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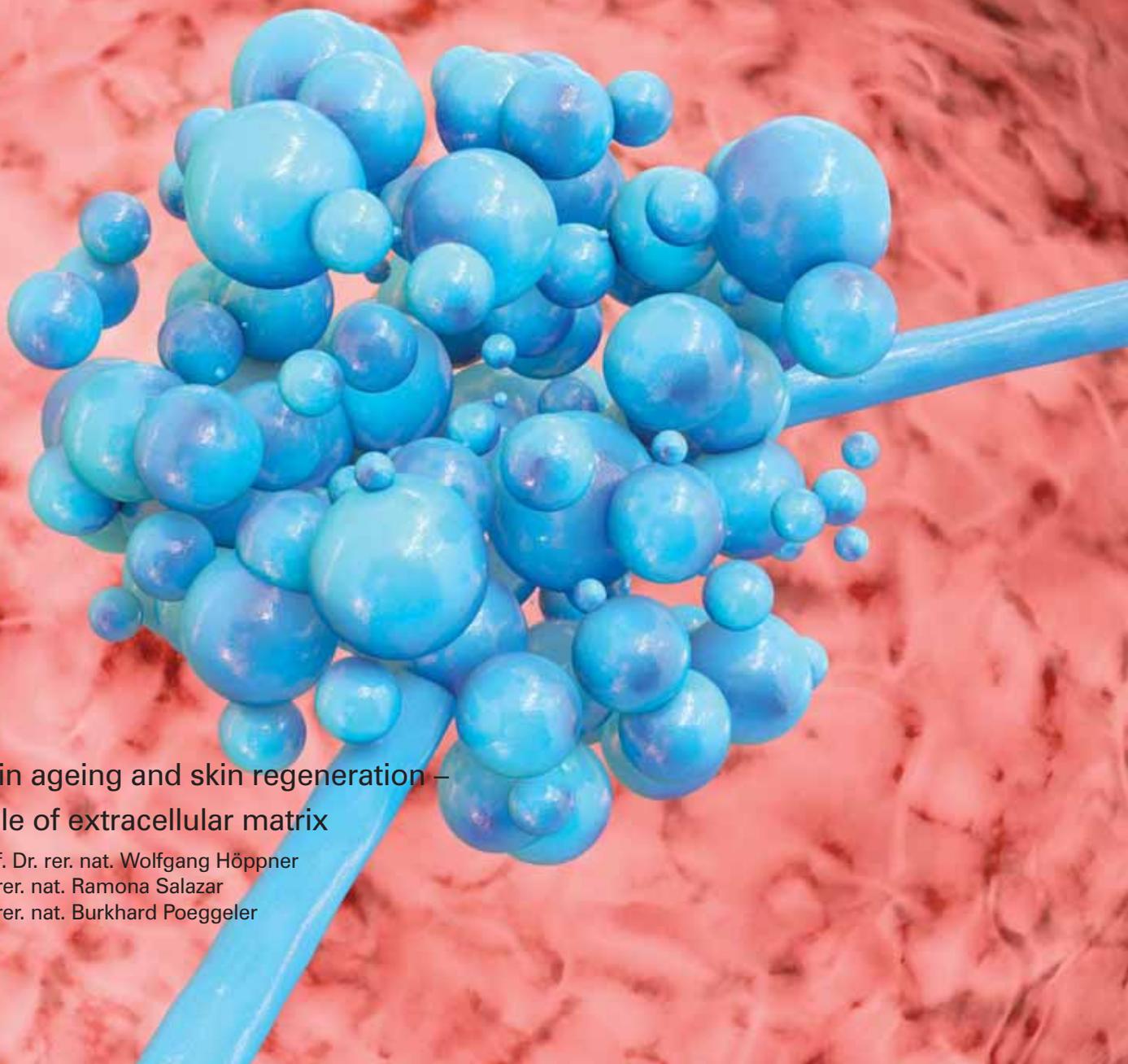
Skin ageing and skin regeneration –
Role of extracellular matrix

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# Skin ageing and skin regeneration – Role of extracellular matrix

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## The ageing process

As the body's largest organ, the skin has various functions (Tab. 1), which are divided among the three layers from which the skin is structured. The layers of skin from the inside to the outside are referred to as subcutis, dermis and epidermis. They each consist of several zones which have specific functions and differ through the existence of different cell types and structural proteins (Fig. 1). When it concerns a youthful, radiant appearance and effective protection against adverse external factors on the physiological signs of ageing, two biological processes in the skin are of special importance: the regeneration of the epidermis and the integrity of the extracellular matrix, the collagen-elastin network in the dermis.

- Barrier (e.g. germs, harmful substances)
- Temperature regulation
- Regulation of water balance
- Sensory perception
- Immune function
- Endocrine function
- Communication (e.g. attractiveness)
- Mechanical protection
- Excretory organ (salts, urea, lipids, protein, harmful substances)

**Tab. 1**  *Skin functions*

The first signs of skin ageing already appear by the mid-twenties. Although ageing is an elementary part of life as a physiological process, the course of ageing can be individually very different. It depends on intrinsic and extrinsic factors (Tab. 2). The extrinsic factors are behaviour-dependent. They seem to be responsible for up to 80 % of skin ageing. Important extrinsic influencing factors are photo ageing (UV and IR radiation of sunlight), nutrition, alcohol consumption and smoking. Intrinsic ageing processes are to be referred to as time-dependent. Genetic disposition plays the most important role here. Since hormonal changes and the biological programme of ageing are ultimately genetically determined, they are a part of genetic disposition in a broader sense. The intrinsic factors are fundamentally not directly influenceable.

But it is assumed that intrinsic and extrinsic factors of skin ageing interact and possibly also intensify each other. Since there are increasing possibilities to determine the genetic dispositions of skin ageing, it makes sense to develop personalised preventive and therapeutic concepts in order to counteract skin ageing through adapted lifestyle, nutrition and targeted skin care. For logical reasons, effective prevention must begin before the symptoms of skin ageing set in and should preferably be purposeful, i.e. personalised. Despite all research efforts and advances of modern molecular and cell biology, the ageing of skin is an incompletely understood, highly complex process. In addition to the visible morphological changes of the skin, there are a series of histological changes which can be visualised in skin preparations under a microscope. There are additional cell biological and metabolic changes which can only be determined with complex laboratory methods [1].

Ageing processes are less pronounced in skin areas which are rarely exposed to external influences, such as the insides of the upper arms and the gluteal region. The influence of intrinsic factors predominates here. The percentage of extrinsic ageing factors is clearly determined with regard to face, décolleté, neck, hands and forearms. In this respect, the skin can serve as a model organ for examinations of endogenous as well as exogenous ageing mechanisms. Various theories which partially also apply to extrinsic mechanisms have been postulated with regard to intrinsic ageing processes (Tab. 3). The greatest difference is that the intrinsic ageing processes continually increase with age, while extrinsic influences are lifestyle-related and can be influenced [2, 3].

