AN INTENSIVE REVIEW OF ROLES OF MATRIX METALLOPROTEINASES IN PHOTOAGING

BY

MISS PAVIDA PITTAYAPRUEK

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE (DERMATOLOGY)
CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE
THAMMASAT UNIVERSITY
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ENTITLED

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Thesis Title	AN INTENSIVE REVIEW OF ROLES OF MATRIX METALLOPROTEINASES IN PHOTOAGING
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ABSTRACT

Matrix metalloproteinases (MMPs) are zinc-containing endopeptidases with an extensive range of substrate specificities. Collectively, these enzymes are able to degrade various components of extracellular matrix (ECM) proteins. Based on their structure and substrate specificity, they can be categorized into five main subgroups, namely (i) collagenases (MMP-1, MMP-8 and MMP-13); (ii) gelatinases (MMP-2 and MMP-9); (iii) stromelysins (MMP-3, MMP-10 and MMP-11); (iv) matrilysins (MMP-7 and MMP-26); and (v) membrane-type (MT) MMPs (MMP-14, MMP-15, and MMP-16). The alterations made to the ECM by MMPs might contribute in skin wrinkling, a characteristic of premature skin aging.

Since wrinkle formation is closely associated with MMPs, their regulation has been targeted as a strategy to prevent photoaging. This is the reason to find agents of being able to protect solar radiation-induced over-production of MMPs.

The objective of this thesis are to review various aspects of photoaging including background, clinical features, pathogenesis (MMPs involvement) and review of treatment (MMPs involvement).

To review literature on roles of MMPs in photoaging, the keywords following terms “matrix metalloproteinase” OR “MMPs” OR “photoaging” OR “skin aging” OR “aging skin” OR “photodamaged” OR “MMP inhibitors” were searched in Pubmed and MEDLINE.

Keywords: Matrix metalloproteinases, MMPs, Photoaging

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Miss Pavida Pittayapruek

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
$^1O_2$	Singlet oxygen
Ach	Acetylcholine
AP-1	Activator protein-1
AST	Astragaloside IV
BTX-A	Botulinum toxin type A
CAE	Coffea arabica leaves extract
CBD	Collagen binding domain
CG	Cultivated gingseng
CSE	Coriander leaf extract
DFUs	Diabetic foot ulcers
ECM	Extracellular matrix
EGCG	Epigallocatechin-3-gallate
EGF	Epidermal growth factor
ERKs	Extracellular signal-regulated kinases
FDA	Food and Drug Administration
GAC	*Galla chinensis*
GPI	Glycosylphosphatidylinositol
GTP	Green tea polyphenols
H_2O_2	Hydrogen peroxide
IFN-γ	Interferon gamma
IL	Interleukin
IPE	*Ixora parviflora* extract
IR	Infrared
JNK	c-Jun NH_2-terminal kinase
KPE	*Kaempferia parviflora* extract
MAE	*Michelia alba* extract
MAPK	Mitogen-activated protein kinase

Symbols/Abbreviations	Terms
MMPs	Matrix metalloproteinases
MT-MMPs	Membrane-type matrix metalloproteinases
Nd:YAG	Neodymium-doped yttrium aluminum garnet
NF- κB	Nuclear factor-kappa B
O_2^-	Superoxide anion
$OH^·$	Hydroxyl radicals
Pump-1	Putative uterine metalloprotease-1
RAR	Retinoic acid receptor
ROS	Reactive oxygen species
RXR	Retinoic X receptor
SCC	Squamous cell carcinoma
TGF-β	Transforming growth factor beta
TIMPs	Tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor alpha
tRA	All-trans retinoic acid
TRPV1	Transient receptor potential vanilloid-1
UV	Ultraviolet
VDR	Vitamin D receptor
β-carotene	Beta-carotene

CHAPTER 1
INTRODUCTION

Skin is the primary means through which an organism interacts with its environment. Accordingly, it is regularly exposed to a direct oxidative environment, including ultraviolet (UV) irradiation. Acute exposure to UV irradiation causes sunburn, connective tissue deterioration, DNA injury, and immune suppression. Chronic or long-term exposure to UV irradiation disrupts the normal skin structure leading to a host of skin issues including premature skin aging (photoaging) and skin cancer (photocarcinogenesis).

UV radiation increases the expression of MMPs in human skin. MMPs are responsible for degrading the ECM proteins including collagen, fibronectin, elastin, and proteoglycans, contributing to photoaging.

In recent years, however, it has been accepted that infrared (IR) and visible light irradiation, which longer wavelengths than ultraviolet spectrum, can cause damaged skin and also leading to photoaging skin. But the biological effects of IR and visible light irradiation are not well-known when compared with the pathological effects of UV.

In the previous studies, there are some journals that wrote about photoaging in many aspects such as a review of UV-induced premature skin aging, IR effects on skin aging, the treatment, and etc. However, there is none that covers all aspects of photoaging. Therefore, it might be useful if there is a study that explains the pathophysiology of photoaging, leading to establishing prevention and treatment for the optimum result.

Nowadays there is more concern about skin aging as a health issue in the general population due to life expectancy is increased and people want to maintain the youthful appearance. It is best described by the sale amounts of cosmetic products that is claimed to prevent, decelerate, arrest or even reverse the aged skin signs. These cosmetic products compose of various molecules with specific biological properties and

are proposed to involved in the skin aging pathogenesis by regulate molecular mechanisms.

According to this study, the knowledge of all aspects of MMP involvement in photoaging and their regulatory pathways, leading to better understand in mechanisms and could be considered the promising targets for unwelcome of aging skin.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Matrix metalloproteinases

MMPs are zinc-containing endopeptidases with a broad range of substrate specificities. They mediate the degradation of the different components of the extracellular matrix (ECM) (1, 2). They are secreted by keratinocytes and dermal fibroblasts in response to multiple stimuli such as oxidative stress, UV radiation, and cytokines (3, 4). To date, at least 28 different types of MMPs have been identified that play an important roles in several pathophysiological processes including photoaging, wound healing, skeletal growth and remodeling, arthritis, inflammation, angiogenesis, and cancer (1, 5, 6).

2.1.1 Structure of MMPs

The domain organization structure of MMPs are quite similar, which is usually consists of: (i) the pro-peptide domain, (ii) the catalytic domain, (iii) the linker peptide domain (or the hinge region) and (iv) the hemopexin-like domain (6, 7).

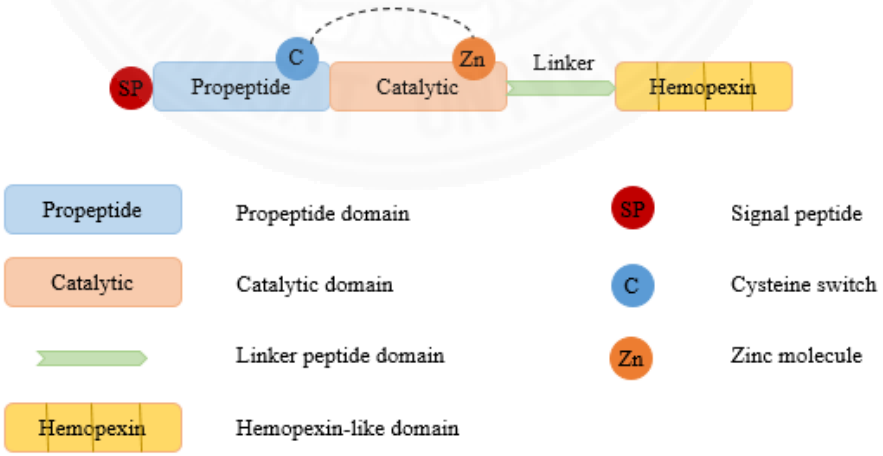

Figure 2.1 The typical MMP structure

2.1.1.1 The pro-peptide domain

The pro-peptide domain, lengths from the N-terminal pro-peptide domain to the catalytic domain, composed of about 80 amino acids. This part is account for the inactivity of enzyme. The cysteine sulphydryl group or Cys-switch (consists of Pro-Arg-Cys-Gly-X-Pro-Asp) located within the pro-peptide domain connects with the Zn^{2+} ion located within the catalytic domain, resulting in inhibition of active site. There are some MMPs possess the specific furin-like serine proteinases sequence in the pro-peptide domain, that is required for degradation and, appropriately, for activation of MMPs (8).

2.1.1.2 The catalytic domain

The catalytic domain contains about 170 amino acids. The morphology of this part is spherical, which consists of (i) three His residues combined with the catalytic Zn^{2+} ion (ii) three His and one Asp residues combined with the structural Zn^{2+}, and (iii) at least two atoms of calcium octahedrally integrated, which responsible for structure stabilization (9).

2.1.1.3 The linker peptide domain

The linker peptide domain or hinge region, consists of variable lengths amino acids, connects the catalytic domain and the hemopexin-like domain. The connecting linker peptide domain is not only acts as the physical spacer, but plays a pivotal functional roles in stabilize enzymes and degradation of substrate, especially for the collagenase subgroups (10, 11).

2.1.1.4 The hemopexin-like domain

The hemopexin-like domain consists of about 200 amino acids. The morphology of this part is a 4-bladed β-propeller, which the first and the last blades are connected with the single disulfide bond (7, 12). This domain mediates interactions with proteolytic substrates, cell-surface molecules and tissue inhibitor of MMPs.

2.1.2 Classification of MMPs

MMPs can be categorized into five main subgroups based on their substrate specificity and structural organization namely (i) collagenases (MMP-1, MMP-8 and MMP-13); (ii) gelatinases (MMP-2 and MMP-9); (iii) stromelysins (MMP-3, MMP-10 and MMP-11); (iv) matrilysins (MMP-7 and MMP-26); and (v) membrane-type (MT) MMPs (MMP-14, MMP-15, and MMP-16). In addition to the five aforementioned subgroups of MMPs, there are few MMPs that are not grouped into any of these categories, such as MMP-12 (or metalloelastase), MMP-19 (or RASI-1), MMP-21, and MMP-28 (or epilysin) (6).

Table 2.1 Classification of human metalloproteinases (MMPs) based on their substrate specificity and structural organization

MMP subgroup	MMP number	Alternate name
Collagenases	MMP-1	- Interstitial collagenase - Collagenase-1 - Fibroblast collagenase
	MMP-8	- Collagenase-2 - Neutrophil collagenase
	MMP-13	- Collagenase-3
Gelatinases	MMP-2	- Gelatinase-A - 72 kDa type IV collagenase
	MMP-9	- Gelatinase-B - 92-kDa type IV collagenase
Stromelysins	MMP-3	- Stromelysin-1 - Proteoglycanase - Transin-1
	MMP-10	- Stromelysin-2 - Transin-2
	MMP-11	- Stromelysin-3

Abbreviations: NA, no reported.

Table 2.1 Classification of human metalloproteinases (MMPs) based on their substrate specificity and structural organization (Cont.)

MMP subgroup	MMP number	Alternate name
Matrilysins	MMP-7	- Matrilysin-1 - Pump-1
	MMP-26	- Matrilysin-2 - Endometase
Membrane-type	MMP-14	- MT1-MMP
	MMP-15	- MT2-MMP
	MMP-16	- MT3-MMP
	MMP-17	- MT4-MMP
	MMP-24	- MT5-MMP
	MMP-25	- MT6-MMP
Other types	MMP-12	- Metalloelastase
	MMP-19	- RASI-1
	MMP-20	- Enamelysin
	MMP-21	NA
	MMP-22	NA
	MMP-23	NA
	MMP-28	- Epilysin

Abbreviations: NA, no reported.

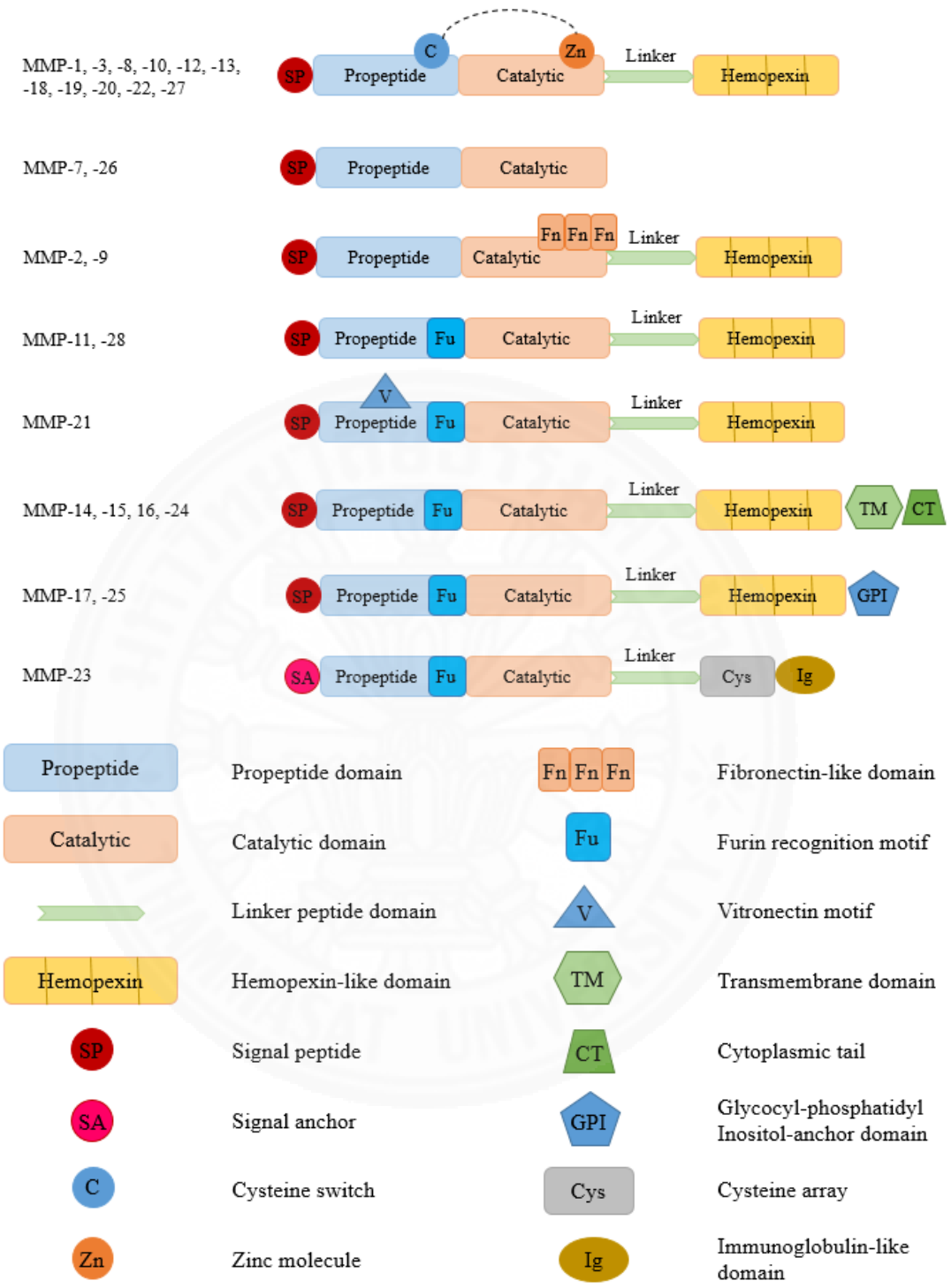

Figure 2.2 The structure of each MMP based on their domain composition. Each MMP is demonstrated in the inactive form of enzyme with the catalytic Zn^{2+} domain connecting with the cystein pro-peptide domain.

2.1.2.1 Collagenases

The term ‘Collagenases’ refers to the ability of this subgroup to enzymatically degrade native collagen with the absent of triple helixes separation. The structure of the active enzyme is compose of the catalytic domain, the linker peptide domain, and the hemopexin-like domain (13). In this subgroups, the hemopexin-like domain usually plays a crucial role in recognize the substrate and correct the location of the MMP enzyme on the substrate (6). Collagenases can cleave fibrillar collagens into the ¾ and ¼ fragments and also destroy other ECM molecules such as aggrecan, versican, gelatin, and casein (14).

Interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13) belong to this subgroup. They are similarities in the structural arrangement and the enzymatic function. But there is slightly differences in the specific substrate recognition (15, 16).

(1) MMP-1 or interstitial collagenase

MMP-1 is also called interstitial collagenase, fibroblast collagenase, and collagenase-1. Although MMP-1 is expressed by a wide range of normal cells in vitro, such as epithelial cells, fibroblasts, endothelial cells, and macrophages, this enzyme is unnoticeable in the normal tissue condition. In vivo, interstitial collagenase is primarily secreted during tissue remodeling, both in physiological and pathological, implying the extensive role in biology (6).

Interstitial collagenase is formed as the single polypeptide and emit as the proenzyme. It consists of a major 57 kDa unglycosylated component and a minor 61 kDa glycosylated component.

The levels of interstitial collagenase are usually undetectable in normal adult cells. In contrast, when the interruption occurs such as wound repair, remodeling, or healing processes as appear in many pathological situations, the expression of interstitial collagenase is increased (17).

Growth factors, cytokines, and hormones can control the interstitial collagenase expression. Activators and inhibitors, for example and tissue

inhibitors of metalloproteinases (TIMPs) and α-macroglobulins, can precisely regulate the proteolytic enzyme activity (6).

Besides the degradation of type I collagen, interstitial collagenase can cleave other ECM proteins such as versican, nidogen, tenascin-C, aggrecan, and casein (18). Thus, interstitial collagenase plays an important role in the ECM remodeling.

(2) MMP-8 or neutrophil collagenase

MMP-8, also called neutrophil collagenase or collagenase-2, was believed to be secreted only in neutrophil during late myeloid evolution. However, recent studies report that neutrophil collagenase can be found in various cell types, such as peripheral neutrophils, mature neutrophils, macrophages, T cells, plasma cells, endothelial cells, keratinocytes, and fibroblasts (6).

Neutrophil collagenase has many substrate specificities. This enzyme is more effective in degrading collagen type I than collagen type III and type II. Neutrophil collagenase also degrades various components of non-collagenous substrates, such as chemokines and serine protease inhibitors. This suggests neutrophil collagenase is involved in biological activities of substrates, due to degradation can either resulting in their activation or in upregulated their biological activities (19, 20). Neutrophil collagenase, the powerful collagenolytic enzyme, also influence the pathogenesis of many inflammatory situations.

(3) MMP-13 or collagenase-3

MMP-13 or collagenase-3, is secreted during development of human fetus by hypertrophic chondrocytes and osteoblasts (21). Collagenase-3 is not secreted by adult cells in normal conditions, whereas its expression is elevated in pathologic conditions such as tissue repair, healing, or remodeling. This enzyme is considered to the promising pharmaceutical target in many diseases due to its capability to cleave collagen type II and other ECM proteins, combined with its limited distribution in normal adult tissue (6).

Collagenase-3 shows higher cleavage specificity for collagen type II, a major collagen present in the cartilage, compared to collagen type I and III. This enzyme is five time less potent than Collagenase-1 in cleaving collagen type I and III; however, it is 5-10 times more potent in cleaving collagen type II. Collagenase-3 is also degrade other ECM molecules, such as collagens type IV, IX, X, and XIV, fibronectin, aggrecan, and gelatin (22-24).

2.1.2.2 Gelatinases

The name of this MMPs subgroup refers to their ability to degraded collagen. There are two enzymes belong to this class, namely (i) MMP-2 or gelatinase-A, and (ii) MMP-9 or gelatinase-B (6).

The structural of this subgroup shares similarities with other MMPs subgroups, which is compose of (i) the N terminal pro-peptide domain, (ii) the catalytic domain, and (iii) the C-terminal hemopexin-like domain. In contrast to other MMPs, there is collagen binding domain (CBD) which is a specific additional exosite located on the catalytic domain (15).

Gelatinases subgroup promptly degrade gelatin by the assistance of repeated domain of three fibronectin type II which binds to gelatin and collagen. This subgroup also cleave various components of ECM proteins such as collagen type IV, V, and XI, elastin, aggrecan core protein, and laminin (7).

(1) MMP-2 or gelatinase-A

MMP-2 (known as gelatinase-A or 72-kDa type IV collagenase) is generally secreted by almost every human cells and primarily by epithelial cells, fibroblasts, and endothelial cells. In physiologic condition, this enzyme is observable in serum concentration, considering its activity is involved in homeostatic functions (25).

As same as other MMPs, the expression of gelatinase-A is also upregulated by the stimuli (26). The activity of gelatinase-A is strictly controlled by TIMPs, especially TIMP-2, TIMP-3 and TIMP-4 (27). Gelatinase-A can degrade a number of substrates, such as growth factor, cytokines, receptors, and binding factors.

Thus, the enzyme activity has involved in the various physio-pathological conditions and essentially neoplastic diseases (6).

(2) MMP-9 or gelatinase-B

MMP-9, known as gelatinase-B or 92 k-Da type IV collagenase, is primarily secreted by immune cells (25). Growth factors, cytokines, and adhesion molecule, especially activated integrins can regulated gelatinase-B expression (28, 29).

Although Gelatinase-A and gelatinase-B share similarities in the substrate specificity, there is little substrate differences. The primarily substrate is basement membrane molecules, but this enzyme also degrade growth factors, cytokines, and receptors (30). Gelatinase-A, not gelatinase-B, can cleave collagen type I, II and III as same as collagenases subgroup (7).

2.1.2.3 Stromelysins

The stromelysins class includes three members, namely (i) stromelysin-1 or MMP-3, (ii) stromelysin-2 or MMP-10, and (iii) stromelysin-3 or MMP-11.

The general structure arrangement consists of the N-terminal pro-peptide domain, the catalytic-domain, and the C-terminal hemopexin-like domain. But MMP-11 shows significant difference in the structure when compared with other MMPs. It expresses an extra pro-protein convertase specific sequence that responsible for a specific activation mechanisms (31).

Numerous studies have been focused about MMP-3, although many aspects are still not fully understood. In contrast to MMP-3, there is little knowledge about the biology of MMP-10 and MMP-11 even though they have been found for many years ago (6).

Stromelysins subgroup has the capability to cleave various molecules of ECM proteins such as collagen type IV and IX, fibronectin, proteoglycans, elastin, and laminin. Growth factors, cytokines, and regulatory soluble molecules also degraded by this subgroup (31).

(1) MMP-3 or stromelysin-1

MMP-3, also known as stromelysin-1, is secreted as the pro-enzyme and then activated by matriptase and serine proteases trypsin-2. They remove the N-terminal pro-peptide domain, resulting in a 43-kDa activated enzyme (32, 33). The structure of activated enzyme consists of (i) the catalytic domain, and (ii) the hemopexin-like domain which is responsible for recognition of macromolecular substrate. The catalytic domain of stromelysin-1 is very similar to the same domain of interstitial collagenase and fibroblast collagenase (34, 35).

Numerous cells can secreted stromelysin-1 such as fibroblasts, keratinocytes, and chondrocytes. Normal fibroblasts and epithelial cells can secreted stromelysin-1 in combination with stromelysin-2 (31, 36).

The primary function of MMP-3 is the activation of pro-MMPs such as collagenases, gelatinase-B, and matrilysins during ECM turnover. In particular, the production of fully active MMP-1 MMP-3 is essential to partially activate pro-MMP-1 (6, 37, 38). In contrast to collagenases subgroup, this enzyme cannot degrade collagen type I. However, it can cleave a broad range of ECM molecules such as collagen type IV, V, IX, and X, fibronectin, fibrillin-1, gelatin, and laminin (39).

(2) MMP-10 or stromelysin-2

The stromelysin-2 structure details are not available. But the structure of stromelysin-2 shares similarities in the structure of stromelysin-1, and generally compose of the pro-peptide domain, the catalytic domain, and the hemopexin-like domain. This enzyme is releases as a 53-kDa pro-enzyme (40).

