# *Molecular aspects of skin ageing: recent data*

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ABSTRACT

**As our body's envelope, the skin acts as a biosensor with the environment and reflects our personality. Skin ageing is therefore an important and interesting topic of study. It results from the combination of intrinsic ageing and photoageing, which is due to the environmental influence, such as reactive oxygen species (ROS). The more recent data are gathered here to remind current knowledge about skin ageing, from a molecular level to the clinical signs, wrinkles and spots mainly. Because knowledge of the preferential biological targets of ageing has recently been making progress, it is possible to delay the manifestation of ageing, by acting on key biological processes.**

KEY WORDS

**skin ageing, DNA, Asian skin, reactive oxygen species, wrinkle, cell senescence**

## *1. Introduction*

Humanity is ageing. The average life expectancy of people living in industrialized nations has doubled since 1900. This will result in social, economic, and healthcare changes that will, in turn, drive public policy worldwide. The cosmetic industry is moving to cater for the ageing population by developing more innovative products. The skin provides a large interface with the environment, and is thus of prime importance. The major changes in the skin that occur with age are a loss of elasticity and a reduction in its protective function. These changes do not just affect the elderly, as they begin when people are younger than thirty. The extent of change depends to a large degree on how much the skin is exposed to sunlight and how stressful is a person's lifestyle.

For years, people have attempted to hide, reverse or control changes in their skin, such as wrinkling, roughness, mottling, blotching and dryness. Skin of elderly people is thin and fragile, due to complex changes very often summarized to reduced dermal collagen and decreased cell proliferation. Skin ageing is thought to result from two processes, intrinsic ageing and extrinsic, or photoageing (1, 2). Intrinsic ageing is believed to be genetically programmed and is thus presented as independent of all external and environmental influences. Extrinsic ageing is due to UV radiation and other environmental insults; that accelerate the skin changes (3). Photoageing leads to a rough/dry/leathery skin, a yellow/dull complexion, lentigines or actinic spots and wrinkles. The study of the biology of ageing has made

rapid progress recently, after decades of stagnation. This paper reviews the most recent data on skin ageing, from the cell nucleus to tissue, and discusses their consequences for the skin.

## *2. Damage due to Reactive Oxygen Species (ROS)*

Most people agree that free radicals are most important agents of ageing. Organic molecules absorb light or UV irradiation and become excited because an electron is transferred to a higher orbital. The excited molecule is a free radical which may cause secondary reactions and damage to various constitutive molecule (4). The energy storage molecule, adenosine triphosphate (ATP) is produced by oxidative phosphorylation in the mitochondria. The energy is produced by the oxidation of reducing equivalents of nutrients via the respiratory chain. However, mitochondria also regulate the intracellular calcium concentration and apoptosis. Abnormaly increased membrane potential is linked to ROS (Reactive Oxygen Species) augmentation and mitochondrial DNA (mtDNA) mutations, constitutive to skin ageing.

ROS are normally produced by mitochondria, but they can also be produced following an external stress. Normal metabolism gives rise to most ROS, primarily via the mitochondrial respiratory chain, in which excess electrons are donated to molecular oxygen ($O_2$) to generate a superoxide anion ($O_2\bullet^-$). The superoxide anion is reduced by the enzyme superoxide dismutases (SOD) to hydrogen peroxide ($H_2O_2$), which, in turn, is reduced to water by catalase, located in the primary peroxisomes, and by glutathione peroxidase (GPx), located in the mitochondria and cytosol. There are three isoforms of SOD (SOD 1 in the cytosol, SOD 2 in the mitochondria and SOD 3 in the extracellular space). Hydrogen peroxide can be converted to the highly toxic hydroxyl radical (OH•) in the presence of transition metals, and all three of the ROS (OH•, $O_2\bullet$, $H_2O_2$) can damage macromolecules directly or indirectly (5).

ROS are responsible for structural and functional alterations of cellular membranes, polyunsaturated fatty acids, proteins and DNA (6). For example, recent studies have shown that mitochondrial aconitase, an enzyme of the citric acid cycle that is critical for controlling the rate of ageing, is a target of oxidative damage (7). Cells also contain antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, and reducing agents like vitamin E and glutathione (8-10). Ageing is associated with a decrease in the plasma concentration of antioxidants, such as glutathione, and with increases in markers of oxidation damage, such as lipid peroxidation products (11,12);. The total glutathione concentration in cultured skin fibroblasts decreases with age, while glutathione reductase activity is unaffected. The antioxidant defenses are less well developed in the early stages of life than in postnatal life. (13).

**Table 1. Reactive Oxygen species.**

| FREE RADICALS | | Half Life (at 37°C) |
|---|---|---|
| Hydroxyl | OH • | 0.3 ns |
| Superoxide | $O_2\bullet^-$ | 0.4 ns |
| Nitric oxide | NO • | Seconds |
| Peroxynitrite | ONOO(-) | 50 ms – second |
| Singlet oxygen | $^1O_2$ | µs |
| Hydrogen peroxide | $H_2O_2$ | Stable |
| Peroxide | LOOH | Stable |

The first changes that occurs in the skin after chronic or acute UV irradiation is the generation of reactive oxygen species, leading to the peroxidation of unsaturated lipids in the cell membrane. Low phototype individuals are more likely to produce large amounts of ROS after exposure to UVA, in particular singlet oxygen that can diffuse across the cell membrane. For example, the oxidation of catalase by singlet oxygen gives rise to more acidic conformers. The integrity of catalase may be a marker of the stress due to exposure to UVA (14). Singlet oxygen, due to its reactivity, appears more and more as a powerful free radical able to damage numerous skin components.

### *Mitochondria: an important target*

The mitochondrial theory of ageing states that mitochondria are the main site for generating free radicals and reactive oxygen in the cell. Thus, mitochondria are vulnerable to oxidative stress and damaged mitochondria can cause an energy crisis in the cell, leading to senescence and tissue ageing. An accumulation of damage decreases the cell's ability to generate ATP, so that cells, tissues, and individuals function less well. There is now considerable evidence that mitochondria are altered in the tissues of ageing individuals and that the damage to mtDNA increases 1000-fold with age. The phagocytic lysosome system for removing mitochondria is also considerably altered in the cells of ageing organisms. Thus, damaged mitochondria play an important role in apoptosis. The survival of the whole cell may depend on the release of caspase activators such as cytochrome C from the mitochondria (15). The extent of this alteration during senescence is still debated (16,17). Oxidative stress increases the production of free radicals and the inner membrane of the mitochondrion is chemically and physically altered. ROS are normally produced in mitochondria because of ubiquinones and the cytochrome b family (Complex III). Complexes I and IV are less vulnerable, while complexes II, III and V are affected by oxidative stress, via damage to lipids

and proteins (18). The electron transport chain is thus compromised, leading to energy supply failure and cell death (19). The mutation rate of mitochondrial DNA is ten-times higher than that of nuclear DNA. Indirect observations suggest there is transport of nucleic acids between mitochondria, which helps to repair the damaged mitochondrial genome (20). Damage to mtDNA will block mitochondrial turnover and replication, leading to decline in ATP production and protein synthesis. The accumulation of 8-hydroxydeoxy guanine (8-oxodG) in mitochondria also indicates great oxidative damage (21-23). Exposure of the human skin to solar radiation leads to an accumulation of mtDNA mutations, caused partly by oxidative damage, and these mutations play an important role in photoageing (24).

### *Genes and cellular pathways*

Alterations in oxidative metabolism and the cell redox state can affect many genes and cellular pathways. The influence of oxidation on mitogenic responses and signal transduction pathways, such as MAP kinase and NF-êB, are well documented (25). Human fibroblasts exposed to several oxidative stresses also develop markers of replicative senescence. Some genes, such as those encoding fibronectin, osteonectin, α1(I)-procollagen, apolipoprotein, SM22 (putative Ca binding protein overexpressed in senescent fibroblasts), and GTP-α binding protein are overexpressed. The mitogenic response to several growth stimuli (serum, PDGF, basic FGF and EGF) is lost (26). The reduction in connective tissue growth factor by UV radiation may contribute to the reduced procollagen synthesis observed in UV-irradiated normal human skin (27).

