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ORIGINAL ARTICLE

In vivo evaluation of skin of children with LC-OCT: An objective assessment

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Abstract

Background: Several non-invasive skin imaging methods have been developed in recent years. Line-field confocal optical coherence tomography (LC-OCT) is one of them, leading to the best compromise in terms of resolution and penetration depth. Skin biopsies are an essential technique in paediatric dermatology, but they are a major stressful event for the child and their parents. Current LC-OCT studies have not been dedicated to a paediatric population. If, however, LC-OCT proves to be helpful in children, it may help guide and decrease a certain number of skin biopsies.
Objectives: (1) To evaluate the feasibility of using LC-OCT in paediatric patients, and (2) to assess the maturation of skin structures in children over time with this method.
Methods: In vivo LC-OCT images were collected on six specific body regions (forehead, forearm, chest, back, dorsum of the hand and palmar surface) and in six age groups (between the ages of 0 and 16 years).
Results: In all body areas and age groups assessed, 9 of 10 images were rated as good-to-excellent, the only exception were the images acquired on the palmar surface. LC-OCT allowed visualizing very well the skin structures up to a penetration of 500 μm. We observed that the body regions located on the upper extremities of the body (forearm, dorsum of the hand and palmar surface) showed both a maturation on their structure and differences in thickness with respect to the other regions evaluated.
Conclusions: LC-OCT can easily be used for non-invasive imaging of children's skin and allows to document progressive skin changes in the different age groups. It may be a useful asset for imaging and diagnosing superficial skin disorders and as such reducing the number of invasive procedures while increasing the speed of diagnosis in the paediatric population.

INTRODUCTION

Non-invasive imaging methods have significantly developed in recent years, in particular for early detection of malignant skin lesions.[1] The reflectance confocal microscopy (RCM) provides images with a spatial resolution comparable to histology (~1 μm). However, horizontal sectional views are typically obtained from these devices, which makes reading the images complex.[2,3] The optical coherence tomography (OCT) has the advantages of a greater penetration (300–1000 μm) into the tissue compared to RCM (200 μm) and an easier image reading due to the vertical orientation of these sections. The OCT produces, however, skin images with a resolution significantly lower than RCM (between 3 and 7.5 μm).[2]

The line-field confocal optical coherence tomography (LC-OCT) is an imaging technique that combines the principles and advantages of RCM and OCT.[1,2,4] This method represents the best compromise in terms of high resolution and deep penetration in the study of the epidermis and the superficial dermis, as it provides vertical sectional views with an image resolution similar to histology (~1 μm) and a penetration depth of 400–500 μm.[2,4–6]

Reports on non-invasive imaging methods to visualize and describe the skin of children are limited.[2,5,7–13] There are no well-structured studies using OCT or LC-OCT in a paediatric population. RCM where conducted either over a limited age group (0–6 months),[12] on a low patient number (around 20 subjects),[7,14] or not dedicated to a paediatric population.[8,13]

Children's skin diseases are not smaller versions of adult's skin diseases. Firstly, there are some conditions that are mainly limited to the paediatric age group.[15] Secondly, lesions can look different in different skin types, ages and body locations.[16] Within this context, the specific objectives of this work were as follows: (1) To evaluate the feasibility of using LC-OCT in paediatric patients and (2) to assess the maturation of skin structures in children of time with this method. To this end, a comprehensive characterization of the healthy skin of paediatric volunteers was conducted by measuring the stratum corneum (SC) and epidermis thickness according to age and anatomical location (i.e. forehead, forearm, chest, back, dorsum of the hand and palmar surface of the hand).

MATERIALS AND METHODS

Clinical protocol

The study was performed at the children's hospital of the University Hospital Lausanne following the approval of the local ethical committee (protocol 2021_00683_2104) and the principles of the Declaration of Helsinki.[17] The study aimed to recruit at least 60 participants divided into six groups (10 participants per group) according to their age group: 0–12 months, 1–2 years, 3–4 years, 5–8 years, 9–12 years and 13–16 years. Adults signed the informed consent for their participating children (0–13 years), whereas participants between 14 and 16 years signed their informed consent for their own.

Inclusion criteria: age <17 years, be followed by the Pediatric Dermatology Unit at the CHUV, have signed parental and/or patient consent for LC-OCT imaging and have two scheduled routine visits (one for the information session and one, if desired, during which the LC-OCT could be performed).

Exclusion criteria: refusal of the patient or his/her parents, presence of skin lesions at specific anatomical locations (forehead, forearm, chest, back, dorsum of the hand and palmar surface of the hand), generalized and/or chronic dermatosis, genodermatoses with structural skin damage, and if the participant or his/her parents do not wish to be informed of any incidental findings.

Image acquisition protocol

In vivo LC-OCT images were collected with an LC-OCT instrument (deepLive™, DAMAE Medical). The device is a CE-marked and EU 93/42/EEC certified product that, among its different acquisition modes (i.e. vertical, horizontal and 3D modes), produces vertical sectional views of the skin (image resolution of 2048 × 680 pixels/1200 × 400 µm; *xz* axis) at a frame rate of 8 frames/s.

