Journal of Pathology
J Pathol 2007; **211**: 241–251
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/path.2098

Review Article

Skin ageing and its treatment

L Baumann*
Department of Dermatology, University of Miami, Miami Beach, FL, USA

**Correspondence to:*
L Baumann, Department of Dermatology, University of Miami, 4701 N. Meridian Avenue, Suite 7450, Miami Beach, FL 33140, USA.
E-mail: lsb@derm.net

No conflicts of interest were declared.

Abstract

The effects of chronic sun exposure on skin are readily apparent when skin not typically exposed to the sun and skin regularly exposed to the sun are compared. While the sun is not the only aetiological factor in the dynamic process of skin ageing, it is the primary exogenous cause among several internal and environmental elements. Thus, photo-ageing, the main focus of this article, is a subset of extrinsic skin ageing. The influence of the sun in extrinsic skin ageing, as well as its role in potentially altering the normal course of intrinsic (also known as natural or cellular) ageing, is discussed. Telomeres, the specialized structures found at the ends of chromosomes, are believed to be integral to cellular ageing as well as in the development of cancer. The ageing process, both intrinsic and extrinsic, is also believed to be influenced by the formation of free radicals, also known as reactive oxygen species. The loss of collagen is considered the characteristic histological finding in aged skin. Wrinkling and pigmentary changes are directly associated with photo-ageing and are considered its most salient cutaneous manifestations. Such photodamage represents the cutaneous signs of premature ageing. In addition, deleterious consequences of chronic sun exposure, specifically various forms of photo-induced skin cancer, are also linked to acute and chronic sun exposure. The only known strategies aimed at preventing photo-ageing include sun avoidance, using sunscreens to block or reduce skin exposure to UV radiation, using retinoids to inhibit collagenase synthesis and to promote collagen production, and using anti-oxidants, particularly in combination, to reduce and neutralize free radicals.

Keywords: photo-ageing; extrinsic ageing; intrinsic ageing; telomeres; free radicals; collagen

Introduction

The contribution or facilitating role of sunlight toward premature skin ageing has been discussed and debated by dermatologists since the end of the 1800s [1], but convincing the public regarding the risks of sun exposure remains an uphill battle, despite success in the use of the skin protection factor (SPF) system in topical products. Still, an inordinate number of patients visiting dermatologists complain about, and seek treatment for, the most salient manifestations of solar exposure — wrinkling and unwanted pigmentation, the tell-tale symptoms of photo-ageing.

While the sun is not the only aetiological factor in skin ageing, it is the primary exogenous cause among several internal and environmental elements. This chapter will focus chiefly on photo-ageing, or the extrinsic skin ageing due to the influence of the sun, but also on the role of the sun in potentially altering the normal course of intrinsic, or natural, ageing. In addition, how both kinds of ageing affect the skin will be discussed, along with strategies aimed at preventing photo-ageing.

Cutaneous ageing

As implied above, there are two primary skin ageing processes, intrinsic and extrinsic. Variations in individual genetic background are thought to govern intrinsic ageing, which results as time passes. By definition, this form of ageing is inevitable and, thus, apparently not subject to manipulation through changes in human behaviour. Conversely, extrinsic ageing is engendered by factors originating externally that are introduced to the human body, such as smoking, excessive alcohol consumption, poor nutrition, and chronic exposure to the sun. Exposure to such elements, which falls within the voluntary realm, although it may sometimes occur under duress, is not inevitable and thus represents premature skin ageing. Of these external factors, sun exposure is considered to be far and away the most significantly deleterious to the skin. Indeed, 80% of facial ageing is believed to be due to chronic sun exposure [2].

Skin that ages intrinsically is smooth and unblemished, and characterized by normal geometric patterns, with some exaggerated expression lines. Histologically, such skin manifests epidermal and dermal

atrophy, flattening of the epidermal rete ridges, as well as reduced numbers of fibroblasts and mast cells [3,4]. In addition, increases are seen in the number of collagen fibrils as well as the ratio of collagen III to collagen I [5].

Exposed areas of the skin, typically the face, chest and extensor surfaces of the arms, display the majority of extrinsically aged skin, which results from the cumulative effects of life-long ultraviolet radiation (UVR) exposure. Rhytides, pigmented lesions (such as ephelides, lentigines, and patchy hyperpigmentation) and depigmented lesions (eg guttate hypomelanosis) comprise the clinical presentation of photo-aged skin. Losses in tone and elasticity are also observed in photo-aged skin, along with increased skin fragility, areas of purpura due to blood vessel weakness, and benign lesions (eg acrochordons, keratoses, and telangiectases). On the Glogau Photo-ageing Scale, which classifies the extent of clinical photodamage, patients with a significant history of sun exposure would likely receive a score that is higher than expected for their age, just as patients with a history of minimal sun exposure would likely score lower than expected for their age.

The histopathological identification of photo-aged skin is made easily, as it is characterized by elastosis. Epidermal atrophy and distinct alterations in collagen and elastic fibres are also associated with photo-aged skin. In particular, skin that is marked by extreme or severe photo-ageing exhibits fragmented, thickened and more soluble collagen fibres [6]. Elastic fibres also experience fragmentation and may exhibit progressive cross-linkage and calcification [7]. Such marked deterioration in the condition of collagen and elastic fibres has been demonstrated to progress with continued exposure to UV radiation. Overall, ageing skin is marked by increased inelasticity, fragmentation and collagen bundle fragility [8].

Intrinsic ageing

Telomeres, the specialized structures found at the ends of eukaryotic chromosomes, have come under increasing scrutiny and are now believed to play an essential role in the intrinsic ageing process at a cellular level. Intact telomeres are integral in extending the lifespan of cells [9]. With age, telomere length shortens. This telomeric erosion has come to be seen as a gauge by which to measure ageing, a veritable internal ageing clock, and the basis for one of the presently favoured theories on ageing [10]. One implication of this theory places ageing and cancer on opposite sides of the same coin. That is, telomerase, the cellular reverse transcriptase enzyme that stabilizes or lengthens telomeres, is expressed in about 85–90% of all human tumours but absent in many somatic tissues [11,12]. Consequently, most cancer cells, unlike healthy ones, are not programmed for apoptosis, or cell death. In other words, the presence of telomerase is associated with telomere stability and tumourigenesis, its absence with telomere shortening and somatic tissue ageing. It is important to note that the epidermis is one of the few regenerative tissues to express telomerase [13]. Further, in a recent investigation of progressive telomere shortening, in 52 specimens of normal human epidermis and 48 specimens of lingual epithelium collected at autopsy from subjects who died between 0 and 101 years of age, researchers determined that the telomere shortening associated with ageing is characterized by tissue-specific loss rates [14]. Indeed, the natural, progressive shortening of telomeres may be one of the primary mechanisms of cellular ageing in skin [15]. Telomeres and other cellular constituents also sustain low-grade oxidative damage as a result of aerobic cellular metabolism, which contributes to intrinsic ageing [16]. Currently, there are no available topical skin care products, systemic drugs or other treatment options that target telomerase because current data do not adequately demonstrate that extending telomere length can be safely performed. One argument for eventual telomerase-based therapies is the belief that inhibiting telomerase may also have antiproliferative and apoptosis-inducing effects not related to the role this ribonucleoprotein plays in shortening telomeres during cell division [17].

