Indonesian Journal of Pharmacy

VOL 34 (3) 2023: 357–371 | REVIEW ARTICLE

Vitamin D3 and Molecular Pathway of Skin Aging

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Article Info

Submitted: 24-06-2022
Revised: 26-03-2023
Accepted: 27-05-2023

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ABSTRACT

Skin aging has been a concern for most people, not only women but also men. Signs of aging, such as lines, wrinkles, dry skin, hyperpigmentation, and loss of elasticity, affect skin appearance and self-confidence. Two domain factors that stimulate skin aging are intrinsic (age, lifestyle) and extrinsic (mostly UV irradiation) factors. Those factors could generate reactive oxygen species (ROS) production, which becomes the primary cause of collagen breakdown through matrix metalloproteinases (MMPs). Collagen and elastin are proteins that play a pivotal role in skin aging because they maintain skin integrity, strength, and resilience. Several studies have reported the effects of vitamin D as a promising agent for skin aging prevention, particularly its effect on collagen synthesis and MMPs inhibition. This review focuses on identifying and assessing the molecular pathways of vitamin D effects on skin aging. Vitamin D3 has a contradictory effect on MMPs, TGF-β, and estrogen. Several studies have reported that vitamin D increases collagen synthesis, but insufficient level of this vitamin could clinically decrease skin elasticity. Overall, vitamin D3 positively affects skin aging through comprehensive mechanisms of inflammatory responses, epidermal barrier preservation, antioxidative activities, DNA damage prevention, DNA repair, and photoprotective actions on the skin. Future research could investigate the other forms of vitamin D3 (polymorphisms, analogs, and metabolites) as a supplement for antiaging of the skin or even senomorphic therapy.

Keywords: elasticity, collagen, molecular mechanism, skin aging, vitamin D3

INTRODUCTION

Skin aging manifests as wrinkles, sagging, and lack of moisture (Philips *et al*., 2019; Stojiljković *et al*., 2014; Tobin, 2017). It is defined based on two main factors which act simultaneously over the lifespan. Intrinsic aging occurs naturally with age and is triggered by oxidative stress, mitochondrial dysfunction, and telomeres shortening (Bocheva *et al*., 2021; Gragnani *et al*., 2014). Those factors participate in cellular senescence as a part of skin aging mechanisms. Telomere shortening acts as a de-protection of chromosome ends from DNA damage response (Srinivas *et al*., 2020). It is known that progressive telomere shortening is one of the main factors in intrinsic aging (Lago & Puzzi, 2019; Tobin, 2017). Meanwhile, extrinsic aging is caused by UV irradiation, air pollution, and lifestyle (for example, nutritional choices and smoking) (Bocheva *et al*., 2021; Gragnani *et al*., 2014).

Both intrinsic and extrinsic factors are characterized by a decrease in elastin and collagen which forms elongated, decayed, inflamed fibroblasts and the disorganization of fibroblast collagen into connective tissue. Elastin and collagen are the majority of extracellular matrix (ECM) components that have a pivotal role in skin aging and are synthesized by dermal fibroblasts (Philips *et al*., 2019; Stojiljković *et al*., 2014; Tobin, 2017). Collagen is a protein that confers tensile strength and supports skin integrity, while elastin fibers provide skin elasticity and resilience (Gragnani *et al*., 2014; Tobin, 2017).

In extrinsic causes, particularly photo-aging, there are decreases in mature collagen and substantial increases in elastic fibers to substitute collagenated dermal matrix components. On the contrary, the degradation of mature collagen is more stable in intrinsic aging (Ramos-e-Silva *et al*., 2013). Skin at a younger age synthesizes significant amounts of procollagen rapidly. The turnover of collagen develops until adulthood and then remains in a quiescent state by age. It increases again to compensate for the disorganized structure and decreasing levels of collagen associated with photodamaged or chronologically aging skin (Reilly & Lozano, 2021).

The escalation of customer interest in skin care, including skin aging, brings up the emergence of various kinds of antiaging. Exploration and research of the most effective antiaging are promising. One prospective agent that might work on the antiaging pathway is vitamin D3. Currently, vitamin D3 is used in sunscreen as a skin differentiation-enhancing agent and as a psoriatic therapy. Previous studies have shown the efficacy of vitamin D as an antiaging, particularly through its mechanisms of protection against UV exposure and preventing double-strand breaks of DNA structure (Bocheva *et al*., 2019; Gordon-Thomson *et al*., 2012; Philips *et al*., 2022). This review describes comprehensive factors inducing aging and the critical points that vitamin D3 works on.

Collagen: Key Component in Skin Aging

Collagen is a protein produced by fibroblast cells. Alongside fibroblasts, collagen, and elastic fibers form connective tissues in the dermis layer (De Araújo *et al*., 2019; Reilly & Lozano, 2021). Collagen supports the skin matrix, thus maintaining the firmness and elasticity of the skin. Elastin provides flexibility to stretch and allows the skin to return to its original conformation. Fibroblast cells tend to be sensitive to several stimulations, such as physical tension and biochemical or signaling pathways that probably activate or induce proliferation. If fibroblasts are activated, collagen and elastin will increase. The biosynthesis of fibroblast components, mainly collagen and elastin, has become the key target of most antiaging strategies (Reilly & Lozano, 2021).

Dermal fibroblasts synthesize collagen through subsequent procollagen-stimulated transforming growth factor-β (TGF-β)/Smad assembly (Liu *et al*., 2019). The production and degradation of collagen were mainly regulated through the TGF-β/Smad pathway. TGF-β1 binds to its receptors (TβRI and TβRII) on the cell surface. The signal is transferred to transcription factors SMADs protein (Smad2 and Smad3 join in complexing with Smad4), which translocate into a nucleus and then regulate the collagen gene transcription (Ding *et al*., 2016; J. W. Shin *et al*., 2019). The pathway has an impact on inducing ECM production and hindering ECM degradation. It is believed that the TGF-β/Smad signaling pathway is a molecular key point in maintaining the integrity of the dermal connective tissue (Shin *et al*., 2019).

Other than that, several factors also participate in collagen synthesis, such as the involvement of calcium, Erk, cyclic adenosine monophosphate (cAMP), and human homolog chromosomes. One experiment using keratinocyte line cells (HaCaT and FEPE1L-8) induced with a calcium concentration of 3.6 to 30 mM, resulted in a proliferation of cells on molecular type I collagen fibrils (gels) on a skin model. The result verified calcium as an essential factor in inducing cell proliferation in collagen. High calcium concentration also prevented keratinocytes apoptosis of type I collagen fibrils. It induced Erk 1/2 activation, substituting Akt activation loss and maintaining type I collagen fibrils in the keratinocytes (Fujisaki *et al*., 2018).

