Glycative Stress Research

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Review article

Glycative stress and anti-aging: 7. Glycative stress and skin aging

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Abstract

Located on the outermost layer covering a living body, skin is an organ which protects the underlying body from the external environment such as shocks, temperature, ultraviolet radiation, chemicals and other threats. Changes to the skin can be visually recognized immediately and can be a trigger factor of a visual impression of aging such as an uneven skin texture, wrinkles, a dull aspect, the decline of elasticity and resilience, the loss of firm and supple skin texture and the decline of skin functions. Influences of aging due to glycative stress appear in the skin, especially in the protein with a long half-life period, such as collagen or elastin. Glycation induces the browning reaction of skin proteins and AGEs (advanced glycation end products) are accumulated. The accumulation of cross-linking AGEs such as pentosidine is a factor of physical damages of the formation of disordered protein cross-linking. Thus, glycation of the skin protein affects the maintenance of fiber tissue stability. Furthermore, the accumulation of CML (N^{ε}-carboxymethyllysine), which is non-cross-linking AGEs, induces the apoptosis to fibroblast tissues, through the medium of RAGE (Receptor for AGEs). Collagen-CML formation induces the migration of keratinocyte, which exists on the epidermis, and decreases the adhesiveness to collagen. In addition, diversified AGEs, which are accumulated in the skin tissues, bind with AGEs receptors to accelerate the secretion of inflammatory cytokine through an intracellular signal transduction. In this manner, the accumulation of AGEs induces not only physical but also physiological damages. Skin glycation due to glycative stress affects changes not only on the microlevel but also on the macrolevel so that the changes on the skin are highly likely to be recognized as a visual impression of aging.

KEY WORDS: advanced glycation end products (AGEs), skin aging, appearance

1. Introduction: Structure and function of the skin

Located on the outermost layer covering a living body, skin is an organ which protects the underling body from external environment such as shocks, temperature, ultraviolet radiation, chemicals and other threats. The skin is composed of three layers, listing from the outside, which are the epidermis, the dermis, and the subcutaneous fat tissues [1].

The epidermis is the outermost layer of the skin with a thickness of 0.2 mm on average. The epidermis can be further subdivided into four layers, beginning with the outermost layer; stratum corneum, granular cell layer, prickle cell layer and basal cell layer (***Fig. 1***). The stratum corneum, which is the outermost layer of the epidermis, has multifarious functions to repel water, acts as a barrier against bacterial and viral intrusion, and protects internal organs such as muscles, nerves, blood vessels and others from external injuries. Therefore, the stratum corneum plays the most important role to sustain the organism.

Keratinocyte accounts for 95% of the cells that constitute the epidermis. Epidermal turnover time is the time taken for the epidermis to replace itself. Keratinocytes are divided and proliferated in the undermost layer, matured, and then migrate to the surface. Through the cell division in the basal cell layer, to the division of daughter cells and shedding from the surface of the epidermis, the turnover time is approximately 40 to 56 days [2, 3].

The stratum corneum, losing their nucleus, constitutes approximately ten layers of a network and exfoliates sequentially as scurf. The stratum corneum is composed of keratin and lipids produced by keratinocytes. The keratinocytes are proliferated in the basal cell layer, produce keratin, differentiate, mature, and migrate to the upper layer. Keratin 5 and keratin 14 are formed in the basal cell layer and keratin 1 and keratin 10 are formed in the prickle cell layer and the granular cell layer.

