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The top echorich band in a 50-MHz ultrasound sonogram reflects epidermal properties

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Synopsis

High-frequency ultrasonography is a useful noninvasive tool to measure the acoustic properties of skin. Due to the ambiguity or confusion over the meaning of the skin entry echo, measurements have been limited to the dermis or full skin thickness with little data on epidermal properties. The purpose of this study was to better understand the nature of the skin entry echo and determine whether it is related to epidermal structure. We approached the problem by dampening the sudden change in material density from the coupling medium to the skin surface using facial tissue as a masking material. The thickness and acoustic density of bare and masked skin sites were measured using dermal ultrasound with a 50-MHz transducer. Results showed that the original thickness and acoustic density of the skin entry echo did not change when the skin was masked up to two layers. A comparison between the epidermal thicknesses measured using ultrasound and confocal microscopy also indicated that the two methods yielded about the same results with no statistically significant difference detected. This study demonstrates that the purported skin entry echo is not just a meaningless artifact, and it reflects useful properties of epidermal structure.

INTRODUCTION

Since first reported in 1979 (1), dermal high-frequency ultrasonography has been shown to be a useful noninvasive tool to understand skin properties. Numerous studies have focused on measuring skin thickness at various anatomical sites from people of different ages, genders, ethnicities, and disease states (2–7). Due to ambiguity or confusion over the meaning of the skin entry echo, many of these measurements were limited to the dermis only or to the full skin thickness, with very little data on the thickness and the echogenicity of the epidermis.

It is evident in the literature that such ambiguity or confusion exists. Most researchers believe that the skin entry echo is an artifact caused by the sudden change in impedance between the coupling medium and the stratum corneum, which makes the epidermis difficult to visualize or measure using ultrasonography (8,9). El Gammal *et al.*, using a 100-MHz

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transducer to visualize normal and psoriatic skin, suggested that for normal glabrous skin, the stratum corneum was sonographically invisible and the first echorich band seen in the sonogram was an artifact and deemed as the entry echo. The viable epidermis and papillary dermis were represented by an echopoor band beneath the entry echo. They further stated that the entry echo remains, no matter whether the horny layer is stripped, occluded with topical agents, or entirely removed (10). Other researchers consider the entry echo band to represent the epidermis without providing adequate supporting data, and have reported correlations between its echogenicity and epidermal hydration or inflammatory states (11–15). Nouveau-Richard *et al*. (16) compared their measurement results between confocal laser scanning microscopy and the dermal ultrasound with a 20-MHz transducer, and concluded that the distance between the first two hyperechoes on an ultrasound A-Scan sonogram represented the epidermal thickness. Their study had provided us with confidence to conduct this study to further support the argument that the sonograms of dermal ultrasound, at least those obtained by using a 50-MHz transducer, contain information to reflect the properties of the epidermis of human skin.

In regard to the epidermal thickness measurement, skin histology has been considered as the "gold standard". Sandby-Moller *et al*. (17) conducted an extensive study to examine the epidermal thickness from 71 Caucasians, and reported that the total epidermal thickness (stratum corneum plus the cellular epidermis) to be about 83.7 (±16.6) μm. This result serves as a good reference to validate the measurement results of instrumental, *in vivo*, noninvasive methodologies although tissue shrinkage in biopsy samples was a concern (10). In a typical 50-MHz ultrasonogram of normal human glabrous skin, there exists a clear, well-defined echorich band on the top section of the B-Scan image. Its thickness is approximately 100 μm, the typical thickness of the epidermis, suggesting there is a possibility that the echorich band may reflect the epidermal thickness. Figure 1A shows a comparison between the images of the histology of human normal skin (18) and the ultrasound B-Scan sonogram. It demonstrates the geometric similarity between these two methods, and the scales

