VALIDATION OF ULTRASOUND PROBE OF DERMALAB COMBO FOR MEASURING SKIN THICKNESS: IN VITRO STUDY

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Abstract

Objectives: DermaLab Combo is an advanced multiparameter skin analysis system developed by Cortex Technology. Though the reliability of the instrument using test-retest and inter-rater reliability was analyzed in previous studies, no studies compared the measurement's accuracy histologically. The study aimed to compare the skin thickness of animal skin measured by DermaLab Combo to the skin thickness measurements obtained from the frozen section and permanent section.

Materials and Methods: This study included 4 healthy male or female (totally 8 sites) rodents weighing at least 160 grams. The outcome assessed was skin thickness. After skin thickness was measured with the ultrasound (USG) probe of DermaLab Combo, an incisional biopsy of the site was done and sent for the frozen section and permanent paraffin section for histological analysis of skin thickness. The data were tabulated and analyzed with SPSS software.

Results: There is no significant difference in the measurement of skin thickness by the USG probe of DermaLab Combo and histological skin thickness assessed by the gold standard permanent paraffin sectioning ($p=0.575>0.05$).

Conclusion: We conclude that the USG probe of DermaLab Combo is reliable in measuring skin thickness for clinical and research purposes.

Keywords: skin thickness, DermaLab Combo, Ultrasound probe, frozen section, permanent paraffin section

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1. Introduction

DermaLab Combo is an advanced multiparameter skin analysis system developed by Cortex Technology. It measures multiple skin parameters, such as skin pH, elasticity, color, thickness, temperature, and hydration. [1] The DermaLab Combo comprises the following components: measurement probes, interface unit, and tables PC. [2] The probes are ultrasound probe, TEWL probe, hydration probe, elasticity probe, skin temperature probe, skin color probe, skin pH probe, video scope probe, and skin strip reader. [3] The ultrasound (USG) probe functions based on the principle of a rotating single element transducer (center frequency 20 MHz, band-width 5-35 MHz, focal distance 13 mm). It provides instant assessment of collagen level, dermal thickness, and low echogenic band (LEB). Options of automatic and manual measurements are available and the system is easy to operate requiring no training. [1]

The device is becoming a popular choice in dermatology and cosmetology practice as it quantitatively assesses the quality of the patient's skin and helps track the prognosis. [4] The device is also being employed in research to measure the quality of skin and scar tissues. [1] Though the reliability of the instrument using test-retest and inter-rater reliability was analyzed in previous studies, no studies compared the measurement's accuracy histologically. Our team has extensive knowledge and research experience that has translate into high quality publications[1–10].The study aimed to compare the skin thickness of animal skin measured byDermaLab Combo to the skin thickness measurements obtained from frozen sections and hematoxylin and eosin (HNE) staining.

2. Materials and Methods

This in-vitro study was conducted at the Department of BRULAC and Department of Oral and Maxillofacial Surgery, Saveetha Dental College and hospital. The approval of the study was given by the "Institutional Ethical committee, SIMATS Review Board" [IHEC/SDC/OSURG-1902/21/345]. The animal ethical committee approval was also obtained for this study [BRULAC/SDCH/SIMATS/IAEC/04-2022/103].

The study included 4 healthy male or female (totally 8 sites) rodents weighing at least 160 grams. The outcome assessed was skin thickness. It was measured using the USG probe of DermaLab Combo (Agaram Industries, Chennai), frozen section, and permanent paraffin section.

Preparation of site

Under general anesthesia, the dorsum of the rodent was shaved and two markings placed were on each rodent for the sites to be measured.

Skin Thickness Measurement - DermaLab Combo

The ultrasound probe of DermaLab Combo was prepped and the system was set up as per user instructions and the system. The lubricant was applied over the probe and gently placed over the markings on the rodent's skin. The probe was moved in a circular motion for 2-3 secs over the site to spread the lubricant before coming to a stop. The "START" option on the laptop monitor was selected to activate the probe. The skin thickness as measured by the USG probe in microns and a USG image of the same was displayed on the monitor. The recordings were noted (Figure 1, Figure 2).