Inside the epidermis, the following exists: melanocytes,

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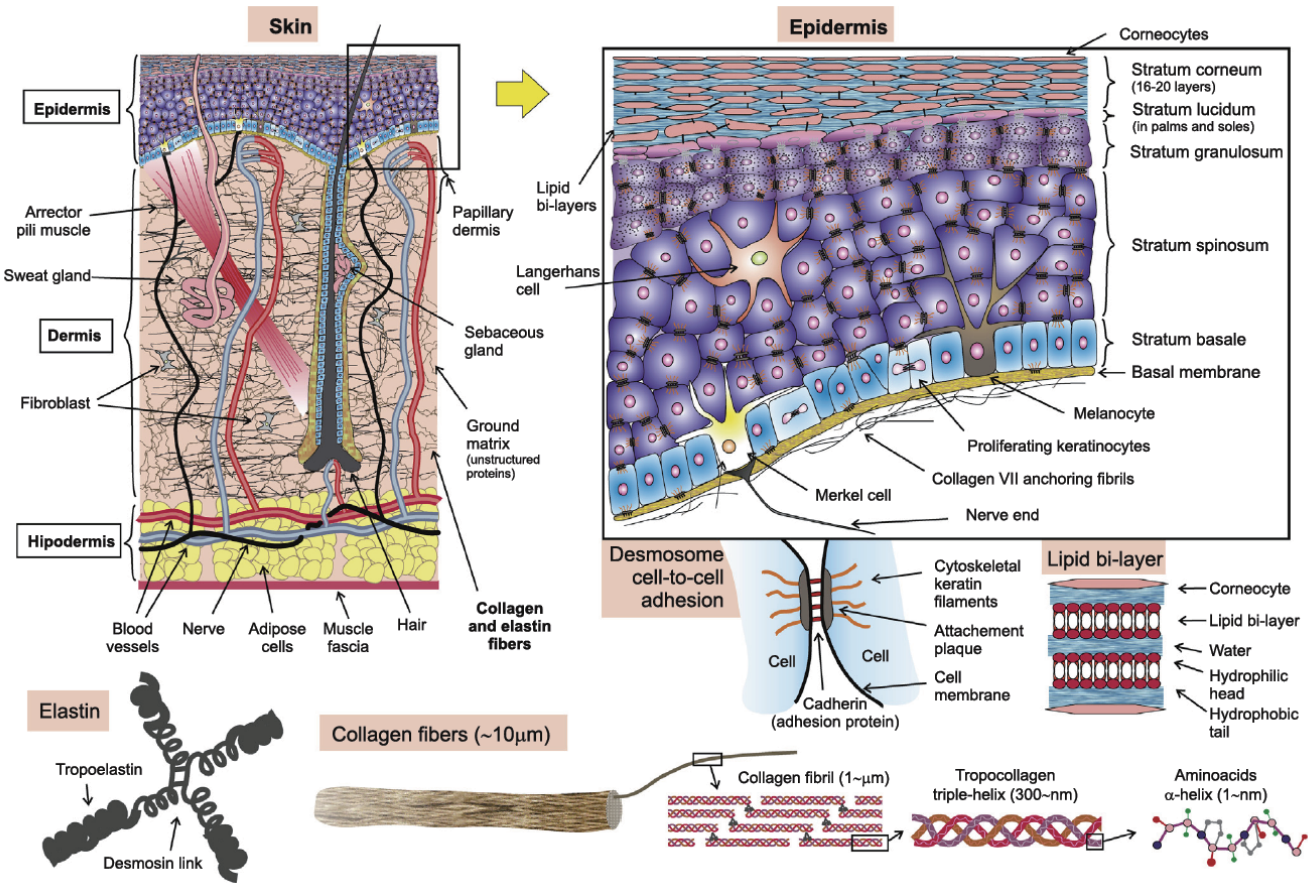

Fig. 1. **Structures of the skin.**
The figure is adapted from adapted from Reference [1].

which are responsible for dark skin color, Langerhans cells, which are related to the immune function of the skin, and Merkel cells, which are sensory recipient cells.

The dermis tissues with a thickness of 2.0-3.0 mm, are located under the epidermis and separated by the epidermis and the basement membrane. Anatomically the dermis has a three-layer structure consisting of the papillary layer, subpapillary layer and the reticular layer. The dermis provides elasticity and strength to the skin. Substances that composes the dermis are interstitial components (extracellular matrix), which compose fibrous tissues, and its productive cells. The main component of extracellular matrix is collagen fiber (mainly type I collagen and type III collagen). Other components are elastic fiber (elastin fiber), proteoglycan (hyaluronic acid, chondroitin sulfate and others) and others.

Collagen accounts for 70% of the dry weight of the dermis and provides firmness to the skin. Elastin fiber with a crosslinked structure accounts for 1-2% and provides elasticity to the skin. Proteoglycan forms colloidal gel holding abundant water and provides moisture to the skin. Inside the dermis, sensory nerve endings sense feelings, comfortability and temperature. Further, the dermis has hair follicles (folliculus pili), blood vessels and secretory glands (sweat glands and sebaceous glands), controlling body temperature, providing moisture to the skin and maintaining a resilient condition.