Collagen in skin tissue is mainly represented by type I (80-90%) and type III (10%) collagen (Reilly & Lozano, 2021). Collagens can be categorized into seven families and eighteen types, but only several types are involved in skin aging. Type I is the primary collagen-forming skin tissue (80% of dermal collagen) and is incorporated with type III collagen (15%) (Avila Rodríguez *et al*., 2018; Tobin, 2017). As skin ages, type I collagen declines, and types IV and VII change. Type IV collagen is an inherent part of the dermo-epidermal junction, particularly in mechanical stability, while types IV and VII are implicated in wrinkle formation (Tobin, 2017).

Matrix Metalloproteinases (MMPs): Collagen Degradation Inducer

MMPs are a family of nine or more endopeptidase enzymes with zinc or calcium ions on their active site. MMPs cause the degradation of collagen, elastic fibers, and other components of the dermal extracellular matrix (Lee *et al*., 2015; Stojiljković *et al*., 2014). MMPs are generated from several cell types, such as mast cells, fibroblasts, neutrophils, and epithelial cells (Philips *et al*., 2011). Dermal fibroblasts usually produce MMPs in intrinsic aging, but MMPs in extrinsic aging are

secreted from epidermal keratinocytes. MMP-12, produced by fibroblasts and macrophages, contributes to solar elastosis and a decrease in elastic fibers (Shin *et al*., 2019). Solar elastosis is abnormal elastic tissues lying deep in the dermis. Expression of elastin increases after UV exposure, resulting in elastolysis. Meanwhile, elastin is degraded by MMP-2, MMP-3, MMP-7, MMP-9, and MMP-12 (Zhang & Duan, 2018).

The upregulation of MMPs in cultured fibroblast cells is primarily caused by UV irradiation, which is related to photo-aging. The expression of MMP-1, MMP-3, and MMP-10 is highly stimulated by UVB, while MMP-1, MMP-2, and MMP-9 are induced by UVA (Pittayapruek *et al*., 2016). On the other hand, the TGF-β/Smad signaling pathway from collagen synthesis downregulates MMPs and upregulates tissue inhibitors of matrix metalloproteinases (TIMPs) (Shin *et al*., 2019). TIMPs, including TIMP-1, TIMP-2, TIMP-3, and TIMP-4, regulate the inhibition of MMPs activity. As a result, TIMPs tend to support collagen synthesis (Lee *et al*., 2015; Philips *et al*., 2011).

MMPs are classified into four predominant subsets. These are collagenases (MMP-1, MMP-8, and MMP-13, MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), membrane-type MMPs (MT-MMP: MMP-14, -15, -16, -17, -24, and -25), matrilysins (MMP-7 and -26), and elastase (MMP-12) (Lee *et al*., 2015; Philips *et al*., 2011; Quan & Fisher, 2015). Each group has its specific role in collagen. The collagenases (primarily MMP-1) degrade structural collagen. The gelatinases cleave basement membrane collagens and denatured structural collagens, while stromelysins and matrilysins also cleave basement membrane collagens (e.g. proteoglycans, matrix glycoprotein). The membrane-type MMPs activate MMPs. Conversely, the elastase cleaves the elastin (Philips *et al*., 2011).

The movement of MMPs to sites of collagen cleavage can be described as a random diffusion of MMPs on intact collagen fibrils. MMP-1 cannot break intact collagen fibrils as the C-terminal telopeptide protects them. As a result, almost 90% of MMP-1 is blocked by the "monomer" unit of collagen. The type I collagen unit structure is a twisted triple helix consisting of two extended α1 chains and one α2 chain. MMP-2 and MMP-9 (gelatinases) cannot break intact type I collagen (Van Doren, 2015). Therefore, 3α chains of type I collagen cleavage must be initiated first by MMP-1, MMP-8, and MMP-13 into ¾- and ¼-sized fragments of intact collagen. MMP-2 and MMP-9 are then exposed to collagen fibrils cleaved by MMP-1, MMP-8, and MMP-13 to digest them (Reilly & Lozano, 2021; Van Doren, 2015). In addition, MMP-2, MMP-9, and MMP-12 affect elastolysis due to extensive binding sites for elastin (Van Doren, 2015).

Molecular mechanisms of skin aging

Most of the aging process approaches are based on extrinsic than intrinsic factors. Many models of the molecular mechanism of skin aging have been proposed, including oxidative stress, inflammation, telomere shortening, chromosomal abnormalities, and single-gene mutations. Those mechanisms do not stand alone but co-occur with others and affect one another (Cao *et al*., 2020).

Oxidative stress and pro-inflammatory cytokines

Reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radicals, are crucial in dermal extracellular matrix degradation in intrinsic or extrinsic aging (Cao *et al*., 2020; Philips *et al*., 2019; Zhang & Duan, 2018). ROS induces MMPs and inactivates TIMPs to decrease collagen levels (Reddy & Gilchrest, 2011; Tobin, 2017). ROS mainly emerge from UVA/B light, mitochondria, toxins (e.g. tobacco), and inflammation. During the intrinsic process, ROS are continuously produced from 1.5-5% of the consumed oxygen and as side products of the mitochondria aerobic metabolism, predominantly from keratinocytes and fibroblasts mitochondria (Kammeyer & Luiten, 2015; Reddy & Gilchrest, 2011).

Besides the ROS, pro-inflammatory cytokines (e.g. interleukin/IL-1, IL-6, tumor necrosis factor-α/TNF-α) also have a predominant role in skin aging (Cao *et al*., 2020; Kim & Park, 2016). IL-1, IL-6, and IL-18 have been known as cytokines associated with skin wrinkles. Along with cysteine-rich protein 61 (CCN1), those cytokines downregulate collagen and elastin synthesis (Kim & Park, 2016). Both ROS and pro-inflammatory cytokines affect mitogen-activated protein kinase (MAPK), Janus Kinases (JAK), and nuclear factor-κB (NF-κB) signal transduction pathways to induce activator protein-1 (AP-1), triggering the downregulation of ECM (Philips *et al*., 2019; Zhang & Duan, 2018). AP-1 indirectly reduces collagen biosynthesis and induces collagen degradation through several mechanisms. The transcription factor complex consisting of AP-1 and NF-κB affects the balance of MMPs and TIMPs, the determinant

for tissue degeneration. Once MMPs are more dominant than TIMPs, collagen and other fibrillar structures will be degraded, and vice versa. When fibroblasts are exposed to UV, MMP transcription increases. Similarly, TIMP-1 tends to rise, while TIMP-2 remains slightly steady (Kammeyer & Luiten, 2015). AP-1 affects the activation of MMP-1, MMP-3, MMP-9, and MMP-12 in dermal fibroblasts, while NF-kB plays an essential role in regulating MMP-1 and MMP-3 (Shin *et al*., 2019). Also, MMP-9 is induced by TNF-α in fibroblasts, inhibiting collagen production (Kim & Park, 2016).

