CHAPTER 2

LITERATURE REVIEWS

2.1 Skin and skin structure

Skin is a complex organ made up with multiple layers of tissues, which covers most of our bodies. It is much more than wrapping since there are many beneficial functions [8, 9].

2.1.1 Skin functions

The physiological functions of skin are including [8-10];

1. *Protection*: protect the body from pathogens, microbial infections and external injuries from the environment (sunlight, chemicals)
2. *Thermoregulation*: adjust body temperature by sweating or increasing blood perfusion for heat loss, and vessel constriction for heat retaining
3. *Sensation*: transmit sensation of the surrounding stimulants (pain, pressure, heat, cold) by skin nerve endings
4. *Control of evaporation*: produce the vapor semi-permeable barrier [11]
5. *Storage*: store body lipids and water
6. *Synthesis*: synthesize vitamin D for our bodies
7. *Water resistance*: prevent environmental water which affects body balance of vitamins, minerals and nutrients
8. *Health indicators*: indicate conditions of health, mental, illness and life-style

2.1.2 Skin structure

The skin consists of three main layers – the epidermis, the dermis and the hypodermis (or subcutaneous tissue).

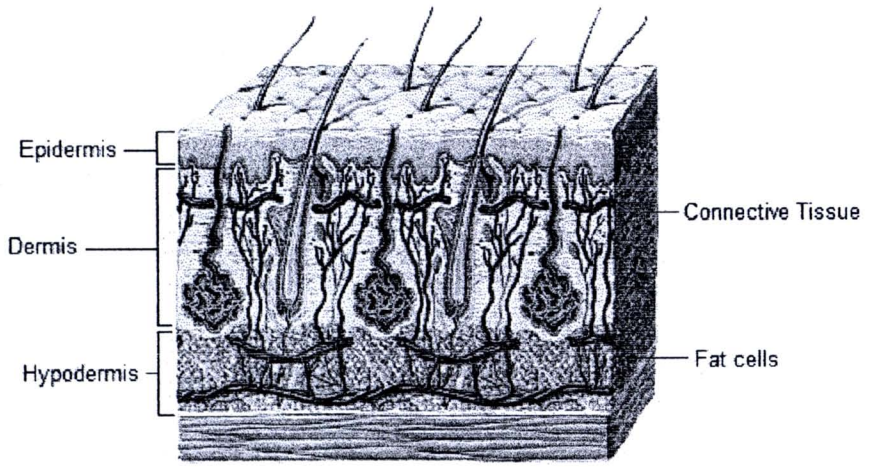

Figure 2.1 Structure and layers of skin [12]

a. Epidermis [1, 8, 10]

Epidermis is the topmost layer of the skin. It also composes of many stratum layers (Figure 2.2);

1. *Stratum corneum* (or *horny layer*) is the outermost layer exposed to the environment. In this layer there are many lifeless cells called 'corneocytes'. These cells arrange like bricks in the surrounding lipid (ceramide) and form the prime barriers to the transdermal delivery.
2. *Stratum lucidum* (or *transparent layer*) is a thin layer of keratin sheets (Rein's barrier) as semi-permeable barrier of aqua and minerals.
3. *Stratum granulosum* (or *granular layer*) is a viable layer containing enzymes (e.g. lipases, glycosidases, phosphatases) that degrade cell organelles.

4. *Stratum mucosum* is the next viable epidermal layer consisting of mature polygonal keratinocytes. The lower level of section also obtains melanin granules produced by melanocytes in basal layer. *Stratum mucosum* is also known as *stratum spinosum, prickle cell layer* or *Malphigian layer*.
5. *Stratum basale* (*stratum germinativum* or *basal layer*) is the only layer that capable of cell division. It contains various cell types, i.e. keratinocytes, melanocytes (produce melanin that protects skin from ultraviolet radiations), Langerhans' cells (uptake exogenous antigens as skin immune system) and Merkel cells (response for cutaneous sensation).

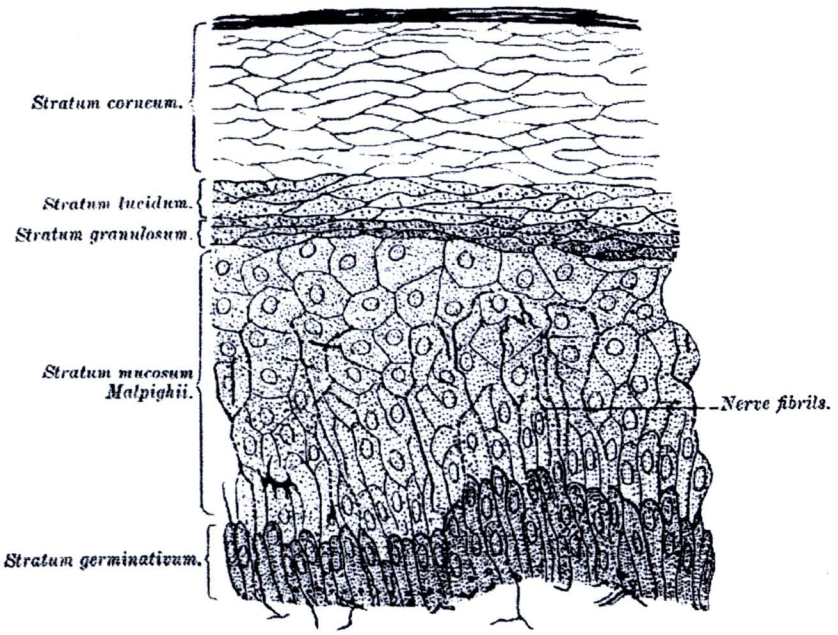

Figure 2.2 Sections of skin epidermis [13]

b. Dermis [1, 8, 10, 14, 15]

Dermis is the main component of the skin and consists of the connective tissue, which provides firmness and elasticity of skin, and maintains skin structure. The

dermis is divided into a papillary layer of loose connective tissue and a reticular layer of dense connective tissue. A papillary layer joints with epidermis by dermal-epidermal junction in basement membrane, and a reticular layer supplies the skin with a reticulate network of blood vessels, lymphatic vessels, nerve endings and numerous appendages (hair follicles, sebaceous glands, apocrine glands, eccrine sweat glands).

Connective tissue, a major element of dermis, is formed of collagen and elastin with hyaluronic acid filled in their gaps. It accommodates several cells such as fibroblasts, macrophages and mast cells.