Peroxynitrite ($ONOO^-$) is generated from the transducer molecule nitric oxide (NO) and superoxide anions ($O_2^{\bullet -}$) under pathological conditions. MMPs and pro MMPs have been shown recently to be activated by peroxynitrite *in vitro* (28). The activation of poly(ADP-ribose) polymerase by peroxynitrite is also implicated in the pathogenesis of various inflammatory conditions and injuries. Tyrosine nitration is mostly mediated by peroxynitrite, a cytotoxic oxidant derived from nitric oxide that can cause DNA breaks. Peroxynitrite inhibits cell proliferation and high concentrations are also cytotoxic. Peroxynitrite and poly(ADP-ribose) polymerase also seem to be involved in the regulation of keratinocyte function and death in contact hypersensivity (29). Nitrogen dioxide and carbonate radical anion must also be taken in consideration. Nitrogen dioxide can be produced from excessive nitric oxide autoxidation in hydrophobic environments such as cell membranes (nitrated lipids) and the interior of proteins (nitrated proteins); carbonate radical anions are produced in strong oxidative conditions and can oxidize nucleic acid guanine residues, GSH and proteins (30).

## *3. Proteins Oxidation and its consequences*

Thin, wrinkled skin is very often attributed to a lack of collagen. The dermis and overlying epidermis of ageing skin are profoundly altered (31). Slower protein synthesis is one of the most common events observed during ageing. The synthesis of both structural proteins, such as collagen, and enzymes that repair and maintain the normal metabolic functions of the cell, is slowed down. This leads to the inefficient removal of damaged molecules and decreased intra-and intercellular signaling pathways. The age-related increase in oxidized proteins may also be linked to modifications of proteins caused by lipid peroxidation products (32- 35). Age-related changes in fibroblasts due to the metal-catalyzed oxidation of proteins lead to an exponential increase in the concentration of protein carbonyl groups in tissue samples taken from people aged 10 to 80 years.

Oxidative damage to proteins may be most important in ageing, because oxidized proteins become inactive and can accumulate in the cell, thereby triggering programmed cell death. ROS increase the carbonyl content of proteins by forming aldehydes and ketones from certain aminoacid residues (36,37). The concentration of adenine nucleotide translocase, a protein in the inner mitochondrial membrane that is tightly bound to six molecules of cardiolipin, which contains highly unsaturated fatty acids, also decreases with age. This protein is the primary intracellular site for the generation of superoxide anion and exhibits adducts of the lipid peroxidation product, 4-hydroxynonenal, a powerful oxidative aldehyde. The concentration of cardiolipin may be a marker of the real age of the cell, based on its energetic capacity (38).

### *Proteasome involvement*

Protein oxidation *in vivo* is a natural consequence of aerobic life and the proteasome complex is responsible for the selective degradation of oxidized proteins. The age-related increase in the concentration of oxidized proteins is partly due to the cell's decreased capacity to degrade them. Lysosomal proteases and the proteasome complex normally degrade oxidized proteins. The 26S proteasome units selectively recognize and degrade oxidized proteins in the cytoplasm, the nucleus and the endoplasmic reticulum. The proteasome activity (multicatalytic proteinase MCP) involved in degrading oxidized proteins may be reduced (39). One of the lipid peroxidation products, 4-hydroxy-2-nonenal (HNE), can cross-link proteins via their lysine residues. The accumulation of oxidized protein, lipofuscin and/or ceroid pigments during ageing may be due to the changes produced in proteins by HNE and their subsequent inhibition of the proteasome unit

(proteasome 20S). This would lead to a vicious circle of cytotoxic protein oxidation products. (34). Oxygen stress (especially 4-hydroxy-2-nonenal) may attack proteins directly or through lipid peroxidation, to inhibit enzymatic activity. Oxidative damage to membrane transport proteins leads to alteration of the intercellular concentrations of calcium and potassium. The activity of the cytosolic proteasomal system also declines during the proliferative senescence of human fibroblasts (40). UVA and UVB irradiation both alter proteasome function in human keratinocytes (41, 42). While UV-induced skin damage is ameliorated by retinol and all-trans retinoic acid, UV irradiation blocks retinoid signaling in human skin through the ubiquitin/proteasome-mediated degradation of nuclear retinoid receptors (43, 44). In the future, it would be interesting to focus on the existence of preferential protein targets.

### *Glycation*

Cross-links can also form between proteins by coupling glucose carbonyl group to aminoacids such as lysine. These compounds called Advanced Glycation End products (AGEs) bind covalently to other proteins, and can cause extensive damage. The collagen lattice formed by cross-linked type I collagen is undeformable (unglycated collagen is fully compactible). Cross-linking collagen fibrils also alters the physical and mechanical properties of the extracellular matrix and changes the organization of the intracellular actin cytoskeleton (45). Glycated collagen may modify normal cell adhesion (46). As adhesion is a fundamental cell function, each alteration can damage cell behaviour (apoptosis, etc) and then change tissue homeostasis. The dermis and elastic fibre network become glycated in people over 35 years of age and solar irradiation appeared to enhance it (47). The fluorescence of epidermal tryptophan moieties and collagen cross-links in the dermal matrix can also be considered to be good *in vivo* markers of photoageing (48). While there are very high concentrations of antioxidant enzymes (catalase, SOD) in the epidermis, the concentrations are much lower in the dermis. Protein glycation and advanced glycation may be inhibited by antioxidant components (49). Photoaged skin has significantly reduced concentrations of antioxidant enzymes in the stratum corneum and the epidermis, while the concentration of oxidized proteins in the upper dermis is increased. Acute exposure to UV irradiation depletes the catalase activity in the skin and increases protein oxidation (50).

## *4. Dermal Matrix alteration*

### *Fibroblasts*

The importance of cytokines, and immune cell homeostasis for ageing and its clinical signs is still not clear. Normal concentrations of cytokines seem to be required for skin cell homeostasis. A disturbance leads to defects or wrinkle formation. Histological studies of chronically sun-exposed skin show that the dermis contains inflammatory infiltrates (51), mostly perivascular and perifollicular. Mast cells are more abundant in photodamaged skin than in normal skin. They synthesize and release mediators that modulate directly or indirectly extracellular matrix production and degradation (including TNFα, TGFβ and prostaglandin 2). They release proteases that can degrade the ECM or activate the proenzyme forms of metalloproteinases. Ultrastructural studies have also shown infiltration of the epidermis by macrophage/dendritic-like cells. These studies have recently been confirmed, showing more epidermal dendritic cells, but fewer Langehans cells in sun-exposed skin (52).

The balance of cytokines in the skin is altered during ageing and fibroblasts become less responsive to growth factors or cytokines. Physiological ageing in human fibroblasts seems to be particularly associated with an altered response to interleukin-1- β, a cytokine produced by monocytes, macrophages and other transitory cells involved in inflammation (53).

Transforming growth factor (TGF)-β1 is a cytokine involved in the differentiation of fibroblasts to myofibroblasts. These myofibroblasts are very important for dermal strength and may be responsible for the contraction of the dermis (54). It acts on fibroblast collagen, fibronectin, glycosaminoglycans, elastin production, all

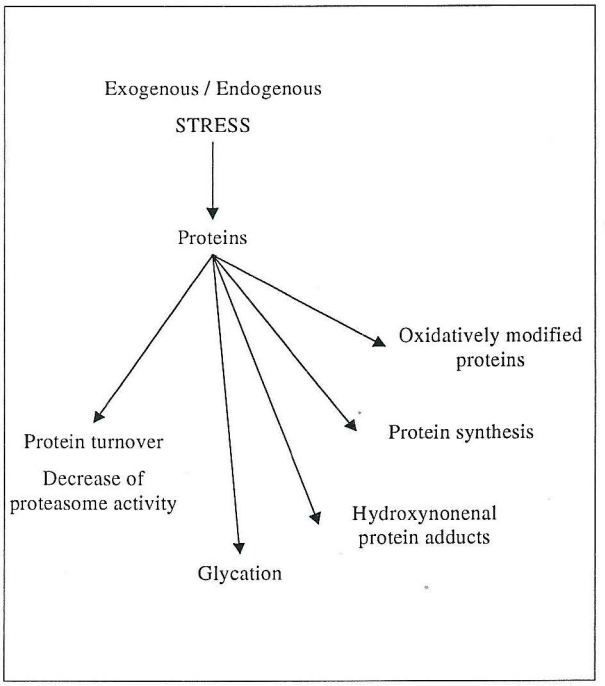


**Figure 1. Cellular consequences of stress on proteins.**

of which are important for the mechanical properties of the skin. The expression of the TGF-β1 gene in epidermal keratinocytes does not decline with increasing cell age. Hence, TGF-β1 does not appear such as the message from epidermis to dermis affected by age (55).