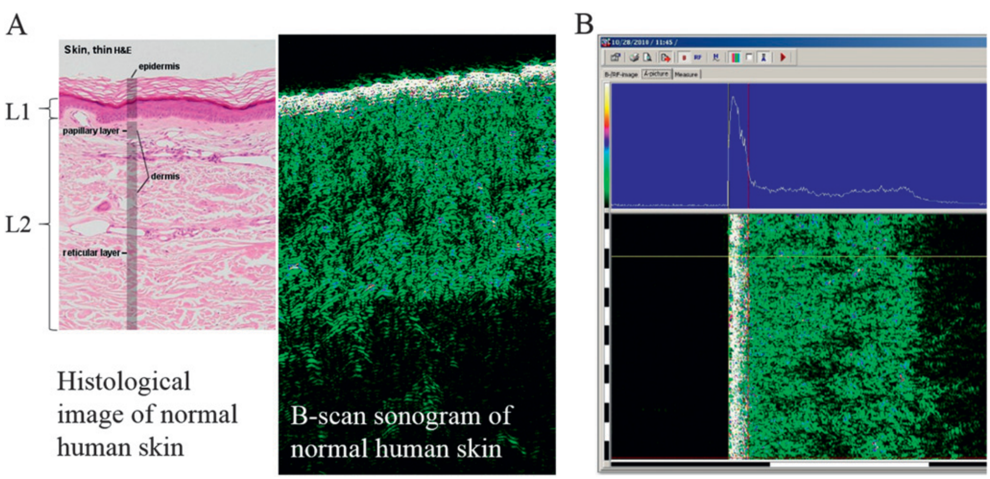

Figure 1. (A) Comparison of skin thickness scales between images of histology and the dermal high-frequency ultrasound. In the histological image, L1 = epidermis and L2 = dermis. In the ultrasound image it shows a B-scan sonogram of normal human skin. {Source of histology: School of Anatomy and Human Biology, The University of Western Australia (18).} (B) Graphical representation of epidermal thickness measurement method in DUB*plus* software. The location of the red vertical line is generally set to include the bottom of the echorich band.

of the epidermal and dermal thickness appear to match well in this example. Furthermore, this echorich band from a 50-MHz ultrasonogram appeared in a very different shape from that of a 100-MHz ultrasonogram in El Gammal and colleagues' report (10). There was no echopoor region shown in the epidermal area. These facts prompted us to believe that the ultrasound results from a 50-MHz sonogram warranted a specific investigation. In an effort to determine whether or not the skin entry echo is solely a meaningless artifact, we devised a thorough experiment. Instead of using topical agents such as a cream to occlude the skin, we altered the sudden density change between the coupling medium and the skin by masking the skin surface with a variety of materials ranging from paper tissue to scotch tape. Our argument was that, if the entry echo is only an artifact, its thickness and acoustic density should change when the skin has a masking material on it during an ultrasound measurement. Otherwise, it would indicate that the skin entry echo is not an artifact, and it may reflect certain properties of the skin.

MATERIALS AND METHODS

MASKING EXPERIMENTS

A device of dermal high-frequency ultrasound, DUB*plus* (Taberna Pro Medicum, Germany) with a 50-MHz transducer, was used to capture cross-sectional images of human volar forearm skin *in vivo*. Thickness (in μm) and acoustic density (in arbitrary unit, a.u.) of different regions in a sonogram were measured using the device's analysis software. Epidermal thickness was measured from the surface of the skin to the bottom edge of the echorich band, as illustrated in Figure 1B.

Various materials such as scotch tape, copy paper, cotton pad, aluminum foil, paper tissue, etc., were tested to mask the skin surface. Each masking material was placed directly on the skin and the ultrasound scanning head was positioned to cover equal portions of bare and masked skin so that both sites were scanned simultaneously (Figure 2A). The thicknesses of the epidermis and the masking layer were measured using the calculation scheme shown in Figure 2B. To produce effects of varying thickness, multiple masking layers (2×2 cm^2) were laid flat on the test site one layer at a time up to eight layers. The

A

B

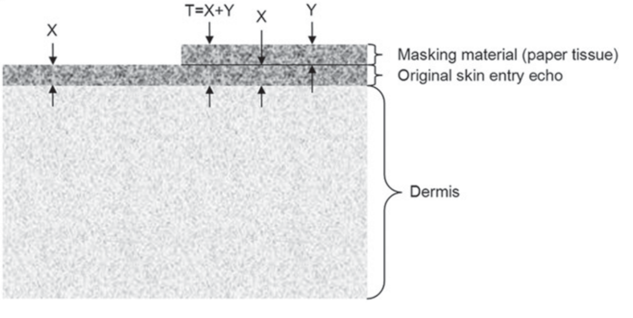

Figure 2. (A) Illustration of ultrasound transducer (scanning head) position. It is placed in such a way to cover the masked and the bare skin sites in one measurement. (B) Calculation scheme for entry echo thickness. The thicknesses of bare skin, masking material, and the masked skin (skin + mask) are represented by the dimensions of X, Y, and T (= $X + Y$), respectively.

thickness of the masking material on skin was obtained by measuring the total thickness of the echorich band and then dividing the quantity by the number of mask layers to calculate the average thickness of a single layer. This quantity was compared with the skin entry echo thickness for significance using Student's *t* test.

