Collagen glycation triggers the formation of aged skin in vitro

Glycation products accumulate during the aging of many slowly renewing tissues, including skin. We have developed an in vitro model of chronological aging of skin based on reconstructed skin modified by artificially glycating the collagen used to prepare the dermal compartment. The morphology of the modified skin is close to the morphology usually observed except that the dermis is altered in its fibrillar structure. Moreover, the analysis of skin markers revealed several unexpected biological and morphological modifications, which reflect in vivo aging and could be related to glycation per se. These include the activation of fibroblasts, increase of matrix molecules (collagen type III and collagen type IV) and metalloproteinase production (MMP1, MMP2 and MMP9), thickening of the basement membrane zone, and more strikingly, the modification of α6 and β1 integrin patterns especially in epidermis, in a way closely resembling aged skin in vivo. We also found that these effects could be related to the production of putative diffusible factors by the dermal fibroblasts activated by glycation. Finally, we show that all these effects are likely to be glycation specific since they could be inhibited by aminoguanidine, a well-known glycation inhibitor. We conclude that the reconstructed skin model modified by glycation of the collagen closely mimics chronological aging of skin in vivo. Taken together, these results strengthen the importance of glycation reactions in skin aging.

Key words: Glycation, collagen, fibroblasts, keratinocytes, aging, reconstructed skin

Aging has recently gained considerable interest because of the astonishing fact that life expectancy has considerably increased in the last decades [1]. Aging is a complex process in which several mechanisms operate simultaneously. These include accumulation of mutations in the genome, accumulation of toxic metabolites, hormonal deprivation, increased formation of free radicals (oxidative damage), and cross-linking of macromolecules by glycation [2]. Skin is an important model for aging studies since it is submitted to both extrinsic influences from the environment (mostly exposure to sunlight but also chemicals and allergens) and intrinsic factors that are presumed to be mostly of genetic origin [3]. Intrinsic aging of skin is characterised by changes in the dermal matrix which becomes less elastic [4] and thinner with age [5] so that the dermis is the main cause for the appearance of "old" skin [6]. A very attractive characteristic of skin and skin cells is that it is possible to reproduce in vitro the three dimensional architecture of skin by reconstructing serially the dermal and epidermal compartments. Many different ways to do this have been proposed [7]. For instance it is possible to produce a dermal equivalent by mixing collagen and fibroblasts in certain conditions to form a dermal tissue in culture and then to obtain a reconstructed skin model by growing epidermal keratinocytes on this substrate provided the culture has been raised at the liquid air interphase and air-exposed. We have extensively used this approach in our laboratory to investigate several aspects of skin physiology including detailed studies of the effects of UV light [8, 9]. The purpose of the present study was to modify the dermal matrix (namely collagen) in a way which mimics the effect of chronological aging in vivo in order to produce aged skin in vitro. Glycation seemed to us the way to achieve this goal.

Glycation is a non-enzymatically driven reaction between free amine groups like those of amino acids in proteins and reducing sugars like glucose. This reaction, also called the Maillard reaction [10], eventually leads to the formation of Advanced Glycation End products (AGEs) such as carboxymethyl lysine (CML), pentosidine and others, which

Abbreviations:
- ECM: Extracellular matrix
- BMZ: Basement Membrane Zone
- DEJ: Dermal Epidermal Junction
- MMP: Matrix metalloproteinase
- AGE product: Advanced Glycation End product
- CML: Carboxymethyl-lysine
can be responsible for the formation of cross-links between macromolecules by covalent bonding. This reaction therefore preferentially affects tissues in which macromolecular structures have a slow turnover rate, and is therefore thought to play an important role in aging [11]. For instance, glycation has been shown to affect skin, hair, lens, basement membrane (kidney), vessel walls, circulating albumin and hemoglobin [12]. Moreover, accelerated accumulation of AGE products has been clearly shown in diabetes mellitus and premature aging of tissues such as in end stage renal disease [12]. Recently, it has been proposed that pentosidine accumulation serves as a marker for aging since it significantly increases with age in healthy individuals [13].

Moreover a correlation between life span of several species and pentosidine accumulation rate was observed [14]. In skin it is thought that accumulation of cross-links between macromolecules of the dermal extracellular matrix is in part responsible for the alterations of the mechanical properties of skin (stiffness) that take place as a function of age. Finally, AGE product formation and accumulation in skin [15] as well as collagen crosslinking were correlated with the severity of diabetes mellitus.