Stromelysin-2 can cleave many ECM proteins and also involved in the activation or pro-MMPs. The proteolytic activity of this enzyme to degrade collagen type IV and V is less strong when compared with the stromelysin-1 activity (7, 41).

(3) MMP-11 or stromelysin-3

MMP-11 is also known as stromelysin-3. Although the structure of stromelysin-3 is less likely related to other stromelysins, this enzyme is also take in to this stromelysin subgroup (6). Stromelysin-3 is extensively expressed in both physiological and pathological conditions, including embryonic implantation, epithelial proliferation, wound repair, inflammatory process, and benign tumor development (42-44). In both physiological and pathological conditions, fibroblasts is mostly released stromelysin-3.

The proteolytic activity of stromelysin-3 is very weak, but it has been reported that stromelysin-3 can degrade protease inhibitors, including α_2 macroglobulin and α_1 proteinase inhibitor (6).

2.1.2.4 Matrilysins

Matrilysin subgroup has two members namely (i) MMP-7 or matrilysin-1 and (ii) MMP-26 or matrilysin-2. Although the structure of these proteolytic enzymes share similarities in specific sequence with stromelysins and collagenases subgroups, matrilysins subgroup is absence of the hemopexin-like domain (45).

(1) MMP-7 or matrilysin-1

MMP-7, also known as matrilysin-1, was initially defined as putative uterine metalloprotease-1 or Pump-1 (46). Matrilysin-1 is extensively secreted in human cells and primarily in the epithelial cells.

Active matrilysin-1 can degrade various ECM proteins and basement membrane molecules, for example collagen type I, III, IV, and V, elastin, fibronectin, entactin, laminin, and gelatin. In addition to ECM degradation, this enzyme also stimulate the collagenases subgroup, resulting in activated collagenases (47).

(2) MMP-26 or matrilysin-2

MMP-26, also known as matrilysin-2 or endometase, is widely secreted in normal cells, for example endometrial cells, and in some tumor cells (7, 45). To date, this enzyme is the smallest MMP. The structure of matrilysin-2 consists

of the signal sequence domain, the pro-peptide domain, and the catalytic domain. Nevertheless, the structure doesn't display the linker peptide domain and the hemopexin-like domain (6).

Matrilysin-2 is exclusive among other MMPs, because it is the only one that lacks the cysteine switch function to preserve the inactivity form of pro-enzyme. There is a histidine sequence instead of cysteine, suggest that it is the specific characteristic of matrilysin-2 (48, 49).

Matrilysin-2 degrade collagen type IV, gelatin, fibrinogen, and fibronectin, but it cannot cleave elastin and laminin (50). Besides the ECM degradation, matrilysin-2 has ability to activate other pro-MMPs, such as pro-MMP-9 (6).

2.1.2.5 Membrane type-MMPs (MT-MMPs)

The structure of membrane type-MMPs subgroup consists of the single peptide domain, the pro-peptide domain, the catalytic domain containing zinc-binding site, the linker peptide domain, and the C-terminal hemopexin-like domain. Differ from other MMPs structure, this MT-MMPs subgroup shows the hydrophobic sequence in the C-terminal hemopexin-like domain and associated in cell membrane (51).

Membrane type-MMPs can be divided into two types based on the additional structure of C-terminal hemopexin-like domain: (i) type I transmembrane proteins type, such as MT1-MMP, MT2-MMP, MT3-MMP and MT5-MMP, which have an extended hydrophobic residues combined with a short cytoplasmic tail, and (ii) glycosylphosphatidylinositol (GPI)-type, such as MT4-MMP and MT6-MMP, which have a short hydrophobic sequence embedding to GPI and not combined with a cytoplasmic tail. In type I transmembrane proteins type, the cytoplasmic tail is involved in various biological actions, including sub-cellular localization, cell signaling, and MT-MMP transportation (52-54).

(1) MMP-14 or MT1-MMP

MT1-MMP (also called MMP-14) is the original type of membrane-type MMP subgroup. MT1-MMP has the proteolytic activity function and also activate other MMPs such as gelatinase-A and collagenase-3 (55).

MT1-MMP can cleave many components of ECM proteins, such as collagen type I, II, and III, fibrin, fibronectin, proteoglycans, vitronectin, fibronectin, and laminins 111 and 332 (56). Moreover, this enzyme can degrade other cell surface molecules, including transglutaminase, syndecan-1, and CD44. The broad range of substrate specificities of MT1-MMP make this proteolytic enzyme becomes an important regulator of the multiple biological functions (57-59).

(2) MMP-15 or MT2-MMP

MT2-MMP is mainly secreted by human placenta during first trimester pregnancy. MT2-MMP can degrades various components of ECM, such as gelatin, aggrecan, tenascin, and vitronectin. Besides the ECM degradation, MT2-MMP can also activate other pro-MMPs such as pro-MMP-2 (7, 60).

(3) MMP-16 or MT3-MMP

The structure of MT3-MMP, a catalytic domain connects with the hydrozamic acid inhibitor, is similar to the structure of MT1-MMP. MT3-MMP can degrades collagen type III, gelatin, aggrecan and casein, suggesting it has a wide range of substrate specificity (6, 7). MMP-16 also activate pro-MMP-2 to the active form.

(4) MMP-24 or MT5-MMP

Differ from other MT-MMPs, MT5-MMP mainly secreted as a soluble proteinase, suggesting the capability to function both as a cell bound and a soluble enzyme in the processes of ECM remodeling (61).

MT5-MMP can cleave various components of ECM proteins, for example vitroonectin, proteoglycans, and fibronectin (62). In addition, this enzyme is able to degrade cell-adhesion protein, such as N-cadherin (63).

MT5-MMP is primarily secreted by neurons which are located in both peripheral and central nervous system. Moreover, inflammatory cells also expressed MT5-MMP (64, 65).

(5) MMP-17 or MT4-MMP

MT4-MMP is generally secreted by monocytes, lymphocytes, and unactivated eosinophils (but not by neutrophils). This enzyme has less or no capability to degrade ECM components. Only fibrinogen, fibrin, and gelatin are able to cleave by soluble MT4-MMP (66, 67).

(6) MMP-25 or MT6-MMP

MT6-MMP is also called as leukolysin because of this enzyme is mainly expressed by leukocytes (68).

MT6-MMP proteolytic activity is similar to stromelysin-1, which has the ability to degrade collagen type IV, fibrin, fibronectin, and gelatin (6). Nonetheless, MT6-MMP differs from MT1-MMP and stromelysin-1 due to it cannot degrade laminin-1 and unable to stimulate pro-MMP-9 to an active form. It has been reported that MT6-MMP might play a crucial role in basement membrane and ECM invasion, and cell transportation. The activity of MT6-MMP is strictly controlled by all TIMP members (69).

To date, the physiological functions and the molecular mechanisms of MT6-MMP are still not fully understood.

2.1.2.6 Other MMPs

In addition to the five aforementioned subgroups of MMPs, there are few MMPs that are not grouped into any of these categories, such as metalloelastase (MMP-12), RASI-1 (MMP-19), MMP-21, and epilysin (MMP-28) (6).

(1) MMP-12 or metalloelastase

MMP-12, also known as metalloelastase or macrophage metalloelastase, is the most effective MMP in elastin degradation (70). Metalloelastase is principally secreted by smooth muscle cells, alveolar macrophages, and respiratory epithelium (71). This enzyme is regulated by serine proteases (e.g., plasmin and

thrombin), other ECM molecules (e.g., hyaluronan fragments), growth factors (e.g., EGF), and cytokines (e.g., IFN-γ and TGF-β) (72).

In addition to elastin degradation, metalloelastase can degrade various ECM proteins, for example collagen type IV fragments, fibronectin, vitronectin, fibrillin-1, and laminin (73, 74). This enzyme also associated with stimulation of other MMPs, for example pro-gelatinase-A and pro-stromelysin-1, which resulting in activation of pro-colaagenase-1 and pro-gelatinase-B (75).

(2) MMP-19 or RASI-1

MMP-19, also called RASI-1, is the soluble 54 kDa protease enzyme and the structure of MMP-19 are not grouped into any of these classifications (6). Although the 3D structure details of MMP-19 are not available, some studies reported that the structure of MMP-19 consists of (i) the catalytic domain with an extra cysteine sequence, which is no obvious biological role, (ii) the acidic linker peptide domain, and (iii) the C-terminal hemopexin-like domain with the N-glycosylation sites (76).

The expression of MMP-19 is essentially found in human adult organs, such as pancreas, intestine, placenta, ovary, and lung, while its expression is less found in macrophages, monocytes, and leukocytes. Only TNF-α has been identified to upregulate the MMP-19 expression (77). MMP-19 can degrade many ECM components, for example collagen type IV, laminin, fibrinogen, and fibrin (6).

(3) MMP-21

To date, there is no reported about the MMP-21 structure details. However, it is known that MMP-21 structure is composed of (i) the pro-peptide domain, (ii) the catalytic domain, and (iii) the hemopexin-like domain (78).

(4) MMP-28 or epilysin

The recently discovered of MMP is MMP-28, also called epilysin. It is widely secreted in normal human organs, for example skin, lung, heart, brain, intestine, and testis (79, 80) as well as in tumor cells (48).

Epilysin, the 59 kDa protein residue, is quite similar to MMP-19 in the amino acid consequence (81). Epilysin consists of the furin-like serine proteinases sequence in the pro-peptide domain (76), whereas it is not the same domain of the transmembrane type of MT-MMPs or other furin-dependent MMP subgroups (80).

Although the interest in MMP-28 biological role is rising, there are few studies have knowledge of the substrates.

2.2 Skin aging

Nowadays, the aging society is increasing and the concern about aging sequence have started to get special attention. The primary target of the aging study is to increase the quality of elderly life and to prevent age-related diseases (82). Especially, aged skin is more interested due to the skin is the most noticeable sign of the aging mechanism and demonstrate the human health which seems to prognosticate the systemic illness and prognosis (82).

The multifactorial process of aging described as the collection of impairment. The collection is likely formed by the gradual turnover of the damaged molecules and by the loss of accurate healing processes (83). Same as other organs, the collection of impaired molecules occur in human skin and resulting in continuous decreasing functions (84).

Aging skin is the complicated mechanisms and is likely associated with genetic predisposition and several environmental components. Thus, aging changes in the skin can be categorized in two groups: (1) intrinsic, or chronologic aging, an inherent degenerative process due to declining physiological functions and capacities; and (2) extrinsic aging, a distinctive deteriorating process caused by environmental factors (85, 86).

2.2.1 Intrinsic skin aging

Chronologic or intrinsic skin aging is an irrevocable and unavoidable process which happens as the time goes by. Intrinsic aged skin is associated with the

genetic susceptibility and alters both covered (such as inner side of upper arm, thigh) and uncovered-areas (such ad face and neck). The clinical features of intrinsic aging alone reveal thin skin, dryness, fine rhytides, soft tissue rearrangement, atrophy of fat, and bone resorption (84, 87).

About skin aging pathogenesis, numerous hypothesizes have been introduced such as the loss of function in cellular DNA repair, the cellular senescence and the reduction of proliferative function, loss of telomeres, oxidative stress, gene mutations, and hormones (87, 88).

2.2.2 Extrinsic skin aging

In contrast to chronological skin aging, extrinsic skin aging is involved in the environmental factors, lifestyle and health. It is related to the personal style, including exposure of sun light, exercise, diet, and smoking. The most significant extrinsic factor in skin aging is accumulative exposure of sun light (84).

The clinical features of extrinsic (or environmentally-induced) skin aging are solar elastosis, coarse rhytides, irregular pigmentation, and rough skin texture (84, 89, 90). These features add on the intrinsic aged skin features at continuous uncovered areas.

Uncover areas such as face and neck undergo the most external factors including sun light exposure and longtime exposed areas might lead to premature aged skin and skin disorders, for example chronic ulcerations, benign tumors (such as actinic keratosis), and malignant tumors (such as basal cell carcinoma, squamous cell carcinoma, and malignant melanoma) (82).

2.3 Photoaging

Extrinsic aging occurs due to the environmental exposure and the most relevant factor is solar irradiation (89). Photoaging is a term coined by Kligman and Kligman to contrast a special divergent skin aging from chronological skin aging process (89, 91). This type of aged skin is a result of long time exposure of the skin

from the sunlight that results in a clinical, histological, and functional divergent features (92).

2.3.1 Epidemiology

While intrinsic skin aging process occurs similarly across the world population regardless of the race, photoaging is evidently different among populations in terms of development time and prominent skin aging signs (Table 2.2) (89). Among the races, the effects of sun exposure on aging skin are extended studied among Caucasians. On the other hand, epidemiological studies in Asians, including Japanese, Koreans, and Chinese are few and rarely among the African American populations (93).

Table 2.2 Epidemiological studies on the photoaging effects

Study populations	Characteristics of photoaged skin
Caucasians (82, 84, 89, 91, 94)	- Earlier onset and more noticeable skin wrinkles and sagging than other populations - Severely atrophic with multiple telangiectasis - Various precancerous lesions (i.e. actinic keratosis) - Solar lentigo and seborrheic keratose are more common in Caucasians - Gradual darkening of skin
Asians (82, 84, 89, 91, 95, 96)	- Pigment changes are the major clinical features - Earlier onset of pigment spots than other populations - Delayed wrinkle onset by about 10 years as compared with Caucasians - Unnoticeable skin wrinkles until 50 years old and that even then its severity is less than Caucasians - Smaller pore areas than other populations
African American (82, 84, 89, 94)	- More pigmentary problems - More severe structure deterioration around facial pores than other populations - Gradual lightening of skin

Skin characteristics varies ethnically among races. No one is spared from skin wrinkling, sunspots, and uneven skin color, yet these symptoms manifest differently according to the ethnic origin (95). Interestingly, although exposed to the same risk factors, development of skin wrinkling varies greatly within a race. Some people of the same race develops severe skin wrinkling while others manifest only mild wrinkling. Further investigation should be done to better understand the reasons of these differences (91). Genetic determination of skin type that results to the difference in melanin content, composition, and location within the ethnic origin is one of the major factor that influences ethnic-specific skin aging manifestation. Other influencing factors of these ethnic-specific skin aging variations can further be explained beyond skin type and unique exposure habits to other environmental factors of specific individuals (89, 95).

2.3.1.1 Caucasian population

Caucasian is a term used to describe people with origins from Europe, North Africa, and Southwest Asia. They have lightly pigmented skin due to aggregation of small melanosomes that results in lesser melanin production. Among the races, Caucasians manifest photodamaged signs earlier than other races due to their relatively less melanin contents. This less pigmentation predisposes them to higher burn response and lower tanning capability (84).

Furthermore, Caucasians manifest earlier onset of skin aging together with more obvious skin wrinkles and sagging signs than other races (82, 89). The typical aging signs of Caucasians have specific lower face features of fine periorbital and perioral wrinkles, sagging skin, and neck jowls with the loss of cervicomental angle due to skin laxity. On the other hand, their upper face manifest skin aging signs as fine and deep wrinkles in the glabella and forehead (84). Multiple telangiectasis, varied premalignant skin lesions like actinic keratosis and severe skin atrophy also found in fair-skinned individuals (89).

A study that compared 500 women of the same age from France and Japan revealed no difference in self-reported lifetime solar exposure and

smoking habits. However, the study revealed earlier onset of solar damage, rhytidosis, and increased severity in French women than the Japanese counterparts (84).

Skin desquamation prevalence is higher among Caucasians with increasing age. More skin desquamation was present in the dorsum of forearm compared to the upper inner arm. The same is also true when comparing Caucasian and African American subjects. Skin dryness is also higher among aged African American and Caucasian subjects (82).

2.3.1.2 Asian population

Majority of the world's population comprises of people with colored skin. Furthermore, more than half of the world populations are Asians (82). However, majority of skin aging studies were performed to Caucasian subjects rather than the Asian counterparts (87, 93). Although the Asian population is diverse, skin aging studies was performed only to specific countries like China, Japan, and Korea. Furthermore, only few studies assessing facial structure were performed to the South East Asian population despite its large population (84).

Clinical manifestation of skin aging among Asians differs considerably compared to Caucasians (91). In Asia population, pigmentation changes are the most frequent and significant manifestation of skin aging. Accordingly, these pigment changes occur earlier in life. In terms of age, erythema index remained the same while melanin index increased significantly in the said population. As the Asian skin ages, it becomes darker and yellower compared to its Caucasian counterparts which becomes darker and redder (84). It is generally understood that the number of melanocytes remain the same after UV exposure regardless of ethnicity. However, among Asians, exposure to UV increases melanin content significantly in their skin (95). Skin color heterogeneity is not fully understood and varied mechanisms could be involved in this process.

With regards to skin wrinkling, the Asian population does not manifest this sign until around 50 years old. When compared to a similarly aged Caucasian, the degree of skin wrinkling is not as prominent as those of the Caucasians (91). A study comparing Japanese women from Nagoya in Japan and German

Caucasian women from Dusseldorf revealed that the Japanese has less facial wrinkling than the comparison group (89). Moreover, other study comparing age-matched Chinese and French Caucasian women revealed that the French manifest facial wrinkling 10 years earlier than the comparison group. However, the Chinese group has higher pigment spot intensity than the French (82, 97). In terms of skin wrinkling patterns, Asians have a coarser, deeper, and thicker rhytides especially in the forehead, Crow's feet, and perioral area. Caucasians, on the other hand, has relatively fine rhytides in the Crow's feet and cheeks area (91). Thus, Asian skin wrinkling patterns varies considerably from the Caucasians.

With increasing age, skin elasticity in the Asian population declined dramatically with concurrent increasing skin fatigability. These changes are attributed to a combination of an increase in collagen crosslinking and decrease in epidermal cell turnover. Also with increasing age, Asians have decreased skin surface moisture and transepidermal water loss, with the exception of adolescents (84). Lastly, skin pores of Asian women are relatively smaller compared to the other races (82).

2.3.1.3 African American population

African American population is a mixture of African, Native American, and Afro-Caribbean race (84). In this population, an almost nonexistent scientific data exists on photoaging.

Melanin is the pigment that gives the skin its distinctive color. In dark skinned types, melanin pigment concentration in melanosomes are double than lightly skinned types. In the African American population, melanosomes are found to be more numerous. Moreover, the individual melanosomes are relatively larger in size and more disperse compared to the other races. Melanin quantity and type, and melanosome arrangement variation contributes to the differences in the skin pigmentation (82, 84).

It is generally understood that higher epidermal pigment concentration and more disperse melanosomes provides higher protection against the damage from solar radiation. Thus, the African American populations are more protected against photoaging and carcinogenesis. However, in this population, they are

more susceptible to dyspigmentation. It is the sign that is defining characteristic of a photodamaged skin in this population (84).

Skin pigmentation changes among African Americans vary considerably against Caucasians in terms of increasing age and level of sun exposure. Acute UV exposure is attributed to an automatic skin darkening among Caucasians and African American. Chronic UV exposure as the age increase, however, is attributed to gradual skin lightening among African Americans and gradual skin darkening among Caucasians (94). Thus, any pigmentation inconsistencies like hyperpigmentation and hypopigmentation is a defining characteristic of photoaging among dark skinned people.

Other than pigmentation differences, African Americans has also thicker stratum corneum with higher fibroblast activity than the Caucasians. Higher fibroblast activity results to a more compact and parallel oriented collagen bundles creating a highly structured and longer lasting youth-like appearing skin. It also makes facials lines among African Americans less visible than the Caucasians. Moreover, intrinsic facial aging is less severe in this population with onset 10 years later. However, this population may manifest a more pronounced malar fat pads sagging, soft-tissue laxity, and jowl formation of the mid-face (84).

Dark skinned people in general have less rhytides. However, they are not spared from rough skin, seborrheic keratoses, and solar lentigines (84). Lastly, they manifest a more significant architectural impairment around facial pores than any other races (82).

2.3.2 Diagnosis

In spite of molecular mechanisms presentation, invasive technique tests are considered the highest standard, photodamaged can be clinically diagnosed to a person who has history of long time exposure from the sun. In connection with this, many scores of skin aging have been created which are primarily descriptive, which may associate photographic grading scales and can be utilized as global indicators of aged skin, for the photodamaged evaluation or the single clinical aged skin signs

evaluation (98, 99). That is why it is wondering to observe that there is no existing validated score of skin aging.

Multiple attempts have been made in determining techniques for examining skin condition quantitatively which includes imaging methods (88). To assess the clinical manifestations of photodamaged skin in female Caucasian populations, Griffiths et al. and Larnier et al. worked on photographic scales. They recommended that photodamage in Far East Asians should not be evaluated by scales used in female Caucasians (91).

A separate grading photographic scales for skin rhytides and dyspigmented skin for both sexes for Koreans was designed by Jim Ho Chung (Figures 2.3-2.6). This grading scale can possibly be used to people in Asian countries (91, 100) and considered as an important assessment tool for skin aging.

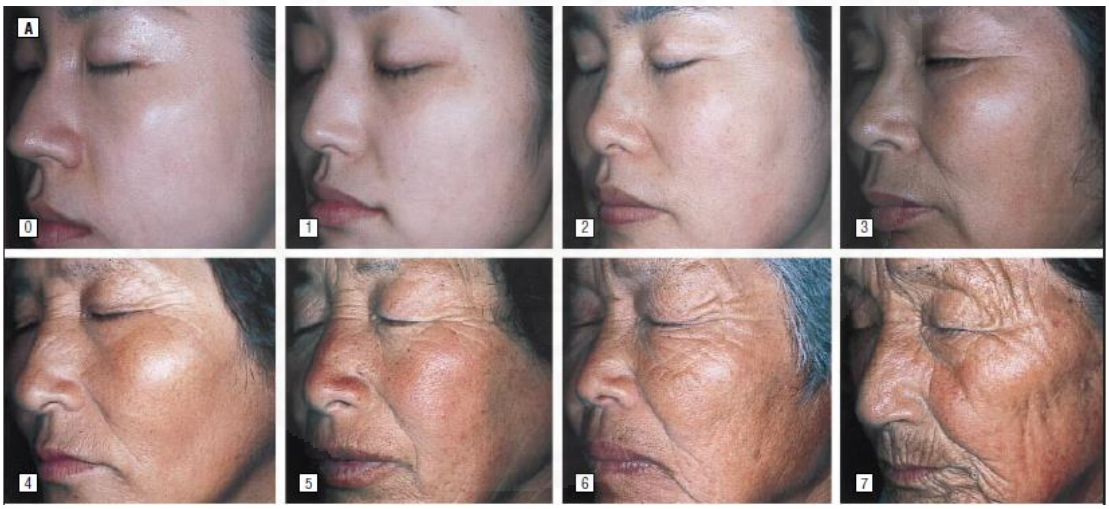

Figure 2.3 Photographic scale shows grading the overall facial skin wrinkling severity in Asian women. Eight consecutive photographic standards demonstrate increasing in skin wrinkling's severity, where 0 depicts none; 1, none/mild; 2, mild; 3, mild/moderate; 4, moderate; 5, moderate/severe; 6, severe; and 7, very severe (100).