The theory of limited cell division is attributed to the loss of telomeres at the ends of chromosomes. Skin cells are among the somatic cells which divide most frequently. Fibroblasts as well as corneocytes have the enzyme telomerase and can therefore divide

| Intrinsic factors of skin ageing |
| --- |
| Genetic disposition |
| Hormonal status |
| Ageing programme |
| **Extrinsic factors of skin ageing** |
| Nutrition |
| Smoking |
| Alcohol consumption |
| Skin care |
| UV light, sun exposure |
| Physical and mental stress |
| Lack of sleep |

**Tab. 2**  *Factors of skin ageing*

| Mechanisms of skin ageing (modified according to) [1] | Intrinsic skin ageing | Extrinsic skin ageing |
|---|:---:|:---:|
| Telomere shortening | + | + |
| Reactive oxygen species (ROS) | + | + |
| Mutations of mitochondrial DNA | + | + |
| Adverse alleles in key genes (SNPs) | + | – |
| Altered hormone level | + | – |
| Altered cell metabolism and signal transduction | + | + |
| Water loss | + | + |
| Loss of collagen | + | + |
| Deceleration of cell regeneration | + | – |
| Collapse of cell metabolism | + | + |

*Tab. 3*

with minimal telomere shortening *[4]*. DNA repair is extremely important, especially for cells which divide frequently. The effectiveness of DNA repair decreases in the course of life. With skin cells there is also the fact that they are particularly exposed to reactive oxygen species (ROS) due to light exposure and environmental toxins. These ROS can destroy intracellular and extracellular structures. Among other things, mitochondrial and nuclear DNA as well as the components of the extracellular matrix is affected by

this. Since the antioxidant capacity (i.e. the protection against oxidation) also decreases with age, the likelihood is increasingly greater that DNA mutation, but also other cell damage, will accumulate with increasing age. The preservation and repair mechanisms can no longer keep up. Eventually more skin cells perish than are regenerated, the skin atrophies and is susceptible to diseases.

Cell biology studies have revealed that the signal transduction pathway via MAP kinase (mitogen-acti-
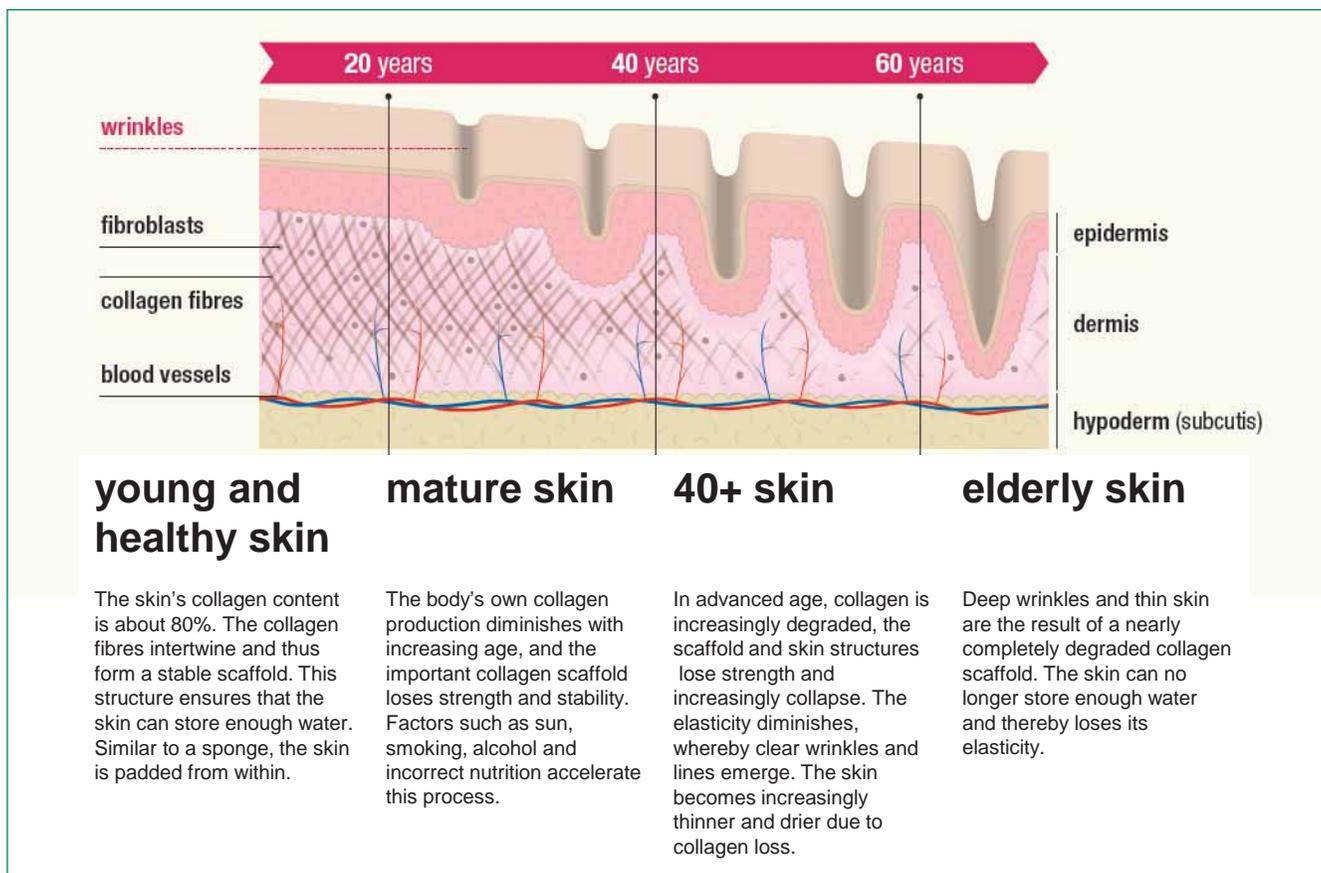


## young and healthy skin

The skin's collagen content is about 80%. The collagen fibres intertwine and thus form a stable scaffold. This structure ensures that the skin can store enough water. Similar to a sponge, the skin is padded from within.

## mature skin

The body's own collagen production diminishes with increasing age, and the important collagen scaffold loses strength and stability. Factors such as sun, smoking, alcohol and incorrect nutrition accelerate this process.

## 40+ skin

In advanced age, collagen is increasingly degraded, the scaffold and skin structures lose strength and increasingly collapse. The elasticity diminishes, whereby clear wrinkles and lines emerge. The skin becomes increasingly thinner and drier due to collagen loss.

## elderly skin

Deep wrinkles and thin skin are the result of a nearly completely degraded collagen scaffold. The skin can no longer store enough water and thereby loses its elasticity.