NF-κB itself induces the infiltration of neutrophils through the transcription of IL-1, IL-6, and TNF-α. Those pro-inflammatory cytokines activate MMP-8 release and induce matrix degradation. On the other hand, activated AP-1 reduces collagen biosynthesis through the deterioration of the TGF-β/Smad signaling pathway, causing a decrease in the precursor of type I and type III collagen (Kammeyer & Luiten, 2015).

Inflammation and ROS production are correlated in dermal tissues. Physiologically, inflammatory cells and keratinocytes release NADPH oxidase (Nox) enzymes to produce a low concentration of ROS as a mediator to kill bacteria. UV-induced inflammation could aggravate the increase of Nox (Kammeyer & Luiten, 2015).

Advanced glycation end (AGE) products

Another factor of skin aging comes from advanced glycation end (AGE) products. AGEs accumulate in the skin during chronological aging and are highly detected in photo-aging, causing tissue to stiffen and elasticity to decrease. AGEs result from the glycation process from excess sugar (such as glucose or fructose) formed covalent bounding with protein or fat. UV irradiation, smoking, or fried diet seem to highly accelerate the AGEs formation (Cao *et al*., 2020; Gkogkolou & Böhm, 2012; Zhang & Duan, 2018). It is different from normal glycosylation because it impairs the normal function of molecules. Glycated collagen is highly resistant to MMP degradation. AGE products could attach to receptors for AGEs (RAGEs) on the cell surface and activate MAPKs/ NF-kB/ extracellular signal-regulated kinases (Erk)/ phosphatidyl-inositol-3-kinase (PI3K) signaling pathway. It activates MMPs in collagen deterioration (Zhang & Duan, 2018).

Telomere shortening

The excessive UV-induced ROS promotes telomere shortening due to mutation, senescence, or cell death (Cao *et al*., 2020; Zhang & Duan, 2018). Telomeres consist of DNA sequences at the end of a chromosome. They maintain the chromosomal integrity and protect it from damage. The telomeres become shorter with cell division and senescence. ROS is one of the factors that induce telomere mutation (Cao *et al*., 2020).

DNA damage

UV light can cause DNA damage directly and indirectly. UVB light causes direct DNA damage through the change in the nucleotide sequence, leading to gene deletion or DNA strand mutation. Meanwhile, UVA light has an indirect effect on DNA damage., These are probably due to the difference between the penetration depth and biological impact between UVA (320-400 nm) and UVB (290-320 nm) on the skin layer. UVA has a less physical impact, such as sunburn, skin photodamage, or photo-aging, than UVB (Cao *et al*., 2020).

However, UVA still affects photo-aging due to its deeper penetration. UVB light only reaches the epidermis, while UVA light can penetrate the dermis (Kammeyer & Luiten, 2015). The absorption of UVA light by the DNA stimulates the transfer of electrons and energy to oxygen molecules, resulting in free radicals forming and causing DNA damage. UVB exposure also alters the expression of microRNA levels in the skin. The miR-34 in human dermal fibroblasts regulates MMP-1, type I collagen, and elastin, while miR-217 and miR-23a-23p regulate cellular senescence (Cao *et al*., 2020).

ROS cause damage either in cellular structural components (e.g. lipid bilayer membrane) or subcellular components (DNA, lipids, and proteins) (Reilly & Lozano, 2021). Along with inflammatory cytokines, ROS degrade cell integrity by targeting DNA, lipid, and protein, resulting in cell damage and oxidized lipids (Philips *et al*., 2019; Zhang & Duan, 2018). Those results are recognized by the complement system, as a part of the immune system, to be cleaved and lead to inflammation. The damaged cells and oxidized lipids activate macrophages to release MMPs, resulting in extracellular matrix degradation (Zhang & Duan, 2018). When the macrophages have reached their ability to clear up, they produce pro-inflammatory factors and ROS. They precipitate deleterious effects on dermal inflammation (Figure 1).