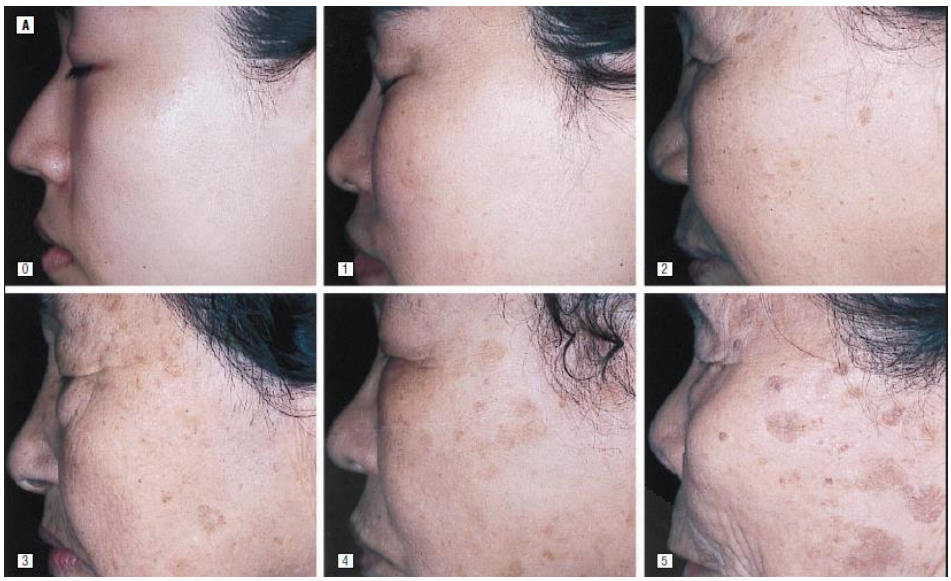

Figure 2.4 Photographic scale shows grading the overall facial dyspigmentation severity in Asian women. Six consecutive photographic standards demonstrate increasing in facial dyspigmenation's severity, where 0 depicts none; 1, mild; 2, mild/moderate; 3, moderate; 4, moderate/severe; and 5, severe (100).

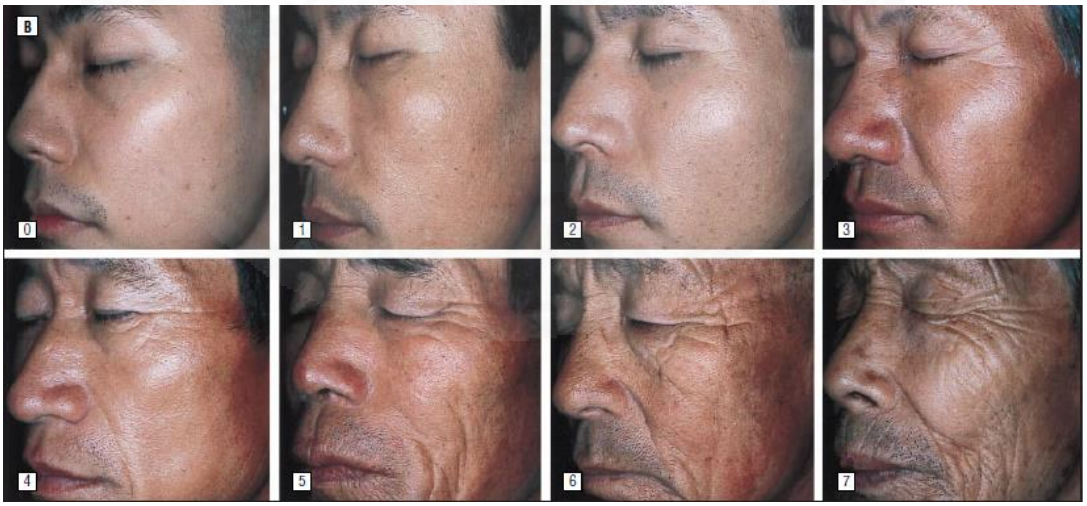

Figure 2.5 Photographic scale shows grading the overall facial skin wrinkling severity in Asian men. Eight consecutive photographic standards demonstrate increasing in skin wrinkling's severity, where 0 depicts none; 1, none/mild; 2, mild; 3, mild/moderate; 4, moderate; 5, moderate/severe; 6, severe; and 7, very severe (100).

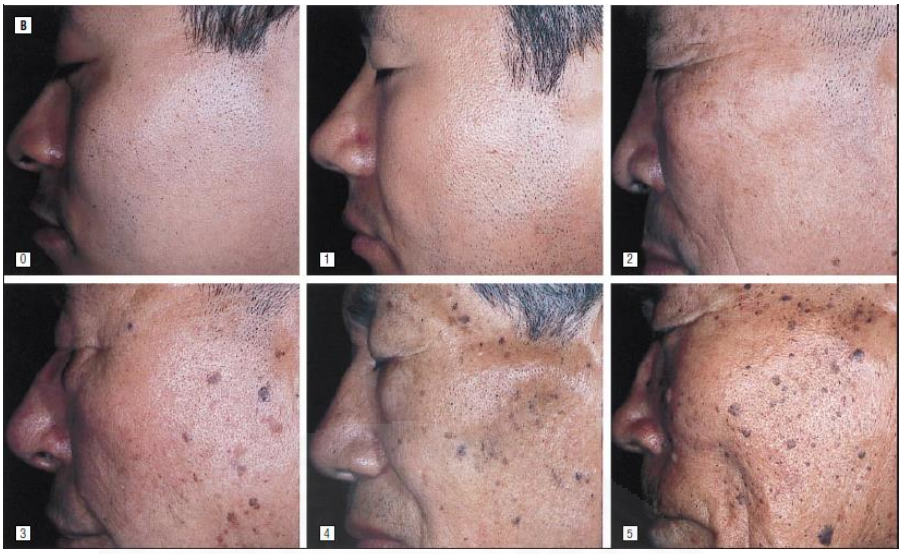

Figure 2.6 Photographic scale shows grading the overall facial dyspigmentation severity in Asian men. Six consecutive photographic standards demonstrate increasing in facial dyspigmenation's severity, where 0 depicts none; 1, mild; 2, mild/moderate; 3, moderate; 4, moderate/severe; and 5, severe (100).

There are intrinsic and extrinsic factors that results to skin aging. Chronological or intrinsic aged skin and extrinsic aged skin, which is mainly by cause of long time sunlight exposure and therefore is also called photoaging, can be clinically and histologically identified, associate distinct pathogenic caused and mechanism. A novel score of skin aging, the 'SCINEXA', based on the evaluation of 5 skin signs which are highly demonstrate intrinsic skin aging and18 skin signs which are greatly demonstrate extrinsic skin aging, has been developed (Table2.3). SCINEXA aging score is the non-invasive, easily done, and sensitive method for the evaluation of extrinsic and intrinsic aged skin signs, simultaneously. This score can be proven applicable in researches on the distinct pathogenetic background of extrinsic versus intrinsic skin aging (98).

Table 2.3 Skin aging symptoms included in the skin aging score ‘SCINEXA’ (98)

Intrinsic skin aging items	Extrinsic skin aging items
- Uneven pigmentation - Fine wrinkles - Lax appearance - Reduced fat tissue - Benign skin tumors	- Sunburn freckles - Lentigines solaris - Pigment change - Change of skin phototype - Yellowness - Pseudo scars - Coarse wrinkles - Elastosis - Cutis rhomboidalis nuchae - Favre racouchot - Dryness - Comedones - Telangiectasias - Permanent erythema - Actinic precancerous - Basal cell carcinoma - Squamous cell carcinoma - Malignant melanoma

Even though correct measurements of facial skin characteristics are required to examine the relation between skin condition and different factors, many methods are subjective, which they are rely on visual grading by experienced examiners (88).

2.3.3 Clinical features

Chronic skin exposure to solar radioactivity has been shown to be concerned in photoaging which is marked by distinct degradation of dermal ECM components. Clinical consequence are the appearance of characterized cutaneous

modification including rough wrinkles, laxity and sagging, mottled dyspigmentation, telangiectasia, sallowness, increased fragility, and rough skin texture (1, 101).

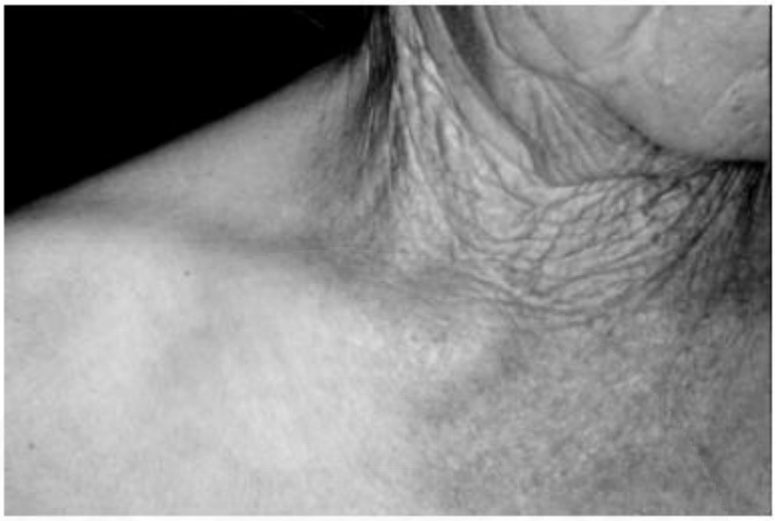

Figure 2.7 Aging skin in Asians. Solar-irradiated skin demonstrating the clinical features of severe photodamage, i.e., dry skin, wrinkling, irregular dyspigmentation, and loss of elasticity, which is in contrast to the sun-protected skin (91).

Solar elastosis is the hallmark of photoaging skin, which is a degenerative state of elastic fibers in the dermis caused by prolonged exposure from the sun (102, 103). Solar elastosis is generated by a cycle of elastic fiber destruction succeeded by ECM production and reassembly into an organization not the same as the first structure. Solar elastosis has various clinical features. In its general form of solar elastosis, shown as yellow discoloration, skin thickening, and roughly skin wrinkles (103, 104).

In fact, histopathologic changes in degenerative dermal elastic tissue that takes place in photodamaged skin refers to solar elastosis. The predominant histological characteristic of solar elastosis is disordered masses of degraded elastic fibers that degenerate to produce an formless mass, consist of tangled tropoelastin and fibrillin and appear basophilic degeneration of dermal elastic fibers, set apart from the

epidermis by a constricted band of normal-appearing collagen with collagen fibers aligned horizontally (103).

2.3.4 Histology

As a consequence of growing age, skin loses its architectural, morphologic features, and most of their cellular functions (105-107). Decreasing and degeneration of collagen, an accumulation of elastotic fibers, and deposition of glycosamicoglycans are manifested in a photoaged skin based in histological studies. These damages to the skin is considered leading to obvious changes, like skin wrinkling and sagging (108, 109).

In histopathologic studies, photodamaged skin may possess a loss of regularly differentiation of keratinocytes or polarity of epidermis. Each of keratinocytes is manifested with atypia, especially in the lower layers of epidermis (110). Moreover, the sustained elevated stratum corneum and granular layers possibly contributes to the formation of skin wrinkling. In epidermal aged skin, the transformation from granular layers to corneum layers, and the desquamation of the corneum layers might be impeded because of disrupted keratinocytes maturation, and thereby halted the stratum corneum compositions building (108). As a result, the hindrance of corneum layers creation may proceed to a loss of tightly fitting characteristics of the skin. There is a flattening of the dermo-epidermal junction that result to manifestation of atrophy (108, 110).

In general, photodamaged dermis cell population increases; a lot of fibroblasts and hyperplastic, and inflammatory infiltrates abound. Elongated and collapsed fibroblasts in photoaged skin are evident too (110). It's interesting that photoaged fibroblasts has ability to synthesize procollagen type I. Therefore, a reduction in procollagen type I synthesis in photoaged skin is not an outcome of irrevocable damages to collagen synthetic capability of fibroblasts (111). However, the collection of partially degraded collagen fibers by the much more MMPs in photoaged skin hinders procollagen type I production (112).

The quantity of elastin decreases with age, but in a skin exposed to sun, the amount of elastin elevated in proportion to the amount of exposure to sun. The

deposited elastin in the skin displays atypical and appears to cover the area originally located by collagen fibers. The microvasculature is also changed and vessel walls are thickening with the accumulation of a basement membrane-like material (110).

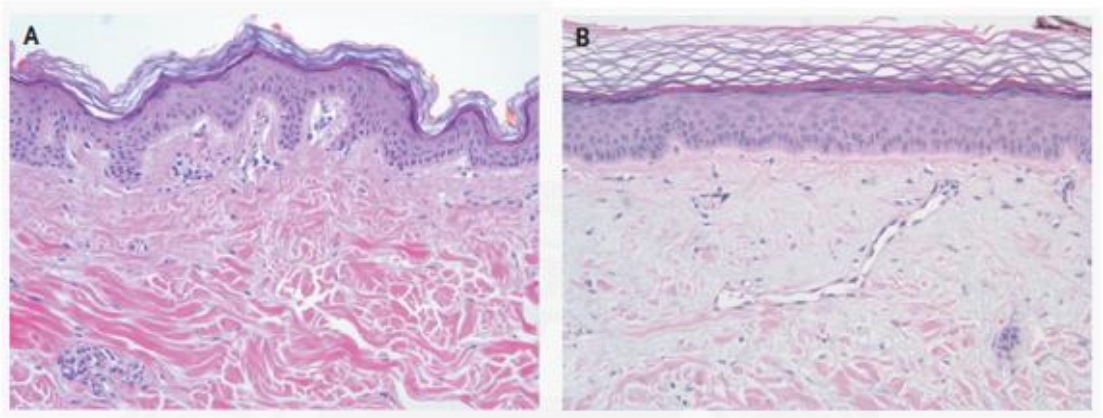

Figure 2.8 Histology of normal skin (Picture A) and Photodamaged skin (Picture B). Picture B shows epidermal atrophy along with the spinous layer narrowing and loss of rete ridges. Underneath the basement membrane area, collagen is compressed and elastic fibers are proliferated, which are curled, thickened and complicated. In superficial dermis, telangiectasia is show up in the elastotic area. In deep dermis, collagen fibers show early basophilic deterioration (113).

2.3.5 Pathogenesis

2.3.5.1 Solar irradiation and skin aging

Solar irradiation is the major environmental factor that causes photoaging. From the full-spectrum of solar iradiation, both UV-B (290-320 nm wavelengths) and UV-A (320-400 nm wavelengths) along with infrared (IR) A with 760-1,400 nm wavelengths and visible light with 400-760 nm wavelengths have capability to activate the process of photoaging skin (89).

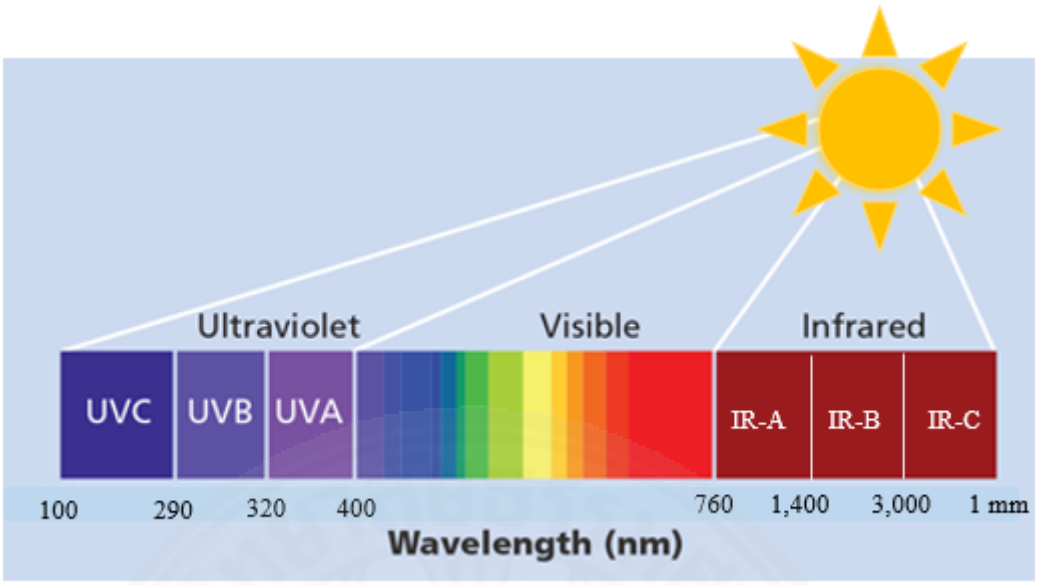

Figure 2.9 The whole spectrum of solar irradiation

UV-A rays, approximately 95% of the UV irradiation, is reaching the Earth's surface and is only slightly affected by ozone levels. 80% of UV-A touches the dermo-epidermal junction and go through the papillary dermis. Only 10% of UV-A is able to absorb by the hypodermis. In contrast to UV-A, UV-B rays is essentially absorbed by the epidermis and approximately 70% of UV-B is obstructed by the stratum corneum (114). The amount of UV-B reaching the earth's surface is lesser than that of UV-A; however, its intensity is high enough to cause photoaging and skin cancer (115, 116).

Infrared irradiation, with wavelengths from 760 nm to 1 mm, is divided 3 parts, namely (i) near IR-A (760-1,400 nm wavelengths), (ii) middle IR-B (1,400-3,000 nm wavelengths) and (iii) far IR-C (3,000 nm - 1 mm wavelengths). Only IR-A rays, approximately 30% of the IR irradiation, is reaching the human skin. And about 65% of IR-A part can penetrate into the dermis (117, 118).

Visible light irradiation, with 400-760 nm wavelengths, are about 45% of solar rays. About 20% of visible light irradiation can penetrate into the hypodermis (119).

This review focuses on human MMPs in relation to photoaging. The first part summarizes the roles of UV-induced MMPs in photoaging, the second part focuses on the involvement of MMPs in the pathophysiology of IR induced photodamage, and the last part studies on the effects of visible light.

(1) Ultraviolet radiation

The action spectrum for UV-induced skin damage is divided into UV-A (320-400 nm) and UV-B (290-320 nm). The amount of UV-B reaching the earth's surface is lesser than that of UV-A; however, its intensity is high enough to cause photoaging and skin cancer (115, 116). Nonetheless, both UV-A and UV-B irradiation can induce oxidative stress in human skin, leading to temporal and persistent genetic impairment, up-regulation of activator protein (AP)-1 activity, and increased MMP expression (106, 120).

UV irradiation induces excess intracellular reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$) (107). ROS, a secondary messenger, activates the mitogen-activated protein kinase (MAPK) family. MAPKs are a family of proline-directed Ser/Thr kinases comprising extracellular signal-regulated kinases (ERKs), p38, and c-Jun NH_2-terminal kinase (JNK). ERK is important to stimulate the expression of c-Fos, whereas p38 and JNK activation are crucial for the expression of c-Jun. c-Jun in combination with c-Fos forms the transcription factor AP-1, which plays an essential role in the transcriptional regulation of MMPs such as MMP-1, MMP-3, MMP-9, and MMP-12 resulting in the degradation of collagen and elastin (85, 120, 121). Additionally, AP-1 inhibits transforming growth factor-β (TGF-β) signaling, a major regulator for the production of procollagen type I in human skin. Impairment of the TGF-β pathway leads to decreased synthesis of procollagen (121-123).

Besides AP-1, nuclear factor-kappa B (NF-κB) is another important transcription factor that is activated in response to UV irradiation. NF-κB is a universal transcription factor that regulates the gene expression of growth factors, chemokines, cytokines, and cell adhesion molecules, in healthy as well as numerous diseased states. Generation of ROS induces NF-κB-mediated transcriptional activation

and regulation of MMP gene expression. Thus, this factor is important to mediate the responses of UV irradiation. NF-κB activity is reported to be responsible for the up-regulation of MMPs such as MMP-1 and MMP-3 in dermal fibroblasts (124-126). Thus, both AP-1 and NF-κB are involved in the process of photoaging.

(2) Infrared radiation

In the same manner with UV-B or UV-A radiation, it is currently agreed that IR radiation may wield profound biological effects on skin of human skin. Still, it remained largely unknown the primary molecular mechanisms underlying the pathological effects of IR irradiation (117, 127).

As stated above, IR wavelengths are much extensive than those within the UV spectrum and therefore perforate into the skin more deeply. This produces additional chromophores and dermal fibroblasts, which are significant for skin's structural integrity and dermal elasticity, main object of this kind of radiant energy (118).

The generation of ROS in mitochondria of dermal fibroblasts starts IR radiation-induced skin damage (127, 128). Essentially, mitochondrial ROS synthesis is of functional importance for IR radiation-mediated the expression of gene by upregulation of MAPK signaling pathways. Due to IR irradiation, activated MAPKs move into the nucleus, where they phosphorylate and stimulate transcription factors including c-Fos, c-Jun, and p38 resulting to the formation and activation of the transcription factor AP-1, which plays an essential role in the transcriptional regulation of MMPs expressions, including interstitial collagenase, collagenase-3, gelatinase-B, and stromelysin-1 (118, 129, 130).

Moreover, activation of other signaling cascades regulating gene expression may also triggered by IR radiation (117). When skin temperature is over 39°C, heat shock-mediated ROS are produced in human skin at generous amounts and caused the quick activation of MAPK signaling pathways resulting in MMPs production including interstitial collagenase, stromelysin-1, and gelatinase-B, and subsequent degeneration of ECM proteins (131, 132).

As a whole, these results means that IR-irradiation is capable of changing gene expression which demonstrates a pro-aging phenotype of the skin (129).

(3) Visible light radiation

Contrary to several studies done to investigate the effects of UV exposure and IR irradiation on human skin, very few studies are done concentrating on visible light and skin. Visible light spectrum starts from 400-760 nm wavelengths and comprise an estimate of 45% of solar rays. Visible light is the most penetrating and an approximate of 20% goes through the hypodermis. Visible light are absorbed via various skin chromophores including melanin, hemoglobin, and even bilirubin (119).

Visible light wavelengths may produce biological effects on human skin that involves higher ROS production and that are of significance for photoaging. Exposure to visible light may also add to photoaging by developing collagen breakdown. It was known to increase the synthesis of interstitial collagenase in epidermal keratinocytes (127). Actually, there are no study exist about the molecular mechanisms in charge of visible light-induced premature skin aging.

2.3.5.2 Inflammation and skin aging

Immunosenescence refers to undergoing profound age-related changes of an individuals' immune system as they age. With age, there are gradual increase in the secretion and circulation of pro-inflammatory cytokines including interleukin (IL)-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ with age, resulting to a systemic chronic low-grade state of inflammation. So, the term "inflamaging" means continuous, low-grade inflammation related to aging (133, 134). Such chronic inflammation could accumulate with time and slowly resulting to tissue damage. It is recognized as one of the trigger factors for various age-associated diseases, including atherosclerosis, diabetes, and especially skin aging (134). In skin, there is chronological (or intrinsic) skin aging and UV-induced skin aging (or photoaging). Both inflammation and ROS accumulation are generally accepted to be the driving causes in both types of aged skin.

In photodamaged skin, UV radiation changes dermal ECM contents and also elevates the inflammatory symptoms such as upregulation of inflammatory mediators and the infiltration of inflammatory cells (135). 'Inflammaging'-involved cell types can be natural immune cells, like monocytes or macrophages, neutrophils, and non-immune cells like keratinocytes (133).

Skin inflammation is nearly related to skin aging because the cytokines and inflammatory mediators have a critical role in the exhibition of these characteristics of aged skin (135). In addition, age-related changes slowly elevates the production and circulation of pro-inflammatory cytokines, resulting to a chronic low-grade state of inflammation (133).

2.3.6 Prevention

The skin serves as cover and protection from the organism against the negative effects of the environment such as sunlight. It has defenses such as endogenous systems for shielding from solar radiation including production of melanin, thickening of epidermis and an antioxidant network (119). The main determinant in response to oxidative stress-mediate damage is the endogenous antioxidant capacity of the skin (109). Thus, exogenous ways are very essential. Avoidance from sun, utilization of photoprotective clothing and enough broad-spectrum sunscreens application are called exogenous protection and are considered the most efficient ways to protect the skin (119).