Werner syndrome (WS) is an autosomal recessive disorder in which the causative gene, *WRN*, encodes a member of the *RecQ*-like subfamily of DNA helicases [18]. The Werner protein (Wrn), a multifunctional nuclear protein exhibiting $3'–5'$ exonuclease and ATP-dependent helicase activities, is known to play a role in numerous DNA metabolic pathways and to be available in WS patients at reduced levels [19]. Patients with WS exhibit signs of accelerated ageing and the premature onset of various age-related disorders; among these, the incidence of sarcomas and other tumours of mesenchymal origin is higher than in the population at large [20]. In one study, mouse embryo fibroblasts derived from homozygous WS embryos exhibited premature loss of proliferative capacity [21]. In a more recent mouse model study, investigators found that exhaustion of telomere reserves elicited WS and telomere dysfunction provoked various Werner-like symptoms, including hair greying, alopecia, cataracts, osteoporosis, type II diabetes and premature death [22]. In addition, accelerated replicative senescence, chromosomal instability, especially non-epithelial tumours often associated with WS, were exhibited in this model, with telomere shortening implicated as the primary cause. Given the association of WS and telomere shortening and the roles of telomeres and telomerase in cellular ageing, further research into these interconnected phenomena will likely have an impact on further elucidating our understanding of the ageing process, as well as potential therapeutic approaches.

Extrinsic ageing

Extrinsic ageing is largely preventable, by nature and by definition. Factors with clearly exogenous origins,

including smoking, poor nutrition and especially solar exposure, are the main causes of extrinsic, premature, cutaneous ageing. Moreover, sun exposure, as stated previously, is believed to account for 80% of facial ageing [23]. Skin damage results from ultraviolet exposure through several mechanisms, including the formation of sunburn cells as well as thymine and pyrimidine dimers, collagenase production, and the induction of an inflammatory response. Sunburn cells, or UV-induced apoptotic cells, have long been used as markers by which to evaluate skin damage caused by sun exposure. UV-induced apoptosis is mediated by caspase-3, high levels of which are thought to be good indicators of the presence of cellular apoptosis [24]. Activation of apoptosis occurs in a pathway that involves caspase-7 [25]. Mast cells and macrophages are found in greater numbers in photo-aged skin and are also thought to be involved in its causative mechanism [26].

Signalling through p53 after telomere disruption is also linked to ageing in addition to photodamage, in association with UVB more so than UVA [27,28]. Histological examination has demonstrated that more infiltrating mononuclear cells are found in skin chronically exposed to the sun in comparison to protected skin [29]. While much more remains to be understood regarding the mechanisms through which a cascade of adverse health effects are induced by UV exposure, it is well known that photo-ageing, photocarcinogenesis and photo-immunosuppression are sequelae of UV exposure, particularly the UVA range (320–400 nm) [30].

Interestingly, telomeres do not appear to play a central role in extrinsic ageing. In a recent study, in which telomere length was measured in 76 specimens of epidermis from sun-protected sites, 24 specimens of epidermis from sun-exposed sites and 60 specimens of dermis, comparisons showed telomere length to be shorter in the epidermis. Intrinsic senescence was evidenced by reduction in telomere length in the epidermis and dermis with age. Telomere shortening was not associated with photo-ageing in this study, as telomere length was not shown to be significantly different between sun-exposed and sun-protected sites [31].

Currently, sun avoidance and the use of sunscreens are the only defence against sunburn cell formation; they can also protect against thymine dimer formation. Theoretically, the fewer sunburn cells present, the lower the skin damage level due to UV exposure.

Characteristics of ageing skin

The key difference between intrinsic and extrinsic ageing is that the latter falls within the volitional control of the individual. Nevertheless, there are salient features exhibited by aged skin, regardless of the cause(s) of skin ageing. The changes undergone by skin as it ages occur throughout the epidermis, dermis and subcutaneous tissue and can manifest in discrete and broad alterations in skin topography.

Epidermis

When considering the visually obvious nature of skin, it seems counter-intuitive to suggest that the age-related changes in the dermis are more pronounced than those in the epidermis, but this is nonetheless the case. That said, the epidermis does manifest some important changes related to ageing. While some studies indicate that aged skin is characterized by a thinner epidermis [32], other studies do not point to such a finding [33]. There is general agreement, however, that the thickness of the stratum corneum does not change with age. In a study comparing the effects of intrinsic and extrinsic ageing, histopathological examination of 83 biopsies from sun-exposed and protrected skin in healthy volunteers aged 6–84 years revealed epidermal thickness to be constant across the decades in both sun-exposed and -protected skin, with the thickness found to be greater in sun-exposed skin [34]. In a different study, the spinous layer of a wrinkle was shown to be thinner at the base than at the flanks [35]; in addition, according to this study, fewer keratohyaline granules are present in the wrinkle base as compared to the flanks.

In aged skin, the intersection of the epidermis and dermis, known as the dermal–epidermal junction (DEJ), is known to be altered, ie aged epidermis manifests a flattened DEJ with a correspondingly diminished connecting surface area. In a study of abdominal skin, DEJ surface area was shown to be reduced from 2.64 mm^2 in subjects aged 21–40 years to 1.90 mm^2 in subjects aged 61–80 [36]. It is thought that such a loss of DEJ surface area may contribute to the increased fragility of the skin associated with age and may also lead to reduced nutrient transfer between the dermal and epidermal layers.

Decreased cell turnover

Other important age-related changes occur in the epidermal layer. Between the third and eighth decades of life, the epidermal turnover rate slows from 30% to 50% [37]. Stratum corneum transit time was shown by Kligman to be 20 days in young adults and 30 or more days in older adults [38]. Such a cell cycle lengthening in older adults coincides with a protracted stratum corneum replacement rate, epidermal atrophy, slower wound healing and often less effective desquamation. Indeed, older patients have been demonstrated to require double the time to re-epithelialize following dermabrasion resurfacing procedures in comparison to younger patients [39]. The cascade of changes related to decelerated cell turnover results, in older skin, in the development of heaps of corneocytes that render the skin surface rough and dull in appearance. In response to these phenomena, many cosmetic dermatologists

use products (eg hydroxy acids, retinoids) to accelerate the cell cycle, in the belief that a faster turnover rate will yield improvements in skin appearance and speed wound healing following cosmetic procedures.

Dermis

Approximately 20% of dermal thickness disappears as people become elderly [40]. Aged dermis has been shown through structural examination to be relatively acellular and avascular [41]. Changes in collagen production and the development of fragmented elastic fibres also characterize normal aged dermis. Dermis that is also photo-aged exhibits disorganized collagen fibrils and the accumulation of abnormal elastin-containing material [42,43]. As the three primary structural components of the dermis, collagen, elastin and glycosaminoglycans have been the subjects of the majority of anti-ageing research pertaining to the skin.