Subcutaneous tissues are a fatty layer with a thickness of several mm located under the dermis. The thickness of the layer is different, depending on which part of the body it is located. The functions of the subcutaneous fat tissue are to protect the body from the heat or the cold of outside air and to absorb a shock as cushioning. Furthermore, it plays the role of energy storage, where fat is stored in adipose cells of the subcutaneous tissues.

2. Aging in the skin

Changes in the skin can be recognized as visual information and are associated with the visual impression of aging. Factors of aging in visual impressions from the viewpoint of beauty are the decline of skin texture, fine lines and wrinkles, a dull aspect, the decline of elasticity and resilience, the loss of firm and supple skin texture and the decline of skin functions.

Perceived age for aging skin appearance

People tend to judge others' age by physical appearance. Facial skin appearances could be an influential information source for perception, such as the conditions of wrinkles, firmness, sagging, complexion, radiance and texture [4]. Perceived age does not necessarily correspond to chronological age. Perceived age could be greatly different from chronological age, depending on life environments, life styles, eating habits and other factors. Especially parts of the body like the face, which is usually visible to others, are easily recognized as signs of aging.

The decline of smooth texture

Area cutanea on the surface of the skin contributes to aging. Sulcus cutis are ridges on the surface of the skin and runs lengthwise and breadthwise extending radially from follicles as the center. Crista is surrounded by sulucus cutis and area cutanea is formed from crista. The skin texture of the young is smooth and even, and has a structure with a clear and well-ordered concavity and convexity, which forms a smooth skin texture with the feel that is delicate and dense.

However, the sulcus cutis becomes shallow and unclear with age, and the follicles become bigger. The cutanea area changes and the quality of the texture declines in advanced ages. The skin feels and looks rough and sandy. These conditions of the skin provide visual impressions of aging.

The increase of wrinkles

Wrinkles show morphological changes on the macro level in comparison to the skin texture. Wrinkles begin to appear, around thirty years old, around eyes and mouths and on foreheads and necks [5]. Wrinkles increase in number and become deeper with aging. The appearances of wrinkles on the face and neck are related to the movement of muscles and the exposure to ultraviolet light. Skin tissue fibers are ruptured by ultraviolet sun light, lose elasticity and reduce resilience against deformation.

The increase of dull aspect

The color tone of the skin is determined mainly by melanin (brown), carotene (yellow) and hemoglobin (red or blueish red). Further, light reflection and absorption affects the color tone due to the thickness of stratum corneum or the condition of the skin surface of texture and wrinkles. The changes in the tone of the skin accompanied by aging causes a decrease in redness, an increase in yellowness and the decline of brightness of color. As a result, the color of the skin looks dark and dull [6]. The factors of dullness of the skin are diversified such as pigmentation, the decline of blood flow, thickened stratum corneum, and glycation, oxidation and carbonylation of skin proteins [7].

The decline of firmness and elasticity

Denatured collagen or elastin in the dermis due to glycation forms a protein cross-linking disorder. As collagen and elastin are associated with firmness and elasticity of the skin, the elasticity and resilience are lost.

The decline of the skin function

The epidermis decreases the proliferation of basal cells and becomes thin as aging progresses. Then, its turnover time is prolonged [8]. The fibroblast of dermis is reduced in proliferation functions and synthesis capability of matrix components. Thus, the dermis atrophies and the skin become less supple and radiant. One of the factors of reduced functions of tissues is that hormones and growth factors have insufficient reaction to skin tissues. The changes of skin functions induce the delay of barrier function recovery of stratum corneum, the decline of water retention function (dryness), the changes of the amount of sebum secretion due to the changes of sex hormone secretion, the decline of skin blood flow, and the changes in the metabolism of lipid and carbohydrate.

3. Glycation and aging of the skin

The glycation of the skin affects skin aging. Aging, glycation of skin protein due to glycative stress, and the formation of AGEs (advanced glycation end products) induce the yellowing of skin tone which results in a dark and dull skin tone.

