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Age related changes of human skin investigated with histometric measurements by confocal laser scanning microscopy in vivo

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Background/aims: The confocal laser scanning microscope Vivascope (Lucid, Henrietta) allows skin to be studied in real-time with a resolution of 0.5 μm horizontal and 1.3 μm vertical in vivo. In this study, we present the results of a comparison between the skin of an older and a younger group of volunteers by in vivo histometric measurements.
Methods: To investigate changes caused by age, 13 young (18–25 years) and 13 older (> 65 years) volunteers were examined. The following parameters were measured using the Vivascope at the volar forearm: minimal thickness of the epidermis (E_{min}), size of cells in the granular layer (A_{gran}), thickness of the horny layer (DSC), thickness of the basal layer (DSB) and number of dermal papillae per area (PapI). The image analysis program image tool was used to measure the size of the cells and the thickness of the basal layer.
Results: The older group of volunteers showed a significant increase in E_{min}, no significant change in DSC, a significant decrease in dermal papillae and in the thickness of the basal layer, and an increase in A_{gran} compared to the younger group.
Conclusions: Histometric measurements by in vivo confocal laser scanning microscopy are a sensitive and non-invasive tool for characterizing and quantifying histological changes of the epidermis and papillary dermis due to ageing.

Key words: Confocal laser scanning microscopy – skin ageing – in vivo histometry

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In the past decade, advances have been made in imaging human skin in vivo by confocal laser scanning microscopy (1, 2). The confocal laser scanning microscope system, Vivascope 1000, is a commercially available instrument that allows skin to be studied in vivo and in real-time imaging. Numerous studies comparing and identifying structures imaged by conventional histological sectioning and the confocal laser scanning microscope have been conducted by M. Rajahyaksha and S. Gonzales (3–5).

Skin that ages chronologically, or enhanced by environmental effects such as UV irradiation (6), shows signs, such as wrinkles, that deepen and align in a parallel pattern, heterogenecity in pigmentation, and a dry, scaly aspect (7). When these phenomena are investigated by biopsy and histological sectioning and staining, dermal elastosis (8), a thin mean epidermal thickness and a flattened epidermal-dermal junction, can be seen (9). The corneocytes shed from the horny layer increase in size as a result of a lower proliferation rate and turn-over of the epidermis (10). In a study on effects of tretionin on chronologically aged skin using ultrastructural analysis by transmission electron microscopy and light microscopy, Kligman described keratinocytes of the basal and spinous layer in aged skin as irregular in size and shape (11).

The aim of this study was to develop histometric parameters to investigate and quantify ageing processes on human skin in vivo, non-invasively, using the confocal laser scanning microscope.

Material and Methods

Subjects

To investigate changes caused by ageing, sites in the middle of the volar forearm of healthy Volunteers, skin type I to III, were imaged. The volunteers were assigned to one of two groups: one group of volunteers of greater than 65 years of age (mean 72.5 years, 11 female, 2 male), and a younger group, of 18–25 years (mean 23.1 years, 11 female, 2 male), each group consisting of 13 volunteers. All investigations were

performed in winter. Informed consent was obtained prior to confocal imaging.

Instrument

The Vivascope 1000 is a confocal laser scanning microscope, built by the Lucid Company to examine skin in vivo. The system uses a laser source with a wavelength of 830 nm, an illumination power up to 20 mW on the object, a water immersion objective and an imaging rate of 20 per second. The field of view shows 128 µm × 260 µm. Its lateral resolution is 0.4 µm, the vertical resolution is about 1.9 µm. The microscope allows imaging of the epidermis and papillary dermis on a cellular level. As Millind Rajahyaksha has shown in 1995 (2), melanin provides a strong contrast in the images of the epidermis, especially between the basal and the spinous layers.

Parameters

All investigations were performed at 21 °C ± 1 °C and 50% ± 5% relative humidity. In healthy volunteers, the horny layer of the forearm appears as a relatively homogeneous, strong, reflective tissue without visible cellular structures. The thickness of the horny layer (DSC) was measured by focusing through the epidermis and measuring the difference between surface and the depth, at which living cells can be observed for the first time, with the micron screw. These cells, the granular layer of the epidermis, appear as bright circles of intercellular matrix and cytoplasm with numerous granules surrounding dark nuclei. The measurement of the DSC was repeated at least five times at different skin sites.