A drop of a paraffin oil was placed on the acquisition site before placing the handheld probe. The paraffin oil provides refractive index matching between the LC-OCT and the skin, which diminishes the specular back-reflection from both surfaces, and allows to achieve optimal image quality.[2] Moreover, given that live images are continuously generated by the instrument, the operator could move the probe to avoid the presence of artifacts that may compromise the image evaluation (i.e. skin imperfections or air bubbles).

The acquisition of one image per body site was usually enough to perform the evaluation. However, involuntary movements or lack of cooperation from the participants might make necessary to repeat the image acquisition. In these cases, a maximum of three images per body site were acquired, with the aim of minimizing frustration for the participants. When more than one image was acquired on a body-site of a participant, the image with the best sharpness and image depth was selected for the evaluation.

The procedure was repeated on six specific body locations: forehead (usually on the temple because it allowed for more stable acquisition of images), forearm (on the middle part), chest (in front of the sternum, to avoid gender bias), back (upper and middle part as this area allows for more stable acquisition of images), dorsum of the hand and palmar surface of the hand. No differences were made between the right or left side of the body.

Image evaluation

LC-OCT images were evaluated by two dermatologists (CG and SV, proficient in OCT image evaluation[18]) to establish the quality of the image according to the visualization of the keratinocyte nuclei, the epidermis and the dermoepidermal junction (DEJ). The qualitative scale used to evaluate the visualization those structures was as follows: 3—excellent, 2—good and 1—bad/not visible.

Besides, three regions of interest (ROI) were randomly selected on each image. Then, both dermatologists independently measured the thickness of the SC and the entire epidermis on those ROI. Measurements were performed using the software integrated in the device (deepLive™, DAMAE Medical).

Statistical analysis

To study the maturation of the skin at the different anatomical locations between the six age groups (0–12 months, 1–2 years, 3–4 years, 5–8 years, 9–12 years and 13–16 years), results were compared statistically using either the ANOVA one factor test or the Kruskal–Wallis test depending on whether the data

verified the normality and equality of variances hypotheses (Shapiro–Wilk test and Levene test). If the test was significant, a pairwise comparison between the six age categories was performed using either the Tukey method (for ANOVA) or its non-parametric alternative (for Kruskal–Wallis).

Besides, to study the differences of skin structures between body locations, results were compared statistically using either the ANOVA on repeated measures or Friedman test depending on whether the data verified the normality and equality of variances hypotheses (Shapiro–Wilk test and Levene test). If the test was significant, pairwise comparison between the six body locations was performed using either paired *t*-tests (for ANOVA on repeated measures) or Wilcoxon tests (for Friedman test). The level of significance was fixed at $\alpha = 0.01$.

RESULTS

Qualitative evaluation of the visualization of the different epidermal structures according to age and body area

Sixty-seven subjects participated on the study, 64 of whom had all body areas evaluated. As a result, a total of 399 images corresponding to six different age groups (8–15 subjects per group), and six different body areas, were acquired and evaluated.

The interobserver agreement (assessed as intraclass correlation coefficient; ICC) for the manual measurements conducted by the dermatologists was >0.98 (that is, excellent agreement). The visualization of the different structures of the epidermis was rated as excellent in 21.7% of the images, good in 68.7% of cases and bad/not visible in 9.6% of the acquisitions. When grouping the data with respect to the area of the body evaluated, it was observed that the number good-to-excellent images was similar on all of the areas. However, the visualization of the epidermal structures on the palmar surface of the hand was bad/not visible in 17% of cases. Interestingly, when grouping the data according to the age of the volunteers, it was observed that age had no significant influence on the ratings.

Impact of body area and age of the infant on the SC and epidermal thickness

Figures 1–6 show a representative image of the different body areas evaluated for each of the age groups included in the study. The SC was the thickest for the palmar surface of the hand, followed by the back of the hand, the forearm and the other areas evaluated (Figure 7). Similar results were observed when evaluating mean epidermal thickness, as it was much thicker on the palmar surface of the hand, than on the back of the hand, forearm and any other area (Figure 8). Gender does not seem to have an impact on the mean thickness of the SC or the epidermis.

Regarding the maturation of the different skin structures with age, no significant differences were observed for the forehead, chest or back. However, a correlation between age and mean SC thickness was observed for the dorsum of the hand ($p < 0.01$), the palmar surface of the hand ($p < 0.001$) and the forearm ($p < 0.001$), whereas a correlation between age and mean epidermis thickness was observed for the dorsum of the hand ($p < 0.01$) and the palmar surface of the hand ($p < 0.01$; Figures 7 and 8).