Altered cytoskeleton function may play a key role in the age-related changes in several cell types, because it is involved in a variety of functions that are altered with ageing (immunological, endocrine and neurological changes) (56). The age-related changes in the cytoskeleton, due to its involvement in metabolic processes and cell surface receptors expression, can indicate defective signal transduction. Aged fibroblasts, which very often contract collagen gels poorly and do not migrate well, have a disordered actin microfilament cytoskeleton and a reduced α-2-β-integrin. (57).

Postmitotic and mitotic cells age differently. Postmitotic cells never divide, such as nerve, muscle and fat cells. Mitotic cells, such as keratinocytes and fibroblasts, divide. Replicative senescence is the process that limits the number of cell divisions. As senescent fibroblasts and keratinocytes accumulate with age in human skin, this could explain the deterioration of the appearance and properties of the skin. Senescent cells secrete degrading enzymes that modify the cytokine / interleukin balance, causing the loss of functional integrity (58).

Lipofuscin, or age pigment, accumulates in cells within the lysosomal vacuoles, especially in fibroblasts. Lipofuscin can also accelerate ageing and senescence under mild hyperoxia (59). The accumulation of lipofuscin may also be involved in spot formation. A lack of glucose-6-phosphate dehydrogenase (G6PD), an enzyme involved in the cell redox balance, may also accelerate fibroblast senescence (60). It has been proposed that phospholipid hydroperoxide glutathione peroxidase (PHGP) helps to protect fibroblasts against UVA-induced lipid peroxidation and the activation of metalloproteinase 1 (MMP1) by UVA.

## *Receptors*

The number, affinity and rate of internalization of epidermal growth factor (EGF) receptors are different in young and old fibroblasts, explaining the loss of responsiveness to EGF with age and the impaired wound healing in the elderly (61). Extracellular matrix is also diminished during ageing and the amount of collagenase in the skin increases with age. Collagenase production is controlled by protein kinase C via the members of the AP1 transcription factor family and can be inhibited by α-tocopherol (62). Down-regulation of ligand-activated receptors is important for normal cell functioning. Receptors bearing their ligand move to specialized regions in the plasma membrane. The resulting vesicles are transported through the cytoplasm by microtubules and fuse with endosomes and lysosomes, where they are degraded. ROS can also alter receptor function. For example, oxidase stress caused by hydrogen peroxide rapidly inhibits the internalization of receptor-bound EGF in human fibroblasts, so that the breakdown of the EGF-receptor complex is inhibited. Hydrogen peroxide also alters negative feed-back within the cell, and attenuates growth factor-induced signal transduction, leading to altered cell metabolism (63). Photo-damage increases the tenascin in cells, which might cause competition for the α2β1-integrin receptor, reducing cell-collagen binding. α2β1-integrin is the major collagen receptor, but is also a receptor for tenascin (64). Tenascin C is a large extracellular matrix glycoprotein whose production by keratinocytes is increased in wound repair; it is also found in normal adult skin. It is often distributed discontinuously in the upper papillary dermis adjacent to the EDJ, close to capillary basement membranes. The concentration of tenascin is increased in photodamaged skin and its distribution is different from that of skin protected from the sun. Tenascin is found along the dermio-epidermal junction in a continuous pattern and extends further into the papillary dermis. The pattern of gene expression in senescent fibroblasts is different from that of their still-dividing counterparts. Presenescent fibroblasts have low concentrations of metalloproteinases (MMPs) and high concentrations o MMP inhibitors (TIMP-1 and TIMP-3). The concentration of MMPs increases as cells become senescent, while that of TIMP decreases. The MMP production is stimulated by activation of the redox-regulated transcription factor NFκB and protein kinase C via activator protein 1 transcription factor (AP1) (65).

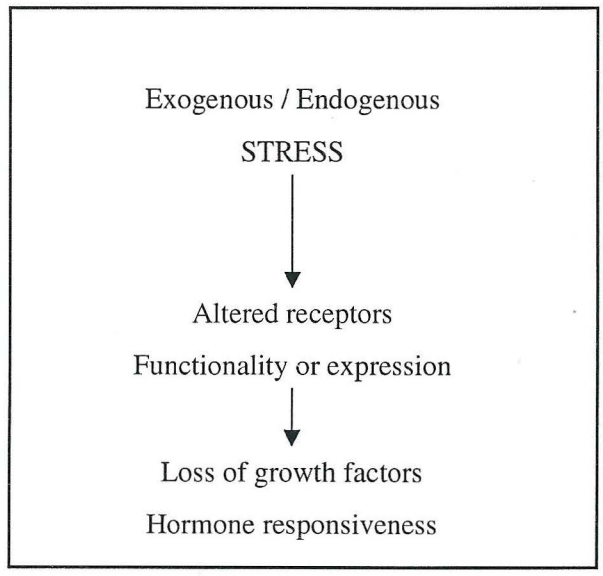


**Figure 2. Cellular consequences of stress on receptors.**

## MMP

Some of the MMP, a family of at least 16 enzymes that digest matrix macromolecules, are activated by UV irradiation (66). Thus, metalloproteinases 1, 3 and 9 (MMP-1, 3 and 9) in the epidermis are activated by UVB, while UVA stimulates MMP-1 *in vivo* and MMP-2 and 3 *in vitro*. The MMPs in the skin are responsible for breaking down macromolecules of the skin ECM, which ensures the skin's three-dimensional integrity. The balance between MMPs and MMP inhibitors is perturbed by environmental factors, such as light. This leads to collapse of the EMC and the visible effects of UV damage: wrinkling, loss of elasticity. Besides chronological ageing, actinic ageing, also called photodamage, causes premature skin ageing: thinning of the dermis, a loss of collagen content and protein organization and a breakdown of the ECM (67, 68).

Type 1 MMPs (interstitial collagenase) and type 9 MMP (gelatinase) break down skin collagen fibers, particularly during photodamage (69, 70). MMP-2 (gelatinase) acts on collagen types I, IV, and VII. Gelatin, elastin and fibronectin are all substrates for MMP-2, whose activity increases with age (71, 72). MMP-1 degrades collagen, which accounts for at least 70% of the dry weight of the dermis. Smoking increases the activity of MMP-1 in the skin *in vivo*. It leads to an imbalance between MMP-1 and the tissue inhibitor of metalloproteinase 1 (TIMP-1), which could be important for ageing (73). The MMP-1 produced by epidermal keratinocytes and dermal fibroblasts in response to various stimuli (74-78) appears to play a key role in dermal remodeling (79-81). Skin fibroblasts produce MMP-1 in response to UVB irradiation and keratinocytes play a major role through an indirect paracrine mechanism involving the release of epidermal cytokine after UVB-irradiation (82). MMP are produced in response to UVB irradiation *in vivo,* and are considered to be involved in the changes in connective tissue that occur in photoageing (83). They are associated with a variety of normal and pathological conditions that involve degradation and remodeling of the matrix (84-87). Several MMPs are produced during wound healing, such as MMP-3 in epidermis repair (88, 89).

UV rays and ageing lead to excess proteolytic activity that disturbs the skin's three-dimensional integrity. These proteinases are important for breaking down the extracellular matrix during chronic wound repair, in which there is re-epithelialization by keratinocyte migration (90). Thus, MMPs are continuously involved in the remodeling of the skin after chronic aggression.

Thrombospondin 2 (TSP2), a secreted extracellular matrix glycoprotein, is an adaptator and modulator of cell matrix interactions (91). It binds to heparan sulfate proteoglycan, low-density lipoprotein receptor-related protein (LRP), and the integrin $\alpha v\beta 3$ (92, 93). Increased MMP-2 activity (gelatinase A) leads to reduced fibroblast adhesion which could contribute to abnormal collagen fibril structure in the skin and the release of angiogenic factors. New data show that TSP2 binds to and inhibits MMP-2 indirectly and therefore plays a role in cell-matrix interactions (94).