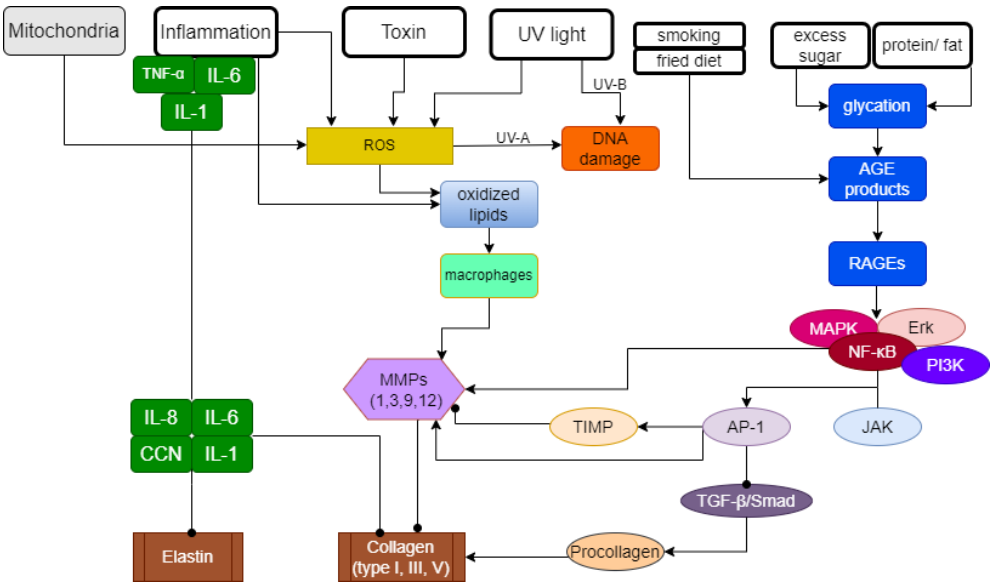

Figure 1. Schematic representation of factors contributing to skin aging through intrinsic factors (mitochondria) and extrinsic factors (UV light, toxins, inflammation, and AGEs products). The factors that affect ROS and DNA damage are involved in the aging cell. Both factors have an impact on collagen, elastin, and matrix metalloproteinase as the predictors in skin aging, whether inducing (→) or inhibiting (●)
Abbreviations: UV (ultraviolet), IL (interleukin), TNF-α (tumor necrosis factor-α), ROS (radical oxygen species), DNA (deoxyribonucleic acid), AGE products (advance glycation end products), JAK (Janus Kinases), MAPK (mitogen-activated protein kinase), NF-κB (nuclear factor-kappaB), RAGEs (receptor for AGE products), Erk ½ (extracellular regulator-signal kinase ½), PI3K (phosphatidyl-inositol-3-kinase), AP-1 (activator protein-1), TIMP (tissue inhibitor of metalloproteinase), MMP (matrix metalloproteinase), TGF-β (transforming growth factor-β), CCN (cysteine-rich protein 61)

Vitamin D3: biosynthesis, metabolites, and its biological activities

Vitamin D is a lipophilic steroid hormone with a physiological and pathophysiological role in the body, including the skin (Meza-Meza *et al.*, 2020; Nair & Maseeh, 2012; Saponaro *et al.*, 2020). There are two forms of vitamin D. The first form of vitamin D is called vitamin D2. It is obtained from UV irradiation exposed to ergosterol of the yeast, hence its name ergocalciferol. The second is called vitamin D3 (cholecalciferol), the natural form of vitamin D, obtained from animal sources and synthesized in the skin (Antal *et al.*, 2011; Meza-Meza *et al.*, 2020; Nair & Maseeh, 2012). UV light (295-300 nm) is an inducer of vitamin D3 synthesis in the skin (Combs Jr, 2008; Franco *et al.*, 2022). UV-B light contributes to more than 90% of vitamin D3 production for the body's daily requirements (Bocheva *et al.*, 2021). When the body is exposed to UV light for 15-20 min, as much as 10.000 IU of vitamin D can be produced (Mostafa & Hegazy, 2015).

7-dehydrocholesterol is a product of cholesterol and precursor to vitamin D3, called pre-vitamin D. Only 5-15% of the 7-dehydrocholesterol has been converted, depending on skin characteristics and the climate condition of the environment. The 7-dehydrocholesterol is in the epidermal layer, mainly in the deeper Malphigian layer. In consequence, this has participated in the loss of UV due to absorption in the stratum corneum (Combs Jr, 2008). 7-dehydrocholesterol undergoes photolysis after UVB exposure resulting in pre-vitamin D synthesis in suprabasal keratinocytes and dermal fibroblasts (Combs Jr, 2008; Mostafa & Hegazy, 2015; Nair & Maseeh, 2012). Vitamin D, produced from UV light or food intake, is biologically inactive. It needs two hydroxylation mechanisms to provide its biological activity (Piotrowska *et al.*, 2016). Pre-vitamin D undergoes hepatic metabolism by 25-hydroxylase (CYP27A1), resulting in calcidiol [25(OH)D], the circulating metabolite of vitamin D, which is detected in serum (Bikle, 2012; Meza-Meza *et al.*,

2020). Calcidiol released to the extracellular space is captured by Vitamin D binding receptor (VDBP) (Meza-Meza *et al*., 2020; Piotrowska *et al*., 2016). Furthermore, it is transported to the kidney to be converted to its biologically active metabolite, calcitriol [1α,25$(OH)_2$D], by 25-hydroxyvitamin D3 1-α-hydroxylase (CYP27B1) (Meza-Meza *et al*., 2020). CYP27A1 and CYP27B1 are also detected in dermal fibroblasts and keratinocytes (Bikle, 2012; Tieu *et al*., 2012). The generic denomination of vitamin D can be applied for naming vitamin D2 and vitamin D3. They are 25(OH)D2 and 25(OH)D3 for the circulating form, while 1α,25$(OH)_2$D2 and 1α,25$(OH)_2$D3 are for the active form (Meza-Meza *et al*., 2020). The active metabolite of vitamin D3, 1,25$(OH)_2$D3, interacts with the vitamin D receptor (VDR), resulting in several biological activities (Franco *et al*., 2022; Nair & Maseeh, 2012). VDRs can be found in skin cells such as keratinocytes, fibroblasts, and melanocytes, with the highest concentration in the stratum basale, where the production of vitamin D occurs (Bikle *et al*., 2012; Combs Jr, 2008).

The VDR protein consists of three domains: the N-terminal dual zinc finger DNA-binding domain, the C-terminal ligand-binding activity domain, and an extensive-unstructured region linking the two functional domains. The domain on VDR is a macromolecule receptor for 1,25$(OH)_2$D3 response. That dual zinc finger DNA-binding is typical in steroid receptor gene families such as androgens, estrogens, glucocorticoids, thyroid hormone, retinoic acid, and other lipophilic regulators (Pike & Meyer, 2012). Therefore, the similarity in dual zinc finger DNA-binding between VDR and those endogenous hormones provides the possibility of correlating vitamin D with endogenous hormones. Activator protein (AP-1), a heterodimer upregulating MMPs in the skin, and NF-kB, which is related to infection and inflammation, are transcription factors that might interact with VDR (Kwon *et al*., 2019; Qian *et al*., 2017).

As it is known for its effect on bone health, vitamin D provides the scaffolding underlying the skin by preserving the facial bones' integrity. It prevents gingival bone demineralization and indirectly avoids wrinkles formation around the mouth (Muzumdar & Ferenczi, 2021). 1,25$(OH)_2$D3 induces epidermal differentiation and inhibits keratinocyte proliferation to maintain cutaneous immunity, skin barrier function, and inflammation recovery (Franco *et al*., 2022; Meza-Meza *et al*., 2020; Mostafa & Hegazy, 2015).

