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Review Article

A new wrinkle on old skin: the role of elastic fibres in skin ageing

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Synopsis

Cutaneous ageing is the result of two distinct, biological processes which may occur concurrently: (i) the passage of time, termed intrinsic ageing and (ii) environmental influences, termed extrinsic ageing. Intrinsic ageing of the skin is a slow process which causes changes in tissue structure and impairs function in the absence of additional biological, chemical and physical factors. The clinical features of intrinsically aged skin are not usually evident until old age when, although smooth and unblemished, the skin surface appears pale and is characterized by fine wrinkles with occasional exaggerated expression lines. Functionally, intrinsically aged skin is dry and less elastic than more youthful skin. In contrast, extrinsically aged skin is exemplified by deep, coarse wrinkles, mottled hyperpigmentation and a marked loss of elasticity and recoil. The two major environmental influences which induce extrinsic ageing are: (i) chronic exposure to solar ultraviolet (UV) irradiation (termed photoageing) and (ii) smoking. This review discusses the changes associated with the ageing process in the skin, with particular emphasis on the role played by the elastic fibre network in maintaining dermal function. The review concludes with a discussion of a short-term assay for independent assessment of the efficacy of anti-ageing cosmetic products using the elastic fibre component fibrillin-1 as a biomarker of extracellular matrix repair.

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Résumé

Le vieillissement Cutané est le résultat de deux processus biologiques distincts, qui peuvent se produire concurremment : i) le passage de temps, désigné comme vieillissement intrinsèque et ii) les influences environnementales, désignées comme vieillissement extrinsèque. Le vieillissement intrinsèque de la peau est un processus lent provoquant des changements de la structure et détériorant la fonction tissulaire sans facteurs biologiques, chimiques ou physiques supplémentaires. Les caractéristiques cliniques de la peau intrinsèquement âgée sont peu visibles avant la vieillesse où, bien que lisse et impeccable, la surface de la peau apparaît pâle et marquée par des rides notables et des lignes d'expression exagérées. Au niveau fonctionnel, la peau intrinsèquement âgée est sèche et moins d'élastique que la peau plus jeune. Au contraire, la peau extrinsèquement âgée est caractérisée par des rides profondes, grossières, une hyperpigmentation en taches et une perte marquée d'élasticité. Les deux influences environnementales majeures à l'origine du vieillissement extrinsèque sont : i) l'exposition chronique aux ultra-violets (UV) et ii) l'exposition tabagique. Cette revue envisage les changements associés au processus de

vieillissement cutané, avec une attention particulière sur le rôle joué par le réseau élastique dans le maintien de la fonction dermique. Cette analyse se termine par une discussion à propos d'un essai d'évaluation de l'efficacité de produits cosmétiques anti-âges utilisant un composant de fibre élastique la fibrillin-1 comme bio marqueur de la réparation de la matrice extracellulaire.

Structural organization of the skin

In human skin, an outermost avascular layer, the epidermis, receives nourishment via diffusion from the underlying vascularized dermis. A basement membrane marks the interface between these layers and this region is termed the dermal–epidermal junction (DEJ). In most anatomical sites, the epidermis is subdivided into four cell layers: *stratum corneum*, *granulosum*, *spinosum* and *basale*. Palmar-plantar skin also contains a *stratum lucidum* layer, which is found between the *stratum granulosum* and *stratum corneum*.

The dermis is subdivided into an uppermost papillary dermis and an underlying reticular dermis. The papillary dermis comprises loose connective tissue that forms strong links with the basement membrane. In humans, finger-like projections called rete ridges interdigitate with the dermis to strengthen this connection between the two compartments. In common with other dense connective tissue, the extracellular matrix (ECM) of the dermis provides strength, extensibility and elasticity to the skin. Fibroblasts secrete the majority of this ECM.

The dermal extracellular matrix

The dermal ECM has a dynamic and complex structure composed of an interlocking mesh of fibrous proteins (both collagens and elastic fibres) and glycosaminoglycan-rich proteoglycans. These ECM proteins can form large complexes that collectively dictate a tissue's mechanical property and assist in cell adhesion and regulation of cell signalling. The ECM is a dynamic structure, with some components under constant modification. The main constituent of the dermal ECM is collagen, particularly collagens I and III, which provide skin with tensile strength.