In Western countries, for more than four decades, application of sunscreens have become the most famous way of protection against UV radiation. There is an existing organic and inorganic filters with various absorption spectrum. They have a filtering or scattering function to UV radiation. Protection from UVB is measured as a minimal erythema dose-based sun protection factor. Compared to UVB protection, protection to UVA is less standardized: the latest methods that are currently utilized are persistent pigment darkening and critical wavelength. There are large differences between European and US sunscreen market like the undergoing of marketing and labeling of sunscreen products by national regulation. Skin cancers, UV-

induced immunosuppression, and skin aging can be prevented by the application of enough amount of broad spectrum UVB and UVA sunscreen protection (136).

Protection against UV-A- and UV-B-induced skin damage and photoprotection of human skin have long been considered almost the same. Within the last ten years, this general belief has been challenged. Human skin photoprotection should not be restricted to UV protection but should include visible light and IR radiation protection, too. Since 2006, availability of sunscreens and daily-care products claiming IR and visible light protection have been existing. First in Europe, then it spread to other conteinents including North and South America and also Asia (127). Truly, latest sunscreen products have not been checked, and are unlikely to contain the efficacy against IR (118). To be able to attain the scientific basis for the development of strategies to prevent IR-induced skin damage, future studies should discover the photocheminal details because the actual photophysical and photochemical mechanisms induced by IR are still unknown (117).

Protecting yourself from the sun is very valued not only for your appearance, but your health. No matter what the age is, protecting oneself from the exposure from the sun reduces the risk of having actinic keratosis, squamous-cell cancer and the development of photoaging. Less exposure to sun during childhood can lessen the risk of having basal-cell cancer. Other treatments are less effective and can be harmful without the adequate protection from the sun (113).

2.3.7 Treatment

Nowadays there is more concern about skin aging as a health issue in the general population due to life expectancy is increased and people want to maintain the youthful appearance. It is best described by the sale amounts of cosmetic products that is claimed to prevent, decelerate, arrest or even reverse the aged skin signs. These cosmetic products compose of various molecules with specific biological properties and are proposed to involved in the skin aging pathogenesis by regulate molecular mechanisms (94).

According to this study, the knowledge of all aspects of MMPs involvement in photoaging and their regulatory pathways, leading to better understand in

mechanisms and could be considered the promising targets for unwelcome of aging skin.

2.3.7.1 MMPs inhibitors

Cutaneous exposure to solar radiation causes the up-regulation of several different MMPs that virtually impair the various components of the ECM. These alterations in the ECM are known to cause skin wrinkling, a major characteristic of premature skin aging. Evolution of novel MMP inhibitors is promising as targets to combat photoaging.

Table 2.4 and 2.5 shows summarize MMP inhibitors that have been reported in photoaging treatment.

Table 2.4 Demonstrate the MMP inhibitors of other treatment modalities

Group	Treatment modalities	Mechanisms
Conventional topical therapies	All-trans retinoic acid (137, 138)	• Suppress UV-mediated MMP-1, MMP-2, and MMP-9 secretion
	Seletinoid G (139)	• Decrease MMP-1 expression
LASERs	Long-pulse Nd:YAG laser (140)	• Decrease MMP-1 expression at low energy • Increase MMP-1 expression at high energy
Toxin	Botulinum toxin type A (141)	• Diminish the photoaged fibroblasts-derived MMP-1, MMP-3, and MMP-9 expression.

(1) Topical therapies

All-trans retinoic acid

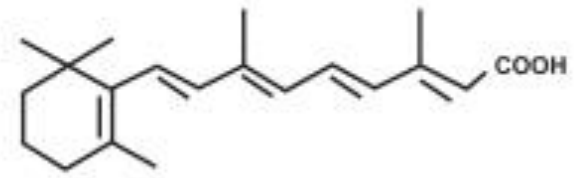

Figure 2.10 All-trans retinoic acid or tretinoin structure (142)

The most active retinoids compound is all-trans retinoic acid (tRA) which regulates many biologic processes by mediate the expression of genes, including retinoid X receptor (RXR) and retinoic acid receptor (RAR). To our knowledge, the promising gene targets of retinoids are about 500 genes, including MMPs-regulated genes (such as collagenases, gelatinases, and stromelysin-1) (137).

In the last two decades, the potency of topical tRA or tretinoin in photodamaged treatment is well confirmed (143). Topical retinoids treatment decreases skin rhytides by stimulating collagen synthesis and diminishing MMPs-regulated ECM proteins destruction (139, 144).

tRA can protect against UV-induced the expression of interstitial collagenase (138, 145) and decrease gelatinases expressions in dermal fibrolblasts (137). Furthermore, tRA also regulate TIMP-1 activity, which subsequently suppresses interstitial collagenase expression. As a result, the relative MMP-1/TIMP-1 expression ratio may be helpful in the recognition of powerful anti-wrinkle components (144, 145). Moreover, tRA can suppress the expression of c-Jun and transcription factor AP-1, leading to decrease UV-induced ECM degradation (139).

tRA treatment can upregulate collagen synthesis in both intrinsic and photoaging skin. This activation promote the better function and appearance of skin. Even though the exactly biological mechanisms of tRA mediates the synthesis of collagen in both intrinsic and photoaging skin remain unclear. It has

been reported that tRA essentially increased procollagen type I, fibrillin-1, and tropoelastin production (138, 139).

Although retinoids has the anti-aging components for decreasing skin rhytides, treatment of retinoids can cause unwanted photo-sensitive side effects such as skin irritation, burning, itching (144).

Seletinoid G

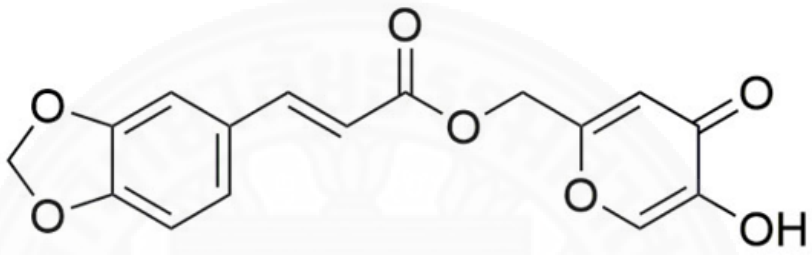

Figure 2.11 Seletinoid G structure (142)

Despite retinoids are effective in aging skin treatment, their unwanted effects are still a problem. Seletinoid G, the newly synthetic retinoid, which has few or no unwanted effects after treatment. Seletinoid G can regulate ECM proteins in elderly skin and suppress UV-induced loss of collagen in youth skin (139).

Topical 1% seletinoid G application can upregulate procollagen type I, fibrilin-1, and tropoelastin expression and suppress interstitial collagenase secretion same as tRA. The suppression of interstitial collagenase by seletinoid G can inhibit the lately biosynthesized collagen destruction, and consequently upregulate the production of procollagen. Despite the exactly molecular protective processes of seletinoid G on UV-mediated interstitial collagenase and procollagen expression has not been explain in detail, it might associated with the inhibition of c-Jun expression and signaling pathway (139).

As a result, seletinoid G, the newly synthetic retinoid, can be treated in chronological aging and photoaging skin, which has few or no side effects to skin, compared to tRA (139).

(2) LASER therapy

Long-pulse Nd:YAG laser

Various laser machines promote improvement of aging skin. Non-ablative lasers are able to stimulating the production of collagen in dermis whereas reserve the epidermis. 1,064 nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser, also called long-pulse Nd:YAG laser, is one type of non-ablative laser which is used for rejuvenated aging skin. However, despite 1,064 nm Nd:YAG laser have been extensively used for rejuvenated aging skin, less studies have researched the molecular mechanisms in collagen rearrangement of 1,064 nm Nd:YAG laser (140).

Histopathological assessment revealed that TGF-β expression increases along with alteration of collagen in the dermis after laser. The MMPs expression after laser show variable results. The MMPs expression is diverse because of the different between fluence of energy and between MMPs subtypes. One month after treated with low fluence 20 J/cm^2 1,064 nm Nd:YAG laser, interstitial collagenese was marked decreased. Whereas interstitial collagenese was mild increased after 1 month after treated with low fluence 20 J/cm^2 1,064 nm Nd:YAG laser. This upregulation of interstitial collagenase may account for the destruction of senescent collagens (140).

(3) Botulinum toxin

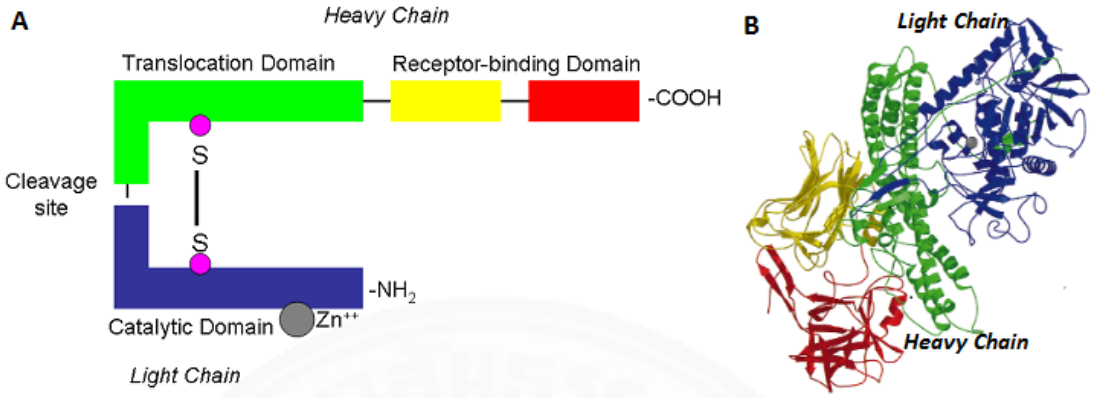

Figure 2.12 Botulinum toxin type-A (BTX-A) structure. (A) Schematic BTX-A structure. (B) Crystal BTX-A structural view. Blue showed the light chain catalytic domain. Green showed translocation domain of the heavy chain. Yellow and red showed N-terminal and C-terminal of the heavy chain, respectively (146).

Botulinum toxin type A (BTX-A), one of seven neurotoxins produced by *Clostridium botulinum*, is a potential agent for decelerate obvious skin aging by diminishing the static and dynamic facial lines and rhytides (141, 147). The mechanism of botulinum toxin action is able to promote chemodenervation on presynaptic neurons, impeding the acetylcholine (Ach) secretion and resulting in denervated striated muscle for 2-6 months after injection. This leads to an atrophy of muscle fibers and consequently characterized by flaccid paralysis (141). Thus, BTX-A is widely used in cosmetic procedures to treat facial rhytides or lines that made by the hyperactivity of muscle.

Besides the neuromodulator, BTX-A can accelerate the type I and type III collagen production. BTX-A also concurrently diminish the photoaged fibroblasts-derived MMP-1, MMP-3, and MMP-9 expression. This suggests that BTX-A plays an important role in the inhibition of solar-induced premature skin aging, and proposing that BTX-A has the ability in anti-photoaging (141).

(4) Botanical extracts for MMPs inhibitors

In recent years, there has been more interest in the use of botanical supplements for the treatment of solar radiation-induced skin photodamage. Botanical extracts are another emerging trend in cosmeceutical and pharmaceutical segment because people have more concern in using chemical or conventional treatment.

In this review, the studies about plant or botanical extracts, herbal preparations, and also isolated plant-derived compound have been collected. Table 2.5 summarizes botanical extracts/compounds for MMPs inhibitors that have been reported in treatment of photodamage skin.

Table 2.5 Demonstrate the MMP inhibitors of natural compounds or extracts

Botanical compounds/extracts	Mechanisms
Astragaloside IV from *Astragalus membranaceus* (123)	• Suppress UV-induced MMP-1 expression
Baicalin extract from *Scutellaria lateriflora Georgi* (Huang Qin) roots (148)	• Diminish the secretion of MMP-1 and MMP-3
Brazilin extract from *Caesalpinia sappan L*. (124)	• Prevent UV-induced MMP-1 and MMP-3 expression
Coffea Arabica leaves extract (149)	• Caffeic acid suppress UV-induced MMP-1 and MMP-9 expression • Cholorogenic acid decrease only MMP-3 expression

Table 2.5 Demonstrate the MMP inhibitors of natural compounds or extracts (Cont.)

Botanical compounds/extracts	Mechanisms
Galla chinensis (GAC) (121)	• 5% GAC revealed inhibit MMP-1 secretion
Gynura procumbens (2)	• Decrease UV-induced MMP-1 and MMP-9 secretion
Ixora parviflora (86)	• Suppress MMP-1, MMP-3, and MMP-9 expression
Kaempferia parviflora (126)	• Inhibit UV-induced MMP-2, MMP-3, MMP-9, and MMP-13 productioin
Macelignan extract from *Myristica fragrans Houtt.* (150)	• Reduce UV-induced MMP-9 expression via MAPKs pathways
Magnolol extract from *Magnolia officinalis* (151)	• Suppress MMP-1, MMP-9, and MMP-13 activity
Myricetin (5)	• Decrease UV-induced MMP-1 and MMP-9 production
Polyphenols from green tea (152)	• Suppress UV-induced MMP-2, MMP-3, MMP-7, and MMP-9
Saponins extracts from *Platycodon grandiflorum* roots (115)	• Diminish UV-induced MMP-1 and MMP-9 secretion
β-carotene (143)	• Decrease UV-induced MMP-1, MMP-3, and MMP-10

Astragaloside IV

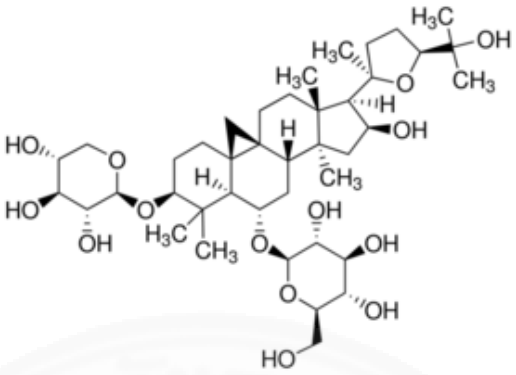

Figure 2.13 Astragaloside IV structure (153)

Astragaloside IV (AST), the small saponin molecule, is a principle active ingredients isolated from *Astragalus membranaceus.* AST has various biological functions such as anti-inflammation, anti-infarct, anti-hypertensive, and inotropic property. It has been shown that AST acts as the powerful suppressor of UV-mediated interstitial collagenase production. Moreover, AST also downregulates UV-mediated inhibitioin of procollagen type I by activating TGF-β/Smad signaling pathway (123).

Baicalin extract

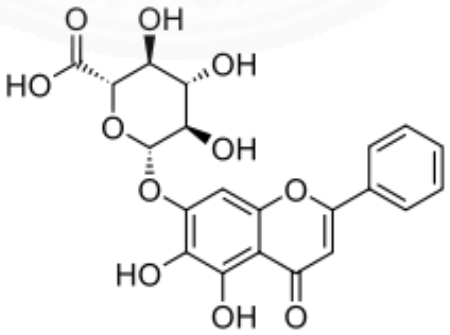

Figure 2.14 Baicalin structure (154)

Baicalin is extracted from *Scutellaria lateriflora Georgi* (Huang Qin) roots. Baicalin exerts various biological functions. This active ingredient is useful in treatment of many diseases, including rheumatoid arthritis, hepatic cytotoxicity, myocardial injury (155).

Topical treatment of baicalin extract can inhibit UVB-mediated epidermal hypertrophy of mouse skin. It can upregulate collagen type I and type III production and downregulate interstitial collagenase and stromelysin-1 expression, suggesting that baicalin is candidate for the powerful treatment of photoaging (148).

Brazilin extract

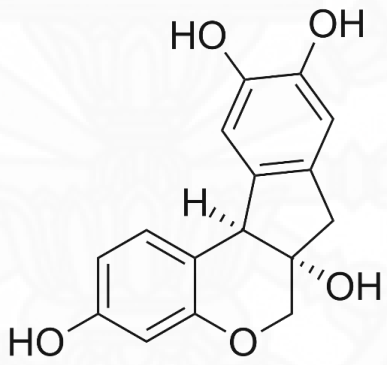

Figure 2.15 Brazilin structure (156)

Brazilin is the principal ingredients isolated from *Caesalpinia sappan L.*. It gives the natural red color that used for histological staining (157, 158). Many researches revealed that brazilin extract exerts many biological functions, including anti-inflammation, anti-platelet aggregation, anti-hepatotoxicity, and regulate immune functions (159).

In anti-photoaging effects, brazilin can prevent UVB-regulated the expression of interstitial collagenase and stromelysin-1. Brazilin also inhibit UVB-induced ROS production in dermal fibroblasts. In addition, UVB-mediated NF-κB expression is totally inhibited by brazilin treatment (124).

Coffea arabica

Figure 2.16 Coffea arabica leaves (149)

Polyphenols are mostly found in vegetables, fruits, green tea and wine. Coffea arabica is a member of *Rubiaceae* family, which is highly in polyphenols compounds. The ingredients of Coffea arabica consist of organic acids (i.e. chlorogenic acid and caffeic acid), alkaloid (i.e. caffeine), and diterpenoid alcohols (i.e. kahweol and cafestol) (160). Both catechin and chlorogenic acid belong to polyphenols compounds, which made the Coffea arabica as a powerful anti-photoaging agents (161).

Coffea arabica leaves extract (CAE) and its components, both caffeic acid and chlorogenic acid, can decrease UVB-mediated photoaging via suppressing the production of MMPs. Caffeic acid diminishes interstitial collagenase and gelatinase-B, but not stromelysin-1. In addition, cholorogenic acid diminishes only stromelysin-1, but not diminishes interstitial collagenase and gelatinase-B. This results in the variety on MMPs suppression, which could downregulate interstitial collagenase on the collagen type I and type III destruction. Additionally, CAE could downregulate gelatinase-B to protect against the destruction of collagen fragments produced by interstitial collagenase and moreover downregulate stromelysin-1 to decrease the expression of pro-intersitial collagenase. Besides MMPs inhibition, CAE and its components can stimulate the production of procollagen type I (149).

Galla chinensis

Figure 2.17 *Galla chinensis* (121)

Galla chinensis (GAC), a natural plants, has been widely used in traditional medicine for long time ago because of its curative effects. GAC has several biological functions such as antimicrobial, antifungal, and antiviral properties. The principle active ingredients of GAC extracts are methyl gallate and gallic acid, which act as the potent antioxidant (162).

Treatment of GAC greatly protected photodamaged skin by decreasing the interstitial collagenase expression and activity. Topically applied 1% GAC is more advantageous than 5 % GAC based on procollagen type I and TGF-β1 expression. Whereas topically applied 5% GAC displayed more effective in interstitial collagenase inhibition. Nonetheless, the most advantageous concentration of GAC to keep balance between interstitial collagenase and procollagen type I expression needs more investigation (121).

Green tea

Figure 2.18 Green tea (152)

Camellia sinensis or green tea is highly in polyphenols, which are more attractive because of their powerful antioxidant and photoprotective functions (163). It has been reported that oral green tea polyphenols (GTP) can suppress UVB-mediated MMPs secretion, including gelatinases, stromelysin-1, and matrilysin-1 in hairless mice. In vivo studies, GTP exerts photoprotective effects at least, by (i) suppression of MMPs expression that destroy ECM components, and (ii) diminution in oxidative stress (152).

Gynura procumbens

Figure 2.19 *Gynura procumbens* (164)

Gynura procumbens (Lour.) Merr., a member of *Asteraceae* family, is extensively used in Southeast Asia such as Thailand, Malaysia, and Indonesia. In conventional medicine, this herb are used to treat many diseases, including viral infections, inflammation, rheumatism, and even cancers (2).

In photoprotective effects, *Gynura procumbens* extract greatly suppressed UVB-mediated interstitial collagenase and gelatinase-B production. Besides MMPs inhibition, *Gynura procumbens* extract also greatly suppressed the other signaling molecules, including cytokines and ROS molecules, leading to efficiently decreased the MMPs expression (2).

Ixora parviflora

Figure 2.20 *Ixora parviflora* (86)

Ixora parviflora, belongs to the *Rubiaceae* family, is highly in polyphenols compounds and extensively used in the traditional Indian (165). Polyphenols ingredients are mostly found in vegetables, fruits, green tea and wine. *Ixora parviflora* extract (IPE) can decrease the expression of interstitial collagenase, gelatinase-B, and stromelysin-1. Additionally, IPE can suppress the MAPKs phosphorylation pathways induced by UVB exposure. Moreover, IPE acts as a powerful inhibitor of UVB-mediated oxidative stress and, consequently, may be a good candidate for anti-photoaging treatment (86).

Kaempferia parviflora

Figure 2.21 *Kaempferia parviflora* (126)

Kaempferia parviflora, also called black ginger, is a member of the *Zingiberaceae* family. In tropical countries, it has been utilized as supplements and conventional treatments for many diseases (166).

In photoprotective effects, oral *Kaempferia parviflora* extract (KPE) can greatly suppress several MMPs expression, including collagenase-3, gelatinases, and stromelstion-1, by decreasing c-Jun and c-Fos activities. In hairless mice studies, oral KPE can upregulate collagen production via increasing procollagen type I, III, and VII expression (126).

Macelignan extract

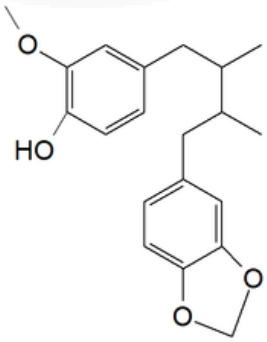

Figure 2.22 Macelignan structure (150)

Macelignan, extracted from *Myristica fragrans Houtt.*, has several biological functions such as anti-hepatotoxicity, anti-carcinogenic, antioxidant, and anti-inflammatory properties. In photoaged skin, treatment of macelignan extract can suppress MAPKs signaling pathway induced by UVB, and subsequently reduce the expression of gelatinae-B (150). Thus, macelignan acts as a MMP-inhibitor which can diminish aged skin.

Magnolol extract

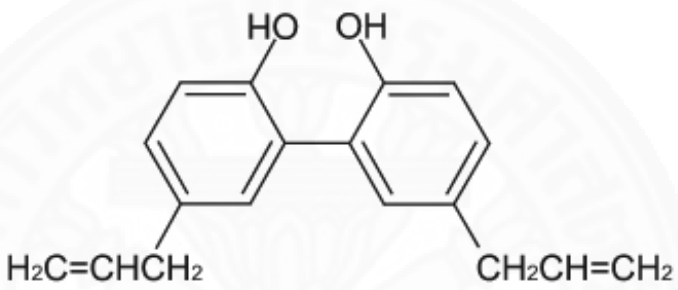

Figure 2.23 Magnolol structure (167)

Magnolol, a Chinese traditional herb that is isolated from *Magnolia officinalis*, has various biological activities, including anti-inflammation, antidepressant, and anti-arthritic (168). In photoaged skin, magnolol extract can reduce UVB-mediated epidermal hypertrophy, skin rhytides, and loss of collagen bundles by significantly diminished the expression of interstitial collagenase, collagenase-3, and gelatniase-B (151).

Myricetin

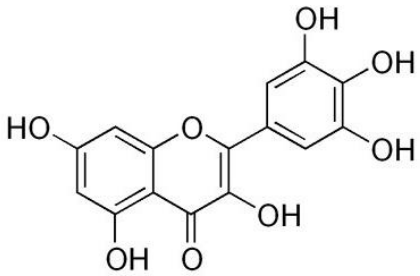

Figure 2.24 Myricetin structure (169)

Myricetin (3,30,40,5,50,7-hexahydroxyflavone) belongs to flavonoids family. It is generally found in foods such as grapes, berries, red wine, and onions (170). Myricetin exerts many biological activities, including anti-inflammation, antioxidant, and anti-carncer.