Collagen

The primary structural component of the dermis and the most abundant protein found in humans, collagen is responsible for conferring strength and support to human skin. Over time, the structural proteins and main components of the skin deteriorate, resulting in the cutaneous signs of ageing. Intrinsically aged skin is characterized by epidermal and dermal atrophy as well as flattening of the rete ridges [44]. Knowledge of the role of collagen in the ageing process over 30 years ago helped to establish the use of bovine collagen as a filling agent to temporarily replace collagen lost with age in soft tissue augmentation procedures. Injectable human-derived products, such as Zyderm and Zyplast, have also emerged during the last decade for these purposes. Other products that contain ingredients such as vitamin C and glycolic acid, and labelled as 'anti-wrinkle creams', are promoted in some cases for their claimed capacity to enhance collagen synthesis. Such products are not harmful, but cannot yet truly match the desired effects. In fact, little is known even about the pathogenesis of wrinkles [45]. The fact that neither an animal nor an *in vitro* model of wrinkling has yet been established may help to explain this gap in knowledge. It is well known, however, that alterations in collagen play an integral role in the ageing process. This, in turn, partly explains the popularity of collagen-containing products intended for 'anti-ageing' purposes.

Of the dry skin mass, 70% is comprised of collagen [46]. In aged skin, collagen is characterized by thickened fibrils, organized in rope-like bundles, that appear to be in disarray in comparison to the pattern observed in younger skin [47]. In addition, lower levels of collagen are synthesized, *in vivo* and *in vitro*, by aged fibroblasts. The ratio of collagen types found in human skin also changes with age. In young skin, collagen I comprises 80% and collagen III comprises about 15% of total skin collagen; in older skin, the ratio of Type III to Type I collagen has been shown to increase, due, significantly, to an appreciable loss of collagen I [48]. In addition, the overall collagen content per unit area of skin surface is known to decline approximately 1%/year [49]. In irradiated skin, collagen I levels have been shown to be reduced by 59% [50]; this reduction was found to be linked to the extent of photodamage [51]. Although collagen I is the most abundant and significant collagen type found in the skin, the effects of ageing are seen in other types of collagen in human dermis.

An integral constituent of the DEJ, collagen IV imparts a structural framework for other molecules and plays a key role in maintaining mechanical stability. No significant differences have been found in collagen IV levels in sun-exposed skin compared to unexposed skin, but significantly lower levels of collagen IV have been identified at the base of wrinkles in comparison to the flanks of the same wrinkles. The mechanical stability of the DEJ may be adversely affected by this loss of collagen IV, thereby contributing to wrinkle formation [52].

Collagen VII is the primary constituent in anchoring fibrils that attach the basement membrane zone to the underlying papillary dermis. In one study, a significantly lower number of anchoring fibrils were identified in patients with chronically sun-exposed skin in comparison to normal controls. It was theorized by the researchers that wrinkles may form as a result of a weakened bond between the dermis and epidermis, due to anchoring fibril degradation [53]. A more recent study showed such a loss of collagen VII to be more marked in the base of the wrinkle (as seen with collagen IV in the same study) [54].

In the last 15 years, the pathogenesis of UVR-induced collagen damage has been well understood and characterized. For instance, it is known that UVR exposure significantly up-regulates the synthesis of several types of collagen-degrading enzymes known as matrix metalloproteinases (MMPs). First, UV exposure leads to an increase in the amount of the transcription factor c-jun; c-fos, the other transcription factor involved in this mechanistic chain, is already abundant without UV exposure. Activator protein-1 (AP-1) is then formed by the combination of c-jun and c-fos. In turn, AP-1 activates the *MMP* genes, which stimulate the production of collagenase, gelatinase and stromelysin. Collagen degradation is mediated by AP-1 activation and by inhibition of transforming growth factor (TGF)β signalling [55]. Research in humans has shown that within hours of UVB exposure, MMPs, specifically collagenase and gelatinase, are produced [56]. Multiple exposures to UVB engender a sustained induction of MMPs [57]. Given that collagenase attacks and degrades collagen, long-term elevations in the levels of collagenase and other MMPs likely yield the disorganized and clumped collagen identified in photo-aged skin. Notably, these MMPs may represent the mechanism through which collagen I levels decline in response to UV exposure. By characterizing

the wide-ranging effects of UV in activating cell surface growth factor and cytokine receptors, researchers have been able to ascertain that skin ageing (extrinsic and intrinsic) is marked by elevated AP-1 activity and MMP expression, inhibited TGFβ signalling, as well as reduced collagen synthesis and greater collagen degradation [58]. These changes are likely to be exacerbated by photo-ageing.

Elastin

Alterations in elastic fibres are so strongly associated with photo-aged skin that 'elastosis', an accumulation of amorphous elastin material, is considered pathognomonic of photo-aged skin. Indeed, UV exposure induces a thickening and coiling of elastic fibres in the papillary dermis. These changes also occur in the reticular dermis as a result of chronic UV exposure [59]. Examination by electron microscopy of elastic fibres in UV-exposed skin has revealed a reduction in the number of microfibrils and increases in interfibrillar areas, the complexity of the shape and arrangement of the fibres and the number of electron-dense inclusions [60]. In addition, small amounts of sugar and lipids and an abnormally high level of polar amino acids have been found in elastin extracted from the skin of elderly patients [61]. The underlying aetiology of age-related changes in elastin is not as well understood as such changes in collagen; however, matrix metalloproteinases are thought to play a role because MMP-2 has been demonstrated to degrade elastin [62].

The initial response of elastic fibres to photodamage is understood, however, to be hyperplastic, resulting in a greater amount of elastic tissue. The level of sun exposure determines the magnitude of the hyperplastic response. In aged elastic fibres, a secondary response to photodamage occurs but is degenerative, with decreases observed in skin elasticity and resiliency [63,64]. Older skin that has experienced this degenerative reaction is characterized by changes in the normal pattern of immature elastic fibres, called oxytalan, that are located in the papillary dermis. These fibres form a network in young skin that ascends perpendicularly from the uppermost section of the papillary dermis to just beneath the basement membrane. This network gradually disappears with age, however [65]. Consequently, skin elasticity is also gradually lost with age [66]. The phenomenon of sagging skin often observed in the elderly may, in fact, be due in large part to this loss of elasticity.

Glycosaminoglycans (GAGs)

GAGs, along with collagen and elastin, are among the primary constituents of dermal skin and are responsible for conferring the outward appearance of the skin. These polysaccharide chains, with repeating disaccharide units attached to a core protein, are also important molecules because they exhibit the capacity to bind water up to 1000 times their volume. There are numerous members in the GAG family, including hyaluronic acid (HA), dermatan sulphate (both of which are two of the most prevalent GAGs) and chondroitin sulphate. These compounds render normal skin plump, soft and hydrated, and are believed to assist in maintaining proper salt and water balance. Several studies suggest that GAGs, particularly HA, have been found to be reduced in amount in photo-aged skin [67]. Some studies offer conflicting reports, however, suggesting no changes in the level of GAGs in aged skin [68]. The fact that HA is synthesized in the epidermis as well as the dermis likely accounts for this discrepancy in findings. In skin that ages intrinsically, the total HA level in the dermis remains stable; however, epidermal HA diminishes almost completely [69].

Hyaluronic acid

Photoaged skin has been shown to be characterized by reduced levels of hyaluronic acid (HA) and elevated levels of chondroitin sulphate proteoglycans [70]. Such patterns, intriguingly, are also observed in scars. HA is found in young skin at the periphery of collagen and elastin fibres and where these types of fibres intersect. In aged skin, such connections with HA disappear [71]. It is possible that the decreases in HA levels, which contribute to its disassociation with collagen and elastin as well as reduced water binding, may be involved in the changes noted in aged skin, including wrinkling, altered elasticity, reduced turgidity and diminished capacity to support the microvasculature of the skin.