1,25$(OH)_2$D3 has been proven to protect against UV-induced cell death in cultured human skin fibroblasts and keratinocytes. The photoprotection effect occurs in a nongenomic signal transduction-mediated pathway through increased nuclear p53 expression and decreased nitric oxide (NO) products (Bocheva *et al*., 2021; Dixon *et al*., 2013; Philips *et al*., 2019). The nuclear p53 could repair DNA, while NO acts as a reverse; it tends to inhibit DNA repair. High levels of NO could be more toxic as it stimulates oxidative and nitrosative damage to DNA. UV radiation upregulates NO production and causes DNA photolesions, forming cyclobutane pyrimidine dimers (CPDs), which are mutagenic and carcinogenic. 1,25$(OH)_2$D3 also decreases CPD and sunburn cells, as observed in Skh:hr1 mouse skin. Vitamin D also boosts antioxidant systems (e.g. metallothionein) that enhance UV protection (Dixon *et al*., 2013; Reichrath, 2007). Vitamin D is anti-inflammatory by reducing TNF-α, IL-1β, IL-6, and IL-8. Those mechanisms commonly involve psoriatic therapy but contribute to photo-aging prevention (Kocic *et al*., 2019; Philips *et al*., 2019).

In addition, to maintain human epidermis viability, 1,25$(OH)_2$D has an essential endocrine system function, including arranging calcium metabolism (Mostafa & Hegazy, 2015). It induces several critical elements in the calcium signaling pathway, such as calcium-sensing receptor (CaSR), and causes other cells, including phospholipase C (PLC), to be more responsive to calcium (Bikle, 2016; Bikle *et al*., 2012). Calcium activates G protein-coupled calcium-sensing receptors (CaSR) and phospholipase C (PLC). They produce second messengers, diacylglycerol (DAG) and inositol trisphosphate (IP3), because of phosphatidylinositol biphosphate (PIP2) hydrolysis. Simultaneously, IP3 induces intra-cellular free calcium release (Cai). Subsequently, DAG and Cai activate protein kinases (PKC). Consequently, PKC stimulates transcription factors (AP-1 family), which regulate gene expression, encoding the proteins entangled in the cornified envelope formation: involucrin, transglutaminase (TG), and loricrin (Figure 2) (Bikle *et al*., 2012; Mostafa & Hegazy, 2015). Keratin, filaggrin, loricrin, and involucrin are keratinocyte structural components that maintain skin barrier integrity (Chieosilapatham *et al*., 2021). AP-1 and cornified envelope are responsible for the keratinocyte differentiation pathway (Bikle *et al*., 2012). Blocking PLC and calcium receptors (CaR) expression prevents calcium-stimulated

differentiation (Bikle, 2016). In an in vivo experiment, calcium formed mainly in the stratum granulosum with a variable concentration in the stratum basale (Bikle *et al*., 2012). Calcium concentration must exceed 0.1 mM to lead intercellular contacts to CaR (Bikle *et al*., 2012).

CaSR activation increases both CaSR itself and VDR gene expression. The feed-forward mechanism occurs when $1,25(OH)_2D3$ inhibits the expression of the parathyroid hormone (PTH) gene. CaSR causes the intracellular breakdown of PTH under elevated calcium and inhibits PTH secretion in hypocalcemia (Brown, 2013). PTH released by the parathyroid gland (PTG), tumor necrosis factor-α (TNFα) secreted by keratinocytes, and interferon-(IFN) secreted by macrophages all induce the formation of $1,25(OH)_2D3$ (Bikle, 2014; Gil *et al*., 2018; Mostafa & Hegazy, 2015).

Vitamin D can regulate the innate immune response by enabling the antimicrobial defence of epithelial surfaces. Moreover, vitamin D also modulate the adaptive immune responses by the role of VDR in activated dendritic cells and macrophage cells. Additionally, vitamin D has an inhibitory effect on the adaptive immune system, specifically T-cell proliferation. As a result, Th1 cells inhibit IFN-, IL-2, and macrophages (Bikle, 2012). Vitamin D may inhibit macrophage MMP secretion in response to UV radiation. T helper and other immune cells have VDR, through which vitamin D can inhibit the release of pro-inflammatory cytokines from these cells, including mast cells and keratinocytes in the skin (Caraffa *et al*., 2016).

The skin's immune system is the stratum corneum, immune cells (macrophages, monocytes, neutrophils, natural killer/NK cells), and antimicrobial peptides (AMPs). AMPs' primary function is to prevent microbial invasion of the skin. Skin injury or bacterial infection will increase enzyme 1α-hydroxylase (CYP27B1) locally and AMPs levels in response to barrier disruption, inflammation, or infection. Consequently, vitamin D levels increase locally (Umar *et al*., 2018).

At the same time, CYP27B1 activates $1,25(OH)_2D3$ in monocytes and keratinocytes, stimulating the expression of toll-like receptor 2 (TLR2) and co-receptor CD14 under inflammatory stimuli. Lastly, it modulates cathelicidin to eliminate invasive species (Bikle, 2012; Umar *et al*., 2018). Cathelicidin is a direct transcriptional target of vitamin D and one of the AMPs which promotes keratinocytes proliferation in skin wound healing and plays a vital role in the pathogenesis of infections and inflammatory cascades (Umar *et al*., 2018).

Besides producing vitamin D3, pre-vitamin D3 is also transformed into the other two photoisomers: tachysterol (T3) and lumisterol (L3) (Franco *et al*., 2022). Oral administration of high-dose vitamin D3 after UVB exposure prevents skin damage and inflammation and maintains the epidermal barrier. When active vitamin D3 is combined with L3, the activated Nrf2 attenuates photo-aging through cytoprotection and detoxification (Bocheva *et al*., 2021).

The correlation between Vitamin D3 and MMPs

Vitamin D, VDR, and MMPs correlate in pathogenesis, such as keloid tissue and hypertrophic scars, lung fibroblast, cardiac tissue, and uterine fibroid cells. However, several studies have observed the effect of vitamin D3 on MMPs in the skin. The attenuation of MMP-9 production by calcitriol has occurred in keratinocytes in an inflammation setting. HaCaT cells treated with TNFα have proven to increase the MMP-9 output, then MMP-9 is markedly attenuated by calcitriol. Calcitriol only attenuates, not abolishes, MMP-9, which is still essential in the damaged tissue renewal process. Calcitriol is only partially reserved for quantitative change in TNFα-induced AP-1 DNA binding. It explains a plausible molecular basis for VDR ligands' action, mostly in psoriasis therapeutics due to the AP-1 role (Bahar-Shany *et al*., 2010).

Research conducted in Seoul, the Republic of Korea, using samples from Korean volunteers, reported that exposure to 100 nM $1α,25(OH)_2D3$ for 72 hours increased MMP-1 expression in human epidermal keratinocytes. Other data showed that MMP-1 increased in dermal fibroblasts, but no data was displayed. They also examined the correlation of $1α,25(OH)_2D3$ with the stimulation of AP-1 activation through the MAPK signaling pathway. The data showed no significant effects on those interactions in epidermal keratinocytes. However, they indicated an effect on MMP-1 upregulation. The study stated that vitamin D has a pro-apoptotic effect on human epidermal keratinocytes. This result may represent the mechanism of action of vitamin D in psoriasis treatment (Shin *et al*., 2019).

