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Running head: Wrinkle changes in the early 30s

Figure count: 8 figures

Table count: 3 tables

Word count: 3210 words

Key words: wrinkle – aging – morphology – compression – mechanical property

Background/purpose: Does the morphology of wrinkles alter gradually with aging or suddenly at a certain age? Based on the theoretical wrinkle simulation of ideal skin, we have suggested that the wrinkle morphology suddenly changes from stratum corneum wrinkling to epidermis wrinkling; the former induces shallow fine furrows, and the latter induces deep prominent wrinkles. To examine the existence of drastic change in wrinkling morphology, we developed a new measurement system for facial skin wrinkling test.

Methods: The mechanical compression test of facial skin was carried out for 102 Japanese women aged 25–56 years. The test was performed on the right temple area skin, and the area of wrinkles induced by the compression was measured by a digital video camera. The rate of increase in wrinkle area during compression was defined as the skin wrinkling rate, and it was calculated for all subjects automatically by image processing.

Results: The test results showed that the skin wrinkling rate underwent a step increase at the age 33, which means that the wrinkling morphologies of young and old skins are completely different, so it changes suddenly in the early 30s.

Conclusion: A new skin measurement system was developed to validate our theory of wrinkle formation mechanism with aging. The results demonstrated the wrinkling morphology changes suddenly at early 30s.

Wrinkles are a typical indicator of aging, and can suddenly become pronounced during middle age. Persistent wrinkles are clearly visible in older skin (Fig. 1), but the chronological process of their formation remains unclear. Many clinical studies have measured age-related changes in the physiological or mechanical properties of the skin, such as pH, transepidermal water loss, and viscoelasticity (1-4). Since the measured parameters gradually alter with aging, it would be reasonable to assume that skin wrinkles gradually increase in thickness and visibility with aging. However, our theoretical simulations of skin wrinkling (5, 6) indicate that wrinkling properties change suddenly in middle age due to the transition in mechanical balance among layers of the skin, even though the mechanical parameters alter gradually (Fig. 1). This study examined the correlation of this theoretical prediction with the actual changes in wrinkling properties. To investigate the age-related changes in wrinkling properties, we carried out a clinical test and examined the correlation with our theory of wrinkle formation.

Many researchers have examined how the mechanical properties of the skin change with age by means of physical testing, such as suction (7-11) or torsion (12-16). Since their results show a prominent correlation between the viscoelastic properties of skin and age, there is no doubt that the mechanical parameters depend on age. The mechanical properties of the layers of the skin (11, 17, 18) have been quantified through detailed experiments and simulations as described below. Skin wrinkling has also been theoretically studied in terms of mechanics (5, 6, 19-22). Nevertheless, even though the mechanical properties of the skin have been clarified qualitatively and quantitatively, much remains unknown due to the complexity of the skin structure. The wrinkling grade measurement (16) is a different type of measurement: whereas other methods measure the resistance to or recovery from mechanical stimulation such as extension or shearing, the wrinkling grade measurement quantifies the shape, that is, the width and number of skin folds under 42% compression, which represents a mechanical *balance* between skin layers. The measured wrinkle is a temporary one induced by external compression, and indicates the capacity for mechanical collapse. This wrinkling property will affect the formation of a persistent wrinkle over time. The present study used an automatic testing system with image processing to measure the quantity of wrinkles in temporary wrinkles under external compression, and clarified the age-related changes in the skin's wrinkling properties.

On the other hand, physical simulation via finite-element analysis (FEA) (23) is now widely used in biological and medical studies to evaluate the deformation behavior of biological tissues and to understand its biomechanical properties (24). For cutaneous mechanics, the FEA has also been used to identify the material properties of layered skin tissue (10, 11, 17), predict skin deformation by surgical operation (25), and simulate the wrinkling behavior of multilayer skin (21, 22), which is called buckling in the structural mechanics (26). We have also elucidated the three basic modes for wrinkling (buckling) of multilayer skin using FEA (5, 6), and theoretically showed that wrinkling properties change suddenly caused by the buckling mode switch (BMS) (6, 27). In this study, we utilize this mechanics theory to describe the clinical test results, which validate the drastic change in wrinkling properties. We then identify the process by which wrinkles quickly become pronounced.

Mechanical Wrinkle Theory

Mechanics of skin wrinkling

When a thin structure such as skin is subjected to compression, it exhibits the characteristic deformation shape of buckling mode (26). Buckling is a structural instability by which a thin structure subjected to compression collapses and bends. Buckling mode is a type of undulating deformation with a characteristic wavelength, with a critical compression ratio (CCR) at which the deformation changes from compression to bending. In this study, the buckling mode is referred to as the wrinkling mode. Since the wrinkling mode determines the spacing of wrinkles, the wavelength is referred to as the specific wrinkle size (SWS) (5). The skin is compressed by the contraction of underlying muscles, and becomes wrinkled when the compression ratio exceeds the CCR. The flat multilayer skin model has three basic wrinkling modes (Fig. 2): stratum corneum wrinkling (Mode 1), epidermis wrinkling (Mode 2), and dermis wrinkling (Mode 3) (5, 6). Each mode has its respective CCR and SWS.

