WORLD FIRST

STEMZYME™

THERAPY

DMK MD™

StemZyme™ Therapy

As one ages, epidermal stem cells start declining. To keep the stem cell population above the optimal level in the skin, one needs to constantly regenerate them. Only having stem cells in the skin is not sufficient, unless they differentiate into other younger skin tissues like keratin, collagen, elastin etc. and that's why epidermal stem cells are so important in maintaining biologically young and bountiful skin.

DMK StemZyme™ Therapy does both, the regeneration of epidermal stem cells and their differentiation into biologically young tissues. This is done in a non-invasive way and using one's own stem cells. **DMK StemZyme** is a self-personalised treatment because your own stem cells are getting utilised in the treatment. Due to such a unique approach, there is no hazard of stem cell rejection in the skin, and it guarantees they will transform into young tissues.

In simplistic terms, think of your skin like an old house, as it ages, the foundation begins to weaken. Even if we do a constant renovation on the house, if the foundation is not renovated properly, the house will eventually collapse.

So, we need to strengthen the foundation first and then start the house that sits on it. The skin is the same, we must renovate and restore the foundation before we work on the surface issues. In this context, epidermal stem cells are the skin's foundation building blocks because all skin tissue can originate from them. **DMK StemZyme Therapy** also prevents inflammation, reduces oxidant radical damage, strengthens immunity, and improves blood supply to the skin.

This manual will delve into the background of the **StemZyme** formulation as well as the science of skin with the DMK concept.

Contents

Foundation Knowledge

Understanding Foundational Knowledge

Skin plays a pivotal role in maintaining homeostasis in the body through processes like thermoregulation, but the skin also has a built-in homeostatic mechanism for its own structural and functional integrity. In this sense, skin is indeed a highly advanced, functional bio-interface. It exists between our internal and external universes, operating through mechanical, thermal, biochemical, and electromagnetic modes. The skin is a complex heterogeneous adaptive structure that varies according to body location, quality of health, diet, age, lifestyle, environmental conditions (e.g., temperature, humidity, pollution level, water quality, sun exposure, contact with external surfaces), and internal environmental conditions (e.g., hormones, pregnancy, metabolic and osmotic balances).

Homeostasis

The pathway to skin homeostasis is through various positive and negative physiological feedback loops. A physiological feedback loop typically involves an input, a control centre, an output, and an intermediary process.

For example, take UVA Exposure as the skin's input. Melanocytes become the control centre the input passes through. The output is higher melanin production and its transfer to keratinocytes (to negate photodamage). The intermediary process is the biochemical and epigenetic route through which melanin is synthesised.

Positive Feedback

In a positive feedback loop, the system's output stimulates the intermediary process, increasing the output until the system is in balance again. For example, if UV damage continues, melanocytes will keep synthesising melanin and transferring it to keratinocytes until they are deemed safe from photogenic damage.

Negative Feedback

Most biological systems maintain homeostasis through negative feedback loops. In a negative feedback loop, the output is decreased in proportion to the input until the system stabilises. For example, when UV damage stops becoming an input, melanocytes are told to stop producing melanin by keratinocyte epigenetic and biochemical signalling.

The Primary Line Of Defence

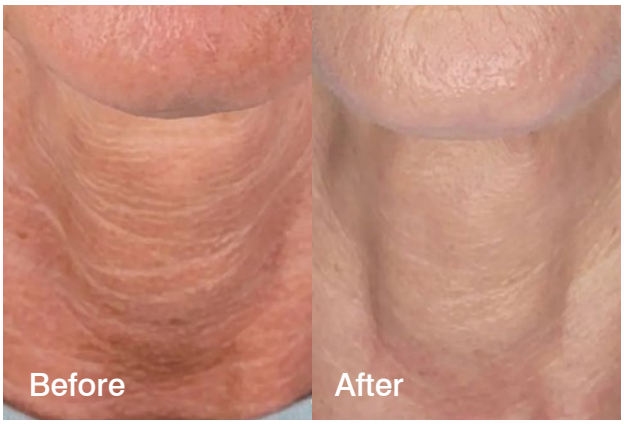
The epidermis is a terminally differentiated stratified squamous epithelium about 200 μm (micrometres/ microns) thick. It is avascular. 95% of the cells in the epidermis are keratinocytes that undergo mitosis at the 0.5–1 m thick epidermal basement membrane. Cells subsequently migrate towards the surface from this basement membrane and form four main, well-delineated layers during their transit, namely the stratum basale (also called the stratum germinativum), the stratum spinosum, the stratum granulosum, and the stratum corneum. The latter consists of a one- to three-cell layer of dead keratinocytes, representing a thickness of 15–30 m. The epidermis includes other cells such as melanocytes, Langerhans cells, and Merkel cells. The complex formed by the living epidermis (excluding the stratum corneum) is called the living or viable epidermis.

The stratum corneum is the primary line of defence against external threats such as mechanical, thermal, chemical, electromagnetic, or biological attacks. In particular, the mechanical properties of the stratum corneum are fundamental in conditioning the transmission of loads and subsequent deformations of the other underlying skin layers across several spatial scales. These pure mechanical aspects are essential for specific biophysical processes, such as the stimulation of mechano-receptors that transduce mechanical energy into neural signalling (e.g., tactile perception and pain) or mechanobiological transduction involved in metabolic processes (e.g., homoeostatic regulation of the skin barrier function).

Any variation in the mechanical properties of the stratum corneum (like those occurring daily because of fluctuations in internal and external environmental conditions of relative humidity and temperature) is likely to affect the material mechanical response and the subsequent altered external surface topography. This has obvious consequences for the skin's tribological (the study of skin friction) response.

The Dermoepidermal Junction

The living epidermis is connected to the underlying dermis through a three-dimensional interlocking wavy interface called the dermoepidermal junction (DEJ), which is the basal lamina explained earlier. Papillae are the protrusions of the papillary dermis into the epidermis. These finger-like structures increase the contact surface area between the reticular dermis and the living epidermis and are thus believed to favour biochemical mass exchanges between these layers (e.g., the transport of oxygen or nutrients.) The DEJ controls the transit of biomolecules between the dermis and epidermis according to their dimension and charge. It allows the passage of migrating and invading cells under normal (e.g., melanocytes and Langerhans cells) or pathological (e.g., lymphocytes and tumour cells) conditions. The DEJ influences the behaviour of keratinocytes via modulation of cell polarity, proliferation, migration, and differentiation.



The Dermis & Papillary Layers

The dermal matrix is composed of an extracellular matrix; a gel-like amorphous phase mainly constituted of proteoglycans, glycoproteins (e.g., fibronectin), blood, and lymph-derived fluids involved in the transport of substances crucial to cellular and metabolic activities. Proteoglycans are composed of multiple glycosaminoglycans (i.e., mucopolysaccharides) interlaced with backbone proteins. Dermal fibroblasts produce glucosamine which is rich in hyaluronic acid and therefore plays an essential role in moisture retention.

An essential aspect of skin mechanics is that In vivo, the skin is in a state of complex in-plane heterogeneous tension patterns that depend on phenotype, age, body location, and position. Principally constituted of a dense array of stiff collagen fibers, the dermis becomes the main load-bearing structural component of the skin when subjected to tension-inducing loads (i.e., in-plane tension, out-of-plane indentation, and suction). The dermis is 15–40 times as thick as the epidermis, varying depending on location; for example, it is much thinner on the eyelids than on the back.

The dermis can be segmented into three main layers: the papillary layer juxtaposed to the epidermis, the sub-papillary layer beneath, and the reticular layer connected to the underlying subcutaneous tissue. The papillary layer is defined by the rete ridges protruding into the epidermis and contains thin collagen fibres, a rich network of blood capillaries, sensory nerve endings, and cytoplasm. The sub-papillary layer, the zone below the epidermis and papillary layer, features similar structural and biological components to the papillary layer. The sub-papillary layer is composed of 80% types I and III collagen (15% of the total collagen content), while the reticular layer is innervated, vascularised, and contains elastic fibres (e.g., elastin). The dermal matrix is made of cells in the interstitial space. Cells present in the reticular dermis include fibroblasts, plasma cells, macrophages, and mast cells.

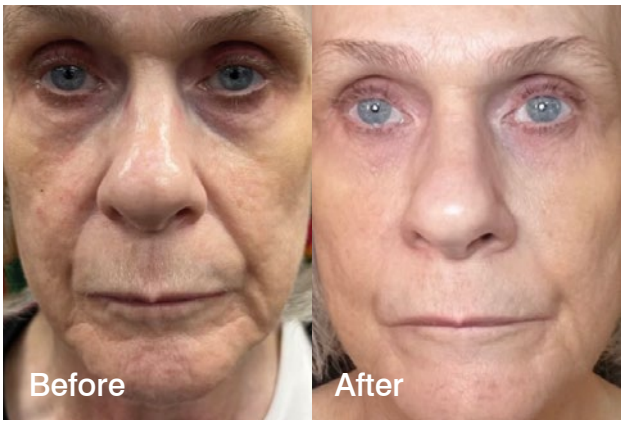
Collagen Fibres

Collagen fibres account for approximately 70% of the weight of dry dermis. Collagen fibres in the papillary and sub-papillary dermis are thin (because of their low aggregate content of fibrils) and sparsely distributed, while reticular fibres are thick, organised in bundles, and densely distributed. Fibrils are typically very long, 100–500 nm in diameter, featuring a cross striation pattern with a 60–70 nm spatial periodicity (a pattern repeating itself in equal distances). The diameter of thick collagen bundles can span 2–15 μm.

Unlike collagen fibres, elastin fibres are highly flexible and can fully recover from strains of more than 100%. Their diameter ranges between 1 and 3 μm. Their mechanical entanglement with the dermis collagen network gives the skin its resilience and recoil ability. This is evidenced by the correlation between the degradation of elastin/abnormal collagen synthesis associated with ageing and the apparent stiffening of the dermis. The diameter of elastic fibres in the dermis is inversely proportional to their proximity to the papillary layer, where they tend to align perpendicularly to the DEJ surface.

The Subcutaneous Tissue

The subcutaneous tissue is the layer between the dermis and the fascia, a band of connective tissue (primarily collagen) that attaches, stabilises, encloses, and separates muscles and other internal organs. The thickness of subcutaneous tissue is highly variable intra- and inter-individually. For example, the subcutaneous fat around periorbital areas is extremely thin, but in zygomatic areas, it is thick. This layer is mainly composed of adipocytes. The role of fascia and subcutaneous fat is to provide mechanical cushioning, heat generation, insulation, and a nutrient reserve for the body and developing cells.



Relationships Among Different Skin Cells

In Homeostasis

Keratinocytes/Langerhans Cells

When Langerhans cells (LCs) “sense” danger in the form of microbial components, they participate in rapid innate antimicrobial responses with the epidermal keratinocytes (KCs). More critically, they initiate the power and specificity of the T-cell components’ adaptive response. LC function can be profoundly modified by cytokine signals from the structural cells of the epidermis, such as keratinocytes, resulting in the alteration of the type of adaptive immune responses induced. For example, cytokines like thymic stromal lymphopoietin (TSLP), released by keratinocytes in atopic dermatitis, alter the LC’s ability to induce adaptive immune responses. Homeostatic cytokines, such as transforming growth factor beta (TGF-), inhibit LC maturation in situ. This is critical for LC retention in the epidermis.

Keratinocytes/Merkel Cells

Merkel cells (MCs) in human skin have been described as post-mitotic because dividing Merkel cells have never been detected. It was suggested that Merkel cells in human skin might originate from a population of undifferentiated keratinocytes or common progenitor cells. Merkel cells are long-lived and rarely produced in adult skin under homeostatic conditions. However, touch dome keratinocytes (TDKC) induce Merkel cell differentiation to increase their numbers upon mild skin injury.

Keratinocytes/Merkel Cells/Fibroblasts

Merkel cells form complexes with sensory nerve afferents (signals that go towards the central nervous system). They possess typical neuroendocrine features, including dense core granules and the expression of chromogranin A, chromogranin B, and synaptophysin. More importantly, they synthesise an array of neuroactive polypeptides/hormones such as vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), neuroendocrine protein B2, preproorexin and orexin receptors, serotonin, and somatostatin. These signal ligands strongly suggest some form of paracrine or autocrine signalling within the skin. MCs directly contact touch dome keratinocytes, and VIP is known to enhance keratinocyte proliferation; thus, MCs may be endocrine regulators of the surrounding epidermis. MCs may also affect dermal fibroblasts by producing Substance P, a peptide that can activate fibroblast proliferation. However, the neuropeptides produced by MCs have diverse functions in other systems, and their exact function and targets in the skin remain to be seen. They likely influence the local fibroblasts, keratinocytes, immune cells, partner neurons, and vasculature. This would mimic the diverse functions of neuroendocrine cells in other organs. Intriguingly, neuropeptide release from MCs can be calcium-independent, suggesting it may be a separate process from the Ca2+-dependent neurotransmitter release involved in mechano- sensation.

Keratinocytes/Merkel Cells/ Langerhans Cells

The finding of robust colony differentiation 200 (CD200) expression in MC and TDKCs raises a question about these cells' covert role in immunity. CD200 is a transmembrane protein that signals through the CD200 receptor (CD200R) to weaken inflammatory reactions and promote immune tolerance. CD200 is normally expressed on thymocytes, T and B lymphocytes, neurons, and endothelial cells; CD200R is expressed on cells of the monocyte/ macrophage lineage and T lymphocytes. CD200 is also highly expressed on keratinocytes of the murine hair follicle outer root sheath (ORS). Skin lacking CD200 is highly susceptible to hair follicle-associated inflammation and immune-mediated alopecia. CD200 expression in the TDKC is likely to represent a second immune-privileged compartment in the skin.

MCs are also implicated as a part of the immune system by their relationship with Langerhans cells, the dendritic antigen-presenting cells that reside in the epidermis. MCs are known to interact with LCs in the skin. Hair follicles normally function as portals for LCs to enter the epidermis, but it is unknown how LCs enter glabrous skin. The dense clusters of MCs in acral skin may allow LCs to enter the hairless epidermis. At the same time, MCs produce CGRP, a neuropeptide that can inhibit LCs' antigen presentation.

MCs may also be involved in modulating inflammatory responses in the skin. Normal MCs produce met-enkephalin (INN), enhancing immune responses at low doses and suppressing responses at high doses.

In addition, MCs can react to histamine or activation of the transient receptor potential cation channel subfamily V member 4 gene (TRPV4). In response MCs release VIP, which can decrease the production of pro-inflammatory cytokines.

In hyperproliferative conditions like psoriasis, MC numbers are higher in lesioned skin than in normal skin. Moreover, MC levels of neuropeptide expression (such as somatostatin, thought to be involved in skin immunology) differ between psoriatic lesions and controls. The MC hyperplasia observed in psoriasis implies that MCs respond to and possibly regulate pathological skin inflammation. Occasionally, leukocytes are observed closely abutting dendritic Merkel cells (DMCs), suggesting another potential functional linkage with the immune system. TDKCs also potentially contribute to regulating epidermal immunity by expression of Keratin 17 (K17), which can function to polarise the cutaneous inflammatory response. The features of MCs and TDKCs predicate their potential involvement in cutaneous immunity.

Keratinocytes/Melanocytes

Keratinocytes, the predominant cell type in the epidermis, are also implicated in regulating skin colour. After UVB irradiation, keratinocytes can secrete cytokines that stimulate facultative (discretionary) melanin synthesis. Cytokines that enhance melanogenesis in melanocytes include basic fibroblast growth factor (bFGF), endothelin-1 (ET-1), alpha-melanocyte-stimulating hormone (□-MSH), proopiomelanocortin (POMC), and stem cell factor (SCF). Keratinocytes from dark skin (e.g., of African descent) contain melanosomes

distributed individually throughout their cytoplasm. Keratinocytes from light skin (e.g., of Caucasian descent) contain melanosomes primarily clustered into aggregates. Keratinocytes in the skin of Asian descent contain a melanosome distribution pattern between these two conditions.

The differential distribution pattern of melanosomes in the epidermis between racial groups appears to be regulated by the keratinocyte. The phagocytotic activity of keratinocytes is also associated with skin colour differential. Protease-activated receptor-2 (PAR-2) is a seven-transmembrane G-coupled receptor known to regulate phagocytosis in keratinocytes. Darker skin exhibits a higher expression of PAR-2 compared to lighter skin; inhibitors of PAR-2 lighten skin effectively. These observations suggest that the PAR-2-mediated transfer of melanosomes may regulate skin colour.

Melanocytes/Langerhans Cells

In epidermal cells, melanocytes and Langerhans cells are morphologically classified as dendritic cells. As antigen-presenting cells (APCs), Langerhans and dermal dendritic cells have powerful antigen-presenting abilities. APCs take up foreign antigens in cells by endocytosis and phagocytosis. APCs then express a glycoprotein called mast histocompatibility class II (MHC class II) and present the antigen to T-cells by forming a complex with foreign antigen peptides.

Although melanocytes are not considered APCs in their primary function, melanocytes express MHC class II through Interferon (IFN) stimulation. Le Poole et al. observed that melanocytes also have the ability of phagocytosis and demonstrated that melanocytes could function as target cells for T-cells by processing and presenting the phagocytosed antigen. Melanocytes express intercellular adhesion factor (ICAM-1), which is responsible for the cell-to-cell interactions of the immune system, and colony differentiation 40 (CD40), which is mainly observed in mature dendritic cells and adaptive immune systems. Melanocytes could also produce cytokines that modulate immune response like APCs, such as Interleukin (IL)-1 alpha, IL-1 beta, IL-8, and transforming growth factor (TGF). The relationship between the toll-like receptor (TLR)-mediated immune response and melanogenesis is the most studied feature of TLR4. TLR4 recognises lipopolysaccharide (LPS) derived from Gram-negative bacteria. TLR4 is also implicated in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and skin sclerosis. In melanocytes, the expression of TLR4 and its adapter molecules colony differentiation 14 (CD14) and Myeloid Differentiation 88 (MyD 88) has been confirmed in cultured human melanocytes. The TLR4 agonist lipopolysaccharide (LPS) increases the expression of TLR4, CD14, and MyD88, and induces pigmentation accompanied by inflammatory cytokine induction. TLR4 activation by LPS induces the expression of tyrosinase and its transcription factor Melanocyte Inducing Transcription Factor (MITF) in melanocytes via the activation of p38 MAPK (mitogen-activated protein kinase).

In human neonatal melanocytes, repeated UV irradiation increases TLR4 expression and pigmentation. LPS stimulation with repeated UV irradiation induces IL-6 secretion from human neonatal melanocytes. This suggests that melanocytes participate in UV-induced immune modulation. Indeed, TLR4 causes a reaction like UV irradiation in Langerhans cells, suggesting

that TLR4 is involved in the recognition of bacteria and an immune regulation similar to UV-induced cutaneous immune regulation.

Candidiasis is an infectious disease that accounts for over half of all systemic mycoses. TLR4 can recognise Candida albicans infection and shows protective effects against candida infection. In human melanocytes, Candida albicans induce pigment production via TLR4. Melanocytes use TLR4 to reduce the infectivity of Candida albicans. Darker melanocytes have higher levels of TLR4 expression and different levels of inflammatory response compared to lighter melanocytes; this contrast suggests that melanocyte pigment levels influence the magnitude of the inflammatory response in human skin. These examples indicate that pigmentation and its process by TLR4 stimulation play roles in immune defences and inflammatory responses.

Fibroblasts/Melanocytes

Dermal fibroblasts are the structural framework for tissues, helping to bring thickness and firmness to the skin. They are responsible for synthesising, remodelling, and depositing collagen and the extracellular matrix. Fibroblasts act on melanocytes directly and indirectly through neighbouring cells by secreting many cytokines. These cytokines include:

- Stem Cell Factor (SCF)
- Dickkopf-Related Protein (DKK1)
- Secreted Frizzled Related Protein (sFRP)
- Semaphorin (Sema7a)
- Cellular Communication Network Factor (CCN)
- Fibroblast Activation Protein alpha (FAP-)
- Keratinocyte Growth Factor (KGF)
- Hepatocyte Growth Factor (HGF)
- Fibroblast Growth Factor (bFGF)
- Neurotrophin (NT-3)
- Neuregulin (NRG-1)
- Transforming Growth Factor (TGF-)

These factors bind to receptors and modulate intracellular signalling cascades (MAPK/ERK, cAMP/PKA, Wnt/-catenin, PI3K/Akt) related to melanocyte functions. It causes the expression of melanin-producing enzymes and melanosome transfer. These factors influence the growth, pigmentation, dendritic mobility, and adhesive properties of melanocytes. Thus, fibroblasts are implicated in both physiological and pathological skin pigmentation. In addition, UV exposure, a significant factor in pigmentary disorders, may affect the secretory crosstalk between dermal and epithelial cells. Four components strongly affect this process:

1. Transforming Growth Factor-b (TGF-b)
2. Platelet-Derived Growth Factor (PDGF)
3. Interleukin-1 (IL-1)
4. Keratinocyte Growth Factor (KGF)

In addition, the proliferation and migration of keratinocytes drive re-epithelialisation.

The proliferation and migration of fibroblasts are crucial to successful dermal wound healing, as they replace lost collagen. Fibroblasts have been observed to migrate increasingly in chemotactic response to PDGF and Transforming Growth Factor. Migration depends

on an appropriate proximate substrate, such as the newly formed collagen-based matrix. It is stimulated by the presence of mature collagen and fibronectin. Proliferation is upregulated by KGF and downregulated by TGF-b. Keratinocytes have a complex effect on the behaviour of fibroblasts: while they secrete IL-1, which upregulates fibroblast proliferation, they also physically control the growth of fibroblasts by forming colonies. In addition, interleukin-1 can downregulate collagen production, and TGF-b can upregulate this process.

Mast Cells/Keratinocytes/Melanocytes/ Langerhans Cells

Mast Cells (MCs) are strategically positioned beneath the epidermal barrier and attached to the endothelial barrier. They are situated to translate potential pathogenic and nonpathogenic danger signals into systemic signals and recruit other immune effector cells to counteract them. This communication axis depends on histamine, cytokines, chemokines, growth factors, and proteases. Keratinocytes play an essential role in mast cell maturation since they also produce stem cell factors. On the other hand, mast cells have both inhibitory and activating effects on KCs. For example, they can express keratinocyte growth factor and platelet-activating factor, which activate KCs; MCs release histamine, heparin, and other MCs mediators, which inhibit KC proliferation and control epidermal regeneration. Tryptase and chymase, which are produced by MCs, promote fibroblast (FB) proliferation while inhibiting KC proliferation. Additionally, Sehra et al. have proven that MCs can regulate epidermal differentiation complex (EDC) genes, suggesting MCs have a protective role in regulating epidermal barrier integrity.

Angiogenesis is an essential process for normal skin development, homeostasis, and remodelling. Skin mast cells can spontaneously secrete several angiogenesis-related factors and exhibit an intrinsic role in vascular development. MC-derived tryptase additionally promotes angiogenesis by degrading the basement membrane. Among all vasoactive mediators that MCs release, MCs impact blood endothelial cells (BECs) via:

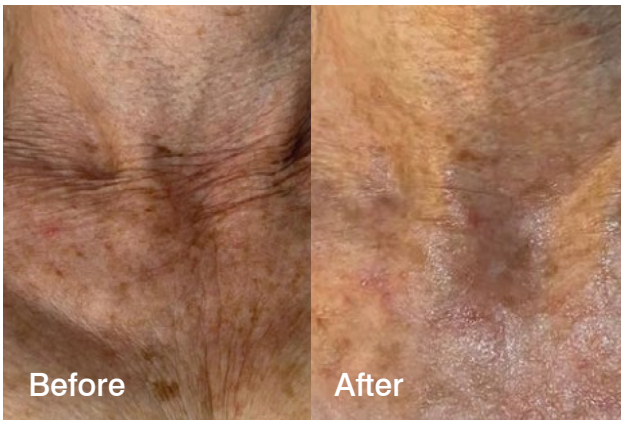
- Histamine
- Tumor Necrosis Factor (TNF)
- Leukotrienes
- Prostaglandin D2 (PGD2)
- Platelet Activating Factor (PAF)
- Vascular Endothelial Factor (VEGF)-A and VEGF-B
- Interleukin 1 (IL-1) and IL-13

MCs impact Lymphatic Endothelial Cells (LECs) via histamine, vascular endothelial growth factor (VEGF)-C, and VEGF-D. A bidirectional mode also characterises these interactions. MCs are not only a source but a target of angiogenic and lymphangiogenic factors. VEGF-A expressed by Endothelial Cells (ECs) can regulate MC proliferation and maturation within the skin. IL-33/ST2 and ATP/P2X7 signalling lead to the activation and degranulation of MCs.