In photoprotective effects, myricetin inhibits UVA-mediated the expression of interstitial collagenase in dermal fibroblasts. In addition, myricetin also inhibits gelatinase-B secretion and activity. Myricetin may be a good candidate for anti-photoaging treatment, but the exactly molecular mechanisms of myricetin associated in anti-photoaging is still not clearly understood (5).

Saponins extract

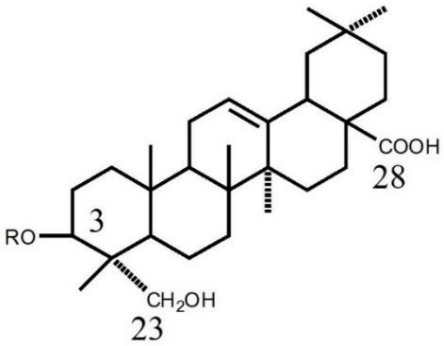

Figure 2.25 Saponins structure (171)

Saponins, isolated from *Platycodon grandiflorum* roots, exerts various biological activities, including antioxidant, anti-inflammatory, and even anti-cancer properties. In anti-photoaging effects, treatment with saponins extract prior UVA exposure can reduce UVA-mediated interstitial collagenase and gelatinase-B expression. Moreover, saponins extract also decreases MAPKs phosphorylation and NF-κB expression induced by UVA irradiation (115).

β-carotene

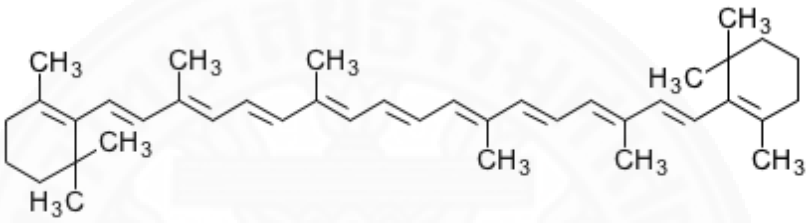

Figure 2.26 β-carotene structure (172)

β-carotene stores in skin from diets and supplementations, which the epidermis has greater concentrations than the dermis (173). It has been reported that β-carotene can decrease UVA-induced MMPs expression, including interstitial collagenase and stromelysins. Moreover, β-carotene acts as the precursor molecule that can be metabolized to retinoic acid and their retinoid pathways. Thus, β-carotene may be a good candidate for anti-photoaging treatment (143).

2.3.7.2 Free radical scavengers

Another strategy to diminish the damaging effects of solar radiation on skin is the use of antioxidants or free radical scavengers.

In this review, the studies about antioxidants or free radical scavengers that used in photoaging treatment have been collected. Table 2.7 summarizes free radical scavengers that have been reported in treatment of photodamaged skin.

Table 2.6 Demonstrate the free radical scavengers of natural compounds or extracts

Botanical compounds/extracts	Study
Coriander leaf extract (122)	• Reduced UV-induced ROS
Galla chinensis (GAC) (121)	• Decrease ROS levels
Neonauclea reticulate (107)	• Scavenge the intracellular ROS

Coriander leaf extract

Figure 2.27 Coriander leaves (122)

Coriandrum sativum L. (CS), also called coriander, belongs to *Apiaceae* (*Umbelliferae*) family which has been widely planted for long time in many countries, including Asia, Middle East, Africa, and Latin American. Naturally, both aerial parts and grains of coriander have been used for food components. Coriander leaves have various principle chemical ingredients, including flavonoid glycosides, caffeic acid, and essential volatile oils (174).

Coriander leaf extract can act as antioxidants to guard against oxidative stress induced by UV. It can essentially inhibit UVB-induced interstitial collagenase secretion and upregulate the production of type I procollagen by quenching

ROS in vitro studies. Moreover, Coriander leaf extract can prevent the elastic fibers and collagen degradation by stimulating TGF-β1 expression and inhibiting interstitial collagenase expression in vivo studies (122).

Galla chinensis

Galla chinensis (GAC), a natural plants, has been widely used in traditional medicine for long time ago because of its curative effects (162). In addition to inhibition of MMPs, *Galla chinensi*s has the ability to reduce the ROS levels, resulting in decreasing harmful effects of UV-mediated photodamaged skin (121).

Neonauclea reticulata

Figure 2.28 *Neonauclea reticulata (Havil.) Merr* (107)

Neonauclea reticulata (Havil.) Merr belongs to *Rubiaceae* family which is high in flavonoid constituents (149). *Neonauclea reticulata* extract can act as the ROS scavenger which quench free radicals induced by UVB exposure. This suggests that *Neonauclea reticulata* extract is a powerful compound for photodamaged treatment (107).

2.3.7.3 Cell signaling inhibitors

In addition to inhibition of MMP expression and activity, the use of cell signaling inhibitors is one strategy to reduce harmful effects of solar irradiation.

In this review, the studies about cell signaling inhibitors that used in photoaging treatment have been collected. Table 2.8 summarizes cell signaling inhibitors that have been reported in treatment of photodamaged skin.

Table 2.7 Demonstrate the cell signaling inhibitors of natural compounds or extracts

Group	Botanical compounds/extracts	Mechanisms
MAPKs signaling inhibitor	Cultivated ginseng (4)	• Suppress UV-induced MAPKs and NF-κB activation, leading to low levels of MMP-1 and MMP-13
	Epigallocatechin-3-gallate (EGCG) from green tea (14)	• Inhibit UV-induced MAPKs, resulting in decrease the expression of MMP-1, MMP-8, and MMP-13
	Magnolol extract from *Magnolia officinalis* (151)	• Suppress the phosphorylation of MAPKs family
	Mangiferin extract from *Anemarrhena asphodeloides* (175)	• Decrease UV-induced MMP-9 expression via MAPKs signaling pathway
	Michelia alba leaves extract (116)	• Inhibit UV-induced MMP-1, MMP-3, MMP-9 via MAPKs pathway

Table 2.7 Demonstrate the cell signaling inhibitors of natural compounds or extracts (Cont.)

Group	Botanical compounds/extracts	Mechanisms
MAPKs signaling inhibitor	*Neonauclea reticulata* (107)	• Block the phosphorylation of MAPKs family (ERK, JNK, and p38), leading decrease MMP-1, MMP-3, and MMP-9 levels
NF-κB signaling inhibitors	Decursin extract from *Angelica gigas Nakai* roots (85)	• Block UV-induced NF-κB activation, resulting in decrease MMP-1 and MMP-3 expression
	Quercitin (125)	• Inhibit UV-indcued NF-κB signaling pathways, leading to decrease pro-inflammatory cytokines secretion

(1) MAPKs signaling inhibitors

Cultivated ginseng

Figure 2.29 *Panax* gingseng (4)

The most extensive medical herbs used in conventional Chinese medicine is *Panax ginseng C.A. Meyer* (176). It has various pharmacologic and biologic functions such as anti-oxidative, anti-aging, and anti-inflammatory activities (177). Phenolic and Ginsenosides ingredients have been extracted from Korean cultivated ginseng. In Japan and China, it has been extensively used to cure fatigue and other diseases (178).

Cultivated ginseng can inhibited NF-κB and MAPKs signaling pathways induced by UVB, consequently suppressed the secretion of MMPs (such as collagenase-1, and collagenase-3) levels and activity (4).

Epigallocatechin-3-gallate

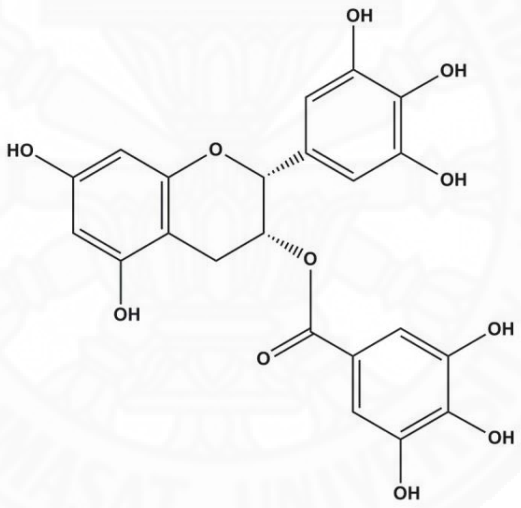

Figure 2.30 Epigallocatechin-3-gallate structure (179)

Camellia sinensis or green tea consists of polyphenols, which are more attraction because of their powerful antioxidants properties (180). The most important ingredient of green tea is epigallocatechin-3-gallate (EGCG) which is a polyphenolic component. Pre-treatment of EGCG in dermal fibroblasts can suppress UVB-induced the secretion of collagenases subgroup, including interstitial collagenase, neutrophil collagenase, and collagenase-3, via the inhibition of MAPKs signaling pathway (14).

Magnolol extract

Magnolol is extracted from *Magnolia officinalis*, which is a Chinese herb. *Magnolia officinalis* has several biological activities, including anti-inflammation, antidepressant, and anti-arthritic (168). In addition to MMPs inhibitors, Magnolol can suppress the phosphorylation of MAPKs family, leading to decrease the expression of MMPs (151).

Mangiferin extract

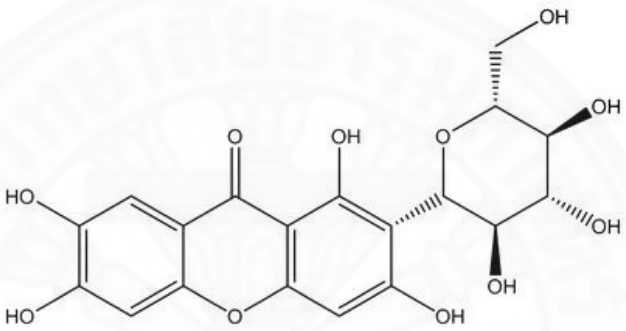

Figure 2.31 Mangiferin structure (181)

Mangiferin is extracted from *Anemarrhena asphodeloides*. In Japan, Korea, and China, *Anemarrhena asphodeloides* roots have been used for antipyretic, antidepressant, antidiabetic, anti-inflammation, and anti-pletelet aggregation (182). For anti-photoaging studies, Mangiferin extract can inhibit UVB-induced epidermal hypertrophy, rhytides formation, and collagen bundles degradation by inhibiting gelatinase-B expression through MAPKs blockage (175).

Michelia alba leaves extract

Figure 2.32 *Michelia alba* (116)

Michelia alba (MA), which belongs to the *Magnoliaceae* family, is a flower that renowned for perfume components and biological functions. For example, Some extracts of *Magnoliaceae* family leaves have an extended spectrum of antifungal and antimicrobial functions (183). MA flower, which has charming odor, is useful in prostatitis and bronchitis treatment. MA bark can also treat fever, malaria, and syphilis (184).

Pre-treatment of MA leaves extract can inhibit UVB-induced degradation of ECM proteins by suppressing MMPs expression such as interstitial collagenase, stromelysin-1, and gelatinase-B. In addition, MA leaves extract can prevent UV-induced photodamaged and skin cancers by suppressing UV-induced ROS production (116).

Neonauclea reticulate

Neonauclea reticulata (Havil.) Merr belongs to *Rubiaceae* family which is high in flavonoid constituents. Flavonoids are mostly found in vegetables, fruits, red wine, and green tea. They have capability in various biological functions such as suppress MMPs expression and antioxidants activities (149).

Pre-treatment with *Neonauclea reticulata* extract can inhibit the expressions of interstitial collagenase, stromelysin-1, and gelatinase-B via suppressing MAPKs pathways, including ERK, JNK, and p38 molecules. In addition to suppress MMPs expression, *Neonauclea reticulata* extract can stimulate collagen synthesis by upregulate fibroblasts proliferation and secretion of TGF-β (107).

(2) NF-κB signaling inhibitors

Decursin extract

Figure 2.33 Decursin structure (185)

Decursin, a coumarin compound, is extracted from *Angelica gigas Nakai* roots. In Korean folk medicine, decursin has been conventionally treated in anemia and other diseases (186). In aging research, decursin extract can prevent photoging by inhibited UV-induced NF-κB signaling pathway, consequently decreases MMPs expression including interstitial collagenase and stromelysin-1. Nonetheless, dercursin has no effect on AP-1 or MAPKs signaling pathway (85).

Quercetin

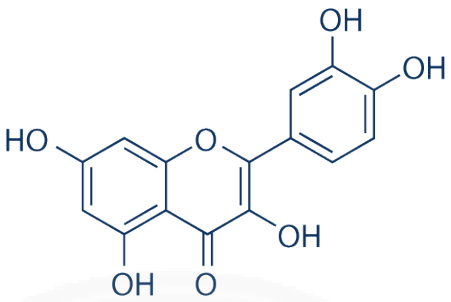

Figure 2.34 Quercetin structure (187)

Quercetin, belongs to flavonoid family, is found in various vegetables and fruits. Many researchers reported that quercetin have many biological effects, such as cell cycle regulation, anti-mutagenesis, apoptosis, and anti-angiogenesis. In photoaged research, quercetin can decrease UV-induced NF-κB activation. Subsequently, quercetin inhibited UV-induced inflammatory cytokines production including TNF-α, IL-1β, IL-6, and IL-8. However, MAPKs or AP-1 induced by UV radiation was not inhibited by quercetin (125).

CHAPTER 3
RESEARCH METHODOLOGY

The keywords following matrix metalloproteinases OR MMPs OR photoaging OR skin aging OR aging skin OR MMPs inhibitors were searched in Pubmed and MEDLINE.

The studies reporting in skin involvement, from year 2000, and published in English were included. Clinical trials or case studies, Experimental studies, and Systematic Reviews that they allowed the quantitative evaluation of the pathogenesis and treatment of photoaging were also included. Studies published in other languages or duplicated studies were excluded.

The abstracts of all chosen papers were reviewed to remove some papers that were not important or were not involved. The rest of them were read in full texts to find out whether they met the inclusion criteria.

As such, the search of the databases resulted in 7,564 journals. After checking the inclusion and exclusion criteria, 2,612 papers were remained. After reading the abstracts, 429 studies were selected to read in full.

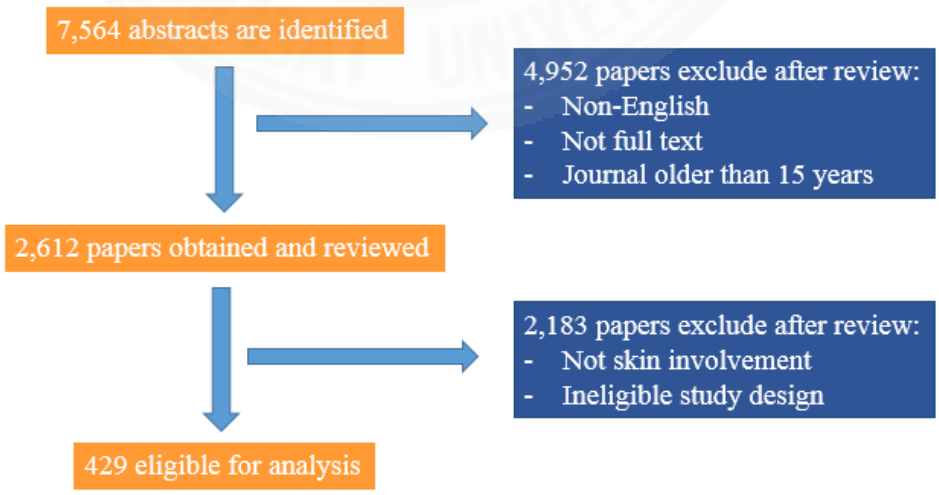

Figure 3.1 Methodology of research

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Results and discussion

4.1.1 Pathogenesis of UV-induced photodamaged skin

The action spectrum for UV-induced skin damage is divided into UV-A (320-400 nm) and UV-B (290-320 nm). The amount of UV-B reaching the earth's surface is lesser than that of UV-A; however, its intensity is high enough to cause photoaging and skin cancer (115, 116). Nonetheless, both UV-A and UV-B irradiation can induce oxidative stress in human skin, leading to temporal and persistent genetic impairment, up-regulation of activator protein (AP)-1 activity, and increased MMP expression (Table 4.1) (106, 120).

Table 4.1 Role of UV-A and UV-B in photoaging induced MMPs (120, 188-190)

MMPs	UV-A	UV-B
Collagenases		
MMP-1	+	++
MMP-8	NA	NA
MMP-13	+	+
Gelatinases		
MMP-2	+	+
MMP-9	+	+
Stromelysins		
MMP-3	+	++
MMP-10	+	++
Matrilysins		
MMP-7	+	+
MMP-26	NA	NA

Abbreviations: ++, highly upregulated; + upregulated; NA, no reported.

Table 4.1 Role of UV-A and UV-B in photoaging induced MMPs (120, 188-190) (Cont.)

MMPs	UV-A	UV-B
MT-MMPs		
MMP-14	NA	NA
MMP-15,16	NA	NA
Other types		
MMP-12	++	+

Abbreviations: ++, highly upregulated; + upregulated; NA, no reported.

Photoaging is caused by an imbalance in equilibrium between the accumulation and degradation of ECM components that provide structural and functional support to the skin tissue. Cumulative exposure to the sun results in continuous degradation of ECM proteins such as collagen and elastin, and a decreased rate of renewal/synthesis of collagen (86, 107, 116). Collagen is the primary insoluble fibrous protein in the ECM and in connective tissue. Type I collagen is the most abundant subtype of collagen found within connective tissue of the skin, followed by small amounts of type III collagen. Fibroblasts, located within the dermis, mainly synthesize collagen, which imparts strength and elasticity to the skin (86, 107, 116).

Degradation of collagen and elastin is normally regulated by MMPs and by the activity of their natural inhibitors, tissue inhibitor of metalloproteinases (TIMPs). Increased MMP activity is an important factor influencing the development of age-related changes in skin (Table 4.2) (191).

Table 4.2 Classification of human MMPs and their function in relation to photoaging

MMP subgroup	MMP number	Alternate name	Role in photoaging
Collagenases	MMP-1	- Interstitial collagenase -Type I Collagenase	- Collagen type I and III degradation
	MMP-8	- Neutrophil collagenase	- Limited role
	MMP-13	- Collagenase-3	- Limited role
Gelatinases	MMP-2	- Gelatinase-A - 72 kDa type IV collagenase	- Collagen type IV degradation
	MMP-9	- Gelatinase-B - 92-kDa type IV collagenase	- Collagen type IV degradation
Stromelysins	MMP-3	- Stromelysin-1 - Proteoglycanase - Transin-1	- Activate MMP-1, MMP-7, and MMP-9
	MMP-10	- Stromelysin-2 - Transin-2	- Activate pro-MMPs
	MMP-11	- Stromelysin-3	NA
Matrilysins	MMP-7	- Matrilysin-1 - Pump-1	- Elastin degradation
	MMP-26	- Matrilysin-2 - Endometase	NA
Membrane-type	MMP-14	- MT1-MMP	NA
	MMP-15	- MT2-MMP	NA
	MMP-16	- MT3-MMP	NA
	MMP-17	- MT4-MMP	NA
	MMP-24	- MT5-MMP	NA
	MMP-25	- MT6-MMP	NA

Abbreviations: NA, no reported.

Table 4.2 Classification of human MMPs and their function in relation to photoaging (Cont.)

MMP subgroup	MMP number	Alternate name	Role in photoaging
Other types	MMP-12	- Metalloelastase	- Elastin degradation
	MMP-19	- RASI-1	NA
	MMP-20	- Enamelysin	NA
	MMP-21	NA	NA
	MMP-22	NA	NA
	MMP-23	NA	NA
	MMP-28	- Epilysin	NA

Abbreviations: NA, no reported.

In the skin, epidermal keratinocytes and dermal fibroblasts mainly secrete MMP-1(interstitial collagenase or collagenase 1), a collagenase that degrades fibrillar collagens type I and III into specific fragments at a single site within the central triple helix. Other MMPs such as gelatinases, further hydrolyze these fragments, ultimately impairing the function of the collagen-rich dermis (2, 5, 188, 192).

UV irradiation induces increased synthesis and expression of MMP-1 by dermal fibroblasts, which is stimulated by the generation of excess reactive oxygen species (ROS), and plays a critical role in photoaging. UV irradiation induces excess intracellular ROS such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$) (107). ROS, a secondary messenger, activates the mitogen-activated protein kinase (MAPK) family. MAPKs are a family of proline-directed Ser/Thr kinases comprising extracellular signal-regulated kinases (ERKs), p38, and c-Jun NH2-terminal kinase (JNK). ERK is important to stimulate the expression of c-Fos, whereas p38 and JNK activation are crucial for the expression of c-Jun. c-Jun in combination with c-Fos forms the transcription factor AP-1, which plays an essential role in the transcriptional regulation of MMP-1, MMP-3, and MMP-9

resulting in the degradation of collagen (2, 107, 126). Additionally, AP-1 inhibits transforming growth factor-β (TGF-β) signaling, a major regulator for the production of procollagen type I in human skin. Impairment of the TGF-β pathway leads to decreased synthesis of procollagen (121-123). Besides AP-1, nuclear factor-kappa B (NF-κB) is another important transcription factor that is activated in response to UV irradiation. NF-κB is a universal transcription factor that regulates the gene expression of growth factors, chemokines, cytokines, and cell adhesion molecules, in healthy as well as numerous diseased states. Generation of ROS induces NF-κB-mediated transcriptional activation and regulation of MMP gene expression. Thus, this factor is important to mediate the responses of UV irradiation. NF-κB activity is reported to be responsible for the up-regulation of MMPs such as MMP-1 and MMP-3 in dermal fibroblasts (124-126). Thus, both AP-1 and NF-κB are involved in the process of photoaging.

UV-induced AP-1 activation enhances the expression of MMP-1, MMP-3, and MMP-9. MMP-3, known as stromelysin-1, differs from collagenases because of its inability to digest collagen type I. However, it does degrade a large number of ECM proteins, such as type IV, V, IX, and X collagens, gelatin, fibrillin-1, fibronectin, laminin, and proteoglycans. The primary function of MMP-3 is the activation of pro-MMPs such as collagenases, gelatinase B, and matrilysins during ECM turnover. In particular, the production of fully active MMP-1 MMP-3 is essential to partially activate pro-MMP-1 (6, 37, 38). MMP-10, known as stromelysin-2, cleaves various ECM proteins and is involved in the activation of pro-MMPs. However, the catalytic function of collagen type IV and type V is quite weak compared to the MMP-3 activity (6, 189).

MMP-9, known as gelatinase B or 92-kDa type IV collagenase, is a member of the gelatinase subgroup of MMPs, whose expression is largely dependent on the activation of AP-1. MMP-9 is produced by human keratinocytes and can digest collagen type IV, an important component of the basement membrane in skin. The epidermal basement membrane is responsible for the epidermal-dermal adhesion, which is crucial for epidermal integrity. It is also important in controlling epidermal differentiation (5, 6, 175, 189). Like MMP-9, MMP-2 (known as gelatinase A or 72-

kDa type IV collagenase) is able to cleave collagen type IV (193). Additionally, both these gelatinases can degrade other substrates such as collagen type V, VII, and X, fibronectin, and elastin. They are essential in degrading fibrillar collagen fragments after their initial degradation by collagenases (37, 86, 152).

Collagenases refer to a class of MMPs with the ability to degrade native collagen without unwinding the triple helical assembly of the substrate. Interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase 3 (MMP-13) belong to this group (6). They have similar configuration and enzymatic functions, despite small differences in substrate specificity. As mentioned above, MMP-1 plays an important role in the photoaging process. Recent studies suggest a limited role for MMP-8 in UV-mediated collagen damage in the skin. Although this enzyme was found to be induced by UV light, it is up-regulation was minimal (194). MMP-13 shows higher cleavage specificity for collagen type II, a major collagen present in the cartilage, compared to collagen type I and III. MMP-13 is five times less potent than MMP-1 in cleaving collagen types I and III; however, it is 5–10 times more potent in cleaving collagen type II (6). Hence, during photoaging, MMP-8 and MMP-13 probably contribute very little to the overall structural damage to collagen.