Mechanism of wrinkle formation with aging

In young skin, Mode 1 has the lowest CCR and Mode 3 has the highest CCR (5), and so Mode 1 is most easily induced by a small compression. SWS follows the same order as CCR, so Mode 1 is shallowest and Mode 3 is widest. The frequent wrinkling of Mode 1 damages the stratum corneum by repeated folding, and the accumulation of repetitive damage leads to the formation of persistent wrinkles (Fig. 3) (28). Thus, the SWS determines the spacing of persistent wrinkles. As the SWS of Mode 1 is small, the formed wrinkles are also fine in young skin. This skin condition, in which Mode 1 is dominant and the formed wrinkles are fine, is called Stage 1.

However, due to deterioration of the skin structure with aging (2, 7, 29-33), the magnitude of CCR of Modes 1 and 2 switches (5, 6). This phenomenon is called the buckling mode switch (BMS). The changes in mechanical parameters of layers such as elastic modulus and thickness are continuous, but the BMS from Mode 1 to Mode 2 is discrete and caused by the disruption of mechanical balance among skin layers. After BMS, Mode 2 can be induced by weak compression, and the formed wrinkles quickly become wider because SWS of Mode 2 is considerably larger than that of Mode 1. In general, wider wrinkles yield deeper furrows (21, 22). Consequently, the frequently damaged portions change along with the change in SWS from Mode 1 to Mode 2 (Fig. 3). Thus, the newly formed persistent wrinkles are wide and deep, and they quickly become pronounced in aged skin. This skin condition, in which Mode 2 wrinkling dominantly affects the formation of pronounced persistent wrinkles, is called Stage 2.

This scenario of wrinkle formation and drastic increase in pronounced wrinkles was deduced from FEA of aging skin (6), and the theory was validated by a parametric study considering the possible range of variations in the elastic modulus and thickness of viable epidermis (VE) (27). The effects of stiffening of stratum corneum (SC) by dehydration (2, 3) and weakening of upper dermis by photoaging (7, 29, 31) were also simulated, and the results showed that BMS can occur in human facial skin due to aging effects in the elastic modulus and thickness of skin layers.

Materials and Methods

Subjects

To evaluate the wrinkling properties of facial skin and age-related changes, a skin compression test was conducted on the right temple area for 102 healthy Japanese female volunteers evenly distributed in age from 25 to 56. Subjects with a BMI in the range of 18–25 were selected to exclude excessively fat or thin subjects. Since the skin around the temple is easily affected by photoaging, the test results must include the effect of photoaging. All subjects gave written informed consent after receiving a complete explanation of the study protocol and purpose of the investigation. The study was monitored to ensure compliance with the Guidelines for Good Clinical Practice. Before participating in this clinical study, each subject signed a consent form that contained all the basic elements outlined in the Code of Federal Regulations (21 CFR) 50.25.

Compression device and video system

To quantify the wrinkling capacity of facial skin, we developed a facial skin compression imaging system (Fig. 4). The system is composed of a skin compression unit equipped with a micro-compressor and a CCD video camera. Two silicone rubber probes are connected to the micro-compressor by rigid arms separated by a probe tip distance of 30 mm. Each volunteer was carefully positioned in a relaxed state using the facial positioning device, and the two probes were gently attached on the skin of the right temple region by double-sided adhesive tape. The facial skin was vertically and intermittently compressed by 1-mm steps up to 10 mm (0.5-mm steps up to 5 mm for each probe). The compression ratio (CR) was calculated by dividing the compressive displacement (probe traveling distance) by the initial probe tip distance (30 mm). Thus, the CR was controlled from 0 to 33.3% at 3.33% pitch. The compressed skin was captured as a digital movie by the CCD video camera under single controlled LED illumination, and an image at each compression step was extracted from the movie and used for the wrinkle measurement.

Image processing

The images of compressed skin were analyzed using image processing software originally developed by P&G Innovation GK. The region of interest (ROI) was manually selected from near the middle of the two probes in the captured image prior to image processing. The image processing detected the shadow areas based on the local contrast, which we assumed to be valleys of the wrinkling surface (Fig. 5). The number of detected shadow pixels was normalized by the number of ROI pixels and quantified as a percentage. This quantity is referred to as the wrinkle area fraction (WAF). However, since the WAF must depend on the threshold value in brightness between shadowed pixels and bright pixels, the threshold was empirically determined and fixed to appropriate constant values for all subjects.

Calculation of wrinkling parameters

As a result of WAF scattering due to measurement errors in CCD images, the mean increase rate of WAF within 20% compression was introduced to eliminate the effect of data scattering and stabilize the evaluation results, and is referred to as the skin wrinkling rate (SWR). SWR represents the slope of the relationship between WAF and CR, and is evaluated by least-squares approximation within a small range of compression (CR of 20%). Moreover, SWR means the compliance to wrinkling, hence the inverse parameter of SWR can be defined as the resistance to wrinkling and is referred to as the skin power quotient

(SPQ). SWR and SPQ can be utilized as new skin condition parameters for investigating the skin wrinkling capacity, and can also be affected by aging. In this study, we used SWR for the correlation to SWS.