While histamine drives vasodilation and vascular permeability, MC-derived TNF primes and recruits' neutrophils. MC-derived TNF and MC granules promote dendritic cell maturation and migration to the skin-draining lymph node (LN), priming T-cells. T-helper type 1 (TH1) and cytotoxic T-cells (TC1) amplify

inflammation. Upon re-exposure MCs initiate vascular responses, neutrophil recruitment, and nerve fiber elongation. Interferon-gamma (IFN) released by type 1 CD8 (TC1) cells activates KCs, which produce T-cell recruiting chemokines, interferon-induced protein 10 (IP-10), and monokine-induced gamma Interferon (MIG). In turn, inflammation is enhanced in a feedback loop by the MCs. Cytokine production is counter-regulated by regulatory T-cells (Treg) via the release of Transforming Growth Factor beta. MCs induce fibroblast proliferation via IL-4, IL-13, VEGF, and basic fibroblast growth factor (bFGF). Moreover, bidirectional communication is necessary for maintaining skin barrier homeostasis. Fibroblast expression and secretion of SCF, the MC Growth Factor, promotes MC differentiation and controls MC activation. Moreover, fibroblasts inhibit MC activation by secreting the enzyme Cyp26b1, which locally downregulates P2X7 expression on skin MCs. This mechanism is a unique skin barrier homeostatic network inhibiting ATP-dependent MC activation. Additionally, MCs continuously secrete TNF, IL-1, IL-4, fibroblast growth factor (bFGF/FGF-2), TGF 1, and VEGF. These mediators also influence fibroblast functions.

MCs can indirectly affect adaptive immunity by modulating dendritic cell functions. MCs and DCs reside near environmental interfaces, allowing for intense intercellular communication. This communication can be based on soluble MC mediators, such as Histamine and TNF, or on the uptake of intact MC granules by DCs, promoting DC migration, DC maturation, and T-cell priming capacity. Moreover, direct MC–DC interactions, including synapse formation, modulate DC functions and fine-tune adaptive immunity. There is a recent finding that MC–DC synapse formation culminates in Mast Histocompatibility Complex (MHC) class II transfer from DCs to MCs, thereby equipping MCs with antigen-presentation capacities that contribute to effector T-cell activation.



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Cellular Signalling

Long Distance Vs. Short Distance Signalling

Cells interact with their immediate micro-environment and respond to signals originating further away. Signalling pathways are classified according to the source of a signalling molecule or ligand.

Endocrine Signalling

Endocrine signalling is an example of long-distance communication between hormone-producing cells, tissues, glands, and cells that express hormone receptor molecules. Hormones are small molecules or glycoproteins usually secreted into the bloodstream before being distributed throughout the body. Endocrine signals often originate from the brain; however, other tissues, including the thyroid gland, stomach, pancreas, liver, kidneys, and reproductive organs, also produce hormones (e.g., Sebaceous glands reciprocating to Testosterone, Estrogen, Cortisol, etc.).

Paracrine Signalling

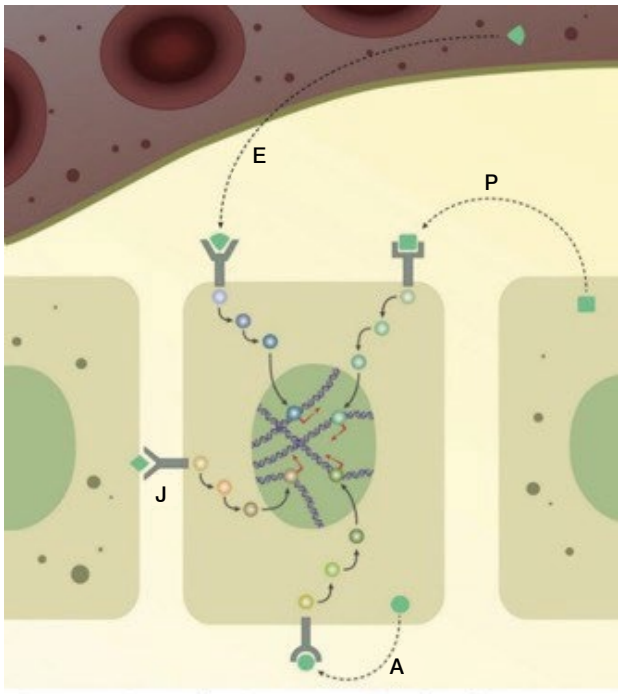
Paracrine signalling occurs between cells near each other. A soluble signalling molecule secreted by one cell diffuses to another cell in the local neighbourhood. For instance, neurotransmitters secreted by neurons diffuse a few nanometers before binding to receptors on target neurons or muscle cells; macrophages produce and transmit cytokines to keratinocytes.

Juxtacrine Signalling

Juxtacrine signalling occurs between neighbouring cells that are in physical contact with each other. In this mode, the signalling molecule is bound to the cell membrane. It may then interact with a receptor on the membrane of an adjacent cell. One example of juxtacrine signalling in the epidermis is the interaction between the Notch receptor and its ligand in keratinocyte proliferation.

Autocrine Signalling

In autocrine signalling, the signalling molecule originates from the target cell itself. This occurs when cells express receptors to a ligand they secrete. For example, in keratinocytes, cognate ligands activate the epidermal growth factor receptor, which mediates most of the autonomous replicative capacity of these cells and is necessary to inhibit differentiation and apoptosis.



A – autocrine mechanism
E – endocrine mechanism
P – paracrine mechanism
J – juxtacrine mechanism

– ligand
– receptor
– gene transcription

Cellular Communication Pathways

Cells talk to each other through small molecules such as amines, peptides, and nucleotides. The molecules either disperse across the cell membrane to bind to the correct target within the cell, or (more commonly) interact with a receptor on the cell's surface. This interaction triggers changes in cell activity. The pathway between targets and receptors is known as signal transduction. Proteins, molecules, and ions make up this pathway; some pathways share the same protein and affect each other's activities. Quick cellular communication responses occur around protein activity (contracting muscles, metabolism). Slow responses require the synthesis of new proteins.

Electrical Signalling

A high-speed method of cellular signalling communication that involves the flow of charged ions (mainly Na+, K+, Ca2+, and Cl-) across a membrane, in a process known as action potential propagation. Cells that mediate electrical signalling are interwoven with low resistance pores such as gap junctions, which allow the passage of ions and other small-sized solutes between cells. These cells often exist in networks, where the electrical signal of one cell is quickly propagated to neighbouring cells. For example, in epidermal wounds, endogenous electrical fields and currents arise spontaneously, and keratinocytes migrate towards the wound site in the direction of these electrical fields.

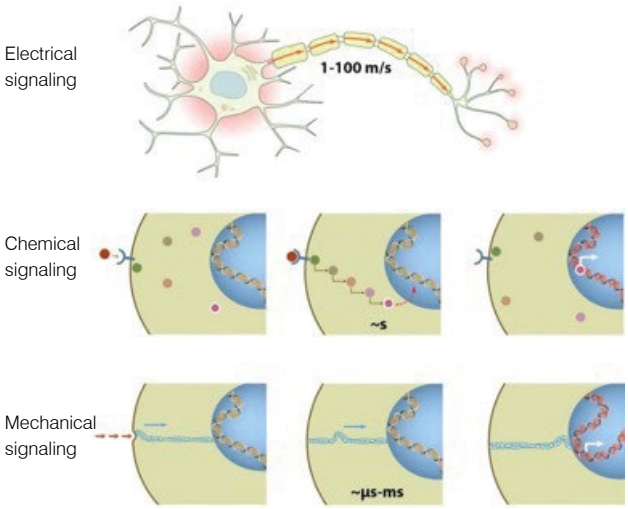
Chemical Signalling

Involves the binding of a ligand or signalling molecule to a receptor located at or near the cell plasma membrane. Chemical signalling involves inducing signalling cascades, where one protein activates another protein, which subsequently activates another, and so on. This method of intracellular signal transduction involves many biomolecules from lipids and proteins. For instance, desmosomes and associated intermediate filaments (Ifs) are critical regulators of mechanical polarisation in the epidermis through the chemical signalling of cadherin desmoglein.

Mechanical Signalling

Mechanical signalling refers to a process where a signal is triggered by a mechanical force such as shear stress or a pull/push applied to biomolecules. Usually, applying such force would induce a conformational change in the protein/receptor, thus exposing functional domains to the environment. Such stretching can trigger many other signalling mechanisms in the cell, leading to cytoskeletal reorganisation and modulations in cellular and nuclear shape and gene expression. Mechano-signalling can often trigger cellular signalling processes much faster than a purely chemical means of activation. For instance, classical cadherins form actin-linked adherens junctions critical for cell-to-cell attachment, force generation, and mechanotransduction. Mechanical environmental cues ultimately alter gene expression programs to regulate cell fate in keratinocytes. Transcriptional regulation is mediated via mechanosensitive transcription factors, notably actin-regulated transcription coregulators MAL–Serum Response Factor (SRF) and Yes Associated Protein / Tafazzin Transcyclase Activity (YAP/TAZ). YAP/TAZ transcriptional activity is dependent on dephosphorylation and subsequent nuclear translocation. YAP phosphorylation is regulated,

among others, by Proto-Oncogene (Src) and Large Tumor Suppressor (LATS) kinases. Its regulation occurs in response to changes in monolayer density – sensed by adherens junctions and ECM stiffness through integrins – to specify stem cell fate under differential mechanical tensions.



Receptors

Receptors are proteins that interact with specific ligands and transmit the resulting signals to the cell's interior. These proteins are often transmembrane, with an extracellular domain for ligand binding and an intracellular domain chemically linked to a downstream signaling pathway. The binding of a ligand to the extracellular region of a receptor often initiates a conformational change within the cytosolic domain, which initiates a series of biochemical reactions known as signal cascades. These cascades pass the signal from one molecule to another before a cellular response is achieved.

Receptors are separated into three broad categories:

G-protein Coupled Receptors (GPCRs)

GPCRs are linked to a monomeric or trimeric G-protein via their cytosolic domain. Activation of GPCRs results from GDP to GTP exchange within a linked G-protein. It subsequently activates a range of kinases that facilitate phosphorylation events on target proteins.

Ion Channels

Ion channel activation also occurs through interactions with a ligand, inducing a conformational change within the protein. In this case, however, the protein will acquire an open conformation that permits the ions to flow into the cell. Ion channels usually transport a specific type of ion.

Na+, K+, Ca2+, and Cl- are the most common ions associated with 'information flow' or signal transduction.

Enzyme-Linked Receptors

Intracellular receptors, such as steroid receptors, are also relevant to signal transduction. These receptors are activated by hydrophobic ligands that can pass through the lipid membrane and enter the cell passively.

Intracellular Messengers

Intracellular or secondary messengers are the intermediate proteins (or small molecules) that carry signals from the receptor to intracellular sensors and effectors. Multiple intracellular messenger molecules are activated for every receptor activated; the signal is amplified at this stage. Examples of intracellular messengers include calcium ions, enzymes such as adenylyl cyclase, and lipids such as inositol triphosphate.

Sensors And Effectors

Sensor and effector proteins are responsible for the cell's response to a signal. They promote exocytosis, endocytosis, migration, actin remodelling, and gene expression processes.

Off Mechanisms

Cells do not respond to any one signal continuously. Instead, "molecular switches" control many signal pathways that can turn off a given response before it becomes detrimental to the required cell state or function. For example, receptors may be desensitised to the ligand, as is commonly seen in ion channels. In addition, intracellular signalling molecules can also degrade. For instance, $Ca2+$ ions are pumped back into calcium stores by pumps, restoring their normal concentrations in the cytosol. These mechanisms ensure that a cellular response is controlled by inhibiting the flow of information and stalling the response, even after detecting a given signal.

Glucose Conjugates

Glycolipids

These are essentially carbohydrate polymers that determine cell shape and offer protection from exterior pathogens, hypotonic conditions, and high internal osmotic pressures, preventing swelling and bursting of the cells. Structural analysis revealed that epidermal lipids containing linoleic acid include acylglucosylceramides. They comprise long-chain ω-hydroxy fatty acids as the amide-linked fatty acids and linoleic acids as the esterified fatty acid linked through the ω-hydroxyl group.

The human epidermis consists of two acylglucosylceramide groups, one containing sphinganine and the other containing phytosphingosine with one double bond. These are termed functional lipids or “Epidermosides.” □-Hydroxylation is the rate-limiting biosynthetic step for expressing these functional lipids, and investigating the hydroxylase active in this process is an important target. In the epidermis of terrestrial vertebrates, lipid lamellae between the horny cells are thought to form a barrier to water loss. Living cells extrude the lipids after assembly in lamellar granules. This assembly might be promoted by recently identified 1-(3′-O-acyl)-β-D-glucosyl-N-(ω-hydroxyacyl) sphingosines, which have 30- and 32-carbon hydroxy acids as amides and linoleic acid esterified to glucose. Such a role for these molecules could explain the effects of essential fatty acid deficiency, in which the lamellar granules fail to assemble, and the barrier to water diffusion is lost.

Glycoproteins

Many proteins, especially those destined for secretion or insertion into membranes, are post-translationally modified by the attachment of carbohydrates. Carbohydrate modifications on the protein appear to be involved in recognition of other binding molecules, prevention of aggregation during protein folding, protection from proteolysis, and increased half-life of the proteins. In contrast to a protein sequence determined by a DNA template, sugars attach to proteins by enzymes that recognise appropriate sites on proteins and attach the sugars. Since many sugars contain many functional groups that can serve as potential attachment sites, the oligosaccharides attached to proteins are enormously varied, complex, and hence "information rich" compared to linear or folded polymers like DNA and proteins.

Proteoglycans

Some proteins are so modified with Chinese Hamster Ovary Cells (CHOs) that they contain more CHOs than amino acids. Proteins linked to glycosaminoglycans are together called Proteoglycans (PGs). PGs can be soluble and are found in the extracellular matrix or as integral membrane proteins.

Phospholipids

Phospholipids are not present in the Stratum Corneum (SC) but are distributed throughout the viable epidermis and dermis. Phospholipids are amphiphilic molecules with polar head groups derived from phosphoric acid and long hydrocarbon chains, which impart hydrophobicity to the molecule. They are the main subclass of lipids in viable keratinocytes. Phospholipids are sub-classified into two

main groups: phosphoglycerides derived from glycerol and sphingolipids, which have a dihydroxyamine (sphingosine) backbone. Phosphoglycerides (glycerophospholipids) have a general structure consisting of two fatty acids linked via ester bonds to two of the hydroxyl groups of glycerol, while the third hydroxy group is esterified with a phosphate side chain. Phospholipids are (relatively) homogenously distributed across the viable epidermis. Eleven phospholipid subtypes have been detected in whole skin tissue, including:

- Phosphoglycerides Phosphatidic Acid (PA)
- Phosphatidyl Ethanolamine (PE)
- Cardiolipin (CL)
- Phosphatidylserine (PS)
- Lysophosphatidylcholine (LPC)
- Phosphatidylinositol (PI)
- Alkylacylglycerophosphocholine (AAPC)
- Ethanolamine plasmalogen (Eplas)
- Phosphatidylcholine (PC)
- Sphingolipids dihydrosphingomyelin (DHSM)
- Sphingomyelin (SM)

PC was the most abundant phosphoglyceride in both epidermis and dermis, followed by the sphingolipid SM. PE intensity increases in deeper layers of the epidermis, consistent with their presence in viable cell membranes.

An Integrated Science & Technology Approach

Piezoelectricity Of The Skin

Piezoelectricity is a linear electromechanical coupling phenomenon. This means that when mechanical stress is applied to a piezoelectric material, it deforms and generates an electrical charge. When certain biological materials are subjected to mechanical stress, it creates an electrical polarisation in them, wherein the crystalline faces of biological materials become electrically charged.

The process of converting mechanical stress into a bio-electrical charge is known as the direct effect. In contrast, the converse effect occurs when an external electrical charge is applied across a piezoelectric material, causing the material to deform mechanically. For example, the epidermis of live human skin has a permanent electric dipole moment perpendicular to its surface. As a result, human skin presents piezoelectric responses, meaning it generates voltage from mechanical stress, and pyroelectric responses generate a voltage when rapidly heated or cooled.

The study of mechanoelectrical conversion properties and their effects on dry human skin is investigated in the dermis, epidermis, and horny layers. Shear stress (perpendicular mechanical force) piezoelectricity exists in the epidermis, dermis, and hypodermis. Piezoelectric properties of the dermis can be ascribed to its collagen-rich structural network. In contrast, the piezoelectric properties of the epidermis appear to originate from partially oriented α-helical tonofibrils (the keratin-like supportive framework structures in epithelial cells which line body surfaces). The highest piezoelectric coefficients are found in the stratum corneum. Although thermally generated currents are observed during thermal pulse experiments, no evidence of relevant contributions from pyroelectric (polar) responses has been noticed in all cutaneous compartments. Due to this bioelectric potential, it can also generate subtle muscle movement in the internal organs such as the esophagus and trachea, joint motion, and arterial pressure by recognising strains on human skin. Piezoelectric properties also exist in the skin's elastin, collagen in tendons, collagen in ligaments, actin, and myosin in skeletal muscles, and even some individual amino acids and DNA molecules.

Each collagen molecule is a strong dipole with two oppositely charged ends. The head is larger with a slightly larger positive charge, while the tail is smaller with a slightly smaller negative charge. Thus, the overall charge of each collagen molecule is positive. Collagen molecules unite to form different anatomical structures (tendons, ligaments, bones, structural frame of the inner organs, etc.). In combination with other electrically active proteins, all collagen molecules generate the fixed electric charge of each organ and tissue.

All electrically active molecules in the tissue cause a cumulative, fixed bioelectric charge in the skin. This charge constantly changes under normal conditions due to an individual's physical activity, diet, stress level, etc. However, despite these constant fluctuations, the changes in fixed electric charge stay within the physiological range assigned to skin tissue by the charged molecules.

The situation changes dramatically if the soft tissue or inner organs are traumatised or develop inflammation. Any soft tissue trauma increases the positive fixed electric charge, especially the swelling, rupture, and twisting of collagen fibres. Therefore, the healing process after initial trauma always includes the slow restoration of the standard electric charge in the affected area.

During DMK Enzyme Therapy, external mechanical stimuli (the repeated application strokes of Enzyme Masque) deform damaged collagen molecules and generate piezoelectricity, increasing the negatively fixed electric charge. A negative electric charge significantly impacts the tissue's proliferation, growth, and regeneration, so it is vital to maintain a negative electrical charge across the skin. In addition, the increased negative charge in the affected area is a critical factor in the correct alignment of procollagen proteins before their maturation into fully developed collagen fibers. Delaying this process slows the

local healing and regeneration of tissues. Thus, restoring the fixed electric charge is critically important in reducing tension in the soft tissue and eliminating physical congestion and neuronal stress in the skin. It is equally important in speeding up the healing process and age management of the cells and tissues.

Anisotropic Properties Of The Skin

The skin is a continuous heterogenous-type structure in terms of its anatomical and spatial scale. It is defined as a membrane and multi-layer shell structure because it possesses a non-negligible bending stiffness (resistance to deformation) apart from sustaining membrane strains.

The epidermis is the layer that protects the body from dehydration and both pathogenic and nonpathogenic assault. It consists of a cohesive stratified structure resulting from the differentiation of keratinocytes from the basal layer to the stratum corneum.

The hypodermis primarily consists of loose connective tissue and lobules of fat. The dermis comprises a network of collagen and elastin fibres, blood vessels, and lymphatic channels immersed in an interstitial fluid composed of proteoglycans, ions, and water. Elastin fibres are responsible for skin elasticity. Collagen fibres, the most abundant protein in the human body, act on skin resistance. This layer is indicative of the skin's primary mechanical behaviour.

The dermis is the main contributor to the tensile mechanical properties of the skin, owing to its high collagen fibre content and significant thickness compared with the stratum corneum and viable epidermis. The intricate architecture of the dermis and its close mechanical connectivity to collagen and elastin fibres lead to anisotropic and nonlinear macroscopic mechanical properties, consistent with the strain stiffening effect (increased stiffness with deformation) typically observed in biological soft tissues under tension.

A constant interplay between heterogeneity and anisotropy is required to regulate the function of biological materials. Heterogeneity relates to biomaterial properties' spatial variation (point to point). On the other hand, anisotropy is directly related to the directional dependence of a given biomaterial, meaning its physical properties are different when measured in different directions.

It is important to note that the measured mechanical anisotropy of the skin is not only due to the structural characteristics of the skin layers and their microstructural constituents. It is also the result of its mechanical interplay with the Langer lines (a map of skin tension across the body). In-plane anisotropy of the skin is correlated with the distribution and orientation of Langer lines while out-of-plane (or across-the-thickness) anisotropy is due to the distinct mechanical properties and complex 3D structure of the skin's layers. It has also been

determined that there is a relation between the relaxed skin tension lines on a human face and the directional dependency of skin stiffness. Various methods have been used to measure the mechanical properties of skin: e.g., uniaxial, biaxial, tensile, and multi-axial tests, application of torsion loads, indentation, suction, and bulge testing. Given the soft tissue's complex hierarchical structure, the skin exhibits a wide range of viscoelastic phenomena, including creep, relaxation, hysteresis, and strain-rate dependency.

DMK Enzyme Therapy conserves the skin's heterogeneity, both point-to-point and across its length. It also protects the spatial orientation of the skin and its directional dependence. This is particularly important in DMK Body Enzyme Treatments because the human body is constantly under mechanical stress in different directions concerning the earth's rotation and magnetic fields.

Pleiotropy In The Skin

Ageing describes the decline of an organism's ability to maintain tissue homeostasis over time. At the molecular level, ageing leads to accumulated cellular damage caused by various agents, such as reactive oxygen, nutritional stress, and spontaneous errors in DNA processing. However, even the most basic unicellular organisms possess DNA repair machinery and antioxidant systems to protect from such damage. Pleiotropy is the random expression of a single gene that generates two or more distinct phenotypic traits; determined by complex physiological evolution from the unicellular state. Pleiotropic outputs emerge through the recombination and permutation of cell-to-cell signalling exercised during reproduction, based on both past and present physical and physiologic conditions in service to the future needs of the organism for its continued survival. Functional homologies ranging from the lung to the kidney, skin, brain, thyroid, and pituitary exemplify the mechanistic evolutionary strategy of pleiotropy.

Pleiotropy occurs deterministically rather than by chance, based on specific physiologic principles, revealing the true nature of evolution. We can deduce that pleiotropy has fostered evolution through iterative interactions between the first principles of physiology and the ever-changing environment. Thus, we understand senescence as the loss of cellular communication due to the natural decline in bioenergy resulting from selection pressure for optimal reproductive success earlier in the organism's life cycle.

Suppose molecular homology is applied between the skin's stratum corneum and alveolar surfactant. In that case, the lipid barrier function of the stratum corneum is much like the alveolar surfactant, which forms tubular myelin as a membrane barrier in both cases. The epithelium secretes lamellar bodies composed of lipid-protein complexed with antimicrobial peptides; that means genes expressed in the skin to form the lipid barrier are the same in the alveolar tissues, but their phenotype presentations are different. The skin and brain are structurally, functionally, phylogenetically, and pathophysiologically homologous. For example, the skin and brain share common lipodystrophies in certain neurodegenerative diseases like Gaucher's. In this case, the excessive myelination of axons in the brain causes tandem skin lipid lesions associated with brain neuronal pathology.