The description above explains the contradictory results of the effect of vitamin D on MMPs on the skin, particularly keratinocytes. Specifically, vitamin D attenuates MMP-9 and yet upregulates MMP-1. Figure 2 portrays the correlation between vitamin D3 and MMPs.

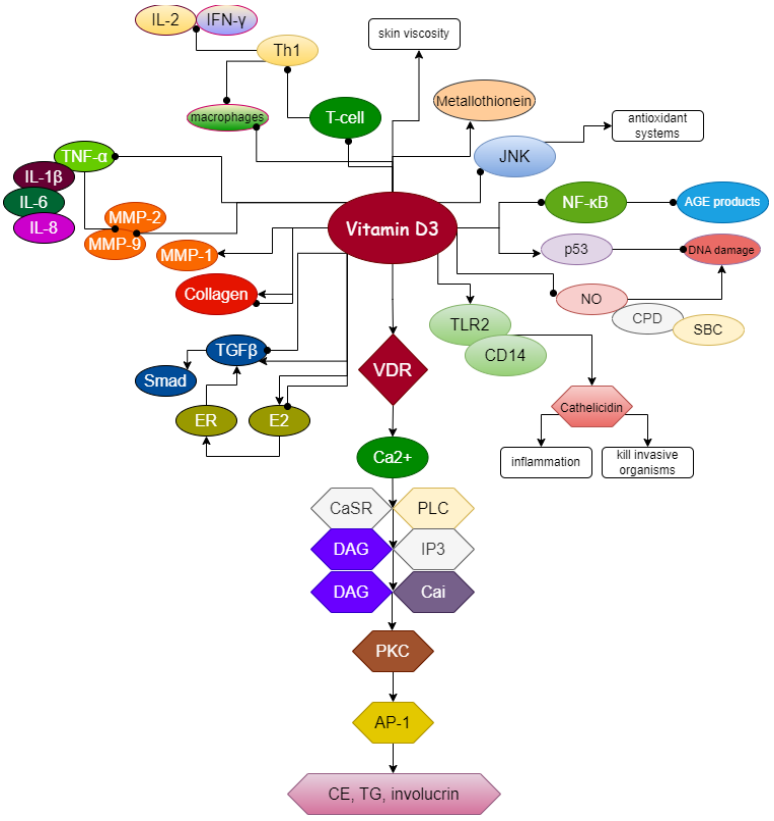

Figure 2. An illustrative overview of the impact of vitamin D3 in association with the signs and causes of skin aging, such as inflammatory mediators (macrophage, T cell, TLR2, CD14, NF-κB), AGE products, DNA damage, skin viscosity, the antioxidant system, metallothionein, collagen, MMPs, TGF-β/Smad pathway, estrogen receptor (ER), skin barrier (cornified envelope, TG, involucrin) and photoprotective effect determinants (NO, SBC, CPD, and p53). (→: induce, ●: inhibit). Abbreviations: IL (interleukin), IFN-γ (interferon-γ), TNF-α (tumor necrosis factor), JNK (c-Jun N-terminal kinase), MMP (matrix metalloproteinase), NF-κB (nuclear factor-kappa B), AGE products (advance glycation end products), NO (nitric oxide), CPD (cyclobutane pyrimidine dimers), SBC (sunburn cells), TLR2 (toll-like receptor 2), TGF-β (transforming growth factor- β), ER (estrogen receptor), VDR (vitamin D receptor), CaSR (calcium-sensing receptor), PLC (phospholipase C), DAG (diacylglycerol), IP3 (inositol trisphosphate), Cai (calcium intracellular), PKC (protein kinases), AP-1 (activator protein-1), CE (cornified envelope), TG (transglutaminase)

The Impact of Vitamin D3 on Skin Elasticity

Treating topical 1.0 μg/day 1,25$(OH)_2$D3 to hairless mice has shown to affect skin elasticity and increase skin viscosity. After six weeks of treatment, coarse and deep wrinkles appeared on the backs of the mice. Those effects showed that 1,25$(OH)_2$D3 stimulates morphological changes and skin sagging. However, 1,25$(OH)_2$D3 was also observed to improve skin mechanical properties. It was found that immediate distention (Ue/elasticity measurement) remains constant after topical application, but delayed distention (Uv/skin viscosity factor) significantly increases. Uv represents an epidermal component (Fujimura *et al*., 2000). These results suggest that 1,25$(OH)_2$D3 cause physical changes in the epidermis, particularly an increase in its thickness and viscosity elements. However, the result is unrelated to the dermis-associated elasticity element.

A similar result has been reported by Cho *et al*. (2019) about wound healing. They compared vitamin D-deficient group with non-deficient group to find the correlation between vitamin D status and biomechanical scar properties. The vitamin D-deficient group showed significantly lower Uf (final distensibility), Ua/Uf (gross elasticity), and Ur/Uf

(biological elasticity) but higher Uv/Ue than the non-deficient group. Those results pointed out that the vitamin D-deficient group had firmer fibrous scars. The lower Uf indicates skin stiffness, while Ua/Uf and Ur/Uf indicate the elastin fibers' ability to restore their shape after distension. Furthermore, the higher Uv/Ue demonstrates that the interstitial fluid moves well through the scar network (Cho *et al*., 2019). The result underlines the role of vitamin D in improving skin's mechanical properties through the epidermal approach and resulting in the low distensibility and elasticity of the skin.

Besides observing the skin elasticity and wound healing progress, the study also measured the serum level of vitamin D. It showed that vitamin D deficiency disturbs epidermal keratinocytes and dermal fibroblast communication pathway and collagen and elastin production (Cho *et al*., 2019).

Vitamin D Increases Collagen Production

An experiment using fibroblast cells treated with 10^{-7} M $1,25(OH)_2D3$ was conducted to observe collagen production. The supernatant of cultured cells was examined on the 3rd-5th days of the experiment. It showed a significant reduction in proliferation cells based on ELISA measurement. There was also a substantial increase in type I and type III collagen accumulation after $1,25(OH)_2D_3$ on day 5 (Dobak *et al*., 1994). However, no further study was conducted to determine the mechanism of $1,25(OH)_2D3$ increasing collagen. Vitamin D can induce collagen expression against glucocorticoid-mediated decreasing collagen synthesis but inhibits elastin expression in specific cell types. Vitamin D has been reported to increase the transcription of type I collagen in UV-radiated and non-irradiated fibroblasts but not in UVB-radiated fibroblasts. It also inhibits cellular elastase activity directly in dermal fibroblast. Vitamin D may show beneficial effects in preventing intrinsic aging and photo-aging (Philips *et al*., 2019). It might be correlated with the photoprotection mechanism of vitamin D.