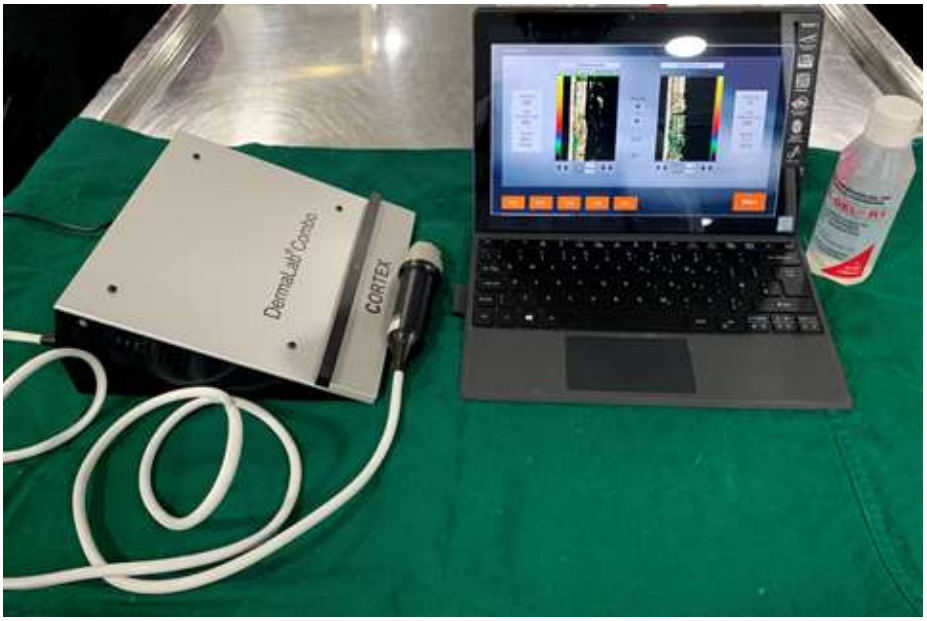

Figure 1: DermaLab Combo Device

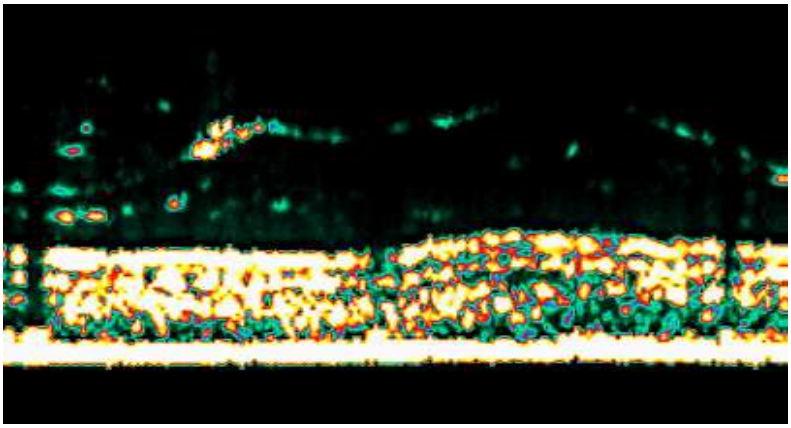

Figure 2: USG image of skin thickness

Surgical Procedure

The surgical procedure was performed under a sterile condition in the operating room of the animal laboratory. The selected healthy rodent was anesthetized with ketamine hydrochloride (i.p) at the dosage of 70mg/kg body weight and xylazine (i.m) at the dosage of 10mg/kg body weight. The lower half of the dorsal part of the trunk was shaved thoroughly and aseptically prepared with a solution of Betadine. A 1x1 cm dimension skin incision was made on the right and left side of the vertebral column, exposing the fascia and muscles underneath. The overlying skin of 1x1cm was excised and given for histological analysis(Figure 3, Figure 4).

