NO induced metabolic processes cause a relaxation of the mimic muscles and a stimulated collagen synthesis by the fibroblasts.

Anti-wrinkle Treatment
After the injection treatment of the wrinkles, a mixture of 4 ml of the cell suspension, 4 ml of Liposome NMF complex and 2 ml of Boswellia nanoparticles (KOKO GmbH & Co. KG, Leichlingen, Germany) is sparingly applied on the skin and then irradiated with blue light for about 10 minutes. The process is followed by radiofrequency treatment (2.2 MHz, 15 Watt, 5 minutes, temperature of 38-40°C, applied with RadioSurg 2200, Meyer-Haake GmbH, Ober-Mörlen, Germany) [9]. About one week after this specific wrinkle treatment, a mesotherapy will be applied.

Mesotherapy
After the skin has been cleansed, it is irradiated with blue light for about 5 minutes. A mixture of 2 ml of Anti wrinkle serum, 1 ml of Liposome concentrate Plus with azelaic acid and 1 ml of Boswellia nanoparticles in a 20 ml DMS® Vitamin mask (KOKO GmbH & Co. KG, Leichlingen, Germany) is applied on the skin. Subsequent to a micro needling treatment (Dermaroller®, 0.25 mm), a lymphatic drainage will follow in order to massage the active agent residues into the skin.

KorneoBioLift comprises 3 treatment sequences with anti-wrinkle treatment and mesotherapy. The corneobiology-based skin treatment is a completely natural approach developed to sustainably revitalize the skin, smoothen out wrinkles and restore the elasticity and hydration. The collagen synthesis in the fibroblasts of the skin is stimulated. Already shortly after the treatment an improved skin condition with smoothed wrinkles is visible and measurable. In contrast to the Dracula therapy, the KorneoBioLift treatment additionally activates the enriched mononuclear cells and mesenchymal stem cells with vital substances and blue light radiations.

KorneoBioLift is a safe and natural anti-wrinkle treatment and revitalization of the skin, using stem cells and growth factors gained from the own blood. Allergies and intolerances hence are avoided. Granulomas as known from traditional filler treatments have not been observed. Almost immediately after the treatment the patients are again fit for socializing. Only in very rare cases, hematomas may appear on the injection spots which however are quickly reabsorbed.

KorneoBioLift is an innovative medical procedure applied by the esthetic dermatologist and utilizes the natural biochemical metabolic processes in the skin for skin rejuvenation and anti-wrinkle treatment. The specific treatment requires detailed knowledge on the skin aging process, particularly of the facial skin in order to select a holistic treatment approach.

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