The age modulated hypoxia response causes an imbalance between MMP-1 and MMP-9 and TIMP. Hence, there may well be altered MMP and TIMP gene expression at wrinkle sites (95, 96). Photodamage also results in the accumulation of abnormal elastin in the superficial dermis, and several MMPs have been implicated in this process. The quantities of matrilysin (MMP-7) and human macrophage metalloelastase (MMP-12), which have broad substrate specificities, are two key parameters that can be used to evaluate long term antiageing treatments (97). They are increased in the abnormal elastic fibers of chronically photoaged skin and contribute to the remodeling of elastic areas in sun-damaged skin. Human metalloelastase also aids macrophage migration, in addition to degrading elastic tissue, so amplifying the disturbance of the inflammatory homeostasis of the tissue. Ultrastructural and histopathologically studies have demonstrated that sun-exposed skin contains accumulated insoluble material and the normal elastic fiber architecture is lost, resulting in a loss of skin resilience and elasticity and probably wrinkle formation.

## Fibrillin and Elastin

Elastic fibers in the dermis form an amorphous matrix of elastin and intertwining bundles of microfibrils, which measure 10-14 nm in diameter. The oxytalan fibers are rich in microfibrils and are orientated perpendicularly to the basal lamina of the epidermis. A study on photoaged skin has shown that UV irradiation increases the tropoelastin mRNA in keratinocytes and fibroblasts (98). Selective inhibition of skin fibroblast elastase could be one way to fight wrinkle formation following cumulative ultraviolet B irradiation (99). Lysozyme may alter the elastic fibers in the surface, preventing further degradation and the accumulation of altered elastic fibers.

Photoaged skin contains elastic material in the reticular dermis, and the fibrillin deposits in the reticular dermis are enlarged. Elastic fibers have a central core of hydrophobic cross-linked elastin surrounded by fibrillin-rich microfibrils. The papillary dermal microfibrillin-rich microfibril network is truncated and depleted in photoaged skin. There are fewer fibrillin-rich microfibrils in wrinkled photoaged skin, probably due to inflammatory cell proteinases (neutrophil elastase), or activation of matrix metalloproteinase (100). Cross-linking causing decreased elasticity could also be involved in wrinkle formation (101).

The fluorescence of tryptophan and collagen cross-links in the dermal matrix may serve as i*n vivo* markers

**Table 2. Main Matrix Metalloproteinases and their substrates.**

| Name | Enzyme | Selected substrates |
|---|---|---|
| **MMP-1** | Matrix collagenase (fibroblast collagenase) | Collagens I, II, III, VII and X |
| **MMP-8** | Neutrophil collagenase | Collagens I, II, III, Link protein, Aggrecan |
| **MMP-13** | Collagenase 3 | Collagens I, II, III |
| **MMP-18** | Collagenase 4 | Collagens I |
| **MMP-2** | Gelatinase A | Gelatins, Collagens I, IV, VII and XI, Fibronectin, Laminin, Elastin |
| **MMP-9** | Gelatinase B | Gelatins, Collagens IV, V and XIV Aggrecan, Elastin |
| **MMP-3** | Stromelysin 1 | Aggrecan, Gelatin, Fibronectin, Laminin, Collagens III, IV, IX and X |
| **MMP-10** | Stromelysin 2 | Aggrecan |
| **MMP-14** | (membrane type) | Collagens I, II and III, Laminin |
| **MMP-7** | Matrilysin | Aggrecan, Fibronectin |
| **MMP-11** | Stromelysin 3 | Fibronectin |
| **MMP-12** | Metalloelastase (Macrophage) | Elastin |

of skin aging, photoaging, and as a way of assessing exposure to UVA radiation (48). The morphology of elastic fibers changes significantly during life. The number of elastin microfibrils (mainly composed of fibrillin) gradually decreases during ageing, and the degenerative process is accelerated by exposure to sunlight. Amyloid P and lysozyme are deposited in thickened fibers, while amyloid P alone is deposited in oxytalan vertically oriented fibers in the papillary dermis. Deep wrinkles are linked to the degeneration of collagen and the deposition of abnormal elastic material (102). Wrinkles are formed by major changes in the dermis matrix and at the dermio-epidermal junction. The content of fibrillin, a component of the elastic fiber network, is increased by prolonged clinical doses of topical retinoic acid. This is why fibrillin-1 has been proposed as a "reporter" molecule for the efficacy of photoageing.

## *Water and GAG*

The altered skin texture and structure of elderly people is caused by changes in proteins, lipids and water, leading to altered mechanical properties, such as wrinkling, sagging, loss of elasticity and apparent dryness. Water structure is important because water can bind to various proteins and is important for maintaining the structural and mechanical properties of proteins. Their natural interaction is diminished in photoaged skin, leading to decreased collagen stability and the fragmentation of collagen fibrils (103). The distribution of glycosaminoglycans (GAG) in the dermis seems to be modified in sun-damaged skin and could be linked to alterations of deep protein. Studies using immunoperoxidase staining of hyaluronic acid and chondroitin sulfate and confocal laser scanning microscopy have shown increased dermal GAGs in sun-damaged skin. The GAGs are deposited on the elastic material of the superficial dermis and not between collagen and elastic fibers, as in normal skin (104). Hyaluronan is a major constituent of the skin extracellular matrix. Hyaluronan polymers become more tissue-associated with advancing age (105). Together with changes in proteins, this contributes to the pronounced alteration of the skin mechanical properties in the elderly.

## *Lipids and cell membrane*

The skin barrier is linked to the lipids of the intercorneocyte space. Intercellular lipids consist of an organized mixture of ceramides, sterols and fatty acids (106, 107). The lipids in intercellular membranes form short- and long-periodicity lamellar phases (108). A recent X-ray diffraction and electron macroscopy study showed no correlation between differences in the organization of stratum corneum lipids and ageing, despite the changes in skin properties often observed in the elderly (109).

Vitamin A (retinol and retinyl esters) is present in the epidermis as free and esterified retinol. Acute exposure to UVA completely depletes the epidermis of vitamin A and causes lipid peroxidation. In contrast, exposure to UVB results only in the loss of vitamin A (110).

The human sebaceous gland undergoes both extrinsic and intrinsic ageing (morphological changes in the sebaceous gland activity). The highly androgen-dependent sebum secretion in neonates reaches its maximum in young adults. The number of sebaceous glands remains unchanged throughout life, but sebum production tends to decrease after menopause in women and after the eighth decade in men. The age-dependent

decrease in androgen leads to a slower cell turnover in the sebaceous glands, resulting in hyperplasia of the facial sebaceous glands. UV may contribute to this process. Molecular studies have shown that overexpression of the ageing-associated gene *Smad7* and parathormone-related protein are linked to hyperplasia of the sebaceous gland, but overexpression of the *c-myc* gene is associated with enhanced sebum production (111). Decreased sebum production is also responsible for skin dryness in the elderly.

## *5. DNA Damages and consequences*

The DNA of the skin is constantly submitted to environmental damage and has developed mechanisms to repair this damage. The balance between damage and repair has a major impact on ageing. The most common cause of premature skin ageing is UV irradiation, which damages DNA through photoproducts. DNA repair is essential for maintaining the functional integrity of DNA. Selective repair, such as the removal of pyrimidine dimers, occurs in the transcribed strand. DNA is repaired by a variety of mechanisms, such as direct reversal DNA damage for thymin dimers, base excision and mismatch repair. Nucleotide excision repair is very important for damage caused by UV. A repair complex binds the damaged DNA, then an endonuclease cuts it on either side of the damaged nucleotide. The original DNA sequence is resynthesized by a DNA polymerase and a ligase.

The most reactive oxygen free radical, OH•, reacts with DNA bases to give altered bases, such as 8-hydroxydeoxy guanine. These are eliminated by the DNA repair enzyme complex, but some accumulate with ageing (112). Ageing may also result from the injury to mitochondrial DNA and peroxidation of the inner mitochondrial membrane lipid. Many studies have suggested that mtDNA suffers more from oxidative DNA damage than does nuclear DNA.