This study was undertaken to investigate the effects of glycation in the reconstructed skin model by pre-glycation of the collagen. Several skin markers of interest were modified in a way mimicking in vivo aging and in addition these modifications were prevented by aminoguanidine, a well-known inhibitor of glycation. We propose that this modified reconstructed skin therefore represents a novel promising model of skin aging. This also suggests that in vivo glycation of dermal extracellular matrix molecules like collagen may actually participate to the biological mechanisms of skin aging.

Materials and methods

Skin samples, keratinocyte and fibroblast cultures

Normal human skin was obtained from mammary plastic surgery. Human epidermal keratinocytes were obtained and cultured as described by Rheinwald and Green [16] on a feeder layer of Swiss 3T3 fibroblasts. Human adult dermal fibroblasts were isolated after spreading from skin explants made in the pieces used for isolation of epidermal keratinocytes. The reference strain of our laboratory [8, 9] was used throughout this study. Human skin samples from donors of various ages (young age 20-30, 5 samples vs old age 55-70, 8 samples) were also used for comparison with reconstructed skin (normal vs young skin and glycated vs old skin respectively).

Preglycation of the collagen

Bovine skin collagen (mostly collagen I) from Gattefossé SA or Coletica SA or Symatèse SA (all unpepsinized collagens and all from Lyon, France) at approx. 4-5 mg/mL in 0.5 N acetic acid solution was incubated in the presence of 10 mM ribose at room temperature during three weeks before extensive dialysis against acetic acid 0.5 N (twice instead of once usually) and then for 24 hours against acetic acid 0.017 N or 1:1000 v/v acetic acid/water) as usually performed for standard collagen preparations. The glycation of collagen in solution was monitored as follows: an aliquot of the type I bovine collagen was solubilized by pepsin digestion (Sigma, 100 μg pepsin in 0.5 ml 0.5 M acetic acid) at 37 °C for 14 hours. After the end of the incubation, the pH of the digested material was adjusted to 7.0 with 0.5 N NaOH. After centrifugation for 5 minutes at 10,000 g, the supernatant containing digested collagen was used for fluorescence measurement. Fluorescence was measured at λem 440 nm (λex 370 nm) for total AGE and at λem 378 nm (λex 328 nm) for pentosidine. HPLC analysis was also performed to demonstrate the presence of AGE products at the biochemical level like CML, a very representative AGE product in skin. This control was also performed at the end of the cultures after enzymatic digestion of reconstructed skin control samples.

Reconstructed skin in vitro

Dermal equivalents (fibroblasts contracted collagen gels) and reconstructed skins were prepared as described in detail previously [17]. Briefly after contraction of the lattice, adult human keratinocytes were seeded on the lattice and kept submerged for 7 days allowing the cells to form a monolayer. The culture was then raised at the air liquid interface and kept 1 week to allow the keratinocytes to stratify and differentiate completely. Collagen lattices modified by glycation of the collagen (and consequently reconstructed skins made with glycated collagen) were prepared by using a 1:1 untreated collagen and glycated collagen solutions mixed prior to use to obtain a homogeneous solution, instead of using normal untreated collagen. Nine independent series of both normal and glycated reconstructed skins were made and studied without observing significant variations in the results. In some experiments it was necessary to have normal unglycated collagen and glycated collagen in two separate dermal compartments. This could be obtained by making two successive half gels, one above the other, provided the first gel was allowed to take before the second one was made. In other experiments the fibroblasts embedded in the collagen gels were lysed by an osmotic shock after stabilization of the gel contraction. This could be obtained without altering the dermal structure by replacing the culture medium of the dermal equivalent by distilled water during 24 hours, with several changes before being left overnight as previously described and successfully used by others [18]. Aminoguanidine (hemisulfate salt, from Sigma), 10 mM in water, was eventually added during preincubation of the collagen.

Histology

Samples were fixed in neutral formalin and treated for histology. Paraffin sections were stained with hematoxylin, eosin, safron (HES) or Van Gieson stain.

Immunohistochemistry

Immunolabelling was performed on air-dried vertical 5 μm cryosections as described [10] mostly using immunofluorescence techniques except for AGE products in human skin where immunoperoxidase staining was preferred because of autofluorescence [19].