The size of cells in the granular layer (A_{gran}) was evaluated by taking pictures of the upper granular layer (Figs. 1, 2). In confocal images, this is the most apical plane of focus showing nucleated cells. The size of cells was evaluated using an image analysis program (IMAGE TOOL).

The minimal thickness of the epidermis (E_{min}) was measured, in a similar manner to that of the horny layer, as the distance between the surface and the level showing the first dermal tissue inside the circles representing the basal layer of the epidermis. Eight measurements per area were taken.

As a water immersion objective was used, care was taken to ensure that all data relating to the horny layer and the minimal thickness of the epidermis were obtained during the first five minutes of adapting the objective and the water immersion to the skin, to avoid changes caused by swelling.

The thickness of the basal layer (DSB) was evaluated by taking pictures of the epidermal-dermal junction at sites where the basal layer was orientated vertically compared to the skin surface (Figs. 3, 4) and analysing them using the image analysis program image tool. Images of at least 5 dermal papillae were captured. The basal layer of each papilla was measured at 5 sites (Fig. 5).

The epidermal-dermal junction of the forearm appears as bright shining circles of basal layer, containing melanin and therefore reflecting strongly, with dark centres, the papillary dermis. These circles are surrounded by spinous layer cells. The lumen of capillary loops can be seen in the centre of the dermal pap-

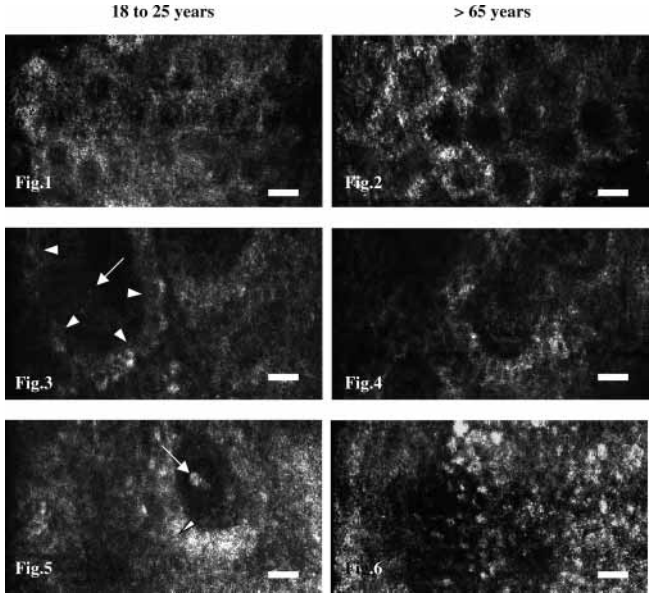


Fig. 1. The granular layer appears as large cells with dark nuclei and strong reflecting cytoplasm containing numerous granules. The size of cells was measured in the most apical layer of the Str. granulosum (A_{gran}). This picture shows the granular layer of a volunteer aged 23 years.

Fig. 2. In the skin of the older group of volunteers, the cells in the granular layer are bigger in diameter than in the younger group.

Fig. 3. The epidermal-dermal junction appears as bright reflecting circles of basal layer (melanosomes are strongly reflective) (arrowheads) around darker round areas which represent the dermal papillae (arrow). Between these circles, spinous layer forming a typical honeycomb-like pattern can be observed. The basal layer in the skin of the young volunteers appears broad and regular.

Fig. 4. In the skin of older volunteers, the basal layer shows a more fringed, irregular aspect than in younger volunteers with varying sizes of the basal cells.

Fig. 5. The bright structure (arrow) in the centre of the dermal papillae represents blood cells flowing in a capillary. At the epidermal junction, images were captured and the thickness of the basal layer (arrowhead) was measured by means of an image analysis program.

