Bioengineering Methods and Skin Aging

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Abstract

Skin aging is an uneven process characterized by epidermal and dermal disorders, accompanied by many clinical signs such as skin dryness, color changes, loss of elasticity, wrinkles, and risk of developing skin cancers. The elderly appearance of the skin depends on a combination of intrinsic or chronological aging, modulated by genetically predisposing factors and extrinsic aging or photoaging. This chapter reviews data that have emerged from technologies aiming at quantitatively assessing the effects of aging on the skin.

Introduction

During the last decade, skin aging has become an area of increasing research interest, because of the prolongation of life span in modern society.

Skin aging is an uneven process characterized by epidermal and dermal disorders, accompanied by many clinical signs such as skin dryness, color changes, loss of elasticity, wrinkles, and risk of developing skin cancers. The elderly appearance of the skin depends on a combination of intrinsic or chronological aging, modulated by genetically predisposing factors and extrinsic aging or photoaging, due to environmental factors, mainly UV exposure, and also wind, relative humidity, pollution, and so on. The effects of the UV radiations on sun-exposed sites are superimposed on the morphological, biochemical, and functional

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changes occurring with aging, making distinction between the two phenomena hard.

Besides genetic aspects, all these environmental factors are responsible for the great interindividual and intraindividual variations and the site-dependent variations of the aging process. A precise and noninvasive quantification of aging is of utmost importance for in vivo studies in skin gerontology and for cosmetic research. Several bioengineering methods have been proposed to objectively, precisely, and noninvasively measure skin aging and to detect early skin damage, which is rather difficult to demonstrate clinically.

This chapter reviews the data that have emerged from recently introduced technologies aiming at quantitatively assessing the effects of aging on the skin.

The variations in biophysical parameters such as hydration and trans-epidermal water loss, which have been evidenced in the skin in elderly subjects, will be discussed in detail in other chapters.

To date, high-frequency ultrasonography has been used most extensively to visualize and quantify age-related skin changes. This chapter focuses on ultrasound findings in intrinsic and extrinsic skin aging. In the last decade, a new noninvasive technique has been developed to examine the epidermis and the papillary dermis at a resolution approaching histological detail: confocal scanning laser microscopy. Review literature data on the application of this promising technique for the study of skin aging have also been included at the end of this chapter.

pH

Cutaneous acidity plays a role in skin barrier homeostasis, in stratum corneum desquamation, and in skin defense against microbiological or chemical insults. In order to measure cutaneous pH, instruments like a glass planar electrode are primarily used. They create a potential difference between the two environments separated by the glass slide, that is, the skin surface and the reference solution contained in the electrode. This potential difference is linearly linked to the difference in H^+ concentration.

Limited data are available on skin pH and age. pH is relatively constant from childhood through age 70. Fluhr et al. did not find a significant difference in pH measured on volar forearm between 44 adults aged 21–44 and 44 children aged 1–6 [1]. These data were confirmed on the forehead in 500 female patients aged 20–70 [2]. In contrast, skin acidity decreases significantly in subjects older than 70 [3, 4]. Wilhelm et al. measured pH on 11 skin sites in 14 young volunteers (mean age 27 years) and in 15 aged volunteers (mean age 71 years) and noted significant differences at the ankle and thigh only [4]. Therefore, the authors attributed the higher pH to stasis and reduced oxygen supply frequently observed in the lower limbs in elderly patients.

Sebum

Sebum production is controlled by the levels of circulating hormones and varies according to the anatomical distribution of sebaceous glands. It is generally measured by an instrument allowing a semiquantitative evaluation of sebum excretion (Sebumeter, Courage and Khazaka Electronic, Köln, Germany). This method is based on photometric measurements of light transmission through a transparent plastic film, which is pressed against the skin in order to obtain adhesion of skin lipids. The recorded values are expressed in arbitrary units, which can be converted into microgram per square centimeter, according to the manufacturer's calibration table.

The sebum excretion rate has been demonstrated to decrease with age by different authors [4–7], more markedly at sites of elevated sebum production. When measuring the casual level of sebum in 63 healthy subjects aged 12–60 and in 24 older subjects at 14 body sites [5], a marked decrease in elderly volunteers was observed, with significantly lower values on the forehead and the upper back (Fig. 1).