COMPARISON BETWEEN ULTRASONOGRAPHY AND CONFOCAL LASER SCANNING MICROSCOPY

Epidermal thicknesses of human forearm skin were measured using confocal laser scanning microscope from 25 dorsal and ventral sites of 10 Asian and Caucasian volunteers. A Vivascope 1500 (Lucid Inc., Henrietta, NY) in the reflection mode was used to collect the optically sliced skin views in a stack of 164 images. Each stack was obtained by scanning a skin site from the skin surface down to the rete ridge structure, with increments of 1.52 μm. A stack of 164 images were obtained from each measurement site. A cross-sectional image was then constructed and the epidermal thickness was measured by plotting the *z*-axis profile of pixel intensity distribution of each stack using ImageJ, an image analysis software developed by National Institute of Health, Bethesda, MD. Each measured result was then verified by visually observing the surface of the skin and the bottom of the rete ridges from the 164 images of a Vivastack. Figure 3A shows an optically reconstructed cross-sectional image of skin, and Figure 3B shows how the epidermal thickness measurement was performed. For the epidermal thickness measurement using ultrasound, sonograms were obtained from the cheek skin of 102 female Asian and Caucasian volunteers using the ultrasound method described in Figure 1B. The results from these two methods were compared.

RESULTS AND DISCUSSION

Of the various conditions tested, most masking materials produced visible artifact by either blocking too much the detection signal or by distorting the B-mode images, and

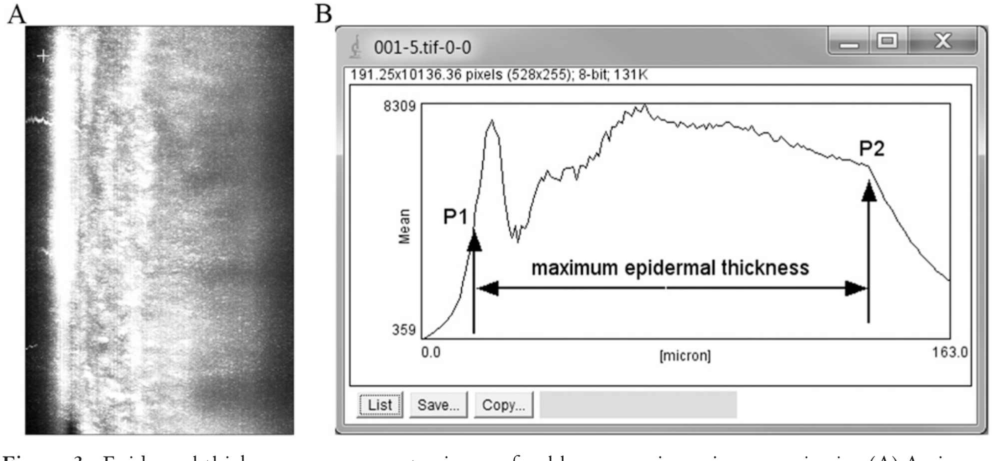

Figure 3. Epidermal thickness measurement using confocal laser scanning microscope *in vivo*. (A) An image of cross-sectional view of skin constructed from a Vivastack of 164 images. (B) Epidermal thickness measurement scheme based on the intensity distribution of image (A). P1 and P2 represent the points at the skin surface and at the bottom of rete ridges, respectively.

they were considered not applicable to the study. Paper tissue (Kleenex® facial tissue), however, was found to be a meaningful masking agent, which we believed to have reflected the sound waves the similar way as that of the cellular epidermis due to its porous structure when soaked in the coupling water during a measurement. The ultrasonogram of skin with a single mask layer placed on it is shown in Figure 4A in which the thickness of the entry echo, T, is nearly doubled at the masked site on the right-hand side of the image when compared to that of the bare skin, X, on the left-hand side. We can see that this increased thickness is a combination of the original entry echo of the skin and that of the masking material on top of it. Figures 4B–I show the sonograms, especially the changes in the entry echo thickness of the masked skin sites. With additional layers of masking material placed on skin there was a corresponding increase in thickness T.