Fig. 6. Little interdigitation can be seen in aged skin. Large parts of the epidermal-dermal junction consist of plane basal layer covering the dermis. The number of dermal papillae per area decreased compared to the younger group of volunteers. (scale bars = 25 µm)

illae as black holes (Fig. 3), often showing bloodflow, with bright erythrocytes moving through the capillary (Fig. 5). The number of dermal papillae per area (PapI) was evaluated by counting each papilla showing the capillary in each field of view; 20 fields of view were checked. As the dermal papilla structure is big compared to the field of view, it often had to be decided whether or not a papilla should be counted, as only parts of it could be seen. Therefore, as there is normally only one small capillary loop in the centre of the papillae in the body region investigated, (Fig. 5), the presence of the capillary in the area of view was taken as the criteria for counting the papillae. (This parameter was measured in only 20 out of the 26 volunteers.)

Image analysis

The captured images of the skin at the height of the epidermal-dermal junction and at the height of the apical parts of the granular layer were analysed using the image analysis program IMAGE TOOL (UTHSCA, San Antonio, USA). The program was calibrated using a sputtered scale, imaged with the same instrument that was used to examine the skin. The thickness of the basal layer was measured as the thickness of the circle of bright reflecting cells (Fig. 5). Five measurements per papilla, with at least 25 measurements per volunteer were performed.

The size of cells in the granular layer was measured with the same program. As skin is wrinkled, the plane of focus and the granular layer were often not parallel through the whole field of view. Sometimes areas showing deeper layers were apparent in images showing mainly the granular layer. As the size of the keratinocytes decreases from the granular to the basal layer due to the different polarity of the cells, care has to be taken not to measure spinous cells instead of cells of the granular layer by mistake. To avoid this, only cells with a broad cytoplasm containing granules were chosen and measured. At least 20 cells per area were measured.

Statistical analysis

Morphometric results from confocal imaging are expressed as mean ± SD. A two-tailed student's *t*-test was used to compare the two groups of volunteers. *P*-values of less than 0.05 were considered significant.

Results

The thickness of the horny layer was 10.4 µm ± 3.2 µm in the younger group and 11.2 µm ± 1.9 µm in the older group. The difference was not statistically significant.

The minimal thickness of the epidermis was significantly higher in the older group than in the younger one (mean E_{min}: 40.0 µm ± 5.6 µm vs. 35.5 µm ± 4.15 µm, respectively; Fig. 7).

The basal layer in the older skin was significantly thinner than that of the younger volunteers and the

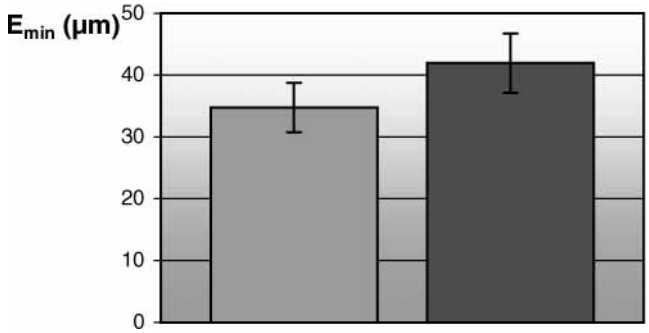


Fig. 7. The distance between surface and the top of the dermal papillae, the minimal thickness of the epidermis, increases with age. Light grey = aged 18 to 25 years, dark grey = aged >65 years.

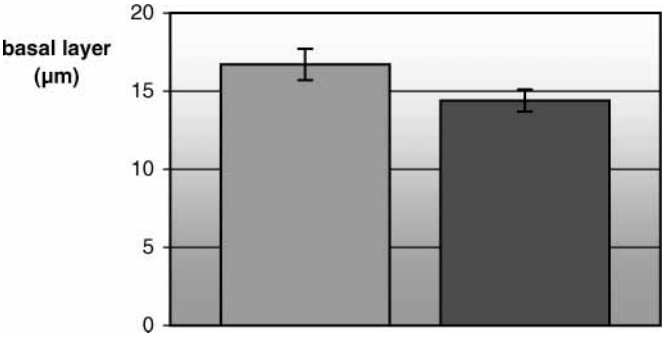


Fig. 8. The thickness of the basal layer measured by image analysis decreases with age. Light grey = aged 18 to 25 years, dark grey = aged >65 years.