Statistical analysis

Both parameters of SWR and SPQ measured with the skin compression system were compared by age groups using one-way ANOVA (significance level $P < 0.001$) to examine the statistical significance of age-related change in the measured parameters. Five age groups were determined from age 25–33, 34–39, 40–43, 44–48 and 49–56 assuming the equivalent base size of 19, 24, 22, 21 and 16, respectively.

Results

Measurement of SWR and SPQ

Figure 6 shows the skin wrinkling of younger and older subjects from the initial state to 20% compression. The photographs are rotated from the natural view so that the bottom of each photo faces the corner of the right eye. A considerable difference in appearance can be seen even at the initial stage. The younger subject's skin is smooth and bright, while the older subject's skin is rough and relatively dark. However, the brightness of the skin surface did not affect the WAF, because all deformation images were filtered by subtraction of the initial baseline image before compression to measure the wrinkling area. The younger subject's skin indicated no significant change in surface appearance except for some small indistinct wrinkles, and the skin stayed smooth throughout the compression process. On the other hand, the older subject's skin exhibited distinct parallel lines of temporary wrinkles that clearly increased with compression. By performing image processing of the compressed skin images of these two subjects, we obtained the relationship between WAF and CR (Fig. 7). The WAF increased almost linearly until 20% compression, and became saturated beyond 23.3% compression. The gradient of this linear increase was evaluated as SWR by least-squares fitting to seven data points within 20% compression. SPQ was calculated as the inverse of SWR. Although the WAF of some subjects was scattered due to unexpected movement of the subject's head or nonuniform wrinkling in the ROI, the tendency towards a linear increase within 20% compression was observed for all subjects.

Age-related change in SWR and SPQ

Figure 8 shows the measured SWR and SPQ of all subjects related to their age. Subjects younger than 33 years obviously differed from older subjects: the SWR of young subjects was quite low at lower than 0.12. This means that the shadows of wrinkling lines did not appear during small skin compression or they were too fine and shallow to be detected by the CCD video camera. The difference in SPQ and SWR between younger and older groups was statistically confirmed by one-way ANOVA and multiple comparisons (Tables 1–3). The data were divided into five groups of age 25–33, 34–39, 40–43, 44–48, and 49–56 to make each group almost the same size. Statistical analysis showed that the difference of the youngest group from the older groups was statistically significant (significance level $P < 0.001$). On the other hand, there was no significant difference among the four older groups.

Furthermore, chronological changes were not clear in subjects younger than 33 years old. The SWR of subjects older than 33 was higher than 0.12 and randomly scattered in the range from 0.12 to 0.43, possibly due to photodamage which depends on the person. The chronological aging effect could not be identified also in aged subjects. The SPQ had the reverse tendency, because it was the inverse parameter of SWR. The SPQ was higher than 8.4 and randomly fluctuated in younger subjects, while it was lower than 8.4 and indicated no significant dependency on aging in older subjects. Consequently, age-related changes were not identified individually in the younger and older subjects, but the difference between younger and older subjects was clear, and there was no ambiguity about the tipping point in SWR and SPQ at around age 33 years. The young skin and aged skin were divided at around 0.12 in SWR and 8.4 in SPQ. This result suggests that the overall chronological change in SWR with aging is as follows: (1) remains at lower values during young age with high SPQ, (2) suddenly jumps to the aged skin region of SWR at the tipping point by the drop of SPQ, and (3) remains at higher values or varies within the aged SWR region during older age after the tipping point. Note that the boundary between young and aged skin is not yet clearly identified, because the number of measured data was not large enough, especially for subjects in their 20s.

Discussion

The drastic increase in SWR observed in the clinical test results is consistent with the jump in SWS predicted by the FEA (Figs. 1 and 8). Of special interest is the causal relationship between the experimental data on SWR and theoretical prediction on SWS. The SWR indicates the rate of increase of the detected shadow area with respect to the compression. The shadow is produced mainly by the undulation of the skin surface, so the wrinkling lines are detected as shadows, and the spacing of shadowed wrinkling lines should correspond to the SWS. Unfortunately, however, the spacing of actual wrinkling lines was not uniform as in the theoretical simulation, and was difficult to measure, especially for young subjects, because the wrinkling lines were too shallow and fine to produce a reasonably detectable shadow area. However, wider wrinkling geometrically makes deeper furrows (21, 22), and then the wrinkling lines can be clearly detected as thick shadowed lines. Therefore, a larger area of detected wrinkling lines qualitatively corresponds to a larger SWS. Consequently, a higher SWR value implies a higher potential to produce wide and deep wrinkling, and the discrete quick increase in SWR at the tipping point can be interpreted as a drastic increase in deep and wide wrinkles, that is, the drastic enlargement of SWS caused by the BMS. Note that the drastic change at the tipping point was common between SWR and SWS, but the mechanism of SWR change remains unclear. In other words, the wrinkling mode of actual skin cannot be observed by *in situ* observation in the *in vivo* compression test, because the detailed deformation inside the skin is invisible. To identify the wrinkling mode and its effect on SWR, we would need to measure the undulating deformation of viable epidermis and upper dermis beneath the stratum corneum. Moreover, to investigate the existence of BMS, it is at least necessary to measure the thickness and mechanical properties of each layer of the skin, and to theoretically examine the relationship between the mechanical properties and compressive deformation.