As one of the primary GAGs, HA can bind 1000 times its weight in water, and may help the skin retain and maintain water. It is found in all connective tissue and is produced mainly by fibroblasts and keratinocytes in the skin [72]. HA is localized not only in the dermis but also in the epidermal intercellular spaces, especially the middle spinous layer, but not in the stratum corneum (SC) or stratum granulosum [73]. Aged skin, which is less plump than youthful skin, is characterized by decreased levels of HA. The role of HA in skin hydration is not clear and HA does not penetrate the skin upon topical application [74]; however, this has not stopped many companies from putting HA in topical skin care products and claiming efficacy. HA is used successfully, however, as a temporary dermal filling agent in soft tissue augmentation procedures.

Melanocytes

With age, there is a reduction in the number of melanocytes in the range 8–20%/decade. Clinically, this decrease is observed as a reduction in the number of melanocytic nevi in older patients [75]. The skin of older patients is less able to protect itself from the sun because melanin, which is reduced in the elderly, absorbs carcinogenic UV radiation. Therefore, older people are more susceptible to developing sun-induced cancers. For this reason, sun protection remains important even for elderly patients, despite the fact that

the majority of an individual's harmful sun exposure occurs during the first two decades of life. It is not 'too late' for healthy elderly people to begin adding a sunscreen to their skin care regimens.

Vasculature

Aged skin has been shown through numerous studies to be relatively avascular. In one study a 35% reduction in the venous cross-sectional area in aged skin was demonstrated in comparison to young skin [76]. Such a loss in the vascular network is especially noticeable in the papillary dermis, with the disappearance of the vertical capillary loops. Reduced blood flow, depleted nutrient exchange, inhibited thermoregulation, decreased skin surface temperature and skin pallor are associated with the reduction of vascularity.

Subcutaneous tissue

Site-specific changes, including gains and losses, are known to occur in subcutaneous tissues that also influence the appearance of the elderly and their skin. Subcutaneous fat diminishes in the face, dorsal aspects of the hands and the shins. Fat amasses with ageing, though, in other regions, particularly the waist in women and the abdomen in men [77].

The role of free radicals in photo-ageing

The ageing process is believed to be at least partially due to the formation and activity of free radicals, also known as reactive oxygen species (ROS). Free radicals are composed of oxygen molecules with an unpaired electron and are engendered by several exogenous and endogenous factors, including UV exposure, pollution, stress, smoking and normal metabolic processes. Further, some evidence suggests that free radicals induce alterations in gene expression pathways, which in turn contribute to the degradation of collagen and the accumulation of elastin emblematic of photo-aged skin [78]. Anti-oxidants neutralize free radicals by supplying another electron, delivering an electron pair to an oxygen molecule and stabilizing it in the process.

Changes in skin appearance

Dry skin

Dry, scaly skin is frequently seen in the elderly. The degradation or loss of skin barrier function with increasing age is partly accountable for this manifestation. The recovery of damaged barrier function has been demonstrated to be slower in aged skin, resulting in greater susceptibility to developing dryness. This is a multifactorial process due, in part, to lower lipid levels in lamellar bodies [79] and a decrease in epidermal filaggrin [80]. Increased trans-epidermal water loss (TEWL) is also exhibited by aged skin, leaving the stratum corneum more susceptible to becoming dry in low-humidity environments. In addition to dryness, aged skin is often characterized by roughness, wrinkling, skin pallor, hyper- or hypopigmentations, laxity, fragility, easy bruising and benign neoplasms.

Benign neoplasms in ageing skin

With age, the appearance and surface texture of skin can change dramatically, as represented by the development of acrochordons (skin tags), cherry angiomas, seborrheic keratoses, lentigos (sun spots) and sebaceous hyperplasias, among other lesions and cutaneous alterations. Patients of dermatologists and plastic surgeons often request removal of these benign neoplasms. Various destructive treatment modalities are available, including hyfrecation and sundry laser options.

Treatment

Photoaged skin is treated with various in-office procedures and numerous topical agents, most of which are intended to 'resurface' the epidermis. Essentially, this translates to removing the damaged epidermis and, in some cases, dermis, and replacing the tissue with remodelled skin layers. Several anti-oxidants are incorporated into topical skin care products, including vitamins C and E, co-enzyme Q10, ferulic acid, green tea, idebenone, pycnogenol and silymarin. Resurfacing procedures have been shown to sometimes spur the formation of new collagen with a normal staining pattern, as opposed to the basophilic elastotic masses of collagen characteristic of photo-aged skin [81]. It is possible that the potential of growth factors, cytokines and telomerase will eventually be harnessed via technological advancement and innovation in the burgeoning fields of tissue engineering and gene therapy [82].

Although there are several treatments available for aged skin, prevention of extrinsic ageing remains the best approach and should be encouraged to all patients. Of course, this entails avoiding exposure to the sun, using sunscreen when sun avoidance is impossible, avoiding cigarette smoke and pollution, eating a diet high in fruits and vegetables, and taking oral anti-oxidant supplements or topical anti-oxidant formulations. The regular use of prescription retinoids can also help prevent or treat wrinkles.

Prevention

The formation of rhytides is considered the most conspicuous and common manifestation, and nearly a *sine qua non* feature, of skin ageing. Wrinkles appear as a result of changes in the lower, dermal layers of the skin. It might come as a surprise to many consumers, given the ubiquity of advertising that touts the newest topical formulations to eliminate wrinkles and the related expenditure of millions of dollars by consumers

on these 'anti-ageing' products, that few skin care product ingredients have the capacity to penetrate far enough into the dermis to ameliorate deep wrinkles. Prevention of wrinkle development, therefore, has assumed a fundamental status in anti-ageing skin care [83]. To prevent the formation of wrinkles, it is necessary to halt the degradation of the skin's three primary structural constituents, collagen, elastin and HA, since all three components are known to decline with age. Consequently, most anti-ageing procedures and products are designed or formulated with the intention of salvaging at least one of these basic cutaneous substances. Because the technology required to suitably deliver these compounds into the skin has not yet been developed, topical products containing collagen, elastin or HA are unable to serve as adequate replacements for what is lost from the skin through ageing. Although no products replenish these key skin components, some products do promote the natural synthesis of these substances. For example, collagen production has been shown to be stimulated by the use of retinoids [84], vitamin C [85] and copper peptide. Collagen synthesis may also be brought about through the use of oral vitamin C [86]. In animal models, retinoids have been shown to increase production of HA [87] and elastin [88]. HA levels are also thought to be augmented with glucosamine supplementation [89]. There are no products yet approved for increasing the production of, or enhancing, elastin.