It has been proposed that changes in sebum excretion during aging reflect the decrease in the endogenous production of androgens occurring in men and women. In menopausal women receiving hormonal replacement therapy, the sebum

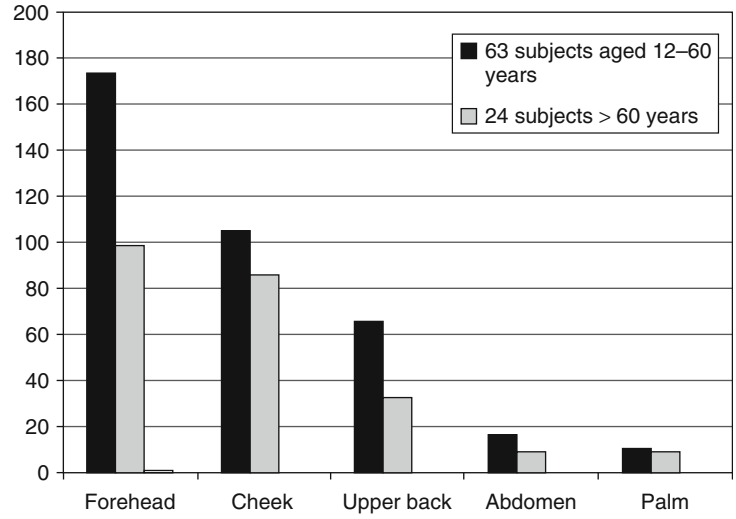

Fig. 1 Casual level of sebum in relation to age ($p < 0.05$ at forehead and upper back)

excretion rate shows a 35 % increase, because of the stimulatory effect of the progestagen component [6]. Caisey et al., by measuring the amount of sebum produced over 1 h on the forehead of 20 young women, 19 premenopausal women, 21 postmenopausal women, and 20 postmenopausal women receiving hormonal replacement therapy, did not find a correlation between age and sebum excretion rate [7]. However, the values in postmenopausal women were significantly lower than in the other groups showing a 35–40 % decrease compared with females receiving estrogen and progesterone. The authors concluded that sebum production is more likely related to hormones than to aging.

Skin Color

Skin color can be determined by a chromameter (Minolta, Osaka, Japan) according to a three-dimensional L* a* b* system. L* represents an attribute on the luminance scale, a* on the red-green color scale, and b* on the yellow-blue one.

Photoaging is clinically characterized by yellowish skin with erythematous areas, associated with telangiectasia and heterogeneity of skin pigmentation. By examining the skin of sun-exposed and adjacent unexposed sites, Richard et al. reported significant differences in L* values and a* values with larger standard deviations at exposed skin sites, indicating a decrease in brightness, an increase in the red component, and color heterogeneity in photodamaged skin in the elderly [8]. In contrast, Warren et al. observed no change in L*a*b* values [9]. In order to study photodamaged skin, Kikuchi-Numagami et al. measured skin color of the dorsum of the hands in 12 middle-aged Japanese golfers, playing golf frequently for the past 4–25 years [10]. By comparing the right hand, exposed to sunlight many hours a day, to the left hand that is protected by a glove from the outer environment, he found that whereas L* value was significantly lower, a* and b* values were significantly higher on the right hand in comparison with the left hand protected by the glove. The differences in L* values were dependent on the length of past golf-playing history.

A negative correlation between L* values and age was found on the lower lips in 80 postmenopausal women, whereas no correspondence was observed between age and the other two color components [7]. L* values were significantly lower in the group of postmenopausal women in comparison with two groups of younger and postmenopausal women receiving hormonal replacement therapy. The authors concluded that menopause may result in a slight darkening of

the lip, which could be prevented or corrected by hormonal treatment. Also Guinot et al. reported that menopausal subjects treated with hormonal replacement therapy showed redder lips than untreated menopausal women [11].

Skin Blood Flow

Skin blood perfusion can be quantified by Laser Doppler Flowmetry and Laser Doppler Velocimetry. A helium-neon laser light is transmitted to the skin via an optical fiber to an estimated depth of more than 1 mm. Light reflected from moving erythrocytes is Doppler shifted. The frequency-shifted signal is proportional to blood flow and can be extracted and measured by the instrument in arbitrary units.