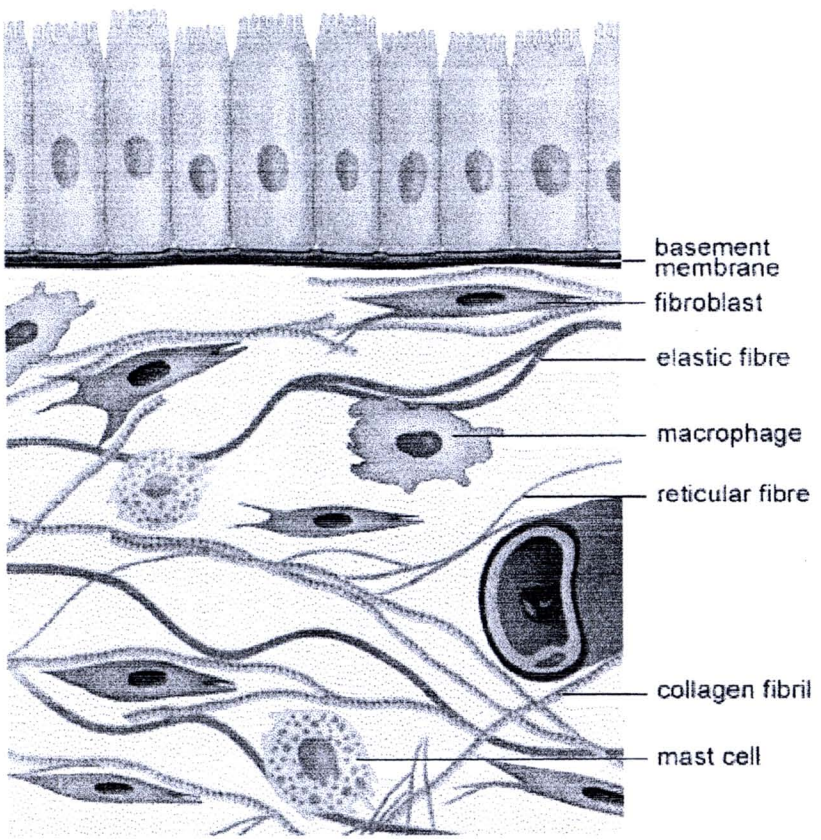

Figure 2.3 Components of skin dermis [14]

1. *Fibroblasts* are the most important parts for skin structure found in young connective tissues. They involve in synthesis of procollagen molecules, which reorganized in collagen fibers by procollagen peptidases (collagen fibrillogenesis). They also play in a role of cutaneous wound repair.

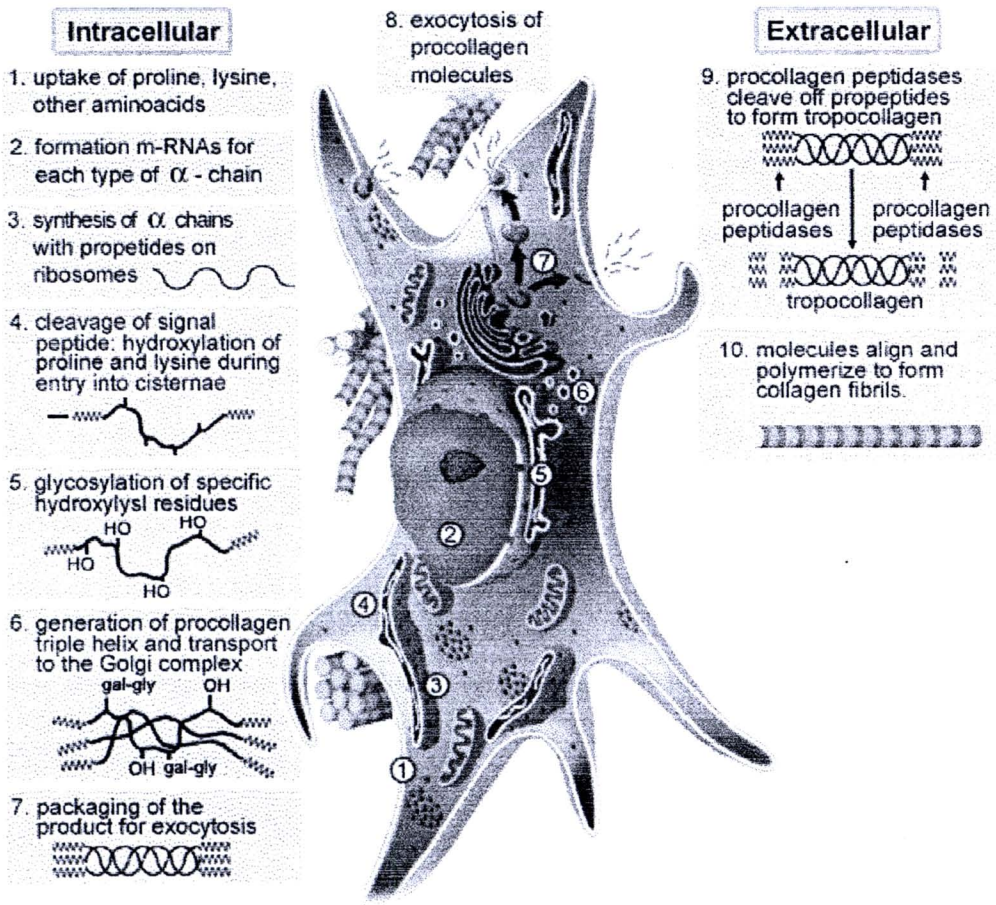

Figure 2.4 Fibroblast and its metabolism [14]

2. *Macrophages* are derived from precursor cells of the bone marrow that differentiate into 'monocytes' in blood system, and differentiate in the dermis as 'macrophages'. They eliminate the foreign antigens by phagocytosis, which makes them called large-eaters.

3. *Mast cells*, the specialized secretory cells, recognize the foreign antigens and response by secreting the histochemical – histamines. They are, additionally, responsible for immediate-type hypersensitivity reaction.

c. Hypodermis

Hypodermis (or subcutaneous tissue) is the deepest part of the skin consisting of fatty tissue. It acts an important role in thermoregulation, storage of energy, and protection from mechanical injury. Adipocytes are the main cells locating in the hypodermis, and produce the fatty tissue named as 'adipose tissue'. The nerve vessels and lymphatics are also found in this layer [8, 10].

2.2 Skin aging

Skin aging is the progressive deterioration of physiological functions in skin organisms, leading to the undesirable visual appearances (e.g. fine lines, wrinkles, sagging, dry skin, freckles, melasma). With age, sebaceous glands and sweat glands are lower in production, causing dry skin or flaky skin. Water retention and permeability of stratum corneum are reduced due to decline of ceramides in lamellar sheets, which present in the intercellular space of corneocytes. Melanocytes produce melanin abnormally, providing freckles and melasma. The connective tissues are loosened owing to decrease of fibroblast procollagen synthesis, and increase of collagen degradation by the matrix metalloproteinase enzymes (MMPs) up-regulation, causing wrinkles [1, 16].