Because inflammation is a known contributor to the degradation of collagen, elastin and HA, reducing inflammation is another integral approach to preventing wrinkle formation. Anti-oxidants, all of which display various distinguishing characteristics and activities, are believed to be an important focus in this endeavour, as these free radical scavengers protect the skin via several mechanisms that are just beginning to be elucidated. Skin inflammation is a known sequela of free radicals directly acting on cytokine and growth factor receptors in dermal cells and keratinocytes. Cytokines and growth factors are known to play a role in skin ageing, but the exact nature of their significance has not yet been clarified. Presently, these compounds are understood to function synergistically in a complex cascade of events requiring the inclusion of several types of cytokines and growth factors [90]. The process is thought to be induced by UV exposure, which affects growth factor and cytokine receptors in keratinocytes and dermal cells, contributing to downstream signal transduction by spurring mitogen-activated protein (MAP) kinase pathways (specifically, extracellular signal-regulated kinase, c-jun N-terminal protein kinase, and p38), which collect in cell nuclei and form cFos–cJun complexes of transcription factor AP-1 and trigger the MMPs collagenase, 92 kDa gelatinase and stromelysin, which attack collagen and other connective tissue of the skin [91,92].

The direct effects of free radicals on the ageing process and cutaneous ageing are understood with greater clarity and certainty, however. For example, free radical activation of the MAP kinase pathways has been shown to stimulate collagenase production, which leads to the breakdown of collagen [93]. Specifically, Kang *et al* showed that pretreating human skin with the anti-oxidants genistein and N-acetyl cysteine inhibited the UV induction of the cJun-driven enzyme collagenase. The use of anti-oxidants to hinder these pathways is thought to inhibit photo-ageing by preventing collagenase production and its resulting detrimental influence on collagen. Interestingly, this study showed that, although genistein and N-acetyl cysteine exhibit anti-oxidant activity, these anti-oxidants exerted no effect on UV-induced erythema. Previous work has also pointed to the likelihood that using anti-oxidants in combination confers synergistic benefits. In a randomized, double-blind, parallel-group, placebo-controlled study of the effects of an oral combination of vitamins C and E, carotenoids, selenium and proanthocyanidins, participants who took the anti-oxidant combination before exposure to UVB exhibited a difference in MMP-1 production in comparison to the placebo group ($p < 0.05$) [94]. Similar to the study by Kang, there were no significant differences between the oral anti-oxidant group and the placebo group regarding minimal erythema dose of the skin.

In yet another recent study of the effects of combined anti-oxidants, one group of subjects was treated daily with a base cream containing 0.05% ubiquinone (co-enzyme Q10), 0.1% vitamin E and 1% squalene, and 50 mg co-enzyme Q10, 50 mg D-RRR-α-tocopheryl acetate and 50 μg selenium were orally administered, while the second group was treated with base cream only [95]. Patients treated with the topical anti-oxidant cream alone exhibited significant increases in the concentration of co-enzyme Q10, D-RRR-α-tocopherol and squalene in the sebum (although not in the stratum corneum or plasma). The group treated both topically and orally also demonstrated higher levels of vitamin E and co-enzyme Q10 in the stratum corneum.

In terms of preventing the effects of photo-ageing, it is not yet known which anti-oxidants are the most effective. Using topical and oral anti-oxidants in combination will likely be the favoured recommendation in the near future. Anti-oxidants should also be used in combination with sunscreens and retinoids to enhance their protective effects. Indeed, it is worth remembering that not all sunscreens have an anti-oxidant effect and not all anti-oxidants have a sunscreen effect. However, a recent Duke University study has demonstrated that vitamins C and E combined with ferulic acid impart both a sunscreen effect and an anti-oxidant effect [96].

Some notable anti-oxidants

Conceivably, volumes could be written on the plethora of natural compounds found in recent years to exhibit

anti-oxidant activity. That is to say, an exhaustive survey of green tea, tea tree oil, grape seed extract, vitamins C and E, ferulic acid, etc. would far exceed the limits of this chapter. A few of the anti-oxidant ingredients that have recently gained favour and attention are briefly discussed here.

Coenzyme Q10 or ubiquinone is a fat-soluble anti-oxidant found in all cells as part of the electron transportation chain responsible for energy production, and has been shown to exhibit antiapoptotic activity [97]. It also naturally occurs in fish, shellfish, spinach and nuts. Like the key constituents of the skin, co-enzyme Q10 has been shown to diminish with age in animals and humans [98]. While UV light is known to remove vitamins C and E, glutathione and co-enzyme Q10 from the epidermal and dermal layers of the skin, co-enzyme Q10 is consistently the first anti-oxidant to be depleted in the skin.

Derived from tropical fern, *Polypodium leucotomos* (PL) extract has exhibited potent anti-oxidant activity. The incidence of phototoxicity was demonstrated to decrease after oral PL administration in subjects receiving psoralen–UVA (PUVA) treatment [99], as well as in normal healthy subjects [100]. In another study, PL-treated keratinocytes and fibroblasts exposed to UV displayed significantly ameliorated membrane integrity, mitigated lipid peroxidation, increased elastin expression and inhibited MMP-1 expression [101].

Silymarin, a naturally occurring polyphenolic flavonoid or flavonolignans compound derived from the seeds of the milk thistle plant *Silybum marianu*, has been shown in several animal studies to exhibit anti-oxidant, anti-inflammatory and immunomodulatory properties that may contribute to preventing skin cancer as well as photo-ageing [102]. The beneficial influence of silymarin is primarily ascribed to silybin, which has been demonstrated to be bioavailable in skin and other tissues after systemic administration [103]. In addition, topical application of silybin before or directly after UV exposure has been found to confer potent protection against UV-induced epidermal damage, by depleting thymine dimer-positive cells [104].

Pycnogenol, a plant-derived substance found in many plant extracts, such as pine bark, grapes and apples, is rich in the potent free radical-scavenging group of compounds known as procyanidins (also called proanthocyanidins). Procyanidins are also contained in several other plants or parts thereof known for conferring anti-oxidant activity, including grape seed, grape skin, bilberry, cranberry, blackcurrant, green tea, black tea, blueberry, blackberry, strawberry, black cherry, red wine and red cabbage. In one study, Skh : hr hairless mice pretreated with pycnogenol concentrations of 0.05–0.2% demonstrated a dose-dependent reduction of the inflammatory sunburn reaction (oedema) following minimally inflammatory daily exposures to solar-simulated UV radiation [105]. In another study, oral supplementation with pycnogenol appeared to ease the effects of UV radiation on the skin, specifically reducing erythema, in 21 volunteers [106]. The UV radiation level necessary to reach one minimal erythema dose (MED) was significantly elevated during supplementation. In addition to its anti-oxidant activity, pycnogenol is known to impart anti-inflammatory effects, which are believed to result, at least in part, from its inhibition of IFNγ-induced expression of ICAM-1 [107].

