Investigation of applicability of high-frequency ultrasonography for analysis of dermal structure in pig skin

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Abstract

Biopsy sampling can cause wound infection and pain in older or diabetic patients, although histological examination of biopsy samples is the gold standard of structural analysis of the skin. High-frequency ultrasonography is a promising tool for noninvasive skin assessment. However, its applicability for structural analysis of the skin is unknown. In the present study, we compared the images of high-frequency ultrasonography with histological examination of pig skin, which is the most validated model of human skin. Ear and dorsal skin were harvested from a pig cadaver, and scanned in three positions with a 20-MHz linear ultrasonography probe. The scanned position was accurately marked and trimmed for histological examination with Mallory–Azan staining. The distribution of hypoechoic regions in the ultrasonographic images mostly corresponded to the sweat glands and hair follicles in the histological sections in the ear skin. Sweat-duct- and blood-vessel-like structures and the papillary dermis were partially depicted. These results suggest the applicability of high-frequency ultrasonography for structural analysis of normal skin.

Keywords: collagen, dermis, pressure ulcer, pig, high-frequency ultrasonography

Introduction

Pressure ulcers are localized injury to the skin and/or underlying tissue as a result of pressure, or pressure in combination with shear stress¹. Pressure ulcers have a serious effect on the physiological, psychological and social aspects of patients, thus, their prevention is an important aspect of nursing care.

Continuous or repetitive loading of external forces and decreased tolerance of skin contribute to the development of pressure ulcers². Therefore, measurement of external force and tissue tolerance are effective for preventing pressure ulcers. Although there are several devices for measuring interface pressure or shear stress³⁴, the direct measurement of tissue tolerance is difficult in patients. It is thought that tissue tolerance mainly depends on the structure of the skin, such as the papillary structure of the dermal-epidermal junction and the collagen network of the dermis. Therefore, structural analysis of skin is important for evaluating tolerance.

The gold standard for structural analysis of the skin is histological examination of biopsy specimens. Biopsy sampling, however, can cause wound infection in patients with a high risk of pressure ulcers, such as older and diabetic patients, because their immunity is often weakened⁶⁷. In addition, biopsy sampling can cause pain⁸.

High-frequency ultrasonography can provide high-resolution images of superficial organs⁹. Therefore, it is expected that high-frequency ultrasonography can be used for noninvasive structural analysis of skin. To date, it has been used for evaluating epidermal structure, function and pathology¹⁰¹¹. It has also been shown that the echoic signals reflect the collagen content in the skin¹²¹³: however, its applicability for dermal structural analysis is unknown.

In the present study, we compared images from
high-frequency ultrasonography with histological examinations to determine the applicability of the former for structural analysis of skin.

Methods

1. Materials

Skin tissues were harvested from a pig cadaver, which had received an intravenous drip of normal saline before euthanasia, as a control animal in a blood transfusion experiment (Figure 1A). We chose ear and dorsal skin as samples for this study, because the structural condition of the dermis was expected to differ according to the overgrowth of subcutaneous fat tissue. Ibuki et al. reported that overgrowth of subcutaneous fat tissue promotes degradation of dermal collagen fibers and decreases the mechanical strength of skin. The hair was shaved with clippers and an electric razor. After scanning with ultrasonography, tissues were trimmed for histological analysis.

2. Ultrasonography

The dermal structures were scanned at three sites by ultrasonography with a 20-MHz linear probe (DermaScan; Cortex Technology, Hadsund, Denmark). Its resolution was 60×130μm, and its observational depth was 10 mm. Gain and gain key were fixed at 3 and 13, and scanning mode was gray scale. The long axis and direction of the scanned position were marked as accurately as possible (Figure 1B).

3. Histological analysis

Each tissue was divided into three pieces and trimmed at the long axis of the scanned position. After fixation with 4% paraformaldehyde, the tissue pieces were embedded in paraffin, and cut into 3-μm-thick sections. Mallory–Azan staining of the sections was performed by the conventional method, including incubation with Mallory’s azocarmine G solution (Muto Pure Chemicals, Tokyo, Japan) at room temperature for 1 h, 5% phosphotungstic acid (Muto Pure Chemicals) at room temperature for 1 h, and Mallory’s aniline blue-orange G solution (Muto Pure Chemicals) at room temperature for 25 min. The histology of the tissue sections was recorded by inverted microscopy (Leica, Wetzlar, Germany) at 10× magnification. Twenty to thirty images were taken from each section, and they were combined using image processing software (Photoshop Elements version 12: Adobe systems, San Jose, CA, USA).

4. Comparison between ultrasonographic and histological images

Rearrangement of image size was required for the ultrasonographic images. The width of the ultrasonographic images was adjusted to 6 mm, according to the manufacturer’s recommendation. In the two ultrasonographic images of ear skin, which clearly showed the border between the cartilage and skin tissue, the depth was adjusted to that of the histological images. The depth of the other images was changed in a similar manner. The hypoechoic area was identified, and its distribution was compared to the structure of the histological images.

Results

1. Histology of ear skin

Macroscopic observation of ear cross-sections revealed flat cartilage under the skin. The subcutaneous adipose layer was not recognized (Figure 1C). Azan staining also showed that the surface of the cartilage was mostly flat, and the subcutaneous adipose layer was limited. The well-developed apocrine glands included a small number of adipocytes. There was no panniculus carnosus or sebaceous glands (Figure 2A−C). Abundant sweat ducts and blood vessels were distributed throughout the dermal layer (Figure 2D). The papillary structure of the dermal–epidermal junction was similar to that of human skin (Figure 2E). The tissue harvested from the earlobe side showed condensation of collagen fibers, compared with the other two ear sections (Figure 2F, G).