*Fig. 1   Age-related skin changes*

vated protein kinase) plays an important role for cell regeneration. Extrinsic triggers of skin ageing such as UV radiation lead to the fact that the transcription factor AP-1 (complex of c-Jun and c-Fos) at the end of the MAP kinase signal chain dysregulates the expression of transforming growth factor beta (TGF-ß), a cytokine that is responsible for the differentiation of numerous cells and tissues. For the extracellular matrix this means an inactivation of the new synthesis of collagen as well as an activation of collagen-degrading metalloproteinase (MMP-1) and the decrease in the synthesis of hyaluronan. Due to intrinsic mechanisms, the skin's collagen content declines with age by an average of 60%. If extrinsic stress factors are added, the collapse of skin structures can be even more dramatic.

### The role of the extracellular matrix with regard to skin ageing

Type I collagen is the quantitatively most frequent protein of the human dermis with a proportion of 80% in the dry matter. Just like collagen type III and type IV, it is synthesised by fibroblasts. The collagen fibres form a 3D network that generates the skin's structural strength. Collagen type I is subject to a natural, but very slow decomposition through metalloproteinase 1 (MMP-1), which is dependent on zinc and calcium. Fibroblasts have matrix receptors, so-called "integrins", which connect the intracellular actin cytoskeleton with the extracellular collagen fibres by means of specific binding to type I collagen. As a result, tension is exerted on the fibroblasts, and this is of great importance for their physiological function. Fibroblasts additionally synthesise elastin, hyaluronan and the proteoglycans decorin and versican. These proteins in the reticular layer of the dermis form a structure-giving network with the collagen fibres that generates strength, elasticity and resilience (Fig. 3). The storage of water is additionally enabled through the hyaluronan and thereby contributes to the biomechanical integrity of the skin. In addition, the components of the extracellular matrix perform physiological functions such as cell adhesion, migration and proliferation as well as the inhibition of apoptosis. It is noteworthy that signals which initiate the regeneration of keratinocytes are also emitted. Hyaluronan is synthesised on the surface of fibroblasts through three membrane-bound enzymes (hyaluronan synthase 1-3) and directly released in the extracellular space. Transforming growth factor beta (TGF-β) is an important inducer of hyaluronan.

Among the various extrinsic triggers of skin ageing, photo-ageing is best examined experimentally, because it can be studied relatively well in vivo as well as in vitro, and shows strongly significant effects on the skin's signs of ageing. If results of research on photo-ageing are compared with other extrinsic stressors (e.g. oxidative stress) and intrinsic triggers of skin ageing (e.g. advanced age or oestrogen deficiency), a number of cell biological commonalities can definitely be determined.

### The power of collagen

Like the other substances in the skin's biomatrix, collagen is a very complexly structured molecule with hierarchically arranged individual building blocks. The basic building block is the collagen molecule. It is reminiscent of a rope: three chain-like strands wind around each other and form a triple helix. In turn, many of these individual building blocks combine to form complex units, the collagen fibrils. These fibrils are only about 100-500 nanometres in size, but have enormous strength and tensile force. The single neighbouring collagen molecules in the collagen fibrils are not located directly, but offset next to each other so that a staggered arrangement with high tensile strength results [5]. These fibrils form fibres, which in turn bundle and eventually form collagen fibres with other molecules (Fig. 2).
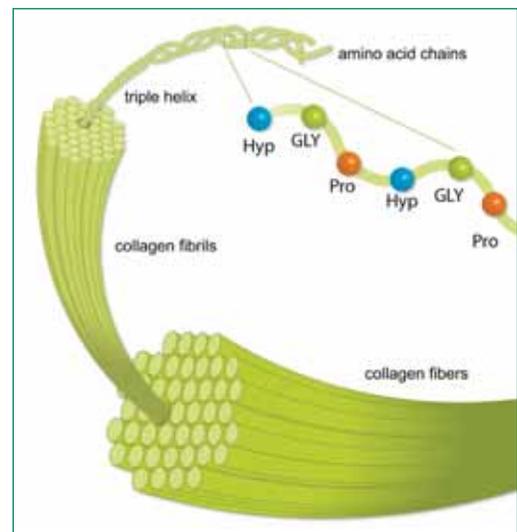


***Fig. 2*** *Structure of collagen fibrils*

Water, the elixir of life, is a decisive element of collagen and makes it an extremely dynamic and powerful molecule in the skin's biomatrix. Water constitutes about 60% of the natural content in collagen, and the water molecules fit seamlessly in the collagen fibres. The high water content explains why dehydration always has a dramatic effect. Even with a slight decrease in the skin's moisture content the collagen molecules contract by 1.2%. The corresponding fibrils contract by 2.4% and the complexly structured collagen fibres can shrink by nearly 5%. Even this relatively slight appearing shortening brings about a tension of 120 megapascals, which is over 300 times more than a muscle generates in terms of tension [5]. Consequently, collagen can develop enormous force
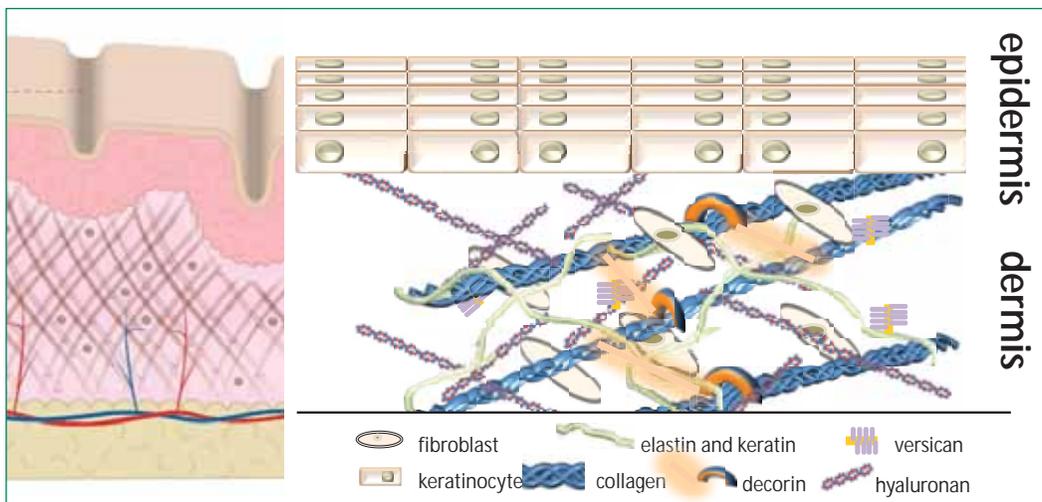
epidermis    dermis

fibroblast     elastin and keratin     versican
keratinocyte     collagen     decorin     hyaluronan

***Fig. 3***    *The extracellular matrix*

and decisively determine the elasticity and strength of the skin through absorption and release of water. Collagen is the decisive protein that gives the skin its structure and first enables its diverse functions. Through binding of water collagen ensures smooth, firm and taut skin with a strong, powerful and dynamic biomatrix *[5-8]*. The elasticity of the tissue is ensured through these endogenous fibres, and the skin's function as the body's flexible surface is therefore safe-guarded. Collagen is with over 80% by far the skin's most important structural element and together with other fibres such as elastin and versican thereby determines the density, strength and volume of this largest human organ. Moisture, elasticity and rough-ness of the skin are decisively influenced by its col-lagen content.