We used essentially mouse monoclonal antibodies against CML (clone 6D12 from Wako, Richmond, VA, USA) but also, polyclonal rabbit antibodies against pentosidine [20], and rabbit polyclonal antibodies against AGE products [21]. Monoclonal antibodies against vimentin (clone 9) were from Monosan, Uden, The Netherlands. Monoclonal
antibodies against and procollagen III were both from Chemicon, Temecula, CA, USA. Rabbit polyclonal antibodies against collagen IV were from Novotec, Lyon, France and Chemicon respectively. Monoclonal antibodies against α6 (clone GOH3), β1 (clone K20) were all from Immunotech, Marseille, France. FITC-conjugate rabbit anti-mouse immunoglobulins or FITC-conjugate swine anti-rabbit immunoglobulins (Dako, Denmark) were used as second antibodies.

Enzyme - Linked Immuno Assays (ELISA)
The matrix metalloproteinases MMP1 (or institional collagenase 1) and MMP2 and MMP9 (respectively 72 kD and 92 kD) content of the tissue culture medium was determined using ELISA essays (Biotrak kit from Amersham Pharmacia, Orsay, France) according to the manufacturer's instructions.

Zymography
Precast zymogram gels from NOVEX (Prolabo, France) were used to study metalloproteinases through the detection of proteolytic activity. Culture medium (20 µL) was used without heating or reduction for SDS-PAGE containing blue casin or gelatin. After electrophoresis, gels were washed twice in zymogram renaturing buffer (Biorad, Ivry sur Seine, France). Gels were incubated for 72 h at 37 °C in a zymogram developing buffer (Biorad), then fixed and stained in gel code blue (Pierce, Bezons, France).

Results
Morphogenesis of skin in vitro using preglycated collagen and related modifications of skin markers
Histology mostly revealed that the dermis appeared slightly different when preglycated collagen was used and was characterized by more apparent collagen fibres (figure 1). It was possible to relate these dermal modifications to actual glycation of the collagen used as matrix, in particular CML was clearly identified using specific antibodies (figure 1). The staining obtained also confirmed the modification of the extracellular matrix organisation, especially the presence of unusual thick collagen bundles likely to correspond to the presence of glycated collagen fibres. Epidermis however in the context of glycation of the dermis was histologically normal looking (figure 1) and comparable to control epidermis with all stages of differentiation including granular and horny layers, although slightly thicker suggesting increased keratinocyte proliferation. This could be confirmed by Ki67 labelling (data not shown).

Immunohistochemistry, in general, provides the means to look more in detail at markers of the different compartments of reconstructed skin and additional changes were revealed. In keeping with histological findings, immunostaining of human type III collagen suggested an increase in the production of dermal collagen by fibroblasts (figure 2) as well as an increase of the staining of basement membrane components like collagen IV (figure 2) or laminin (data not shown). Vimentin labelling (figure 2) also revealed that fibroblasts appeared more spindle-shaped looking and they were more frequently close to the epidermis as well as increased in number, an observation likely to be relevant especially because glycated dermal equivalents were

Figure 1. HES histological staining (A, B) and CML labelling (C, D) of skin reconstructed in vitro using untreated collagen (A, C) or pre glycated collagen (B, D). Note that the histological pictures look similar except that the dermal organization seems to be modified by glycation (bar = 100 µm).

slightly larger (slightly less contracted). In addition, an increase of Matrix Metallo Proteinases or MMPs (mostly MMP1 or collagens 1 and MMP2 or 72 kD gelatinase in its processed form) detected in the tissue culture medium was observed (figure 3), indicating activation of the fibroblasts and degradation of the dermal matrix. Therefore, it was tempting to relate this increase in MMPs to a decreased thickness of the dermal compartment as seen by means of histology (figure 1D). Interestingly, β1 integrin, a broad integrin able to bind not only collagen but also laminin and tenasin, as well as α6 which also binds laminin were seen in most suprabasal epidermal layers or at least in the epidermal basal layer, respectively (figure 4). This labeling pattern was different from that observed in controls where β1 staining appeared restricted to the epidermal basal layer and α6 to the basement membrane (figure 4), as described [22]. Taken together, these observations suggest that not only the collagen of the extracellular matrix and dermal fibroblasts but also epidermal keratinocytes were affected by glycation of the collagen. Interestingly, similar modifications were seen in epidermis in vivo as a function of age when the distribution of β1 and α6 integrin was studied in samples from young and old skin (figure 4). Moreover it was possible to relate the "aged skin" phenotype to the presence of glycation products in the dermis (figure 4). The immunoreactivity of these AGEs can be related to numerous studies based on chromatographic techniques which have documented their three- to five-fold elevation in old vs. young skin [33, 34].