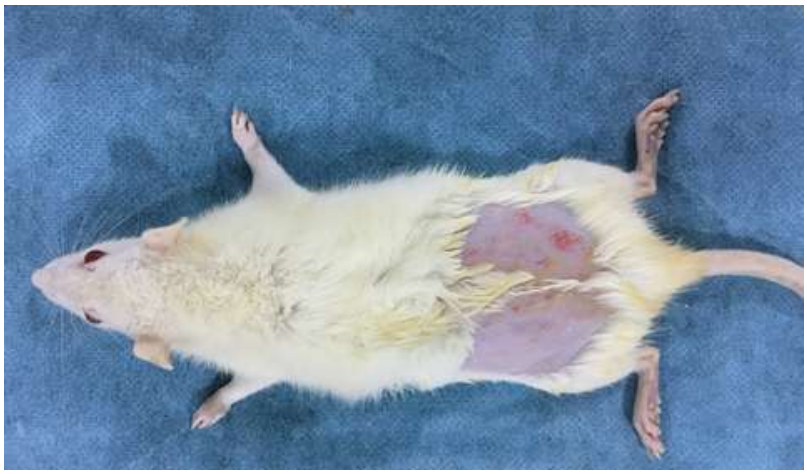

Figure 3: Dorsum of the rat with skin exposed after shaving the hair

Figure 4: Excision of the site to be histologically measured for skin thickness Histology

Thetissue was sent for the frozen section and permanent section to the Department of Oral and Maxillofacial Pathology at Saveetha Dental College. A part of tissue was used for the frozen section while the other part was used for the permanent section.

Frozen section: The tissue was frozen at -30 degrees, sectioned to 3-10 microns in thickness using a cryostat, the slide was stained using hematoxylin and eosin, mounted using dpx, and thickness was measured under the microscope at 4X 10x and 40x magnification (Figure 5).

Permanent section: The tissue was fixed in 10% neutral buffered formalin for 24 hours and in grades of alcohol for dehydration followed by acetone and xylene for 1 each. Paraffin wax impregnation was done for 24hours, and further, the paraffin-embedded wax was sectioned using Leica microtome to 3-to-10-micron thickness, hematoxylin, and eosin stained and seen under a microscope to measure skin thickness (Figure 6).

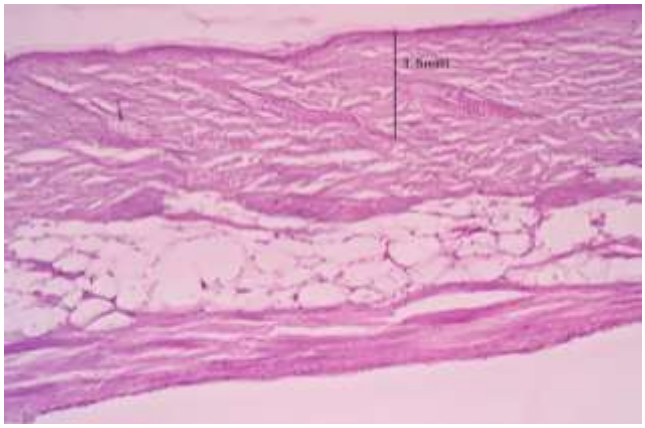

Figure 5: Frozen section and skin thickness

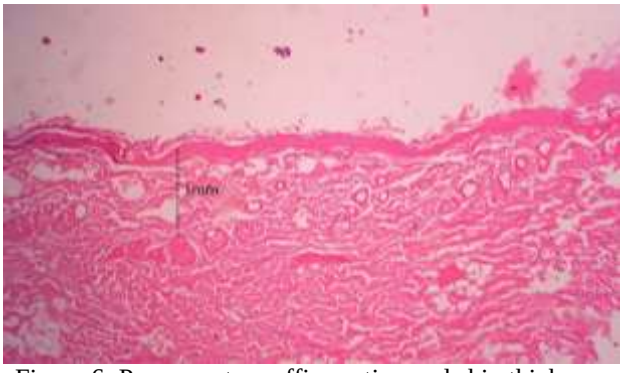

Figure 6: Permanent paraffin section and skin thickness

Statistical Analysis

The collected data were analyzed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. To describe data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & S.D were used for continuous variables. To find the significant difference in the multivariate analysis for repeated measures the Friedman test followed by the Wilcoxon signed-rank test was used and represented by Whisker's box plot. In both the above statistical tools, the probability value of .05 is considered a 'significant' level.