In the epidermis, Notch signalling controls the epidermal cell proliferation, differentiation, migration, and apoptosis by only interacting with other cellular pathways. Any disruption of Notch signalling, whether due to direct mutation or aberrant regulation of genes involved in the signalling route, might lead to hyper- or hypo-activation of signalling molecules and target genes, ultimately inducing the onset of skin diseases. There are multiple poorly understood mechanisms through which Notch signalling contributes to the pathogenesis of skin diseases. So far, Notch signalling alterations have been implicated in the pathogenesis of five human skin diseases: Hidradenitis Suppurativa, Dowling Degos Disease, Adams-Oliver Syndrome, Psoriasis, and Atopic Dermatitis. A single Notch signalling pathway yields five different phenotypic diseases through various expressions.

Applied Biochemistry

Collagen Fibers

Collagen fibres comprise most of the dermis and form a highly organised three-dimensional scaffold surrounding cells. There are various water-binding macromolecules, such as glycosaminoglycans, fibronectin, tenascin, fibronectin, epimorphin, and others. Collagen fibres have a uniform orientation and provide passive tension, which causes internal skin tension along Langer's lines. Elastin fibres connect collagen bundles and perform the function of adaptation to deformation (returning collagen fibres to their original state after the termination of the load).

In addition to its mechanical function, collagen plays a crucial role in regulating cell migration and differentiation. Collagen interacts with cell surface proteins through receptors recognising amino acid sequences on the collagen molecule. A signalling function activates as proteins of the cell surface bind to it. Moreover, specific proteins can bind to collagen and integrins, promoting cell adhesion and proliferation. When collagen fibres disintegrate, peptide regulatory factors are released to affect further collagen regeneration.

Types Of Collagen

We have identified 29 types of collagen in humans and other vertebrates, encoded by at least 45 different genes. The composition of collagen fibres varies in different organs depending on the functions of the corresponding organ. For example, the skin, which bears mechanical stress, is dominated by fibrillar collagens, including a large amount of type I collagen and a small number of types III and V.

Collagen types I, III, and V belong to the group of fibrillar collagens. Collagen type I is distributed in many tissues, such as the skin, bone tissue, the cornea and sclera of the eye, and the walls of blood vessels. Collagen type I, in addition to its mechanical function, has a signalling function and is involved in the organisation of the extracellular matrix. In turn, this affects the organisation of the epidermis and dermis. Cells can directly bind to collagen monomers through integrins α1β1, α2β1, α10β1, and α11β1. Furthermore, this complex is involved in cell signalling, cell adhesion, cell migration, and remodelling of the collagen matrix.

Collagen III is the primary collagen type in foetuses, while less of it exists in adult skin. It is present in the reticular organs and the walls of blood vessels and is often found in fibres with type I collagen fibrils. Collagen type III is most important in hollow organs. However, it also

interacts with platelets during blood coagulation (through specific glycoproteins and non-integrin receptors) and plays a vital role as a signalling molecule in tissue regeneration. Collagen III participates in cellular adhesion, migration, proliferation, and differentiation through interaction with receptors on the cell surface, including integrins. Throughout a person's life, the ratio of collagen types I and III shifts towards an increase in collagen I.

Collagen type V plays a valuable regulatory role in soft tissues, the placenta, blood vessels, and chorion. Compiling type I collagen fibre without collagen type V is impossible. Collagen type V is in the region of the N-terminal domain on the fibril surface. It is believed to determine the site of the beginning of fibril assembly in vivo.

Collagen types IV and VII and laminin form the basis of the epidermal basement membrane, providing anchoring sites (anchoring of endothelial cells and keratinocytes) and performing barrier functions in the epidermis. Collagen type IV belongs to the network-forming collagens in the basement membrane and lens capsule. It is part of the collagens that form filament beads in soft tissue and cartilage microfibrils.

Collagen type VII belongs to the group of collagens that form anchor fibrils located in the dermo-epidermal junction and is responsible for the strength of this junction. Collagen type XIV belongs to fibril-associated collagens and is typical for various soft tissues; it interacts with the surface of fibrils, regulating fibrillogenesis. Collagen type XVII belongs to transmembrane collagens on the surface of epidermal cells. It is a component of hemidesmosomes (multi-protein complexes located on the basement membrane), which mediate the attachment of keratinocytes to the underlying membrane.

Collagen Synthesis and Degradation

Many signalling molecules and proteins influence the synthesis and assembly of collagen fibre. Most important are:

- N-propeptides of collagen type I
- Fibronectin
- Lysyl oxidase
- Tenascin-X
- Thrombospondin
- Matrilins
- Perlecan
- Decorin
- Biglycan
- Fibromodulin
- Lumican

For example, a mutation of the gene encoding tenascin-X leads to the development of Ehlers-Danlos syndrome. In this syndrome, collagen fibrils are of unusual size and shape, with a lower packing density; as a result, the total collagen content in the skin is reduced by 30%. In addition, collagen and N-propeptides inhibit further procollagen synthesis through negative feedback regulation.

Fibronectin is one of the most common extracellular matrix glycoproteins, which plays a vital role in development, cell growth, differentiation, adhesion, and cell migration through integrin-mediated signalling. For the fibrillation of collagen type I, the presence of collagen type V is required because of its role as a central nucleus during the formation of collagen type I fibres.

Transforming growth factor β1 (TGF-β1) also plays a role in regulating collagen gene expression. It binds to the extracellular matrix through binding to latent TGF-β binding protein 1, which is associated with fibronectin-1 and fibrillin microfibrils. However, it should be noted that TGFβ1 stimulates myofibroblast differentiation, resulting in pathological fibrosis (scarring) during tissue regeneration. Additionally, the mechanical tension (stiffness) of tissues that supports profibrotic activation affects myofibroblasts. Various cytokines may be involved in suppressing TGFβ1 activity, including interferon-gamma (IFNγ), interleukin-1 (IL-1), and basic fibroblast growth factors (bFGF, FGF-2). As a result of their actions, collagen deposition decreases, and apoptosis occurs. Hypoxia can lead to a decrease in the level of mRNA and collagen type III protein in chondrocytes and, on the contrary, to their increase in the lungs, leading to alveolar fibrosis. In response to hypoxia, trauma, or metabolic stress, ATP and ADP form adenosine, and purine. They are released into the skin. In fibroblasts, adenosine activates the expression of the COL3A1 gene through its receptors. Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) also increase the expression of COL3A1 mRNA and protein in human skin fibroblasts through Mitogen-Activated Protein Kinase (MAPK) signalling.

The physiological process of renewal of collagen fibres takes 40 to 60 days on average. Collagen degradation occurs in two stages: in the first stage, collagen fibres and fibrils are fragmented; in the second, phagocytosis caused by macrophages and fibroblasts occurs, with subsequent cleavage of fragments in the lysosomes to amino acids and peptide sequences.

Elastic energy storage is consistent with the stretching of charged pairs located in flexible regions of the collagen molecule. Shear thinning, or thixotropy of skin is hypothesised to reflect the breakage of bonds between collagen fibrils. Collagen and elastin are complex macromolecules, hybrids of flexible and rigid regions. The flexible areas reversibly store elastic energy during stretching by breakage of secondary bonds. After stretching, the flexible spaces become extended and transfer stress to the rigid regions of these molecules. This protects collagen and elastin fibres from premature mechanical failure.

Reactive oxygen species (ROS) arising from oxidative cell metabolism play a significant role in both processes. ROS affects extrinsic and intrinsic skin ageing and induces the transcription factor c-Jun via mitogen-activated protein kinases (MAPK). This leads to an overexpression of matrix metalloproteinase (MMP)-1, MMP-3, and MMP-9 and prevents procollagen-1 expression. Therefore, elevated levels of degraded collagen and reduced collagen synthesis are pathologies in intrinsically aged and photoaged skin.

The Extracellular Matrix

The extracellular matrix (ECM) is the general term for the large proteins and polysaccharides secreted by cells in a multicellular organism. It acts as connective material to hold cells in a defined space. Cell density can vary significantly between different tissues of an animal: from tightly packed muscle cells with many direct cell-to-cell contacts to liver tissue in which some of the cells are only loosely organised, suspended in a web of extracellular matrix. The ECM's polysaccharides and proteins include collagen, fibronectin, laminin, and proteoglycan, all secreted by cells. The proportions of these components can vary greatly depending on tissue type. Two very different examples of ECM are the basement membrane underlying the epidermis, a thin, almost two-dimensional layer that helps organise the skin cells into a barrier to repel external dangers, and the massive three-dimensional matrix surrounding each chondrocyte in cartilaginous tissue. The ability of knee cartilage to withstand the repeated shock of footsteps is due to proteins in the ECM, not the cells embedded in the matrix, which are few and sparsely distributed. Although both types of ECM share some components, they are distinguishable in function or appearance but the proportions and identity of the constituent molecules.

Hyaluronic Acid

HA is a non-sulphated glycosaminoglycan (GAG) composed of repeating polymeric disaccharides D-glucuronic acid and N-acetyl-D-glucosamine, linked by a glucuronic □ (1□3) bond. In aqueous solutions, HA forms specific stable tertiary structures. HA polymers have many configurations and shapes depending on their size, salt concentration, pH, and associated cations. Unlike other GAGs, HA is not covalently attached to a protein core but may form aggregates with proteoglycans. HA is most abundant in the skin, accounting for 50% of HA in the body. HA also exists in the vitreous of the eye, the umbilical cord, and synovial fluid, as well as all tissues and fluids of the body: skeletal tissues, heart valves, lungs, the aorta, the prostate, tunica albuginea, corpora cavernosa, and the corpus spongiosum of the penis. HA is produced primarily by mesenchymal and other cells.

Functions of HA include hydration, joint lubrication, filler, and the framework through which cells migrate. HA synthesis increases during tissue injury and wound healing as it regulates several aspects of tissue repair, including the activation of inflammatory cells to enhance immune response and the response to the injury of fibroblasts and epithelial cells. HA also provides the framework for blood vessel formation and fibroblast migration that may be involved in tumour progression. Correlation between HA levels on the surface of cancer cells with the aggressiveness of tumours has also been reported.

The size of HA appears to be of critical importance for its various functions described above. HA of large molecular size, usually more than 1,000 kDa, is present in intact tissues and is antiangiogenic and immunosuppressive. In contrast, smaller polymers of HA are distress signals and potent inducers of inflammation and angiogenesis.

Ha Synthesis

HA is synthesised by specific enzymes called HA synthases (HAS). These membrane-bound enzymes synthesise HA on the inner surface of the plasma membrane. Then, HA is extruded through pore-like structures into the extracellular

space. There are three mammalian enzymes, HAS-1, HAS-2, and HAS-3, which exhibit distinct enzymatic properties and synthesise HA chains of various lengths.

HA has a dynamic turnover rate. It has a half-life of 3 – 5 minutes in the blood, less than a day in the skin, and 1 – 3 weeks in cartilage. HA is degraded into fragments of varying size by hyaluronidases (HYAL) by hydrolysing the hexosaminidic (1–4) linkages between N-acetyl-D-glucosamine and D-glucuronic acid residues in HA. Six HYALs have been identified in humans: HYAL-1, -2, -3, -4, PH-20, and HYALP1. Mutations in the HYAL-1 gene are associated with HYAL deficiency and mucopolysaccharidosis type IX. HYAL-2 has very low activity compared to plasma HYAL-1 and specifically hydrolyses HA of high molecular weight, yielding HA fragments of approximately 20 kDa, which are further degraded to small oligosaccharides by PH-20.

HA can also be degraded non-enzymatically by a free-radical mechanism in the presence of reducing agents such as ascorbic acid, thiols, and ferrous, or cuprous ions, a process that requires the presence of molecular oxygen. Thus, agents that could delay the free-radical-catalysed degradation of HA may be useful in maintaining the integrity of dermal HA and its hydrating properties.

Applied Biophysics

Tissue shape emerges from individual cells' collective mechanical properties and behaviour and how they integrate into the surrounding tissue. Tissue architecture and its dynamic changes subsequently provide feedback to guide cell behaviour. The skin is a dynamic, self-renewing barrier, subjected to large-scale extrinsic mechanical forces throughout its lifetime. Withstanding this constant mechanical stress without compromising barrier integrity requires compartment-specific structural specialisation and the capability to sense and adapt to mechanical cues.

Cells generate and sense forces to control their (structural & functional) fates. This is particularly important for adjusting skin surface area to changing body size. The mechanical transition of the monolayer from a fluid- to a solid-like state coincides with skin switching between the lateral expansion of the monolayer and delamination events. In vivo, work further indicates that the basal layer of the actively stratifying embryonic epidermis exists in such a solid-like state. Proliferation in this jammed cell layer causes crowding and the lateral compression of cells, which is sufficient to trigger differentiation. This is consistent with studies showing that individual human epidermal stem cells cultured on micropatterned surfaces undergo differentiation when cell spreading is restricted.

Restriction of cell spreading triggers a reduction in cortical cellular tension, and increased cell-to-cell adhesion subsequently triggers cell delamination from the basal layer, allowing epidermal cells to couple cell fate, mechanics, and position. Consistent with the idea of cell size dictating cell fate, space liberated by delamination triggers the division of a neighbouring cell in the adult epidermis, where cell divisions are rare. Thus, cell shape and size balance the division and differentiation of stem cells in the embryonic and adult epidermis. Stem cells in the embryo constantly cycle to provide sufficient material for the lateral expansion of the epidermis, whereas stem cells only divide on demand to replace delaminating cells during adult homeostasis.

Surface Instability

Expressions (or temporary wrinkles, also called expression lines) are associated with skin and facial movement. They can originate from facial muscular activation (e.g., smiling) or simple mechanical actions on the skin's surface, such as twisting, shear force, or compression. Gravitational folds on the face arise from the combined effect of constant gravitational loads applied to a microstructurally age-altered epidermis, dermis, and hypodermis.

Wrinkling, ubiquitous in nature, is a multi-scale spatial phenomenon spanning over eight orders of magnitude. From a physical point of view, wrinkles result from a complex interplay between material and structural properties, boundary, and loading conditions, the exact nature of which remains obscure. Wrinkles can be induced in a variety of ways, including residual strains in bi-layer structures, differential growth in multi-layer biological structures, and mechanical loads combined with geometric constraints (i.e., inextensibility, geometrical coupling with a thicker foundation) in thin structures (i.e., membranes and shells). Wrinkles may also be induced by rebalancing the material and structural properties in these single or multi-layer systems.

Upon in-plane compression of both layers, the top (thin) layer will favour large wavelength bending deformations due to its higher bending stiffness, while the bottom (soft) layer will penalise these wavelengths and promote smaller wavelengths. The energy-minimising structural response to competition between bending energies causes length scales to emerge in the form of wrinkles. If compression continues after the formation of wrinkles, the latter will evolve into folds.

Bio-Electromagnetism

Physiology interacts with its environment via ambient fields such as light, sound, electricity, magnetism, and all other living organisms to generate massive amounts of information in energy fields. They are based on measurements of magnetic field profiles associated with human brain waves and the heart.

It is hypothesized that picotesla range magnetic fields are physiologic. These measurements were made by superconducting quantum interference detectors or atomic magnetometers. Various basic science experimental studies were accomplished to test the initial hypothesis, including but not limited to nerve regeneration, wound healing, cardiovascular studies, and cancer cell studies. Studies utilising extremely low frequency picotesla range magnetic fields revealed that these non-ionising fields might be physiologic. The determination of specific flux densities was made using a novel particle wave equation mc2=BvLq known as Jacobson Resonance.

Additionally, it is speculated that various biological structures may be piezoelectric, ionising, and non-ionising radiant as the effect of these low-level magnetic energies produced by photon/phonon transductions. Various biological structures (e.g., keratin, collagen, genes, alpha and beta sheaths of proteins) may be piezoelectric. It has been hypothesised that when conformational states of protein and/ or DNA are altered, this information is transmitted to the rest of the DNA through non-linear lossless vibration waves, or solitons, based on biological piezoelectricity. Interatomic communications via electromagnetic forces are at the root of all signal transductive coupling mechanisms. When the binding protein for

telomeres undergoes conformational changes in concert with incomplete lagging strand DNA synthesis (mechanical error), electromagnetic signals are sent to the rest of the DNA and inhibit standard genetic information transfer mechanisms.

The target-specific restoration of physiologic electromagnetic profiles may induce particle jumps, such that transposed immunogenic quantum domains may be normalised, restoring biological order and atomistic/molecular coherent communications and cooperativity. This means that a biological system, defined as reversible, does not change macroscopically as a state function. Instead, it changes slowly on a quantum level in small volumes of tissue space via normal ageing processes.

The skin is in a state of complex in-plane heterogeneous tension patterns that depend on individuals, their age, body location, and position. The behaviour of keratinocytes is influenced by the dermoepidermal junction (DEJ) via modulation of cell polarity, proliferation, migration, differentiation, non-linear behaviour, structural non-homogeneity, and mechanical anisotropy.

Ceramides

Ceramides (CERs) consist of a hydrophobic fatty acid chain linked to an amino containing sphingoid base. The linkage is achieved via anamide bond between the carbonyl group of the carboxylic acid of the fatty acid chain and the amino group of the base. In human SC, there are many ceramide subclasses in varying quantities. The different ceramide subclasses are continually expanding, with even more species identified in the last decade. CERs were originally classified into nine subclasses: [NS], [AS], [EOS], [NP], [AP], [EOP], [NH], [AH] and [EOH]. These CERs contained one of sphingosine [S], phytosphingosine [P], or 6-hydroxysphingosine [H] as the sphingoid base linked to one of non-hydroxy fatty acid [N], alpha-hydroxy fatty acid [A], or esterified omega-hydroxy fatty acid [EO] as the fatty acid chain.

Short-chain ceramides do not maintain the barrier properties of long-chain ceramides, despite having the same polar headgroups and hydrogen bonding ability. The ceramides containing four and six-carbon fatty acid chains proved to have the highest permeability. The short aliphatic chain may not be long enough for effective interactions with other lipid chains, paramount in the lamellar architecture of the SC. Lipid membrane models indicate that the sphingoid base of omega-O-acyl ceramides, with ultra-long carbon chains ≈32, significantly affects membrane architecture and permeability. The many ceramide subclasses and the unique arrangement of potential fatty acid hydrocarbon chains have led to a multitude of heterogeneity between the ceramide molecules found in the SC (>400 ceramide species have been identified); hence, CER composition is challenging to predict.

The ceramide subclasses interact (both intermolecularly and intramolecularly) with other lipids forming the characteristic densely packed three-dimensional structures known as the lipid lamellae. The highly ordered architecture is vital for the skin's structural integrity and barrier function. Lamellar bodies and the multilamellar barrier are mainly comprised of ceramides. Intracellular ceramides act as second messengers for numerous signaling pathways, including apoptosis, cell growth, and differentiation. In addition, the metabolism of ceramides may suppress the apoptotic process.

Therefore, ceramides have important intracellular and intercellular roles in the skin's barrier function.

Free Fatty Acids

Most barrier non-esterified fatty acids (NEFAs) and fatty acids bound to ceramides in the SC are saturated and unbranched, hydrocarbon chains of length between C16−26. The main NEFAs in this study include palmitic acid (C16:0), stearic acid (C18:0), behenic acid (C22:0), lignoceric acid (C24:0), and hexacosanoic (cerotic) acid (C26:0). The terminal carboxylic acid functional group (COOH) contributes to the acidity of the skin. Lignoceric acid (C24:0) and hexacosanoic acid (C26:0) make up 50% of the net weight of the NEFA content in SC. The shorter-chain fatty acids, such as palmitic acid (C16:0) and stearic acid (C18:0), are located closer to the external surface. Monounsaturated NEFA such as oleic acid (C18:1, n-9) and polyunsaturated NEFA such as eicosapentaenoic acid (C20:5, n-3), docosahexaenoic acid (C22:6, n-3) and linoleic acid (C18:2, n-6) are present in smaller quantities (25 % of total NEFA). Several longer-chain fatty acids from C21:0 to C28:0 are also present. The composition of fatty acids in the SC, as with other lipids, varies by anatomical site, with a higher percentage in the face and abdomen compared to leg and plantar human SC. The concentration of NEFA varies throughout the regions of the skin. The amount of epidermal NEFA is decreased in areas with higher densities of sebaceous glands.

Cholesterol

Cholesterol and its derivatives are lipophilic steroids with a rigid, four-ring (ABCD) structure. Cholesterol has a free alcoholic group on ring A. It comprises approximately 27% of the SC's lipid content and is essential for the fluidity and rigidity of the SC. The sterol contributes to the correct lamellar and lateral structure. Cholesterol esters contribute 10% to the SC lipid content. Cholesterol sulphate is a sulphated derivate of cholesterol. It is a minor component, comprising 2–5% of the total lipids in the SC. Cholesterol sulphate is generated in the viable epidermis by cholesterol sulfotransferase. However, it is also desulphated in the viable epidermis by steroid sulfatase, creating a 'cholesterol sulphate cycle.' The function of the sulphate ester is not fully understood; however, it is believed to be involved in the SC cohesion and regulation of desquamation. The level of cholesterol sulphate decreases in desquamated material and changes in recessive conditions like X-linked ichthyosis.

Sebaceous Gland Lipids

In addition to the three main lipid classes (ceramides, fatty acids, and cholesterol), other lipids known as skin surface lipids are present on the SC. These are either epidermal or sebaceous in origin, with the composition varying depending on anatomical location. According to the lipidomic analysis of the skin, supplementary lipids are supplied to the SC via sebum. Sebum is a lipid-rich substance containing squalene, triacylglycerols (TAGs), and wax esters produced in the sebaceous glands. Several of these lipids, notably squalene and wax esters, are only in sebum at present and not at any other anatomical location. Sebum also delivers glycerol and vitamin E to the SC, which hydrates the skin's surface. It is unevenly

distributed across the skin, with the highest concentrations at the forehead, upper chest, and dorsum. The density of sebaceous glands varies by body site, which influences the SC lipidome. For example, sebaceous NEFA increased in areas with a high density of sebaceous glands, while epidermal NEFA decreased. Sebum protects the skin by acting as a barrier against bacteria and other xenobiotics, and there is emerging evidence for an immunomodulatory role. Sebum composition undergoes significant changes in many skin diseases, including acne, atopic and seborrheic dermatitis.

The predominant lipid subclass in sebum is the Triacylglycerols (TAGs), and most wax esters contain a fatty acid C16:1 acyl group. In addition, the polyunsaturated hydrocarbon squalene is an essential component of sebum (10–15%) and 50−125 mg (variation due to age, gender, and ethnicity) of sebum present on the face daily. Squalene is formed through the condensation of two molecules of farnesyl pyrophosphate, catalysed by squalene synthase.

NEFAs make up a significant proportion of sebum. NEFAs in sebum have a chain length ranging between 12 and 30 carbons with a degree of unsaturation between 0 and 6 double bonds. NEFAs are essential for the skin's structural integrity and serve as endogenous antiseptics. When secreted in sebum from the sebaceous glands, NEFAs have 'self-sterilising' properties on the skin's surface. Sapienic acid, an isomer of palmitoleic acid, is a unique sebaceous monounsaturated fatty acid found only in sebum, comprising over 20% of the total fatty acid content.

Skin As An Endocrine And Exocrine Hormone Organ

Several hormones have targeted effects on the skin. For example, hair follicles and sebaceous glands are the targets for androgen steroids secreted by the gonads and the adrenal cortex. In addition, hormones play an essential role in the development and physiological function of human skin tissues; hormones are also produced in the skin. For example, the circulating androgens dehydroepiandrosterone (DHEA) and androstenedione are converted in the skin through different pathways to testosterone, androstenedione, or potent androgen 5α-dihydrotestosterone (5α-DHT). Thus, the skin is not only the recipient of signals from distant transmitters but is also an endocrine organ. Hormones exhibit pleiotropic biologic effects on the skin and act through paracrine, autocrine, intracrine, and endocrine mechanisms. growth factor-1, neuropeptides, sexual steroids, glucocorticoids, retinoids, vitamin D, peroxisome proliferator-activated receptor ligands, and eicosanoids are all growth hormone/insulin-like examples of active hormones on the skin.

The skin produces hormones released in the circulatory system that are important for the functions of the entire human organism. Significant examples include sex steroids, where a large proportion of androgens and estrogens in men and women are synthesised locally in peripheral target tissues from the inactive adrenal precursors DHEA and androstenedione. DHEA and androstenedione are converted to testosterone or 5α-DHT by different pathways. This conversion occurs with the involvement of the three isotypes of the intracellular enzyme 5α-reductase and the isotypes of 17β-hydroxysteroid dehydrogenase, thus

making the skin responsible for considerable amounts of circulating 5α-DHT levels.