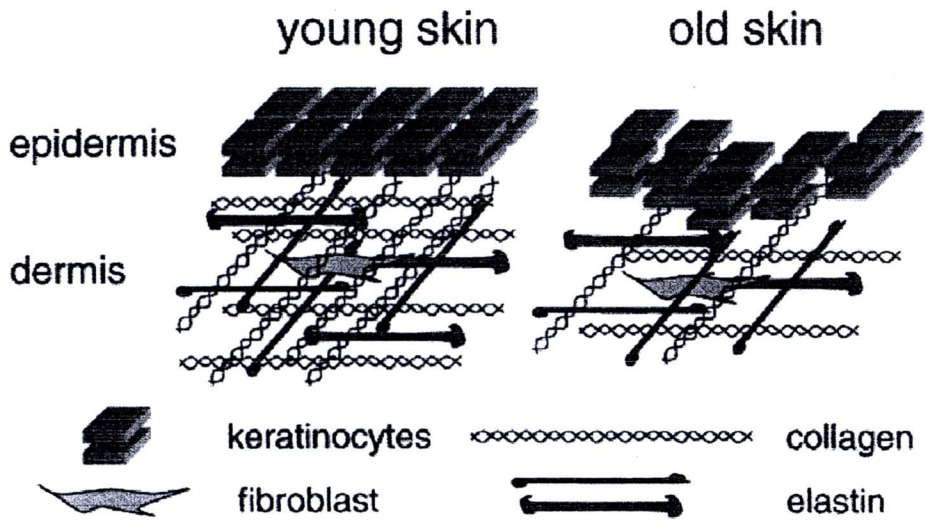

Figure 2.5 Schematic drawing of young and old skin [17]

The MMPs are a large family of enzymes responsible for degrading connective tissue. They can be classified into four subfamilies; the collagenases, the gelatinases, the stromelysins, and the membrane MMPs. In human, there are three collagenases; MMP-1 (interstitial collagenase or collagenase 1), MMP-8 (neutrophil collagenase or collagenase 2), and MMP-13 (collagenase 3). MMP-1 operates in normal collagen turnover and matrix remodeling (wound healing), and expressed by keratinocytes and fibroblasts. MMP-8 is synthesized by neutrophil leukocytes, and released during inflammatory process. MMP-13 is expressed in various epithelial cancers and also in fibroblasts in chronic cutaneous ulcers. The combined actions of MMP-1, MMP-2 (gelatinase A; 92 kDa), MMP-9 (gelatinase B; 72 kDa) and MMP-3 (stromelysin 1) can fully degrade skin collagen and components of the elastic network [4, 18].

2.2.1 Type of skin aging

The skin aging are divided into two types – intrinsic aging (or chronological aging) and extrinsic aging (or premature aging) [2, 10].

a. Intrinsic aging

The intrinsic aging is a natural occurrence toward increasing of our life span. Skin cell turnover decreases, and causes thinner skin. Collagen and elastin production are slow down from diminution in fibroblast growth, making loss of skin flexibility. The amount of MMPs gradually elevates in decades, and brings connective tissue degradation, leading to wrinkles and fine lines. The visual results of intrinsic aging are dry skin, lax skin, fine lines and wrinkles [1, 2, 10].

b. Extrinsic aging

In contrary to intrinsic aging, the extrinsic aging is caused by external factors, resulting in the premature aging, such as stress, smoking and pollution, but the most common reason is ultraviolet (UV) radiation over exposure. They extremely damage connective tissue by inducing over up-regulation of MMPs to degrade collagen. They also deteriorate skin functions by DNA damage. The extrinsic aging provides the visually appearances; aging spot, rough skin, dry skin and deep wrinkles [2, 10].

2.2.2 Theory of aging

There are several of notable theories that relate to skin aging, and some of them are briefly summarized and presented in Table 2.1.

Table 2.1 Summary of Aging Theories

Theory Name	Theory Explanation
Wear and tear theory (August Weissman, 1882)	Years of damage to cells, tissues, and organs wears and kill them. This damage begins at the molecular level within our cells to DNA. Even our bodies have capacity to repair DNA, but not all repairs are accurate or complete; hence, the progressively accumulative damage brings signs of aging such as wrinkles and sagging skin [2].
Cross-linking theory (Johan Bjorksten, 1941)	Cross-linking (or glycosylation) is binding of sugars to protein. The glycosylated proteins are impaired and unable to perform as efficiently. They also implicate in many diseases; diabetes, Alzheimer's disease, atherosclerosis. Cross-linking of DNA may cause malformed cells and cancer. Cross-linking of skin collagen leads to wrinkles [2, 17].
Neuroendocrine theory (Vladimir Dilman, 1954)	With age, the hypothalamus which controls many hormones declines in secretion and the effectiveness of those hormones are reduced. Increase of cortisol level (the hormone responsible for stress regulation) provides hypothalamus damage and breakdown of muscular tissue and collagen [2, 19].
Free radical theory (Denham Harmon, 1954)	Free radicals attack our cell membranes, creating metabolic waste products interfere DNA & RNA synthesis, inhibit protein synthesis, and destroy cellular enzymes. With age, the accumulated radicals slow down cell function therefore reducing the body's self-repair capabilities, and leading to wrinkles, sagging skin and aging spots [2, 19, 20].

Table 2.1 (continued)

Theory Name	Theory Explanation
Immunologic theory (Roy Walford, 1969)	The immune system is in role of various pathologies of aging. With age, an imbalance of immune system from dysregulated immune function and excessive inflammation can bring many diseases and more signs of aging [16, 21].
Telomere theory (Alexei Olovnikov and John Watson, 1972)	Telomeres are sequences of nucleic acids extending from the ends of chromosomes. They shorten each time of cell division by imperfective duplication. And this shortening of telomeres leads to cellular dysfunction and aging [2, 19].
Oxidative stress theory (R.S. Sohal and R.G. Allen, 1990)	Aging is heavily influenced by external oxidative stresses which influence the genetic program through modulation of redox sensitive genes [4]. The ROS activate cell surface receptors, causing in reduced procollagen production and excessive MMPs regulation [22].

2.2.3 Free radicals and skin aging

Free radicals are any simply molecules with unpaired electrons that make them unstabilized and highly reactive to acquire the electrons. The free radicals steal paired electrons from neighboring molecules (oxidation), thereby converting them into the other radicals, or altering their chemical structures [2, 23]. Many types of free radicals associate with biology (Table 2.2).

Table 2.2 Type of free radicals associating with biology [23]

Type of free radicals	Examples
1. Reactive oxygen species (ROS)	superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$), hydroperoxyl radical ($HO_2^{\cdot}$), peroxyl radical ($RO_2^{\cdot}$), alkoxyl radical ($RO^{\cdot}$), carbonate anion ($CO_3^{\cdot -}$), carbon dioxide anion ($CO_2^{\cdot -}$)
2. Reactive nitrogen species (RNS)	nitric oxide radical ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$, $NO_2^{\cdot -}$)
3. Reactive chlorine species (RCS)	Atomic chlorine ($Cl^{\cdot}$)
4. Transition metals	Many transition metals can become radicals.