Diffusible factor(s) are likely to be responsible for the "aged skin" phenotype obtained with preglycated collagen in reconstructed skin
The findings above indicate that among the integrin family members, β1 and α6 which are normally restrictively expressed in basal keratinocytes, were affected and enhanced by glycation. Since they also bind collagens or laminin present at the dermal epidermal junction or in the dermis of reconstructed skin, it was therefore tempting to attribute
Figure 2. Comparison of the human collagen content of the dermal equivalents of normal reconstructed skin and reconstructed skin prepared with preglycated collagen. Immunolabelling of human procollagen III (A, B), human collagen IV (C, D) and vimentin (E, F) either in normal (A, C, E) or glycated (B, D, F) reconstructed skin. Note an increase in collagen output when preglycated collagen was used. Also note that the fibroblast orientation is characterized by a more elongated shape when embedded in glycated collagen (bar = 100 μm).

These results to direct contact between epidermal keratinocytes and glycated collagen molecules of the dermis. To test this hypothesis reconstructed skins were modified by introducing an upper normal dermal layer made of untreated collagen in order to separate epidermis from the glycated dermal substrate (see materials and methods). Surprisingly, this modification did not suppress the previously observed increase of β1 and α6 integrin labelling in epidermis (figure 5), suggesting that one or more diffusible factors most likely stemming from the fibroblast layer were involved, rather than direct contact between keratinocytes and the glycated matrix. This hypothesis was confirmed by making reconstructed skins in which fibroblasts were lysed (see material and methods). β1 integrin labelling in epidermis was expanded in the presence of glycated collagen only when living fibroblasts were there (figure 6).

Anti-glycation effect of aminoguanidine in reconstructed skin

In order to confirm the relationship between the altered patterns observed in reconstructed skin made with preglycated collagen to glycation of the collagen, we examined in this system the effect of aminoguanidine, a well-known inhibitor of glycation [23], both on the presence of AGE products like CML and the distribution of β1 integrin as a skin marker reflecting the effect of glycation. Figure 7 shows that the amount of detectable glycation products was decreased and that the distribution of β1 integrin tended to normalize when the incubation of collagen was performed in the presence of aminoguanidine. These findings suggested that not only specific chemical inhibition of glycation itself was obtained but also prevention of at least some of the biological effects of glycation observed in the skin model.

Discussion

The reconstructed skin as a model of aging

This study demonstrates for the first time that some of the phenotypic changes that were observed in aging skin can be reproduced in skin reconstructed in vitro using collagen modified by glycation. Histology revealed that the dermal structure itself was only slightly modified in its fibrous appearance while a normal-looking epidermis was formed. The changes in the dermal matrix were related to the presence of advanced glycation products revealed by immunolabelling and the fact that glycation is known to alter collagen fibril organisation [24], expand molecular packing of collagen [25] and may inhibit collagen lattice contraction [26, 27]. All these phenomena are likely to be related. This modified phenotype was also associated with an increase in fibroblast number suggesting "activation" of these...
Figure 4. Distribution of specific integrins in epidermal keratinocytes and dermal fibroblasts of reconstructed skin or skin in vivo. Immunolabelling of α6 (A, B), β1 (C, D) in normal (A, C) and glycated reconstructed skin (B, D) as indicated by white arrow heads or white arrows respectively. Note that both α6 and β1 extend respectively to a few more or to numerous suprabasal layers in reconstructed epidermis when preglycated collagen was used. Also note an increase in intensity and number of β1 positive dermal fibroblasts in glycated reconstructed skin. Staining pattern of α6 and β1 integrins in epidermis of specimens from young (yr 23) and old (yr 61) human skin in vivo characterized by their glycation product content. Immunolabelling of integrins: α6 integrin (E, F), β1 integrin (G, H) in young (E, G) or old (F, H) human skin as indicated by white arrow heads or white arrows respectively. Note increased α6 and β1 staining in the epidermal compartment of older human skin. Immunolabelling of CML (I, J) in young (I) or old (J) human skin serves as control for the human specimen used to verify that the 23 years old and the 61 years old had actually a "young" (no CML labelling) and an "old" (CML labelling) phenotype, respectively (bar = 100 µm).

Figure 5. β1 integrin distribution in reconstructed skin comprising two dermal layers made with preglycated collagen restricted to the lowest compartment (indicated by [G] Glycated) and normal collagen in the upper compartment (indicated by [NG] Not Glycated) is strikingly similar to reconstructed skin completely made with preglycated collagen rather than reconstructed skin made with normal collagen. β1 integrin immunolabelling (A, B) indicated by white arrows, CML labelling (C, D) in two dermal layered reconstructed skin made either normal collagen in the 2 dermal compartments (A, C) or normal and preglycated collagen in the upper and lower dermal compartment, respectively (B, D) (bar = 100 µm).