Collagen peptides, which are naturally formed during the decomposition of protein or which can be pro-duced through (bio) technical hydrolysis, promote the new synthesis of collagen and support the regenera-tion of skin. The skin's thickness, density and volume are decisively determined through the binding of col-lagen and versican *[6-10]*. Due to their increased syn-thesis rate after administration of collagen peptides and other "dermonutrients", regeneration which lifts the structure of the skin with renewal of all its constit-uent fibres back to the level of healthy young skin can even be triggered in advanced age. A stimulation of these essential elements through collagen peptides leads to a causal repair of age-related deficits in the extracellular matrix.

## Photo-ageing as a model of extrinsic skin ageing

With regard to photo-ageing, a differentiation is to be made between short-term acute and chronic UVB irradiation. The effects on regulatory signalling systems and the restructurings of the extracellular matrix differ considerably.

An increase of hyaluronan synthase 3 (responsible for the synthesis of medium length hyaluronan chains) can be determined in skin acutely irradiated through UVB rays. The hyaluronan content increases propor-tionately. Hyaluronan activates hyaluronan recep-tors, among other things CD44 (HCAM: homing cell adhesion molecule) and the receptor for hyaluronan mediated mobility (RHAMM) *[11]*. Signalling pathways which lead to proliferation and migration of fibro-blasts, inhibit apoptosis and adhesion and stimulate the regeneration of keratinocytes are also triggered. The proteoglycan versican is also upregulated under the conditions of acute UVB irradiation and protects against oxidative changes. The versican splice variant V1 generates a protective effect against apoptosis. An overall environment that enables repair and remodel-ling processes is created *[12]*. In particular, fibroblasts are stimulated for regeneration and the new synthesis of collagen is also thereby facilitated. Short-term UVB exposure triggers inflammatory processes in the skin which result in repair and restructuring.

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In contrast, protective mechanisms are dysregulated through chronic UVB irradiation, and radicals which induce the degrading enzymes hyaluronidase 1 and 2 are created and lead to a direct fragmentation of hyaluronan chains [11].

The hyaluronan fragments themselves activate toll-like receptors and thereby modulate inflammatory responses. Chronic UVB exposure of the skin is characterised by a reduction of its hyaluronan content, the decrease in water content of the dermis and the



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emergence of high-molecular collagen fragments [11, 12] which are referred to as collagen neoepitoptes. It is very likely that this is associated with a reduction of TGF-ß concentration [13]. Since only about 5% of UVB radiation can penetrate into the dermis under in vivo conditions, it must be assumed that crosstalk with the epidermis occurs and the effect of UVB radiation on the keratinocytes triggers effects in the dermis by means of a modified cytokine release [13]. The emerging high-molecular collagen fragments inhibit the new synthesis of hyaluronan. The effect is mediated via bioactive RGD sequences (= Arg-Gly-Asp, peptide epitopes) which bind to integrins and thereby downregulate hyaluronan synthase 2. The matrix loses the capacity to bind moisture. In the course of chronic photo-ageing, versican is subject to molecular changes which impair the binding to other matrix molecules. A lasting destruction of the extracellular matrix arises with chronic UVB exposure. The inoperable fragments of the matrix structure are only slowly degraded and impede restructuring [11].

## Genetic disposition of skin ageing

Our individuality is predefined to a not inconsiderable part by our genetic blueprint. And so it is not surprising that the process of skin ageing also has a "personalised genetic note". The exact knowledge of this genetic individuality helps to understand why people age differently and why various measures for skin care are effective in different ways.

The cosmetics industry has recognised that there is a need for personalised skin care products. Although many studies have been implemented in recent years which aimed to research the individual genetic disposition of skin, the results have not been implemented in practice up to now.

In the meantime, genetic variants (single nucleotide polymorphisms: SNPs) in key genes of skin metabolism have been identified and their effects on metabolic processes, quality and regeneration capacity of the skin has been examined in clinical trials.

Consideration of the following processes is of decisive importance for determining the individual disposition of skin ageing:

- Oxidative radical stress
- Detoxification of metabolic products, medications and environmental substances
- Regeneration capacity of the skin

### Oxidative radical stress in the skin

Oxidative stress occurs when the formation and neutralisation of reactive oxygen species (ROS) in the metabolism no longer balance each other and the cell is inundated with aggressive oxygen radicals. Most ROS have unpaired electrons and are therefore free radicals. Among these are the superoxide anion, hydroxyl radical, nitric oxide and lipid radicals. Molecules such as hydrogen peroxide and peroxynitrite are themselves not free radicals, but damage cellular structures and substances through their strong oxidising properties and thus also contribute to oxidative stress.

Free radicals and ROS are themselves created physiologically in the organism:

- During cellular respiration
- In the cell metabolism
- During immune response and inflammatory processes
- As products in enzymatic reactions („Fenton" reaction, xanthine oxidase, NADH/NADPH oxidase reaction, nitric oxide synthase reaction)
- During oxygen transport

On the other hand, to a not inconsiderable extent they are a consequence of our lifestyle (Tab. 2).

| Enzyme | Gene | Detoxification function | Cofactors |
|---|---|---|---|
| Superoxide dismutase 2 | SOD2 | Superoxide | Manganese |
| Catalase | CAT | Hydrogen peroxide | Iron |
| Glutathione peroxidase 1 | GPX1 | Hydrogen peroxide | Selenium, glutathione |

***Tab. 4*** *Characterisation of antioxidant enzymes for decomposition of superoxide radical and hydrogen peroxide in the skin*

About 1-3% of the oxygen required in the cellular respiratory chain is permanently converted into superoxide in the mitochondria. Since this radical irreversibly damages the sensitive iron-sulphur proteins in the respiratory chain, it must be detoxified completely and as quickly as possible.

For this purpose our organism has an efficient enzymatic defence system that detoxifies reactive oxygen species (Tab. 4.). But genetic variants in these genes lead to a large individual fluctuation range in the enzymatic antioxidant capacity.

The manganese-dependent superoxide dismutase in the mitochondrion converts superoxide into hydrogen peroxide, which is diffused through the mitochondrial membrane and degraded into nontoxic end products in the cytoplasm by the enzymes catalase and peroxidase (glutathione peroxidase).

The sun's UVA and UVB radiation particularly leads to an increase in the concentration of oxidative radical compounds in skin cells. In vivo studies of human skin were able to show that the expression of the effective antioxidant catalase declined within 24 hours after UV irradiation in the epidermis as well as in the dermis by 50% compared to the control and only reached the base level again after 72 hours [14]. Whereas if the enzymatic level of catalase was raised through overexpression in human keratinocytes, the formation of ROS could be prevented after UVB irradiation [15].