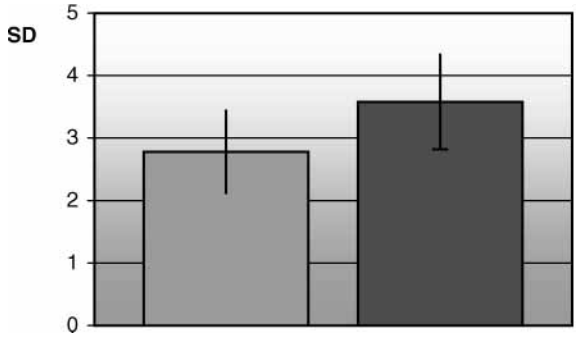


Fig. 9. The standard deviation of the single measurements of the thickness of the basal layer in each individual is significantly bigger in the older volunteers, reflecting the irregular aspect of the basal layer observed in their skin. Light grey = aged 18 to 25 years, dark grey = aged >65 years.

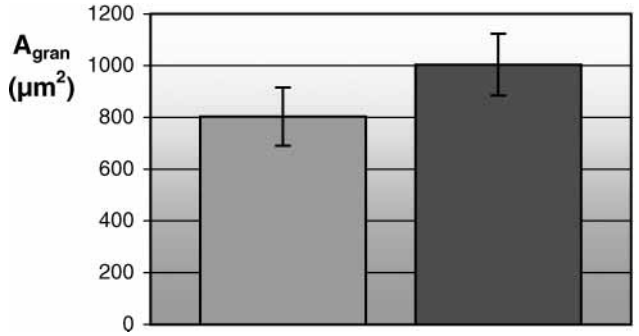


Fig. 10. The size of cells in the granular layer is bigger in the skin of the older group of volunteers compared to the young group, similar to the known increase with age in the size of corneocytes. Light grey = aged 18 to 25 years, dark grey = aged >65 years.

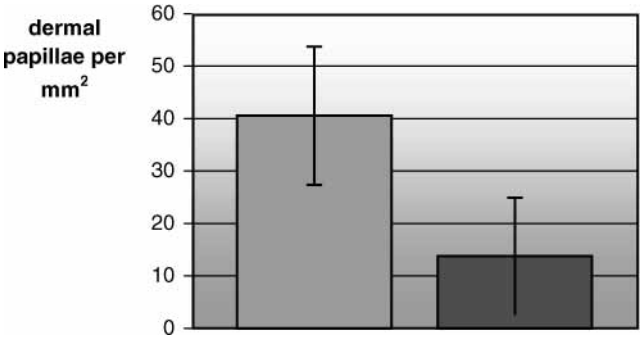


Fig. 11. The number of dermal papillae per area decreases dramatically with age. Light grey = aged 18 to 25 years, dark grey = aged >65 years.

mean standard deviation of the 25 measurements of each volunteer was greater in the older than in the younger group (Figs. 8, 9). This reflects the irregular, fringed aspect of the basal layer in aged skin (Fig. 4). The mean thickness of the basal layer in the aged group of volunteers was 14.4 µm ± 0.7 µm, compared to 16.7 µm ± 1.0 µm in the younger group, and the mean standard deviation of the thickness of the basal layer in the data of each volunteer was 2.78 µm ± 0.66 µm in the younger group and 3.58 µm ± 0.76 µm in the older group.

The cells in the upper granular layer (A_{gran}) were significantly larger in the older group of volunteers: 1004 $\mu m^2 \pm 120$ μm^2 compared to 803 $\mu m^2 \pm 113$ μm^2 (Figs. 1, 2, 10).

The number of dermal papillae per area was significantly lower in the aged group than the younger group (Fig. 11).

In the younger skin, mean papillae containing capillaries was 40.56 ± 13.19 per mm^2 compared to only 13.82 ± 11.11 per mm^2 in the aged skin. In the older group of volunteers, regions of totally plane basal layer often filled the whole area of view (Fig. 6).

Discussion

The thickness of the horny layer did not show any changes in our study, confirming similar findings in previous studies (Kligman 1986, Grove 1983).