The dermal elastic fibre network

Elastic fibres of the dermis are composed of an elastin core and a microfibrillar scaffold, which in adult tissues comprises mainly the glycoprotein, fibrillin-1. This network of fibres imbues skin with elasticity [1]. Elastic fibre formation is under tight developmental control. During early development, tropoelastin is first deposited perinatally onto a pre-formed scaffold of fibrillin-rich microfibrils [2] which define the direction of fibre growth. These microfibrils persist in adult tissue on the periphery of mature elastic fibres and as independent structures.

The elastic fibre network forms a distinctive arrangement within the dermis. In the reticular dermis, the elastic fibre network comprises thick elastin-rich fibres which run in parallel to the DEJ. The lower papillary dermis contains a finer network of elaunin fibres, that have a reduced elastin content and connect to candelabra-like cascades of discrete, fibrillin-rich microfibrillar bundles called oxytalan fibres at the DEJ [3, 4] (Fig. 1). Elastic fibres are required to maintain their elastic function for a lifetime; however, various enzymes, for example matrix metalloproteinases (MMPs), are able to cleave elastic fibre molecules [5].

The importance of elastic fibres in connective tissues is highlighted by the severe heritable

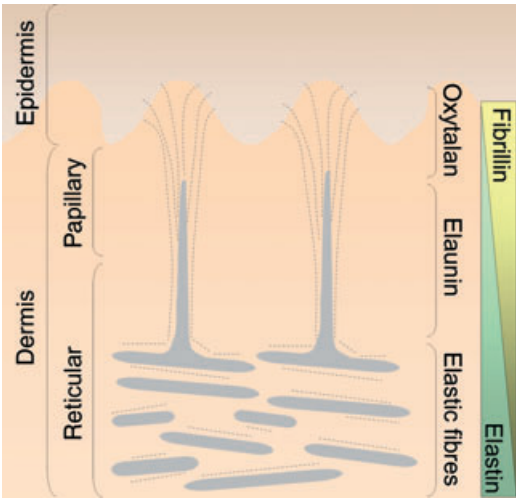

Figure 1 The elastic fibre network consists of elastin (solid grey lines) and fibrillin (grey dotted lines) and forms a distinctive arrangement within the dermis. The fibres of the upper papillary dermis form candelabra-like cascades of discrete, fibrillin-rich microfibrillar bundles, called oxytalan fibres that intercalate with the DEJ. The lower papillary dermis contains a finer network of fibres, or elaunin fibres, with reduced elastin content running perpendicular to the basement membrane. Within the reticular dermis, the elastic fibre network comprises thick elastin-rich fibres running in parallel to the DEJ.

diseases caused by mutations in fibrillin-1 and elastin. Fibrillin-1 mutations cause Marfan syndrome, which is associated with cardiovascular, ocular and skeletal defects [6], whereas elastin mutations cause Williams syndrome, supravalvular stenosis and cutis laxa [7]. Given the importance of both elastin and fibrillin-1 in the formation and function of elastic fibres; the following sections of this review will take an in-depth look at both these molecules with particular emphasis on fibrillin-1 structure and assembly.

The structure and function of elastin

Elastin with an approximate molecular mass of 64–66 kDa, is a key ECM protein, critical to the recoil capacity of many vertebrate tissues [8]. It constitutes around 2–5% of the skins dry weight [9] and acts passively in the dermis to return the skin to its original state following application of an external force. Although dermal fibroblasts maintain the ability to secrete elastin [10], synthesis is repressed in the majority of adult tissues by post-transcriptional mechanisms [11]. Mature elastin is extremely stable and does not turnover rapidly in healthy tissue. The half-life of elastin is estimated to be approximately 70 years [12] and is therefore considered to persist for the lifespan of the host.

Tropoelastin, with an approximate molecular mass of 67–70 kDa, is the secreted soluble precursor of elastin. The length of tropoelastin varies as a result of alternative splicing, resulting in the translation of multiple heterogeneous isoforms [13]. Alternative splicing is thought to tailor the structural function of tropoelastin/elastin in different tissues [14]. Tropoelastin consists of alternating repetitive hydrophobic domains of variable length which confer elasticity, interspersed with cross-linking domains [13]. The hydrophobic domains are dominated by proline, alanine, valine, leucine, isoleucine and glycine residues, with glycine and valine being the most abundant. Mature elastin is formed through the lysine-mediated cross-linking of tropoelastin by the enzyme lysyl oxidase [15]. During the formation of the elastic fibre, the microfibrillar component works as a scaffold for the deposition of tropoelastin. In addition, it is thought that the scaffold helps to align the cross-linking domains of tropoelastin, enabling the formation of mature elastin [16]. Indeed, the interaction of the N-terminal part of the microfibrillar-associated glycoprotein with the C-terminal end of tropoelastin is required for correct elastogenesis [16].