The best estimate of the intracrine formation of estrogens in peripheral tissues in women is 75% before menopause and close to 100% after menopause, excepting a small contribution from ovarian and/or adrenal testosterone and androstenedione. In postmenopausal women, an intracrine mechanism makes almost all active sex steroids in target tissues. Moreover, there is more insulin-like growth factor (IGF)-binding protein-3 message in the skin than in the liver and circulating IGF-binding protein-3 concentrations increase by growth hormone and IGF-1. GH directly regulates IGF-binding protein-3 synthesis, and the response of skin IGF-binding protein-3 mRNA levels to both GH and IGF-I suggests that dermal fibroblasts could be more important than the liver in the regulation of circulating reservoir IGF-binding protein-3 in certain circumstances.

The skin produces hormones released in the circulation that are important for functions of the entire human organism, such as vitamin D. The skin is the unique site of cholecalciferol synthesis derived from cholesterol. Epidermal keratinocytes contain the machinery needed to produce calcitriol and vitamin D receptors. In addition to its capacity to produce hormones, the human skin can metabolise hormones to activate and inactivate them. These steps are overtaken in most cases by different skin cell populations in a coordinated way indicating the endocrine autonomy of the skin. Characteristic examples of this kind of endocrine skin function are the metabolic pathways of the corticotrophin-releasing hormone/ proopiomelanocortin axis, sex steroids, vitamin D, and retinoids.

Epigenetics & Molecular Biology – The Skin's Perspective

The accumulation of senescent cells over time probably reduces tissue regeneration and contributes to skin ageing. Keratinocytes and dermal fibroblasts undergo senescence in response to several intrinsic or extrinsic stresses, including telomere shortening, overproduction of reactive oxygen species, unhealthy diet, and excessive sunlight exposure. Epigenetic mechanisms directly regulate skin homeostasis and regeneration but also mark cell senescence and the natural and pathological ageing processes.

Various signaling and transcriptional pathways regulate the expression of genes implicated in epidermal SC homeostasis, proliferation, differentiation, and ageing in a stage-specific manner. The critical requirement for maintaining the survival of the skin SC population throughout life is the repression of p16INK4a. This explains the tight regulation in skin cells of p16INK4a encoding gene Cyclin-Dependent Kinase Inhibitor Kinase (CDKN2A), which belongs to the INK4a/ARF locus. In contrast, p63 is a master regulator of epidermal morphogenesis. It acts as a transcription factor implicated in maintaining keratinocyte self-renewal and/ or cell fate decisions. The Tumor Protein (TP) 63 gene encodes several isoforms of p63 due to alternative promoters, different translation initiation sites, and alternative splicing events.

In the human epidermis, DNp63 is the predominant isoform and plays a crucial role in keratinocyte proliferation and differentiation process through an aMyc-regulated gene network and the interaction with several other transcription factors such as Activator Protein (AP-1), Krueppel Like Factor (Klf4), Aryl Hydrocarbon Receptor Nuclear Translocator

(Arnt), and Peroxisome Proliferator-Activated Receptor Alpha (PPAR-alpha). Specifically, DNp63 and the protein encoded by its transcriptional target (Regulated in development and DNA damage responses 1) (REDD1) gene are essential for progenitor cells' proliferative capacity and differentiation. Furthermore, DNp63 promotes keratinocyte proliferation by suppressing the expression of senescence-inducing miRNAs. Thus, the regulation of p63 expression is fundamental to skin regeneration.

Transcription factor-dependent and epigenetic regulatory mechanisms tightly collaborate to ensure proper epidermal homeostasis. Indeed, several epigenetic networks preserve keratinocyte stemness and promote proliferation by repressing the transcription of the p16INK4a-encoding gene, other cell-cycle inhibitors, and the unscheduled activation of genes associated with non-lineage- or terminal differentiation. Unbalancing opposite epigenetic enzymatic activities drives the transition from epidermal SC quiescence to activation. On the contrary, specific epigenetic networks may promote terminal keratinocyte differentiation by acting through p63-regulated networks on Epidermal Differentiation Complex (EDC) genes. In dermal fibroblasts, the epigenetic networks are involved in the repression of INK4a/ARF locus and inflammatory genes to fight against senescence and paracrine pro-inflammatory processes. Finally, the deregulation of epigenetic pathways directing epidermal homeostasis can induce epigenomic instability and, in turn, skin ageing.

Ageing is characterised by the accumulation of macromolecular damages, impaired tissue renewal, and the progressive loss of physiological integrity. One of the hallmarks of ageing is cellular senescence triggered by several intrinsic (e.g., telomere shortening, ROS overproduction) and extrinsic (e.g., UV radiation, nutrient deprivation, inflammation) stimuli, leading to growth arrest and phenotypic alterations such as chromatin and secretome changes. Cellular senescence prevents the uncontrolled proliferation of damaged cells and induces the clearance and regeneration of the tissue. Skin ages by intrinsic or chronological ageing and extrinsic or photo-ageing, which are superimposed in sun-exposed areas of the body.

Epigenetic regulatory networks consist of three major events: DNA modifications (mainly DNA methylation), histone modifications (mostly histone methylation or acetylation), and recruitment of higher-order chromatin remodelers. DNA and histone modifications affect gene transcription by altering histone–DNA and histone–histone interactions, thus regulating the accessibility of transcription factors and components of the transcriptional machinery to the chromatin. Histone modifications occur mainly at the amino-terminal portion of histone tails and act cooperatively and synergistically to repress or activate transcription.

Phenotype vs. Genotype

What makes one individual's skin so genetically different from another is still a mystery because the answer is nonlinear. The concept of phenotype describes the observable skin attributes of an individual, as opposed to genotype, the inherited material transmitted by ancestral genes. A gene alone cannot cause an observable phenotypic trait or the emergence of observable skin characteristics; genes need a cellular environment, the combined action of multiple other genes, and

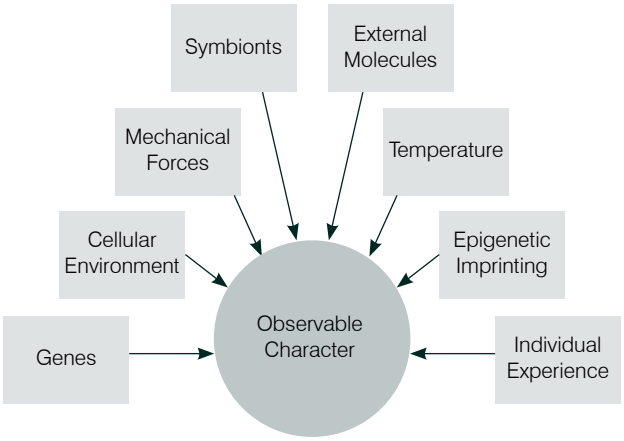
certain physicochemical conditions to have an observable effect on organisms. The relationship between the genetic and the phenotypic level is quite abstract, and can correspond to three distinct differences within the living world:

- The difference in skin phenotypes between two bioidentical individuals, like twins.
- The difference in skin phenotypes within a given ethnic population
- The difference in skin phenotype that first appeared during evolution, between an organism harbouring the ancestral allele/trait and its direct descendant, which evolved the new allele/trait.
- Of note, the variation in phenotype does not always immediately follow the emergence of the new mutation. Instead, it can appear later from the singular assortment of alleles segregating in the population.

Phenotypic differences appear to fall under two major categories: the presence/absence of something in the skin or the shift between two present alternative organoleptic (sense organ) characteristics. Similarly, on the genotype side, a mutation can correspond to the presence/absence of a relevant DNA sequence or nucleotide polymorphism (having different aspects).

Classical Genetic Reductionism

Integrative Approach of Development



The differential perspective makes it evident that the loss of phenotype is not necessarily associated with a loss of genetic material and vice versa. For example, the evolutionary gain of dark pigment in animal coats is often associated with the loss of the Mc1R gene. The gain or loss of a phenotype is also subjective. For example, hair loss might also be considered a gain of the naked epidermis. Similarly, there is a fallacy in the distinction between permissive and instructive signals. A permissive signal is associated with the presence/absence of a phenotype, and an instructive signal indicates the shift between two present alternatives.

Errors during transcription and translation are phenotypic, while errors during DNA replication are genotypic. Most phenotypic errors are introduced during translation when ribosomes translate RNA sequences into amino acid sequences. Therefore, the skin needs continuous protection from phenotypic mutations/errors occurring during protein synthesis. These errors can lead to amino acid substitutions that produce abnormal proteins. Abnormal proteins are difficult to detect and usually degrade within minutes.

A piece of functional protein machinery built from genetic information is central to every live physiological act. Errors in this process (phenotypic mutations) are more frequent than errors during DNA replication (genotypic mutations). However, even flawless genetic information is useless if the cell cannot synthesise functional proteins. Transcription and translation, the two processes involved in decoding DNA, must be sufficiently accurate to allow a cell to build reliable protein machinery. Genes and proteins can decrease their phenotypic mutation rate by using preferred codons or increasing their robustness against amino acid substitutions.

All the cells in the skin work in harmony with each other, forming specialised tissues and organs that respond to environmental factors (e.g., the Stratum Corneum, Granulosum, Sebaceous glands, Basement Membrane, Merkel Cells, etc.). Intercellular mechanisms enable cells to influence the function of their neighbouring cells or modulate specific properties of their surroundings. Like many cellular processes, signalling must be tightly regulated, often by other signalling mechanisms.

DMK Therapy & Products

DMK Macro-Operating Principles of Skin Revision

Remove: Targeted Components For Elimination

Premalignant Cells

Multicellular organisms maintain homeostasis by balancing cell proliferation and cell death. There are two common forms of cell death: apoptosis and necrosis. Apoptosis, often equated with programmed cell death, is a physiological form of cell death that is responsible for the deletion of cells. Apoptosis is morphologically and biochemically characterised by cell shrinkage, dense chromatin condensation, cellular budding, fragmentation, rapid phagocytosis by nearby cells, and DNA fragmentation into units of approximately 200 base pairs. Apoptosis can be triggered by various stimuli such as cytokines, hormones, drugs, and viruses. Their signal transduction can be tightly regulated by genes such as B Cell Lymphoma 2 (Bcl-2). In the skin, apoptosis is also responsible for skin homeostasis, such as keratinocyte differentiation and the hair growth cycle. The most common cause of premalignant cells is damage to the DNA inside skin cells. Damage to DNA can cause changes in various genes that usually control cell growth, prolong cell survival, manage cell division, and prevent unwanted cell death. Other common causes include UV rays, infections, inflammation, genetic conditions, etc. Such premalignant DNA fragments must be cleared from cells to reestablish homeostasis.

Abnormal Cell Infiltrates

Numerous factors are implicated in forming epidermal barriers, such as cornified envelopes, corneocytes, lipids, junctional proteins, proteases, protease inhibitors, antimicrobial peptides, and transcription factors. An impaired epidermal barrier can occur from a fragile cornified envelope, reduced epidermal lipid content, aberrant filaggrin processing, inflammatory dermal cell infiltrates (mainly composed of CD4+ T-cells), and loss of epidermal dendritic T-cells. Abnormal cell infiltrates must be removed from the cellular environment to restore physiological functions in the skin.

Extraneous Pollutants & Particulate Matter

The skin is exposed to ultraviolet radiation (UVR) and environmental air pollutants such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), oxides, particulate matter (PM), ozone (O3), and cigarette smoke. Exposure of the skin to air pollutants has been associated with skin ageing and inflammatory or hyper-reactive skin conditions such as atopic dermatitis, eczema, psoriasis, or acne, with skin cancer among the most severe effects. On the other hand, some air pollutants (i.e., O3, nitrogen dioxide, and sulphur dioxide) and scattering particulates (clouds and soot) in the troposphere reduce the effects of shorter wavelength UVR and cause damage to the skin, especially in polluted urban areas. Extraneous particles must be removed from the skin's exterior without disturbing microbiome diversity.

Rebuild: Components Of Restoration

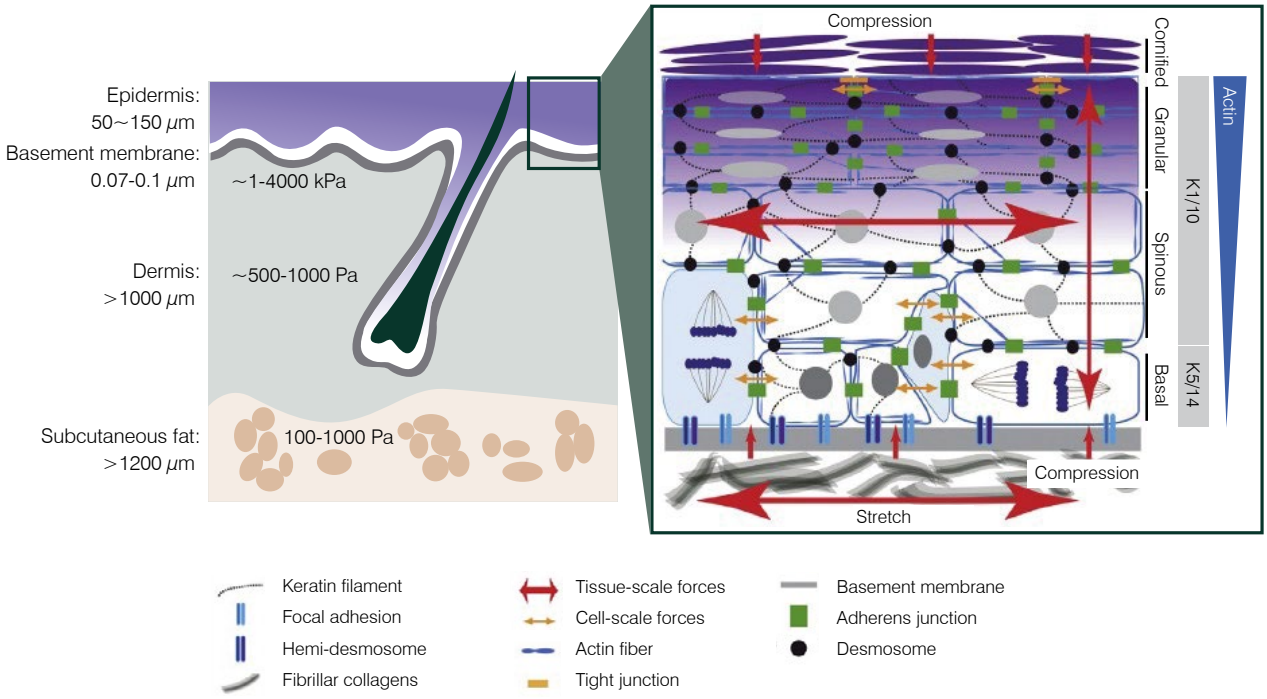
Structural Integrity

The most important biophysical parameters to consider in building structural integrity are the skin's pH, epidermal hydration, transepidermal water loss, the size of corneocytes, the composition of superficial lipids, and sebum excretion.

Epidermal keratinocytes preserve the tensile strength of the tissue and bear variable loads while simultaneously executing dynamic, homeostatic turnover and maintaining a tight, bidirectional barrier. Keratinocytes are tethered to each other via cell-to-cell adhesions and to the BM via cell-matrix adhesions in the basal layer. These adhesions are linked to dense cytoskeletal networks of actin, microtubules, and keratin, which collectively determine the mechanical properties of the cell. Forces within the epidermis transmit within and across layers.

Of the cytoskeletal networks, keratin intermediate filaments exhibit the greatest ability to withstand mechanical load and strain; they are critical for tissue integrity. In contrast, the viscoelastic actin network is critical for force generation and propagation. Microtubules are organised in a centrosomal array in the basal layer but become cortically localised upon differentiation. They modulate epidermal integrity by fortifying cell-to-cell adhesion strength, recruiting myosin to apply tension, and mechanically linking them to the actin cytoskeleton. Classical cadherins form actin-linked adherens junctions, critical for cell-to-cell attachment, force generation, and mechanotransduction. Adherens junctions also coordinate the assembly of the other intercellular junctions, namely desmosomes that associate with keratin intermediate filaments. They provide tight adhesion, junctions, and mechanical resistance to provide additional intercellular cohesion and prevent water loss. The cytoskeleton and cell-to-cell junctions exhibit layer-specific organisation and composition, reflecting layer-specific functional and mechanical requirements.

In contrast to the epidermis, the dermis consists mainly of the Extracellular Matrix (ECM) and sparsely populated fibroblasts. The collagen-dominated dermal ECM has a basket–weave–like structure that provides mechanical strength. The tensile strength and compressibility of fibre-forming collagens make them critical proteins of the dermal ECM. Although we have identified 28 types of collagens, types I and III comprise the bulk of human skin collagen. Whereas fibre-forming collagens and their cross-links define the rigid mechanical structure of the skin, elastic fibres confer extensibility and recoil to collagen, enabling skin stretching. Additional components, such as proteoglycans and glycoproteins, create an osmotically active, hydrated interstitial space. Matricellular proteins, such as tenascins, organise paracrine signalling without contributing to the bulk mechanical properties.



Mechanical properties and force coupling in the skin. The skin consists of the epidermis, dermis, and subcutaneous fat, with compartment-specific mechanical properties and the basement membrane representing the stiffest structure (left panel). The epidermis (right panel) is a loading element subjected to the large-scale forces including compression and stretch (red arrows), which are transmitted within and across layers (red arrows). The epidermis consists mainly of keratinocytes connected via actin-linked adherens junctions and keratin-linked hemidesmosomes. Tight junctions are formed only in the last viable layer through mechanotransduction by high-tension adherens junctions. Differentiating cells depart from the basal layer through perpendicular cell divisions and delamination. Cell shape changes, divisions, and movement within the layers generate forces between cells (orange arrows), modulating cell fate and position. K, Keratin.

Thus, mechanical stress is primarily dissipated across collagen and elastin fibrils within the dermis. Long-range elastin fibres dictate mechanical behaviour at small stresses and strains and during recoiling. Wavy, linearising collagen networks stiffen the skin to dissipate significant stresses and strains.

The dermis can be divided into the upper papillary dermis, which is more densely populated with fibroblasts and contains thin collagen fibrils and elastin, and the underlying reticular dermis, characterised by thick collagen bundles and fewer cells. Fibroblasts are responsible for depositing and remodelling connective tissue matrix during morphogenesis, injury, fibrosis, and scarring. They are recognised as a heterogeneous pool of cells with specialised functions, gene expression profiles, and contractile properties critical for ECM deposition. The subsets of fibroblasts that deposit ECM can be distinguished based on the transient, early embryonic expression of the protein Engrailed-1. Whereas the Engrailed-negative cells produce a provisional ECM made up of fibronectin, Engrailed-positive cells deposit collagens to form mature ECM with high tensile strength. In addition to serving as force-bearing and -transducing entities, skin cells also actively sense the physical properties of their environment and respond by activating signalling cascades to control their

fate and function. An elegant example of skin mechanoresponsiveness is the establishment of global tissue polarity, where mechanical forces within the skin are sufficient to direct the asymmetric localisation of planar cell polarity components.

Functional Integrity

Transcriptional regulation is mediated via mechanosensitive transcription factors, notably actin-regulated transcription coregulators MAL–Serum Response Factor (SRF) and Yes Associated Protein / Tafazzin Transcylase Activity (YAP/TAZ). YAP/TAZ transcriptional activity is dependent on dephosphorylation and subsequent nuclear translocation. YAP phosphorylation is regulated by Src and Large Tumour Suppressor (LATS) kinases (amongst others) in response to changes in monolayer density, sensed by adherence junctions and ECM stiffness through integrins to specify stem cell fate under differential tension. Activating nuclear YAP to coordinate transcription with its partner Transcriptional Enhancer Factor Domain (TEAD) promotes cell growth and inhibits terminal differentiation in the epidermis. SRF is another mechanosensitive transcription factor that works in concert with its coactivator T-Cell Differentiation Protein (MAL) to control cell fate. SRF is maintained in the cytoplasm by binding to G-actin; when F-actin increases via Rho activity or other signals, SRF translocates into the nucleus to activate transcription. MAL-SRF transcription factors regulate the expression of the cytoskeleton and contractility-related genes required for proper cell division and induction of differentiation in response to a restricted cell matrix area.

The propagation of direct mechanical force into the nucleus modifies chromatin state and transcriptional activity. Rapid, direct mechanical loading of the nucleus induces chromatin stretching and transcription. If mechanical force persists, chromatin condenses, leading to suppressed gene expression. In epidermal stem cells, this type of long-term stress results in reduced nuclear actin content, which attenuates global transcription. This allows polycomb repressive complex 2 to condense chromatin at differentiation gene promoters through trimethylation of histone 3 at lysine 27, preventing keratinocyte differentiation. Keratins have also been observed in the nucleus, regulating the cell cycle and transcription. However, these observations were made in states of inflammation; overall, the role of nuclear keratins in homeostasis remains unclear.

Together, different epidermal cells integrate changes in cell density, shape, and actin dynamics to activate specific signalling pathways. The cells carefully tune transcriptional activity and chromatin states to regulate differentiation. This allows the tissue to balance lateral expansion and stratification and adjust the skin's surface area to the body's changing needs.

(Figures 1 and 2). In the living body, oxidative stress exists in lipid, protein, and DNA radicals. Oxidative stress is defined as an increase in the production of ROS and other oxidants that exceeds the antioxidant capacity.

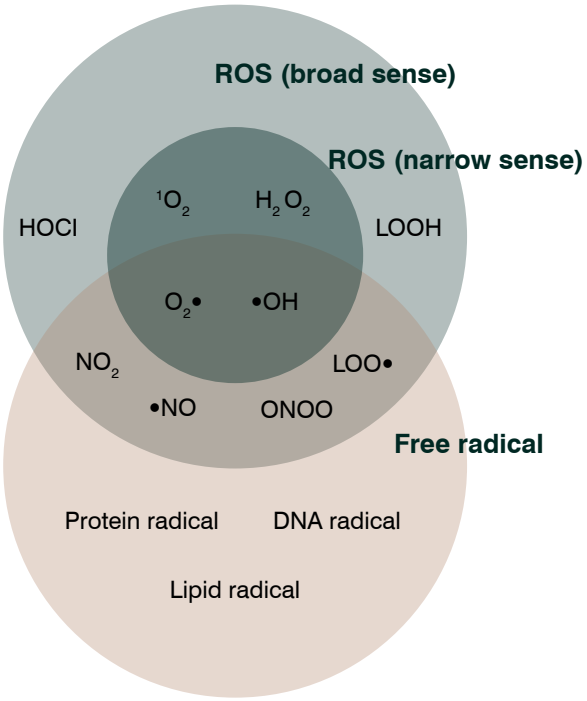


Figure 1

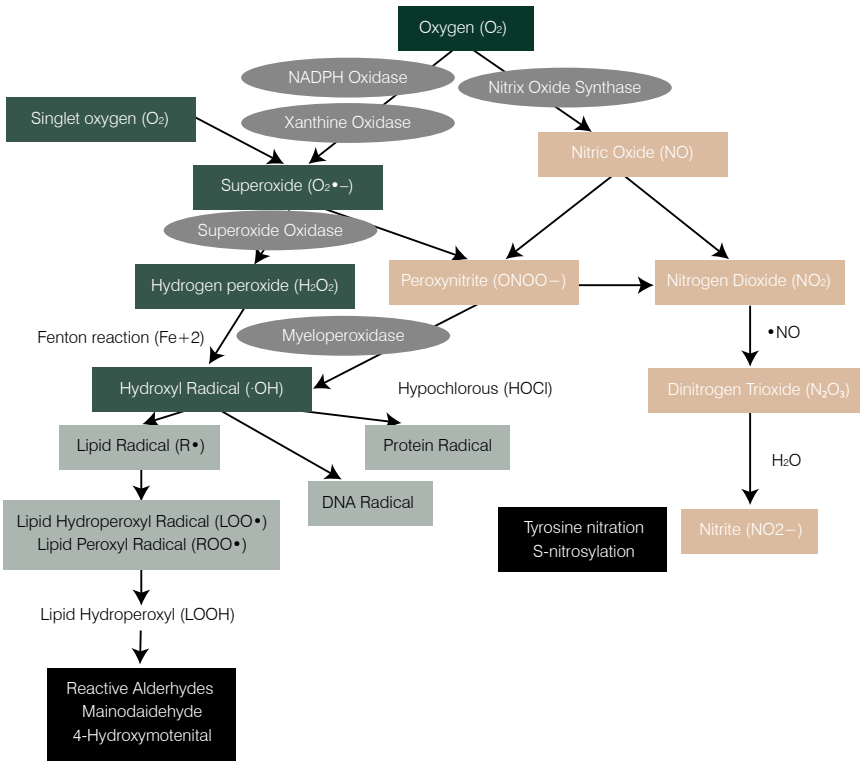


Figure 2

Protect: Potential Skin Detriments

ROS

Oxidative stress plays a significant role in the skin's ageing process for intrinsic and extrinsic ageing. Although the results differ between the dermis and epidermis, extrinsic ageing is largely driven by oxidative stress from UV irradiation. There are six ROS (reactive oxygen species): superoxide (O2), hydrogen peroxide (H2O2), hydroxyl radical (OH), singlet oxygen (1O2), Nitric oxide (NO), and peroxynitrite (ONOO). These ROS are involved in complex and diverse reaction pathways and sometimes form molecules and atoms with unpaired electrons called free radicals. Other sources of ROS include the mitochondrial Electron Transport Chain (ETC), peroxisomal and ER-localised proteins, the Fenton reaction, and enzymes like cyclooxygenases, lipoxygenases, xanthine oxidases, and NADPH oxidases.