How important the avoidance of large quantities of ROS is for the cell is underscored by the results of new studies regarding superoxide dismutase 2 (SOD2). In both studies, mitochondrial stress was induced through experimental SOD2 deficiency. The uncoupling of the respiratory chain and thereby a reduction of ROS formation was observed in the first study [16]. In the second study, the SOD2 deficiency initially led to damage of nuclear DNA and thereby induced the premature ageing of the epidermis in the course by increasing the terminal differentiation rate, yet the number of cells and the density of the epidermis were reduced [17].

Consequently, with adverse genetic disposition in several enzymes of oxidative defence, oxidative stress can even arise due to slight burdens with ROS and metabolic radicals, and the cell's need for antioxidants can increase.

**Detoxification capacity of the skin**
Clinical trials have confirmed that epidermal keratinocytes show an enzymatic pattern for the skin's detoxification function that is similar to the liver [18, 19]. Just like in the liver, the detoxification process of water-insoluble metabolic waste products in the skin proceed in two phases and serve the conversion of these substances into water-soluble products which thus

| Enzyme | Gene | Detoxification phase | Cofactors |
|---|---|---|---|
| Cytochrome P450 oxidoreductase 1A1 | CYP1A1 | Phase I | FAD, NAD |
| Cytochrome P450 oxidoreductase 1B1 | CYP1B1 | Phase I | NAD |
| Cytochrome P450 oxidoreductase 2E1 | CYP2E1 | Phase I | NAD |
| Quinone oxidoreductase | NQO1 | Phase I | NADPH |
| Epoxide hydrolase 1 | EPHX1 | Phase I | - |
| Glutathione S-transferase M1 | GSTM1 | Phase II | Glutathione |
| Glutathione S-transferase T1 | GSTT1 | Phase II | Glutathione |
| Glutathione S-transferase P1 | GSTP1 | Phase II | Glutathione |
| N-acetyltransferase 1/2 | NAT1/2 | Phase II | Acetyl coenzyme A |
| Catechol-O-methyltransferase | COMT | Phase II | S-Adenosyl methionine |
| UDP-glucuronynltransferase 1A1 | UGT1A1 | Phase II | UTP, glucuronic acid |
| Sulfotransferase 1A1 epoxide hydrolase | SULT1A1 | Phase II | PAPS |

***Tab. 5*** *Selected detoxification enzymes in the skin*
*(Abbreviations: FAD: Flavin adenine dinucleotide; NAD: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; UTP: uridine-5'-triphosphate; PAPS: 3'-Phosphoadenosine-5'-phosphosulfate)*

modified can be excreted from the body. In phase 1 of detoxification, reactive and therefore often aggressive intermediate products are generated among other things through oxidation reactions which are coupled to water-soluble low-molecular transfer molecules (glutathione, glucuronic acid, glycine and glutamine as well as methyl, sulphate and acetyl groups) in the enzymatic reactions of phase 2 and then excreted. In contrast to the liver, the phase II enzymes of detoxification dominate in the skin. Important enzymes of the skin are presented in Tab. 5.

Although only about 5-10% of the quantities of enzymes in the liver are found for most phase I enzymes in the skin, there are exceptions. The cytochrome P450 enzymes CYP1A1 and CYP1B1 are not constitutively formed in the skin, but the enzymatic synthesis of both enzymes is increased 100-fold during exposure with polycyclic aromatic hydrocarbons (PAHs) [20, 21]. A gene variant through which the inducibility of the enzyme is enhanced even more is also known for CYP1A1. For the most part, CYP1A1 and 1B1 convert PAHs (among others, dioxin and benzopyrene) into genotoxic epoxides which are subsequently hydrolysed by the enzyme EPHX1. The EPHX enzyme concentration in skin biopsy samples of various test subjects varied by a factor of 2.6. Two frequently occurring genetic variants which can activate as well as inhibit the enzyme are among others causal for this fluctuation range. It is also interesting that the quinone oxidoreductase (NQO1) in the skin, which is also inducible through PAHs, is found to the same extent in the liver. Glutathione S-transferase (GSTP1), glucuronyltransferases and sulfotransferases as well as N-acetyltransferase (NAT1) dominate with the phase II enzymes [14].

The listed enzymes are subject to a high genetic variability, whereby to some extent considerable fluctuations occur in the detoxification capacity of the skin.

Conclusion: our skin is an active metabolic and detoxification organ whose capacity differs individually from person to person.

### Regeneration capacity of the skin

It is assumed that in every cell about 50,000 damages to our genetic material occur every day due to internal and external influencing factors. A majority of these daily arising DNA mutations in the skin cells is attributable to the ionising radiation of sunlight. While short-wave UVB rays only penetrate into the epidermis down to the basal cell layer and are responsible for the development of severe redness of the skin (sunburn), long-wave UVA rays can penetrate deeper into the dermis and in addition to harmful photo-oxidation

reactions also cause damage to the skin's elastic connective tissue fibres. The decrease in the skin's lifting, collagenous or elastic connective tissue fibres is an essential reason for premature skin ageing and wrinkle formation.

In addition to DNA mutations, the high-energy radiation of the sun also causes the increased formation of harmful oxygen radicals which lead to oxidative stress in the cells. This effect has been documented by means of clinical trials. The spectrum of antioxidant enzymes in the skin as well as in the blood's erythrocytes changed after longer UV exposure. The concentration of the enzymes catalase and GPX1 increased considerably, whereas the expression of superoxide dismutase 2 (SOD2) tended to decrease [16, 21]. If the body's own oxidative defence does not manage to eliminate the emerging ROS, damage to the DNA molecules frequently occurs, for example through aggressive hydroxyl radicals, or formation of DNA thymine dimers also occurs through the direct absorption of UV radiation. In both cases, complex DNA repair processes are started in the cells in order to repair the arising DNA defects and to restore the integrity of cell metabolism. If the enzymes of the DNA repair system are not able to completely eliminate the arising mutations, the affected cell is subject to programmed cell death (apoptosis) and must be replaced by a new cell. A selection of important regulatory enzymes and proteins which are relevant for the skin's complex regeneration processes is presented in Tab. 6. For the skin's regeneration processes it is also important that the genetic variability of significantly involved enzymes leads to individual fluctuations in the regeneration capacity of the skin.

### Dermatocosmetic active ingredients against skin ageing

The balance between collagen synthesis and collagen decomposition and the related integrity of the extracellular matrix plays a pivotal role with intrinsic as well as extrinsic skin ageing. As presented above, the hyaluronan content and water storage are therefore also linked. These factors determine the skin volume, elasticity and tightness, and are therefore an important prerequisite for a youthful, radiant appearance of the skin.