2. Histology of dorsal skin

Macroscopic observation revealed a large amount of subcutaneous adipose tissue and large hair follicles. The border between the dermis and subcutaneous adipose tissue was unclear (Figure 1D). These macroscopic characteristics were associated with abundant adipose tissue, which surrounded the hair follicles and apocrine glands, and reached the subcutaneous adipose tissue (Figure 3A−C). The epidermis was thicker and the collagen fibers were more condensed in dorsal than ear skin. The fibrous septa of the subcutaneous adipose tissue were thickened. There was no panniculus carnosus or sebaceous glands.
3. Ultrasonography of ear skin

Two of three ultrasonographic images of ear skin were well developed (Figure 2A’, B’), in which the surface of cartilage was clearly visible. The distribution of hypoechoic regions mostly corresponded to the sweat glands and hair follicles that were identified by Azan staining. It was noteworthy that the sweat-duct- and blood-vessel-like structures were observed as the hypoechoic regions (Figure 2D’). The papillary dermis was also partially visible (Figure 2E’). An ultrasonographic image from the earlobe side failed to identify the surface of cartilage, although the structures of the shallow layer of the dermis were seen (Figure 2C’).

4. Ultrasonography of dorsal skin

The echoic signals were markedly attenuated in the dermis layer, although the epidermal structure was well defined (Figure 3A’—C’).

Discussion

We compared structural analysis of skin by high-frequency ultrasonography and histology. Our results indicated that high-frequency ultrasonography visualized the epidermal and dermal structures in detail.

Pig skin is the most validated animal model for human skin because of several common characteristics, such as sparse hair, thick epidermis with...
papillary structure, firm attachment to the underlying organs, and orientation and distribution of blood vessels, although sebaceous glands are absent\textsuperscript{16,17}. Both ear and dorsal skin examined in this study showed these characteristics resembling human skin. In the dorsal skin, we observed infiltration of adipose tissue into the dermis, especially around the hair follicles and apocrine glands, and condensation of collagen fibers. Similar histological features are reported in the skin of patients with severe obesity\textsuperscript{18,19}. Therefore, ear skin is recognized as a model for normal human skin, and dorsal skin is a model for skin in patients with severe obesity.

High-frequency ultrasonography is used widely for evaluating skin in the cosmetics industry and in research\textsuperscript{9,20-21}. Ultrasound frequency and attenuation coefficient are directly correlated with tissue collagen content\textsuperscript{13,14}. In our examination of ear skin, hyperechoic signals were detected in the dermal area. This observation agrees with previous studies\textsuperscript{20,21}, although we did not measure collagen content.

The distribution of hypoechoic regions mostly corresponded to the distribution of appendages, including hair follicles and apocrine glands in the dermal area. Higher magnification images suggested that the tubular structures, including sweat ducts and blood vessels, were also depicted as hypoechoic regions. The papillary structure of the dermal-epi-
In dorsal skin, echoic signals were markedly attenuated in the reticular dermis, although the collagen content was increased compared with that in ear skin. A possible reason for the attenuation of echoic signals was infiltration of adipocytes into the dermis, which has been reported in severe chronic obesity in humans and mice\(^\text{18,22}\). It is known that adipose tissue considerably attenuates ultrasound\(^\text{23,24}\). Another possibility is the alteration of chemical and mechanical properties of dermal collagen. Enser and Avery reported glycosylation of lysine and hydroxylysine in obese diabetic mice\(^\text{25}\). Glycosylation of collagen results in alteration of crosslinking and architecture of fibrils\(^\text{26}\), and acoustic impedance closely associated with the architecture of scanning materials\(^\text{24}\). Our results suggested that the intensity of echoic signal reflects collagen content and alteration of chemical or mechanical properties of dermal collagen. Skin from the earlobe side also showed attenuation of echoic signals and condensation of collagen fibers in the deeper layer of the reticular dermis, suggesting qualitative alteration of collagen fibers, because there was little subcutaneous adipose tissue.

Recently, some optical devices have been developed for noninvasive and real-time observation of skin morphology. In vivo confocal laser scanning microscopy provides high-resolution images of the epidermis and papillary dermis, in which the morphology of keratinocytes can be visualized\(^\text{27}\). Optical coherence tomography is an imaging technique for skin morphology using infrared light. Tomography can distinguish the corneal layer from the living cell layer in the epidermis; visualize the patterns of hair keratin in the hair shaft; and identify dermal blood vessels\(^\text{28}\). In contrast, high-frequency ultrasonography has some advantages that provide the morphological images of the whole layer of the dermis, and is superior in portability, although image resolution is inferior to that with other tools. These optical devices are promising for investigating skin morphology and an alternative to invasive histological analysis using biopsy samples, which is a gold standard for skin diagnosis.

**Conclusion**

High-frequency ultrasonography visualized the microstructure of the pig ear skin, which is a human skin model, suggesting its applicability for noninvasive evaluation of dermal structure. However, the intensity of echoic signals did not correspond to the collagen content in dorsal skin, which is a model for skin in patients with severe chronic obesity. Although the decreased intensity probably reflected the mecha-
tional or chemical alteration of collagen fibers, further studies are required to apply this method to evaluate skin from obese individuals. The association between the dermal structures evaluated with high-frequency ultrasonography and pressure ulcer development should be examined.

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Conflict of interest

The authors freely borrowed high-frequency ultrasonography equipment from Integral Corporation (Tokyo, Japan). Integral did not contribute to the design and performance of the experiment, data analysis, or writing of this manuscript.

References