The reactive oxygen species (ROS) are the oxygen-containing chemical species that are unstable due to the presence of unpaired electrons [23]. ROS are considered to be the main free radicals for skin aging as they enable to, in excess amount, cause damage to connective tissue components of the dermis [4, 20]. There are four primary sources of these oxidants formed within living organisms, i.e. mitochondria (use oxygen in ATP generation and bring in radicals), peroxisomes (fatty acid degrading organelles), cytochrome P450 enzymes (interact with many substrates – food, toxic, drugs), and white blood cells (secrete many oxidants to eliminate foreign antigens/pathogens). From outside our bodies, pollution, cigarette smoke, metal ions, food, drugs, and UV radiation contribute ROS [20]. Both UVA (320 – 400 nm) and UVB (290 – 320 nm) induce large amount of ROS generation near or within the human skin cells, and result in skin aging, specifically called 'photoaging' [22, 24].

ROS induce cross-linking (glycosylation) of skin collagen and elastin, causing decreased skin elasticity [2, 17]. ROS attack cell membranes which are rich of polyunsaturated fatty acids (PUFA). Oxidation of PUFA gives away lipid peroxides and breakdown products (e.g. malondialdehyde; MDA) that can cross-link proteins (e.g. enzymes), nucleic acids and cellular DNA, bringing more signs of aging [20]. ROS also attack DNA, leading in dysregulation homeostasis (MMPs up-regulation, procollagen down-regulation) [24].

In addition, ROS cause excessive MMPs regulation and diminished procollagen production by activation of cell surface membrane receptors, as secondary messengers, via three distinct signal transduction pathways; mitogen-activated protein kinases (MAPKs) pathway, nuclear factor (NF)-κB pathway and phosphoinositide-3 kinase (PI3K)/AKT pathway [22].

1. *MAPKs pathway* [4, 22, 24, 25]

ROS stimulate two cell surface receptors; growth factor receptors (GFR) and cytokine receptors (CR), activating three MAPKs signaling modules – extracellular signal-regulated kinase (ERK), c-Jun *N*-terminal kinase (JNK), and p38. This activation then elevates expression of a highly active transcription factor complex; activator protein (AP)-1 which composes of c-Jun and c-Fos. AP-1 attracts to the binding site in nucleus, inducing up-regulated MMPs transcription in keratinocytes and fibroblasts, and also reducing type I procollagen biosynthesis in fibroblasts.

2. *NF-κB pathway* [22, 26, 27]

NF-κB is a redox-regulated transcription factor involved in various genes including MMPs, IL-1 and TNF-α. NF-κB normally exists in cytoplasm as inactive state by forming trimer complex with inhibitor κB (IκB). After CR stimulation by ROS, the IκB kinase complex has been activated, and provides IκB phosphorylation. The phosphorylated IκB is then degraded by proteasomes, remaining active NF-κB (p50/p65 dimer). The active NF-κB translocates into nucleus to regulate target genes, and promotes gene expression of MMPs in both keratinocytes and fibroblasts.

3. *PI3K/AKT pathway* [22, 24]

Transforming growth factor-β (TGF-β) is a major regulator of extracellular matrix protein synthesis. It acts through binding to its cell surface receptor which is TGF-β receptor complex. TGF-β binding induces phosphorylation of Smad3 protein by TGF-β type I receptor (TβRI), and the phosphorylated Smad3 forms complex with Smad4 (co-Smad). This complex translocates into the nucleus, then binds to Smad binding element (SBE), and promotes procollagen gene expression.

ROS impairs TGF-β signaling by inhibiting TβRII in TGF-β receptor complex that activates TβRI. Additionally, activation of GFR also stimulates AKT which interacts with unphosphorylated Smad3, preventing Smad3 phosphorylation. Thus, it appears that ROS can depress procollagen synthesis through those two mechanisms.