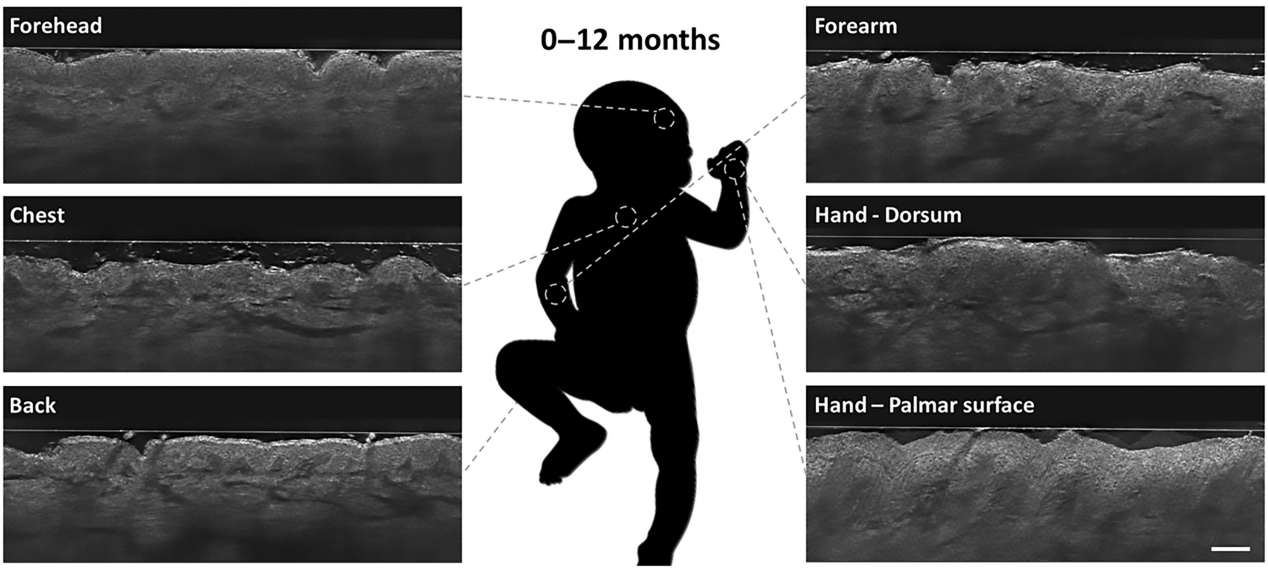

FIGURE 1 Representative LC-OCT vertical skin images of the different body sites evaluated for the age group 0–12 months. The central body schema was adapted from Servier Medical Art by Servier. Original images are licensed under a Creative Commons Attribution 3.0 Unported License. Scale: 100 μm.

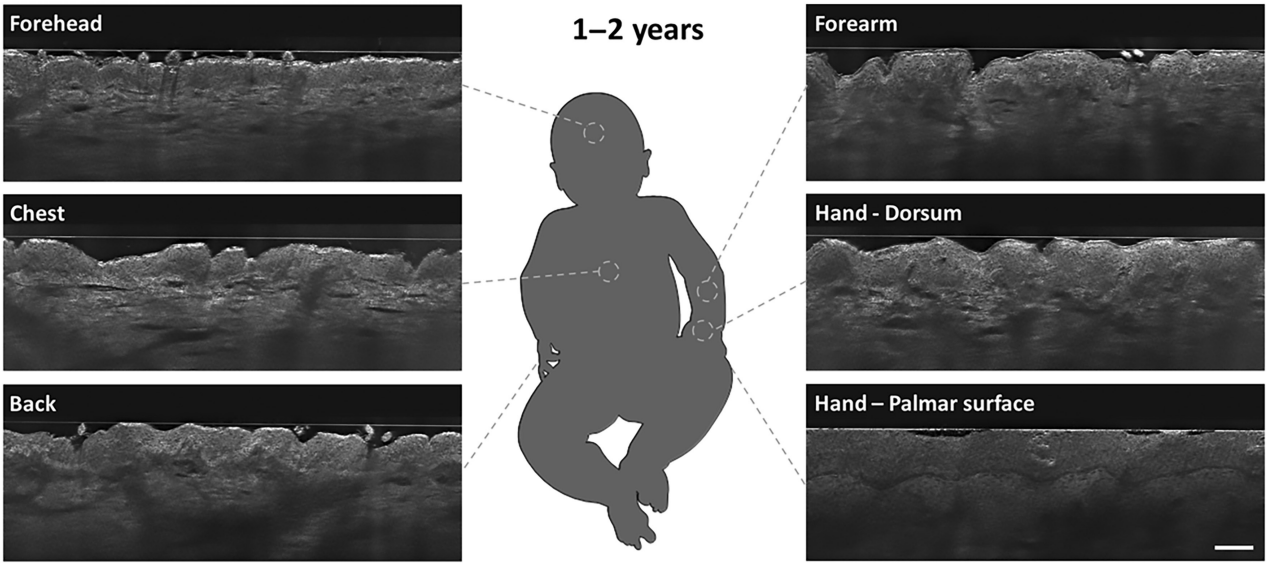

FIGURE 2 Representative LC-OCT vertical skin images of the different body sites evaluated for the age group 1–2 years. The central body schema was adapted from Servier Medical Art by Servier. Original images are licensed under a Creative Commons Attribution 3.0 Unported License. Scale: 100 μm.