The structure and function of fibrillin microfibrils

In man, although there are three highly homologous fibrillin genes, fibrillin-1 appears to be the major structural component of fibrillin-rich microfibrils in adult tissues. Fibrillin-1 is an important component of the dermal elastic fibre network, which fulfils key biomechanical and biochemical roles [17–19]. In the skin, fibrillin-1 is both a product of dermal fibroblasts and keratinocytes [20] and is deposited as an early event during the wound healing response [21]. Regardless of tissue type or species, isolated fibrillin-rich microfibrils exhibit a 'beads on a string' appearance with an average bead to bead distance of 56 nm [22, 23] (Fig. 2a).

Fibrillin molecules are large (340 kDa) and complex multidomain glycoproteins (Fig. 2b). Fibrillin-1 and fibrillin-2 have distinct but overlapping patterns of expression [24], with fibrillin-2 generally expressed earlier in development than fibrillin-1 [25]. The third fibrillin gene has a restricted expression pattern, with fibrillin-3 protein expressed in the brain. However; it is unclear whether fibrillin-3 can form microfibrils [26, 27].

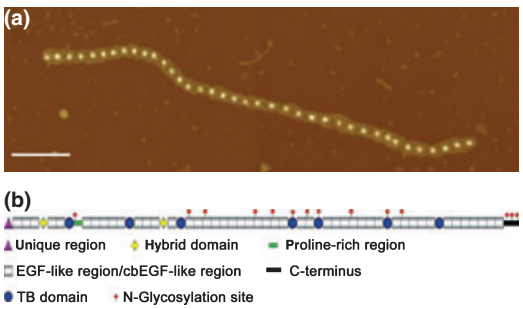

Figure 2 Intermittent contact mode atomic force microscopy (a) reveals the characteristic 'beads on a string' appearance of isolated fibrillin-rich microfibrils. Scale bar 200 nm. (b) Schematic demonstrating the domain structure of human fibrillin-1. The predominant structural motif of the fibrillin-1 is the epidermal growth factor (EGF)-like domain. In addition, there are 7 TB modules, 2 hybrid motifs and a proline-rich region. Fibrillin-1 also contains 14 occupied *N*-linked glycosylation consensus sequences.

Human fibrillin-1 is encoded by the FBN1 gene located on chromosome 15q21.1. It consists of 2871 amino acids and has a predicted unprocessed molecular mass of 347 kDa [28]. There are 14 occupied *N*-linked glycosylation consensus sequences [29] and the signal peptide, consisting of 27 amino acids, is located at the extreme N-terminus. The predominant structural motif of the fibrillins is the epidermal growth factor (EGF)-like domain; of the 47 EGF-like domains in fibrillin-1, 43 bind calcium (cbEGF-like domains). In addition, there are seven transforming growth factor β-binding protein-like (TB) or 8-cysteine modules, two hybrid motifs with similarities to both cbEGF-like domains and TB motifs, and a proline-rich region that may act as a molecular hinge [30]. The amino acid sequence of fibrillin-1 is predominantly hydrophilic [31]. High resolution imaging of fibrillin-1 molecules and molecular fragments has been achieved using rotary shadowing [32, 33]. Using this approach, fibrillin-1 appeared as a relatively flexible rod-like molecule of approximately 160 nm in length [33, 34]. Although the assembly process of fibrillin-1 monomers into highly complex microfibrils remains poorly defined it has been established that, at least in part, assembly is a cell-regulated process that proceeds independently of tropoelastin. Intracellular chaperone associations are likely to play key roles in molecular folding and *N*-glycosylation and help to prevent inappropriate intracellular assembly [35]. Assembly occurs in association with cell surfaces, indicating that cell surface receptors play a key role in this process. The fourth fibrillin-1 TB motif contains an RGD sequence that interacts with the integrins $\alpha v\beta 3$ [29, 36–38] and $\alpha 5\beta 1$ [29]; thus it has been postulated that these receptors might influence microfibril assembly.