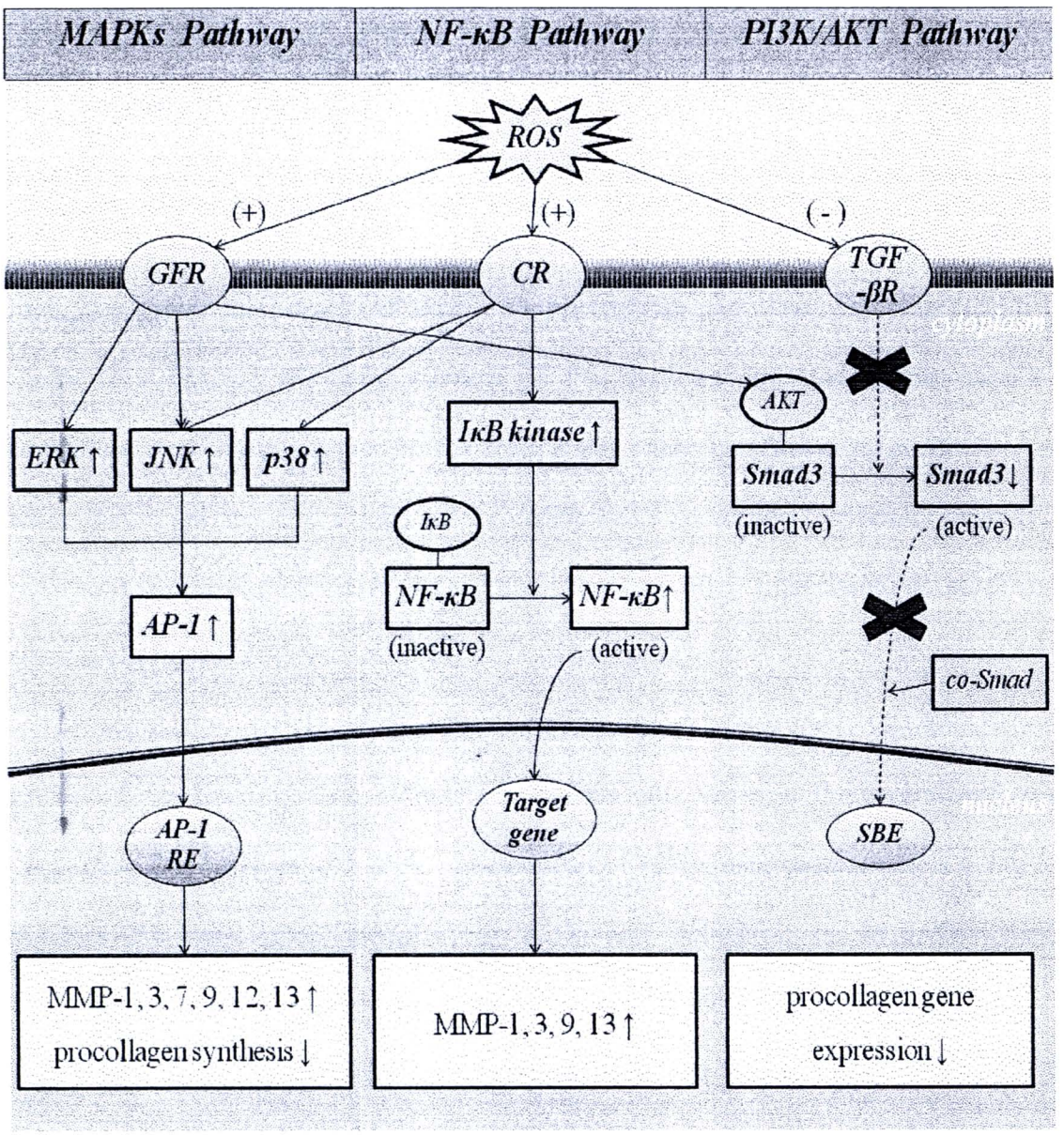

Figure 2.6 Role of ROS on cell surface receptors via three transduction pathways; (MAPKs = mitogen-activated protein kinases, NF-κB = nuclear factor-κB, and PI3K/AKT = phosphoinositide-3 kinase/AKT pathways)

2.3 Natural antioxidants preventing skin aging

Free radicals are at all times generated in organisms from both intrinsic factors (cellular metabolisms) and extrinsic factors (environment and life-style). Our bodies have their natural defense systems existing in enzymatic system (e.g. superoxide dismutase (SOD), catalase) and non-enzymatic system (e.g. glutathione, ubiquinone (or CoQ10), carotenoids, vitamins C and E) which usually occur in many organelles such as plasma membrane, cell membrane, cytoplasm and Golgi complex [16, 20]. There is a balance between creation of free radicals and antioxidant capacity controlling and protecting our bodies from those oxidants. However, declination of antioxidant capacity with aging, and excessive ROS generation (especially from UV radiation) expose the oxidative stress, then bring into many undesirable diseases and also skin aging [4, 28, 29].

Antioxidants are, chemical compounds, capable to inhibit the oxidation reaction promoted by free radicals. They can reduce skin damage by neutralizing the aggressive free radicals and ROS. Restoration of antioxidant compounds is a key to enhance the scavenging capacity for ROS, and utilization of natural/botanical antioxidants is one of the most interesting ways to prevent skin aging [29-31].

Polyphenolic compounds are the important and large group of naturally-occurring antioxidants. They are widely distributed in higher plants, but also occur in mosses, fungi, algae and lichens. They are known as plant secondary metabolites, which appear to be important for human lives. Many polyphenolic compounds show antioxidant activities against ROS, which may provide anti-inflammatory activity and inhibit regulation of MMPs, and also act as metal chelators. Moreover, they can induce the expression of genes encoding anti-oxidative enzymes [29, 30, 32].

2.3.1 Grape and grape products

Grapes (*Vitis vinifera*) are the well-known fruits associating with many health benefits. Grapes and their products (such as wines) contain several kinds of polyphenolic compounds, e.g. flavonoids – catechins, procyanidins, quercetin, rutin, and non-flavonoids like resveratrol [29]. And 60-70 % of grape polyphenolics are found in grape seeds [33].

The mature grape seeds are a rich source of oligomeric procyanidins (OPCs) consisting of a variable number of flavan-3-ol units (catechin and epicatechin) linked by $C_4 \rightarrow C_8$ bonds. Grape seed OPCs are combination of dimers, trimers, oligomers, and polymers (with an average degree of polymerization between 4 and 11) [34-37]. The grape seed extracts (GSE) have reducing activity for lipid peroxidation *in vitro* [35]. For skin aging, GSE appear to have strongly inhibitory effects to several collagen degrading enzymes, and negative effects to regulation of UVB-induced and X-ray-induced inflammatory mediators (MAPKs and NF-κB), due to their potent antioxidant compounds; the OPCs [29, 34, 37]. Furthermore, grape seed OPCs may have a vitamin E-sparing effect [34] by vitamin E regeneration [30].

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a stilbene phytoalexin commonly presented in the skin and seeds of grapes, and in red wines. Resveratrol is a low-molecular weight, conjugated substance with strong antioxidant activity [38, 39]. The topical application of resveratrol reduces UVB-mediated lipid peroxidation, and decreases inflammation responses in human epidermal keratinocytes through inhibition of synthesis or release of inflammatory transcription factors; NF-κB, in a dose- and time-dependent manner [33, 39].

2.3.2 French maritime pine bark

French maritime pine (*Pinus pinaster*) barks are the other important sources of natural polyphenolics such as catechin, epicatechin, procyanidins (OPCs), gallic acid and cinnamic acid, but the main compounds are OPCs with contents of 65-75 % [33, 40]. The pine bark extracts (PBE) exhibit potent antioxidant activity through neutralization of ROS, making them capable to inhibit MMPs release from activation of NF-κB. They also stimulate intracellular anti-oxidative enzymes – catalase, SOD, glutathione peroxidase and glutathione disulfide reductase [40, 41]. In addition, topical application of PBE appear to have dose-dependently astringent properties by promoting wound healing (shortening time for wound healing) and lowering scar formation owing to enhancement of collagen synthesis [42].

2.3.3 Soy bean

Soya or soy bean (*Glycine max*) is an edible legume widely used in Asian diet. It is a natural source of many isoflavones which genistein is a major active substance [29, 33, 43]. Genistein (5,7,4'-trihydroxyisoflavone) protects UVB-induced aging of fibroblasts via maintenance of antioxidant enzyme activities and inhibition of collagenase mRNA transcription through MAPKS pathway [29, 44]. Besides, it exhibits great penetration into the skin without inducing skin erythema or skin irritation, hence topically used of soy bean isoflavones may serve as a route against photodamage and photoaging [43].

2.3.4 Pomegranate

Pomegranate (*Punica granatum*) is an ancient fruit appeared in several antique civilizations – Egyptians, Babylonians and Greeks as a sacred fruit. The pomegranate is native from Himalayas in northern India to Iran but has been cultivated since ancient times over the Mediterranean region. The pomegranate fruit contains the edible seeds separated by white pericarp [45]. The seed oils constitute with fatty acids promoting proliferation of epidermal keratinocytes while the seed peels (juices and seed cakes) contain polyphenolic compounds [46]. Punicalagin, a major compound, is hydrolysable tannin consisting of ellagic acid and gallic acid linked to glucose [47]. The pomegranate seed peel extracts show stimulating effect to type I procollagen synthesis and inhibitory effect to MMP-1 production in dermal fibroblasts [46]. Additionally, it performs protective effects against UVA and UVB by reducing activation of NF-κB, preventing apoptosis, increasing DNA repair and lowering UV-induced ROS levels [48].