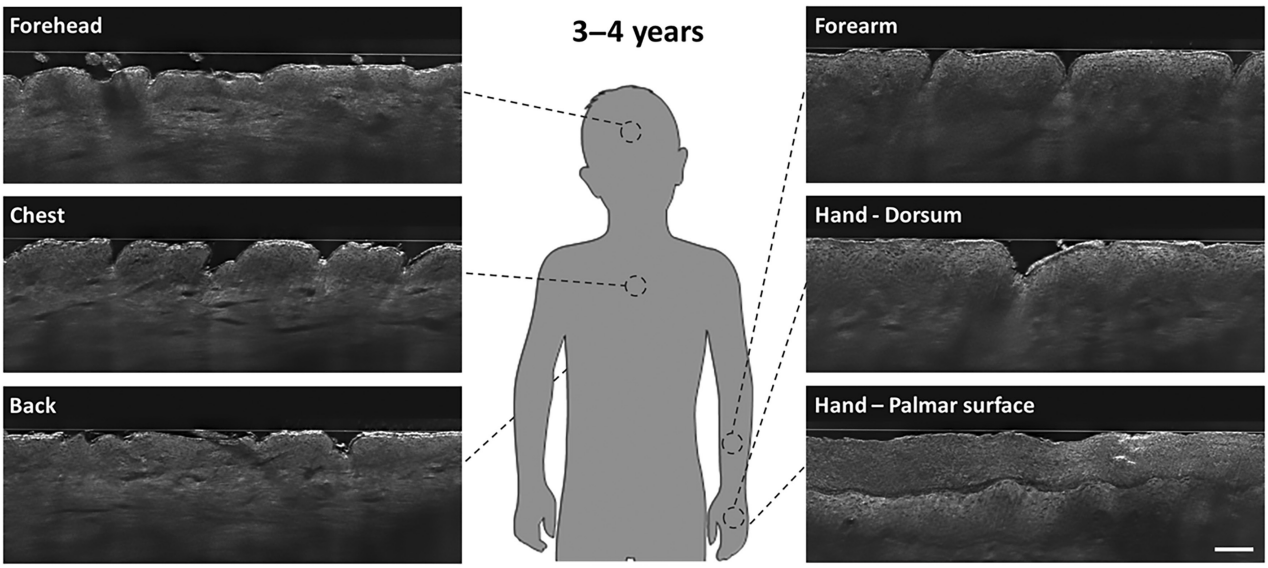

FIGURE 3 Representative LC-OCT vertical skin images of the different body sites evaluated for the age group 3–4 years. The central body schema was adapted from Servier Medical Art by Servier. Original images are licensed under a Creative Commons Attribution 3.0 Unported License. Scale: 100 μm.

DISCUSSION

LC-OCT as a non-invasive in vivo imaging system for paediatric skin

Skin biopsies are tools used by the dermatologist to diagnose a variety of skin conditions. Despite complications may be encountered, it is considered as a safe and routine procedure[19] with discomfort in the biopsy site being the most commonly reported adverse effect in adults. However, this is different in paediatric patients. A skin biopsy is an essential technique in dermatology for the diagnosis of skin diseases, but it is also a major and stressful event for the child as well as the parents, that may result in unnecessary scarring given the healing properties of paediatric skin.[20] As a consequence, there is a need to search for non-invasive alternatives that allow (1) better selection of lesions to be evaluated, (2) more accurate area of interest to be biopsied, (3) to reduce the number of unnecessary biopsies[14] and (4) an early diagnosis of skin diseases in paediatrics.[21] One such method would be LC-OCT, a device capable of visualizing and acquiring images of the skin in real time. Nevertheless, despite