The thickness of the entrance echo from the masked and unmasked skin sites was measured from Figures 4B–I. For the bare skin site, it had an average value of 116.0 ± 9.5 µm. To determine the thickness of each masking layer, we first subtracted X from T in each of the eight sonograms to obtain a total thickness of the masking layers on skin. Cumulative thickness curves when plotted against the number of masking layers are shown in Figure 5A. Then, dividing this total thickness by the number of masking layers in each sonogram, the thickness of a single masking layer was calculated. From the eight sonograms shown in Figure 4, the average thickness of a single masking layer was 90.7 ± 4.5 µm. Figure 5B shows the thickness values of the entry echo measured from the bare skin sites as well as from the masked sites. These two populations were found to be

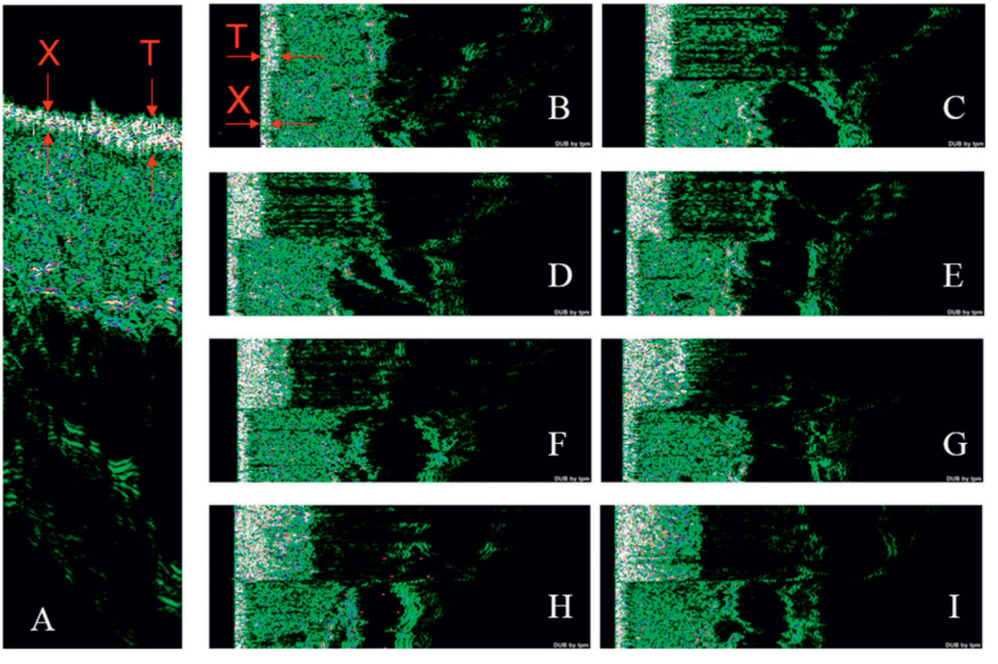

Figure 4. (A) Ultrasound sonogram of ventral forearm skin masked with a layer of paper tissue on the right-hand side showing a wider entry echo band than that on the left-hand side of the image. (B–I) Sonograms after rotating 90° counterclockwise and a phasing operation to align the surface to a vertical line. The thickness of entry echo band increased with increasing layers of masking tissue, from one layer (B) to eight layers (I). The upper half of each image shows the masked skin and the lower half is bare skin. T = total thickness of entry echo; X = bare skin entry echo.