3. Results

Totally 8 sites in 4 rodents were measured for skin thickness using a USG probe of DermaLab Combo, frozen section, and histological staining. The descriptive statistics showed the mean skin thickness from all the three modalities of measurements tested in this study to be as follows: 719 microns (USG), 750 microns (HNE), and 1162 microns (frozen section) (Table1) (Figure 7). The results of test statistics revealed a significant difference between the measurements in the frozen section and USG while no significant difference was found in the measurements between hematoxylin and eosin (HNE) staining and USG. Though away from the scope of this study, we also assessed the difference between the measurements from the frozen section and permanent paraffin section and found it to be significant (Table 2).

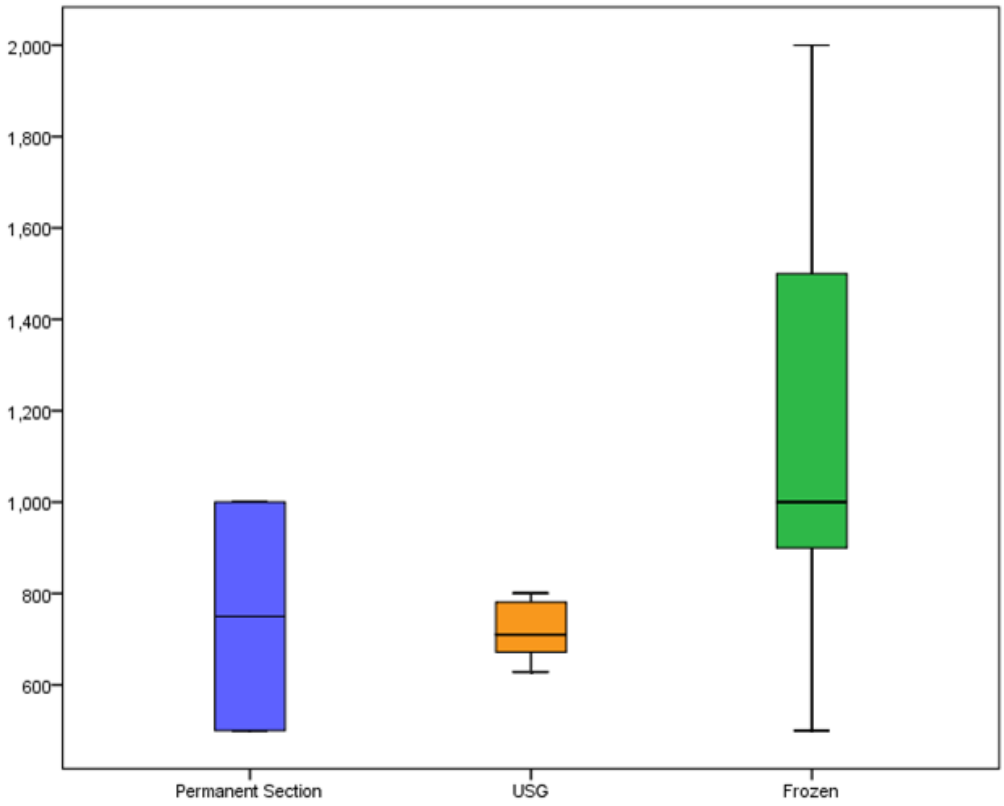

Figure 7: Graph representing a comparison of mean between the measurements from permanent section (HNE), frozen section, and USG probe

Table 1: Descriptive statistics

	Mean	**SD**	**N**
Permanent section	750.00	267.261	8
USG	719.25	63.450	8
Frozen section	1162.50	474.906	8

Table 2: Table showing test statistics where USG-ultrasonography, PS- permanent section, FS- frozen section.

	Z	**p-value**
USG - PS	-.560[b]	.575
FS - PS	-2.264[c]	.024

FS - USG	-2.240[c]	.025

a.Wilcoxon Signed Ranks Test
b. Based on positive ranks.
c. Based on negative ranks.