It is also important to note that the shadowed area is affected by the initial skin roughness, such as enlarged pores or persistent large wrinkles, which are pronounced in older subjects. Enlarged pores and persistent wrinkles quickly produce a large area of surrounding shadows with a small compression, and are easily and widely detected by image processing. For this reason, the tipping point of SWR can also be interpreted as a drastic change in the magnitude of skin roughness. Persistent wrinkles in the early stage after BMS are not sufficiently deeply developed to be visible, so they were not pronounced under normal relaxed conditions (28). However, once the skin was compressed, the wide and deep wrinkling of Mode 2 immediately appeared from the decreased CCR. Therefore, the compression test is useful for visualizing the implicit change in the wrinkling capacity and measuring it as the SWR. Moreover, persistent wrinkles in the late stage after BMS are already affected by Mode 2 wrinkling and are sufficiently developed (28), so they are easily detected without compression, and the rapid increase in shadowed area is also easily measured by the high SWR.

The effect of prestress, which is the tension in the collagen and elastic fiber networks in dermis (29), should also be considered. In general, the prestress is strong in young skin, and decreases with aging. The wrinkling in the compression test occurs by compressive stress, and so tensile prestress inhibits wrinkling. However, this is for dermis wrinkling, which is Mode 3 and independent of the BMS, and the large wrinkling corresponding to Mode 3 was not observed up to 20% compression in the test. In the epidermis, the prestress is likely to be weak because there is no long continuous fiber network, and so the prestress hardly affects the measured SWR.

In summary, the SWR not only expresses the increase in the quantity of wrinkles in temporary wrinkling caused by compression, but also the changes in wrinkling mode even when apparent persistent wrinkles are not sufficiently developed, and therefore the SWR is a promising parameter for evaluating the skin wrinkling capacity and its change with aging. Moreover, the SPQ is also useful for assessing the wrinkle resistance of skin. Both our theoretical and experimental results showed a drastic change in the skin wrinkling capacity, and therefore the mechanism of formation of wrinkles with aging, which we proposed based on the BMS, was qualitatively validated.

Acknowledgment

We are grateful to Ms. Yasuko Inoue (P&G Innovation GK) for her significant technical and clinical support, and Ms. Akane Marubayashi (Former graduate student, The University of Tokyo) for her technical assistance.

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Figure Legends

Fig. 1. Relationship between wrinkles and age. (a) Photographs of the tail of the left eye of four Japanese female subjects. The difference in skin morphology with respect to age can be recognized in terms of persistent wrinkles. (b) Variation in the specific wrinkle size (SWS) with respect to the degree of aging predicted by finite element simulations (6). The wrinkle size increased drastically due to the buckling mode switch (BMS), which means the selective transition of the wrinkling mode from Mode 1 to Mode 2, as shown in the inset figures. Wrinkling depth and width differ between wrinkling modes.

Fig. 2. Three basic wrinkling modes of the four-layer skin model. The flat skin undergoes upper-layer bending against compression beyond the critical compression ratio. This phenomenon is called buckling. The four-layer skin model has three specific buckling modes. Mode 1 is stratum corneum wrinkling (buckling of superficial layer), Mode 2 is epidermis wrinkling (buckling of upper two layers), and Mode 3 is dermis wrinkling (buckling of upper three layers). Each mode has an individual critical compression ratio. These wrinkling modes are simply the categorization of the compressive deformation of skin, and differ from the actual deformation shape.

Fig. 3. Wrinkle formation mechanism based on the multistage buckling (wrinkling) theory. Upper and lower figures show young and aged skin, respectively. The left column shows the condition of the skin, the middle column the dominant wrinkling mode corresponding to the minimum critical compression ratio (CCR), and the right column the

resultant deformation as persistent wrinkles. In young skin, the CCR of Mode 1 is lower than that of Mode 2; in aged skin, the CCR of Mode 2 is lower than that of Mode 1 (5, 6). The dominant wrinkling mode determines the furrow spacing, and a wider wrinkle yields deeper damage to the skin (21, 22). Moreover, repetitive damage by wrinkling results in the formation of persistent wrinkles (28). Young skin forms fine and shallow furrows by Mode 1 wrinkling, while aged skin forms coarse and deep persistent furrows as aged pronounced wrinkles.

Fig. 4. Facial skin compression imaging system. (a) Schematic illustration of the testing system consisting of the face positioning table and compression arms equipped with CCD video camera and LED illumination. (b) Photograph of the testing system. (c) View of testing.

Fig. 5. Example of analyzed image for detection of wrinkles. The left figure shows the original image and the region of interest (ROI). The areas outlined in blue in the right figure are the detected shadows of the wrinkle lines.