The proteins of collagen and elastin, which are main components of the dermis, have a long half-life period and are subject to the influences of glycation [9, 10]. Collagen and elastin form a cross-linking fiber in the process of forming fiber in tissues through lysine and hydroxylysine residue. The formation of cross-linking AGEs such as pentosidine forms a protein cross-linking disorder and then stiffens the proteins. Thus, the glycation of the skin protein influences maintaining the stability of fiber tissues as factors of physical damage, such as the decline of firmness and elasticity. Further, elastin with the accumulation of CML (N^{ε}-carboxymethyllysine), a type of non-cross-linking AGEs, which is difficult to degrade by neutrophil elastase, accelerating aggregation, increasing fiber diameter, and reducing elasticity and extensibility. Similarly, protein with an accumulation of AGEs advances the formation of cross-linking and is difficult to be degraded by protease, prolonging the turnover time of the skin.

In an examination with healthy subjects, face skin tissues were stained with CML antibody. It was shown that the accumulation of CML in elastic fibers began between the ages of 30 to before 50. Then, the accumulation of CML expands over all elastic fibers in the elderly [11]. CML are produced in the skin collagen glycation progresses, which accounts for 33% of collagen during the age of 20's to 80's [12]. The parts such as a nose, the middle of the forehead, the chin, and head parietal region parietalis, which are susceptible parts of the sunlight, form more AGEs than the parts such as the chest region, the back and the thighs, which are less exposed to the sunlight (***Fig. 2***) [13]. Therefore, the glycation of elastic fibers such as collagen and elastin induce the decline of firmness and elasticity of the skin.

The exposure to ultraviolet light and oxidative stress of the skin promotes the AGE formation [14]. Solar elastosis refers to the condition of the accumulation of aberrant elastin fiber on the dermis of the parts exposed to sunlight. This disease is considered to occur, due to the long-term ultraviolet light-exposed skin and the produced AGEs from elastin, which is related to wrinkles and hanging folds of loose skin.

Contrarily, the accumulated AGEs in the skin bind with RAGE (receptor for AGEs,) which exists on the surface of the skin, which promotes the secretion of pro-inflammatory cytokine such as TNF-α, IL-1 and IL-6 through an intracellular signal transduction [15]. Therefore, the formation and accumulation of AGEs in the skin triggers physiological damages due to these factors. Collagen with the accumulation of CML induces apoptosis of fibroblast through an intracellular signal transduction [16]. In addition, the progression of glycation in the skin tissues induces CML formation in lysine residue of proteins. Carboxymethyl lysine-collagen induces the migration of keratinocyte, which exists on the epidermis, and decreases the adhesiveness to collagen [17, 18].

The accumulation of AGEs is shown in the epidermis, which has a shorter turnover time than the dermis. CML is accumulated in keratin-10 contained in the epidermis [19]. Furthermore, CML also accumulates in the stratum corneum of the outermost layer of the skin. The smooth texture of the skin is reduced in the stratum corneum with abundant CML [20]. The accumulation of CML in the stratum corneum is related

to the decline of isotropy of the sulcus cutis and the decline of arithmetic roughness index of the surface of the skin. The accumulation of CML in the stratum corneum induces the decline of the smooth texture and causes facial appearance to look older. In addition, the accumulation of CML in the stratum corneum increases with aging (*Fig. 3*) [21].

People with a high level of glycative stress look older than those who don't. With higher value of fasting blood glucose level [22] or the abundant accumulation of AGEs [23], they are recognized to have an older perceived age, even if they are in a good health, in comparison to people at the same chronological age without glycative stress.

The glycation of the skin influences changes in the skin, not only at a micro-level but also at a macro-level and it contributes to the visual impression of aging.

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Conflict of Interest Statement

The authors claim no conflict of interest in this study.