It is increasingly recognised that oxidative stress is to be considered the most important cause for the disturbance of collagen homoeostasis. A burden with reactive oxygen species is the result of numerous known extrinsic skin ageing factors such as UV light, tobacco consumption, environmental toxins and ozone (Tab. 2). The burden due to oxidative stress also increases with the decrease in the oestrogen level during menopause. The classic ingredients of

| Enzyme | Gene | Function | Cofactors |
|---|---|---|---|
| X-ray repair, complementing defective, in chinese hamster, 1 | XRCC1 | DNA repair processes, among other things | - |
| Excision repair cross-complementing rodent repair deficiency, complementation group 2 | ERCC2 | After UV exposure | Magnesium, iron-sulphur cluster |
| Tumor protein p53 | TP53 | Cell cycle regulation | Zink |
| Vitamin D receptor | VDR | Control of differentiation processes in the skin | Zink, Vitamin D3, Retinoid-X-receptor-alpha |
| Matrix metalloproteinase-1 | MMP1 | Collagen decomposition | Zink, Calcium |
| Matrix metalloproteinase-3 | MMP3 | | |
| Methylentetrahydrofolat reductase | MTHFR | DNA synthesis and DNA repair processes | B-Vitamine (B6, B12, Folsäure) |
| Collagen, type I, alpha 1 | COL1A1 | Most frequent collagen, among other things in bones, skin, connective tissue | Vitamin C |

***Tab. 6***   *Important regulatory enzymes of skin regeneration*

cosmetic care products usually serve for reduction of symptoms, in which case they support water storage, diminish dehydration and preserve the skin's fat content. Antioxidants have also been among the ingredients of cosmetic products for a long time. However, it is important that the active ingredients reach the depth of the skin where the desired effect is intended.

**Modern galenics for dermatocosmetics**

The active ingredients used in skin care products are normally applied to the skin surface, i.e. topically applied or inserted into the layers of skin by means of injection, needling, ultrasound or other physical methods. The problem is to reach the dermis and thereby the most important layer for the regeneration of the organ.

That is why modern dermatocosmetics rely on new galenic approaches with which the deeper layers of skin are also reached and the targeted release of active ingredients is possible. The most common modern galenic preparations which make it possible to transport the active ingredients so that the barrier can be penetrated without damage and the release to the desired target structures can occur are summarised in Tab. 7.

**Dermatocosmetic active ingredients against skin ageing**

More and more specific active ingredients which can protect, repair and regenerate structures are being used to specifically influence skin ageing. In addition to defending against oxidative stress, the aim is to directly or indirectly influence collagen metabolism and to support the regeneration of fibroblasts and keratinocytes.

Up to now, a secured and sustainable optical and physiological improvement of skin aged through chronic UVB irradiation only seemed to be achievable through the topical application of retinoid derivatives. The influence which these treatments have on composition and structure of the extracellular matrix has been the subject of numerous studies. The direct stimulation or inhibition of the transcription of genes via nuclear receptors is best researched as the mechanism of action of retinoids. By these pathways retinoids lead to synthesis of new type I collagen in aged skin with simultaneous deactivation of the synthesis of MMP-1. The new synthesis of hyaluronan is also promoted by retinoids. This takes place through activation of the transcription of the hyaluronan synthase 2 gene promoter. A structural improvement in the histology is visible with regard to elastin. A quantitative alteration of the elastin content has not been determined. The extent to which non-receptor-mediated effects of retinoids play a role in skin generation is not reported. The use and effect of other common dermatocosmetic active ingredients is summarised in Tab. 8.

**Therapy with components of the extracellular matrix**

An important therapeutic option is the injection of components of the extracellular matrix such as collagen or hyaluronan into the dermis. Hyaluronan is preferred today because it does not lead to allergic reactions. Cross-linked hyaluronan is preferred because the effects last longer. The injective of native hyaluronan leads to pericellular integration and lastingly improves elasticity and skin surface properties. This effect is attributable to the stimulation of the proliferation of fibroblasts. A significant increase of collagen

| Liposomes and lamellar lipid double membrane systems | Spherical vesicles with a lipid double membrane, usually from phospholipids, size approx. 20 nm | Hydrophilic substances can be transported inside, lipophilic and amphiphilic substances can be transported in the membrane. |
|---|---|---|
| Nanoemulsions | Oil-in-water emulsion with droplet diameter of 50–500 nm | Very well suited for the transport of lipophilic substances |
| Lipid nanoparticles | Similar to nanoemulsion, but lipid components in solid state, size 50–100 nm | Active ingredients distributed in lipid matrix, sensitive substances very well protected |
| Oleosomes | Similar to liposomes, but with lipophilic phase inside, outer shell features emulsifying lipids | Transport of lipophilic active ingredients |
| Surfactant-free formulations | Polymeric and solid matter emulsifiers are used instead of classic amphiphilic emulsifiers. Stabilisation through macromolecules. | Transport of lipophilic and hydrophilic active ingredients |

**Tab. 7**  *Nanodisperse systems for topical application of dermatocosmetics*

and an increased release of TGF-ß can be demonstrated after 3 months.

### Regeneration of the extracellular matrix from within

Actually, the topical application of cosmetics alone often only leads to a superficial, short-term and symptomatic treatment of ageing skin. In order to be able to causally counteract the skin ageing processes, it would be desirable to systemically administer the nutrients or active ingredients so that they can lastingly reach the deeper layers of skin by natural means. That is why a healthy, balanced diet which supplies all necessary vital substances to the skin's biological processes is an important prerequisite for delaying skin ageing. The intake of highly effective dermatocosmetic active ingredients as foodstuffs is an interesting and promising new concept.

### Dermonutrients against skin ageing

The idea for therapeutic skin rejuvenation was virtually born with the introduction of dermonutrients in cosmetic products which can topically improve the nutrition and regulation of the skin. Natural active ingredients and nutrients which have more of a "foodstuff character" as well as highly concentrated, nature-identical, semisynthetic or synthetic active ingredients as well as their derivatives, which are to be classified as medicinal products and therefore as "dermatopharmaceutics", are used. The artificial term "cosmeceutical" inspired by the terms "pharmaceutical" and "nutraceutical" reflects the desire to link a therapy against skin ageing either in the nutritive or in the pharmaceutical sense with cosmetic use. Skin care products such as creams, serums and lotions as well as the active ingredients used in them solely cannot causally and lastingly influence the skin ageing processes from inside the depth of the skin. In order to be able to counteract skin ageing processes at the root and to achieve a regeneration of the extracellular matrix from within, the nutrients or active ingredients must be administered so that they can also reach the deeper layers of the skin such as the dermis.

### Drinkable collagen rejuvenates the extracellular matrix

The active ingredients in drinkable collagens, short-chain collagen peptides, also penetrate into the deep layers of the skin after oral intake and can therefore counteract the skin ageing processes where they start [7–9]. Short-chain collagen peptides are extracted in an elaborate and lengthy process through an enzymatic hydrolysis of collagen and have a very high bioavailability of nearly 100%. The collagen peptides reach the small intestine after oral intake. Dipeptides, tripeptides and tetrapeptides as well as free amino acids are formed from this during digestion [7–9]. These can easily be absorbed by the mucosa of the small intestine and then released in the blood circulation by means of which they reach the skin.