Fig. 6. Images of compressed facial skin from 0 to 6 mm displacement (20% compression). The left series of photos is of a younger subject aged 28 years, and the right series is of an older subject aged 39 years. In older skin, distinct parallel wrinkle lines are seen developing step by step as the skin compression proceeds.

Fig. 7. Relationship between compression ratio (CR) and wrinkle area fraction (WAF)

obtained by image processing of the photograph of compressed skin. Test results of a younger subject (age 28) and older subject (age 39) are indicated. The CR means (probe traveling distance) / (initial distance between probe tips) and the WAF is defined by (total number of wrinkle pixels) / (total number of pixels in the ROI). The slope within 20% compression is defined as the skin wrinkling rate (SWR).

Fig. 8. Measurement results of the skin compression test conducted on 102 Japanese female subjects. (a) Relationship between age and skin wrinkling rate (SWR). (b) Relationship between age and skin power quotient (SPQ). The data points could be divided into young and old groups around the age of 33 years, SWR 0.12, and SPQ 8.4.

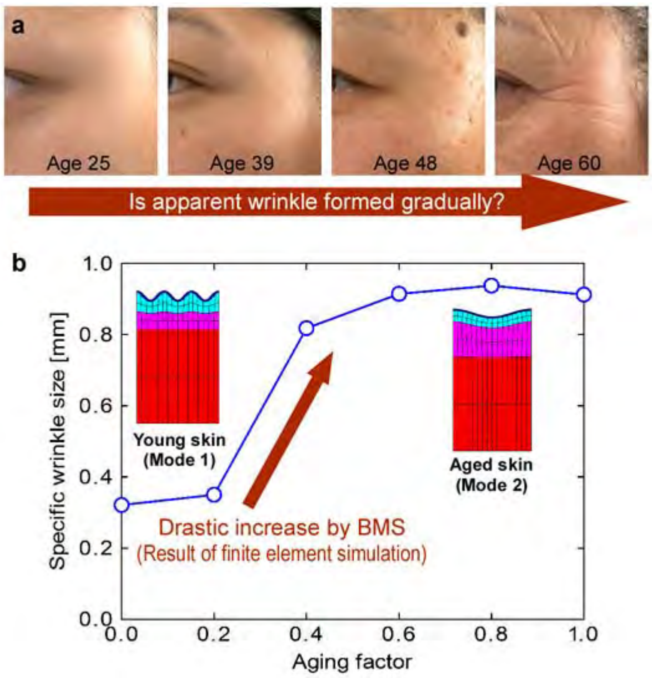

a
Age 25
Age 39
Age 48
Age 60
Is apparent wrinkle formed gradually?
b
Young skin
(Mode 1)
Aged skin
(Mode 2)
Drastic increase by BMS
(Result of finite element simulation)
Specific wrinkle size [mm]
Aging factor
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 1

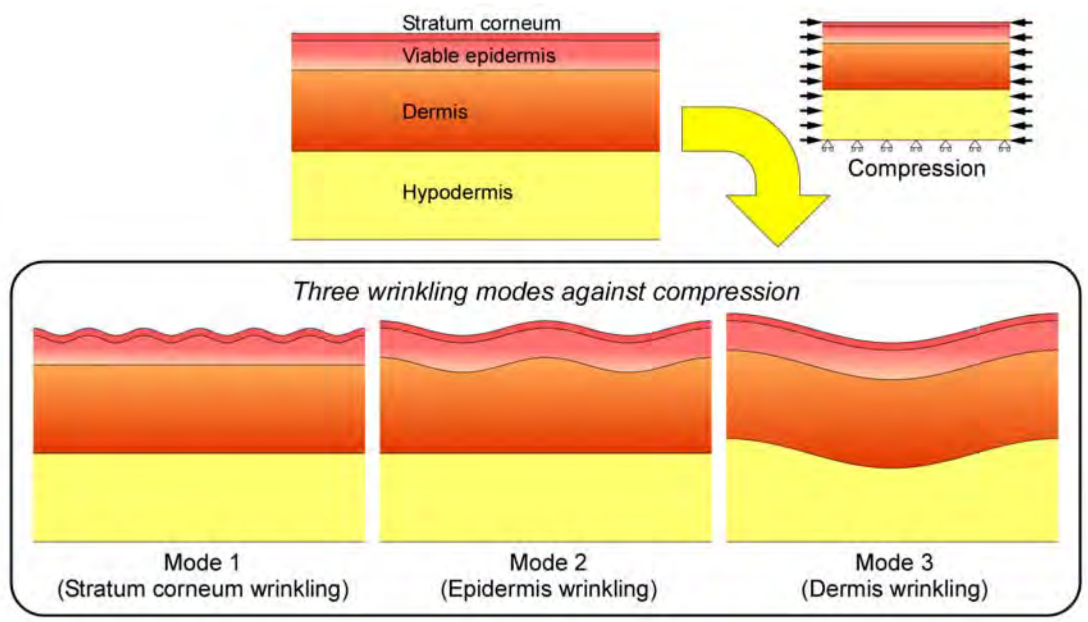

Stratum corneum
Viable epidermis
Dermis
Hypodermis
Compression
Three wrinkling modes against compression
Mode 1
(Stratum corneum wrinkling)
Mode 2
(Epidermis wrinkling)
Mode 3
(Dermis wrinkling)