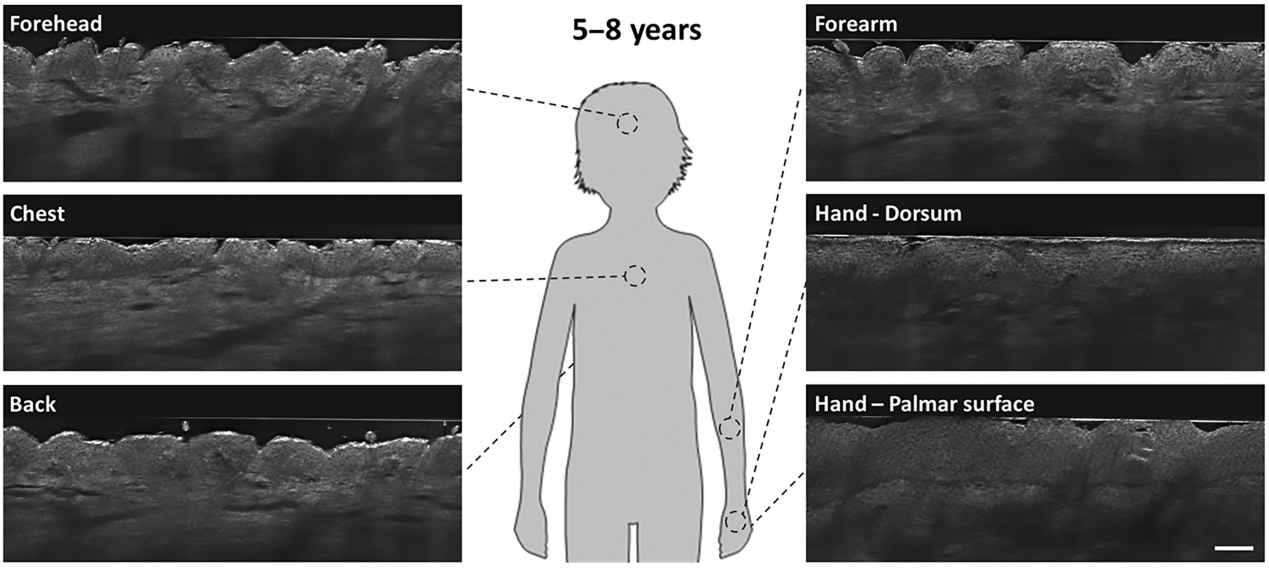

FIGURE 4 Representative LC-OCT vertical skin images of the different body sites evaluated for the age group 5–8 years. The central body schema was adapted from Servier Medical Art by Servier. Original images are licensed under a Creative Commons Attribution 3.0 Unported License. Scale: 100 μm.

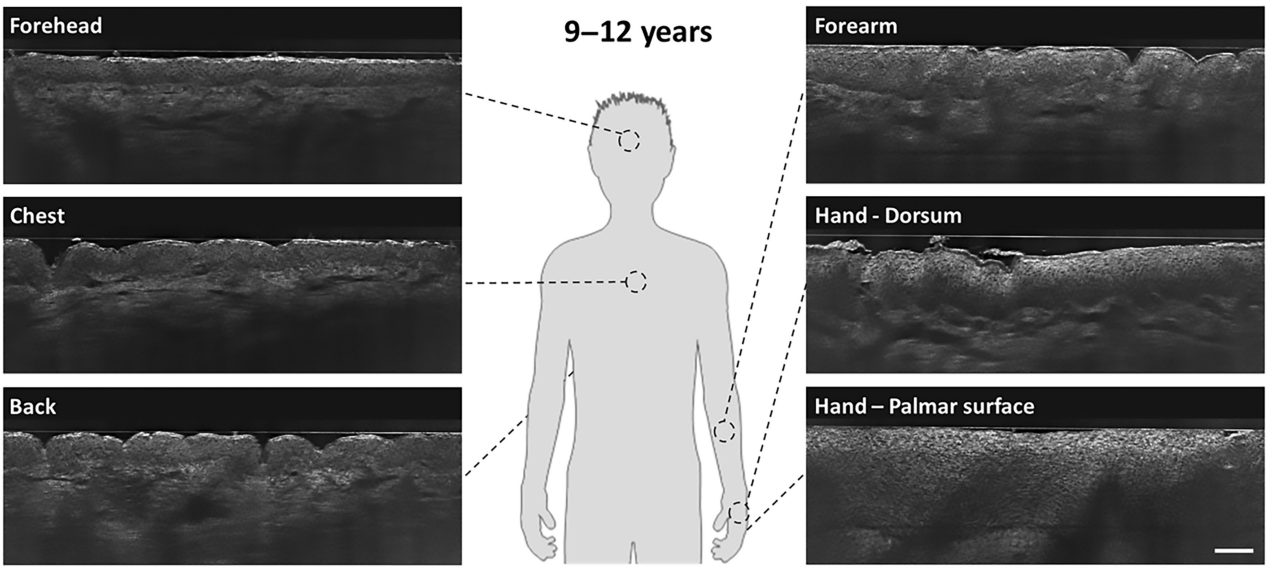

FIGURE 5 Representative LC-OCT vertical skin images of the different body sites evaluated for the age group 9–12 years. The central body schema was adapted from Servier Medical Art by Servier. Original images are licensed under a Creative Commons Attribution 3.0 Unported License. Scale: 100 μm.

different groups have evaluated its use in adult patients, none have done so in paediatric patients.

In this study, the skin was evaluated in six different stages of the passage from infancy to adulthood: infancy (0–12 months, 1–2 years and 3–4 years), childhood (5–8 years), pre-pubescent period (9–12 years), and puberty and adulthood (13–16 years). In addition, six different regions of the body were selected according anatomical and practical criteria in the aim to cover different regions, different skin characteristics and different skin types. The forehead and the palms were selected because they are two regions where it is expressly recommended not to perform skin biopsies in infants, either because of the subsequent scarring that may remain or because it is a particularly sensitive and painful area.[20] Also, the palms present a completely different quality of skin compared to the rest of the integument. The back and the chest were selected because they are the largest areas of the body, which increases the odds of using the device in these regions. Additionally, the chest area was also chosen to evaluate the impact of infant respiratory motion on the quality of the images. Finally, the dorsum of the hand and the forearm were selected because they are two areas in which