Genomic and mitochondrial DNA are both intimately involved in the process of ageing. There is some decline in DNA repair capacity with age. Increased DNA fragility or DNA strand breaks, chromosomal aberrations based on cytogenetic examination, decreased DNA methylation and changes in ploidy all increase with age. Rearrangements, translocations, and sequence alterations also increase with age (113). The main type of damage generated by apparently all types of reactive oxygen species (ROS) is oxidative changes to guanine (114).

Free radicals produce a number of lesions in DNA, damaging bases, sugar lesions, DNA-protein cross-links, causing single-strand breaks, double-strand breaks, and abasic sites by different mechanisms (115). A recent review relates the contributions of stress-induced damage to cellular DNA:

- by damage to nuclear DNA and its repair mediated by poly(ADP-ribose) polymerase1 (PARP 1)
- damage to telomeric DNA and its contribution to telomere-driven cell senescence
- the accumulation of mutations in mitochondrial DNA (116).

The effects of oxidative stress can be direct or indirect. For example, some cell constituents (flavins, phorphyrin), many dyes (acridines, methylene blue, neutral red) and drugs can act as photosensitizers inside cells. The excited state of a photosensitizer can be thought of as genotoxic species similar to other free radicals, because they can directly or indirectly cause DNA modifications (4). Highly selective changes to guanine are also caused by photosensitizers that modify DNA via singlet oxygen (type II photoreaction), or one-electron oxidation (type I photoreaction) (117-120). Endogenous and exogenous oxidative stress can cause serious damages to mitochondrial DNA. Deletions of mitochondrial DNA may be used as markers of skin ageing and exposure to UV irradiation (121-124). Direct evidence for the increased presence of UV-induced damage in mt DNA was obtained recently. Ray *et al* (2000) used a PCR method to show that the number of mtDNA deletions in the epidermis is significantly associated with increased exposure to UV radiation. UV radiation may directly or indirectly act via free radicals to cause mutations at labile sites in mtDNA, enhancing intra genome recombination, and increasing deletions. Mutations of mt DNA accumulate during ageing and in photoaged skin; the most common mutation is a 4977 base pair deletion (called common deletion). Chronic exposure of human skin to sunlight results in more mtDNA mutations than in un-exposed skin. UVA–irradiation produces singlet oxygen that generates the common mutations of mitochondrial DNA that occur in photoageing (125).

It is difficult to precisely measure oxidative DNA damage, because extraction and sample treatment may cause oxidation. Various analytical techniques can be used to measure oxidative damage to DNA: gas chromatography (GC) and liquid chromatography (LC) with mass spectrometry (MS) provide positive identification and accurate quantification. Modified nucleosides have been measured recently by methods using LC/tandem MS (LC/MS/MS) and LC/MS (112).

## *6. Telomerase involvement*

The closest thing to a cellular clock resides at the tips of chromosomes. The chromosome ends, the telomeres, do not contain genes that program hereditary traits, but are functional complexes. The telomeres at the ends of eukaryote chromosomes protect them from

degradation or fusion. The telomeres shorten each time human cells divide. The cell may finally fall into a senescent state. Telomerase is a ribonucleoprotein that synthesizes the repeated sequences at chromosome ends and helps DNA polymerases to complete the replication. New data indicate a new way to link telomeres to senescence. The telomere is a dynamic nucleoprotein complex that can switch stochastically between an uncapped state and a capped state, which preserves the physical integrity of the telomere and allows cell division to proceed (126-130).

It has been suggested that cell senescence may be good because it is a defense against cancer, which is marked by uncontrolled cell division. Cells unable to regrow their telomeres stop dividing before they can cause too many mistakes. As there is no telomerase in many somatic tissues, telomere erosion may well be a major factor in cell ageing (131). Telomerase therapy might one day help generate a new supply of cells to treat age-related diseases. It has been showed that there is a telomerase activity in the skin.

It is possible to distinguish between ageing and longevity. Telomere shortening is probably more involved in regulating cell longevity than in ageing *stricto sensu.* Ageing is due to accumulation of molecular disorders and a lack of cell energy. This loss of energy is involved in the deterioration and a gradual loss of the functional integrity of the tissues. Nevertheless, telomere shortening (or more precisely telomerase dysfunction), oxidative damage and hormones could all be signals involved in ageing.

The regulation of telomerase in mammalian cells is multifactorial, involving telomerase gene expression, post-translational protein-protein interactions, and protein phosphorylation. Several proto-oncogenes and tumor suppressor genes are involved in the regulation of telomerase activity (132). Several physiological factors, like EGF and/or amphiregulin, and growth factors, can also influence telomerase (133). Telomere length and telomerase activity may determine cell senescence. Hyperoxia accelerates telomere shortening by causing oxidative stress. A reduction of stress, for example by the action of free radical scavengers, delays replicative senescence. Telomere act as a "sentinel" for oxidative damage to the genome and replicative senescence may be triggered by telomeres as a consequence of DNA damage. It is thus very important to ensure that cells have sufficient telomerase (134). Guanine of the telomerase 3' overhang (TTAGGG) can be considered as a target for reactive oxygen species or UV irradiation (135). Estrogen activates telomerase via direct and indirect effects. There may be hormonal control of telomerase activity. Sex steroids may thus influence cell senescence and ageing (136). The activity of telomerase may be also regulated by the tumor suppressor protein p53; a lack of this protein may lead to increased telomerase activity in cancer cell development (137). Mutations of the p53 gene and telomerase activity are linked, and these mutations are considered to be UV specific (138). The transcription factor NF-κB may act at a specific site to influence the activity of the telomerase catalytic subunit (hTERT) T (139). More recently,

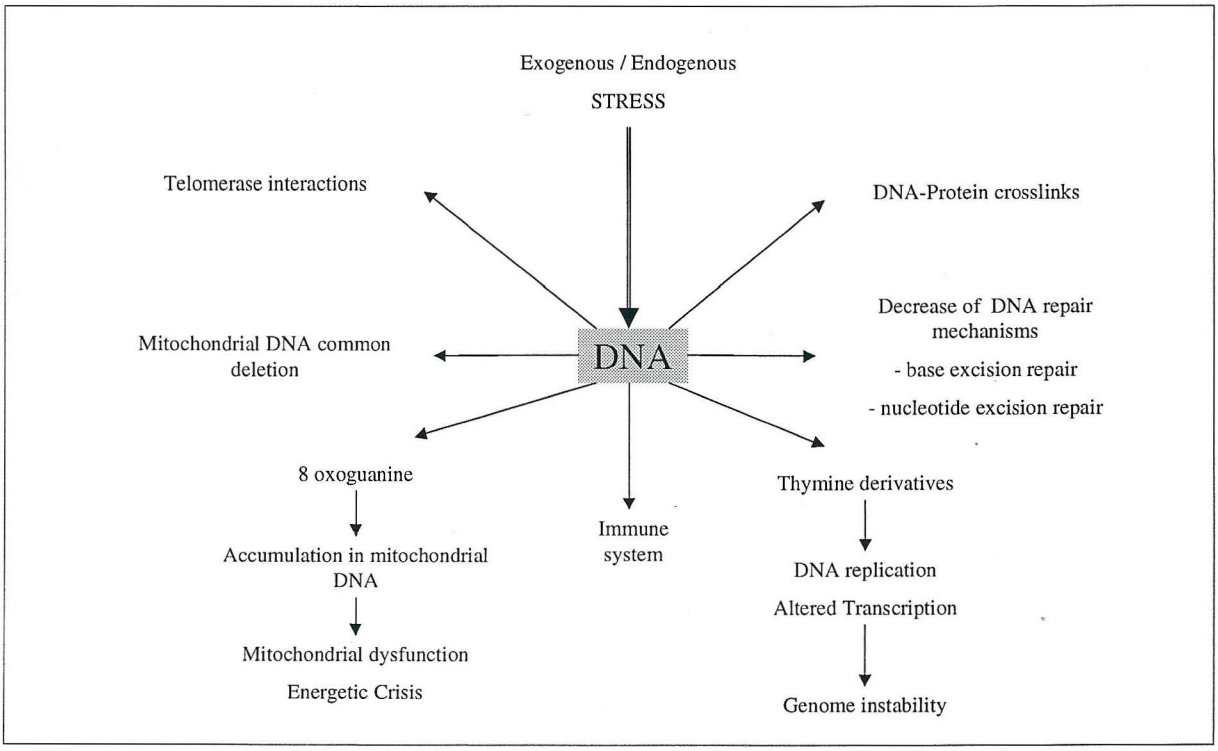


**Figure 3. Consequences of external stress on the DNA.**

telomerase and its catalytic subunit hTERT have been shown to be involved in oxidative stress, and UV irradiation disturbs the telomerase activity in human keratinocytes (140).

The nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP 1) participates in the regulation of both DNA repair and transcription. Moderation of PARP following DNA damage has also been proposed to protect skin cells from UV induced acute and chronic photodamage (141). The ageing and survival of endothelial cells are linked to molecular mechanisms that control cell proliferation, quiescence, apoptosis and senescence. The activation of telomerase in human dermal microvascular endothelial cells also seems to affect their durability both *in vitro* and *in vivo* (142).

### *Apoptosis*

Cell senescence could be, like apoptosis, a part of the body's defenses, a natural mechanism to prevent cells from accumulating mutations with their physiological consequence, malignancy (143). The differentiation, apoptosis and senescence of keratinocytes share some molecular pathways. Epidermal differentiation and apoptosis lead to cell death and the removal of cells by transglutaminase activation and proteolysis. Regulators of apoptosis, such as Bcl-2 (suppressor) or Bax (promotor), are also produced during differentiation. Senescent cells are cells which can no longer replicate, but can still respond to growth factors. They have lost their ability to perform correctly tissue homeostasis. Cells lose their ability to divide early in differentiation.

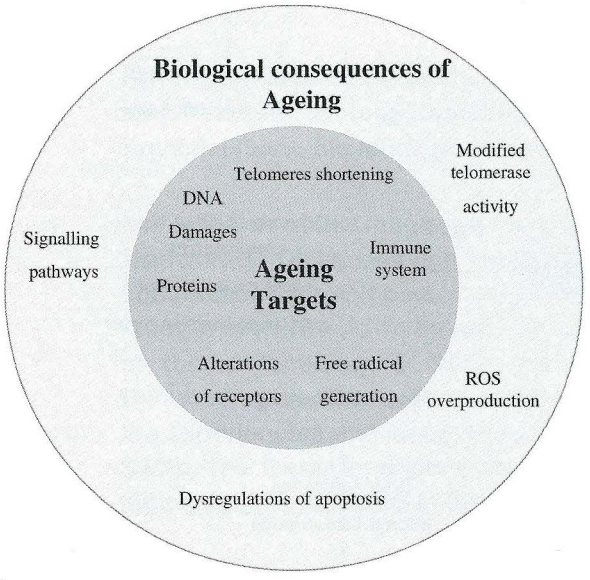


**Figure 4. Consequences of ageing on main biological targets.**

Some authors think that senescence is not directly linked to epidermal differentiation, due to differences in the responses to cell cycle inhibitors (144).

Apoptosis is a cellular end point of the stress response. Apoptosis removes damaged cells from UV-irradiated tissues. The genes that control apoptosis in the epidermis, such as the bcl-2 gene, are disregulated during ageing. The decreased efficiency of apoptosis may contribute to chronological ageing and extrinsic skin ageing. Only epidermal stem cells escape cellular senescence. It appears that epidermal terminal differentiation, apoptosis and cell senescence are all triggered by stimuli. Nevertheless, keratinocytes will undergo classical epidermal differentiation or will irreversibly enter into senescence or apoptosis, depending on their state and on the nature and strength of the stimuli. Oxidative damage is a cellular stress that can cause senescence like growth arrest, or even apoptosis. Ras-induced senescence is also mediated by ROS, but is not clearly associated with telomere shortening. The tumor suppressor P16 accumulates as fibroblasts approach senescence, and by inhibiting its degradation, P19 indirectly mediates the growth arrest or apoptosis. P (145).

## *7. Morphological Variations*

The skin becomes thicker until maturity and then becomes thinner in women over 50-60 years old. Measurements of skin physical properties show that it becomes thinner, stiffer, less tense and elastic with ageing (146).

### *Mechanical Properties*

Young's modules (elasticity modules) of the skin, a ratio between stress and deformation, increases linearly with age. This is in agreement with data indicating that skin becomes more rigid and less able to stretch in response to stress with age. This has to be correlated with the increased crosslinking of collagen, the disorganization of the fibril network and the large amount of free water in the dermis. Ageing decreases skin function and causes clinical changes such as wrinkling, color changes (yellowish, patches, pigmentation), and a loss of elasticity (146). Sagging is one of the major age-related morphological changes in the face. While wrinkles and general changes in the face have been well studied, a recent method using photostandards and 3 D analysis of replicas shows that women's cheeks begin to sagging when they reach 40 (147). Recently measurements of site-related and age-dependent variations in facial skin show that there is an overall increase of skin echogenicity and thickness with age. The skin on the upper and lower lips, on the chin and infraorbital regions is thicker than that on the central forehead, lateral forehead and cheeks. The facial skin thickness be-

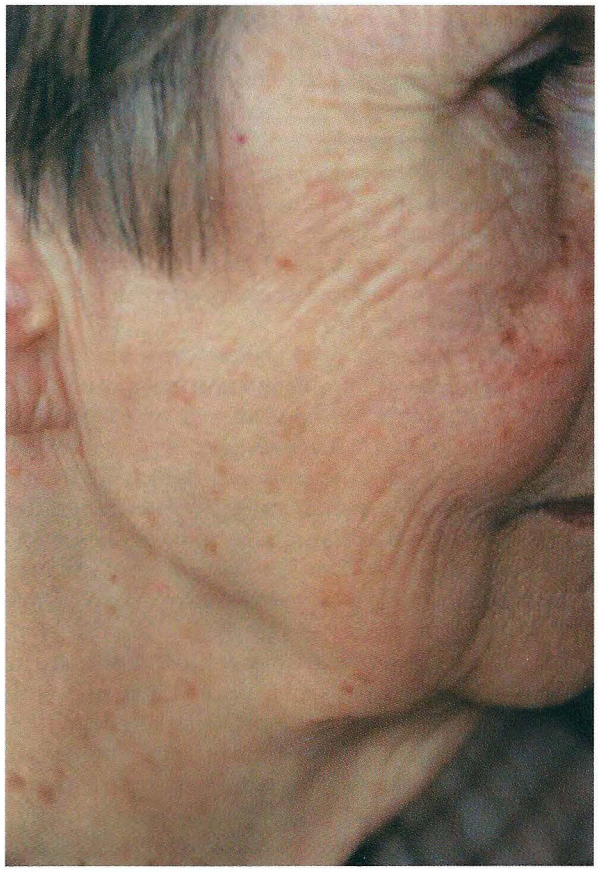

**Figure 5. Clinical signs of ageing on a Caucasian woman of 75 years old: presence of wrinkles, spots and sagging.**

comes greater over the lateral regions of the forehead, lips and nose in elderly subjects, and becomes thinner over the infraorbital regions (148). Fine lines are due to the gradual breakdown of collagen and elastin fibers, and they are exacerbated by sun damage. Very deep wrinkles are associated with the muscle below the skin surface. Muscles contract more with age to compensate for the loss of volume. Excessive exposure to sunlight and smoking can cause major changes in the skin (73). The skin may darken; develop very fine wrinkles, spots, and sag, all of which are symptoms of photoageing. This is a very serious concern for middle-aged women, especially women in Asia. Studies using the two point gap discrimination method plus microneurographic recording in response to mechanical stimuli have also revealed changes in tactile spatial discrimination in the elderly (149).

The subcutis is also concerned in ageing. Ageing results in larger fat cells in the subcutaneous tissue. Hormone changes linked to ageing may also cause difference in body fat distribution (150). Energy metabolism is also regulated via leptin, a fat cells-derived hormone, in adults. The concentration of leptin in the blood varies during the menstrual cycle. Leptin binding activity is low at birth and high in the pre-pubertal years, but it is stable during adult life and does not vary with ageing (151-153). The adipocytes also act as estradiol stores. The circulating concentration of this hormone varies with age, and is most important in mature skin. Menopause, the physiological cessation of menstruation caused by decreased function of the ovaries, leads to thinning of the dermis, mainly due to a decrease in the collagen content, atrophy of subcutaneous tissues and increased skin dryness (154, 155).