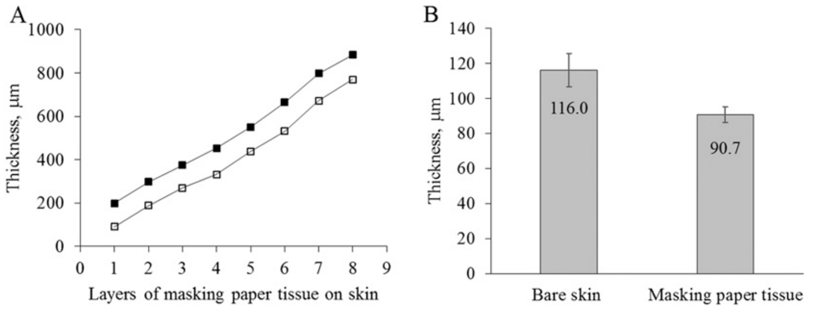

Figure 5. (A) Cumulative thickness of the entry echo band in sonograms of skin with various masking layers. Solid square line shows the combined thicknesses of the original skin entry echo band and the band produced by the masking material. The hollow square line displays the total thickness of the entry echo band produced by the masking material alone. (B) Comparison of the thickness values between the bare skin site and a single layer of masking paper tissue on skin. The two data sets were statistically different.

significantly different (p < 0.05) indicating they describe the thickness of two distinctly different structures, i.e., a layer of skin and a layer of masking material. From the literature we know that the commonly accepted thickness of epidermis is between 70 and 140 μm (16,17). Thus, the average thickness of the entry echo measured from 50-MHz ultrasonograms on the bare skin site (116 μm) fits within the reported thickness range of the epidermis. This result suggests a strong possibility that the skin entry echo may reflect the thickness of the epidermis.

The effect of masking material on the acoustic density of the skin entry echo further supports our argument. When the skin was masked with up to two layers of paper tissue, the acoustic density of the original skin entry echo (underneath the entry echo caused by the masking material) was about the same as that of the bare skin. As shown by the hollow bar in Figure 6, the acoustic density of the skin entry echo on the bare skin site was 147.8 (±7.13 a.u.) while the acoustic density of the original skin entry echo on the masked sites (underneath that of the masking material) was 152 and 145 a.u. when the layers of masking paper tissue was one and two, respectively. This result could not be explained if one believes that the skin entry echo is only an artifact. If that was true, one would expect a

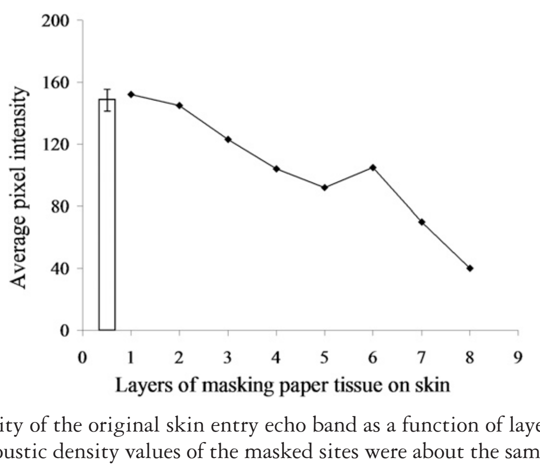

Figure 6. Acoustic density of the original skin entry echo band as a function of layers of masking paper tissue. It is seen that the acoustic density values of the masked sites were about the same as that of the bare skin site when the skin site was masked with up to two layers of paper tissue.

corresponding change in acoustic density after the skin was masked since the sudden shift in impedance from the coupling medium to the surface of skin had been dampened. In other words, the acoustic density of the original skin entry echo would have been dramatically reduced if the impedance change from the masking material to the skin surface had become much less. Therefore, this result further supports our argument that the skin entry echo, commonly purported to be an artifact, may represent properties of the epidermis.

COMPARISON BETWEEN ULTRASONOGRAPHY AND CONFOCAL LASER SCANNING MICROSCOPY

The epidermal thickness measured using confocal microscopy was very similar to that of the ultrasound method. The average thickness value obtained from 10 people (25 skin sites of dorsal and ventral forearms) was 108.2 (±16.8) μm while the value measured from the sonograms of ultrasound was 104.6 (±11.9) μm. Plotting the results of epidermal thickness measured using ultrasound method from the 102 volunteers in a histogram we obtained a quite normal distribution. The thickness values ranged from 70 to 150 μm with the mode of the distribution falling between 100 and 110 μm as shown in Figure 7A. Comparing this chart to the results obtained using confocal microscope in Figure 7B we can see that these two distributions are very similar. Student's *t* test on these two data sets showed no statistical difference between them ($p = 0.329$), and therefore it is difficult to believe that it is just a coincidence when these two sets of data match so well.