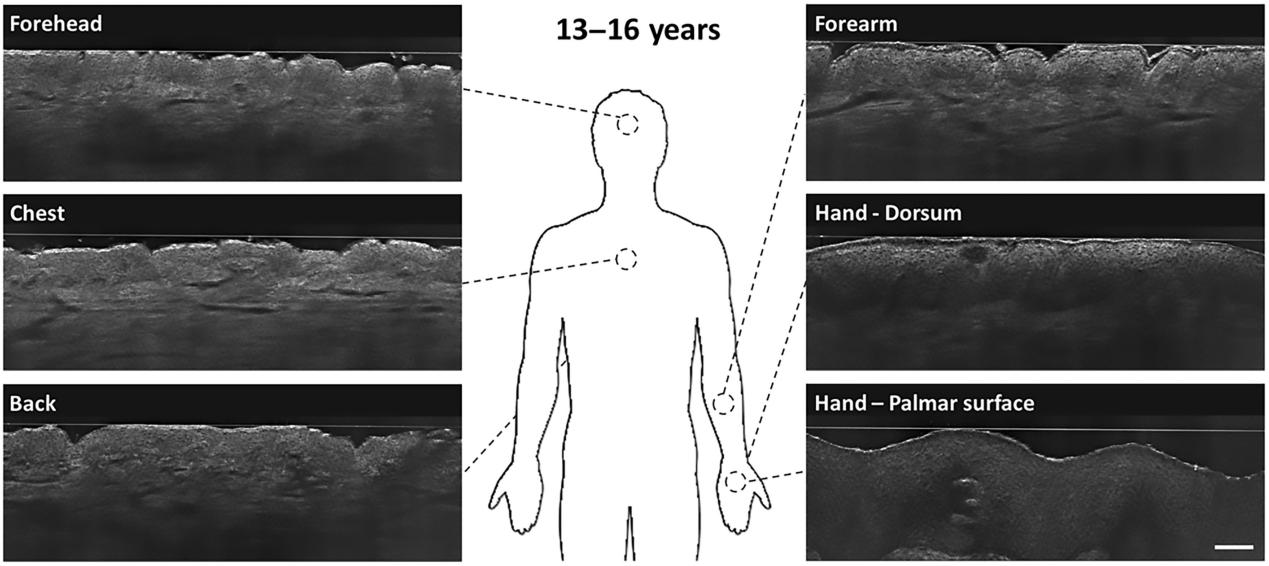

FIGURE 6 Representative LC-OCT vertical skin images of the different body sites evaluated for the age group 13–16 years. The central body schema was adapted from Servier Medical Art by Servier. Original images are licensed under a Creative Commons Attribution 3.0 Unported License. Scale: 100 μm.

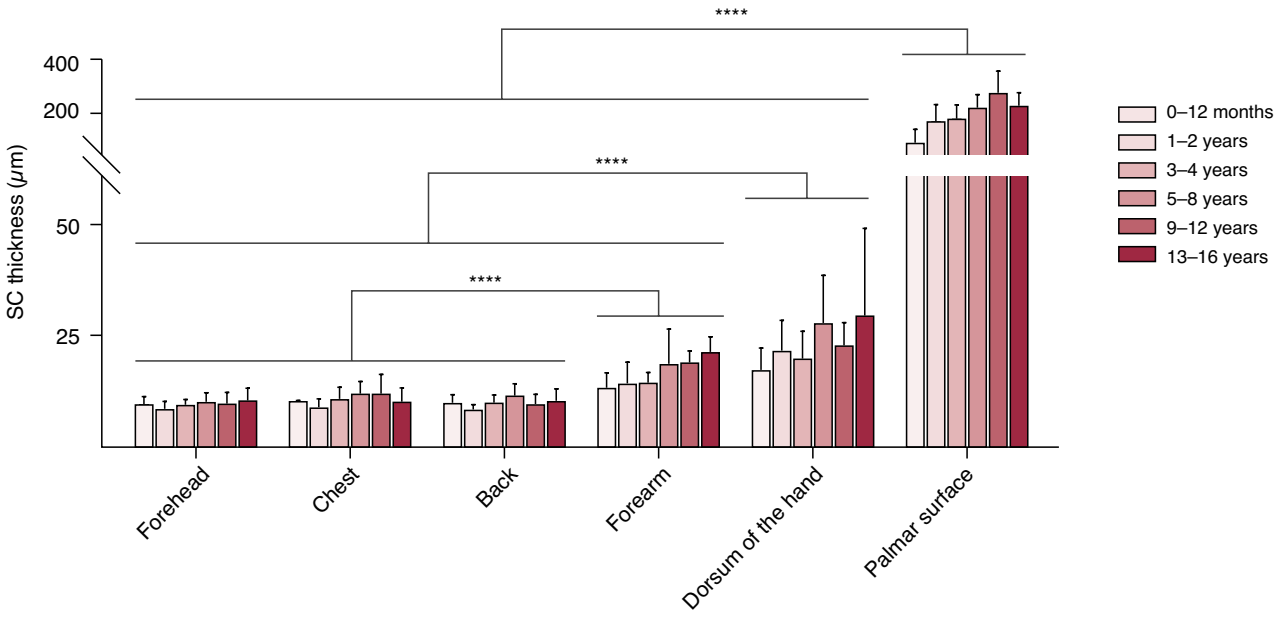

FIGURE 7 Evaluation of the SC thickness on different skin areas and age groups. Forehead, chest and back sites: thin SC that does not increase with age. Forearm: statistically significant thicker SC than the previously mentioned areas; its thickness increases with age of participants. Dorsum of the hand: statistically significant thicker SC than the previously mentioned areas; its thickness progressively increases with the age of participants. Palmar surface: thickest SC than any other skin area evaluated; its thickness increases with the age of participants. ****(p-value < 0.0001).

the child's collaboration is particularly important, since they tend to retract them as a measure of self-protection.