Data regarding age-related changes in blood perfusion are often conflicting, probably because of the small sample sizes and the varying age ranges of the studies [1, 3, 12–15]. By using Laser Doppler flowmetry on the volar forearm in 44 children and 44 adults aged 21–44, Fluhr et al. found higher blood flow values in children. On the contrary, Kelly et al. did not observe any significant differences in blood flow perfusion both in ventral forearm and forehead in a small study population comprising ten subjects aged 18–26 and ten subjects aged 65–88 [13]. Likewise, in a study comparing only nine elderly and ten young volunteers, the skin vascular response to heat and cold challenge measured by Laser Doppler Velocimetry was delayed in elderly subjects [14]. This may be due to a reduced vessel density in aged skin. Also in a study population of 201 people aged 10–89, the blood flow measured after immersion in water at 10 °C was lower in subjects over 50 and the restorative ability poorer in subjects over 70 compared with younger ones [15]. The basal blood flow decreased with age in all the areas with high blood flow, such as the lip, finger, nasal tip, and forehead.

In conclusion, trends indicate that aging is associated with a decrease in cutaneous blood perfusion, in particular in photo-exposed areas [1, 3, 12, 14, 15].

Skin Surface Roughness

The morphological study of the skin surface can be performed by optical, mechanical, laser, and transparency profilometry. The first method is based on skin replicas and evaluation of the black-and-white reflections by light irradiation depending on topography of skin furrows. The image is processed using a special image processing software by a CCD camera or a high-resolution black-and-white video and a connected computer. Mechanical profilometry offers a two-dimensional quantification of the absolute height and depth of wrinkles and furrows. Laser profilometry is an optical technique based on the principle of light amplification and reflection from a cutaneous replica. Recently, the visiometer method or transparency profilometry (Skin Visiometer SV600, Courage and Khazaka, Cologne, Germany) has been developed with some advantages, as short processing time and direct visual control of the skin surface topography on the computer monitor. This technique uses a thin silicone gel print of the skin surface, which allows parallel light to pass through and is registered as a change of transparency by a CCD video camera [16].

Aging is characterized by fine and coarse wrinkling, whose estimation is surely of great interest especially in the field of cosmetic research.

By performing laser profilometry on the dorsal surface of the hands in Japanese golfers, Kikuchi-Numagami et al. observed that roughness parameters were increased on the right hand exposed to sun in comparison with the left hand protected by a glove when playing golf [10]. The differences became larger in golfers with lower handicap and longer golf history, probably due to elastogenesis in the dermis of the photoaged skin [9]. Likewise, Quan et al. found significant differences in skin roughness between sun-exposed and sun-protected areas by using mechanical profilometry [17]. However, this difference was significant only in the group of older subjects. To study photodamage and the effect of tretinoin on it, Marks et al. performed optical profilometry on crow's foot areas, associated with other noninvasive and invasive technique, concluding that there is no

single method to quantify the degenerative changes due to photodamage [12]. In a study evaluating the crow's feet of 95 women aged 30–50 by transparency profilometry and ultrasonography, an increment of all roughness parameters was observed, as age increases [18]. Moreover, a correlation between skin roughness and dermal density and thickness was found.

Skin Thickness

In order to measure cutaneous thickness under a variety of normal and pathologic, ultrasonography has been widely used for about 30 years. Since penetration depth of the ultrasound waves is inversely related to its frequency, the optimal frequency for achieving a higher resolution for skin examination is 15–20 MHz. The ultrasonic wave (velocity 1,580 m/s) is partially reflected at the boundary between adjacent structures, generating echoes, whose amplitudes are characteristic of the nature of the media. The ultrasonographic image can be evaluated either manually or with computer assistance to quantify skin thickness.

Two forms of ultrasonography, A and B modes, are available. The first gives an unidimensional representation of skin echogenicity and is easier and quicker than the B-mode. However, by A scanning, the determination of the dermis-subcutis interface is based on the observation of a peak corresponding to the impedance jump between adjacent parts of the tissue, making determination of the dermis-subcutaneous tissue interface difficult, whereas B-scan measurement of skin thickness represents the mean of consecutive A-scan lines composing the whole bidimensional image. Thus, the reproducibility of B-mode assessment is higher, enabling the production of bidimensional images of cross sections of the skin. By means of the B-scan method, skin thickness values are approximately 15 % greater than A-scan measurements [19].