2.3.5 Marine algae (Seaweeds)

In these recent years, the research of natural sources of antioxidants has been expanded not only in plants or herbs, but also in other sources, like seaweeds. Seaweeds or marine algae are well-known sources of dietary fibers (i.e. carrageenans) and mucilage polysaccharides [49, 50]. Dulse algae (*Palmaria palmata*) are red seaweeds harvested on east coast of USA and Canada, and consumed as snack food or seasoning. They also show radical scavenging activity and inhibit peroxidation of linoleic acid since containing of flavonoids (e.g. catechin, epigallocatechin-3-gallate (EGCG)) [50, 51], which make *P. palmata* be an interesting choice for anti-aging.

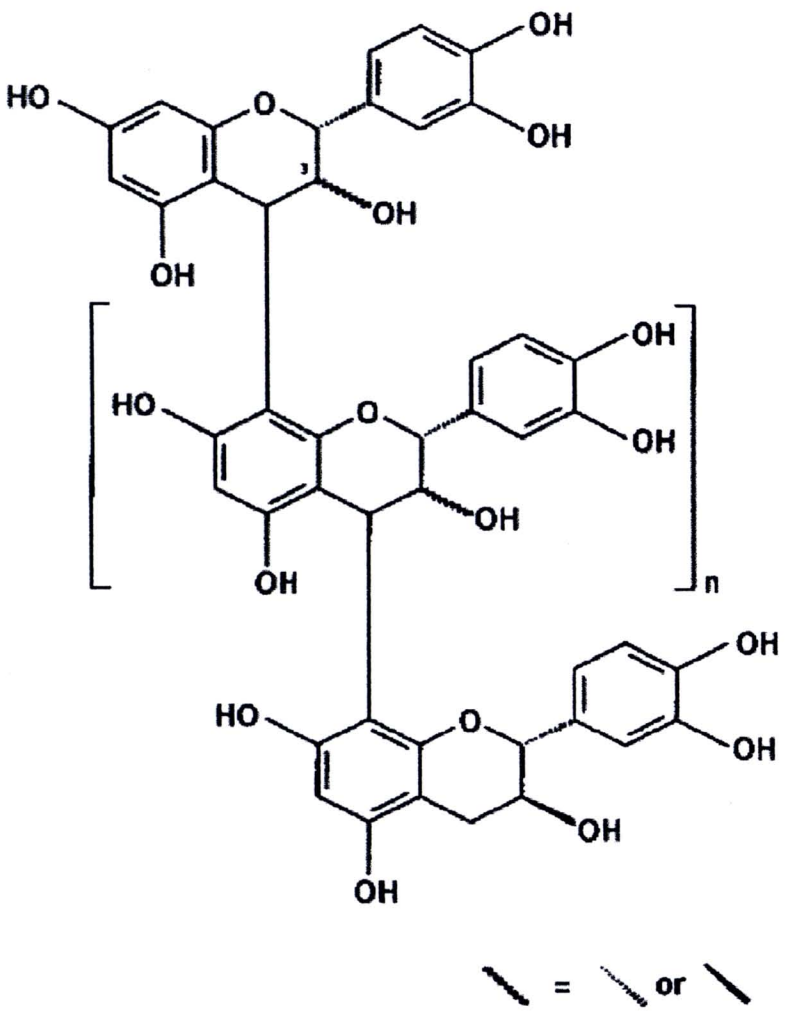

Figure 2.7 Chemical structure of Procyanidins [36]

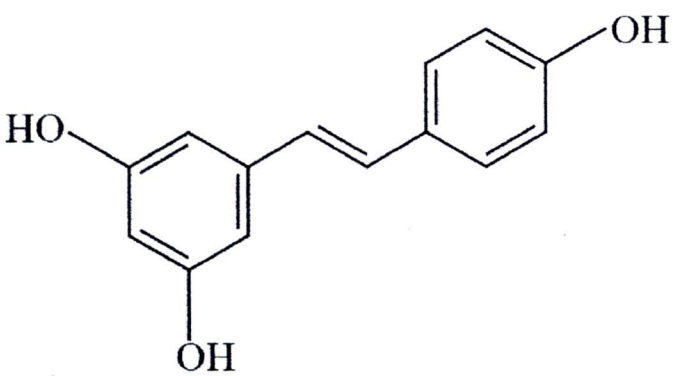

Figure 2.8 Chemical structure of Resveratrol [33]

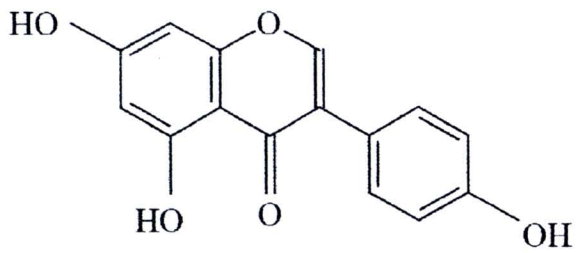

Figure 2.9 Chemical structure of Genistein [33]

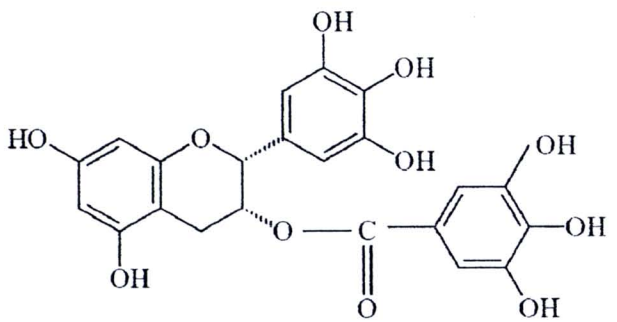

Figure 2.10 Chemical structure of Epigallocatechin-3-gallate (EGCG) [33]

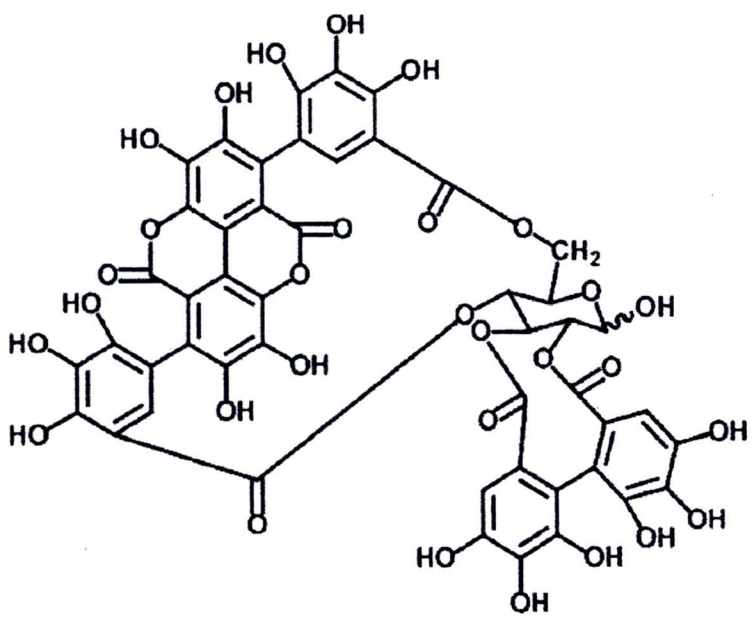

Figure 2.11 Chemical structure of Punicalagin [47]

2.4 Testing Methodologies

In this part the principles of testing methods used in this study will be described along the *in vitro* antioxidant activity tests and the clinical study (performance test) for wrinkle-reducing capacity of the final products.