In addition to the degradation of collagens in skin, changes in the level of elastin have also been well documented in the process leading to photoaging. Elastin is a major component that contributes to the function of recoil and resilience, although it constitutes only 2%-4% of the total protein content of the skin. Reduced levels of elastin are associated with various diseases such as atherosclerosis and arthritis. Degradation of elastin results in an aged appearance of the skin (195-197). MMP-12, known as macrophage metalloelastase, is the most effective MMP against elastin. Macrophages and fibroblasts secrete MMP-12 in response to acute UV radiation. MMP-12 plays a crucial role in the development of solar elastosis as indicated by the association between the expression of MMP-12 and the amount of elastotic material in the upper dermis of photodamaged skin (193, 195, 197). The process of solar elastosis refers to the collection of dystrophic elastotic material in the dermis (120, 195). In addition to elastin, MMP-12 can cleave many other substrates belonging to the ECM, such as collagen type IV fragments, fibronectin, fibrillin- 1, laminin, entactin,

vitronectin, heparin, and chondroitin sulfates. MMP-12 is also responsible for the activation of other pro-MMPs, such as pro-MMP-1, MMP-2, MMP-3, and MMP-9 (6, 196). In addition to MMP-12, MMP-7 (called matrilysin) can efficiently degrade elastin. Upon UV irradiation, MMP-7 can cleave not only elastin but also many other substrates of the ECM, such as collagen type IV, entactin, fibronectin, laminin, and cartilage proteoglycan aggregates (6, 193).

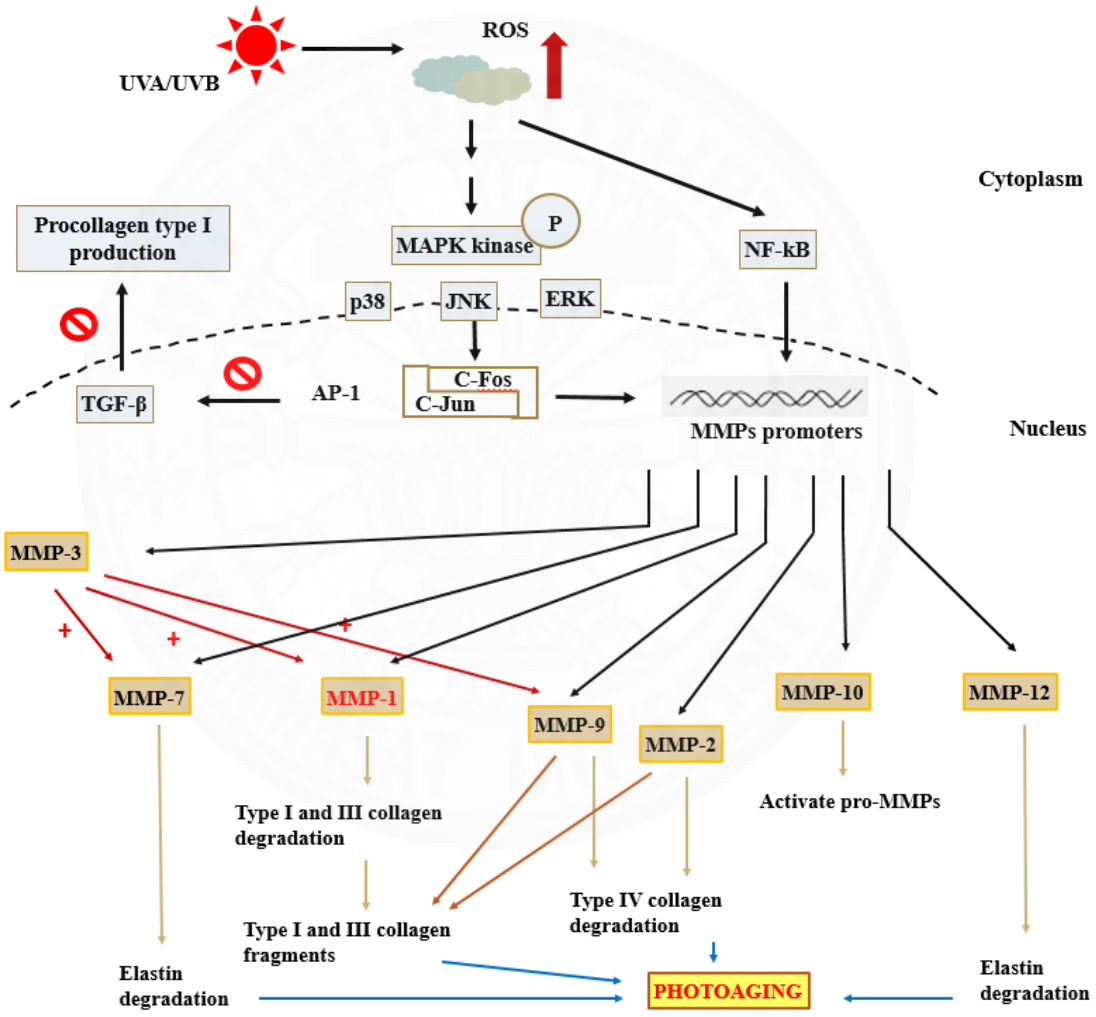

Figure 4.1 Schematic diagram showing the roles of MMPs in UV-induced photoaging

Figure 4.1 summarize the MMPs involvement in UV-induced photodamaged leading to better understand the molecular mechanisms and will benefit in the development of MMPs inhibitors.

4.1.2 Pathogenesis of IR-induced photodamaged skin

In the same manner with UV-B or UV-A radiation, it is currently agreed that IR radiation may wield profound biological effects on skin of human skin. Still, it remained largely unknown the primary molecular mechanisms underlying the pathological effects of IR irradiation (117, 127).

Infrared irradiation, with wavelengths from 760 nm to 1 mm, is divided 3 parts, namely (i) near IR-A (760-1,400 nm wavelengths), (ii) middle IR-B (1,400-3,000 nm wavelengths) and (iii) far IR-C (3,000 nm - 1 mm wavelengths) (117). This partition accords in part with the molecular effects of IR such as the wavelength-dependent absorption in different skin layers. The more increasing of wavelength in the IR spectral region, the less penetration through the skin and subcutaneous tissues. Short wavelength IR-A ray enters the subcutaneous tissue without elevating the skin's surface temperature clearly, while IR-B and IR-C is took in entirely in the epidermal compartment and resulting in skin's surface temperature elevating extending from pleasant warmth to thermal burn (117, 131).

As stated above, IR wavelengths are much extensive than those within the UV spectrum and therefore perforate into the skin more deeply. This produces additional chromophores and dermal fibroblasts, which are significant for skin's structural integrity and dermal elasticity, main object of this kind of radiant energy (118). Any type of radiation must be absorbed by a chromophore to be able to initiate a biological effect. Components of the mitochondrial respiratory chain like cytochrome c oxidase have been recommended as possible photoreceptors for IR radiation (130).

The generation of ROS in mitochondria of dermal fibroblasts starts IR radiation-induced skin damage (127, 128). Essentially, mitochondrial ROS synthesis is of functional importance for IR radiation-mediated the expression of gene by upregulation of MAPK signaling pathways. Due to IR irradiation, activated MAPKs move into the nucleus, where they phosphorylate and stimulate transcription factors including c-Fos, c-Jun, and p38 resulting to the formation and activation of the transcription factor AP-1, which plays an essential role in the transcriptional regulation

of MMPs expressions, including interstitial collagenase, collagenase-3, gelatinase-B, and stromelysin-1 (118, 129, 130). In connection with this, IR radiation-induced MMPs expression is significant for the reason that it can break down the ECM proteins, which would eventually cause the building of coarse rhytides, a clinical hallmark of skin with photoaging (117, 127, 198). IR exposure also suggested to decrease collagen type I production by lessening the secretion of procollagen type I-activating TGF-β in photoaged skin, so the mechanism of elevated MMPs expression and activity is probably not the only reason for IR-induced photodamaged skin (127). In addition, chronic IR irradiation can result to a markedly elastosis, which mimics the damage effects caused by UV (112).

In addition to IR radiation-induced MMPs expression, IR exposure can augmented UV-induced MMPs activity such as collagenase-3, stromelysin-1, and gelatinases. In UV-radiated skin, Collagen and elastic fiber are increased. These increases in connective tissues may elevate the thickness of dermis and skin fold and result to the building of skin rhytides by UV radiation. IR radiation can strengthen the UV-mediated collagen and elastin fibers production, and UV-mediated hypertrophy of the skin. These IR biological effects may worsen skin wrinkling induced by UV exposure. But, the molecular pathogenesis that identify how IR strengthen UV-mediated wrinkle formation continue to be researched (112).

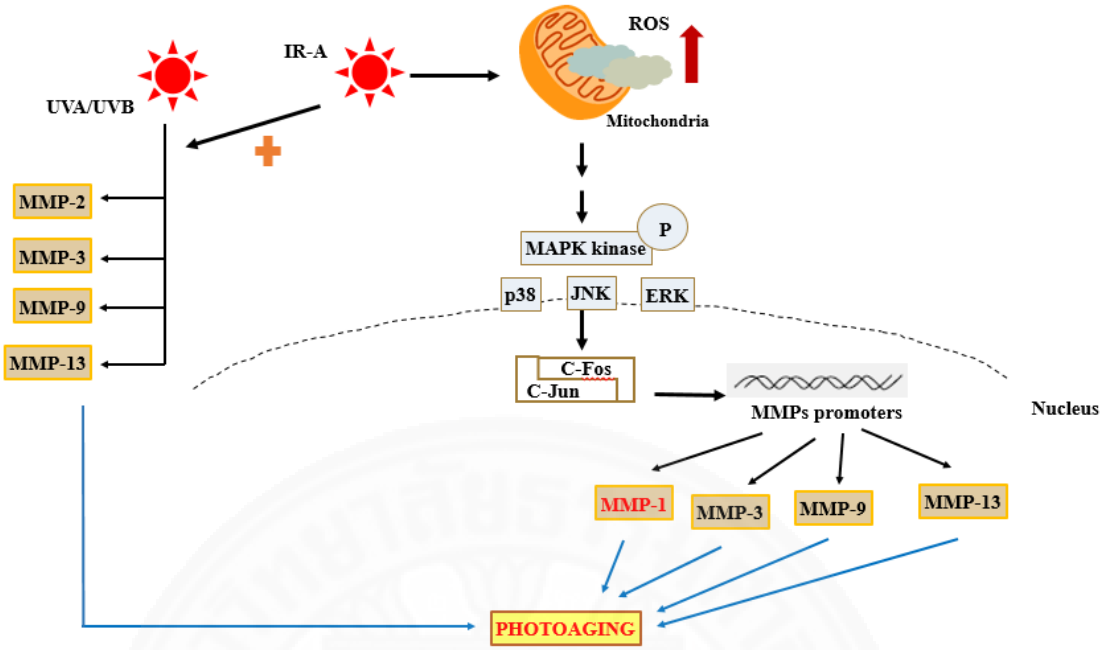

Figure 4.2 Schematic diagram demonstrating the roles of MMPs in IR-induced photoaging and IR augments UV-induced photoaging

Moreover, activation of other signaling cascades regulating gene expression may also triggered by IR radiation (117). When skin temperature is over 39°C, heat shock-mediated ROS are produced in human skin at generous amounts and caused the quick activation of MAPK signaling pathways resulting in MMPs production including interstitial collagenase, stromelysin-1, and gelatinase-B, and subsequent degeneration of ECM proteins (131, 132). Heat is also considered to generate metalloelastase which has a capability of destruction of the preceding elastic fiber network, consequnetly sharing to the building up of elastotic material in photodamaged skin (131).

Transient receptor potential vanilloid-1 (also called TRPV1, or capsaicin receptor) belongs to the nonspecific cationic channel family. TRPV1 acts as the heat sensor, regulates the heat shock-induced the expression of various MMPs (131, 132). Heat-induced TRPV1 activation initiates an influx of divalent cations (such as Ca^{2+}) (199). Ca^{2+} can control MMPs production and activation. It may be associated in regulating the metalloelastase activity. High concentration of extracellular calcium

produce the gelatinase-B secretion in human epidermal keratinocytes. Changing in intracellular calcium concentration can regulate the production of interstitial collagenase from migrating epidermal keratinocytes (200). Therefore, Ca^{2+} influx related with TRPV1 might function as the signaling pathway of photoaging.

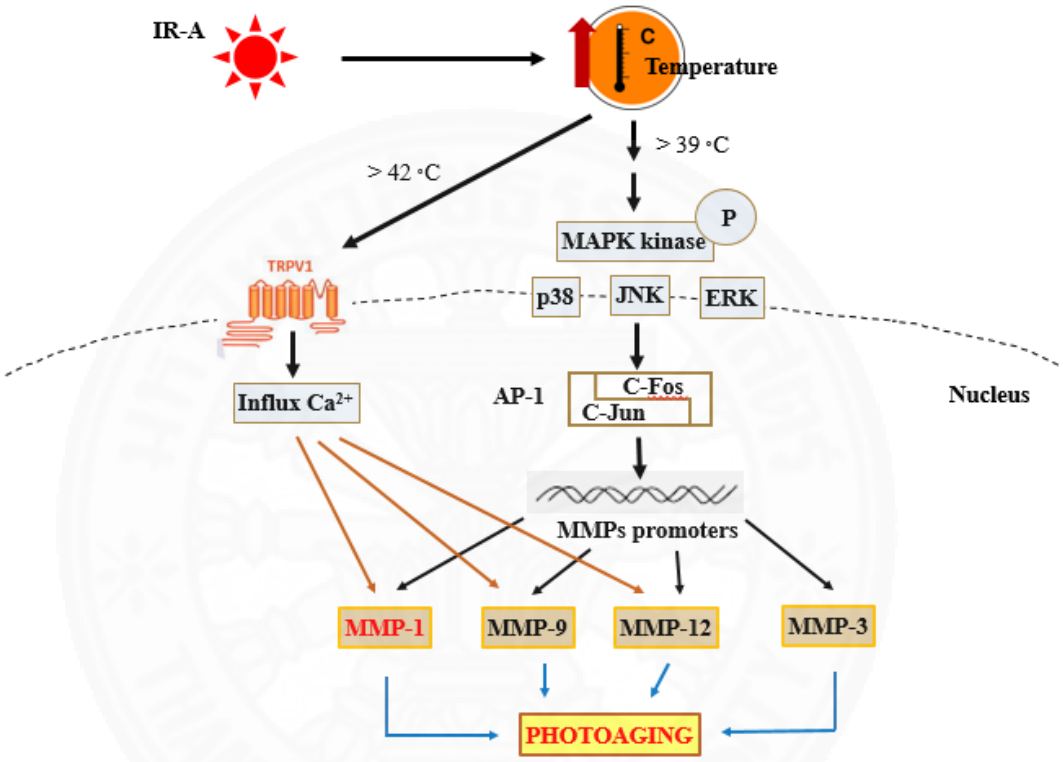

Figure 4.3 Schematic diagram demonstrating the roles of TRPV1 in IR-induced photoaging

As a whole, these results means that IR-irradiation is capable of changing gene expression which demonstrates a pro-aging phenotype of the skin (129). Cumulative exposure of IR and heat, including stoves and fires, leads to the cutaneous lesion termed as erythema ab igne. The clinical characteristics of erythema ab igne are reticular hyperpigmentation and telangiectasia. This skin lesion is histologically characterized by epidermal atrophy, dermal hemosiderin and melanin accumulation, and vasodilation (117, 131, 132). After several years, thermal keratosis are developed which is histologically characterized by epidermal thickening, keratinocyte dysplasia,

and dermal elastosis. The alterations in these lesions are similar to the elastotic changes in photodamaged skin (102, 117, 131). Like actinic keratosis, thermal keratosis are premalignant lesions exhibiting keratinocyte dysplasia, which may progress into skin cancers, including invasive squamous cell carcinoma (117).

4.1.3 Pathogenesis of inflammation and aging skin

Immunosenescence refers to undergoing profound age-related changes of an individuals' immune system as they age. With age, there are gradual increase in the secretion and circulation of pro-inflammatory cytokines including interleukin (IL)-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ with age, resulting to a systemic chronic low-grade state of inflammation. So, the term "inflamaging" means continuous, low-grade inflammation related to aging (133, 134). Such chronic inflammation could accumulate with time and slowly resulting to tissue damage. It is recognized as one of the trigger factors for various age-associated diseases, including atherosclerosis, diabetes, and especially skin aging (134). In skin, there is chronological (or intrinsic) skin aging and UV-induced skin aging (or photoaging). Both inflammation and ROS accumulation are generally accepted to be the driving causes in both types of aged skin.

In photodamaged skin, UV radiation changes dermal ECM contents and also elevates the inflammatory symptoms such as upregulation of inflammatory mediators and the infiltration of inflammatory cells (135). 'Inflammaging'-involved cell types can be natural immune cells, like monocytes or macrophages, neutrophils, and non-immune cells like keratinocytes (133).

Inflammation response is stimulated by UV radiation, resulting to edema and triggering the recruitment of neutrophils. Infiltrating neutrophils are vital players during skin damage after exposure to solar radiation. These cells synthesize huge amounts of ROS and pro-inflammatory cytokines, such as IL-1β/α, IL-6, TNF-α, and IFN-γ, contributing to the inflammatory response (201, 202). These pro-inflammatory cytokines originate the inflammatory signaling pathways by stimulating resident cells to secrete chemokines and by activating cell-adhesion molecules responsible for transendothelial migration of immune cells that required for initiating

inflammatory response. The production of these cytokines functions as the primary role in the recruitment and activation of inflammatory cells, resulting to damage of the skin after solar irradiation (202, 203).

One of the major pro-inflammatory cytokines associated with the pathogenesis of aging skin is TNF-α. It acts as the key regulator which activates many responses such as infiltration and stimulation of inflammatory cells in inflamed skin. TNF-α also be an important mediator in various inflammatory skin situations by the complicated processes such as extrinsic and intrinsic skin aging. There are numerous researches that TNF-α can stimulate MAPKs signaling pathways, resulting to activating interstitial collagenase expression and consequently degradation of the ECM components. TNF-α also induces many other pro-inflammatory cytokines, including IL-1α and IL-6, leading to more activating inflammatory responses (135).

Besides persistent inflammation, oxidative stress is also the major pathologic responses in UV-induced photodamaged skin (201). ROS can oxidize and impair cellular lipids, proteins, and even DNA. It also induce oxidative stress-mediated transcription factors, like NF-κB, the key pro-inflammatory transcription factor which activate various cytokines secretion, such as IL-1α, IL-6, and TNF-α (202). Besides the pro-inflammatory cytokines production, ROS has ability to mediate the expression of genes, such as MMPs gene expression (204).

In addition to ROS and inflammatory cytokines production, the activation of neutrophils is utilized for clearance of UV-regulated apoptotic cells, and to eradicate the skin cells with oxidized surface lipids. The enzymes they produce, such as neutrophil elastase, interstitial collagenase, and gelatinase-B add up to the process of photoaging (134). The combination with oxidative stress and neutrophil elastase stimulated by low-grade inflammation are involved in the destruction of ECM proteins indirectly by MMPs upregulation (205).

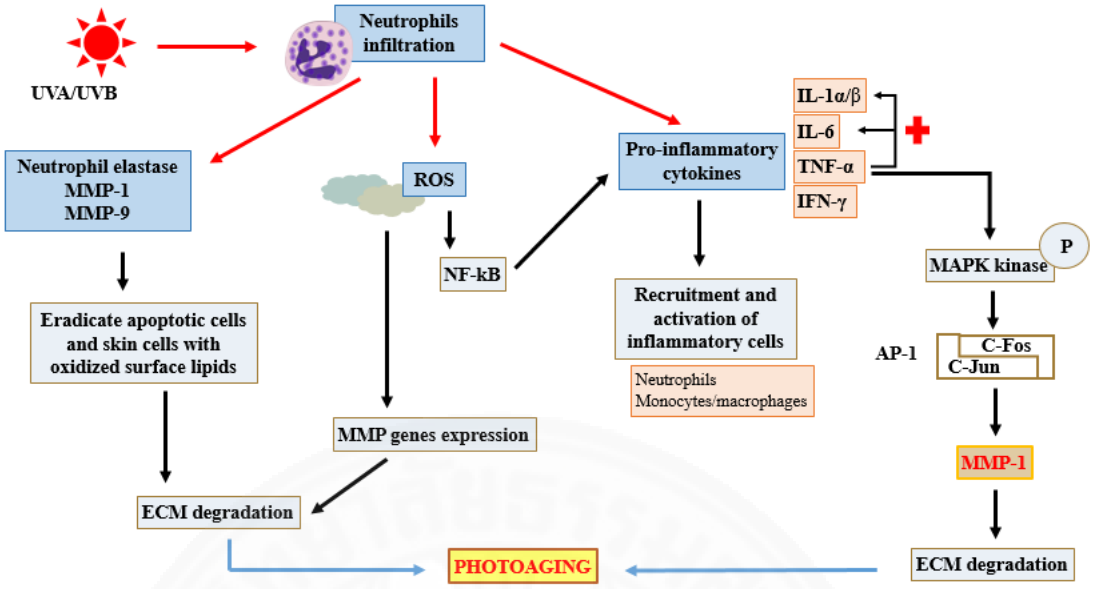

Figure 4.4 Schematic diagram demonstrating the roles of neutrophils in inflammaging

In fact, monocytes/macrophages also play an essential role in this event due to the domination of these to the infiltrates after a few hours to eradicate apoptotic cells and oxidized lipids. Macrophages produce several MMPs that can aid in the migration within the skin by degrading ECM. Macrophages also synthesize ROS, which can initiate the transcription of MMPs in dermal fibroblasts. Multiple UV damage to the skin leads in repeated cycles of macrophages infiltration after every exposure. The multiple macrophages infiltration will result to repeated destruction to the ECM proteins of the dermis because of the production of MMPs and ROS (134, 203). This is worsen in aged skin, where the amount and cellular function of aged fibroblasts decline and in consequence, cannot effectively restore or rejuvenate the ECM components. Moreover, aged macrophages are unable to migrate properly. This results in the accumulation of aged macrophages in the dermis and then more aggravate the impairment (134).

Keratinocytes are the primary target of UV radiation and serve as essential function in numerous responses to the photodamage by producing and upregulating different pro-inflammatory activators, like IL-1β and TNF-α (134, 203). Cytoplasmic Ca2+ increased by UV exposure is necessary for stimulation of caspase-1 or IL-1β-converting enzyme. Consequently, Caspase-1 stimulate proIL-1β into an

active enzyme IL-1β, which secreted by keratinocytes. IL-1β production directly induces inflammatory process and indirectly induces interstitial collagenase expression, which both of these pathways are involved in the pathogenesis of aging (203, 206).

Fibroblasts, originate from mesenchymal cells, are associated with the inflammatory and immune responses because of their ability to secrete growth factors, chemokines, cytokines, and other active signaling molecules. Fibroblasts are involved in the inflamm-aging situation in elderly due to there is mild elevated of basal IL-6 production in old versus young fibroblasts. In contrast to IL-6, the secretion of IL-8 is mild lowered in aged fibroblasts compared to young fibroblasts (133). Moreover, ROS generation can activate lipid peroxidation of cell membrane and deteriorate dermal fibroblasts (201).