These peptides presumably accumulate in all layers of the skin by means of integrins and other binding sites [7, 8]. There they stimulate the synthesis of collagen and other important elements of the biomatrix such as elastin and hyaluronan [7, 8] The collagen peptides can thus stimulate the regeneration of the skin in a natural way and thus lastingly and durably counteract the ageing problems of the entire skin. This includes the compensation of age-related diminished collagen synthesis, which decisively contributes to restoring its moisture and elasticity, and removing its roughness with increased formation of wrinkles and lines [7–9].

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| **Vitamin A and retinoids** | Retinol, vitamin A acid and retinal are used. Effect on gene expression via nuclear receptors. Very good effect on collagen synthesis, inhibition of collagenases. |
| **Vitamin C** | Hydrophilic molecule that directly acts as a radical scavenger and also reduces other oxidised antioxidants. Is essential for hydroxylation of proline during collagen synthesis. Induces collagenase inhibitors and thereby stabilises collagen and elastin. |
| **Vitamin E** | Lipophilic antioxidant that particularly protects membranes against oxidation. Inhibits the gene expression of collagenases and thereby stabilises collagen and elastin. |
| **Coenzyme Q10** | Is formed in the mitochondria, among other things as a coenzyme for transport of electrons during oxidative decarboxylation. Presumably lowers the MMP-1 expression with topical application. |
| **Alpha lipoic acid** | Coenzyme in the mitochondrial energy metabolism. Very high antioxidant effect against peroxide, hydroxyl and superoxide radicals as well as nitric oxides. |
| **Phytoestrogens** | Has revived oestrogen therapy which hardly played a role due to feared side effects. Hormone-like effects of isoflavones, coumestans and lignans. The decrease of wrinkles and skin dryness has been proven in clinical trials. |
| **Catechins (green tea)** | Polyphenols (e.g. epicatechin, epigallocatechin, and epigallocatechin-3-gallate) show a photo-protective effect. Decrease of sunburn cells, Langerhans cells and DNA damages after exposure with UV rays. |
| **Biomelanin** | Biotechnologically produced polyphenolic melamines. Good protection of biomembranes against peroxidation. |
| **Sulforaphane** | Phytochemical that occurs as glucosinolate-glucoraphanin, particularly in crucifers such as cabbage and broccoli. In contrast to conventional antioxidants, the active ingredient does not directly neutralise free radicals, but sets the cell's antioxidant defence mechanisms in motion, in which among other things phase II enzymes of the detoxification system are regulated. |
| **Copper tripeptide complex** | Copper is essential for lysyl oxidation during the cross-linkage of collagen fibres. Copper tripeptide complexes stimulate fibroblasts to synthesise collagen. |
| **Growth factors** | As a growth factor, TGF-ß stimulates the synthesis of procollagen I in fibroblasts after topical application and counteracts a UV-induced reduction of sensitivity for TGF-ß. Plant-based growth factors for use against skin ageing are being discussed and employed. For kinetin (N6-furfuryladenine), a plant cytokine, a reduction of signs of ageing has been experimentally proven on fibroblasts, and is therefore employed as a dermatocosmetic active ingredient. Other plant-based growth factors, primarily from plant-based stem cell extracts, are being tested. |
| **Palmitoyl pentapeptides** | Pentapeptides with partial sequences of collagen stimulate collagen synthesis after topical application. |

***Tab. 8*** *Dermatocosmetic active ingredients against skin ageing*

The stimulation of the synthesis of important structural elements of the skin such as collagen, elastin and aggrecan, biglycan, decorin, versican, loricrin, filaggrin and fibrillin could be clearly demonstrated in biochemical, cytological and clinical studies *[7–10]*. So the dipeptides, tripeptides and tetrapeptides formed from the oligopeptides contained in the drinkable collagens can achieve a visible improvement of skin elasticity in a very natural and physiological way. Other than through the merely external cosmetic application which can achieve superficial and short-terms effect, a regenerative effect is also achieved in the deeper layers of the skin through collagen peptides as a nutrient *[7–9]*. Especially there the bioactive dipeptides, tripeptides and oligopeptides can accumulate and stimulate the collagen, elastin and biglycan formation *[5, 6]*. These skin fibres ensure taut, firm and smooth skin with maximum flexibility and elasticity through adequate hydration. Cosmetics do not ordinarily reach the deeper layers of the skin and that is why the principle "beauty through health" by means of a stimulation of skin regeneration through increased formation of its decisive structural elements cannot be attained. So the skin's moisture, flexibility and elasticity are very specifically improved by means of an oral nutrient supplementation and its premature ageing is reduced through increased skin roughness [7-9]. These stimulating effects of collagen peptides lead to an improved regeneration of the cutaneous biomatrix, also and particularly among elderly people, and can thus permanently improve skin appearance all one's life.

**Rejuvenation effects of collagen peptides**
Compared to placebo in clinical trials, collagen peptides can significantly and positively influence skin moisture as well as skin elasticity. A 15% higher skin elasticity after administration of collagen peptides has been demonstrated. This already occurred after 4 weeks and also continued 8 weeks after starting treatment with collagen peptides. A very relevant result was the sustainability of application: even after discontinuation of intake significantly enhanced skin elasticity could still be determined 4 weeks after the last intake of collagen peptides *[6, 7]*. As a result, they can causally counteract the skin's ageing process more effectively than many other treatments, and lastingly and permanently improve skin moisture and skin elasticity *[6, 7]*.

Collagen peptides contain a high proportion of L-arginine. As a result, bio-energetic stimulation, antioxidant protection and ubiquitous regeneration of the skin occur by means of an increased formation of the messenger nitric oxide (NO) *[6, 7, 9]*. Consequently, collagen peptides not only have nutritive effects, but can also directly or indirectly influence signal transduc-

tion in the skin and accelerate the renewal rate of the epidermis. So other decisive skin parameters such as their regeneration through collagen peptides can also be positively influenced. Various effects could not only be demonstrated on the uppermost layer of skin, the epidermis, but also on all other layers of skin, especially the deeper layers such as the dermis and subcutis. So the unequivocal evidence of the unique effect of collagen peptides on the skin's essential abilities such as its inherent flexibility and elasticity, was provided and it was demonstrated that specific dermonutrients with high bioavailability and bioactivity can still strongly influence the physiology of this organ even at an advanced age *[7–10]*. The skin's increased moisture, flexibility and elasticity improve the entire skin appearance, and its youthful appearance can be restored and permanently preserved.