Nine out of ten images were rated as good-to-excellent in all of the body areas evaluated and age groups assessed. The only exception would be the palmar surface of the hand because, especially in children older than 9 years of age, the visualization of the DEJ was compromised. These results were surprising since the images were obtained in paediatric population (which is in constant movement) and in some regions with attributes that make them particularly complex to acquire. However, in addition to the live images that are continuously generated by the instrument on the computer, a 2D image can be instantly recorded with a simple user click on the probe button. These two factors may explain the good results observed as it minimizes the collaboration needed from the volunteer.

At present, the main weaknesses that we found while using the LC-OCT device were as follows: (1) the weight

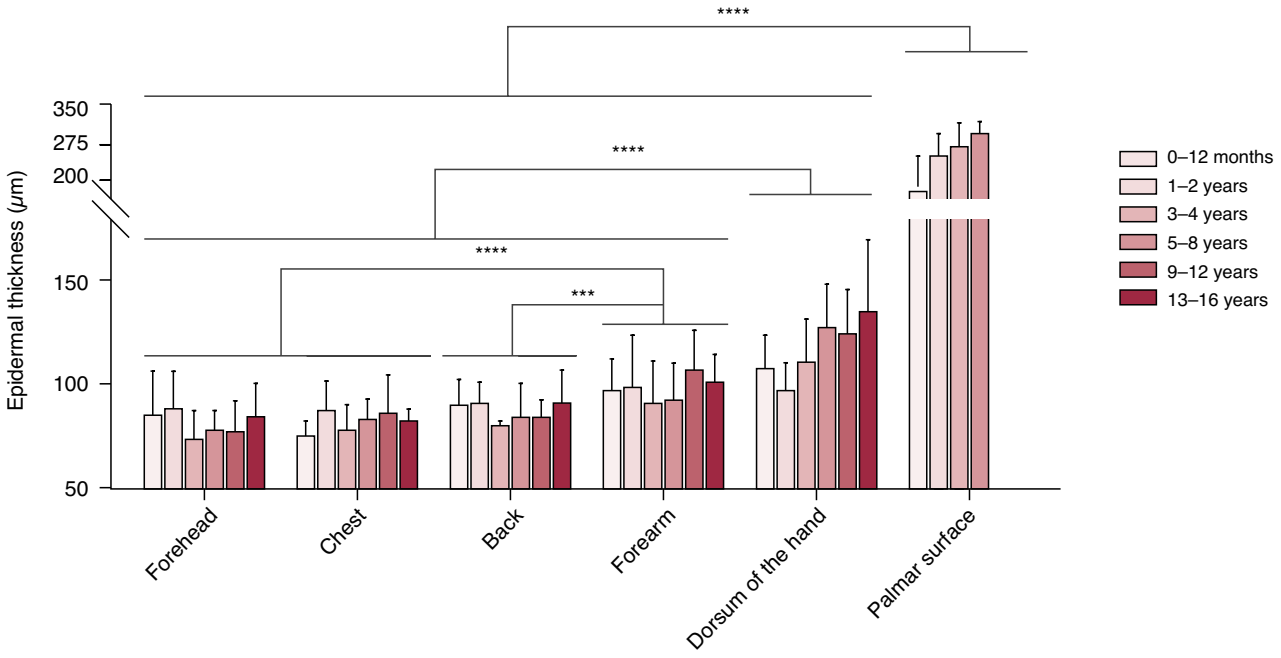

FIGURE 8 Evaluation of the epidermis thickness on different skin areas and age groups. Forehead, chest and back sites: thin epidermis that does not increase with age. Forearm: statistically significant thicker epidermis than the previously mentioned areas; its thickness does not increase with age of participants. Dorsum of the hand: statistically significant thicker epidermis than the previously mentioned areas; its thickness progressively increases with the age of participants. Palmar surface: thickest epidermis than any other skin area evaluated; its thickness increases with age. Due to the limitations of the device, the epidermis thickness on the palmar surface of the hand could not be evaluated in children older than 9 years of age. ***(p-value < 0.001); ****(p-value < 0.0001).

of the machine head and (2) the maximum depth that can be captured. In areas with a low presence of adipose tissue (i.e. the back or forehead regions), the machine head can rest on the bone, which makes the acquisition process easier for the operator. However, in regions such as the forearm, the operator must hold the weight of the head during the entire image acquisition process, which may affect the quality of the image. Besides, in our opinion, the limitations related to the acquisition depth are the main constraint of the device. In the specific case of the palmar surface of the hand, the thickness of the SC causes that the entire epidermis could not be visible in children older than 9 years of age (Figure 8). In any other region evaluated, the LC-OCT seems a very useful asset for diagnosing superficial skin disorders, but not for diseases below the upper dermis.