The molecular form of fibrillin-1 secreted from cells is unclear. Fibrillin molecules are able to undergo limited intracellular assembly to form dimers and trimers that may represent intermediate assemblies [33, 35, 39]. Difficulties in expressing recombinant full-length fibrillin-1 have prevented the detailed assessment of whether fibrillin-1 is secreted as a monomer which is capable of self assembly. Several assembly models have been proposed and all support the concept that microfibrils assemble in a head-to-tail fashion at the cell surface. Two major models now predominate in microfibril assembly, the hinge model and the staggered model (see Kielty *et al.* [30] for detailed review of both models).

Loss of elasticity as a result of elastic fibre degradation is a major contributing factor in ageing of connective tissues including the lungs, the cardiovascular system and the dermis of the skin [40]. The effects of intrinsic ageing and extrinsic ageing on the elastic fibre network of skin will now be examined in detail.

Characteristic features of intrinsically aged skin

Intrinsic ageing of the skin is a slow process which causes changes in tissue structure. The gross clinical features of intrinsically aged skin are not usually evident until old age when, although smooth and unblemished, the surface appears pale and is characterized by fine wrinkles with occasional exaggerated expression lines [41]. Intrinsically aged skin exhibits epidermal and dermal atrophy and reduced numbers of mast cells [42]. In addition, fibroblasts are reduced in number [42], producing less collagen and more MMPs [43]. The DEJ is altered structurally in that the epidermis exhibits a flattened appearance, because of the loss of the rete ridges. In a study using abdominal skin biopsies, 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 years [44]. 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.

Characteristic features of extrinsically aged skin

Extrinsic ageing is caused by environmental influences that are superimposed onto a background of intrinsic ageing. In most individuals, extrinsically aged skin is confined to sites such as the face, chest and extensor surfaces of the arms, because of the accumulation of life-long UV exposure [45]. Chronic UV exposure which is responsible for around 80% of the effects of facial skin ageing [46] is termed photoageing. Acute exposure of human skin to UV radiation causes sunburn, altered pigmentation, inflammation, immune suppression and dermal connective tissue damage

Table I Characteristics of intrinsically and extrinsically aged skin

Intrinsically aged skin	Extrinsically aged skin
Fine wrinkling	Coarse wrinkling
Smooth texture	Roughened texture
Clear complexion	Sallow complexion
Uniform pigmentation	Mottled pigmentation
Gradual loss of elasticity	Marked loss of elasticity

[47, 48]. Chronic UV irradiation over many years disrupts the normal architecture of the skin and ultimately causes premature skin ageing and skin cancer [45–49] (see Table I for a comparison of the clinical features of intrinsic ageing and photoageing).

In contrast to intrinsically aged skin, tissue damage caused by chronic sun exposure is evident in both the epidermis and dermis [50]. Photoaged epidermis exhibits atrophy and disruption of normal epidermal keratinocyte maturation [50]; however, many histological features of photoageing are most apparent in the ECM of the dermis. Indeed, the most profound effect of UV exposure is exhibited by the dermal elastic fibres. The initial response of elastic fibres to photodamage is understood to be hyperplastic, resulting in a greater amount of elastic tissue [51, 52]. The level of sun exposure determines the magnitude of the hyperplastic response. Considerable disruption of the elastic fibre network is seen in chronically photoaged skin with abundant dystrophic elastotic material in the reticular dermis considered to be a defining characteristic. This accumulation of amorphous elastin material that is immunopositive for both tropoelastin and fibrillin-1 is termed 'solar elastosis' [51, 52].

During photoageing, the oxytalan fibres at the DEJ also exhibit degenerative changes. Examination of the upper papillary dermis adjacent to the DEJ reveals that although fibrillin-1-positive fibres are immunohistochemically identifiable in sun-damaged skin, their architecture and abundance is markedly affected in that discrete microfibrillar bundles are rarely observed [53] (Fig. 3). Minimally photoaged skin also reveals a loss of fibrillin-rich microfibrils at the DEJ, which implies that fibrillin-1 is one of the first constituents of the microfibrillar network to be damaged by solar exposure [53]. Fibrillin-1 can therefore be considered as an early marker of photoageing [53, 54].

Examination by both scanning and transmission electron microscopy of the elastic fibres in UV-exposed skin reveals a reduction in the number of microfibrils and an increase in interfibrillar areas. In addition, the complexity of the shape and arrangement of the fibres and the number of electron-dense inclusions is altered [55]. In contrast, during intrinsic ageing, changes in the nanomechanical properties of fibrillin-rich microfibrils also occur in that microfibril periodicity increases whereas microfibrillar length decreases [56].