2.4.1 *In vitro* methods to determine antioxidant activities

a. DPPH radical scavenging method

DPPH (2,2'-Diphenyl-1-picrylhydrazyl) is a stable free radical with deep purple color in alcoholic solution, and the maximum absorbance of DPPH is approximately 515 – 520 nm [52, 53]. The color of DPPH solution changes from purple to yellow as the radical is quenched by the electron donating antioxidants, leading to reduction of absorbance [54]. This assay is to measure the absorbance of remaining DPPH$^{\cdot}$ radical after a certain time which inversely correlates to the radical scavenging activity of the antioxidants [53].

This method is a simple, rapid and convenient method for screening antioxidant activity of many samples, but it has some limitations. The assay can be interfered by carotenoids which also have the nearby maximum absorbance (below 530 nm). Moreover, steric hindrances may occur in large molecules accessing to the radical portion located at the center of DPPH structure. Accordingly, those limitations enable to bring mistranslation of analysis [52, 55].

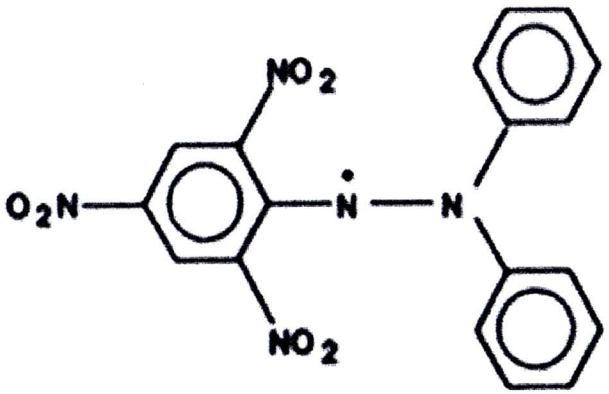

Figure 2.12 Chemical structure of DPPH· radical [56]

b. TBA-reactive substances (TBARS) method

TBARS method is an assay for lipid peroxidation that can be useful for evaluate antioxidant activity of many substances. Lipid peroxidation of polyunsaturated fatty acids (PUFA) generates various reactive products (Figure 2.13) including malondialdehyde or MDA. MDA has been used for many years as a marker for lipid peroxidation because of its reaction with thiobarbituric acid (TBA) to form a strongly pink colored chromogen as performed in the following reaction:

$$\text{MDA} + 2\text{TBA} \xrightarrow{\text{acid, heat}} \underset{\text{(pink color)}}{\text{MDA-TBA}_2}$$

Absorbance measurements at about 532 nm serve as an indicator of the extent of lipid peroxidation [52, 57]. Inhibiting lipid peroxidation by antioxidant reduces MDA generation which also influences vanishing of the absorbance.

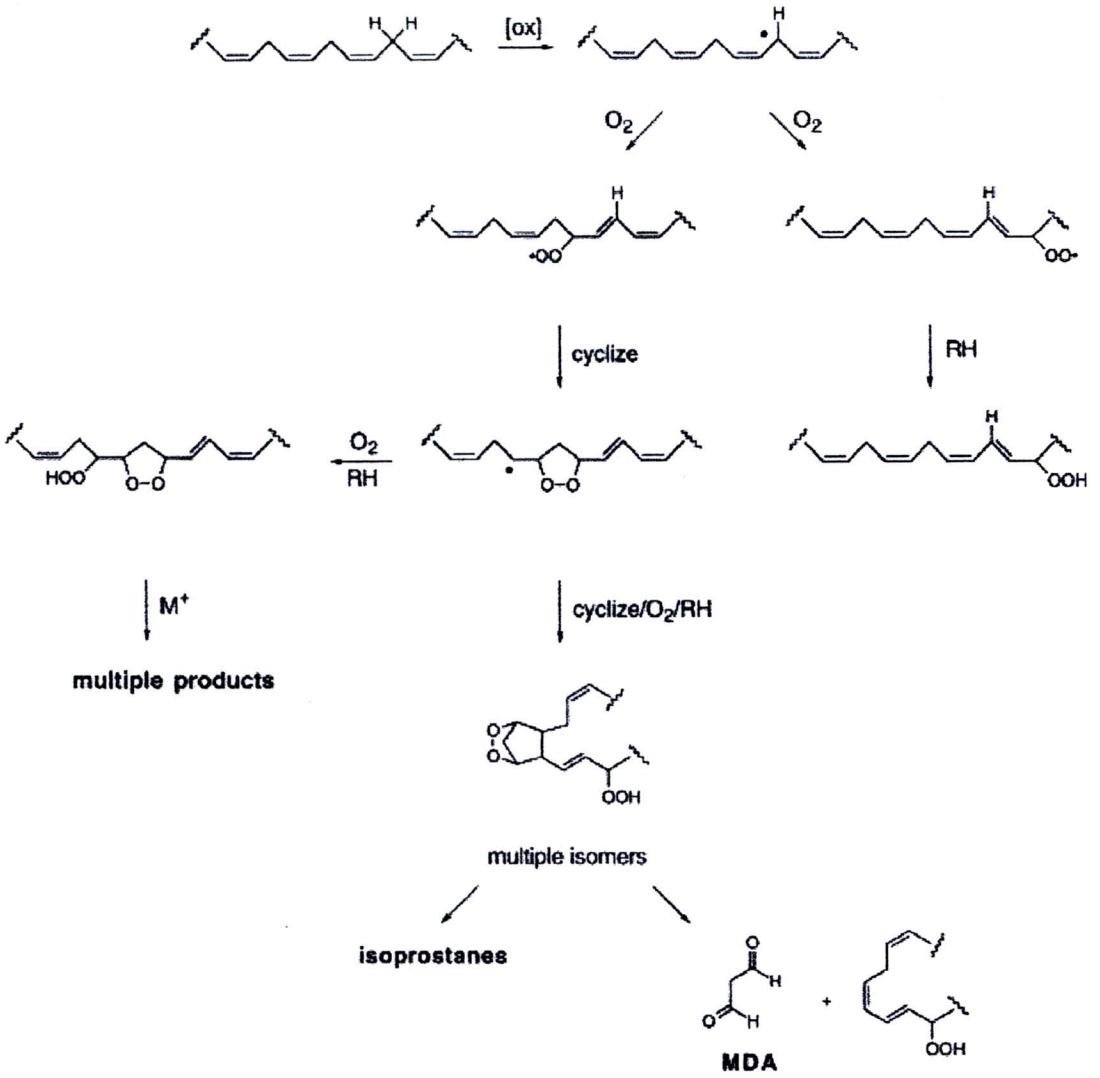

Figure 2.13 Pathways of lipid peroxidation [57]

Although some limitations occurs in this method (e.g. various degrees of saturation (amount of double bonds) in different PUFA providing the distinct TBARS inhibition rate, i.e. the higher degree of saturation shows the greater rate), but it is also a convenient and reliable process to confirm antioxidant activity. Thus the TBARS method is the widely acceptable assay for lipid peroxidation [58, 59].