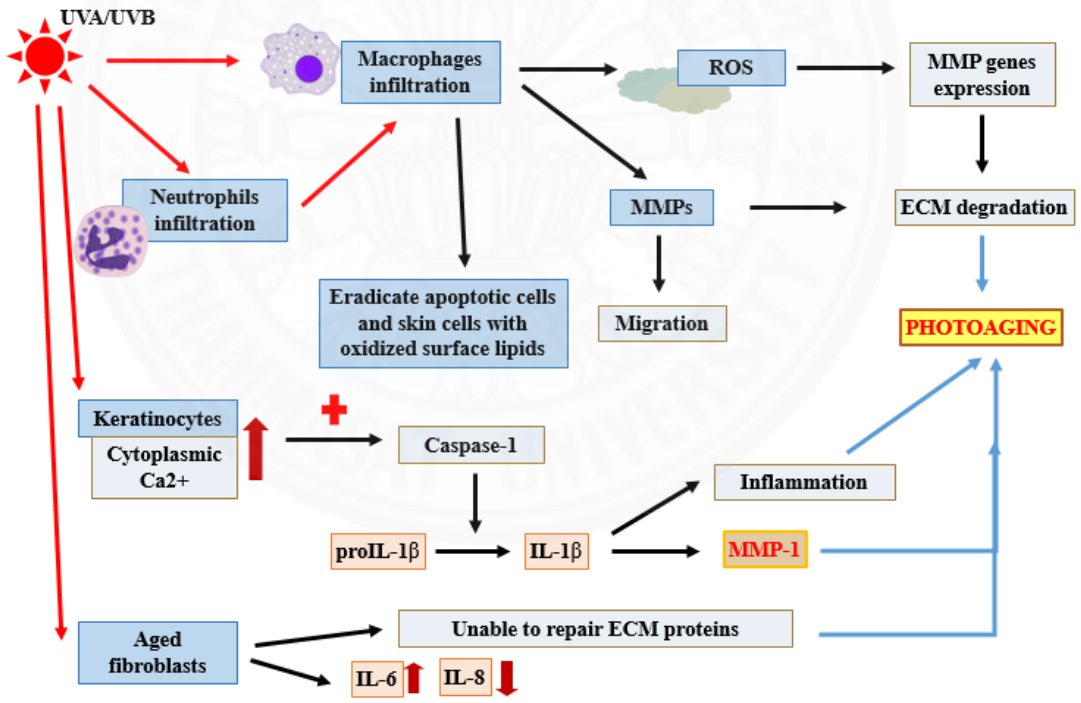

Figure 4.5 Schematic diagram demonstrating the roles of macrophages, keratinocytes and aged fibroblasts in inflammaging

Taken together, skin inflammation is nearly related to skin aging because the cytokines and inflammatory mediators have a critical role in the exhibition of these characteristics of aged skin (135). In addition, age-related changes slowly elevates the production and circulation of pro-inflammatory cytokines, resulting to a chronic low-grade state of inflammation (Figure 4.6) (133).

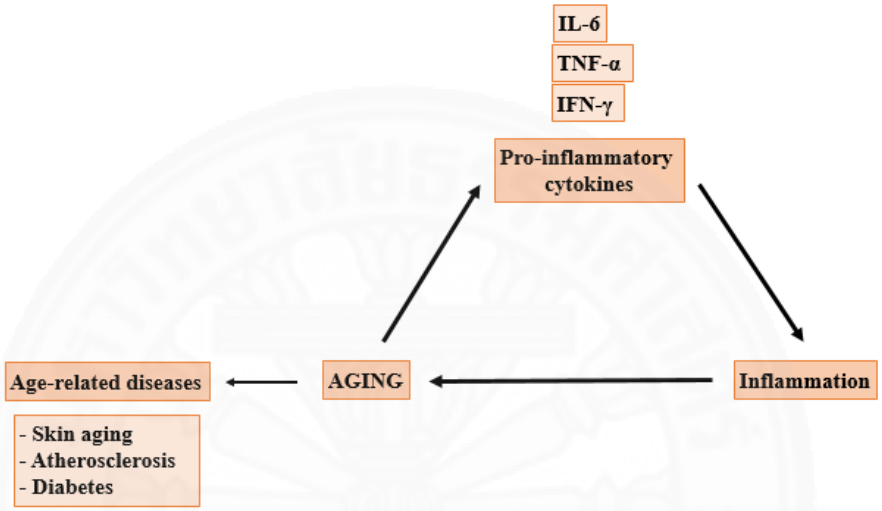

Figure 4.6 Demonstrate the association between aging and inflammation

4.1.4 Solar elastosis

Solar elastosis is the hallmark of photoaging skin, which is a degenerative state of elastic fibers in the dermis caused by prolonged exposure from the sun (102, 103). Solar elastosis is generated by a cycle of elastic fiber destruction succeeded by ECM production and reassembly into an organization not the same as the first structure. Solar elastosis has various clinical features. In its general form of solar elastosis, shown as yellow discoloration, skin thickening, and roughly skin wrinkles (103, 104). In the early stage of solar elastosis, there is an accumulation of insoluble disorganized elastin and microfibrillar proteins (fibronectin), seen clinically as waxy, thickened, and furrowed facial skin. In its advanced stage, deterioration of dermal elastin results in a mottled appearance that is clinically associated with a loss of skin elasticity (120).

In fact, histopathologic changes in degenerative dermal elastic tissue that takes place in photodamaged skin refers to solar elastosis. The predominant histological characteristic of solar elastosis is disordered masses of degraded elastic fibers that degenerate to produce an formless mass, consist of tangled tropoelastin and fibrillin and appear basophilic degeneration of dermal elastic fibers, set apart from the epidermis by a constricted band of normal-appearing collagen with collagen fibers aligned horizontally (103).

Despite the term “Solar elastosis” refers to the collection of dystrophic elastotic material in the dermis, little is known about the exact mechanisms leading to the accumulation of elastotic material in photodamaged skin (120, 195, 207). Repeated exposure of UV radiation leading to dermal MMP accumulation, whether by progressive accumulation of dermally synthesized enzymes or by a diffusion from the epidermis (120).

MMP-12, known as macrophage metalloelastase, plays a crucial role in the development of solar elastosis as indicated by the association between the expression of MMP-12 and the amount of elastotic material in the upper dermis of photodamaged skin (193, 195, 197). Macrophage metalloelastase is secreted by macrophages, fibroblasts, normal keratinocytes, keratinocyte-derived tumors, and activated T cells (120). Macrophage metalloelastase has a broad range of substrate specificity, being able to cleave type IV collagen, elastin, fibronectin, vitronectin, and laminin (208). This enzyme is the most active MMP against elastin. Thus, macrophage metalloelastase has an important role in elastin remodeling in solar elastosis (120, 195).

In addition to macrophage metalloelastase, MMP-7 (called matrilysin) can efficiently degrade elastin too (6). Matrilysin is the smallest MMP due to lacking of the hemopexin domain. Matrilysin has a broad range of substrate specificity, being able to cleave type IV collagen, elastin, fibronectin, vitronectin, laminin, and gelatins (208). Nonetheless, other MMPs cannot rule out the role in the pathogenesis of solar elastosis, because gelatinases subgroup also has elastotic activity (120).

Besides degradation by protease enzymes, repeated UV exposure-stimulated epidermal keratinocytes and dermal fibroblasts can secrete tropoelastin mRNA expression in human skin in vivo, leading to increase synthesis of abnormal elastin and the accumucation of elastotic materials in photoaged skin (195, 207). Under normal conditions, epidermal keratinocytes are not able to be an elastin-producing cells. Furthermore, increased abnormal fibrillin production and accumulation have been reported within the dermis of photodamaged skin. The fibrillins are major components of microfibrils, which are either associated with elastin in elastic fibers or elastin-free bundles (195).

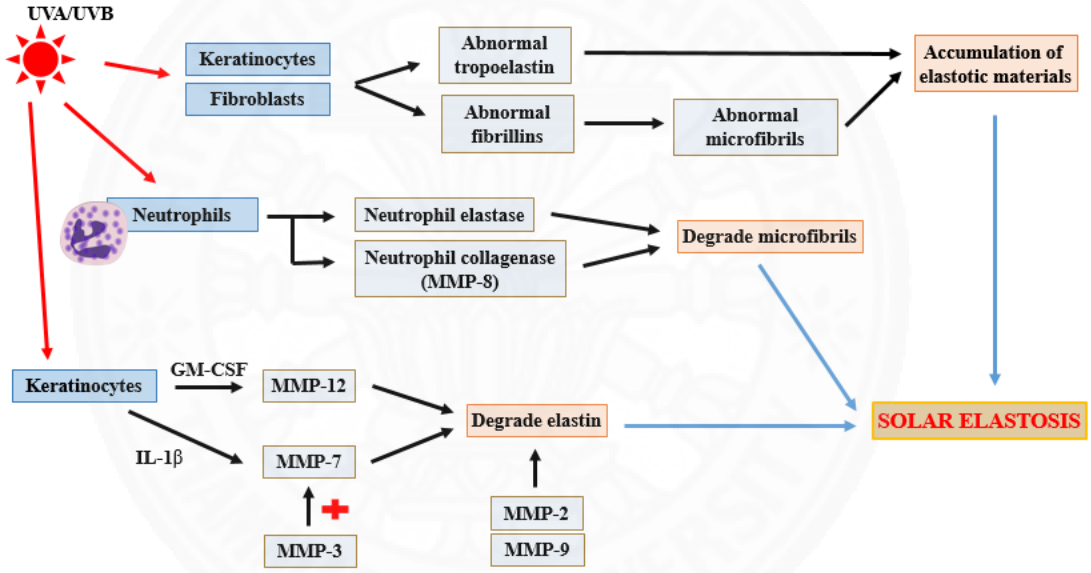

Figure 4.7 Schematic diagram demonstrating the roles of MMPs in UV-induced solar elastosis

As a whole, it may be proposed that the abnormal production of tropoelastin and fibrillins in both keratinocytes and fibroblasts by solar UV exposure, and its deposition and degradation of elastin and microfibrils by several enzymes in the dermis, may partially contribute to the accumulation of abnormal elastotic materials in photodamaged skin (207, 209).

4.1.5 Treatment of photoaging

In fact, the pathogenesis of photoaging is involved in several pathways and they can be the promising targets for developing the novel treatment. Matrix metalloproteinase enzymes are some part of the pathogenesis and they are quite interested. Thus, this review is created to find the proper MMPs treatment.

To our knowledge, there are various treatment modalities for slow down aging processes by regulate MMPs pathways, including topical therapies, laser treatment, toxin usage and botanical compounds/extracts. Although the outcomes are satisfied, only few studies explain about the molecular mechanisms.

Table 4.3 Treatment modalities from researches with their mechanism of actions and effects both in vivo and in vitro studies

Treatment	Study design	Mechanisms	Effects
All-trans retinoid acid (tRA) (137, 138)	• In vivo study, human skin	• Inhibit MMP-1 expression • Increase procollagen type I production	• Decrease MMP-1 secretion by 50%, compare with vehicle control • Increase procollagen type I secretion by 58%, compare with vehicle control
Botulinum toxin type A (210)	• In vitro study, human dermal fibroblasts	Increase procollagen type I production	• Procollagen type I started increased after 12 hours and continue increased over 48 hours in cells culture

Table 4.3 Treatment modalities from researches with their mechanism of actions and effects both in vivo and in vitro studies (Cont.)

Treatment	Study design	Mechanisms	Effects
Botulinum toxin type A (BTX-A) (141)	• In vitro study, human dermal fibroblasts	• Inhibit MMP-1 and MMP-3 expression • Increase procollagen type I and III production •	• At 5 U/10^6 cells BTX-A, decrease MMP-1 and MMP-3 expression by 80% and 68%, respectively compared to UVB-radiated control • At 5 U/10^6 cells BTX-A, increase procollagen type I and III expression by 80% and 78%, respectively compared to UVB-radiated control
L-Ascorbic acid or vitamin C (211)	• In vivo study, female hairless mice	• Decrease MMP-3 and MMP-13 expression • Increase type I procollagen synthesis	• Decrease MMP-3 and MMP-13 expression by 80%, compare with UVB control • Improve skin wrinkling by 35% • Decrease skin thickness by 89%

Table 4.3 Treatment modalities from researches with their mechanism of actions and effects both in vivo and in vitro studies (Cont.)

Treatment	Study design	Mechanisms	Effects
L-Ascorbic acid or vitamin C (212)	• In vitro study, human fibroblasts	• Inhibit MMP-1 expression	• At 50 µM of vitamin C, decrease MMP-1 expression by 10% • At 50 µM of vitamin C, decrease UVA-induced decrease in collagen by 17%
Long-pulsed Nd:YAG laser (140)	• In vivo study, hairless mice	• Decrease MMP-1 expression at low energy • Increase MMP-1 expression at high energy • Increase TGF-β expression	• At 40 and 60 J/cm^2 show more changes in collagen bundles than 20 J/cm^2 • Skin appendages and epidermis has no changes
Seletinoid G (139)	• In vivo study, human skin	• Inhibit MMP-1 expression	• Decreased MMP-1 expression by 65.5% (better than rTA treatment) • No skin irritation in short-term treatment

Table 4.3 Treatment modalities from researches with their mechanism of actions and effects both in vivo and in vitro studies (Cont.)

Treatment	Study design	Mechanisms	Effects
α-tocopherol or vitamin E (211)	• In vivo study, female hairless mice	• Decrease MMP-3 and MMP-13 expression • Increase type I procollagen synthesis	• Decrease MMP-3 and MMP-13 activity by 80%, compare with UVB control • Improve skin wrinkling by 35%
α-tocopherol or vitamin E (213)	• In vitro study, human fibroblasts	• Inhibit MMP-1, MMP-2, MMP-3, and MMP-9 expression • Increase type I and type III procollagen	• Decrease MMP-1, MMP-2, MMP-3, and MMP-9 activity by 0.23-, 0.12-, 0.09-, and 0.14-fold, respectively

Table 4.3 shows treatment modalities which are widely used in nowadays. All of them are reported to suppress MMP-1 expression. As mentioned before, MMP-1 is able to degrade collagen type I that is the most abundant subtype of collagen found within skin. This can be suggested that MMP-1 is the most important enzyme associated with photodamaged skin and also be the most popular in researches.

Although these therapies are generally accepted in treatment photoaging, there are still developing the novel compounds or extracts to use as single or combination with conventional therapies. To date, there are many new emerging botanical extracts or natural compounds from all around the world which have been experimented and evaluated for the efficacy of anti-aging properties. Their inhibiting mechanisms are associated in the pathogenesis of photoaging such as MMPs inhibitors, free radical scavengers, and even cell signaling inhibitors as shown in Table 4.4 and Table 4.5.

Table 4.4 Botanical or plants extracts from researches with their mechanism of actions and effects in vivo studies

Botanical extracts	Study design	Mechanisms	Efficacy
Baicalin extract from *Scutellaria lateriflora Georgi* roots (148)	• In vivo study, hairless mice	• Inhibit MMP-1 and MMP-3 expression • Increase procollagen type I and III production	• Topical 0.5 or 1 mg/cm^2 baicalin can decrease epidermal thickness and prevent loss of dermal collagen
Coriander leaf extract (CSE) (122)	• In vivo study, hairless mice	• Inhibit MMP-1 expression • Increase procollagen type I production	• Inhibit UVB-induced epidermal thickness and collagen rearrangement
Galla chinensis (121)	• In vivo study, male albino hairless mice	• Inhibit MMP-1 expression • Regulate TGF-β1 activity	• Decrease skin wrinkles in hairless mice both topical and oral treatment
Kaempferia parviflora (KP) (126)	• In vivo study, hairless female mice	• Inhibit MMP-2, MMP-3, MMP-9, and MMP-13 expression • Increase procollagen type I, III, and VII production	• At KP 200 mg/kg/day, decrease the expression of MMP-2, MMP-3, MMP-9 and MMP-13 by 44%, 34%, 45%, and 35%, respectively • Oral KP can decrease skin wrinkles and epidermal hypertrophy induced by UVB

Table 4.4 Botanical or plants extracts from researches with their mechanism of actions and effects in vivo studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Magnolol extract from *Magnolia officinalis* (151)	• In vivo study, hairless male mice	• Inhibit MMP-1, MMP-9, and MMP-13 expression • Suppress the MAPKs phosphorylation	• Inhibit UV-induced skin wrinkles, skin hypertrophy, and loss of collagen fibers in hairless male mice
Mangiferin extract from *Anemarrhena asphodeloides* (157)	• In vivo study, hairless male mice	• Inhibit MMP-9 secretion via MAPKs pathway	• Mangiferin improve skin wrinkles and epidermal hypertrophy as compared to UVB-induced control groups • Collagen bundles significantly increase in mangiferin treatment
Myricetin (5)	• In vivo study, hairless mice	• Suppress MMP_1 and MMP-9 expression	• Inhibit skin wrinkles induced by UVB • Decreased epidermal thickness by 38% and 58% at 1 and 5 nmol myricetin, respectively
Polyphenols from Green tea (GTP) (152)	• In vivo study, hairless mice	• Inhibit MMP-2, MMP-3, MMP-7 and MMP-9 expression	• Oral 10-12 mg GTP can decrease the expression of MMP-2, MMP-3, MMP-7 and MMP-9 by 67%, 62%, 63%, and 60%, respectively

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies

Botanical extracts	Study design	Mechanisms	Efficacy
Astragaloside IV from *Astragalus membranaceus* (123)	• In vitro study, human skin fibroblasts from newborn skin	• Inhibit MMP-1 secretion	• Decreased MMP-1 expression by 25% at 40 µg/mL Astragaloside IV, compared with UVA-radiated control
Brazilin extract from *Caesalpinia sappan L.* (124)	• In vitro study, human dermal fibroblasts from foreskin	• Inhibit MMP-1 and MMP-3 expression via NF-κB pathway • Decrease ROS production	• Decreased MMP-1 and MMP-3 expression by 56% and 50% at 50 µM brazilin, respectively when compared with UVB-radiated control • Decreased ROS activity by 36% at 50 µM brazilin, compared with UVB-radiated control
Coffea arabica leaves extract (CAE) (149)	• In vitro study, human foreskin fibroblasts	• Inhibit MMP-1, MMP-3, and MMP-9 expression • Increase procollagen type I production	• At 25 µg/mLCAE, decreased MMP-1, MMP-3, and MMP-9 expression by 50%, 69%, and 41%, respectively compared with UV-radiated control • At 10 µg/mLCAE, increased procollagen type I expression by 60%, compared to control

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Coriander leaf extract (CSE) (122)	• In vitro study, normal human dermal fibroblasts	• Reduced UV-induced ROS activity • Increase procollagen type I expression • Suppress MMP-1 expression	• Decreased ROS levels by 43% at 100 µg/mL CSE, compared with UVB-radiated control • Stimulated procollagen type I production by 50% at 100 µg/mL CSE, compared with control • Decreased MMP-1 expression by 20% at 100 µg/mL CSE, compared with non-radiated cells
Cultivated gingseng (CG) (4)	• In vitro study, human skin fibroblasts	• Suppress ROS production • Inhibit MMP-1 and MMP-13 via suppress MAPKs and NF-κB pathways • Increase procollagen type I activity	• Decreased ROS levels by 19% and 54% at 1 µg/mL and 10 µg/mL CG, respectively compared with UVB-radiated control • At 10 µg/mL CG, decreased MMP-1 and MMP-13 expression by 53% and 43%, respectively compared with UVB-radiated control • Increase collagen contents by 34% at 10 µg/mL CG compared with UVB-radiated control

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Decursin extract from *Angelica gigas Nakai* roors (85)	• In vitro study, human dermal fibroblasts from foreskin	• Suppress MMP-1 and MMP-3 secretion via inhibit the activation of NF-κB • No effect on the activity of MAPKs or AP-1	• At 30 µM decursin, decreased MMP-1 expression by 31.25 % and MMP-3 expression by 50%, compared with UVB-radiated control
Epigallocatechin-3-gallate (EGCG) from green tea (14)	• In vitro study, human dermal fibroblasts	• Inhibit MMP-1, MMP-8, and MMP-13 expression via MAPK pathways • Increase procollagen type I activity	• At 20 µM EGCG, decreased MMP-1, MMP-8 and MMP-13 expression by 80%, 45% and 33%, respectively compared with UVB-radiated control • EGCG > 1 µM can increase procollagen type I expression

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Galla chinensis (GAC) (121)	• In vitro study, normal human dermal fibroblasts	• Inhibit MMP-1 expression • Decrease ROS levels	• MMP-1 expression decreased by 20.3 % after 10 µg/mL GAC, compared with UVB-irradiated control • Removed ROS by 28.6 % at 1 µg/mL GAC and 45.7 % at 10 µg/mL GAC, compared with UVB-irradiated control
Gynura procumbens (2)	• In vitro study, human dermal fibroblasts from neonatal foreskin	• Inhibit MMP-1 and MMP-9 expression • Decrease ROS production	• Decreased MMP-1 expression more efficiently than positive control, retinoic acid • Decreased MMP-9 expression by 73% at 20 µg/mL *Gynura procumbens*, compared with UVB-radiated control • Decreased ROS activity by 36% at 50 µg/mL *Gynura procumbens*, compared with UVB-radiated control (more effective than vitamin C)

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Ixora parviflora (86)	• In vitro study, human foreskin fibroblasts	• Inhibit MMP-1, MMP-3, and MMP-9 expression	• At 50 µg/mL *Ixora parviflora*, decreased MMP-1, MMP-3 and MMP-9 expression by 58.7%, 21.4% and 22%, respectively compared with UV-radiated control
Macelignan extract from *Myristica fragrans Houtt.* (150)	• In vitro study, immortalized human keratinocyte cell line HaCaT	• Inhibit MMP-9 expression via MAPKs pathways	• At 1 mM macelignan, MMP-9 expression was reduced to the level seen in non-UVB exposed control cells
Mangiferin extract from *Anemarrhena asphodeloides* (157)	• In vitro study, human keratinocytes	• Inhibit MMP-9 secretion via MAPKs pathway	• Decreased MMP-9 expression by 40%, compared with UVB-radiated control

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Michelia alba leaves extract (MAE) (116)	• In vitro study, human foreskin fibroblasts	• Inhibit MMP-1, MMP-3, and MMP-9 expression via MAPKs pathway • Decrease ROS production	• Decreased MMP-1, MMP-3 and MMP-9 expressions by 80%, 36% and 22% at 25 µM MAE, respectively when compared with UVB-radiated control • 200 µg/mL MAE inhibit ROS secretion more than at 12.5 µg/mL ascorbic acid
Neonauclea reticulate (107)	• In vitro study, human dermal fibroblasts from foreskin	• Suppress MMP-1, MMP-3, and MMP-9 expressions via the MAPK phosphorylation blockage • Scavenge ROS induced by UV radiation • Increase TGF-β and procollagen type I secretion	• Decrease degradation of collagen • The scavenging activity at 10 μg/mL *Neonauclea reticulate* was similar to 50 μg/mL ascorbic acid • Stimulate collagen synthesis

Table 4.5 Botanical or plants extracts from researches with their mechanism of actions and effects in vitro studies (Cont.)