The loss of elastic fibre integrity in response to solar UV exposure leads to a marked reduction of skin elasticity and manifests as skin wrinkles. A similar loss also occurs during intrinsic ageing but

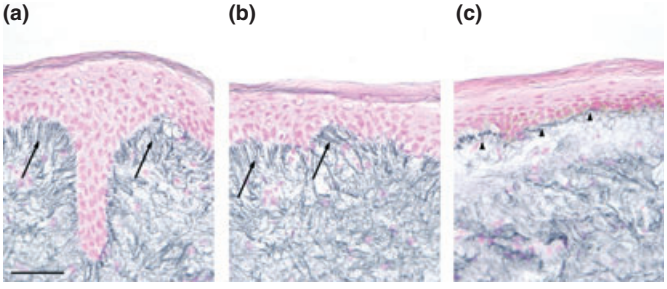

Figure 3 Immunohistochemistry identifies major alterations in the fibrillin microfibrillar network of photoaged skin. Photoprotected buttock skin from a young adult (a) contains a complex system of fibrillin-1-positive microfibrillar bundles (arrows) which emanate from the DEJ and coalesce with elastic fibres within the reticular dermis. Intrinsically aged, photoprotected buttock skin (b) exhibits a similar arrangement of fibrillin-1-positive microfibrillar bundles (arrows) to those seen in photoprotected skin from a young adult. However, there is a distinct flattening of the epidermis and an associated loss of rete ridges at the DEJ. Photoaged skin from the forearm (c) also exhibits a flattened epidermis and is characterized by a significant reduction in fibrillin-1-positive microfibrillar bundles (arrowheads). Scale bar 50 μm.

it is generally associated with a progressive loss of elasticity [57]. The mechanical alterations that take place reflect structural degenerative changes including denudation of the microfibrillar mantle surrounding elastic fibres and exposure of the elastin core to proteolytic degradation [30].

Exposure to tobacco smoke from cigarettes is also an accelerating factor both to the intrinsic and UV-mediated extrinsic ageing processes [58–60]. The underlying mechanism is not well known, but elastic fibres of the dermis seem to be the major target of smoke-derived components. Elastosis caused by the degradation of the elastic fibres is increased in the skin of patients who smoke [61] and a large number of studies have reported a significant increase in facial wrinkles in smokers [59, 62–65].

The wrinkles of cigarette smokers differ from those of non-smokers, in that they are narrower and deeper, with more sharply contoured crows-feet and prominent peri-oral lines. This pattern of wrinkles was first described in 1971 [66] and is now referred to as 'smoker's face'. The facial wrinkles typically radiate at right angles from the lips and eyes; there is a gauntness to the features; an atrophic 'greyed' appearance of the skin and an uneven complexion [67]. The physical movement of pursing the lips and squinting when inhaling the cigarette smoke may lead to the formation of lines around the mouth and crow's feet. Heavy smokers (defined as those who smoke >1 pack per week) are 4.7 times more likely to have facial wrinkles compared to non-smokers, regardless of sun exposure [58].

Elastic fibre remodelling

Remodelling of the elastic fibre network plays an important role in the pathomechanisms underlying wrinkling of the skin. Elastic fibre remodelling in response to UV exposure is predominantly caused by the activation of MMPs [45]. These enzymes, for example: MMP-1 (collagenase); MMP-2 (72 kDa gelatinase); MMP-9 (92 kDa gelatinase) and MMP-3 (stromelysin-1) can act independently or in concert to degrade components of both the collagenous and elastic matrices [5, 68]. Matrix metalloproteinases are zinc-dependent endopeptidases, that are synthesized in an inactive form and are activated by cleavage of the propeptide domain by extracellular proteinases [69]. Inhibition of MMP activity is modulated by four protease inhibitors termed tissue inhibitors of matrix metalloproteinases (TIMPs).

The basal expression of MMPs in 'normal' skin is relatively low; however, lifestyle choices such as cigarette smoking have been shown to increase MMP-1 mRNA levels in the skin [70]. This has led to the suggestion of a link between MMP-1 induction and skin wrinkle formation [70]. It has been demonstrated that MMPs can be markedly up-regulated by UV irradiation both *in vivo* and in cultured cells [45, 49, 71]. *In vitro* studies have demonstrated the ability of MMP-2, -3, -9, -12 and -13 to degrade fibrillin-1 peptides and fibrillin-rich microfibrils [5]. Furthermore, it appears that MMP-9 displays the greatest elastinolytic and fibrillin-degrading activity, whereas MMP-2 shows greater specificity towards degrading constituents of the basement membrane [72]. In conjunction with the up-regulation of MMP activity, UV irradiation also induces expression of the specific inhibitor of MMP activity, TIMP-1, in a dose-dependent manner [49]. Despite this, UV exposure clearly encourages a degradative environment within the dermis resulting in extensive damage to the ECM [73].