RNI

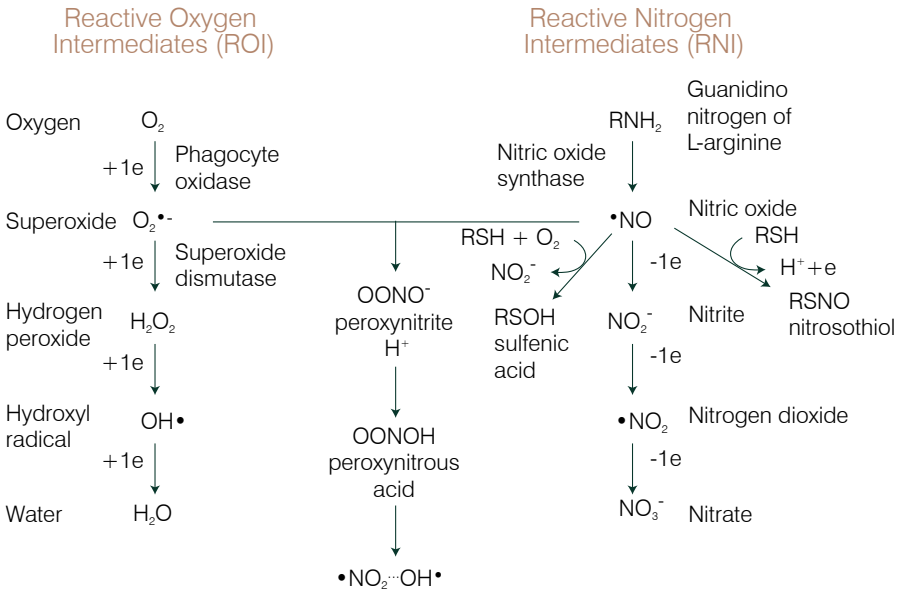
RNI refers to reactive nitrogen intermediates or oxidation states and adducts of the nitrogenous products of nitric oxide synthases. These range from nitric oxide (NO) to nitrate (NO3−), which arise in physiological environments and include NO−, NO2, NO2−, N2O3, N2O4, S-nitrosothiols, peroxynitrite (OONO−), and dinitrosyl-iron complexes.

Hydrophobic RONS (i.e., NO, NO2, O2, O3, and N2O4) can translocate more easily across the SC lipid bilayer than hydrophilic RONS (i.e., H2O2, OH, HO2, HNO2, and HNO3) and ions (i.e., NO2− and NO3−) which experience much higher permeation barriers.

Reactive oxygen (ROS) and nitrogen species (RNI) are dangerous in living organisms due to their destructive effect on biomolecules. However, present studies demonstrate another critical activity of ROS and RNS: their signaling functions in physiological and pathological processes. ROS and RNIs play significant roles in many enzymatic/gene cascades, causing adverse changes during the development of skin diseases and pathological disorders (e.g., skin cancer, the toxic effects of irradiation on the skin, and skin wounding).

UVR

Molecules or regions of molecules that absorb UVR are referred to as UV chromophores. Biological systems are rich in UV chromophores, including DNA and some amino acid residues. In DNA, the nucleotides thymine and cytosine absorb UVB to become electronically excited. In proteins, the amino acid residues tyrosine (Tyr), tryptophan (Trp), and cystine (double-bonded cysteine) absorb UVR from sunlight, reaching an absorbance peak at 280 nm for Tyr and Trp and lower for cystine. Tyr and Trp have a benzene ring structure that facilitates an electronic transition from the ground state to the singlet excitation state that requires photons in the UVB region (180–270 nm). The excited chromophores can transfer their energy or donate an electron to O2, forming several reactive oxygen species (ROS). The excess energy can cleave intermolecular bonds, such as disulphide bonds, or facilitate the formation of pyrimidine dimers (molecular lesions) in DNA during ionising & non-ionising radiation.



The photodynamic production of unstable ROS indirectly mediates UVR damage in biological organisms. UVR exposure generates ROS via the reaction between excited UV chromophores and molecular oxygen (O2). In brief, the excited UV chromophore reacts with O2 to produce either superoxide anion radical (O2) through electron transfer or singlet oxygen (1O2) through energy transfer. Superoxide dismutases, which are present in the cell and the ECM, convert O2 into hydrogen peroxide (H2O2). H2O2 undergoes the Fenton reaction in the presence of Fe(II) to generate hydroxyl radicals (HO). Intracellular ROS can react with and cause damage to both proteins and DNA.

Photo-aged skin is characterised by heterogeneity in epidermal thickness, accumulation of immune cells, and pigmentation alterations associated with the formation of lentigo (senilis lentigines, or age spots). However, significant alterations occur primarily within the dermis: collagen fibres undergo disorganization and partial degradation, which cause wrinkles, the disintegration of elastin fibres, and the accumulation of abnormal elastic tissue that characterizes solar elastosis (thick, bumpy, yellowed skin resulting from long-term sun damage).

Sun-exposed skin is affected by UV-A (320–400 nm) and UV-B (290–320 nm) radiation. UV-A rays are less energetic but permeate deeper into the dermal layer. They cause DNA, protein, and lipid damage, and generate ROS that induce dermal remodelling. UV exposure at a sub-erythemal dose stimulates the production of fibroblast-derived elastase which leads to drastic alterations of dermal structure and lentigo. Moreover, UV-A exposure increases the production of matrix metalloproteinase (MMP-1), which hydrolyses interstitial collagen, leading to the disorganisation and progressive degeneration of dermal ECM. Chronic UV-A irradiation also affects hyaluronan synthesis by downregulating hyaluronic acid synthases (HAS) 1, 2, and 3, altering the dermal proteoglycan composition.

Aged fibroblasts increase melanogenic gene transcription, leading to hyperpigmentation and lentigo. Unlike UV-A, UV-B radiation is mainly

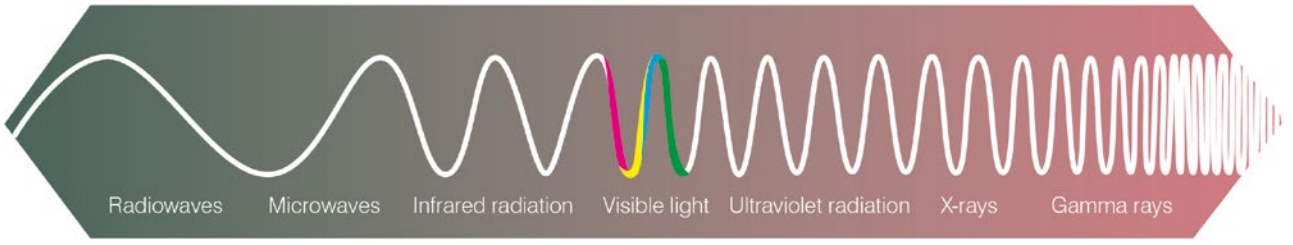
absorbed by the epidermis and directly induces DNA lesions such as cyclobutene pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs) in keratinocytes. DNA photolesions may result in mutations leading to cell senescence, apoptosis, or carcinogenesis. Moreover, UV-B radiation stimulates keratinocytes to release soluble factors such as cytokines, interleukin (IL)-1, and IL-6 that give rise to an inflammatory response, typically known as sunburn. Keratinocytes also induce the secretion of MMP-1 from dermal fibroblasts through a paracrine mechanism. It may be relevant to note that In vitro, human dermal fibroblasts are more susceptible to UV exposure than epidermal keratinocytes.

Photo-dynamically produced ROS may cleave the DNA sugar backbone, causing single-stranded breaks (SSB). They can also oxidise guanine nucleotides to produce another photolesion type, 8-oxoguanine, which can cause mismatched pairing between DNA bases. ROS 1O2 and HO· produced by UVR are oxidising agents that target amino acids vulnerable to oxidation, including histidine, methionine, arginine, and glycine. Oxidation-associated changes in protein structure may, in turn, affect its function. UVR exposure can also break or form intermolecular bonds in proteins. Altered amino acids can affect protein function, with high and low UVR doses decreasing and increasing collagen's thermal stability. UVR can also disrupt the function and structure of lipids via lipid peroxidation, resulting in compromised cell membranes. Extracellularly, ROS may cause damage to abundant ECM proteins, such as collagen and elastin, and UVR-chromophore-rich proteins, such as fibrillin microfibrils and fibronectin.

Electromagnetic Spectrum

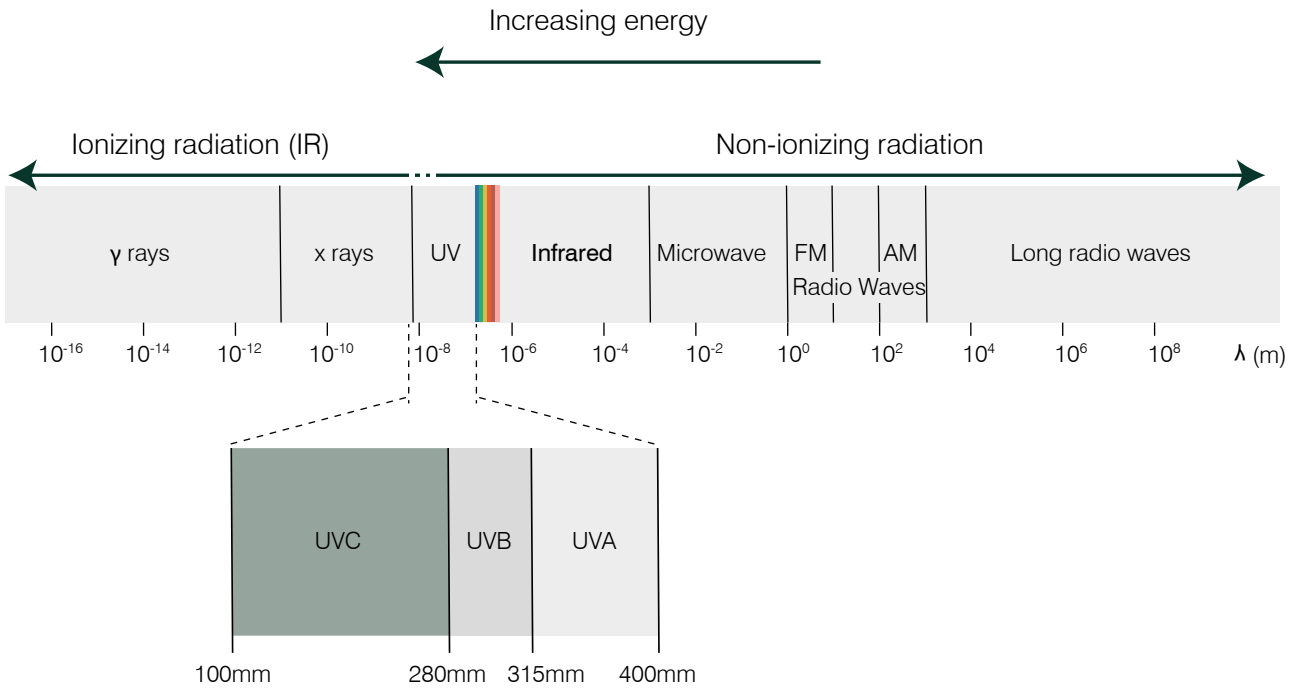
Lower energy

Higher energy



Non-ionizing radiation

Ionizing radiation



Ionising Radiation

In contrast to UVR, photons from ionising radiation are energetic enough to ionise most molecules and atoms, potentially disrupting intermolecular bonds. An abundance of water molecules in biological systems results in a large percentage of ionising radiation being absorbed by water in a process called water radiolysis, producing multiple ROS species. Water radiolysis induces the formation of hydrogen peroxide, superoxide anion, and an abundance of highly reactive hydroxyl radicals. The exposure of DNA to such ionising radiation may directly induce oxidation via deprotonation or electron removal, again producing photolesions such as 8-oxoguanine. Ionising radiation is highly energetic, indicating that electrons ejected from radical formation could potentially increase the probability of single-stranded breaks (SSB) occurring close enough (within 10 base pairs) to promote the formation of double-stranded breaks (DSBs). DSBs are potentially highly cytotoxic due to the risk of failed repair, such as in Non- Homologous End Joining (NHEJ) or homologous recombination, resulting in gene mutations, clastogenic effects, teratogenesis, and carcinogenesis.

Bioinspired & Biomimetic Pharmacology

At DMK, we bridge the gap between biology and design, advancing the adoption of nature-inspired physiological strategies to solve the most prominent skin conditions. Nature provides us with an untapped source of disruptive innovation and knowledge that could translate into solving current healthcare conditions. The design of products following nature's design, popularly known as biomimicry, has played a vital role in pushing technology and product effectiveness to the next level. Humans

have long sought to mimic certain animals' designs and methodology. For example, the walking technique of vertebrates has been effectively mimicked for a quadruped robot to make a system more efficient by consuming less power.

Indirectly, nature acts as a driving factor in pushing technological growth. Biomimicry offers an empathetic, interconnected understanding of how life works and, ultimately, where we fit in. It is a practice that learns from and mimics the strategies used by species alive today—we use principles and functions found in biological systems that have been developed through evolution and apply this knowledge to produce novel and exciting essential technologies and new approaches to solving scientific problems.

The skin exhibits the following functions on its own, and these functional inspirations can be translated into esthetic products and protocols to match precisely natural skin homeostasis.

Level of Understanding	Examples from Skin	Histological Analogy
Molecule	Polypeptide	Nano Structures
Organelle	Intermediate Filament	Single function System
Cell	Keratinocyte	Microsystem
Tissue	Stratum granulosum	Smart Materials
Organ	Skin	Sub System
Organ System	Skin-Gut-Brain Axis	Multifunction System
Organism	Homo Sapiens	Autonomous System
Population	Genotype & Phenotype	Self Evolving Systems
Community	Ethnicity	Co Operative Systems
Ecosystem	Continent	Complex Systems
xBiosphere	Planet	Macro Systems

> Biomimicry is a scientific deduction and translation of naturally occurring structures, functions, processes, and system networks into robust, economical, and highly efficient innovative materials, designs, and systems.
>
> – Dr. Jayant Lokhande MD, MBA-Biotechnology

Exemplary DMK Biomimetic Creations

Here are just a few examples of DMK's biomimetic formulations and what processes they mirror.

1. DMK **Seba-E** + **Herb & Mineral Mist** – Mimic the acid mantle when combined
2. DMK **Enzyme Therapy** – Triggers enzymes to catalyse cell processes
3. **TransGenesis** – Uses polypeptides as vehicles for active ingredients
4. **StemZyme** – Simulates cells replication and differentiation
5. **Enbioment** – Creates phylosymbiosis and poly-diversity

Biomimetic Dermal Pharmacology Industrial Applications –

Skin Functions	Discrete Application	Bio-Inspired Materials (discrete applications)
Radiation Shield	Nuclear Reactor	Construction Coating Materials
Chemical-Bio shielding	Protection against Smoke	Dynamic Polyresins
UV Filter	Agriculture	Advanced Bioprotectants for Plants
Chemical Synthesis	Functional Tattoos	Nuclear Imaging Potentiated Tattoo Inks
Thermal Management	Space Travel Wears	Functional Apparels
Epidermal Folds	Cardiac Stents	Bio resin Composites
Self-Defense & Repair	Trauma Surgery	Personalised Regenerative Tissues
Information Transfer	Algorithms	Robotics

DMK Enzyme Activation Therapy

DMK Enzyme Therapy is employed to restore skin homeostasis and induce biotransformation. Biotransformation makes harmful substances less active and more water-soluble. Contaminants include foreign permeating substances like reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI), also known as free radicals, and internal substances like malformed proteins, inflammatory cell infiltrates, and premalignant cells. When these substances become water-soluble and less active, cells can more easily excrete and eliminate them from the skin's biological environment.

The viable epidermis is the predominant location of biotransformation; though the dermis also shows enzymatic activity, it is much less intense than the epidermis. In addition, active substances usually reside in the dermis for a short time due to their uptake into systemic circulation by the capillaries.

Hydrolysis of free radicals by enzymes mainly occurs in the epidermis, with the stratum basale displaying the highest level of hydrolytic activity. It is assumed that oxygen consumption is proportional to the enzymatic activity of cells; one study testing the activity of esterase enzymes on fluorescein-5-isothiocyanate diacetate in the skin found that the viable epidermis displayed the highest oxygen consumption at 4.53 ±

1.39 μL O2/mg/h. At a far lower level, the dermis consumed 0.49 ± 0.12 μL O2/mg/h, about one-tenth of the viable epidermis oxygen consumption under identical conditions.

The skin metabolises foreign substances in two consecutive phases:

- **Functionalisation Phase** – In the functionalisation phase, a group of polar molecules (molecules with one slightly negative and one slightly positive end due to unevenly distributed electrons) is either generated or unmasked (activated) by oxidative, reductive, or hydrolytic reactions to radical substance groups.
- **Conjugation Phase** – In the conjugation phase, small hydrophilic endogenous molecules covalently attach to damaging radical substances, e.g., glucuronic acid, sulfate, or glycerin. The endogenous molecule gives up some of its electrons, neutralising the free radical. The reaction product will always have an increased molecular weight.

DMK Enzyme Therapy takes 45 minutes to take full effect, cycling through 3 phases in 15-minute increments. Every 15 minutes, a different enzyme cluster is activated to hydrolyse, neutralise, and excrete free radicals.

Phase I – Hydrolysis – The first 15 minutes

Involves the enzymatic modification of oxidants and extraneous substances by cytochrome P450 (CYP), aldehyde oxidases (Aos), aldehyde dehydrogenases (ALDHs), aldo-ketoreductases (AKRs), alcohol dehydrogenases (ADHs), esterases, flavin-containing monooxygenases (FMOs), and cyclooxygenases (COXs).

Phase II – Xeno-Digestion – The second 15 minutes

Involves the activation of enzymes like glutathione s-transferases (GSTs), glucuronosyltransferases (UGTs), sulfotransferases (SULTs), n-acetyltransferases (NATs), and methyltransferases (MTs). They add polar groups to the phase I products to prepare them for excretion.

Phase III – Bio-Drainage – The third 15 minutes

Involves the activation of transporters like ATP-binding cassette (ABC) and solute carrier (SLC), which export altered internal and external oxidants and their metabolites from the cell.

Phase I Enzymes

(Hydrolysis With Reverse Osmosis)

Alcohol Dehydrogenase

There are five known enzyme classes of alcohol dehydrogenase (ADH) in humans: ADH1 with subunits α, β, and γ, ADH2, ADH3, ADH4, and ADH5. The most prominent representative of the ADH family is ADH1, which catalyses the oxidation of primary and secondary aliphatic alcohols to aldehydes. ADH is in both human keratinocytes and hair root cells.

Flavin-Dependent Monooxygenase

Flavin-dependent monooxygenase (FMO) is the main enzyme class for oxidation. It has a high transcription level in human skin, even higher than that of the Cytochrome P (CYP) family. FMO is the main enzyme class in the oxygenation of dermally applied substances and radicals.

Aldo Ketoreductase

Aldo-keto reductase 1C subfamily (AKR1Cs) are primarily responsible for the local levels of active steroid hormones. AKR1C1 and AKR1C2 inactivate progesterone and 5alpha-dihydrotestosterone, respectively, whereas AKR1C3 activates oestradiol and testosterone. AKR1C1-3 are expressed in keratinocytes and fibroblasts, with marginal expression in melanocytes. In human primary keratinocytes, AKR1C1 and -2 are dose-dependent UVB-inducible. AKR1C subfamily genes are stress-inducible and might function as survival factors in keratinocytes.

Cyclooxygenase

Cyclooxygenase (COX), a prostaglandin-endoperoxide synthase (PTGS), catalyses the formation of prostaglandins from arachidonic acid. It exists in three isoforms. Prostaglandins are lipid signalling mediators that play a central role in various physiological and pathophysiological processes, including inflammation, reproduction, nocioception, and gastrointestinal protection.

COX-dependent pathways influence keratinocyte differentiation, hair follicle development, and hair growth. In addition, COX-2 mediates inflammatory processes in the skin, including inflammatory hyperalgesia and nociception. The administration of specific COX-2 inhibitors has cancer chemopreventive properties and can also reduce edema, vascular permeability, and other markers of cutaneous inflammation. Recently, topical COX-2 inhibitors have been formulated as a novel pharmacologic approach for treating COX-2-mediated skin diseases.

Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) converts aldehydes into carboxylic acids. There are twelve classes of ALDH in the human body; however, ALDH1 and ALDH3 are only detected in excised human skin. ALDH1 has a high affinity for aldophosphamide and is vital in detoxifying peroxide aldehydes.

Carboxylesterase

Carboxylesterase (CE) hydrolyses carbon esters by the intramolecular addition of water to an alcohol and acid residue: (carboxylic ester + H2O alcohol + carboxylate). Alcohols are then further oxidised to aldehydes, and the carboxylates are conjugated in phase 2 of biotransformation. The CE family is organized into five classes. However, the CE2 isoenzyme has only been verified in human keratinocytes.

Cytochrome P450

CYPs are the largest group of metabolising enzymes in the skin, which catalyse the transfer of one atom of molecular oxygen onto permeating substrates, generating alcohol and water. The expression of CYP appears to be polymorphic. Its activity shows remarkable interindividual variability depending on age, gender, and the anatomical site of application. CYP2D6, CYP2E1, and CYP3A4 transcription are present in human skin. UV radiation does not activate or inactivate enzymes but generates reactive oxygen species. For example, an increased level of reactive oxygen species leads to the suppression of Matrix Metallopeptidase Inhibitors (MMP), thereby increasing MMP activity in the skin.

Further examples of UV radiation-inducible enzymes in the skin are the CYP enzymes CYP1A1 & CYP1B1, hemoxygenase, cyclooxygenase, and nitric oxide synthase. When particulate matter adheres to skin, these polycyclic hydrocarbons become more permeable. Skin metabolises these harmful molecules by benzo[a]pyrene hydroxylase. Increasing the amount and duration of exposure to polycyclic hydrocarbons on the skin leads to adjusting the benzopyrene hydroxylase level. Enzyme levels are modified not only by environmental molecules such as polycyclic hydrocarbons but also by purposely applied compounds such as drugs or active substances.

Phase II Enzymes

(Inactivation Of Oxidants)

Phase II metabolising enzymes typically play a part in detoxification and cytoprotection against many ROS.

Glutathione S-Transferases (Gsts)

These are one of the largest and best-studied phase II metabolising enzymes native to the skin.

Udp-Glucuronosyltransferases (Ugt)

These enzymes facilitate glucuronidation. Glucuronidation represents a major pathway that facilitates the transformation of many lipophilic xenobiotics and endobiotics into more water-soluble compounds. The UDP-glucuronosyltransferase (UGT) family catalyses the glucuronidation of the glycosyl group of a nucleotide sugar to an acceptor compound (aglycone) at a nucleophilic functional group of oxygen (e.g., hydroxyl or carboxylic acid groups), nitrogen (e.g., amines), sulphur (e.g., thiols), and carbon, with the formation of a beta-D-glucuronide product.