In contrast to our findings, others have reported a decrease in MMP1 when cultivating fibroblasts on glycated collagen lattice while noticing decreased contraction of the gel [36]. This may be due to lack of 3-dimensional environment for the fibroblasts in their culture system. Thus our observation of an increase in MMP1 is likely the direct result of a biological response mediated by AGE products. Assuming...
the age-related decrease in skin thickness is in part due to collagen digestion, our data suggest that such processes might be mediated by glycation. Paradoxically, however, we have also observed increased labelling of basement membrane zone or BMZ molecules like collagen IV or collagen VII (not shown) at the dermal epidermal junction (DEJ), suggesting possible accumulation of these basement membrane components as a consequence of glycation. The increase in laminin and collagen IV [56], or alteration only [37] or increase in collagen IV only [38] in basement membranes of diabetic rats has been shown in several reports [37-39] and related to glycation using cultured cells [40] or even correlated with elevated glucose in humans [41]. An increase in the thickness of the basement membrane in human skin as a function of age has also been reported [42]. A less effective interaction between basement membrane components in aging skin [43] or diabetes mellitus [44] has been proposed to explain these findings and may be related to our observations.

An interesting result of this study is that not only dermal but also epidermal markers were affected by glycation [34]. Among all classical epidermal markers investigated, especially those related to epidermal stratification and differentiation such as desmosomes, filaggrin, and loricrin and others, only integrins, i.e. β1 and α6, specifically and clearly showed altered patterns after glycation of the collagen. The fact that these two integrins rather than others (not shown), were affected by glycation is not surprising. Because of its numerous ligands depending on its alpha partner, β1 integrin is involved in a wide variety of important biological functions such as epithelial cytoskeletal organization, basement membrane biosynthesis, adhesion and migration of fibroblasts and keratinocytes, and collagen synthesis. It is in association with α2 the major collagen receptor on epithelial cells [45] and similar results were obtained for α2β1 (not shown). It is therefore a broad integrin whose substrates include collagen I, collagen IV, laminin and fibronectin which were directly (collagen I and III) or indirectly (collagen IV) affected by glycation. α6 is another integrin which can combine with β1 to bind laminin as a preferential substrate whose production was also increased by glycation in this system (data not shown). In this system, glycation also resulted in increased expression of α6, another integrin which can combine with β1 to bind laminin as a preferential substrate. Cell adhesion and migration have been shown to be altered by glycation, and even mechanical properties like tension [46] and are likely to be modified by the presence of glycation products. Of great interest is the fact that the integrins were more expressed in epidermal layers of gly-

**Figure 6.** HES histological staining (A, B, C, D), vimentin immunolabelling (E, F, G, H) and β1 integrin distribution (I, J, K, L) of skin reconstructed in vitro using normal collagen and living fibroblasts (A, E, I), or normal collagen and lysed fibroblasts (B, F, J), or glycated collagen and living fibroblasts (C, G, K), or glycated collagen and lysed fibroblasts (D, H, L). Note that β1 integrin (indicated by white arrows) is increased in epidermal layers only if both glycated collagen and living fibroblasts were present and that both skin constructs containing lysed fibroblasts (glycated or unglycated) are strikingly similar. Living fibroblasts are indicated by black arrows (bar = 100 μm).
cated reconstructed skins than in the control, especially in
the case of β1 integrin that was observed in most suprabasal
layers, β1 and α6 have both been previously proposed to be
epidermal stem cell markers [47, 48], but the increased
expression of β1 integrin observed here seems unlikely
related to a stem cell property in the context of aging. In
fact, β1 integrin staining (indicated by white arrows) which is normally restricted to the first
suprabasal layers (I) is extended to almost all suprabasal layers (K) when preglycated collagen was used. In contrast staining was
only partially extended to suprabasal layers when preglycation of the collagen occurred in the presence of aminoguanidine (L). Interestingly, CML staining which occurred only when preglycated collagen was used (G) is reduced when aminoguanidine was
added during preincubation (H) (bar = 100 µm).