The underlying aetiology of age-related changes in elastin is not well understood; however, MMPs are thought to play a role as MMP-2 has been demonstrated to degrade elastin [74]. Fibrillin-1 may also be digested by a variety of enzymes secreted by infiltrating immune cells, for example, neutrophil elastase and mast cell tryptase [75]. Therefore, skin inflammation may play an additional and important role in elastic fibre remodelling.

Treatment of photoaged skin

The 'gold standard' pharmaceutical therapy for the treatment of photoaged skin is the application of topical retinoids. Topical all-*trans* retinoic acid (t-RA), a derivative of vitamin A, was first used to treat actinic keratoses and acne in the 1970s [76]. However, the use of retinoids as a treatment strategy for photoageing was discovered purely by chance when patients undergoing treatment for acne observed an improvement in periorbital wrinkles. Subsequently, several studies of topical t-RA for repair of photoaged human skin followed, all confirming that clinical improvement of facial wrinkling could be observed [77, 78].

In 1991, a short-term patch test assay was developed to study the effect of topical retinoids in

normal human skin [79]. In this assay, a single application of 0.1% t-RA cream under occlusion for 4 days produced a consistent erythema and characteristic epidermal changes as noted for prolonged t-RA use. The optimal concentration of t-RA used in the patch test has subsequently been modified from the more conventionally used concentration of 0.1%, to a concentration of 0.025%, because the lower concentration has similar efficacy but results in lower levels of irritancy [80]. Using this short-term patch test protocol, it has been demonstrated that fibrillin-1 mRNA and protein are significantly increased following topical application of t-RA [54]. This increase in fibrillin-1 expression at the DEJ was also reminiscent of the partial restoration of fibrillin-1 that occurs after daily application of t-RA for 4 years [54]. Therefore, fibrillin-1 may be considered a robust biomarker for the repair of photoaged dermis.

A large number of commercially available cosmetic anti-ageing products are designed to target the undesirable clinical features of photoageing such as fine wrinkling, roughened texture and mottled dyspigmentation. It is now widely accepted that the efficacy of these commercially available over-the-counter products should be demonstrated by independent verification. For this reason, the original 4-day patch test protocol [54] was adapted to simulate the effects of long-term, real-life use of the products [81–83].

The effects of the sphingolipid derivative, salicyloylphytosphingosine (SP) have been tested using this adapted patch test protocol [81]. Test products (SP at 0.05% and 0.2%), t-RA (0.025%) and vehicle were applied under occlusion for 8 days prior to biopsy and histological assessment in photoaged volunteers. Increased deposition of fibrillin-1 was observed at the DEJ in skin treated with SP. This study therefore indicated that application of the ceramide derivative, SP, may be a novel agent for the repair of photoaged skin.

More recently, different formulations of a commercially available anti-ageing product have been applied using the patch test assay and examined for the expression and distribution of fibrillin-1 [54, 82]. In this study, it was found that use of the over-the-counter product under occlusion for 12 days could induce deposition of fibrillin-1 in photoaged dermal ECM. The findings of this study have now been extended to test a new formulation of the product under both an occluded patch test protocol and a 6-month randomised, controlled trial [83]. Again, deposition of fibrillin-1 at the DEJ was noted following application of the anti-ageing product for both 12 days and 6 months [83]. This study supported the use of fibrillin-1 as an informative and robust biomarker for assessing efficacy of potential photoageing repair products. It also demonstrated that the short-term patch-test protocol is an effective screening process for modelling the effects of long-term, 'real-life' use of commercial products.

Conclusions

The biology of skin ageing is a complex process and has the end effect of reducing the production of ECM proteins and increasing the level of ECM remodelling. Clearly, one of the most pronounced effects of ageing is the degradation of the elastic fibre network, particularly at the DEJ. The loss of structural integrity of the fibrillin-rich microfibrils can be mitigated by topical retinoid treatment. However, it is now apparent through the use of short-term screening assays that cosmetic products may also be successful at restoring the network of fibrillin rich-microfibrils. This observation suggests that such cosmetic products may alleviate some of the features of photoaged and perhaps aged skin.

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