Fig. 2

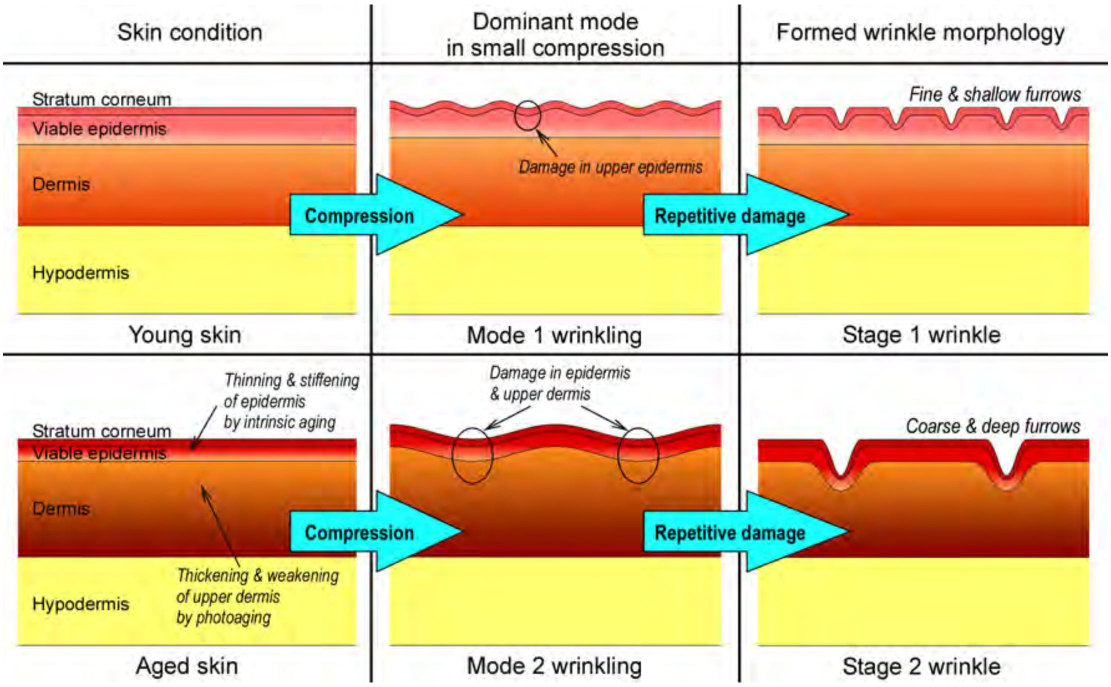

Skin condition
Dominant mode
in small compression
Formed wrinkle morphology
Stratum corneum
Viable epidermis
Dermis
Hypodermis
Compression
Damage in upper epidermis
Repetitive damage
Fine & shallow furrows
Young skin
Mode 1 wrinkling
Stage 1 wrinkle
Thinning & stiffening
of epidermis
by intrinsic aging
Stratum corneum
Viable epidermis
Dermis
Hypodermis
Thickening & weakening
of upper dermis
by photoaging
Compression
Damage in epidermis
& upper dermis
Repetitive damage
Coarse & deep furrows
Aged skin
Mode 2 wrinkling
Stage 2 wrinkle