The changes in minimal thickness of the epidermis were considered to be due in part to changes in the living tissue of the epidermis. In previous studies (Kligman 1986), mean thickness of the epidermis decreased with age. Although this seems to contradict our results, these studies also describe the height of the epidermal-dermal junction decreasing with age. Our results for thickness of the epidermis, immediately above the dermal papillae, could still be consistent with a decrease in the mean epidermal thickness found by histological methods, if the decrease in height of the epidermal–dermal junction was more than twice the decrease in the mean epidermal thickness.

The correlation between size of cells in the granular layer and age is consistent with a documented increase in corneocytes with age. We propose that the size of corneocytes is determined by the size of cells in the upper granular layer and that comparable results can be obtained using both investigation methods, providing the kinetics are excluded.

Changes in the thickness of the basal layer are commonly attributed to flattening of the epidermal-dermal junction, however, this is unlikely to be the case in our study, as this should cause an increase in the thickness of the basal layer, by decreasing the angle between the direction of the basal layer and the plane of focus and increasing its projection, while the thickness of the basal layer in our study was increased. In spite of this, we suggest that this decrease in height and increase in irregularity reflects the decrease in proliferation of the basal layer in aged skin. Kligman, 1993, pointed out that the basal and spinous cells showed an irregular shape and size in electron microscopic images when investigating the effects of topical retinoic acid on chronologically aged skin. As the rate of proliferation is decreased in aged skin (Grove *et al.*, 1983), the irregular shape could be a result of a reduced number of the basal layer cells participating in the cell cycle. The variation in thickness of the basal layer could possibly be studied in vivo, dynamically and non-invasively.

The decrease in the number of dermal papillae per area (PapI) with age reflects the flattening of the epidermal-dermal junction (9). In particular, this demonstrates that not only the height of the dermal papillae decreases with age, but also the number of interdigitations.

The most dramatic age-related changes in this study were seen in the number of papillae per area. As this parameter was closely linked to its function of supplying the epidermis with water and nutrients via the dermal vasculature, it seemed to be a more sensitive measure for qualitative evaluation of the epidermal junction, than the measurement of height in histological sections. It can also be obtained easily, quick and non-invasisely.

In conclusion, histometric measurements by in vivo confocal laser scanning microscopy are a sensitive and non-invasive tool to characterize and quantify histological changes of the epidermis and papillary dermis due to ageing.

References

1. Imbert D, Hoogstraate J, Marttin E, Cullander C. Imaging thick tissues with confocal microscopy. Methods Mol Biol 1999: 122: 341–355.
2. Pierard GE. In vivo confocal microscopy: a new paradigm in dermatology. Dermatology 1993: 186: 4–5.
3. Rajadhyaksha M, Gonzalez S, Zavislan JM, et al. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. J Invest Dermatol 1999: 113: 293–303.
4. Gonzalez S, Gonzalez E, White WM, et al. Allergic contact dermatitis: correlation of in vivo confocal imaging to routine histology. J Am Acad Dermatol 1999: 40: 708–713.
5. Gonzalez S, Rubinstein G, Mordovtseva V, et al. In vivo abnormal keratinazation in Darier-White's disease as viewed by real-time confocal imaging. J Cutan Pathol 1999: 26: 504–508.
6. Kligman LH. Photoaging. Manifestations, prevention and treatment (Review). Dermatol Clin 1986: 4: 517–528.
7. Kligman LH, Kligman AM. The nature of photoaging: its prevention and repair. Photodermatol 1986: 3: 215–227.
8. Miyachi Y, Ishikawa O. Dermal connective tissue metabolism in photoageing. Australas J Dermatol 1998: 39: 19–23.
9. Lavker RM, Zheng PS, Dong G. Aged skin: a study by light, transmission electron and scanning electron microscopy. J Invest Dematol 1987: 88: suppl. (3): 44–51.
10. Grove GL, Kligman AM. Age-associated changes in human epidermal cell renewal. J Gerontol 1983: 38: 137–142.
11. Kligman AM, Dogadkina D, Lavker RM. Effects of topical tretinoin on non-sun-exposed protected skin of the elderly. J Am Acad Dermatol 1993: 29: 25–33.

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