Sulfotransferases (Sults)

Sulfotransferases catalyse the formation of sulfuric acid esters, most often referred to as sulphates, from a wide range of metabolites and various endogenous neurotransmitters, hormones, bile acids, carbohydrates, and proteins. The sulfation reaction is responsible for either detoxication or metabolic activation of many toxins, carcinogens, environmental chemicals, and other xenobiotics.

N-Acetyltransferases (Nats)

Human NAT enzymes can detoxify, rendering many hazardous molecules biologically inactive. N-acetyltransferases (NATs) perform biotransformation of carcinogens as well. They are associated with the endogenous metabolic pathways of cells and can be good targets for deactivating oxidant molecules.

Methyltransferases (Mts)

The methyltransferases use S-adenosyl-l-methionine (Ado-Met) as the methyl donor. The basic methyl transfer reaction (methylation) is the catalytic attack of a nucleophile (carbon, oxygen, nitrogen, or sulfur) on a methyl group to form methylated derivatives of proteins, lipids, polysaccharides, nucleic acids, and various small molecules. Such methyl conjugation is an essential pathway in metabolising many oxidants, ROS compounds, and endogenous neurotransmitters and hormones. Methylation is fundamental to the control of gene transcription.

Phase III Transporters

(Excretion Of Inactivated Oxidants)

Membrane transporters are widely distributed throughout the body, particularly in the epithelia of major organs, such as the liver, intestine, and kidney, and other organs with barrier functions, such as the brain, skin, testes, and placenta. Transporters are responsible for the cellular trafficking of essential nutrients and metabolites in the skin, maintaining cellular homeostasis.

Membrane transport proteins are essential in translocating different endogenous and exogenous components (compounds, nutrients, metals, etc.) across plasma membranes. They are primarily composed of two families: ATP binding cassette (ABC) and Solute Carrier (SLC) transporters. ABC transporters are active transporters and utilise the energy of ATP binding and hydrolysis to facilitate the in- or efflux of their substrates across membranes. On the other hand, SLC transporters utilise ion electrochemical gradients, such as sodium or proton gradients, to transport the substrates. 49 ABC transporters subtypes have been identified in humans, divided into the 7 subfamilies ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG. These transport a broad spectrum of endogenous substrates, including lipids (ABCA1 and AB family), cholesterol (ABCG1), iron (ABCB6–10), organic anions (ABCC2), peptides (ABCB2 and 3), as well as exogenous compounds, such as biocompatible™ molecules across intra- and extracellular membranes.

Approximately 400 SLC transmembrane transporters are divided into six groups with 66 distinct subfamilies (SLC1 to SLC66). Like ABC family members, they transport various compounds facilitating everything from the cellular uptake of nutrients to the absorption of ROS. Their function in the translocation of substrates can be described using four models: Cotransporter or symporter (SLC1 and SLC5), antiporters (SLC4 and SLC26), facilitated (SLC2), and 'orphan transporters,' for which substrates and functions need identification. In humans, ABC transporters mostly perform efflux functions by eliminating ROS from the body, while SLC transport systems can be influxes, effluxes, or antiporters. A few SLC transporters also have the ability for bidirectional transport.

DMK MD Skin Analysis

DMK's Conception Of New Skin Layers

Quantadermis™

> If you want to find the secrets of the universe, think in terms of energy, frequency, and vibration.
>
> Nikola Tesla

The skin interacts with its environment via ambient fields such as light, sound, electricity, magnetism, and all other living organisms to generate massive amounts of information in the form of energy fields. Quantadermis describes an outer skin layer defined by DMK, formed by Biophoton emissions and the Spin Magnetic Moment.

All atomic nuclei consist of protons and neutrons with a net positive charge. Certain atomic nuclei, such as the hydrogen or phosphorus nucleus, possess a property known as "spin," dependent on the number of protons. This can be conceived as the nucleus spinning around its axis, although this is a mathematical analogy. The nucleus does not spin in the classical meaning but induces a magnetic moment through its constituent parts, generating a local magnetic field with north and south poles. The quantum mechanical description of this dipolar magnet is analogous to classical mechanics of spinning objects, where the dipole is a bar with magnetic poles aligned along its axis of rotation. Nuclei that possess spin can be excited with magnetic fields in short pulses, whereby the absorption of energy via the nucleus causes a transition from higher to lower energy levels and vice versa on relaxation, returning the system to thermal equilibrium. Energy absorbed (and subsequently emitted) by the nucleus induces a voltage that can be detected, amplified, and displayed as "free-induction decay," causing each nucleus to resonate at a characteristic frequency when placed in the same magnetic field.

As per quantum physics, there is no difference between energy and matter. From the atomic to the molecular level, the skin is constantly in motion-creating resonance. This resonance is essential to understanding how electromagnetism (radiation/light) can affect the skin differently. While all matter resonates, there are signature resonant frequencies, emitting unique characteristic signals from the nuclei of their respective atoms.

In individual atoms, the direction electrons spin determines magnetism. This is called the Spin Magnetic Moment. Related processes include nuclear magnetic resonance (NMR), wherein nuclei in a strong, constantly vibrating magnetic field are perturbed by a weak oscillating magnetic field. The nuclei in these atoms respond by producing an electromagnetic signal characterised by the magnetic field at the nucleus. Currently, NMR is the only tool at our disposal to study tissue samples at the molecular level.

Multidisciplinary research suggests that the mind and body communicate in a bidirectional flow of hormones, neuropeptides, and cytokines. E.g., In the immune system, protein molecules known as cytokines are the principal mediators of communication between the immune and neuroendocrine systems. Activated immune cells can permeate the blood-brain barrier and secrete cytokine mediators. Cytokines play an enormously important role in system homeostasis during immune challenges.

This results in immune system modulation, particularly regarding inflammation and infection.

Several In vivo and In vitro cell and tissue cultures have demonstrated effects caused by low-frequency or weak electromagnetic frequency (EMF), causing changes in cell proliferation, alterations in membrane structure and function, changes in nucleic acids, protein phosphorylation, and adenosine triphosphate (ATP synthesis) in the skin.

All cells produce EMFs because humans produce complex electrical activity in the body's 210 different cell types. For example, neurons, endocrine cells, and muscle cells are all referred to as "excitable cells." These cells produce a current (via electron transfer), a magnetic field (via moving charges), a pulsed frequency, pH, oxygen, carbon dioxide, and light (via biophotons).

All living creatures, including humans, emanate a weak light called biophoton emission. Fritz-Albert Popp first researched biophoton emission using a type of photomultiplier (which converts photons into electrical signals) to count light, photon by photon. This radiance is linked to excited states within the living system. However, these are not bioluminescent particles; the light emissions are extremely weak and cannot be observed by the naked eye.

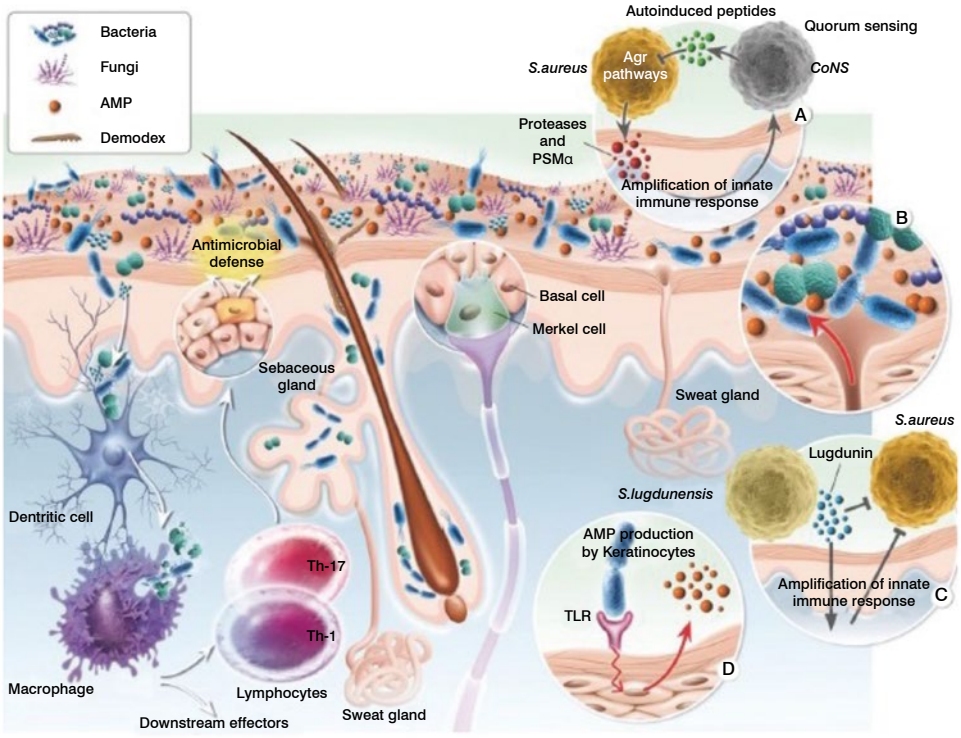
The Quantadermis is a layer formed by the Spin Magnetic Effects of Microbiota and Biophotons emitted by various tissues in the epidermis and dermis. Though research on this subject is in its early stages, some speculate that the skin damage process causes the microbiome and epidermal tissues to emit more biophotons. Through the Quantadermis layer, we observe that these increased biophoton emissions may be important biomarkers for photoageing, dehydration, and UV damage.

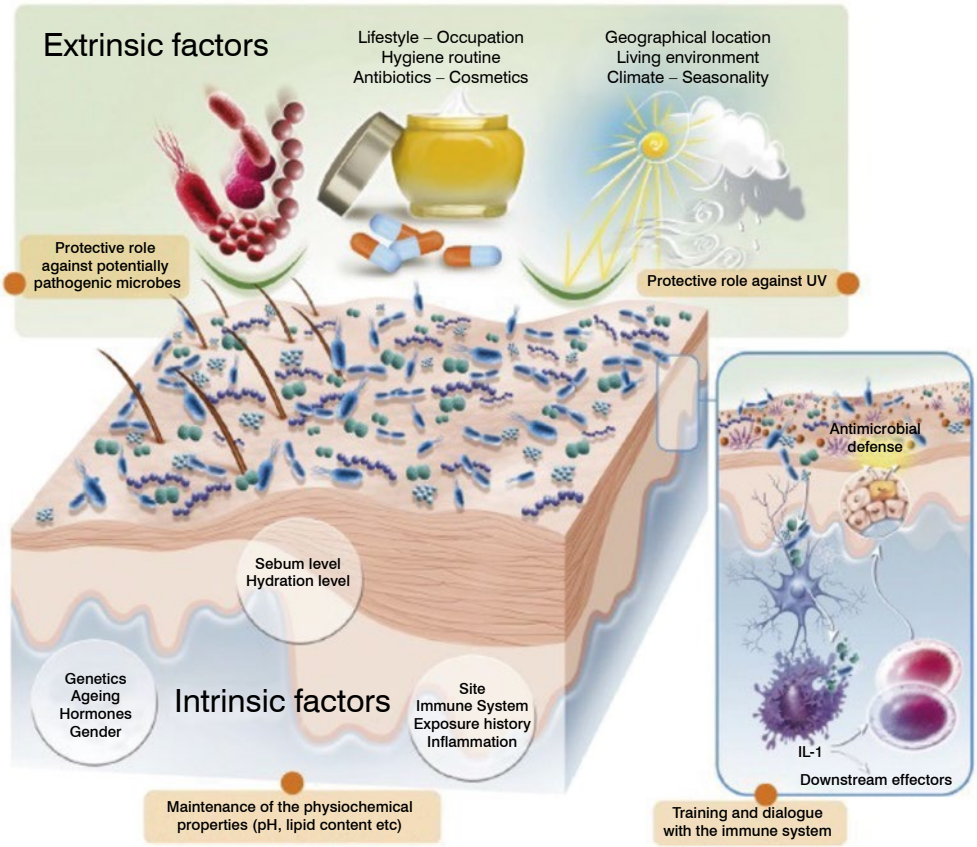
The Microbiome/Microdermis

All microbiotas perform several reactions involving light energy (photons) and chemicals to derive energy for their biological sustainability. To do so, they utilise inorganic compounds like carbon dioxide, water, hydrogen sulphide, etc.

Microbiota in the skin contribute to the skin's barrier function and ensures homeostasis. They have many roles; for example, skin microbes secrete protease enzymes involved in desquamation and stratum corneum renewal; sebum and free fatty acid production are involved in pH regulation, and the secretion of lipase enzymes is involved in lipidic film surface breakdown; urease enzymes are implicated in urea degradation.

Other roles of microbiota include the production of biofilms, bacteriocins, and quorum sensing. Microbiotas also protect against potentially pathogenic microorganisms by competition. For example, commensal bacteria, or Malassezia fungi, produce an antimicrobial peptide (AMP) production with various indoles that inhibit many other yeasts and moulds. A specific strain of Staphylococcus epidermidis was even shown to produce 6-N-hydroxyaminopurine, which may confer protection against skin cancer.


Bacteria
Fungi
AMP
Demodex
Autoinduced peptides
Quorum sensing
S.aureus
Agr pathways
CoNS
A
Proteases and PSMα
Amplification of innate immune response
B
Antimicrobial defense
Basal cell
Merkel cell
Sebaceous gland
Sweat gland
S.aureus
Lugdunin
S.lugdunensis
Dentritic cell
AMP production by Keratinocytes
Amplification of innate immune response
C
Th-17
Th-1
TLR
D
Macrophage
Lymphocytes
Downstream effectors
Sweat gland


Extrinsic factors
Lifestyle – Occupation
Hygiene routine
Antibiotics – Cosmetics
Geographical location
Living environment
Climate – Seasonality
Protective role against potentially pathogenic microbes
Protective role against UV
Antimicrobial defense
Sebum level
Hydration level
Genetics
Ageing
Hormones
Gender
Intrinsic factors
Site
Immune System
Exposure history
Inflammation
IL-1
Downstream effectors
Maintenance of the physiochemical properties (pH, lipid content etc)
Training and dialogue with the immune system

Bacteria present as commensal residents include gram-positive bacteria belonging to the genera Staphylococcus spp., Corynebacterium spp., Enhydrobacter spp., Micrococcus spp., Cutibacterium spp., and Veillonella spp, and gram-negative bacteria (GNB) like Enterobacteriaceae, nonfermenting GNB, Roseomonas mucosa, Pseudomonas spp., Acinetobacter spp., Pantoea septica, and Moraxella osloensis. Autotrophs on the skin can produce their own food. Phylogenetic analysis of Archaea like Thaumarchaeota and Euryarchaeota placed them close to ammonia-oxidising archaea from the soil identified as chemolithotrophs. They can cause ammonia turnover, which could influence the pH regulation of the human skin and, therefore, the body's natural protective barrier.

Bacteriophages are predominant, and the lytic activity of bacteriophages is linked to the modulation of bacterial populations. Bacteriophages participate in the homeostasis of the skin microbiota, and C. acnes play a role in modulating bacterial skin populations. Cutibacterium and Staphylococcus phages are the most abundant skin phages, while Streptococcus and Corynebacterium phages are present in lower quantities. Phages are identified as Acheta domestica; Densovirus; Alphapapillomavirus; Human papillomavirus (β), (γ), and (*μ*); Merkel cell polyomavirus; Molluscum contagiosum virus; Polyomavirus HpyV7; Polyomavirus; HpyV6 RD114 retrovirus; and Simian virus. Fungi, including Malassezia, Cryptococcus, Rhodotorula, and Candida species, have been identified as human skin commensal organisms. Malassezia spp. Is the main genus of commensal skin fungi. Demodex mites in the Demodicidae family live in seborrheic areas of the skin, such as the face and hair. Demodex mites are also widely found on the eyelids and the nasal area.

Bioimaging Techniques

Bioimaging methods are as complex as skin structure and function. Dermatology professionals usually decide on esthetic treatments based on clinical and/or aesthetic skin visualisation. However, with current technological advances in bioimaging, specific instruments can be very well incorporated into age management practices to understand the skin's cellular structure at a deeper level. As a result, skincare professionals can better manage the outcome of various esthetic procedures. Skin bioimaging also offers clues to conditions beyond the esthetic realm and may mandate medical intervention before aesthetic procedures.

The following techniques and instruments can be beneficial in understanding skin conditions and planning aesthetic treatments.

Digital Photographic Imaging

The simple purpose behind the digital camera is to measure light reflection, refraction, and scattering from the skin. Certain inferences are derived based on the permeation and density of light through different skin tissues. Polarising filters attached to the camera's lens separate reflectance and backscattering of light. A computer then breaks this electronic information down into digital data. Such parallel-polarised photography visualizes skin texture and scale. When this technique is used in cross-polarised photography, it enhances imaging of skin vascularity and pigmentation. Thus, one can determine the status and depth of skin pigmentation and properly manage age-related pigmentation issues and treatments.

Dermatoscopy

Dermatoscopy, or epiluminescence microscopy, is the In vivo evaluation of epidermal microstructures, rete pegs, dermal papillae, and papillary dermis. This device can assess skin structures in depth and record skin images for before-and-after comparisons or the evaluation of esthetic procedures.

Confocal Microscopy

Confocal microscopy can provide better cellular resolution of skin and cutaneous structures. Confocal microscopes can create images by utilising the intrinsic differences between refractive indices of cellular structures, especially melanin, collagen, and keratin. Melanin and keratin provide contrast in confocal images. Melanin has the strongest refractive index at 1.7; keratin's refractive index is 1.5. Collagen and inflammatory cells are also highly refractile structures. This investigative method can help point out potential skin areas to treat when targeting the esthetic revision of age-related wrinkles and instilling derma-fillers.

Optical Coherence Tomography

OCT is a micron-scale imaging method that measures the back reflection of infrared light. In skin tissue, the imaging permeation depth is about 2 mm. This can enable visualisation of the stratum corneum, epidermis, upper dermis, appendages, epidermal blood vessels, and microvasculature. In conditions like erythema, rosacea, acne, anisotropic pigmentation, and scars, this method can be beneficial in determining which aesthetic intervention to use.

High-Frequency Ultrasound

HFUS uses the reflection of ultrasound wave impedance and creates images. When the transducer is placed on the skin, a computer interprets its reflected sound waves into images. High-intensity echoes generate hyperechoic images (white), whereas low-intensity echoes generate anechoic images (black). For example, keratin is the primary determinant of hyperechogenicity of the epidermis. Collagen determines hyperechogenicity of the dermis. In the subcutis, fascia and connective tissue are hyperechoic, whereas fat globules are anechoic. When HUFS is coupled with a colour doppler, it can help visualise blood flow through the skin. While treating conditions like uneven keratin and collagen dispersion, relaxed muscles, and cellulite depositions, this method can offer clues on potential areas to localise aesthetic intervention.

Multispectral Optoacoustic Tomography

'MSOT is under critical review for employment in dermal imaging and is not yet widely available. It can generate images based on the identification of endogenous and exogenous chromophores. Chromophores can yield colour (e.g., hemoglobin, melanin, etc.), and exogenous are artificial molecules, like nano-sized dyes. MSOT can provide deeper imaging depths of up to 1cm, as well as in 3D format.

Fluorescence Imaging

Fluorescence imaging creates images with the fluorescent properties of fluorophores. Fluorophores absorb energy from an external light source, like visible or ultraviolet light. The fluorophore becomes excited with

corresponding photon energy (excitation energy), and a photon is emitted when it returns to the ground state. These emitted photons are detected by photomultiplier tubes with a single-photon detection sensitivity and can offer excellent clues on pyridine nucleotides, flavins, aromatic amino acids, proteins, and porphyrins. From an esthetic point of view, this can offer an in-depth analysis of topically applied products and their efficacy, retention, and safety.

Multiphoton Tomography

MPT is another technique that compares excitation differences between two photons: endogenous fluorophores versus second harmonic generated fluorophores (primarily from dermal collagen). Endogenous fluorophores include nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide phosphate, flavoproteins, keratin, lipofuscin, elastin, melanin, and metal-free porphyrins. This method can present in-depth structural and histological information on all endogenous fluorophores that can be skin age markers.

In-Clinic Skin Analysis Techniques

Colourimetry

Measures the colour of the skin with a probe that sends out white LED light, arranged circularly to illuminate a large part of the skin. When the uniformly emitted light hits the skin surface, it is partly reflected and partly scattered. A small proportion travels into the skin and is scattered by the deeper layers. The light reflected from the skin is measured in the probe. The raw data of the probe is corrected with a special colour matrix to adapt it to standard values and expressed accordingly.

Corneofix

A special foil that collects corneocytes (flakes of dead skin cells). The number, size, and thickness of corneocytes on the foil indicate the desquamation/hydration level of the stratum corneum. Many thick, large corneocytes can only be collected when the skin is dehydrated or damaged. However, well-moisturised skin shows small regular flakes on the foil.

Corneometery

The most used instrument worldwide to obtain exact and reproducible values of the hydration level of the skin surface, mainly the stratum corneum. The measurement is based on the capacitance measurement of a dielectric medium. A corneometer measures the change in the dielectric constant, due to skin surface hydration by capacitance differences of a precision capacitor.

Cutometry

This measurement uses negative pressure created by a vacuum pump within the device that draws the skin into the probe's aperture. Inside the probe, the permeation depth is determined by a non-contact optical measuring system. It consists of a light source and a light receptor, as well as two prisms facing each other, projecting the light from the transmitter to the receptor. The light intensity varies due to the permeation depth of the skin. The skin's resistance to being sucked up by negative pressure (firmness) and its ability to return to its original position (elasticity) are displayed as curves in real time.

Mexametery

A quick, easy, and economical tool to measure the two components mainly responsible for skin color: melanin and hemoglobin. This measurement is based on absorption/reflection. The mexameter probe emits 3 specific light wavelengths, and a receiver measures their reflection from the skin. As the quantity of emitted light is defined, it calculates the quantity of light absorbed by the skin.

Sebumetery

Used to measure sebum (oil) on the skin, scalp, and hair. The measurement is based on grease spot photometry. The opaque tape of the machine contacts skin or hair and becomes transparent according to the amount of sebum on the surface. When the tape is inserted into the device's aperture, its transparency is measured by a photocell. The light transmission reflects the sebum content.

Tewametery

Assesses transepidermal water loss (TEWL), an indispensable parameter for the evaluation of the water barrier function of the skin, with utmost accuracy and reproducibility. Water constantly evaporates from the skin and is a part of the body's metabolism. The amount of water lost (TEWL) is expressed in g/h/m^2. 30 sensors inside the hollow cylinder of the probe detect the skin's relative humidity and temperature like a camera. The high amount of data allows the user to not only measure inside the probe with high accuracy but can also show results for the areas right outside the probe, namely the skin surface and ambience above the probe.

Visiometery

Can evaluate the topography of the skin's surface by the light transmission of a very thin, special, blue-dyed silicone replica. The replica is placed between a parallel light source and a b/w CMOS camera. Light is absorbed according to the thickness of the silicone material. The replica reproduces a relief of the skin as a negative, i.e., wrinkles are higher in the replica, absorbing more light where the silicone is thicker.

Lambert and Beer's Law calculate the amount of absorbed light: $\Phi ex = \Phi in \bullet e\text{-}kd$. Outgoing light is proportional to the incoming light, the material's thickness, and constant k.

Different Types of Stem Cells

Embryonic Stem Cells

These cells are present in the blastocyst, the hollow ball of cells from the fertilised egg. They are pluripotent and can give rise to every cell type in the fully formed body except the placenta and umbilical cord.

Embryonic stem cells exercise their activity over a short period during the development of the fertilised egg, giving rise to all the embryonic germ lines (ectoderm, mesoderm, endoderm) that subsequently develop into the tissues of the adult organism.

Adult or Tissue-Specific Stem Cells

These stem cells can generate different cell types for the specific tissue or organ in which they live. For example, blood-forming (or hematopoietic) stem cells in the bone marrow can give rise to red blood cells, white blood cells, and platelets. However, blood-forming stem cells do not generate liver, lung, or brain cells. Likewise, other tissues and organs' stem cells do not generate red or white blood cells or platelets.