Fig. 3

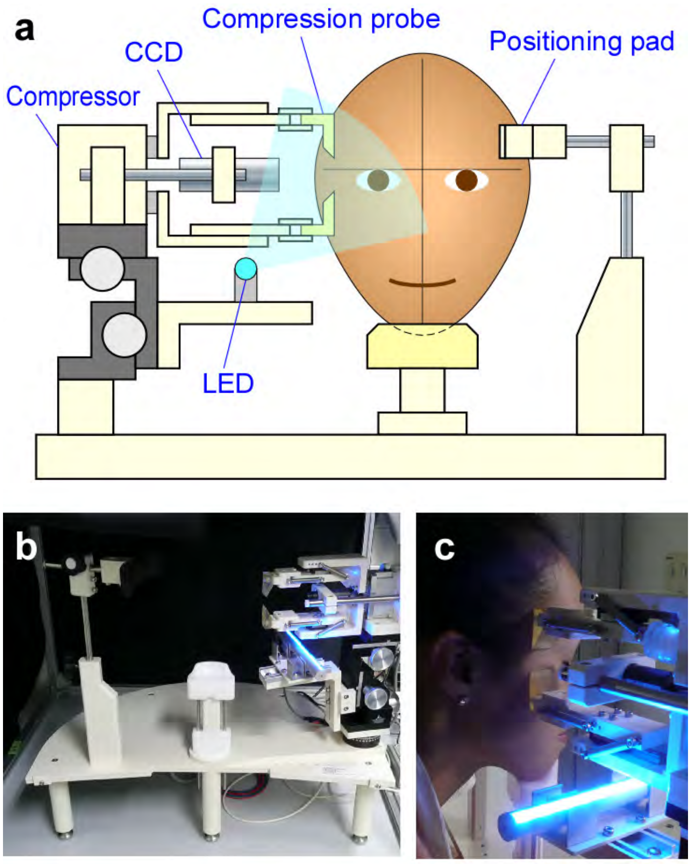


Fig. 4

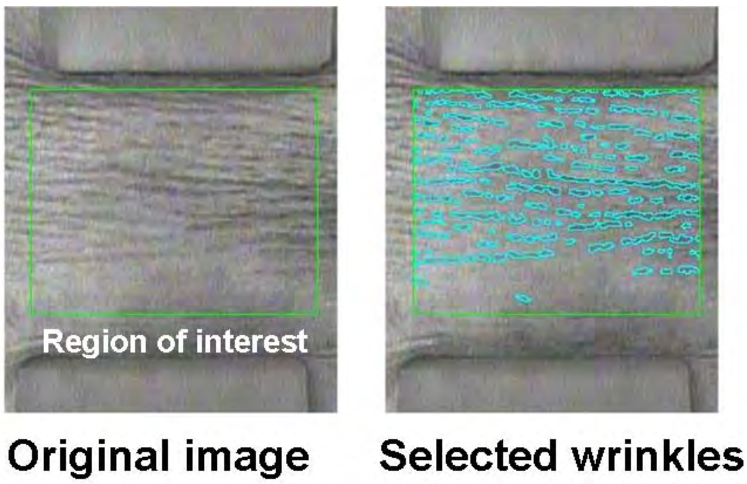


Fig. 5

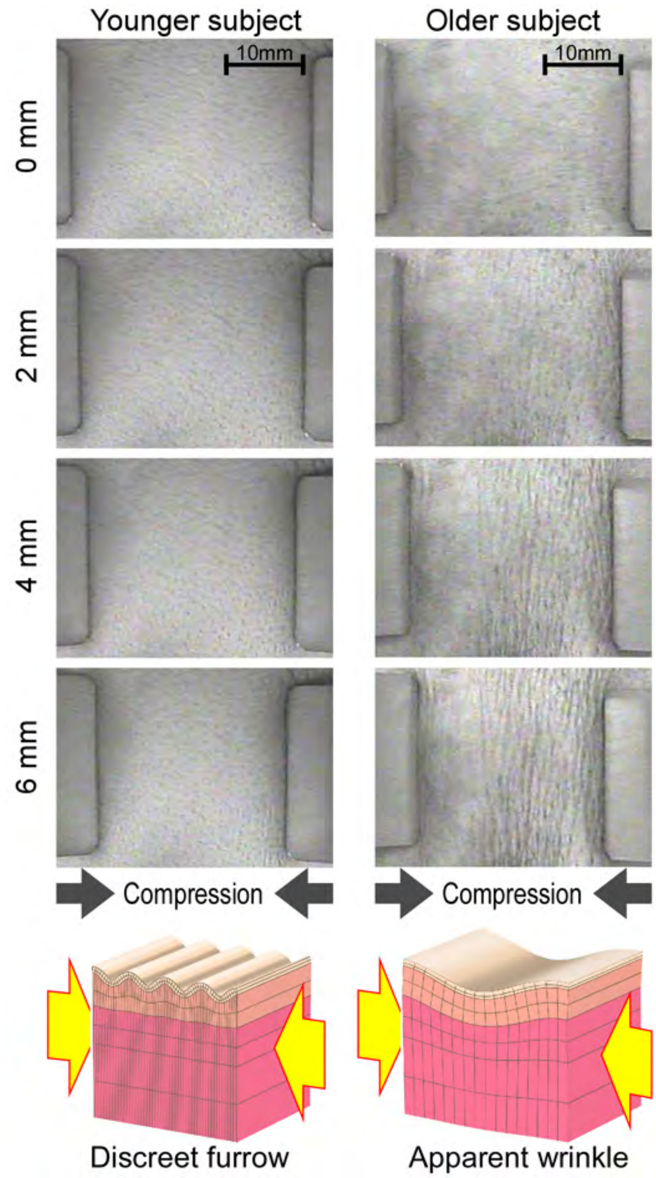
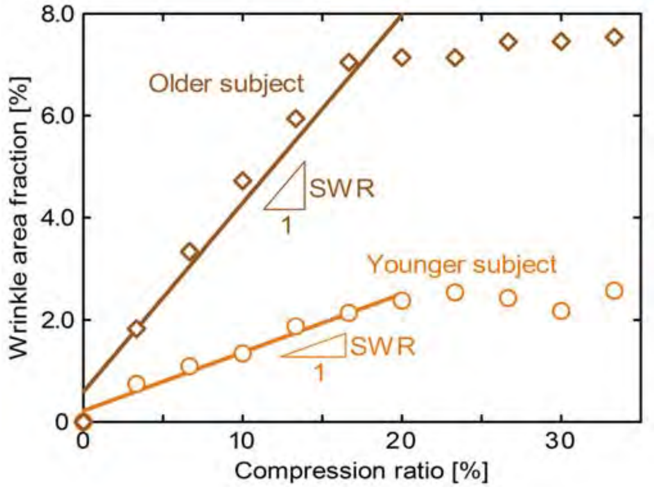