Skin thickness has been a widely used parameter to evaluate the influence of different factors on skin aging, but the measurement of age-related changes in skin thickness yielded conflicting results. In fact, depending on the anatomical site, both thinning and thickening are observed. This poor consensus can be explained by differences in the age range and body site of study populations and also in the frequency, mode, and gain curves of the ultrasound technique [20]. Thus, the adhesion to standardized measurements protocols in reproducible conditions is of utmost importance.

Employing A-scan ultrasound on the volar forearm, Tan et al. found that skin thickness increased progressively up to the age of 20 and decreased subsequently [21], whereas Escoffier et al. observed that skin thickness increases up to the age of 20–30 years, remains constant until 65 years, and then gets thinner, being at 90 years significantly thinner than at 5 years [22]. A similar trend was described by de Rigal et al., employing the B-scan method, in a population of 142 females aged from 0 to 90 years [19]. Skin thickness on both the volar and the dorsal aspects of the forearm thickened up to 15 years (maturation phase), did not vary until the seventh decade of life, and diminished thereafter (atrophy phase). These modifications in skin thickness seemed to be correlated with the age-dependent degree of elastosis.

Site-to-site variations in skin thickness related to aging were reported by many authors [23–27].

In 162 volunteers in the age range of 27–90, skin thickness variations were assessed using both A- and B-mode high-frequency ultrasonography at six different anatomical locations (forehead, cheek, volar forearm, dorsal forearm, upper abdomen, and buttocks) [25]. It was found that subjects over 70 years have thinner skin when compared to young volunteers (age *27–31*), but the differences were significant for sun-protected skin sites (abdomen and buttocks) only.

In a study on 90 Danish subjects aged 18–94, skin becomes thicker with age at the forehead and buttocks, but decreases at the extremities (dorsal and ventral forearm, and ankle) significantly [26]. Since these data cannot be explained by differences in sun exposure, the authors concluded that the effect of aging on axial skin could differ from that on extremity skin.

With regard to photoaging, Leveque et al. reported an increase in skin thickness in sun-exposed areas in a study on cyclists in the Tour de France [28]. Likewise, Adhoute et al. observed skin thickening on face and neck sites induced by solar exposure [29]. In 170 women aged from 17 to 76, Takema et al. measured skin thickness on the ventral forearm, forehead, cheeks, and corners of eyes and mouth by employing a 20-MHz A-mode ultrasound scanner [24]. Skin thickness increased with age on all sun-exposed areas of the face, whereas it seemed to decrease on the sun-protected volar forearm. On the contrary, using B-mode ultrasound on the neck of 30 elderly women (age 81 ± 6 years) with high lifetime sun exposure, Richard et al. found that skin thickness was lower on an exposed area in comparison with an adjacent, anatomically equivalent unexposed area [8]. Especially on facial skin, thickness assessment yielded contrasting results. On the forehead, skin thickness appeared both to decrease [23, 30] and to increase [24, 26, 27] with age, in different studies. Employing A-mode ultrasound, Denda et al. measured skin thickness on the forehead and the cheek and observed a decrease with age [23], whereas Takema found an increase in the same areas and also on eye corners [24]. In contrast, using B-mode ultrasound in 95 Korean women aged 30–50, Lee et al. did not observe changes with age on the crow's feet [18]. In a study population of 20 women aged 25–30 and 20 women aged 60–90, skin thickness was assessed at 12 different facial skin sites using a 20-MHz B scanner [27]. Higher values in skin thickness in the elderly were observed on all assessed facial sites except on the infraorbital region. In particular, the increase in skin thickness was statistically significant on the lateral regions of the forehead, the upper and lower lips, and the nose. The fact that facial skin thickness does not show a decreasing trend as at other skin sites can be explained by the observation that on sun-damaged facial skin, the reduction in collagen and ground substance content, which gradually takes place with aging, is counterbalanced by the overall rearrangement of the dermal collagen network and the accumulation of elastotic material [31, 32].