Some tissues and organs within the body contain small caches of tissue-specific stem cells that replace cells lost from everyday living or injury, such as those in the skin, blood, and gut lining. Adult stem cells are responsible for regenerating or repairing tissue after damage and maintaining homeostatic equilibrium by the renewal rate of different cell types like skin, liver, spleen, etc. These cells, which are located at specific sites known as niches, can differentiate into all cell types in their host tissue. This niche is a critical element in the functional regulation of adult stem cells. The somatic cells that make up the niche of a tissue interact with resident stem cells, establishing molecular signals that regulate their proliferative activity and capacity to differentiate.

Mesenchymal Stem Cells

MSCs or stromal cells are cells isolated from stroma, the connective tissue surrounding other tissues and organs. Various MSCs are thought to have stem cells, and their immunomodulatory properties are being tested as treatments for many disorders, but there is little evidence to date that they are beneficial.

Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPS) were engineered in the lab by converting tissue-specific cells, such as skin cells, into cells that behave like embryonic stem cells. iPS cells can be critical to understanding normal human development, disease onset and progression, and testing new drugs and therapies. While

iPS cells share many of the same characteristics as embryonic stem cells, including the ability to give rise to all the cell types in the body, but they are not the same. iPS stem cells are associated with specific problems as they require the insertion of exogenous genes, which are usually silent in the adult organism. Re-expression in tissues may lead to unpredictable harmful effects, such as tumorigenesis.

The epidermis was the first organ where stem cells were identified in situ through a label retention technique and, along with the hematopoietic system, is the tissue where stem cells are best characterised. In addition, a close relationship has been demonstrated between stem cells, cancer, and ageing.

In pluricellular eukaryotes, stem cells comprise a well-defined cell population characterised by two

unique properties: their capacity for differentiation into one or more cell types and their capacity for self-renewal, giving rise to cells with the same potential for differentiation. In mammals, these cell types can be of embryonic or adult origin. Embryonic stem cells exercise their activity over a short period during the development of the fertilised egg, giving rise to all the embryonic germ lines that subsequently develop into the tissues of the adult organism. Adult stem cells, in contrast, are responsible for maintaining homeostatic equilibrium in the tissues of the adult organism; that is, for maintaining the renewal rate of different cell types in each tissue and regenerating or repairing tissue after damage. These cells, located at specific sites known as niches, can differentiate into all cell types in their host tissue. Niches are a key element in the functional regulation of adult stem cells. The somatic cells that make up a niche interact with resident stem cells, establishing molecular signalling that regulates their proliferative activity and capacity to differentiate.

The biology of stem cells has been a subject of increasing interest in biomedicine. Regenerative Medicine makes clinical use of stem cells, whether of embryonic or adult origin, as a tool for generating new organs or tissues In vitro to replace the damaged tissue of the patient. The possibility of reverting a somatic cell of an individual into the pluripotential state of an embryonic cell (induced pluripotent stem cells or iPS) has attracted significant interest given the potential application in regenerative medicine. However, this technique is associated with certain problems as it requires the insertion of exogenous genes that are usually silent in the adult organism. Re-expression in tissues may lead to unpredictable harmful effects, such as tumour formation. In addition, the use of totipotential embryonic stem cells, that is, cells with embryonic characteristics, are associated with ethical controversy, given the potential for reproductive cloning to create genetically identical individuals. An exciting alternative to overcome these problems is resident stem cells in adult tissues. These cells can differentiate into a variety of cell lines (including cell lines other than those that make up the original tissue) but lack the potential to generate a complete organism.

The homeostatic control of the epidermis is achieved through a small population of multipotential stem cells that rarely divide and have a high differentiation potential. This population responds to the pathophysiologic needs of the tissue, generating numerous progenitor cells with a high replication capacity and a low differentiation capacity. These cells spread through the basal layer of the epidermis, forming a transit-amplifying (TA) cell population. Cotsarelis was the first to demonstrate that the bulge region of the hair follicle is a niche for epidermal stem cells.

Progenitor cells have also been found in the basal layer of the interfollicular epidermis. There is still debate as to why a wide variety of epidermal stem cell populations are localised to different regions of the follicle (bulge, infundibular, and papillary dermis) and the interfollicular epidermis, where they express specific protein markers. Progenitor cells of the bulge region of the follicle contribute to hair regeneration; they also participate in wound healing, as they can emigrate to the interfollicular epidermis and differentiate into keratinocytes. This population of progenitor cells In vitro is strongly multipotent and can differentiate into keratinocytes, neurons, glial cells, melanocytes, and mesenchymal cells. Moreover, In vivo, this population participates in the angiogenic processes in the skin.

StemZyme Concept and Efficacy

DMK StemZyme Treatment is an intelligent biomimicry-based approach that is non-invasive, non- immunogenic, and clinically effective in activating epidermal stem cells. DMK StemZyme is a systematic and enzymatic approach to activate one's epidermal stem cells and guide them to differentiate into various skin cells like keratinocytes, fibroblasts, Langerhans cells, melanocytes, glial cells, Merkel cells, and subcutaneous fascia. DMK StemZyme is customised for the individual, as it activates one's inherited Stem Cells.

This treatment is non-invasive and does not create immunity or inflammatory shock like other approaches. This approach also inhibits the overexpression of genes that otherwise can become active in other stem cell treatments and predispose oncology disorders. Gene overexpression in a stem cell pathway is one of the primary concerns in stem cell therapy.

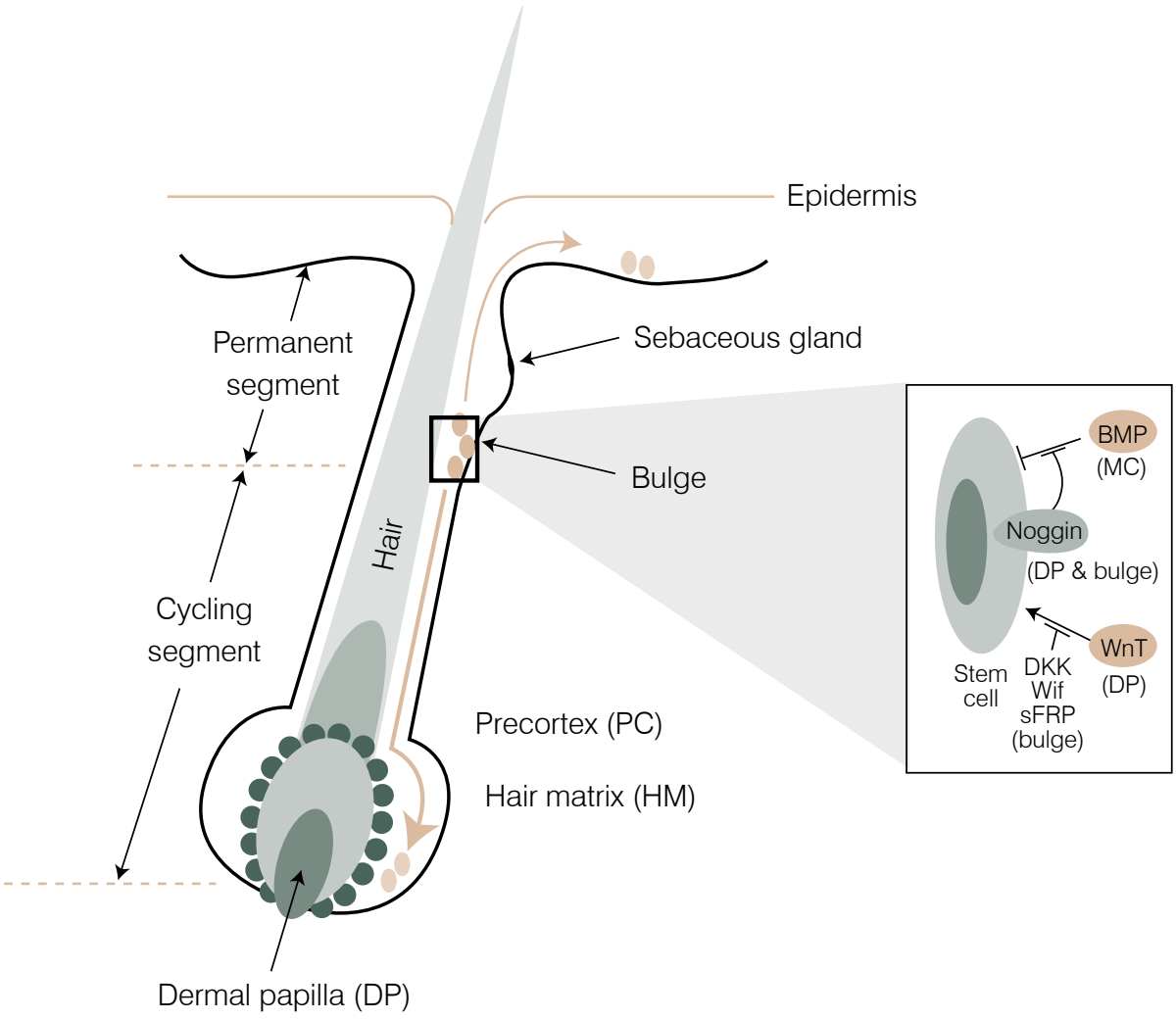
The epidermis achieves homeostatic control through a small population of multipotential stem cells that rarely divide and have a high differentiation potential. This population responds to the pathophysiologic needs of the tissue, generating numerous progenitor cells with a high replication capacity and a low differentiation capacity. These cells spread through the basal layer of the epidermis, forming a transit-amplifying (TA) cell population.

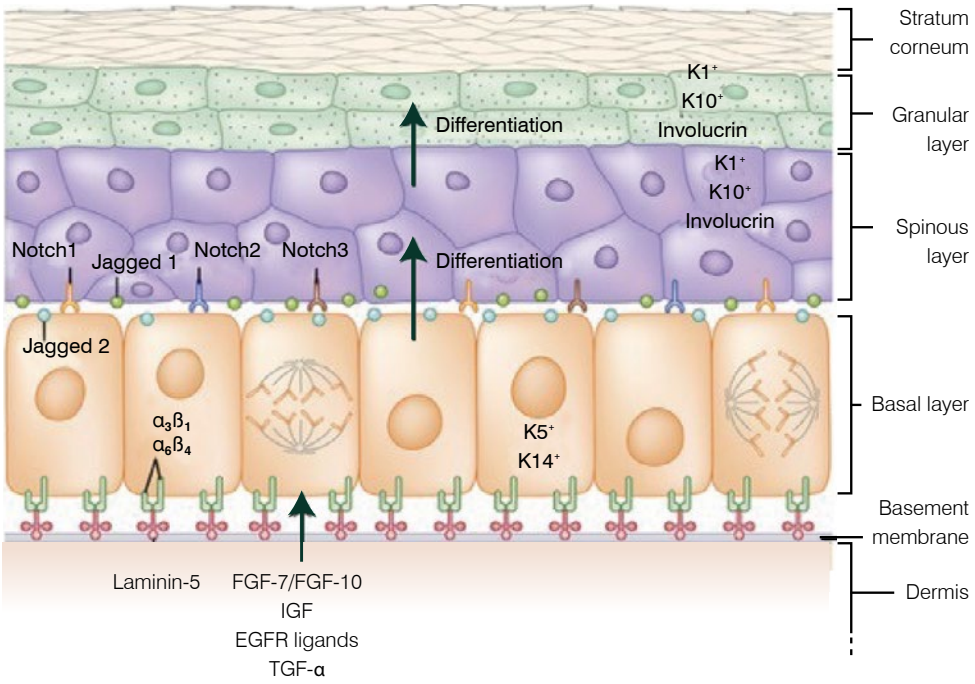
Only the basal layer of the epidermis is mitotically active, meaning it can generate new cells; other layers of the epidermis are not mitotically active. Several scientific experiments have inferred that epidermal stem cells are located in the bulge apparatus and interfollicular epidermis. The adult skin epithelium comprises molecular building blocks, each consisting of a pilosebaceous unit (HF and sebaceous gland) and its surrounding interfollicular epidermis (IFE). The IFE contains progenitor cells to ensure tissue renewal in the absence of injury, and HFs contain multipotent SCs that are activated at the start of a new hair cycle and upon wounding to provide cells for HF regeneration and repair of the epidermis. As cells leave the basal layer and move outward toward the skin surface, they withdraw from the cell cycle, switch off integrin and laminin expression, and execute a terminal differentiation program. The program remains transcriptionally active in the early stages of spinous and granular layer production. However, it culminates in producing the dead, flattened cells of the cornified layer, sloughed from the skin surface and continually replaced by inner cells moving outward.

> The stem cell is indeed a true and practical example of embodied consciousness because consciousness originates everything that exists.
>
> – Dr. Jayant Lokhande
> MD:MBA – Biotechnology

Progenitor cells are also found in the basal layer of the interfollicular epidermis. There is still a scientific debate as to why a wide variety of epidermal stem cell populations are localised to different regions of the follicle (bulge, infundibular, and papillary dermis) and the interfollicular epidermis, where they express specific protein markers. Progenitor cells of the bulge region of the follicle contribute to hair regeneration and participate in wound healing, as they can emigrate to the interfollicular epidermis and differentiate into keratinocytes. This population of progenitor cells is strongly multipotent and can differentiate In vitro into keratinocytes, neurons, glial cells, melanocytes, and mesenchymal cells. Moreover, In vivo, this population participates in the angiogenic processes of the skin.

The IFE, which generates the lipid barrier of adult skin, constantly renews its surface throughout a human being's life and undergoes re-epithelialisation after injury. The renewing and repairing activities of the epidermis imply the existence of stem cells to ensure these critical functions.





The major structural proteins of the epidermis are keratins and a network of 10-nm keratin intermediate filaments (Ifs) that connect toα6β4-integrin-containing hemidesmosomes. They anchor the base of the epidermis to the laminin5-rich extracellular matrix. Keratin Ifs also connect to intercellular junctions called desmosomes, composed of a core of desmosomal cadherins. Together, these connections to keratin Ifs provide an extensive mechanical framework for the epithelium.

The basal layer is typified by the expression of keratins K5, K14, and (embryotic) K15, whereas the intermediate suprabasal (spinous) layers express K1 and K10. Desmosomes connected to K1/K10 Ifs are especially abundant in suprabasal cells, whereas basal cells possess a less robust network of desmosomes and K5/K14. Basal cells utilise a more dynamic cytoskeletal network of microtubules and actin filaments that interface through β-and α-catenins to E-cadherin mediated cell-to-cell adherens (junctions), in addition to the αβ1- integrin-mediated cell-ECM junctions.

Filaggrin and loricrin are produced in the granular layer. The cornified envelope seals the epidermal squames and provides the barrier that keeps microbes out and essential fluids inside. Any skin revision approach targeting stem cells must provide circumstantial safety. The following gene expressions should inform in this regard.

ITGA3 (3) – responsible for Re Epithelisation

ITGA 6 (6) – responsible for Tumour Generation

ITGB1 (1) – responsible for Tumour Metastasis

ITGB4 (4) – responsible for Invasive Carcinoma

JAG1 – Increased activity (expression) of the JAG1 gene has been linked to certain cancers, including breast, head, and neck tumours. The increased expression of the JAG1 gene may promote the development of new blood vessels that nourish a growing tumour. The altered gene

expression may also enhance other cancer-related events such as cell division (proliferation) and the inflammatory response.

NOTCH 1 – The increased activity can lead to uncontrolled cell growth and division, which can result in cancer development.

NOTCH 2 – Overexpression of this gene may lead to uncontrolled cell growth and cell division in immune system cells, which can result in the development of lymphoma.

NOTCH3 – The mutations that cause lateral meningocele syndrome occur at the end of the gene in a region known as exon 33. These gene mutations result in a NOTCH3 protein with an abnormally short (truncated) NICD. The shortened protein is missing the portion that usually causes the breakdown of the NICD after it has performed its function in the cell nucleus and is no longer needed. As a result, the presence of the NICD in the cell is prolonged, and the protein continues to affect the activity of other genes. However, this prolonged NICD activity and its connection to the specific features of lateral meningocele syndrome are not well understood.

OCT4 – Oct4 is re-expressed in different cancer stem cells, tumour cell clusters at the origin of chemotherapy tumour resistance, and cancer recidivation. The precise understanding of the molecular mechanisms of Oct4 regulation are a challenge in applied research fields (particularly its ON/OFF switch in tissues depending upon its microenvironment) for regenerative medicine and cancer therapy.

DMK MD StemZyme Treatment is a six-step biomimetic process based on how epidermal stem cells are physiologically regulated and expressed in the epidermis.

1. Access the Stem Cell Gateways
2. Stem Cells Trigger and Keratin Conformation
3. Stem Cells Activation
4. Stem Cells Fate Determination
5. Stem Cells Differentiation
6. Stem Cells Preservation
7. StemZyme Actives Safety Profile

A gene expression study was conducted where results were analysed in two arms. The first arm was for actives in StemZyme #2 (Stem Cell Trigger Step), StemZyme #3 (Stem Cell Activation Step), and StemZyme #5 (Stem Cell Differentiation Step). The second arm was for Vehicle Control. A qPCR was performed using validated TaqMan gene expression assays. The following genes were analyzed by using the full-thickness In vivo Skin Culture Model (EFT-400, MatTek), and Gene Expression Activity was measured:

Target genes: ITGA3, ITGA6, ITGB1, ITGB4, NOTCH1, NOTCH2, NOTCH3, JAG1, OCT4

(Please refer to the figure to understand the activity location of these genes)

Endogenous control genes: GUSB, PPIA

Conclusion

Ingredient	Purpose/Function	Formula(s)	Biological Activity
ITGA3	1.16	16% increase	Re-Epithelization
ITGA6	-1.58	37% decrease	Tumour Generation
ITGB1	Non Significant	Non Significant	Tumour Spread
ITGB4	-1.14	12% decrease	Invasive Carcinoma
JAG1	-1.37	27% decrease	Head Neck Tumour
NOTCH1	-1.39	28% decrease	Uncontrolled Cell Growth
NOTCH2	Non Significant	Non Significant	Uncontrolled Immune Cell Growth
NOTCH3	-1.34	26% decrease	Lateral Meningocele
OCT4	Non Significant	Non Significant	Cancer Stem Cells

Other Prevailing Approaches in Epidermal Stem Cell Treatments

Plant Stem Cells – Isolating Plant Stem Cells and infusing them in creams, lotions, and serums applied topically.

Pitfall – Plant Stem Cells are dead once they are isolated. Plant stem cells also have different histological fates and do not renew any epidermal skin organelle in Human Skin.

Small Peptides from Plant Stem Cells – Isolating small chain polypeptides and infusing them in cosmetic products.

Pitfall – Small chain Polypeptides are unstable and do not activate the stem cell pathway in human epidermal skin.

Stem Cell Facial or Stem Cell Face Lift with Micro-needling – Isolate stem cells from belly fat and inject through microneedling on the face.

Pitfall – These are not original epidermal stem cells, so they degrade quickly and do not differentiate into epidermal skin organelles. This is also an invasive procedure.

Chemical Exfoliation – Induce exfoliation and irritation to the stratum corneum and activate stem cells indirectly.

Pitfall – Stem cells are located in the interfollicular epidermis and bulge apparatus; simple chemical exfoliation and irritation do not induce mitotic activation in the basal layer.

StemZyme Therapy
Professional Products

StemZyme #1

This poor dilating serum helps loosen surface skin and activate stem cell reservoirs in the follicular bulge and rete ridges at the dermo-epidermal junction. It also clears the pathways for **StemZyme** phytoactive compounds to become bioadoptable. This formula has a moderate thermogenic effect that assists vasodilation, penetration of active ingredients, and stem cell renewal.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** assists in reducing the effects of impaired stem cell function that includes fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

StemZyme #1 serum increases the solubility coefficient of the stratum corneum with a moderate thermogenic effect. Thermogenesis helps enhance the deeper permeation of active ingredients in subsequent **StemZyme** Treatment steps. Thermogenesis also assists the bulge apparatus and rete ridges to start upregulating stem cell self-renewal.

Keratin conformation is the very next step of **StemZyme** Treatment. Because mTOR signalling pathways are essential in keratinocyte differentiation, this step requires thermogenesis 1 to 2 degrees higher than body temperature.

Key ingredients:

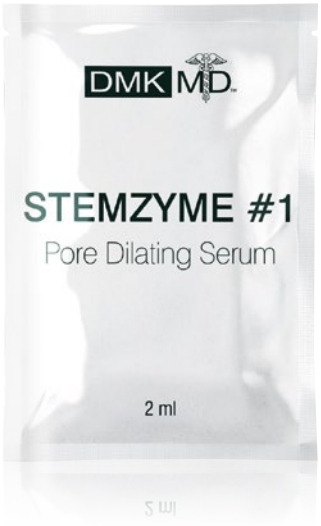
- **Madhuca longiofolia sophorolipids (butter tree)** – absorption enhancer.
- **Rubia cordifolia root extract (Indian madder)** – dilates papillary skin pores for better penetration.
- **Celastrus paniculatus seed extract** – activates bulge apparatus and epithelium at rete ridges.
- **Plumbago zeylanica root extract (ceylon leadwort)** – dilates follicular skin pores for better penetration.
- **Premna mollissima leaf/ root extract** – increases permeability of the corneocyte epithelium.

Prescription notes

- Use **StemZyme #1** during the first steps of the **StemZyme** protocol after completing **Enbioment** cleansing and **Alkaline Wash** treatments.

Professional directions

- Squeeze contents of a **StemZyme #1** packet onto fingertips. Press serum into the face, neck, and décolleté. Set a timer and leave it on the skin for 5 minutes.
- Remove serum from the skin after five minutes with damp 4x4s and proceed to the next steps outlined in the protocol.



StemZyme #2

This serum initiates the Keratin Conformation phase of the **StemZyme Treatment Protocol.** It is applied with the **DMK Hot & Cold Pack** to activate keratin remodelling. Heat and cold exposure increase keratin elasticity, induces molecular folding, and prevents cytolysis (cell disruption). The temperature flux also improves somatosensation (sense of touch), the anchoring of epithelial cells to the basement membrane, and cell-to-cell connections within the epidermis. This reinforces the skin's firmness and ensures healthy keratinocytes mature into healthy corneocytes as they migrate to the skin's surface.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** assists in reducing the effects of impaired stem cell function that includes fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

StemZyme #2 serum increases keratin elasticity. It initiates the precise molecular folding of keratinocytes, so they develop into healthy corneocytes. As keratinocytes are integrated with cell signalling, these cells modify epigenetic pathways and EPSCs to determine keratin's histological fate. Thermo and cryo exposure induces keratin remodelling and prevents cytolysis. Exposure to this temperature gradient improves somatosensation and the quality of keratin filaments. This serum also helps anchor hemidesmosomes and desmosomes to the basal membrane, interlinking them to reinforce the foundation.

Key ingredients:

- **Cyperus rotundus root extract (nutgrass)** – refolds keratinocyte proteins and reduces inflammation caused by improperly folded proteins.
- **Ocimum sanctum leaf extract (tulsi, holy basil)** – assists with restructuring keratinocytes and helps tactile or somatosensation.
- **Emblica officinalis seed extract (amla, Indian gooseberry)** – re-establishes keratinocyte communication and feedback loop.
- **Moringa oleifera seed oil (moringa)** – reduces oxidation and oxidative stress loads on keratinocytes.
- **Piper nigrum seed extract (black pepper)** – loosens up dead keratinocytes.
- **Madhuca longiofolia sophorolipids (butter tree)** – enhances tissue permeability and formula penetration.

Professional directions

- Empty the first packet of **StemZyme #2** onto your fingertips. Apply product to face, neck, and décolleté. Gently effleurage into the application area for 30 seconds.
- Place **DMK Hot Pack** on designated treatment areas for 60 seconds. Remove and set aside.
- Place **DMK Cold Pack** on designated treatment areas for 30 seconds. Remove and set aside.
- Repeat hot (60 sec.) and cold (30 sec.) pack application for one more cycle.
- Empty contents of second packet of **StemZyme #2** on fingers and effleurage into face, neck, and décolleté for 30 seconds.
- Repeat hot (60 sec.) and cold (30 sec.) pack application for two cycles.
- Empty 3rd packet of **StemZyme #2** in the same manner as before to the face, neck, and décolleté, and gently effleurage for 30 seconds.
- Repeat hot (60 sec.) and cold (30 sec.) pack application for two cycles.
- Leave remaining **StemZyme #2** residue on the skin and proceed to the next steps outlined in the protocol.