Younger subject
Older subject
10mm
10mm
0 mm
2 mm
4 mm
6 mm
Compression
Compression
Discreet furrow
Apparent wrinkle

Fig. 6



Older subject
Younger subject
SWR
1
SWR
1
Wrinkle area fraction [%]
Compression ratio [%]
8.0
6.0
4.0
2.0
0
0
10
20
30

Fig. 7

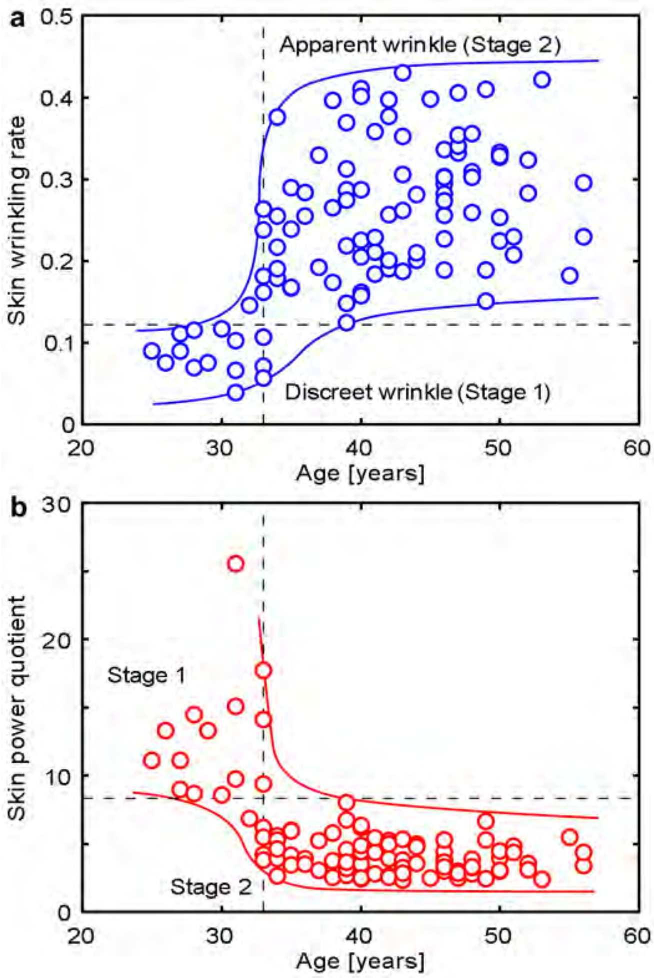

a
Skin wrinkling rate
Apparent wrinkle (Stage 2)
Discreet wrinkle (Stage 1)
Age [years]
0.5
0.4
0.3
0.2
0.1
0
20
30
40
50
60
b
Skin power quotient
Stage 1
Stage 2
Age [years]
30
20
10
0
20
30
40
50
60

Fig. 8

TABLE 1. Age groups and average values of SWR and SPQ

Age	*N*	SWR		SPQ	
		Mean	S.D.	Mean	S.D.
25–33	19	0.1144	0.0585	10.9346	5.0949
34–39	24	0.2498	0.0730	4.3862	1.3819
40–43	22	0.2718	0.0888	4.0789	1.2589
44–48	21	0.2958	0.0582	3.5264	0.7593
49–56	16	0.2741	0.0769	3.9573	1.1539

TABLE 2. Results of one-way ANOVA for SWR

Age	Compared to	Mean difference	Standard error	Significance
25–33	34–39	−0.1354*	0.0227	< 0.001
25–33	40–43	−0.1574*	0.0232	< 0.001
25–33	44–48	−0.1814*	0.0235	< 0.001
25–33	49–56	−0.1597*	0.0251	< 0.001
34–39	40–43	−0.0220	0.0219	0.317
34–39	44–48	−0.0459	0.0221	0.041
34–39	49–56	−0.0243	0.0239	0.312
40–43	44–48	−0.0240	0.0226	0.291
40–43	49–56	−0.0023	0.0243	0.923
44–48	49–56	0.0216	0.0246	0.381

* The mean difference is statistically significant at the $P < 0.001$ level.

TABLE 3. Results of one-way ANOVA for SPQ

Age	Compared to	Mean difference	Standard error	Significance
25–33	34–39	6.5484*	0.7684	< 0.001
25–33	40–43	6.8557*	0.7837	< 0.001
25–33	44–48	7.4082*	0.7923	< 0.001
25–33	49–56	6.9769*	0.8490	< 0.001
34–39	40–43	0.3073	0.7386	0.678
34–39	44–48	0.8598	0.7477	0.253
34–39	49–56	0.4289	0.8076	0.597
40–43	44–48	0.5524	0.7634	0.471
40–43	49–56	0.1216	0.8221	0.883
44–48	49–56	-0.4309	0.8303	0.605

* The mean difference is statistically significant at the $P < 0.001$ level.