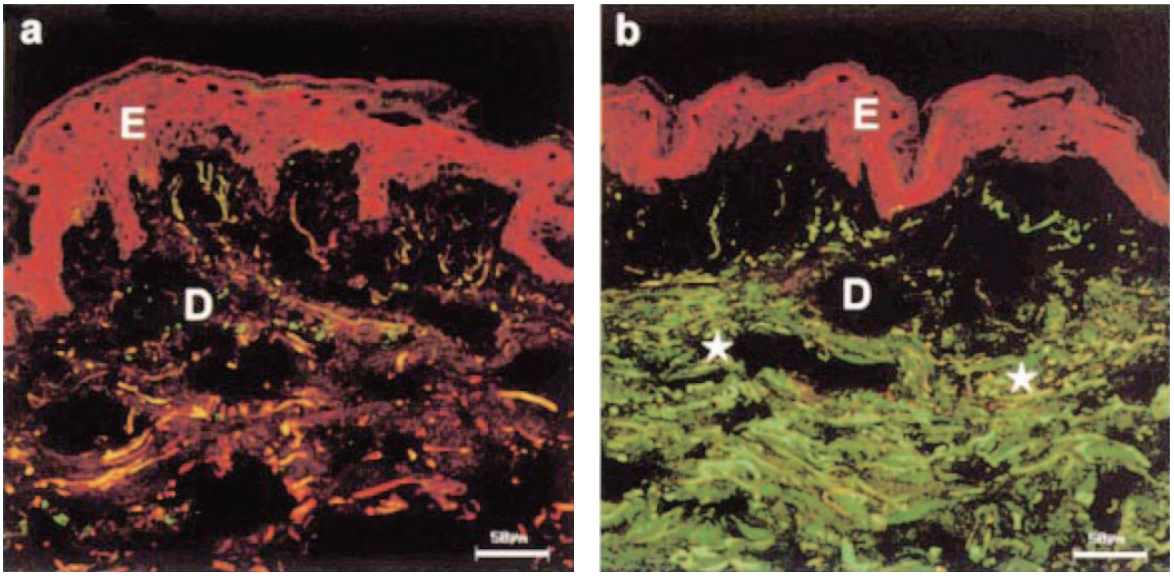

Fig. 2. **Expression of advanced glycation end products (AGEs) in normal abdominal human skin.**
AGEs were revealed by immunofluorescence in human skin obtained from a 34-year-old (**a**) and a 65-year-old (**b**) woman. Immunolocalization was carried out on fresh frozen sections using a monoclonal antibody raised against AGEs. Sections were visualized by confocal microscopy. The staining of AGEs is very intense (stars) in the older skin compared with that in younger skin. E, epidermis; D, dermis. Scale bar 50 mm. The figure is adapted from adapted from Reference [13].

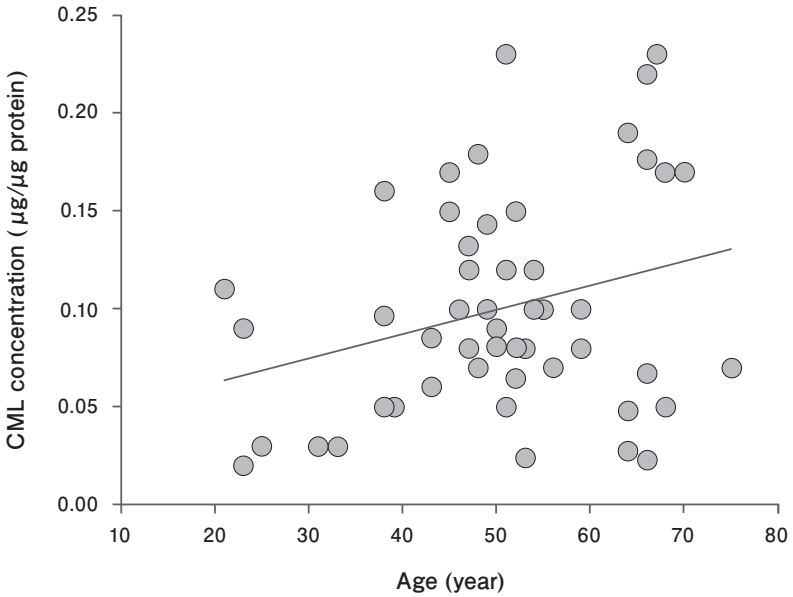

Fig. 3.
Relationship of CML in the stratum corneum and age.
Subject: n = 52. y = 0.0012x + 0.0378, $R^2 = 0.079$, $p < 0.05$. CML, N^{ε}-carboxymethyllysine. The figure is adapted from adapted from Reference [21].

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