Botanical extracts	Study design	Mechanisms	Efficacy
Quercitin (125)	• In vitro study, human keratinocytes	• Inhibit UV-induced NF-κB activation	• Decreased NF-κB activity up to 80% at 2 µg/mL quercitin, compared with vehicle control group
Saponins extract from *Platycodon grandiflorum* roots (115)	• In vitro study, immortalized human keratinocyte cell line HaCaT	• Inhibit MMP-1 and MMP-9 expression via NF-κB and MAPK signaling pathway • Increase procollagen type I production	• At 2 µg/mL Saponins, decreased MMP-1 and MMP-9 expression by 45% and 53%, respectively when compared with UVA-radiated control • Increased procollagen type I secretion by 45%, compared with UVB-radiated control
B-carotene (143)	• In vitro study, immortalized human keratinocyte cell line HaCaT	• Inhibit MMP-1, MMP-3, and MMP-10 expression	• At 1.5 µM β-carotene, decreased MMP-1 and MMP-10 expression by 30% and 33%, respectively compared with UVA-radiated control • At 1.5 µM β-carotene, decreased MMP-3 expression by 45% compared with non-radiated control

Concurrently, botanical compounds or extracts are very famous in various researches and there are continue discovered newly botanical compounds or extracts. This trend appears due to human behavior as they look for natural compound treatments as an alternative to conventional and/or chemical therapies. From natural compounds or extracts all around the world, there are several active ingredients which could reduce harmful effects of photodamaged skin in either direct or indirect pathways.

This review tries to collect the knowledge regarding to active ingredients or compounds from botanical agents and their molecular mechanisms. However, most of these botanical compounds or extracts have been performed in vitro studies and some studies have been conducted in vivo animal studies. To the best of our knowledge, there is no report in clinical trial studies which can evaluate the clinical aging signs improvement. Thus, it is very difficult to compare the effectiveness between each compounds/extracts or compare with conventional therapies. Nonetheless, using several active ingredients, which can inhibit or decrease different signaling pathway of photodamaged processes, is the best solution for the most effective treatment of photoaging.

4.1.6 MMP inhibitors in other diseases

In addition to photodamaged skin, MMPs are involved in several physiopathological conditions, such as wound healing, skeletal growth and remodeling, arthritis, Alzheimer's disease, multiple sclerosis, and even cancer (6). To date, many researchers are developing the novel MMP inhibitors for cure the diseases. Thus, MMPs are viewed as a druggable target in several diseases.

Table 4.6 shows drugs which are developed in nowadays for treatment various diseases. All of them are reported to suppress MMP expression and activity. This can be suggested that MMP enzymes are considered to the promising pharmaceutical target in many diseases due to its capability to cleave ECM components and migrate cells.

Table 4.6 Drugs from researches with their mechanisms of action and effects both in vivo and in vitro studies

Treatment	Study design	Mechanisms	Effects
Calciprotiol (214)	• In vitro study, human SCC cell line	• Inhibit MMP-9 and MMP-13 expression	• At concentration of 10^{-6} to 10^{-8} mol/L of calciprotiol, significantly decrease MMP-9 and MMP-13 expression in a dose-dependent manner
Calcitriol (215)	• In vivo study, 11 patients with diabetic foot ulcers (DFUs)	• Inhibit MMP-1 and MMP-10 expression	• Significant reduce MMP-1 and MMP-10 expression in keratinocytes derived from DFUs
Calcitriol (216)	• In vivo study, patients with chronic rhinosinusitis and nasal polyp	• Inhibit MMP-2 and MMP-9 expression	• Decrease MMP-2 and MMP-9 by 20% and 34%, respectively when compared with control
Doxycycline (217)	• In vivo study, male Wistar rats	• Inhibit MMP-2 expression	• Significant reduce MMP-2 expression in the periodontal ligament of rat incisor without altering the eruption process

Table 4.6 Drugs from researches with their mechanisms of action and effects both in vivo and in vitro studies (Cont.)

Treatment	Study design	Mechanisms	Effects
Minocycline (218)	• In human clinical trials • (10 patients of fragile-X syndrome)	• Inhibit MMP-9 expression	• Decrease MMP-9 expression by 75% after 3 months of treatment • 9 of 10 patients show clinical improvement
Tetracycline (219, 220)	• Review article	• Inhibit collagneases and gelatinases activity	• Successfully in rheumatoid arthritis treatment
Tigecycline (221)	• In vivo study, male Wistar rats	• Inhibit MMP-9 expression	• Significant accelerate wound healing in staphylococcal-infected burns

Calcitriol, also known as 1,25-dihydroxycholecalciferol or 1,25-dihydroxyvitamin D_3, is the hormonally active form of vitamin D. The production of calcitriol occurs in human skin. The enzymatic complex required for the production of calcitriol and its nuclear receptor (called vitamin D receptor or VDR) are located in the epidermis (215). In human skin, calcitriol has various biologic functions, such as keratinocytes proliferation and differentiation (222). Moreover, calcitriol can inhibits MMPs expression. There is reported that calcitriol inhibit collagenase-1 and stormelysin-2 expression in diabetic foot ulcers [VD1]. Additionally, calcitriol can significantly suppress gelatinases in patients with chronicnic rhinosinusitis and nasal polyp (216).

Calcipotriol or calcipotriene, a synthetic form of calcitriol, which is widely use in the psoriasis treatment. Besides psoriasis treatment, calcipotriol can inhibit MMPs expression and activity. There is reported that calcipotriol significantly

decrease gelatinase-B and collagenase-3 in human SCC cell line. These results may proposed the role of calcipotriol in the precancerous lesions (214).

Tetracycline are natural products produced by *Streptomyces* originally identified in the 1940s which were originally characterized as having bacteriostatic activity and are still commonly prescribed for use as antibiotics. Not only used safely as antibiotics, tetracycline has been reported to inhibit MMPs expression and activity, such as collagenases and gelatinases (220). It has been successful in the inhibition of MMPs expression in rheumatoid arthritis trial (219). The molecular mechanism of the suppression remains unknown. Most studies suggest that tetracycline may interact with zinc-binding site of the catalytic domain to destabilize MMP's structure, leading to preventing catalysis (220).

Other drugs of tetracycline antibiotic family that have been reported to suppress MMPs expression such as doxycycline, minocycline, and tigecycline. Doxycycline is an antibiotic which has been widely researched in several diseases associated with elevated MMP activity such as chronic wounds, osteoarthritis, rheumatoid arthritis, and periodontitis (219). In each of these trials, the advantageous effect of doxycycline is not because the direct antimicrobial effect, but the inhibition of MMPs expression such as collagenases and gelatinase-A (217, 220).

Minocycline is widely used in acne vulgaris treatment. Besides antimicrobial effect, minocycline also inhibits MMPs expression. There is reported that minocycline can inhibit gelatinase-B expression and activity in mouse fragile-X syndrome model study (218, 223). Tigecycline also have a beneficial effect on gelatinase-B inhibitors, besides its antimicrobial activity, leading to accelerate wound healing in staphylococcal-infected burns (221).

Although these drugs are usually accepted in treatment other diseases, there are still no reported in photodamaged skin treatment. It might be effective in reducing photodamaged effect due to these drugs can inactivate MMP activity. Further clinical studies are warranted to clarify whether topical or even systemic drugs may prove clinically photodamaged skin by suppressing MMPs expression.

4.2 Conclusion

Cutaneous exposure to solar irradiation causes the up-regulation of several different MMPs that virtually impair the various components of the ECM. These alterations in the ECM are known to cause skin wrinkling, a major characteristic of premature skin aging. Regulation of MMPs is one of the strategies to prevent photodamaged to the skin as their activities contribute to wrinkle formation. Use of free radical scavengers, antioxidants, and cell signaling inhibitors are considered on the therapeutic basis to diminish the damaging effects of solar radiation and to prevent solar-initiated photoaging because of their capability to inhibit the expression and activity of MMPs and their regulatory pathways. Nonetheless, using several active ingredients, which can inhibit or decrease different signaling pathway of photodamaged processes, is the best solution for the most effective treatment of photoaging.

CHAPTER 5
LIMITATIONS AND RECOMMENDATIONS

5.1 Limitations

5.1.1 There is limitation in the publications of epidemiologic of skin aging in African American populations.

5.1.2 There is limitation in the data of botanical treatments of photoaging in human clinical trials.

5.1.3 There is limitation in the gold standard of clinically diagnosis of photoaging.

5.1.4 There is limitation in the publications of pathogenesis of IR and visible light induced photodamaged skin.

5.1.5 There is limitation in the publications of IR and visible light-induced photodamaged treatment.

5.2 Recommendations

5.2.1 Difference of photoaging between each populations should be collected more.

5.2.2 Further publication should be done in human clinical trials for evaluate the clinical aging signs improvement and for compare the novel compounds with conventional treatment.

5.2.3 Further studies should review other signaling pathways that involved in photoaging process, which leading to newly develop the promising target therapies.

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APPENDICES

APPENDIX A
TRPV1

Transient receptor potential vanilloid 1 or TRPV1 or capsaicin receptor is an unspecific cation receptors which is a part of TRP family. The family of TRP consists of at least 30 ion channels, which are primarily passable by both monovalent and divalent cations, such as Na^{+}, Mg^{2+}, and Ca^{2+}. The external stimuli, including temperature, osmotic and mechanical pressures, and light, can regulate these ion channels-induced response. These stimulation can upregulate or downregulate the TRP receptors permeability to specific ions, consequently changing the potential of cell membrane and resulting in depolarization (199).

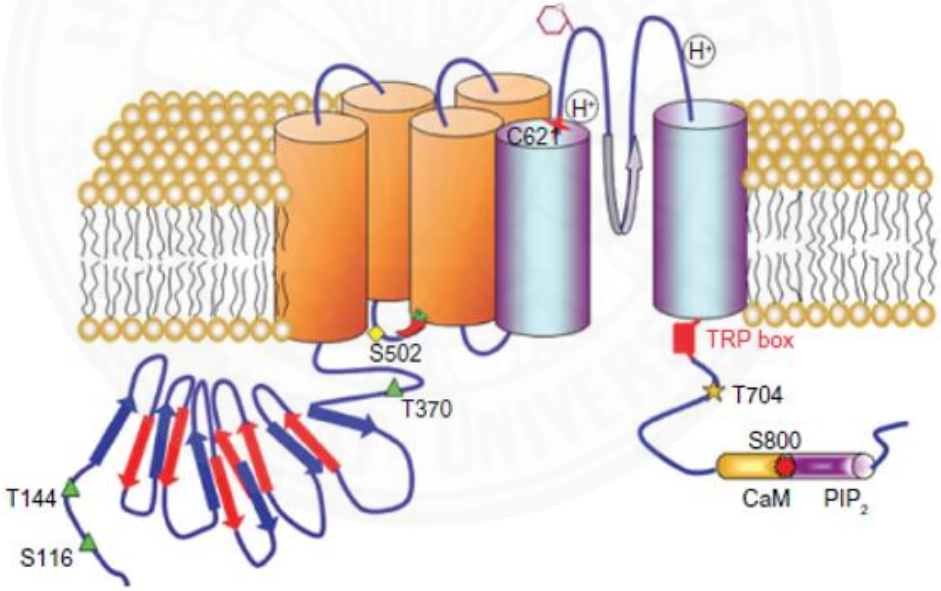


Figure A1 Transient receptor potential vanilloid 1 (TRPV1) strucutre (224)

The receptors of TRP family are widely found in almost tissues and are involved in various functions, including fluid and mineral balance, transduction of pain signaling, gut movement, regulation of blood circulation, tissue survival, cell growth and death. TRPV1 receptor or capsaicin receptor is exclusive in that capsaicin, which

is the principal pungent component of hot chili peppers, can stimulated TRPV1 receptor. Moreover, heat temperature (>42 ◦C), protons (pH reduction), spider toxins and cations can stimulated TRPV1 receptor. This suggests that TRPV1 is an important biological sensor to chemico-thermal activation and tissue damage (199, 200).

APPENDIX B
RETINOIDS

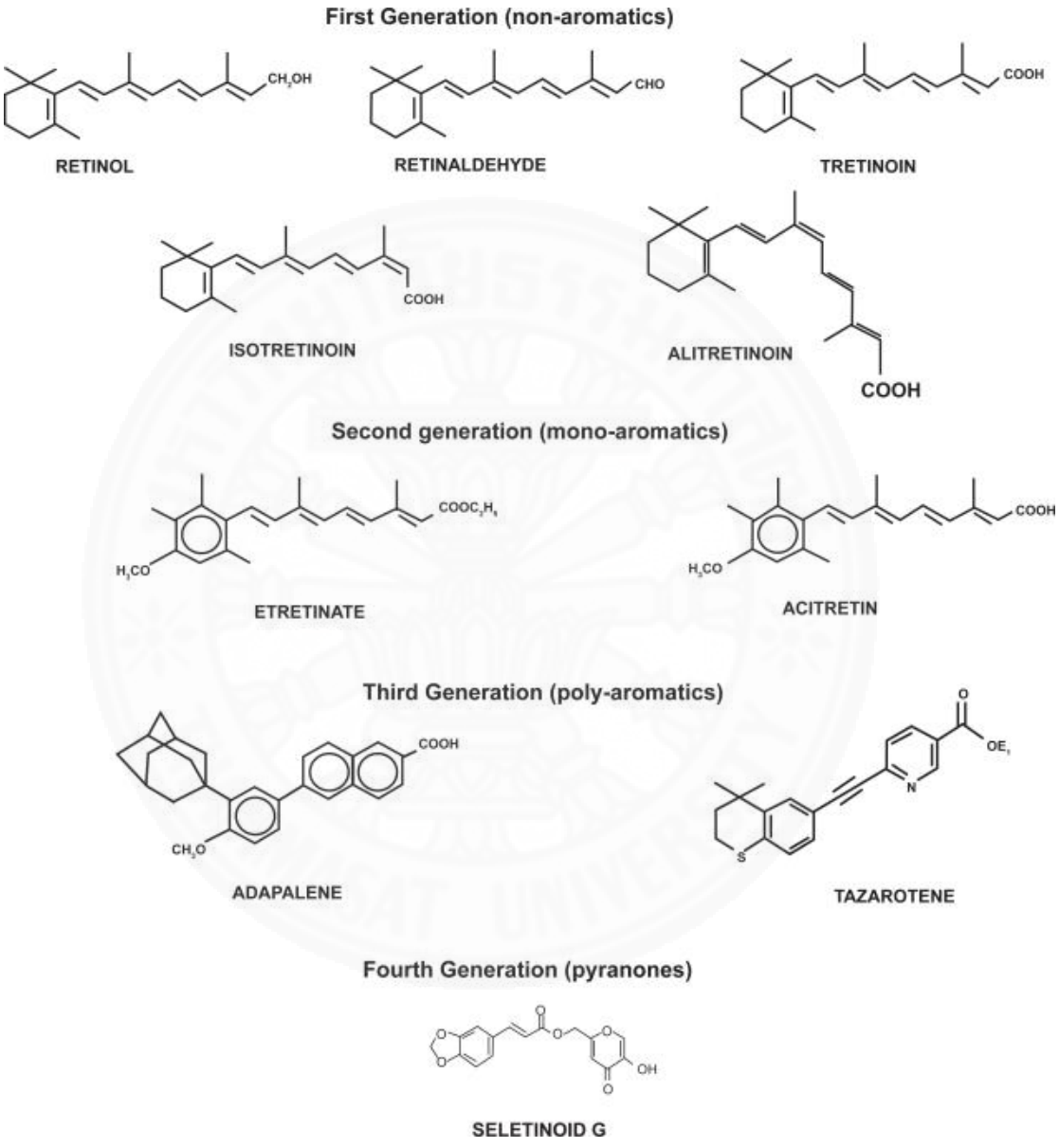


Figure B1 Chemical structures of retinoids (142)

Retinoids belong to the vitamin A-derived compounds family. This family are consists of retinol, all-trans retinoic acid (also called tretinoin), tazarotene, adapalene, β-carotene and other carotenoids. Retinoids are associated with the various

biological functions such as anti-inflammation, adhesion of platelet, thrombosis, and fibrinolysis activation (137).

The most active retinoids compound is all-trans retinoic acid (tRA) which regulates many biologic processes by mediate the expression of genes, including retinoid X receptor (RXR) and retinoic acid receptor (RAR). To our knowledge, the promising gene targets of retinoids are about 500 genes, including MMPs-regulated genes (such as collagenases, gelatinases, and stromelysin-1) (137).

In the last two decades, the potency of topical tRA or tretinoin in photodamaged treatment is well confirmed. tRA can stimulate keratinocytes proliferation, as long as inhibit the differentiation of terminal keratinocytes. Consequently, the transit-amplifying keratinocytes layers is thickening, resulting in the smoother looking of skin. In addition, tRA can impede UV-induced MMPs expression and UV-inhibited collagen synthesis (143).

Although retinoids have an anti-wrinkle,effects, its treatment can produce retinoid dermatitis effects, including itching, burning, and skin irritation. This response restricts the extensive use of retinoids. Various studies have been researched to establish the novel retinoid derivative which have less adverse effects and to establish the additives for decreasing skin irritation induced by retionids (144).

APPENDIX C
BOTULINUM TOXIN TYPE A

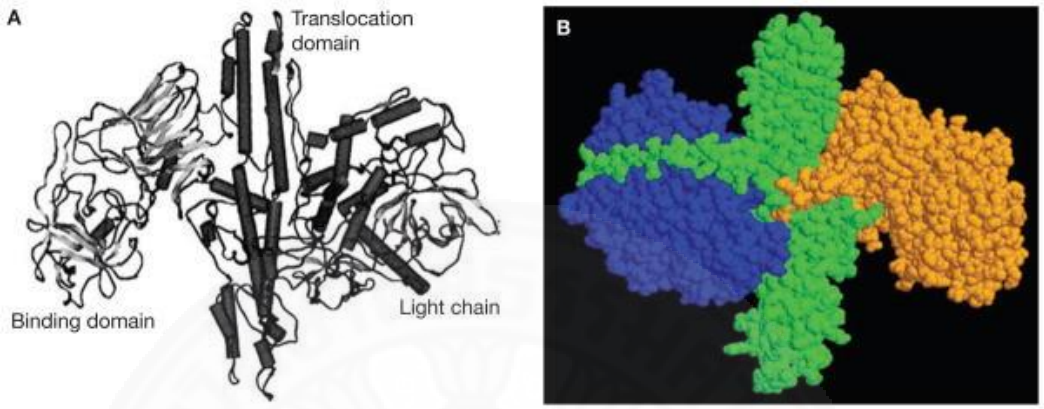


Figure C1 Botulinum toxin type-A structure. (A) 3D crystalline BTX-A structure. (B) Alternative 3D BTX-A structural view. Blue showed the light chain. Orange and green showed C-terminal and N-terminal of the heavy chain, respectively (225).

Botulinum toxin type A (BTX-A) is one of seven *Clostridium botulinum*-derived neurotoxins. The potency of BTX-A in retard obvious aging process by diminishing the static and dynamic facial rhytides and lines is well established (141, 147)]. The mechanism of botulinum toxin action is able to promote chemodenervation on presynaptic neurons, impeding the acetylcholine (Ach) secretion and resulting in denervated striated muscle for 2-6 months after injection. This leads to an atrophy of muscle fibers and consequently characterized by flaccid paralysis (141). Thus, BTX-A is widely used in cosmetic procedures to treat facial rhytides or lines that made by the hyperactivity of muscle.

BTX-A are widely used in medical and nonsurgical treatments, since the Food and Drug Administration (FDA) approved its treatment in glabellar wrinkles in 2002 (210). Presently, the novel BTX-A treatments are continue developed, even though most of BTX-A indications are still “off label”.

Some doctors have noticed the lifting effect of the face after intradermal BTX-A injections at middle and lower face. It has been postulated that this lifting effect occurs due to the activation of collagen synthesis, the less secretion of sebum, and the facial pore size reduction (141, 210).

APPENDIX D
PICTURES OF PLANTS

Table D1 Plants extracts or natural compounds inhibit or downregulate MMPs expression

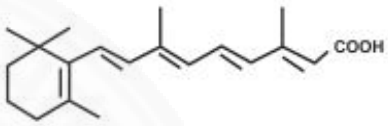
Mechanisms	Compounds/Extracts	Picture
Inhibit/ down-regulate MMPs expression	All-trans retinoic acid (142)	
	Astragaloside IV from *Astragalus membranaceus* (123)	
	Baicalin extract from *Scutellaria lateriflora Georgi* (Huang Qin) roots (148)	
	Brazilin extract from *Caesalpinia sappan L.* (124)	

Table D1 Plants extracts or natural compounds inhibit or downregulate MMPs expression (Cont.)

Mechanisms	Compounds/Extracts	Picture
Inhibit/ down-regulate MMPs expression	Coffea Arabica leaves extract (149)	
	Galla chinensis (121)	
	Gynura procumbens (2)	
	Ixora parviflora (86)	

Table D1 Plants extracts or natural compounds inhibit or downregulate MMPs expression (Cont.)

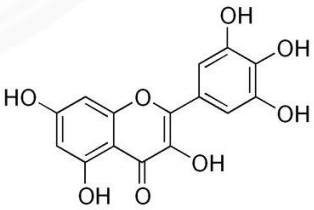
Mechanisms	Compounds/Extracts	Picture
Inhibit/ down-regulate MMPs expression	*Kaempferia parviflora* (126)	
	Macelignan extract from *Myristica fragrans Houtt.* (150)	
	Magnolol extract from *Magnolia officinalis* (151)	
	Myricetin (169)	
	Polyphenols from green tea (152)	

Table D1 Plants extracts or natural compounds inhibit or downregulate MMPs expression (Cont.)

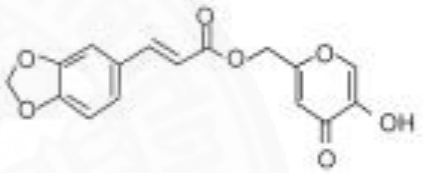
Mechanisms	Compounds/Extracts	Picture
Inhibit/ down-regulate MMPs expression	Saponins extracts from *Platycodon grandiflorum* roots (115)	
	Seletinoid G (142)	
	β-carotene (143)	

Table D2 Plants extracts or natural compounds act as antioxidants or free radical scavengers

Mechanisms	Compounds/Extracts	Picture
Antioxidants/ free radical scavengers	Coriander leaf extract (122)	

Table D2 Plants extracts or natural compounds act as antioxidants or free radical scavengers (Cont.)

Mechanisms	Compounds/Extracts	Picture
Antioxidants/ free radical scavengers	Galla chinensis (121)	
	Neonauclea reticulate (107)	

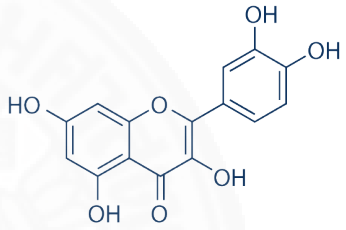
Table D3 Plants extracts or natural compounds act as cell signaling inhibitors

Mechanisms	Compounds/Extracts	Picture
MAPKs signaling inhibitors	Cultivated ginseng (4)	
	Epigallocatechin-3-gallate (EGCG) from green tea (152)	

Table D3 Plants extracts or natural compounds act as cell signaling inhibitors (Cont.)

Mechanisms	Compounds/Extracts	Picture
MAPKs signaling inhibitors	Magnolol extract from *Magnolia officinalis* (151)	
	Mangiferin extract from *Anemarrhena asphodeloides* (157)	
	Michelia alba leaves extract (116)	
	Neonauclea reticulata (107)	

Table D3 Plants extracts or natural compounds act as cell signaling inhibitors (Cont.)

Mechanisms	Compounds/Extracts	Picture
NF-κB signaling inhibitors	Decursin extract from *Angelica gigas Nakai* roots (85)	
	Quercitin (187)	

BIOGRAPHY

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Publications

1. Pittaya P., Meephansan J., Prapapan O., Komine M., and Ohtsuki M. Role of matrix metalloproteinases in Photoaging and Photocarcinogenesis. International Journal of Molecular Sciences. 2016 Jun;17(6):868-88

Work Experiences	2014-2015: Internship at Thammasat University hospital, Thailand