Skin Echogenicity

By providing quantitative data of echogenicity, high-frequency ultrasound permits the noninvasive evaluation of age-dependent modifications in collagen structure, elastosis, and other ultrastructural features of the skin, together with the effects of diurnal and hormonal changes [6, 32–34]. When a B-mode ultrasonographic image of normal skin is generated, a hyperreflective band-like echo is observed between the medium and the skin, the so-called entry echo, corresponding to the epidermis, due to the impedance change from the coupling medium to the stratum corneum. Immediately below the entry echo, there is the corium, rich in collagen fibers, which are the main source of its echogenicity.

In 1989, de Rigal et al. studied 142 females aged 1–90 by B-mode 25-MHz sonography on the volar and dorsal aspects of the forearm [19]. They identified a subepidermal hypoechogenic band, appearing as a relatively homogeneous, echolucent structure, located immediately below the entry echo. This subepidermal low-echogenic band (SLEB), which was invisible in the young, was present in most elderly subjects at the forearm and was located in the upper dermis, in some cases occupying the greater part of the dermis. The thickness of SLEB increased with age progressively and was higher on the dorsal forearm. Comparing two adjacent, anatomically equivalent sites, with and without sun exposure on the neck, Richard et al. noticed that SLEB thickness was greater on sun-exposed sites [8]. The presence of SLEB has been confirmed by many other investigators and was correlated to the severity of photodamage [12, 20, 26, 27, 34]. A Japanese study on 130 women aged 18–83 failed to demonstrate the presence of SLEB on facial areas (forehead, cheeks, and eye corners) probably because of the cultural tendency toward careful facial sun protection [35]. Changes in SLEB have been used to assess the efficacy of antiaging cosmetics [36]. Since the main source of dermal echogenicity is represented by well-arranged collagen bundles, the appearance of SLEB is correlated to the structural changes that occur with age.

In elderly skin, collagen bundles are replaced by a more homogeneously stained material, leading to the dissolution of the regular architecture of the collagen and elastic fibers and to the deposit of a greater amount of hydrated proteoglycans and glycosaminoglycans and of unbound water [37, 38]. Gravitational changes in body water balance throughout the day may explain the diurnal variation in SLEB thickness described by Gniadecka et al. on the volar forearm of 23 subjects aged 75–100 [34]. In 74 % of the study population, a clear diurnal variation in SLEB thickness was found. People who have a thick SLEB in the morning before rising had a thinner SLEB in the afternoon; the opposite was also true. Moreover, a well-developed SLEB was present only in 53 % of cases, and in some volunteers this was irregular with ill-defined borders. In these instances, SLEB thickness measurements were complicated and unreliable. SLEB can be more precisely quantified by image analysis than by visual scoring or thickness measurement. Computer-assisted analysis of the ultrasound image is based on the attribution of arbitrary values by a 0–255 scale to the echoes' amplitudes for each pixel, segments the image by pixel range, and measures regions of echogenicity as specified by the investigators [20]. To calculate echogenicity, the number of low-echogenic pixels, being defined as those with echogenicity 0–30, can be measured and related to the total number of pixels; this ratio is increasing with the decrease in echogenicity.

Ultrasound evaluation of the dermis by means of image segmentation showed that age-related changes are not limited to the upper dermis, characterized by the appearance of SLEB, but also to the lower dermis, which appears more echogenic in elderly subjects at all examined sites [20, 26, 27, 39, 40]. This dermal hyperreflecting band has been reported to become thinner with increasing age on both the volar and the dorsal forearm in 142 females aged 0–90 by de Rigal et al. [19]. In a study population of 90 subjects aged 18–94, Gniadecka et al. studied echogenicity in the different layers of the dermis on regions with different levels of sun exposure (dorsal and volar forearm, forehead, and ankle) and nonexposed buttocks [26]. The echogenicity of the lower dermis increased at all examined sites, including those with little or no sun exposure, suggesting that changes in the dermal echogenic band characterize chronological aging. In contrast, SLEB was present at the sun-exposed dermis alone and, prior to formation of a discernible SLEB a progressive, age-related decrease in echogenicity of the upper dermis was found in sun-exposed areas (dorsal forearm and forehead), but not at moderately exposed sites (ventral forearm and ankle). A significant relationship between skin echogenicity of the upper skin layers, age, and degree of photodamage assessed clinically further indicates that the decrease in subepidermal echogenicity may provide an objective parameter to evaluate solar damage. These results were confirmed in a study on 55 adults aged 18–57, undergoing 20-MHz ultrasonography and image analysis on the volar and the dorsal side of the forearm [40]. The authors demonstrated that skin echogenicity measured as a ratio between the upper and the lower dermis may be used to objectively estimate photoaging.