Figure 7. Prevention by aminoguanidine of the extended β1 integrin distribution in epidermis of reconstructed skin made with
preglycated collagen. HES histological staining (A, B, C, D), CML immunolabelling (E, F, G, H) and β1 integrin labelling (I,
J, K, L) of reconstructed skin made with untreated collagen (A, E, I) or collagen preincubated with 10 mM aminoguanidine (B,
F, J) or collagen preincubated with 10 mM ribose (C, G, K) or collagen preincubated with both 10 mM ribose and 10 mM
aminoguanidine (D, H, L). Note that β1 integrin staining (indicated by white arrows) which is normally restricted to the first
suprabasal layers (I) is extended to almost all suprabasal layers (K) when preglycated collagen was used. In contrast staining was
only partially extended to suprabasal layers when preglycation of the collagen occurred in the presence of aminoguanidine (L).

Similar changes have been noticed when re-programming
of epidermal cells occurs [52] or during alteration of the
differentiation program as in psoriasis [53]. Induction of β1
expression in suprabasal epidermal layers has then been
associated with enhanced migratory activity of human fi-
broblasts [54], which is in agreement with the activation of
these cells in response to glycation of the collagen. In
addition, β1 seems to be involved in the control of collagen
synthesis as suggested by wound studies [55]. This is also
supported by the fact that differentiation may be affected by
glycation in other tissues as well. For instance, collagen
glycation seems to reduce the differentiation of certain cells
like osteoblasts [56], while glycation products would accel-
erate differentiation of pericytes [57]. Finally, in the context
of these studies it is also of interest to recall that β1
knockout mice have increased epidermal thickness [58],
suggesting that β1 may play a general role in the control of
epidermal morphogenesis and homeostasis rather than dif-
ferentiation per se [59]. This observation is relevant since i)
we also found that β1 was increased in aged skin in vivo and
ii) in vivo skin aging is known to be accompanied by
reduction of epidermal thickness.
Role of diffusible factors

Numerous reports suggest that dermal-epidermal interactions are important in skin physiology either in vivo or in vitro [18, 60, 61] in reconstructed skin. These interactions work both ways and diffusible factors can be produced by mutual induction [60, 61], i.e., by fibroblasts which influence keratinocytes [59], and vice-versa by keratinocytes which influence fibroblasts [62]. It was interesting and unexpected, however, that modification of the dermal extracellular matrix (i.e., glycation of the collagen) was only indirectly responsible for the effects observed at the level of the epidermis especially since integrins were affected. On the other hand, our findings do not exclude that the triggering mechanism takes place through cell (fibroblast) matrix interactions, since AGE receptors have been described on the surface of certain cell types [63]. These integrins can be influenced by diffusible factors [64], in particular when the basement membrane is missing or not yet mature like in wound healing [65]. This is an important observation because it suggests that an actual increase of integrins β1 and α6 does occur (rather than a lack of degradation of β1 and α6 integrins by keratinocytes migrating upwards in the epidermis). It also suggests that, through diffusion, a broader biological effect of glycation is produced in the skin model rather than a localized effect restricted to cells in contact with glycated molecules of collagen.

Inhibition of glycation by aminoguanidine in reconstructed skin

The fact that aminoguanidine, although known to inhibit enzymatic activities not related to glycation, was able to prevent some of the most striking biochemical and biological alterations produced in reconstructed skin by glycation of the collagen like the modification of β1 integrin pattern as well as others like collagen or basement membrane molecules, (data not shown) strongly argues in favor of the idea that the modifications seen are specific for glycation. Such functional inhibition of the effect of glycation has been seen in other systems [38]. The functional effect of aminoguanidine on integrin pattern in the epidermis of reconstructed skin made with glycated collagen strengthens the potential importance of glycation in skin aging and hence its prevention.

Organotypic approach of skin aging in vitro

To our knowledge only a few approaches using 3D systems of reconstructed skin have been proposed so far with a view either to looking at the effect of glycation or to reproducing in vivo some aspect of aging in vitro. For instance Jeanmaire et al. have proposed incubating dead de-epidermized human dermis with glucose in vitro to reproduce age-induced glycation [66], while Rittié et al. have cultivated human dermis with glucose in vitro to reproduce age in vivo [59]. These integrins can be found in the liquid-air interface. Exp Cell Res 1985; 159: 536-9. Also in vitro model of reconstructed skin can mimic several features of skin aging when cells were grown onto a glycated matrix. The ability of aminoguanidine to prevent these changes not only supports a role for glycation products in skin aging, but also provides a pharmacological paradigm for the development of drugs with anti-skin aging properties.

Acknowledgements. Financial support: None. Conflict of interest: None.

References