## *Wrinkles*

Wrinkles are modifications of the skin associated with cutaneous ageing and develop preferentially on sun-exposed skin. Clinicopathological features of wrinkles were studied among the different types of skin relief modifications. Four types of skin depressions can be defined according to their depth: folds, permanent wrinkles, reducible wrinkles and skin micro-relief. Development of wrinkles may be secondary to actinic elastosis and to the disappearance of microfibrils and collagen fibers at the dermoepidermal junction. Epidermis is involved with a decrease of the cell renewal, an increase of involucrin, a decrease of integrin β 1, type VII collagen and fibrillin 1 (156). Wrinkles are one of the major concerns for women along with spots and freckles. Principal causes of wrinkling are ageing and excessive exposure to UV rays. Wrinkles are the expression of the accumulation of modifications at different levels of the skin. Development of so-called fine wrinkles begins to take place in the thirties, reaching a peak in the fifties, while deep wrinkles increase in the fifties. Little is known about the exact histological changes underlying wrinkle formation. Changes in collagen type I, III, type IV and VII at the DEJ have been recorded (157-160). Collagen VI, concentrated in the

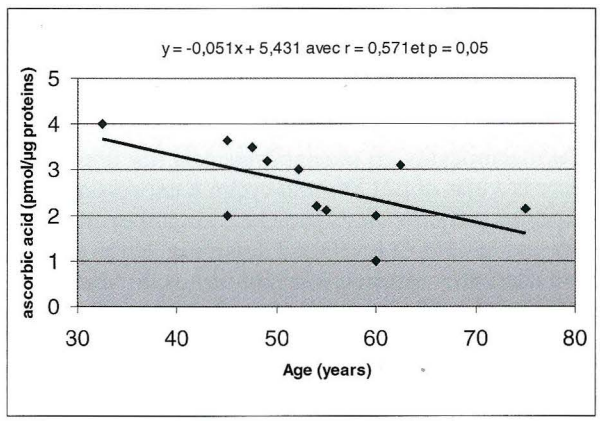


***Figure 6. $^{14}C$ Ascorbic acid intracellular transport in Human Normal Fibroblasts (lifting), n=12 (M. Dumas courtesy).***

papillary dermis immediately below the dermal-epidermal junction is similar in photoprotected and photoaged skin (159). Some fibroblasts which are accumulating damages are less stimulated by surrounding ascorbic acid, resulting in a decrease of collagen and a loss of dermis density. Very recently, in aged fibroblasts from photoexposed zones, a decrease of the intracellular transport of pericellular ascorbic acid has been proposed in wrinkle formation and ellagic acid derivatives have been proposed to overturn the phenomenon (161; Marc Dumas, personal communication).

The balance between the stress activated (SAPK) and mitogen activated (ERK) MAP kinase signaling pathways regulates cell growth and extracellular matrix production (ECM). SAPK activity, measured by c-jun phosphorylation, is increased in the elderly and inhibits ECM production by activating collagenase and inhibiting collagen synthesis (162). Oxidative damage is central to skin ageing, and is particularly involved in wrinkle formation. The extracellular signal-regulated MAP Kinase pathway (ERK), which mediates cell responses to growth factors, is less active in old human skin *in vivo*. At the same time, the activity of the stress-activated MAP Kinase pathway (c-jun, p38 MAP Kinase) increases in old human skin *in vivo*. The amounts of c-Jun mRNA and protein are increased in old skin, but the amounts of c-Fos mRNA and protein are not. It has also been demonstrated that retinol activates the ERK pathway in old skin but does not alter the stress-activated c-jun kinase. (163). TGF β-1 in the extracellular matrix and in keratinocyte may also be an important marker for measuring the efficiency of an "anti-wrinkle" treatment (164).

## *Spots / Freckles*

Melanocytes are specialized cells that are located in the basal layer of the epidermis. They synthesize and transfer melanin pigments to surrounding keratinocytes, thus protecting from UV carcinogenic effects (160, 165, 166). Molecular mechanisms and cell cycle regulatory gene expression leading to melanocyte senescence and transformation differ significantly from fibroblasts. As found in other cell types, progressive telomere shortening appears to trigger replicative senescence in normal melanocytes. In melanocytes and not fibroblasts, there is a loss of p21 $^{Waf-1}$ and cyclin E expression. Melanocytes and fibroblasts present common events as an increase in p16 $^{INK4a}$ levels and down-regulation of E2F1, also shared by senescent keratinocytes. In fibroblasts, the senescent phenotype is linked to the repression of the gene c-fos, upregulation of p21 $^{Waf-1}$, and down-regulation of E2F transcription factors. p21$^{Waf-1}$ may be a major regulator of fibroblast senescence. (167).

Adult melanocytes are able to stop to proliferate and terminally differentiated melanocytes are still metabolically active but postmitotic. The altered differentiated functions of senescent melanocyte are not well known. Alterations of melanosomes, melanin synthesizing enzyme in mitogen activated protein kinase (MAPK), and in cell cycle progression have been reported. All these altered functions may have a real impact in tissue (168). The knowledge of the mechanisms of human skin color is of prime importance to develop skin care increasing the skin radiance and fighting spot formation. The change of the absorbance spectrum from reflectance including the scattering effect has been found not to correspond to the molar absorption spectrum of melanin and blood (169, 170).

Disturbed keratinocytes-melanocytes interactions during melanosome transfer and skin melanosome distribution patterns could be related to spot formation. Melanocytes located in the basal layer of epidermis produce melanin-loaded melanosomes, which are distributed to neighboring keratinocytes. UV radiation lead to an accumulation of melanosomes in melanocytes and treatment with MSH induces exocytosis of melanosomes. UV and MSH increase the phagocytose of melanosomes by keratinocytes (171).

Kinesin and kinectin are motor proteins that are involved in some stage of melanosome transport (166; 172-174). Melanosome transport along the dendrite is mediated in part by microtubule proteins and myosin V, which traps melanosomes at the actin-rich periphery of the dendrite SNARE protein (soluble N- ethylmaleimide-sensitive factor attachment protein receptors) and Rab-3a. Proteins involved in vesicle transport, docking (rab 3) and membrane fusion (SNARE) seem to be involved in the changed melanosome dynamics caused by UV irradiation. This can be thought of as an exocytosis towards the keratinocytes (175). Serine protease inhibitors that interfere with PAR 2 (protease-activated receptor 2) may cause depigmentation by affecting melanosome transfer and distribution (176). The formation of skin spots on photoexposed areas is a very complex problem, but may be viewed as a double problem. The direct and indirect processes stimulated by UV irradiation (endothelin-1, thymin dimers, NO) increase the melanin production (177-179). Their local aggregation can also generate new reactive oxygen species (180). A disturbance of cell-cell signaling (cadherin E, P, etc) can cause melanin to be transferred to basal keratinocytes instead to suprabasal cells, resulting in pigment accumulation (181). Accumulation of pigment in such cells could also be due to local defect of keratinization and to oxidation products, like lipofuscin. Changes due to UV irradiation and ageing could make these cells, pigment-loaded by error, less easy to eliminate. Furthermore, melanocytes getting the information that their protective pigment distribution pattern is abnormal will continue to oversynthesize pigment, leading to more, uncontrolled accumulation.

Other biochemical factors may be responsible for spot formation. Melanogenic stimulatory factors derived from epidermal cells in senile freckles include endo-

thelin and stem cell factor (182). Abnormal sphingomyelin deacylase production leads to high concentrations of sphingosylphosphorylcholine instead of ceramide in the epidermis of patients with atopic dermatitis. The sphingosylphosphorylcholine is associated with the pigmentation defects frequently observed in atopic dermatitis (183). The question of a specific way of spot formation in mature skin linked to the hormonal balance modification is also still open. UV-induced melanogenesis is mediated by nitric oxide radicals. There is a decrease in tyrosinase activity stimulated prior to NO-stimulation (184). Human melanocytes contain the mammalian melanin concentrating hormone (MCH), but human keratinocytes and fibroblasts do not. The MCH is coupled to a G protein receptor, SLC1; the inactivated complex blocks the cyclic AMP second messenger pathway and increases intracellular calcium. Disturbance of this pathway by UV irradiation or other elements can also favor spot formation (185). The hormonal control of skin pigmentation via interaction of POMC peptides (eg α-MSH, ACTH) with other local and circulating hormones may influence melanocyte function, and thus coordinate the pigmentary response of the skin, especially after major changes in hormone concentration (186). Metallothioneine, an intracellular free radical scavenger, could be induced in human melanocytes. Suppression of melanogenesis is partly due to the induction of metallothioneine.