Evaluating echogenicity of the dermis as a whole, some investigators found that it decreases with increasing age [41]. In contrast, using a 20-MHz B-mode scanner on six skin sites on 24 volunteers aged 27–31 and on 24 volunteers over 60, an increase was found in overall echogenicity of the dermis in the elderly [20].

Depending on different skin areas, nonuniform variations in skin echogenicity from childhood to adulthood were observed [42]. A gradual increase was observed in echogenicity on the limbs with increasing age, whereas on the face and the trunk echogenicity was higher in children than in adults. In ultrasound images, facial skin shows scarce reflectivity, both in the young and the elderly, compared to other skin sites, such as the forearm, where the dermis is highly echogenic and the dermis-hypodermis boundary is well outlined. When evaluating overall skin echogenicity at different facial areas in young and elderly women, an increase with age at all examined sites except the infraorbital regions was found [27]. Moreover, age-related changes in skin echogenicity,

consisting in the appearance of a subepidermal band and an enhancement of the lower dermis reflectivity, were present at most facial sites. These findings of increased overall echogenicity in the elderly may be due to an enhancement of the lower dermis echoes, rather than a decreased echogenicity of the upper dermis.

Confocal Scanning Laser Microscopy and Aging

In vivo confocal scanning laser microscopy (CSLM) is a noninvasive technique permitting optical en face sectioning of the skin with good contrast and high resolution, providing cellular and subcellular details. It seems to have a tremendous potential for research and diagnostic purposes in dermatology, since CSLM supplies an open "histological" window to the tissue noninvasively. The commercially available Vivascope microscopes (Lucid, Rochester, NY) use a diode laser source with a wavelength of 830 nm, an illumination power up to 20 mW on the object and water immersion. The penetration depth of imaging allows the visualization of the epidermis and the upper dermis with a good correlation to histologic sections [43, 44].

Sauermann et al. investigated skin aging using CSLM on the volar forearm of 13 young and 13 elderly volunteers [45]. The cells in the granular layer were significantly larger in the older subjects confirming histological findings documenting the increase of corneocytes with age as a result of a lower proliferation rate and turnover of the epidermis. Basal layer thickness decreased significantly, whereas thickness of the epidermis increased in the older volunteers compared to the younger ones. However, the most relevant age-related change in this study was the reduction in the number of dermal papillae per area with age reflecting the flattened epidermal-dermal junction in elderly skin. Histometric measurements by CSLM proved to be a sensitive tool for characterizing histological changes in the epidermis and papillary dermis due to aging and also for cosmetic research. The same authors evaluated the efficacy of a cream containing vitamin C applied twice a day for 4 months on the volar forearm in a study population of 33 women aged 45–67 by using CSLM [46]. Topical vitamin C resulted in a significant increase in the density of dermal papillae and in a reduction of granular layer cell size, indicating relevant effects in correcting the structural changes associated with the aging process.

In CSLM images, Neerken at al. identified a reflecting layer of fibrous structures, whose depth strongly depends on age below the basal layer [47]. In addition, large structural changes, such as the flattening of the dermo-epidermal junction and a thinning of the epidermis, were observed with increasing age.

CLSM was also employed to assess photodamaged skin to study alterations in dermal collagen fibers brought about by long-term sun exposure [48].

Conclusion

Despite the many tools and techniques available for the noninvasive evaluation of age-related changes in skin structure and functions, further work is needed to develop a unified understanding of skin aging. To date, results of some studies on skin aging are often conflicting and difficult to interpret. Instrument and other measurement-related variables can partly explain the differing results. Moreover, the great interindividual and intraindividual variations of the human skin make consensus a challenging objective. Therefore, further studies which include larger study populations and standardized protocols are necessary.

Cross-References

- ▶ Corneocyte Analysis
- ▶ Hydration of the Skin Surface
- ▶ Transepidermal Water Loss in Young and Aged Healthy Humans

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