DISCUSSIONS ABOUT THE TECHNIQUE

The approach illustrated in this study helped us to collect experimental evidence and to show that the skin entry echo may reflect the structural information of skin, not just a meaningless artifact. Use of paper tissue to mask the surface of skin was found to be a convenient way to test the argument that the thickness and pixel intensity should have been changed had the skin entry echo been an artifact caused by the sudden change in echoing property from coupling water to the skin. While the physical masking method

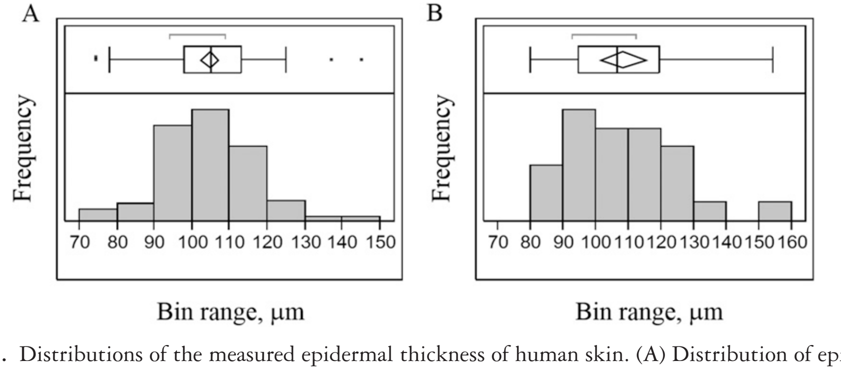

Figure 7. Distributions of the measured epidermal thickness of human skin. (A) Distribution of epidermal thickness of cheek skin by using ultrasound with a 50-MHz transducer. $N = 102$, mean = 104 μm, and standard deviation (SD) =11.9 μm. (B) Distribution of epidermal thickness of dorsal and ventral forearm skin using confocal laser scanning microscope. $N = 25$, mean = 108.2, and SD=16.8 μm.

works, other approaches would be more direct to validate ultrasound measurement of epidermal thickness. One of the approaches would be an *ex vivo* method which measures the thickness directly from an epidermal sheet separated from the cadaver skin using enzyme digestion methodology. We will examine the feasibility of this approach as the next step to this investigation. Additionally, the relevance of this technique to the 20-MHz transducer would need to be addressed. Evolved in the past decades, 20 MHz has been a standard method of ultrasound measurement of skin. When we designed this study, we had chosen the 50-MHz transducer since it had better sonogram pixel resolution and the imaging depth was well within the full thickness of human skin. The more detailed view of rete ridge structure of epidermis in 50 MHz provided us with the ability to more accurately detect the minimum thickness of epidermis (from skin surface to the top of the rete ridge structure) particularly when it is required to compare the results obtained from the confocal microscope. A 20-MHz transducer had resulted in sonograms with a more uniform boundary at the edge of the skin entry echo, which had yielded epidermal thicknesses toward the higher end when compared to the biopsy and confocal results. Adjusting the gain level may be able to improve the resolution and it needs a thorough investigation as the next step to establishing proper conditions which work with transducers of various frequencies.

CONCLUSION

The results of this study demonstrate that the acoustic properties of the skin entry echo correlates to epidermal thickness. Our observations indicate that the presence of the echorich band beneath one or two masking layers is consistent in thickness with the echorich band of adjacent bare skin. The acoustic density and thickness of the purported skin entry echo remained the same despite being masked. If the echorich band known as the skin entry echo is an artifact due solely to a sudden impedance change, the thickness and acoustic density would change accordingly once the skin is masked. In this experiment no drastic changes in acoustic density or thickness were observed after the skin was masked. Thus, researchers should not avoid using this echorich band in 50-Mhz ultrasound sonograms to study relative changes in the thickness or acoustic density of the epidermis. Understanding that the ultrasound method may not have the resolution as high as confocal microscope for accurate measurement of epidermal thickness, this result may provide us with a rough estimate of epidermal properties when other more advanced techniques or methods are not available.

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