Of course, sun avoidance and sunscreen use are well established to be the primary components in anti-ageing regimens, although still underappreciated by many segments of the public. A recent study in the *Journal of the American Medical Association* specifically reinforces the utility of sunscreen; in this study, children with the proclivity to freckle developed 30–40% fewer freckles when daily treated with an SPF 30 sunscreen, as compared to children not treated with a sunscreen [108]. This study buttresses dermatologists' recommendations for sun protection in preventing the development of these pigmented lesions, which not only make the skin appear older but are associated with an increased risk of melanoma. Clearly, sun avoidance is not easy to manage and is often impossible, as well as being an unpopular or poorly received suggestion among many patients. That said, practitioners should gauge the receptivity of their patients and recommend as stringent a sun-avoidance regimen as will likely be accepted. At the very least, patients should be discouraged from any exposure to tanning beds or engaging in unnecessary sun exposure, particularly between 10 a.m. and 4 p.m. Using a Wood's light to show patients the sun damage that they have already incurred can be a useful approach toward persuading them to curb their sun exposure and strive for feasible sun avoidance. This demonstration can also convince patients to incorporate protective measures, such as use of sunscreens, anti-oxidants and retinoids, particularly since many patients mistakenly believe, before being convinced otherwise, that their sun exposure is minimal and that any behavioural modification is unwarranted. Indeed, sunscreen should be recommended for daily use, even when the patient intends to remain indoors. Along these lines, patients should be reminded that UVA rays have the capacity to penetrate glass, so they should also limit their risk at home and at work by not lingering near sun-splashed windows. UVA shields placed on windows can provide some protection, however. Finally, sun-protective clothing, such as a broad-brimmed hat and SPF 45 clothing, should be encouraged for patients as general advice for when sun avoidance is not practical and for those who anticipate prolonged exposure.

Conclusion

Skin ageing is a dynamic, multifactorial process, best characterized and understood in dichotomous

expressions: intrinsic or natural ageing is cellularly determined as a function of heredity, is inevitable and results in cutaneous alterations; extrinsic ageing, which also manifests in cutaneous changes, originates from exogenous sources and is avoidable. In other words, intrinsic ageing is a natural result of the passage of time, and not subject to the realm or whims of human control or behaviour. Extrinsic ageing results from various factors, but exposure to the sun is the primary source. Therefore, photo-ageing is roughly synonymous with, although technically a subset of, extrinsic ageing.

The American Academy of Dermatology, practising dermatologists and other clinicians have been preaching the mantra that 'there is no such thing as a healthy tan', with some portion of the populace absorbing this message. Citing the attendant wrinkling and pigmentary changes associated with photo-ageing and the potentially more serious consequences of chronic sun exposure can be effective approaches for doctors, as this method appeals to an individual's strong concern about appearance. The clinical appearance of photo-ageing is characterized by rough, dry skin, mottled pigmentation and wrinkling. Such cutaneous manifestations, particularly when extensive or severe, can be harbingers of skin cancer. It is important for physicians to impress upon patients that photodamage represents the cutaneous signs of premature ageing. A summary of the role of telomeres in cellular ageing and cancer and/or a brief discussion of the differences between intrinsic and extrinsic ageing might prove useful in altering the behaviour of patients and stemming the tide of photodamage, photo-ageing, and photo-induced skin cancers.

The only known defences against photo-ageing beyond sun avoidance are using sunscreens to block or reduce the amount of UV reaching the skin, using retinoids to inhibit collagenase synthesis and to promote collagen production, and using anti-oxidants, particularly in combination, to reduce and neutralize free radicals.

References

1. Unna PG. *Histopathologie der Hautkrankheiten*. A. Herschwald: Berlin, 1894.
2. Uitto J. Understanding premature skin aging. *N Engl J Med* 1997;**337**(20):1463–1465.
3. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
4. Roupe G. Skin of the aging human being. *Lakartidningen.* 2001;**98**(10):1091–1095.
5. Lovell CR, Smolenski KA, Duance VC, Light ND, Young S, Dyson M. Type I and III collagen content and fibre distribution in normal human skin during ageing. *Br J Dermatol* 1987;**117**(4):419–428.
6. Lavker RM. Structural alterations in exposed and unexposed aged skin. *J Invest Dermatol* 1979;**73**(1):59–66.
7. Yaar M, Gilchrest BA. Aging of skin. In *Fitzpatrick's Dermatology in General Medicine* Vol 2, 5th edn. McGraw-Hill: New York, 1999; 1700.
8. Roupe G. Skin of the aging human being. *Lakartidningen* 2001;**98**(10):1091–1095.
9. Geserick C, Blasco MA. Novel roles for telomerase in aging. *Mech Ageing Dev* 2006;**127**(6):579–583 [Epub 3 March 2006].
10. Boukamp P. Ageing mechanisms: the role of telomere loss. *Clin Exp Dermatol* 2001;**26**(7):562–565.
11. Boukamp P. Ageing mechanisms: the role of telomere loss. *Clin Exp Dermatol.* 2001;**26**(7):562–565.
12. Pendino F, Tarkanyi I, Dudognon C, Hillion J, Lanotte M, Aradi J, *et al*. Telomeres and telomerase: pharmacological targets for new anticancer strategies? *Curr Cancer Drug Targets* 2006;**6**(2):147–180.
13. Boukamp P. Skin aging: a role for telomerase and telomere dynamics? *Curr Mol Med.* 2005;**5**(2):171–177.
14. Nakamura K, Izumiyama-Shimomura N, Sawabe M, Arai T, Aoyagi Y, Fujiwara M, *et al*. Comparative analysis of telomere lengths and erosion with age in human epidermis and lingual epithelium. *J Invest Dermatol* 2002;**119**(5):1014–1019.
15. Roupe G. Skin of the aging human being. *Lakartidningen* 2001;**98**(10):1091–1095.
16. Kosmadaki MG, Gilchrest BA. The role of telomeres in skin aging/photoaging. *Micron* 2004;**35**(3):155–159.
17. Pendino F, Tarkanyi I, Dudognon C, Hillion J, Lanotte M, Aradi J, *et al*. Telomeres and telomerase: pharmacological targets for new anticancer strategies? *Curr Cancer Drug Targets* 2006;**6**(2):147–180.
18. Lebel M, Leder P. A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. *Proc Natl Acad Sci USA* 1998;**95**(22):13097–13102.
19. Ahn B, Harrigan JA, Indig FE, Wilson DM III, Bohr VA. Regulation of WRN helicase activity in human base excision repair. *J Biol Chem* 2004;**279**(51):53465–53474 [Epub 22 September 2004].
20. Poot M, Gollahon KA, Emond MJ, Silber JR, Rabinovitch PS. Werner syndrome diploid fibroblasts are sensitive to 4-nitroquinoline-N-oxide and 8-methoxypsoralen: implications for the disease phenotype. *FASEB J* 2002;**16**(7):757–758 [Epub 12 Mar 2002].
21. Lebel M, Leder P. A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. *Proc Natl Acad Sci USA* 1998;**95**(22):13097–13102.
22. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, *et al*. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet* 2004;**36**(8):877–882 [Epub 4 July 2004].
23. Uitto J. Understanding premature skin aging. *N Engl J Med* 1997;**337**(20):1463–1465.
24. Yao W, Malaviya R, Magliocco M, Gottlieb A. Topical treatment of UVB-irradiated human subjects with EGCG, a green tea polyphenol, increases caspase-3 activity in keratinocytes. *J Am Acad Dermatol* 2005;**52**(3, pt 2):150.
25. Pinnell S, Lin F-Y, Grichnik J. A topical anti-oxidant solution containing vitamin C, vitamin E, and ferulic acid prevents ultraviolet radiation induced caspase-3 induction in skin. *J Am Acad Dermatol* 2005;**52**(3, pt 2):158.
26. Bosset S, Bonnet-Duquennoy M, Barre P, Chalon A, Kurfurst R, Bonte F, *et al*. Photo-ageing shows histological features of chronic skin inflammation without clinical and molecular abnormalities. *Br J Dermatol* 2003;**149**(4):826–835.
27. Kosmadaki MG, Gilchrest BA. The role of telomeres in skin aging/photoaging. *Micron* 2004;**35**(3):155–159.
28. Kappes UP, Luo D, Potter M, Schulmeister K, Runger TM. Short- and long-wave UV light (UVB and UVA) induce similar mutations in human skin cells. *J Invest Dermatol* 2006;**126**(3):667–675.
29. Bosset S, Bonnet-Duquennoy M, Barre P, Chalon A, Kurfurst R, Bonte F, *et al*. Photo-ageing shows histological features of chronic skin inflammation without clinical and molecular abnormalities. *Br J Dermatol* 2003;**149**(4):826–835.