2.4.2 Clinical test of anti-wrinkle products

The subjective assessments of cosmetic products usually take one of three forms: consumer research, competitor analysis and clinical trials. The clinical tests (also called 'performance tests') of anti-aging or anti-wrinkle claimed products are necessary and commonly conducted on human volunteers to assess their efficiency. Many parameters should be considered for the experimental design [60]:

1. *The subject* – age, sex, race, skin site, skin type, medications;
2. *The instrument* – zero setting, calibration, function;
3. *The environment* – temperature, relative humidity, light source;
4. *The test product* – stability, amount/surface unit, frequency of application, duration of usage; and
5. *The control* – placebo control, blank/negative control, blinded design.

The ideal measuring conditions are about 20 °C and about 50 % air humidity [61]. Test site will be analyzed at before test product application and at the test conclusion, or at various designated time intervals during the application period. However, the duration of usage for anti-aging products should be at least 4 weeks [62].

Skin surface topographies are used to determine skin texture or microrelief; a good indicator for the skin aging process (wrinkles, fine lines and roughness). The topographies of the face and forearm correlate with skin surface aging and enable to indicate improvement of skin texture after the product application [60, 61].

The skin profilometry is a method to analyze skin surface by illuminating test area with a light source, and capturing the reflected light with a detector. The device analyzes length and depth of wrinkles with the specific algorithmic software and then shows the results in various parameters including [60, 61];

a. Surface

This parameter is calculated by the size of the wavy surface in comparison to the stretched surface (x : 1). The smoother the area was before stretching it, the closer the two values are together. The result is displayed in % (e.g. 112 % = the stretched area is 12 % larger than the original surface).

b. Volume

The volume parameter implies to the virtual amount of liquid needed to fill the image until the average height of all mountains in the measuring area. The smoother an area before filling up, the less liquid is needed. The result is showed in mm^3.

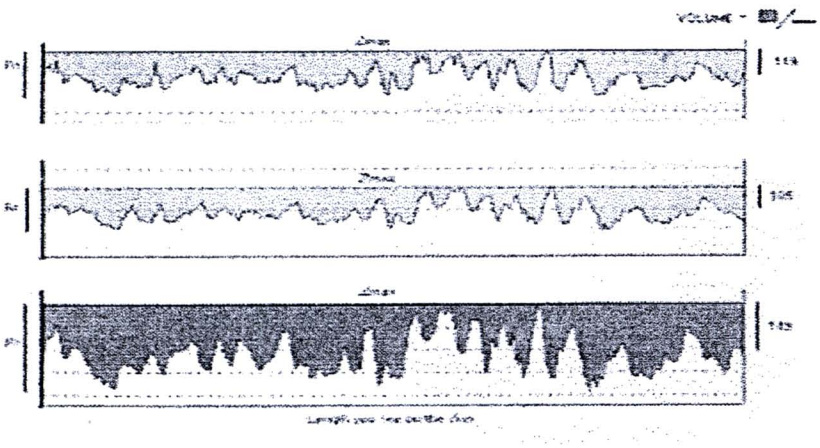

Figure 2.14 Volume parameter [61]

c. Arithmetic average roughness (Ra)

The arithmetic average roughness or the average optical roughness (Ra) is the area between the middle line and the average line of roughness profile.

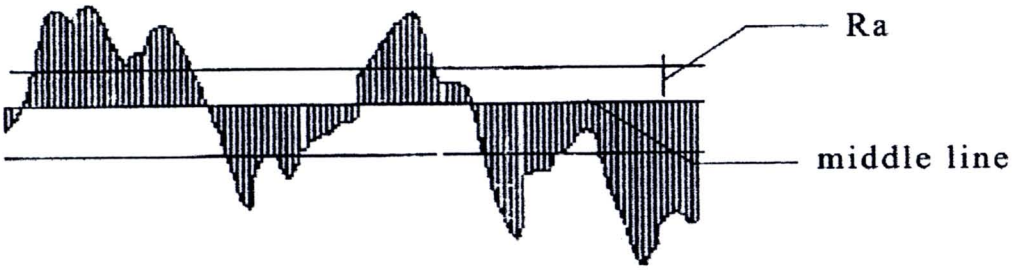

Figure 2.15 Ra parameter [61]

d. Average roughness (Rz)

The average roughness (Rz) is the arithmetic mean of the roughness depths calculated from five segments of the same length.

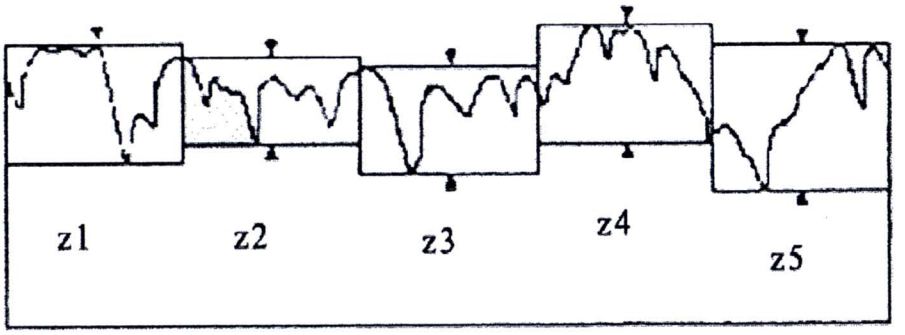

Figure 2.16 Rz parameter [61]

The reduction of these four parameters correlates to an improvement in skin surface (or decreasing of wrinkles, fine lines and skin roughness) [61, 63].

2.5 Cosmetic serum

Cosmetic serum is a popular type of cosmetic formulation in these recent decades. There isn't an absolute definition, but the cosmetic serum always refers to a highly concentrated product based on the water oil emulsion like any other cream or lotion. Serums contain about ten-fold higher active substance concentration than creams and lotions. They are usually water-based which makes them lightweight and quickly absorbed into the skin. Therefore, they are a concentrated way to get anti-aging ingredients into the skin, and most skin types (e.g. dry skin, oily skin) can use them as they tend to be light and water based [64, 65].

However, the stability and the skin compatibility (without irritation or allergic reaction) of cosmetic serums should be considered since they are consisted of a high concentration of active ingredients.