Prescription note

- Use **StemZyme #2** after applying and removing **StemZyme #1**.

StemZyme #3

This skin nutrient paste is used during the **StemZyme Protocol** underneath **Enzyme Masque #1.** The nutritional and bioactive substances in the paste are responsible for optimizing, increasing, and controlling stem cell turnover in the epidermis.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** is the ultimate Age Management treatment, reducing the effects of impaired stem cell function. Effects include fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

To maintain a viable EPSC population, half the daughters of stem cells in each generation must remain as stem cells. One stem cell could generate two similar daughter cells whose fates would be governed by their forthcoming epigenetic exposure. At the other polar end, the stem cell division could be odd, producing one daughter cell that inherits the stem-cell character and another that inherits factors that force it to embark on terminal differentiation into other epidermal cells.

When **StemZyme #3** is used with DMK Enzyme treatment, it creates a favourable epigenetic environment for EPSCs to maintain SC status and favours histological guidance to terminally differentiating SCs.

Key ingredients:

- Elaeocarpus angustifolius fruit/ seed extract (blue quandong, blue marble tree) – initiates epidermal stem cell mitosis.
- Emblica officinalis fruit extract (amla) – maintains epidermal stem cell mitosis and structure.
- Withania somnifera root (ashwagandha, Indian ginseng) – stimulates epidermal stem cell mitosis.
- Boerhavia diffusa rootextract (punarnava, red spiderling, hog weed) – regenerates targeted stem cells in bulge apparatus and dermal papilla reservoirs.

Professional directions

- Empty the contents of the **StemZyme #3** packet onto your fingertips. Effleurage into the face, neck, and décolleté.
- Leave on the skin for two to three minutes and proceed to the next steps outlined in the protocol.

Prescription note

- Apply **StemZyme #3** after **StemZyme #2** protocol. **StemZyme #3** is used under **Enzyme Masque #1.**

StemZyme Therapy
Home Prescriptives

StemZyme #4

This skin stabilising emulsion acts as a 'guide' for newly activated stem cells. It is used immediately after the **Enzyme Masque #1** step of the StemZyme protocol and then again at 8-hour intervals for the first 72 hours (3 days) after a **StemZyme Treatment.** The actives in **StemZyme #4's** Skin Stabilising Emulsion support new cell division and cellular processes of the stem cells, including cell differentiation.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** is the ultimate Age Management treatment, reducing the effects of impaired stem cell function. Effects include fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

After a mitotic trigger, it is vital to stabilize cell division for 72 hrs (the amount of time needed for SCs with neighbour cells to coordinate). **StemZyme #4** helps stabilise and coordinate mitosis until a cell completes its life cycle. It also assures that further cascades of protein signal networks form correctly.

Key ingredients:

- **Amaranthus caudatus seed oil (amaranth)** – provides proper nutrients to stem cells.
- **Asparagus racemosus root (shatavari)** – regenerates stem cells at bulge reservoir in hair follicles.
- **Emblica officinalis fruit extract (Indian gooseberry)** – stimulates and supports stem cell mitotic activity.
- **Hemidesmus indicus extract (Indian sarsaparilla)** – increases blood flow through the capillaries.
- **Madhuca longifolia sophorolipids (butter tree)** – enhances tissue permeability and formula penetration.
- **Nelumbo nucifera seed extract (sacred lotus)** – maintains the genetic coding and integrity of activated stem cells.

Professional directions

- Dispense two pumps of **StemZyme #4** (or one packet) and gently pat into the face, neck, and décolleté with fingertips. Wait 15 seconds and proceed to the next step outlined in the protocol.

Prescription notes

- Use **StemZyme #4** after the **Enzyme Masque #1** portion of the **StemZyme** protocol.
- Clients use **StemZyme #4** as a Home Prescriptive in eight-hour intervals, days one to three following their in-clinic **StemZyme** treatment.

StemZyme #5

Applied 2 minutes after **StemZyme #4 Skin Defining Emulsion,** is used during the **StemZyme Protocol** and as part of the first 72 hours after **StemZyme** clinical treatments. This formula assists in regulating the stem cell differentiation pathways of new daughter cells after mitosis. Transit-amplifying (TA) cells are stem cells that have three possible fates- apoptosis (cell death), senescence (cell aging), or terminal differentiation (maturing into a specialised cell). **StemZyme #5** Skin Defining Emulsion directs stem cells towards terminal differentiation, in which they become epidermal skin cells.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** is the ultimate Age Management treatment, reducing the effects of impaired stem cell function. Effects include fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

StemZyme #5 primes newly formed EPSCs to transform into TA cells through various gene expression profiles and cell cycle regulations. TA cells can have 3 fates: apoptosis, senescence, and terminal differentiation. **StemZyme #5** restricts TA cells programmed for the first two fates.

Key ingredients:

- Trigonella foenum-graecum seed extract (fenugreek) – helps redistribute subcutaneous adipocytes.
- Asparagus racemosus root extract (rhatavari) – to differentiate new stem cells in papillae.
- Ficus lacor bark extract (java fig), ficus racemosa bark extract (cluster fig), ficus religiosa bark extract (sacred fig, pepal), ficus benghalensis bark/ bud extract (banyan), thespesia populneoides bark extract (Indian tulip) – combined, the fig/tree extracts support the differentiation of new daughter cells into keratinocytes.
- Glycyrrhiza glabra stem extract (licorice) – melanin and pigmentation regulator.
- Withania somnifera root extract (ashwagandha) – to differentiate new stem cells in stratum corneum, granulosum, and lucidum.
- Madhuca logifolia sophorolipids (butter tree) – enhances tissue permeability and formula penetration.

Professional directions

- Dispense two pumps of **StemZyme #5** (or one packet) and gently pat into the face, neck, and décolleté with fingertips. Wait 15 seconds to absorb fully.
- Finish with a layer of **DMK Sunscreen** during daylight hours.

Prescription notes

- Use **StemZyme #5** two minutes after applying **StemZyme #4.**
- After applying **StemZyme #5** and DMK sunscreen in-clinic, schedule follow-up **Enzyme #1** Treatments with the client on days 12, 24, 36, and 48 of their StemZyme Treatment.
- Clients use **StemZyme #5** as a Home Prescriptive in eight-hour intervals, days one to three following their in-clinic StemZyme treatment.

StemZyme #6

StemZyme #6 Age Management Skin Lotion is applied to the skin at 12-hour intervals beginning on day four and continuing through the treatment. It assists in renewing the body's turnover rhythm and replenishing epidermal stem cells. Disruption of the skin's natural clock alters the circadian rhythm of DNA repair and replication, stem cell ageing, and tissue repair.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** is the ultimate Age Management treatment, reducing the effects of impaired stem cell function. Effects include fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function:

Ongoing regular regeneration of EPSCs and preservation of their physiological structure is vital to counteracting the age-related modification of the epidermis. **StemZyme #6** aids in efficient EPSC reciprocation to the circadian rhythm of various physiological systems.

Key ingredients:

- **Buchanania lanzan seed extract (chironji, charoli)** – regulates keratinocyte pigmentation.
- **Aegle marmelos fruit extract (bael, stone apple)** – prevents premature apoptosis of stem cells.
- **Mimosa pudica root extract (sleeping grass, touch me not)** – strengthens cells at the basal layer.
- **Gossypium herbaceum root extract (levant cotton)** – generates cells at the basal layer of the epidermis.
- **Madhuca longifolia sophorolipids (butter tree)** – enhances tissue permeability and formula penetration.

Professional directions

- Cleanse the face, neck, and décolleté morning and night using 1 pump of **Enbioment Cleanser.**
- Apply two pumps of **StemZyme #6,** followed by two pumps of **StemZyme #7** to the entire face, neck, and décolleté.

Prescription note

- Clients use **StemZyme #6** as a Home Prescriptive days 4 - 50 following their in-clinic **StemZyme** treatment.

StemZyme #7

StemZyme #7 Skin Refining Lotion is applied to the skin at 12-hour intervals beginning on day four through the remaining course of the treatment period. This formula works in tandem with the **StemZyme #6** Age Management Skin Lotion to support the maturation and differentiation of new epidermal stem cells.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** is the ultimate Age Management treatment, reducing the effects of impaired stem cell function. Effects include fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

Ongoing appropriate terminal cell differentiation of EPSCs (Daughter Cells) is vital to epidermal homeostasis. **StemZyme #7** assists in directing newly formed TA cells in their differentiation pace and final formation in various epidermal organelles.

Key ingredients:

- **Madhuca longifolia sophorolipids (butter tree)** – enhances tissue permeability and formula penetration.
- **Ricinus communis root extract (castor)** – controls stem cell turnover rate.
- **Piper nigrum seed extract (black pepper)** – prevents mutation of stem cells.
- **Tinospora cordifolia stem extract** – prevents immunogenic shock to stem cells.
- **Trigonella foenum-graecum seed extract (fenugreek)** – helps rebuild ridges along the dermo-epidermal border.

Professional directions

- Cleanse the face, neck, and décolleté every morning and night using 1 pump of Enbioment Cleanser.
- Apply two pumps of **StemZyme #6,** followed by two pumps of **StemZyme #7** to the entire face, neck, and décolleté.

Prescription notes

- Clients use **StemZyme #7** as a Home Prescriptive days 4 - 50 following their in-clinic StemZyme treatment.

StemZyme Dietary Supplement

StemZyme Integumentary System Support is a potent blend of botanical extracts. When used with DMK Skin revision treatments, this supplement supports collagen production for firmer, more vibrant skin and provides additional support to new epidermal stem cells.

When used with DMK Skin revision treatments, this supplement supports collagen production for firmer, more vibrant skin and provides additional nutritional support to new epidermal stem cells. The nutrients in this supplement also balance pigmentation and blood flow, improve somatosensation, reduce inflammation, modulate the immune system, and prevent mutation and oxidative damage.

Diagnostic

StemZyme is suitable for anyone ages 10+. The **StemZyme** Treatment System targets aged and declining epidermal stem cells, charging them with new vitality and regulating cellular proliferation. **StemZyme** is the ultimate Age Management treatment, reducing the effects of impaired stem cell function. Effects include fine lines and wrinkles, sagging skin, uneven skin tone, discolouration, uneven texture, dullness, sallow complexions, enlarged pores, dry skin, and hyper-reactive skin.

Function

StemZyme Integumentary System provides the necessary cellular energy to epidermal stem cells and strengthens newly formed epidermal organelles. The nutrients in this supplement also balance pigmentation and blood flow, improve somatosensation, reduce inflammation, modulate the immune system, and prevent mutation and oxidative damage.

Key ingredients
(pending TGA confirmation)

- Asparagus racemosus root extract (shatavari) – This ingredient regenerates stem cells at the bulge apparatus in hair follicles. It also helps differentiate new stem cells in papillae and strengthens the basement layer of the epidermis. Shatavari is antioxidant, reduces wrinkles, and soothes inflammation.
- Centella asiatica leaf extract (gotu kola) – Gotu kola is a herb in the parsley family that contains amino acids and madecassoside, a wound-healing antioxidant. This herbal extract improves texture, fights inflammation, counteracts glycation, and promotes fibroblast proliferation and collagen synthesis. It also improves tactile sensation.
- Curcuma Longa Rhizome Extract (Turmeric) – Curcumin is derived from turmeric. This phytonutrient is antioxidant, anti-inflammatory, antiviral, antibacterial, and antifungal. In addition, it exhibits wound healing benefits, from decreased healing time to increased fibroblast and vascular density. Curcumin reduces the signs of aging and pigmentation by increasing collagen and inhibiting melanin synthesis.
- Tinospora cordifolia stem extract – Giloy strengthens and modulates the skin's immune system, protecting stem cells from immunogenic shock. It also has anti-allergenic properties.
- Withania somnifera root extract (ashwagandha) – Stimulates epidermal stem cell mitosis and helps stem cells differentiate in the stratum corneum, stratum granulosum, and stratum lucidum. Ashwagandha protects the elasticity of both healthy and damaged

keratinocytes. It is also antioxidant, anti-inflammatory, soothes stressed skin, and may protect against screen-emitted HEV light.

- **Piper nigrum seed extract (black pepper)** – Pepper helps loosen dead keratinocytes from the skin's surface. It is also antioxidant, preventing stem cells from denaturing or mutating through oxidative damage.
- **Crocus sativus stamen extract (saffron).**

Professional directions

- Take 4 capsules daily with food – 2 capsules in the morning and 2 capsules in the evening.

Prescription note

- Pregnant or breastfeeding women should not use it without a doctor's approval.
- Consult a physician first if you have high blood pressure, heart disease, or take prescription medication.
- Not recommended for children.

StemZyme Therapy Protocol

StemZyme Protocol

Day 1

- Cleanse the face, neck and décolletage with **Enbioment Cleanser.**
- Remove cleanser residue with two damp cotton pads.
- Using one of the **DMK Applicator Brushes,** apply **Exoderma Peel** on the hairline and eyebrows.
- Apply **Alkaline Wash** to the face, neck and décolletage, leaving on for 30 secs, then remove. This can be done in sections to ensure the solution is left on no longer than 30 seconds if needed.
- Apply **Exoderma Peel** immediately with the other **DMK Applicator Brush,** remove after 5 min with a warm, moist towel.
- Apply **StemZyme #1** with fingertips, pressing into skin, leave for 2-3 minutes then remove.
- Apply **StemZyme #2** with fingertips, lightly effleurage into the skin for 30 secs, do not remove.
- Apply **DMK Hot Pack** onto the face for 1 min.
- Place **DMK Cold Pack** onto the face for 30 secs.
- Repeat **DMK Hot & Cold Pack** (Hot 1 min, Cold 30 secs)
- Apply **StemZyme #2** with fingertips, lightly effleurage into the skin for 30 secs, do not remove.
- Do two more rounds of the **DMK Hot & Cold Pack** (Hot 1 min, Cold 30 secs).
- Apply **StemZyme #2** with fingertips, lightly effleurage into the skin for 30 secs, do not remove.
- Do two more rounds of the **DMK Hot & Cold Pack** (Hot 1 min, Cold 30 secs).
- Apply **StemZyme #3** with fingertips, lightly effleurage into the skin and leave on for 2-3 mins, do not remove.
- Apply **Enzyme Masque #1** with **Aqua D'herb,** remove after 45 mins.
- Apply **Stemzyme #4** with fingertips and gently pat into the skin, wait 15 secs then apply **StemZyme #5** over the top and gently pat into the skin for another 15 secs, do not remove.
- Apply **DMK Sunscreen.**

Please refer to the **Home Prescriptives Manual** and **Protocols Manual** for product references.

Day 12

- Apply **Enzyme #1 Treatment Kit.**

Day 24

- Apply **Enzyme #1 Treatment Kit.**

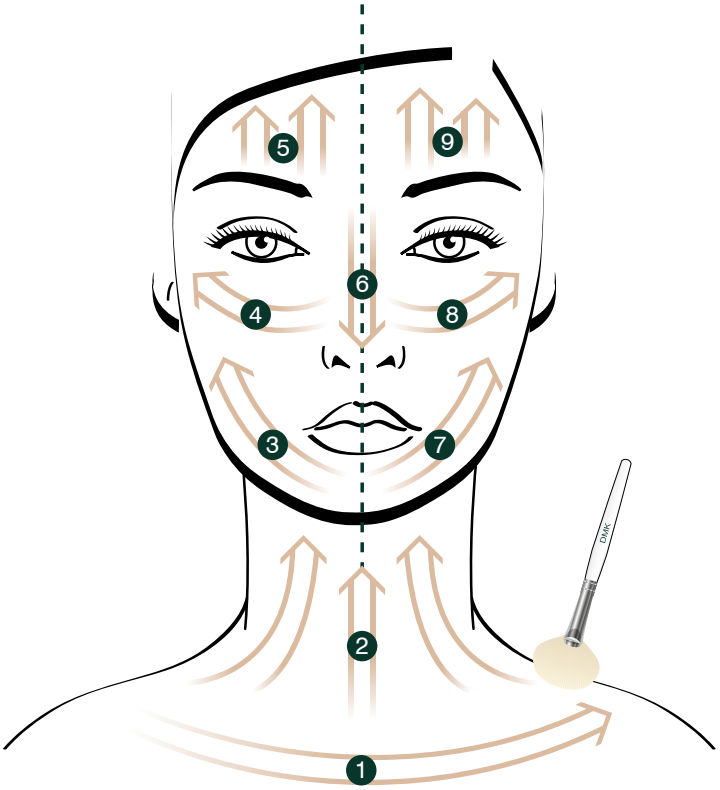
Day 36

- Apply **Enzyme #1 Treatment Kit.**

Day 48

- Apply **Enzyme #1 Treatment Kit.**

Note: More **Enzyme Treatments** can be performed if necessary.

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StemZyme Home Prescriptives

Day 1-3

- Cleanse the face, neck, and décolletage every 8 hours (a total of 3 times daily) using **Enbioment Cleanser.** Pat the skin dry.
- Dispense 2 pumps of **StemZyme #4** and gently pat into the face, neck, and décolleté with fingertips. Wait 15 seconds.
- Repeat the above step with **StemZyme #5.**
- During daylight hours, apply **DMK Sunscreen.**
- Take 4 capsules of the **StemZyme Dietary Supplement** per day – 2 capsules in the morning and 2 capsules in the evening.

Note: Do not apply makeup or any other products, except those listed, for the first 3 days after the initial **StemZyme Treatment.** Advise clients to use more serum than they usually would, saturating the skin. Any leftover product will be ineffective for future treatments and must be thrown out.

Day 4-50

- Cleanse the face, neck, and décolleté with **Enbioment Cleanser** every morning and night.
- Apply 2 pumps of **StemZyme #6,** followed by 2 pumps of **StemZyme #7** to entire face, neck, and décolletage.
- Take 4 capsules of the **StemZyme Dietary Supplement** per day – 2 capsules in the morning and 2 capsules in the evening.

Note: You may apply additional **DMK Home Prescriptives** as usual (excluding specialty products) including **EFA Ultra.**

StemZyme Therapy & Enbioment

StemZyme with Enbioment

Epidermal adult stem cells and the skin microbiome function in a tandem feedback loop pattern. To nullify any underlying sub-clinical inflammation or immune reaction, **Enbioment** is combined with **StemZyme** via the following:

Applications

- Prescribe one week of the **Enbioment Microbiome System** as a preparation phase before **StemZyme Treatment.**
- This will allow the skin to take advantage of activated skin microbes and enhance the outcome of **StemZyme Treatment.**
- Prescribe one week of the **Enbioment Microbiome System** after 50 days of **StemZyme Home Prescriptives.**
- This will allow the skin to use activated microbiotas to stabilize the stem cell feedback loop.

StemZyme & Enbioment with Acne Therapy

Skin microbiota plays an essential role in epidermal infections; epidermal stem cells actively participate in sebaceous gland revival.

Applications

- Prescribe **Enbioment Microbiome System** for one week before starting acne therapy.
- In-clinic, use **Enbioment Cleanser** before **Alkaline Wash.** After neutralisation, apply **Enbioment Mist** and **Serum,** followed by **Enzyme Masque #1.**
- Prescribe **Enbioment Microbiome System** for one week after acne therapy with **Home Prescriptives.**
- Three weeks after completing acne therapy, perform an in-clinic **StemZyme Treatment,** acne and **StemZyme Home Prescriptives** may be used together.

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StemZyme & Enbioment with RP Therapy

Skin microbiota plays a vital role in epidermal proliferation; epidermal stem cell activation triggers epidermal rejuvenation.

Applications

- Prescribe the **Enbioment Microbiome System** for one week before the first day of **Revitosin & Pro Alpha** application.
- Prescribe the **Enbioment Microbiome System** for one week after epilation is complete.
- Perform **StemZyme Treatment** in-clinic one week after the prior **Enbioment System** is complete.

StemZyme Therapy for Aesthetic Procedures

StemZyme with Botox

Botulinum neurotoxins (BoNTs) are metalloproteases that act on nerve-muscle junctions to block exocytosis through a particular and exclusive endopeptidase activity. This block works against soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins of presynaptic vesicle fusion machinery.

Toxins' ability to produce flaccid muscle paralysis through chemical denervation is well-utilized in esthetic medicine. Each commercially available BoNT formulation is unique, differing mainly in molecular size and composition of complex proteins, biological activity, and antigenicity. The nerve terminal intoxication by BoNTs is entirely reversible, and the duration of therapeutic effects of BoNTs varies for different serotypes. BoNTs can block the cholinergic neuromuscular or cholinergic autonomic innervation of smooth muscles.

Localised effects unrelated to the toxin may include bruising at the injection site, local edema, erythema, and transient numbness. Headaches commonly occur within the first 24 hours after BoNT administration but usually become less frequent with repeated injections.

When onabotulinumtoxinA is injected beneath the skin, it paralyses the underlying muscles so that overlying skin wrinkles, rhytides, and deeper creases are tightened and reduced. OnabotulinumtoxinA is also known to inhibit the release of excitatory neurotransmitters from the motor and sensory neurons by preventing vesicle fusion to the cell membrane. OnabotulinumtoxinA also exerts a direct analgesic effect on the skin.

Side Effects of OnaboulinumtoxinA May Include:

- Redness, Infection, Pain, and Bruising
- Skin discolouration, lumps, and scarring at the injection site
- Expressionless' skin, where it becomes difficult to show emotion due to face tightness

These side effects can be avoided or reduced if **StemZyme Therapy** is performed in-clinic at least five days after Botox Injections.

Applications

- Perform **StemZyme Therapy** 5 days before Botox injections for an additional analgesic effect.
- If **StemZyme Follow-up Enzyme Treatments** (day 12th, 24th, 36th) occur around Botox injections, then only perform **Enzyme Masque #1** on the client (Should not include **Muscle Banding with Enzymes #2 and #3**).
- **StemZyme Dietary Supplement** should be taken for at least 120 days after onabotulinumtoxinA is in situ.

StemZyme & Enbioment with Derma Fillers

Derma Filler Parameters	Do StemZyme & Enbioment Influence	StemZyme & Enbioment Therapy Effect
Retention Time	Yes	Can increase treatment retention time
Metabolism	Yes	Helps reduce or prevent the skin from metabolizing (degrading) Derma Fillers
Inflammation	Yes	Helps reduce or avoid inflammation
Distribution	Yes	Can avoid/reduce lumping and enhance equal tissue distribution
Hemorrhage	Yes	Can minimize internal micro capillary hemorrhaging
Collagen Build Up	Yes	Can increase the half-life of skin without building up AGEs

Applications

- **StemZyme Therapy** should be performed 24 hrs after Derma Filler is in situ.
- **StemZyme Dietary Supplements** should be continued 120 days from the Derma Filler is in situ.
- Prescribe **Enbioment Therapy** for 1 week as preparation for Derma Fillers, partly because Microbiota influences all parameters mentioned earlier.


DMK MD.
ENBIOMENT™ CLEANSER
Microbiome System Probiotic Restoring Cleanser
150 ml (5.07 fl oz)
DMK MD.
ENBIOMENT™ MIST
Microbiome System Probiotic Restoring Spray
100 ml (3.4 fl oz)
DMK MD™
ENBIOMENT™ SERUM
Microbiome System Probiotic Restoring Emulsion
30 ml (1 fl oz)

Post-Treatment Side Effects, Hazards, and DMK Corrective Applications

Microneedling & Dermabrasion

Side Effects and Post-treatment Hazards

- Scarring
- Inflammation
- Oedema
- Mutagenicity
- Hyper proliferation
- Microgranulomas
- Micro keloids
- Erythema
- Dysbiosis

Corrective Applications

- **DMK StemZyme Therapy** to reduce scarring, inflammation, and pre-empt epidermal hyperproliferation.
- **Enbioment Therapy** for 4 weeks immediately after the procedure to re-establish eroded skin microbiota and avoid dysbiosis.
- **DMK Home Prescriptives – Wetter Than Water, FirMatrix, TransGenesis, Beta Gel,** and **Contraderm** for 6-8 weeks.

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