### *Cutaneous microvasculature*

The ageing and survival of endothelial cells are linked to molecular mechanisms controlling cell proliferation, quiescence, apoptosis and cellular senescence. The activation of telomerase in human dermal microvascular endothelial cells is linked to their durability both *in vitro* and *in vivo*. Knowledge of telomerase activity and other markers of amplifying dermal perivascular cells may reveal more about the regenerative capacity of the skin microvasculature. Telomerase activity / length seems to be directly linked to the angiogenic potential (130; 142; 187-190).

## *8. Specificity of Asian Skin*

The formation of facial wrinkles is a sign of photoageing. A unique study of over 3000 people from five ethnic groups (Africa, American, East Asian, Caucasian, Indian Asian and Latino) of different ages has revealed age-dependent changes of the skin (wrinkles, hyperpigmentation and pores). The mean fraction of the face area covered with wrinkles is significantly smaller in African Americans than in Caucasians, but East Asians have the smallest wrinkled area at any given age. The authors suggest that racial differences in other genetic factors besides skin pigmentation, such as DNA repair, are important in determining the development of skin wrinkles. African Americans have more hyperpigmented spots and facial pores than other racial groups. Caucasians have significantly les well hydrated skin than African Americans, East Asians and Latinos (191). The relationship between skin phototype and deep and fine wrinkle scores on the faces of 230 Japanese subjects shows that sunlight-sensitive subjects have deeper wrinkles.(192).

A recent study has compared the action of sun protection factor according to COLIPA recommendations on Asian and Caucasian volunteers. The observed difference in SPF is not only due to skin color but also to internal factors affecting response of the skin to UV (193). Asian skins are not all the same. Differences in the minimal dose causing erythema in Chinese and Korean subjects were also linked to differences in skin chromopohores (194). A study of 230 Japanese individuals classified according to their skin phototype (type IV predominant) suggests that deep wrinkles are more severe in phototype I, and that there is no link between phototype and fine wrinkles. Skin phototype does not seem to be related to hair or eye color. Some Japanese with dark hair and black eyes have sun sensitive low phototype (192).

A study of the factors causing dark circles around the eyes in 60 healthy Japanese women indicates that the dark circles become darker as the blood mass increases, showing the importance of hemodynamics in the area (195). A comparison of the cheek skin color of Caucasian and Japanese women shows an increase in the yellow axis with age in Japanese women, whereas there was an increase in the red axis in Caucasians, followed by a decrease around 50 years (196). East Asians living in Los Angeles and Japaneses living in Akita (Japan) had similar degrees of skin wrinkling. East Asians have less facial hyperpigmentation than Latinos, African Americans or Caucasians (191). The facial hyperpigmentation of 56 women (aged 22 to 67 years) and melanin spot distribution showed more melanin granules around the nuclei in chloasma and senile pigmentation (197). Age-related alterations in the echogenicity of the skin in Japanese women (130 women aged 18-83 years) were studied, with measurements on the forehead, eye corners, and cheeks. An increase in the lower layer of the dermis probably due to the accumulation of degraded or disarranged collagen and a decrease in echogenicity in the upper dermal layer indicate a tendency similar to that seen in Caucasians (198). Measurements of the sebum excretion rate and skin surface contours of 662 healthy Korean volunteers revealed age-related changes. The forehead/ cheek sebum excretion rates increased with age while there were changes in the skin pores and skin surface texture. The disappearance of primary lines, secondary lines and increased pore size with age is probably linked more to exposure to sunlight (199). As demonstrated for the Japanese

volunteers, sunlight also modifies lipid peroxidation, and cholesterol 7-hydroperoxide is a good marker (200).

According to JH Chung, the patterns of wrinkles in Asian people differ from those of Caucasians. For example, Korean people have deeply wrinkled forehead and perioral areas. Wrinkles and pigmentary modifications are the two main characteristics of photoageing in Koreans (201). The skin of groups of 100 different Asian women was compared . In Japanese the skin was in a better condition compared to six other populations. Age-related increase of sallowness was more prominent in Chinese and Korean skin, while. in Philippines the highest tendency of combined skin lesions prevailed (202).

There are several independent risk factors of wrinkles, such as age, exposure to sunlight, menopause, skin color and smoking. Pregnancy is another factor of risk for facial wrinkling due to the high levels of sex hormone bound to globulin and low concentrations of free estradiol. Progesterone also acts as an antagonist to estrogen. Lactation delays the return of ovulation and decreases the estradiol concentration. Hormone Replacement Therapy (HRT) decreases the risks of wrinkling. The decrease in skin collagen due to estrogen deficiency in post-menopausal women may aggravate the severity of wrinkles. Men are also subject to pigmentation damage, leading to seborrheic keratosis and solar lentigo, and, as in women, it leads to depigmentation (lentigo, freckles, mottled pigmentation). In 189 Korean women, a significant increase in the risk of wrinkles was found associated with an increasing number of full-term pregnancies and menopausal age (203).

A multicentric study carried out on 3 000 Chinese women showed no crow's feet area prevalence in Northern cities and a higher peri-oral and glabella wrinkle score in Southern cities. Only 203 women were concerned at 21-25 years of wrinkles in crow's feet area, but at 36 years 75 % of women were concerned (204). Comparing 160 French and Chinese women (20-65 years) the onset of wrinkles delayed by around 10 years in Chinese women (205). From 26 to 60 years, 60% of the Chinese women exhibit pigmented spots on their face. Small facial pigmented spots characterize young population (18-40 years), spots of more than 6 mm diameter increase after 30. Whatever the age group, pigmented spots are always more pronounced on the face than on the hands. Climatic factors and chronic exposure of UV are suggested as a main cause of pigmented spots (206).

## Conclusion

The skin acts as a biosensor because it forms a large interface with our environment. It is a dynamic living barrier which is of prime importance in our social life. The recent studies described in this paper clearly show the complex interconnections at all levels of the tissue and provide a more precise picture of the importance of the events involved in ageing and photo-damage. It may become possible to delay the appearance of signs of skin ageing by acting on key mechanisms revealed by research into this fascinating topic

## Abbreviations

| | |
|---|---|
| *AGE* | *Advanced Glycation products* |
| *ADP* | *Adenosine di Phosphate* |
| *ATP* | *Adenosine tri Phosphate* |
| *DNA* | *Desoxyribonucleic acid* |
| *ECM* | *Extracellular Matrix* |
| *EDJ* | *Epidermal-Dermal Junction* |
| *EGF* | *Epidermal Growth Factor* |
| *FGF* | *Fibroblast Growth Factor* |
| *G6PD* | *Glucose-6-Phosphate Deshydrogenase* |
| *GAG* | *Glycosaminoglycans* |
| *GC* | *Gas Chromatography* |
| *GPx* | *Glutathion Peroxydase* |
| *GTP* | *Guanosine Triphosphate* |
| *HME* | *Human Metalloelastase* |
| *HNE* | *4-hydroxy-2-nonenal* |
| *HRT* | *Hormone Replacemnt Therapy* |
| *hTERT* | *telomerase catalytic subunit* |
| *LC* | *Liquid Chromatography* |
| *LRP* | *Lipoprotein receptor-related protein* |
| *MAPK* | *Mitogen Activated Protein Kinase* |
| *MCP* | *Multicatalytic Proteinase* |
| *MMP* | *Matrix Metalloproteinase* |
| *MSH* | *Melanocyte stimulating Hormon* |
| *mt DNA* | *Mitochondrial DNA* |
| *PARP* | *Poly(ADP-ribose) polymerase 1* |
| *PDGF* | *Platelet-derived growth factor* |
| *ROS* | *Reactive Oxygen Species* |
| *SAPK* | *Stress Activated MAP Kinase* |
| *SNARE* | *Soluble N- ethylmaleimide-sensitive factor attachment protein receptors* |
| *TSP2* | *Thrombospondin 2* |
| *UV* | *Ultraviolet* |

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