A significant and visible reduction of lines and wrinkles in the area of the eyes is one of the most striking effects of collagen peptides, which is immediately noticed. This distinct effect was clearly proven in two clinical trials *[7, 8]*. The discovery of fundamental molecular mechanisms and mediators of the anti-wrinkle effect with evidence of the effect particularly also in the clearly visible area of the eyes has revealed that the compensation of age-related reduction of collagen synthesis and a reduction of age-related stimulation of collagen decomposition through matrix metalloproteases expressed specifically in the skin also represent important factors here *[6–8]*. The systematic recording of changes showed the distinct effects of collagen peptides on the depth and volume of skin

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wrinkles in the area of the eyes [7–10]. The volume of wrinkles and lines around the eyes could be reduced by up to one-third in only four weeks [7–10]. These effects were lasting and permanent, because they even persisted 4 weeks after discontinuation of treatment with the collagen peptides [7, 8]. These effects were demonstrated in major randomised, placebo-controlled, double-blind clinical trials. The natural and physiological effect of drinkable collagens, which in stark contrast to cosmetics do not only have superficial and short-term effects, but contribute in the long

also plays an important role. The skin's stabilising collagen fibres also lose strength with increasing age and are partially not only degraded, but even negatively altered, in which case collagen loses its water-binding power as an essential structural element of the skin [5, 7–9]. The intact collagen level is directly related to the thickness, density and volume of the skin, which in turn determines its elasticity, flexibility and strength. The skin's ability to replenish its collagen storage decreases with age, and the skin ages visibly [5, 7–9]. The naturally healthy skin structure and function can



term to an immediately visible improvement of skin appearance, was shown in a very impressive manner. The face and the eyes are crucial mirrors of our health. The efficacy of this unique new, targeted nutrient supplementation for reversal of the skin's ageing process is most impressively shown here. The eye area is especially striking and immediately signalises the stress and health of the organ that is not without good reason referred to as the "mirror of the soul".

Naturally the formation of wrinkles in the face is particularly striking, but other areas of the human body are also affected by the diminished formation of collagen and other elements of the cutaneous biomatrix. In addition to a limited regeneration capacity of cells, the age-related reduction of the subcutaneous layer of fat

only be restored if the collagen deficits in the deeper layers of skin are sustainably balanced again. These impressive effects are also retained when applied for longer periods, which is confirmed by currently ongoing studies on natural nutrient supplementation developing from within the depth of the skin. Therewith, an unequivocal reversal of the skin's ageing process that can be clearly demonstrated in a biochemical, physiological and phenomenological sense is achieved for the first time. An intensified regeneration of the entire skin, including the deeper layers, has been a great challenge for cosmetic medicine, dermatology and pharmaceutics up to now. And so for the first time there is a chance for a sustainable rejuvenation through a targeted nutrient supplementation.

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Additional fields of application for collagen peptides such as tightening the connective and fatty tissue are now being researched, and initial promising results are also already available. Moreover, the new approach can be optimally combined with other methods of cosmetic medicine and cosmetics, such as specifically acting serums for promotion of regeneration which are applied externally. New ongoing studies indicate that therefore synergistic effects can be achieved, and a rapid, permanent and visible improvement of the entire skin appearance can be enabled for the first time.

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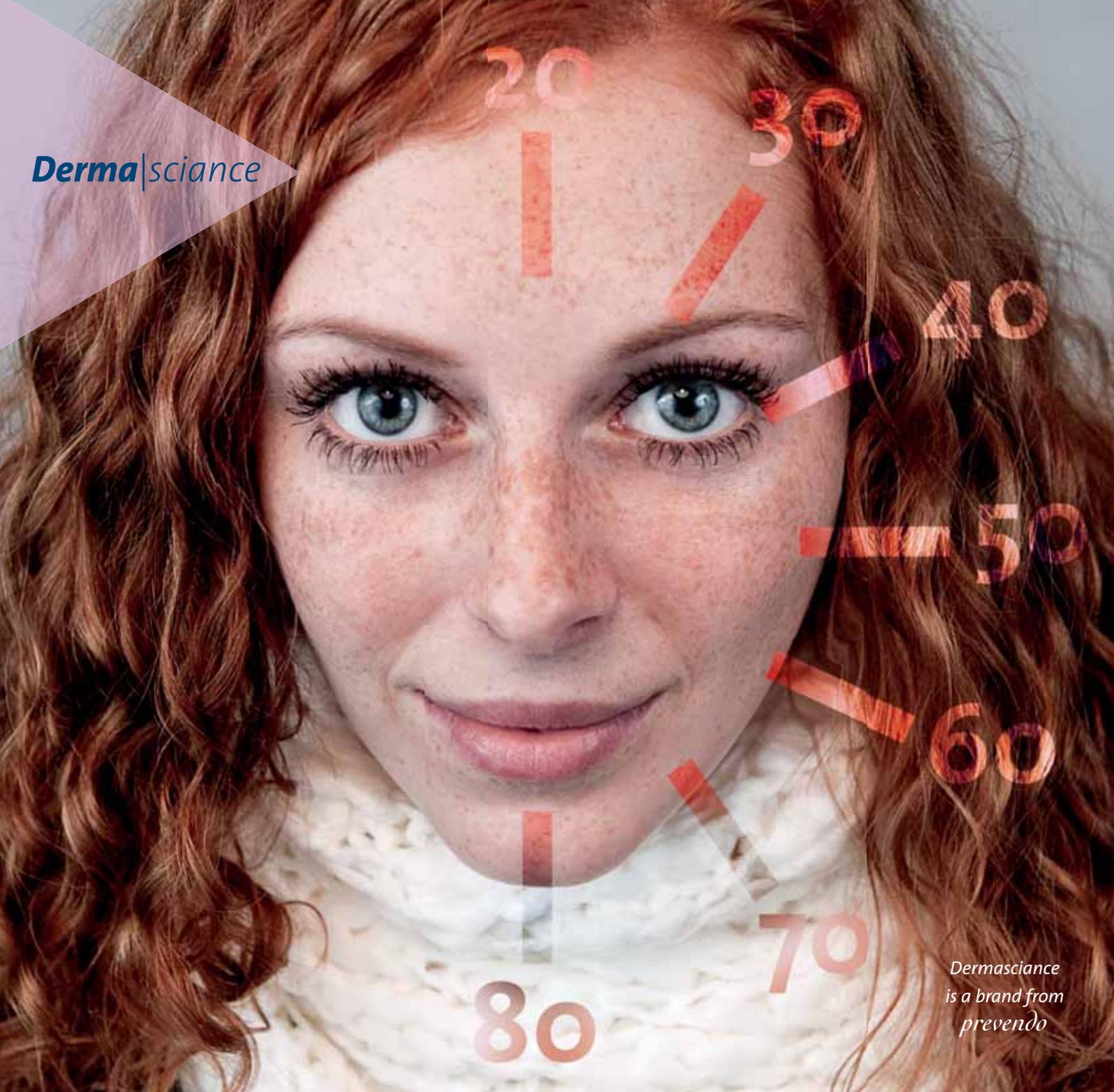
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