The effect of Vitamin D3 on the expression of TGF-β and Estrogen

As described above, TGF-β plays a crucial role in fibroblast collagen production, thereby applying the mechanism in wound healing after activating keratinocytes. After wound closure, the negative feedback of TGF-β inactivates keratinocytes to debilitate collagen production. Vitamin D administration upregulates TGF-β and cathelicidin; on the contrary, it downregulates pro-fibrotic factors and collagen. The dermal wound healing effect was intensified when a low dose of TGF-β1 (2 ng/mL) was added to vitamin D (100 nM). The combination results in a synergistic effect of collagen production on the dermal fibroblast facilitates wound healing process (Cho *et al*., 2019; Ding *et al*., 2016). The other experiments show that vitamin D reduces fibrosis by downregulating TGF-β. It occurs in cancer therapy when TGF-β acts pro-fibrotic, while vitamin D reveals its anti-fibrotic effect (Fischer & Agrawal, 2014). Other data confirm that topical calcipotriol, a vitamin D analog, reduces skin fibrosis due to its immunoregulatory effects in fibroblasts. It decreases TGF-β-induced collagen and fibroblast proliferation and increases metalloproteases (Usategui *et al*., 2014). Other data indicate a reduction in the phosphorylation of Smad2-mediated TGFβ1 by vitamin D. TGF-β performs as a cell growth inhibitor whose signaling is transduced by Smads (Corduk *et al*., 2012; Meredith *et al*., 2015). Vitamin D also affects TGF-β through increasing estrogen receptors (ER). Meanwhile, ER signaling affects decreasing Smad molecules and inhibits the TGF-β expression (Corduk *et al*., 2012). Those two contrasting bodies of vitamin D/TGF-β and collagen interaction signify biphasic effects (Figure 2). It may be caused by the different levels of TGF-β involved in the pathway and the types of tissues/ organs. Vitamin D and TGF-β/Smad signaling pathways synergize upregulatory effects on collagen in specific contexts (Ding *et al*., 2016; Shin *et al*., 2019).

Estrogen and other sex steroids play a role in the structural and biological effects of epidermal keratinocytes and dermal fibroblasts. The estrogen receptor (ER) is vigorously expressed in the epidermis and dermis layers (Farage *et al*., 2012). The ER is a member of the superfamily of nuclear receptors for steroids, retinoic acid, thyroid hormones, and vitamin D3 (Weikum *et al*., 2018). The three types of estrogen are estrone (E1), estradiol (E2), and estriol (E3) (Hong *et al*., 2017). Clinically, a significant decrease in estrogen in menopause affects symptoms of aging, particularly the loss of elasticity. It is lamentably that the levels of estrogen decline with age. A threshold estrogen level is believed to be required to maintain skin integrity. Estradiol levels, one of the estrogen hormones, decline from over 300 pmol/L to approximately 20 pmol/L in menopause. Estrogen can be used as a supplement to prevent or reverse aging in early and late menopausal women by increasing skin collagen. Human Replacement

Therapy (HRT) is one example of antiaging supplement that improves signs of aging in populations with estrogen deficiency, although controversial. A study has demonstrated that while estrogen increases collagen in postmenopausal women, HRT administration for the first five years of menopause shows no significant improvement in skin elasticity (Farage *et al*., 2012). Several distinctions exist between male and female skin aging factors, including the hormone. Women have elevated estrogen levels, whereas men have elevated testosterone levels. Consequently, estrogen loss affects women's aging, while testosterone loss affects the aging of men (Farage *et al*., 2012; Regan & Partridge, 2013). Despite women's high estrogen levels, their skin is less elastic than men's. Testosterone continues to contribute to men's skin suppleness. There is no substantial variation between male and female skin elasticity (Rahrovan *et al*., 2018).

At the transcriptional level, vitamin D3 and estrogen action are associated (Hasan *et al*., 2019; Swami *et al*., 2013). Vitamin D3 increases E2 secretion by expressing mRNA for enzymes involved in E2 production in a granulosa cell-culture medium. Although there are no substantial variations in protein expression, vitamin D3 increases estrogen release in the ovary of pigs. Vitamin D3 has considerably increased E2 synthesis in pig granulosa cells but not in follicular development cells (Hong *et al*., 2017). In treating polycystic ovarian syndrome/ PCOS, vitamin D3 lowers 17-beta-estradiol output via aromatase enzyme activity (Bakhshalizadeh *et al*., 2017). Vitamin D3 inhibits steroidogenic enzyme expression and stimulates estradiol and progesterone production in PCOS granulosa cells via AMP-activated protein kinase (AMPK) signaling (Bakhshalizadeh *et al*., 2018). In these trials, vitamin D3 appeared to boost estrogen in normal skin, but its effects on pathophysiological ovarian cells differed. In another study, high-dose vitamin D (1200-3200 IU daily) reduces estrogen and progesterone (Crew, 2013). Calcitriol simultaneously inhibits MMPs expression and boosts the activity of tissue inhibitor of metalloproteinase 1 (TIMP-1) to reduce angiogenesis in cancer cell (Krishnan *et al*., 2012). The abovementioned vitamin D and estrogen theories present two contradictory vitamin D and estrogen interaction pathways. There were several postulations concerning vitamin D's mechanism related to estrogen, including aromatase gene expression. The research demonstrated that vitamin D generates estrogen biosynthesis by maintaining extracellular calcium homeostasis (Gangula *et al*., 2013; Kinuta *et al*., 2000). That mechanism is not entirely specific regarding the effects on collagen. Hence, the interactions between vitamin D and estrogen-related receptors to dermal fibroblast are still debatable.