4. Discussion

The main purpose of this study was to compare the skin thickness of animal skin measured by DermaLab Combo to the skin thickness measurements obtained from the frozen section and hematoxylin-eosin staining. Many researchers have tried to establish skin thickness measurements by a variety of methods. [15-19]

Previously, all scar assessment tools provided only subjective assessments based on scales (for example- The patient Observer Scar Assessment Scale, Visual Analogue Scale, Manchester Scar Scale, etc.). [20]Though these scales are widely used in research and clinical settings for the assessment of scar quality, these subjective assessments do not provide the actual status of the scar with respect to scar thickness, collagen level, elasticity, hydration, and skin/scar parameters. [21] The introduction of digital skin analysis systems has aided in quantitatively assessing these variables which is a big boon for dermatologic/cosmetology research. [22]

The DermaLab Combo is an integrated skin testing device that was developed for skin testing in the cosmetic field and is becoming increasingly popular in dermatology/ cosmetology. [2]

The DermaLab Combo is a relatively new commercially available integrated skin testing device primarily designed for the R&D departments of manufacturers in the fields of cosmetics, personal care products, pharmaceuticals, and household products. [23] Its use has also extended to clinical research for assessing the skin quality, and scar assessment and has also become increasingly popular in dermatology/ cosmetology clinics. The device is intuitive and easy to use requiring no prior training. [24]

The frozen section was first introduced in 1891 and has been used for many decades as a rapid diagnostic tool used commonly in analyzing tissues obtained intraoperatively. [25] It is used regularly in cancer surgeries (especially head and neck) during intraoperative periods to assess the depth of infiltration and to provide adequate clearance. It has several indications including identification of tissue type, differentiating benign versus malignant nature of the tissue, type of malignancy, determination of surgical margins, the positivity of lymph nodes, and presence of malignant implants in other tissues. [26] Though the frozen section preserves the architecture of the tissue better than other intraoperative diagnostic methods, it is still inferior to the gold standard 'permanent paraffin section' fixed in formalin. [27] The factors that might lead to an inaccurate diagnosis by frozen section analysis are sampling errors, difficulties in histologic determination of the cell types, or errors in identifying the grade of the lesion. [28]

A permanent paraffin section is the gold standard of histopathology as a series of chemical solutions are used to obtain a high-quality slide which allows for providing an accurate diagnosis. [29] The whole process takes up to 2-3days. Almost always, every frozen section is followed by a permanent section to provide the final histopathological interpretations/ diagnosis. [30]

In this study, we compared the skin thickness measurements obtained from USG to those obtained from frozen sections (FS) and permanent sections (PS). The results of our study reveal that there is a significant difference between the skin thickness measurements of USG and FS while no significant difference was found in the measurements between PS and USG. That is, the measurements of skin thickness obtained from the USG probe of DermaLab Combo are consistent

with that obtained from the permanent paraffin section but inconsistent with that obtained from the frozen section. It is worth noting that the skin thickness measurements significantly differed between frozen and permanent sections themselves. This could be because of the difficulty in appreciating the different cells of the epidermal and dermal layers in the frozen section. To add, animal skin being more delicate and thinner than human skin is difficult to section and process. This difficulty was also faced by the pathologists involved in this study. Hence, the skin thickness measurement obtained from the frozen section is likely to be erroneous and can be excluded from the comparison. The skin thickness measured by the USG probe and obtained from performing a permanent paraffin section of the sample, revealed the USG probe to give comparable measurements with that of the gold standard permanent sectioning with no significant difference between the two ($p>0.05$).

We acknowledge our choice of study design (in-vitro) could be a limitation as we are validating an instrument that is only used in humans. But histological validation of skin thickness in healthy humans using biopsy would be ethically rebelling. Since our objective was to compare skin thickness measurements of the instrument with that of histological skin thickness, we chose an in-vitro study design.

5. Conclusion

We conclude that there is no significant difference in the measurement of skin thickness by the USG probe of DermaLab Combo and histological skin thickness assessed by the gold standard permanent paraffin sectioning. We deem the USG probe of DermaLab Combo to be reliable in measuring skin thickness for clinical and research purposes.

Conflict of Interest

The authors declare no conflicts of interest.

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