Otherwise, the LC-OCT deepLive™ medical device is an instrument that has proven to be useful for imaging the skin in the paediatric population regardless of the patient's age.

Maturation of the SC and epidermal thickness in paediatric population

As for the diagnosis of different skin conditions, the evaluation of the skin maturation process has traditionally been carried out by histological skin biopsy studies. However, studies such as that of Fonseca de-Souza et al.[22] question the feasibility of this type of study, both because of a lack of reliable evidence and because of the ethical difficulties associated.

Based on several studies in which skin maturation has been evaluated by noninvasive approaches, there is evidence that children's skin develops in relation to structure, function and composition during the first years of life.[7,23–25] Nonetheless, the functional development of the skin does not seem to be linked to an increase in skin thickness. Indeed, in our study, only three of the six areas included showed both a structural maturation and differences in thickness with respect to the other regions (i.e. forearm, dorsum of the hand and palmar surface; Figures 7 and 8).

The impact of age on skin thickness has been previously discussed in the literature, while authors such as Mogensen et al.[11] (0.5–5.5 vs. 29–59 years), Gambichler et al.[26] (20–40 vs. 60–80 years) or Pedrazzani et al.[4] (22–27 vs. 31–37 vs. 40–49 vs. 52–57 vs. 61–69 years) reported a significant negative correlation between age and skin thickness. Others as Koehler et al.[27] (23.3 ± 1.9 vs. 47.3 ± 3.1 vs. 72.1 ± 6.4 years) or Miyauchi et al.[12] (0.2–3 months vs. adults) found no differences between age groups; or even an increase with age (Fluhr et al.[8]; 0–1 vs. 2 vs. 4–5 vs. 7–8 vs. 20–35 years). According to our results and these previously described (Figure 9), we foresee two differentiated periods when evaluating skin maturation. Between infancy (0–1 years) to adulthood (12–16 years), a continuous development of the skin is noticeable, which translates into an increase in skin thickness. Then, after adulthood, a significant decrease in skin thickness can be observed.

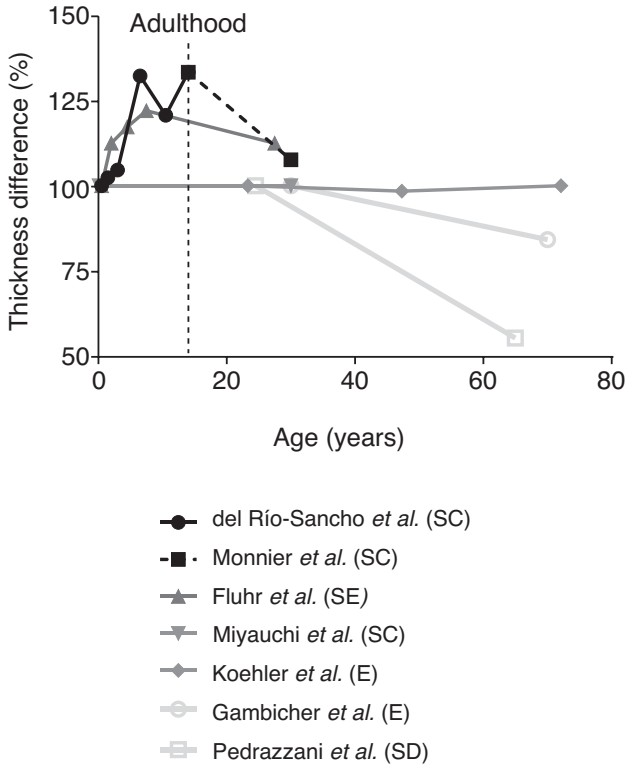

FIGURE 9 Graphical representation of published reports evaluating the impact of age on skin thickness. The value corresponding to the youngest group of those evaluated in the study (>3 months) was considered as the initial thickness value (i.e. 100%). The values represented in 'del Río-Sancho et al.' (i.e. those presented in this manuscript) do not include the measurements conducted on the palmar surface of the hand to allow a comparison with Monnier et al.[5] Dark grey line includes authors that reported a positive relation between age and thickness.[8] Gray lines include authors that reported no differences between age groups.[12,27] Light grey lines include authors that reported a significant negative correlation between age and skin thickness.[4,26] The structure of the skin evaluated on the reports was SC, stratum corneum; SE, suprapapillary epidermis; E, epidermis; and SD, superficial dermis.

CONCLUSIONS

The LC-OCT allows to visualize the healthy skin of the child and to create an iconographic database of children's skin. This noninvasive imaging method seems promising and could help, in the future, in the study and monitoring of the paediatric population. Ideally, this should make it possible to reduce the number of invasive procedures in children (i.e. skin biopsy) while increasing the speed with which the diagnosis is obtained.

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ETHICS STATEMENT

The patients in this manuscript have given written informed consent to publication of their case details.

FUNDING INFORMATION

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of Interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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