30. Marrot L, Belaïdi JP, Meunier JR. Importance of UVA photoprotection as shown by genotoxic related endpoints: DNA damage and p53 status. *Mutat Res* 2005;**571**(1–2):175–184.
31. Sugimoto M, Yamashita R, Ueda M. Telomere length of the skin in association with chronological aging and photoaging. *J Dermatol Sci* 2006;**43**(1):43–47 [Epub 9 March 2006].
32. Lavker RM. Structural alterations in exposed and unexposed aged skin. *J Invest Dermatol* 1979;**73**(1):59–66.
33. Whitton JT, Everall JD. The thickness of the epidermis. *Br J Dermatol* 1973;**89**(5):467–476.
34. El-Domyati M, Attia S, Saleh F, Brown D, Birk DE, Gasparro F, *et al*. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol* 2002;**11**(5):398–405.
35. Contet-Audonneau JL, Jeanmaire C, Pauly G. A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. *Br J Dermatol* 1999;**140**(6):1038–1047.
36. Katzberg AA. The area of the dermo–epidermal junction in human skin. *Anat Rec* 1958;**131:**717.
37. Yaar M, Gilchrest BA. Aging of skin. In *Fitzpatrick's Dermatology in General Medicine* Vol 2, 5th edn. McGraw-Hill: New York, 1999; 1697–1706.
38. Kligman AM. Perspectives and problems in cutaneous gerontology. *J Invest Dermatol* 1979;**73**(1):39–46.
39. Orentreich N, Selmanowitz VJ. Levels of biological functions with aging. *Trans NY Acad Sci* 1969;**31:**992.
40. Ibid.
41. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
42. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997;**337**(20):1419–1428.
43. El-Domyati M, Attia S, Saleh F, Brown D, Birk DE, Gasparro F, *et al*. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol* 2002;**11**(5):398–405.
44. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
45. Kligman AM, Zheng P, Lavker RM. The anatomy and pathogenesis of wrinkles. *Br J Dermatol* 1985;**113**(1):37–42.
46. Gniadecka M, Nielsen OF, Wessel S, Heidenheim M, Christensen DH, Wulf HC. Water and protein structure in photoaged and chronically aged skin. *J Invest Dermatol* 1998;**111**(6):1129–1133.
47. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
48. Oikarinen A. The aging of skin: chronoaging versus photoaging. *Photodermatol Photoimmunol Photomed* 1990;**7**(1):3–4.
49. Shuster S, Black MM, McVitie E. The influence of age and sex on skin thickness, skin collagen and density. *Br J Dermatol* 1975;**93**(6):639–643.
50. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997;**337**(20):1419–1428.
51. Griffiths CE, Russman AN, Majmudar G, Singer RS, Hamilton TA, Voorhees JJ. Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). *N Engl J Med* 1993;**329**(8):530–535.
52. Contet-Audonneau JL, Jeanmaire C, Pauly G. A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. *Br J Dermatol* 1999;**140**(6):1038–1047.
53. Craven NM, Watson RE, Jones CJ, Shuttleworth CA, Kielty CM, Griffiths CE. Clinical features of photodamaged human skin are associated with a reduction in collagen VII. *Br J Dermatol* 1997;**137**(3):344–350.
54. Contet-Audonneau JL, Jeanmaire C, Pauly G. A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. *Br J Dermatol* 1999;**140**(6):1038–1047.
55. Rittie L, Fisher GJ. UV-light-induced signal cascades and skin aging. *Ageing Res Rev* 2002;**1**(4):705–720.
56. Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, *et al*. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* 1996;**379**(6563):335–339.
57. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997;**337**(20):1419–1428.
58. Rittie L, Fisher GJ. UV-light-induced signal cascades and skin aging. *Ageing Res Rev* 2002;**1**(4):705–720.
59. Mitchel RE. Chronic solar dermatosis: a light and electron microscopic study of the dermis. *J Invest Dermatol* 1967;**48**(3):203–220.
60. Tsuji T, Hamada T. Age-related changes in human dermal elastic fibres. *Br J Dermatol* 1981;**105**(1):57–63.
61. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
62. Scharffetter-Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, *et al*. Photoaging of the skin from phenotype to mechanisms. *Exp Gerontol* 2000;**35**(3):307–316.
63. Matsuoka L, Uitto J. Alterations in the elastic fibers in cutaneous aging and solar elastosis. In *Aging and the Skin*, Balin A, Kligman AM (eds). Raven: New York, 1989; 141–151.
64. Lavker RM. Cutaneous aging: chronologic versus photoaging. In *Photodamage* (1st edn), Gilchrest BA (ed.). Blackwell Science: Cambridge, MA, 1995; 128.
65. Montagna W, Carlisle K. Structural changes in aging human skin. *J Invest Dermatol* 1979;**73**(1):47–53.
66. Escoffier C, de Rigal J, Rochefort A, Vasselet R, Leveque JL, Agache PG. Age-related mechanical properties of human skin: an *in vivo* study. *J Invest Dermatol* 1989;**93**(3):353–357.
67. Ghersetich I, Lotti T, Campanile G, Grappone C, Dini G. Hyaluronic acid in cutaneous intrinsic aging. *Int J Dermatol* 1994;**33**(2):119–122.
68. Pearce RH, Grimmer BJ. Age and the chemical constitution of normal human dermis. *J Invest Dermatol* 1972;**58**(6):347–361.
69. Elsner P, Maibach HI. *Cosmeceuticals and Active Cosmetics: Drugs versus Cosmetics* (2nd edn). Marcel Dekker: New York, 2005.
70. Bernstein EF, Underhill CB, Hahn PJ, Brown DB, Uitto J. Chronic sun exposure alters both the content and distribution of dermal glycosaminoglycans. *Br J Dermatol* 1996;**135**(2):255–262.
71. Ghersetich I, Lotti T, Campanile G, Grappone C, Dini G. Hyaluronic acid in cutaneous intrinsic aging. *Int J Dermatol* 1994;**33**(2):119–122.
72. Tammi R, Säämänen AM, Maibach HI, Tammi M. Degradation of newly synthesized high molecular mass hyaluronan in the epidermal and dermal compartments of human skin in organ culture. *J Invest Dermatol* 1991;**97**(1):126–130.
73. Sakai S, Yasuda R, Sayo T, Ishikawa O, Inoue S. Hyaluronan exists in the normal stratum corneum. *J Invest Dermatol* 2000;**114**(6):1184–1187.
74. Rieger M. Hyaluronic acid in cosmetics. *Cosm Toil* 1998;**113**(3):35–42.
75. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
76. Gilchrest BA, Stoff JS, Soter NA. Chronologic aging alters the response to ultraviolet-induced inflammation in human skin. *J Invest Dermatol* 1982;**79**(1):11–15.
77. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;**15**(4, pt 1):571–585.
78. Scharffetter-Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, *et al*. Photoaging of the skin from phenotype to mechanisms. *Exp Gerontol* 2000;**35**(3):307–316.

79. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 1995;**95**(5):2281–2290.
80. Tezuka T, Qing J, Saheki M, Kusuda S, Takahashi M. Terminal differentiation of facial epidermis of the aged: immunohistochemical studies. *Dermatology* 1994;**188**(1):21–24.
81. Nelson BR, Majmudar G, Griffiths CE, Gillard MO, Dixon AE, Tavakkol A, *et al*. Clinical improvement following dermabrasion of photoaged skin correlates with synthesis of collagen I. *Arch Dermatol* 1994;**130**(9):1136–1142.
82. Ostler EL, Wallis CV, Aboalchamat B, Faragher RG. Telomerase and the cellular lifespan: implications of the aging process. *J Pediatr Endocrinol Metab* 2000;**13**(suppl 6):1467–1476.
83. Baumann L. How to prevent photoaging? *J Invest Dermatol* 2005;**125**(4):xii–xiii.
84. Varani J, Warner RL, Gharaee-Kermani M, Phan SH, Kang S, Chung JH, *et al*. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. *J Invest Dermatol* 2000;**114**(3):480–486.
85. Nusgens BV, Humbert P, Rougier A, Colige AC, Haftek M, Lambert CA, *et al*. Topically applied vitamin C enhances the mRNA level of collagens I and III, their processing enzymes and tissue inhibitor of matrix metalloproteinase 1 in the human dermis. *J Invest Dermatol* 2001;**116**(6):853–859.
86. Kockaert M, Neumann M. Systemic and topical drugs for aging skin. *J Drugs Dermatol* 2003;**2**(4):435–441.
87. Margelin D, Medaisko C, Lombard D, Picard J, Fourtanier A. Hyaluronic acid and dermatan sulfate are selectively stimulated by retinoic acid in irradiated and nonirradiated hairless mouse skin. *J Invest Dermatol* 1996;**106**(3):505–509.
88. Tajima S, Hayashi A, Suzuki T. Elastin expression is up-regulated by retinoic acid but not by retinol in chick embryonic skin fibroblasts. *J Dermatol Sci* 1997;**15**(3):166–172.
89. Matheson AJ, Perry CM. Glucosamine: a review of its use in the management of osteoarthritis. *Drugs Aging* 2003;**20**(14):1041–1060.
90. Fitzpatrick RE. Endogenous growth factors as cosmeceuticals. *Dermatol Surg* 2005;**31**(7, pt 2):827–831; discussion, 831.
91. Fisher GJ, Voorhees JJ. Molecular mechanisms of photoaging and its prevention by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin *in vivo*. *J Investig Dermatol Symp Proc* 1998;**3**(1):61–68.
92. Kang S, Chung JH, Lee JH, Fisher GJ, Wan YS, Duell EA, *et al*. Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin *in vivo*. *J Invest Dermatol* 2003;**120**(5):835–841.
93. Kang S, Chung JH, Lee JH, Fisher GJ, Wan YS, Duell EA, *et al*. Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin *in vivo*. *J Invest Dermatol* 2003;**120**(5):835–841.
94. Greul AK, Grundmann JU, Heinrich F, Pfitzner I, Bernhardt J, Ambach A, *et al*. Photoprotection of UV-irradiated human skin: an antioxidative combination of vitamins E and C, carotenoids, selenium and proanthocyanidins. *Skin Pharm Appl Skin Physiol* 2002;**15**(5):307–315.
95. Passi S, De Pita O, Grandinetti M, Simotti C, Littaru GP. The combined use of oral and topical lipophilic anti-oxidants increases their levels both in sebum and stratum corneum. *Biofactors* 2003;**18**(1–4):289–297.
96. Lin FH, Lin JY, Gupta RD, Tournas JA, Burch JA, Selim MA, *et al*. Ferulic acid stabilizes a solution of vitamins C and E and doubles its photoprotection of skin. *J Invest Dermatol* 2005;**125**(4):826–832.
97. Papucci L, Schiavone N, Witort E, Donnini M, Lapucci A, Tempestini A, *et al*. Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property. *J Biol Chem* 2003;**278**(30):28220–28228.
98. Beyer RE, Ernster L. The anti-oxidant role of coenzyme Q. In *Highlights in Ubiquinone Research*, Lenaz G, *et al* (eds). Taylor and Francis: London, 1990; 191–213.
99. Middlekamp-Hup MA, Pathak MA, Parrado C, Garcia-Caballero T, Rius-Diaz F, Fitzpatrick TB, *et al*. Orally administered *Polypodium leucotomos* extract decreases psoralen–UVA-induced phototoxicity, pigmentation, and damage of human skin. *J Am Acad Dermatol* 2004;**50**(1):41–49.
100. Middlekamp-Hup MA, Pathak MA, Parrado C, Goukassian D, Rius-Diaz F, Mihm MC, *et al*. Oral *Polypodium leucotomos* extract decreases ultraviolet-induced damage of human skin. *J Am Acad Dermatol* 2004;**51**(6):910–918.
101. Philips N, Smith J, Keller T, Gonzalez S. Predominant effects of *Polypodium leucotomos* on membrane integrity, lipid peroxidation, and expression of elastin and matrixmetalloproteinase-1 in ultraviolet radiation exposed fibroblasts, and keratinocytes. *J Dermatol Sci* 2003;**32**(1):1–9.
102. Katiyar SK. Silymarin and skin cancer prevention: anti-inflammatory, anti-oxidant and immunomodulatory effects (review). *Int J Oncol* 2005;**26**(1):169–176.
103. Zhao J, Agarwal R. Tissue distribution of silibinin, the major active constituent of silymarin, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention. *Carcinogenesis* 1999;**20**(11):2101–2108.
104. Dhanalakshmi S, Mallikarjuna GU, Singh RP, Agarwal R. Silibinin prevents ultraviolet radiation-caused skin damages in SKH-1 hairless mice via a decrease in thymine dimer positive cells and an up-regulation of p53-p21/Cip1 in epidermis. *Carcinogenesis* 2004;**25**(8):1459–1465.
105. Sime S, Reeve VE. Protection from inflammation, immunosuppression and carcinogenesis induced by UV radiation in mice by topical pycnogenol. *Photochem Photobiol* 2004;**79**(2):193–198.
106. Saliou C, Rimbach G, Moini H, McLaughlin L, Hosseini S, Lee J, *et al*. Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B-dependent gene expression in keratinocytes are modulated by a French maritime pine bark extract. *Free Radic Biol Med* 2001;**30**(2):154–160.
107. Bito T, Roy S, Sen CK, Packer L. Pine bark extract pycnogenol downregulates IFN-gamma-induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression. *Free Radic Biol Med* 2000;**28**(2):219–227.
108. Gallagher RP, Rivers JK, Lee TK, Bajdik CD, McLean DI, Coldman AJ. Broad-spectrum sunscreen use and the development of new nevi in white children: a randomized controlled trial. *J Am Med Assoc* 2000;**283**(22):2955–2960.