Vitamin D and Advanced Glycation End (AGE) products and their interactions

Vitamin D affects AGE products via inhibition of NF-κB activity. AGE receptors are divided into two types. The receptor for advanced glycation end products (RAGE) on the cell membranes regulates inflammation, oxidative stress, and cell apoptosis. The second receptor, the soluble receptor for AGEs (sRAGE), binds to circulating AGEs. It is responsible for preventing the harmful effects of the interaction between AGEs and RAGE. Vitamin D can be beneficial in reducing AGE levels and decreasing sRAGE levels under vitamin D deficient and pathological situations. On the other hand, vitamin D treatment could be harmful when applied under normal conditions. Future studies must assess the correlation between vitamin D levels and AGEs receptors (Kheirouri & Alizadeh, 2020).

The Role of Vitamin D as an Antioxidant

Oxidative stress is the imbalance between reactive oxygen species (ROS) and antioxidants as the cellular defense mechanism (Reilly & Lozano, 2021). It plays a crucial role in skin aging and elevates intracellular levels of ROS, which is a potent inducer of MMPs expression regulation (Gragnani *et al*., 2014). ROS are naturally produced during normal cellular metabolism at low levels. The system of intrinsic antioxidant systems equalizes the redox balance of the cells. Superoxide dismutase is converted to hydrogen peroxide. Catalase acts to convert hydrogen peroxide to oxygen and water, but less enzyme activity might convert superoxide and hydrogen peroxide into highly toxic products. The skin itself contains antioxidants, but ROS inactivates them. 1,25D inhibits c-Jun N-terminal kinase (JNK), which inhibits apoptosis, possibly improves antioxidant systems, and protects UV-induced keratinocytes. As stated, the antioxidant metallothionein is a cysteine-rich protein coupled with an oxygen radical scavenger. Cadmium's enhancement of metallothionein reduces superoxide, hydroxyl radicals, sunburn cells, and cell damage. 1,25(OH)2D3 induces metallothionein

transcription while reducing UV-induced sunburn cells, suggesting that metallothionein also has a photoprotective function (Dixon *et al*., 2013). We would suggest that an antioxidant would be proper to address the issue related to stress oxidative as the initial inducer of the skin aging pathway. It is because the activity of vitamin D as an antioxidant has been proven. Vitamin D also exerts antioxidant actions via Nrf2 or antioxidant enzyme upregulation. It also promotes the renewal of the mitochondria (Sosa-Díaz *et al*., 2022).

In several antioxidant activity experiments, vitamin D increased the level of TAC (total antioxidant capacity) and CAT (catalase) but not SOD (superoxide dismutase), thus improving healthy aging of the subjects: elderly, male adult and pregnant women (Azimzadeha *et al*., 2020; Tagliaferri *et al*., 2019). Besides being induced by UV radiation, ROS can be produced via overburdened macrophages. Vitamin D and macrophages' interaction could hinder oxidative stress (Bikle, 2012).

FUTURE DIRECTIONS OF VITAMIN D3 AND SKIN AGING: SENOTHERAPEUTIC STRATEGY

Most molecular mechanisms of skin aging point out the failure of several basic physiologic maintenances, which leads to cell senescence. It is the terms of controlling the aging process at the cellular level, characterized by the loss of proliferative capacity, dysfunctional mitochondria, and substantially altered expression and secretion patterns of bioactive molecules (Lee & Harries, 2021; Low *et al*., 2021). Cell senescence is linked to telomere attrition, as telomeres in human somatic cells become shorter with each cell division. The telomere loss causes double-strand breaks of the chromosome, which stimulates DNA damage response (DDR) (Csekes & Račková, 2021; Lee & Harries, 2021; Low *et al*., 2021).

Furthermore, senescent cells release many biologically active molecules called Senescence-Associated Secretory Phenotype (SASP), which include pro-inflammatory cytokines and chemokines, matrix metalloproteinases, oxidized lipids, reactive oxygen species, and others (Low *et al*., 2021). Cell senescence is a primary process in skin aging. Senescent cells accumulate in the epidermis and dermis layers and can be exacerbated by DNA-damaging agents and mitochondrial dysfunction (Raffaele & Vinciguerra, 2022). Today, senotherapy is becoming an emerging strategy for healthy skin aging, specifically targeting the senescence cell. Senolytics and senomorphics are two types of senotherapy. Senolytics tend to eliminate the senescence cells, while senomorphics only modulate or attenuate the cells. Only a few studies of senotherapeutic treatment have examined skin aging prevention or treatment (Kim & Kim, 2019; Sosa-Díaz *et al*., 2022). Senomorphics attenuate SASP to eradicate its deleterious effects, not to kill senescent cells. The metabolic pathway of SASP includes NK-κB, MAPK, IL-1, glucocorticoid receptors, and MMPs (Low *et al*., 2021; Sosa-Díaz *et al*., 2022). The building blocks of senescent cells involved in the abovementioned process are related to vitamin D molecular mechanisms (Sosa-Díaz *et al*., 2022). Vitamin D has anti-inflammatory and antioxidative properties, which are related to molecules of SASP. It is suggested that vitamin D can be served as a senomorphic. Another exciting facet is the research of vitamin D polymorphisms, analogs, and metabolites. Each has potential variability effects towards skin aging to be discovered (Franco *et al*., 2022; Sosa-Díaz *et al*., 2022). The vitamin D3 metabolite, novel (CYP11A1-intitated) D3 hydroxyderivatives, attenuates the process of skin aging, either chronological aging or photo-aging. Photoisomers of pre-vitamin D3, vitamin D3 and lumisterol (L3), are promising photoprotective and reparative agents. They can be administered orally, topical, or parenterally (Bocheva *et al*., 2021). Numerous studies are currently trying to develop and uncover the comprehensive mechanisms of vitamin D and its effective route. Vitamin D and the skin will continue to be a topic of attention and significance for the foreseeable future.

CONCLUSION

Vitamin D may promote collagen synthesis in dermal fibroblasts, directly or indirectly inhibit MMPs via macrophages, and activate TIMPs. Further study needs to be conducted on the effect of vitamin D on estrogen on collagen production. Meanwhile, investigations on the relationship between vitamin D, collagen, MMPs, TGF-β, and Smad2/3 have been conducted, but it is important to bear in mind the possible controversy in the results. Thus additional research is required. Vitamin D3 and its active metabolites exert antiaging effects via inflammatory responses, epidermal barrier preservation, antioxidative activities, DNA damage prevention, DNA repair, and photoprotective actions on the skin. Future research may investigate vitamin D3 as a supplement for antiaging skin.

ACKNOWLEDGMENTS

The authors are grateful to the Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, for providing all the facilities for searching all the literature. This review article was a part of research funded by the Thesis Recognition Program 2019, No (2129/UN1/DITLIT/DIT-LIT/LT/2019).

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