Aging Collagen in the Human Papillary Dermis: Comparison of Non Invasive in vivo Methods

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Background: With a share of 90%, collagen is the most abundant protein in the dermis. It forms a fine network of fibers and is largely responsible for the skin's resistance and elasticity. With increasing age, collagen degrades and fibers become cross-linked. Skin elasticity decreases and wrinkles form. A major cause of collagen loss and degradation is sun exposure. UV-A rays penetrate into the dermis and generate free radicals. The skin reacts to this, for example, with the formation of metalloproteinases, enzymes that cut collagen fibers, and the collagen degrades.

Imaging of the collagen network by invasive methods using punch biopsies and histological staining has long been established. However, in cosmetic research, noninvasive methods should be used whenever possible. A number of imaging techniques that can be applied directly to living skin are available. These include ultrasound imaging of the skin, two-photon microscopy, confocal reflectance microscopy, and Line Confocal Optical Coherence Tomography (LC-OCT). Furthermore an indirect method exists to measure collagen density in vivo and depth-resolved with confocal Raman spectroscopy by quantifying dermal water content. Due to different resolution and different measurement principles, the age-related degradation of collagen in the papillary dermis presents differently according to the methods used.

The aim of this work was to compare the age-related collagen degradation as visualized by LC-OCT with the methods described in the literature. In a clinical study, images of papillary collagen were acquired in subjects of different ages using LC-OCT. The suitability of LC-OCT for in vivo collagen imaging, its advantages and disadvantages are discussed based on our results, and the current literature on existing techniques.

Methods: In 43 male and female subjects aged 7-70 years with fair skin and no signs of pronounced photoaging, images of papillary collagen were obtained with LC-OCT. Images were taken on the inner, sunlight-protected forearm and on the sun-exposed dorsal forearm

respectively. Collagen content was evaluated by image analysis. Main criterion was the optical attenuation as a measure for the density and reflectiveness of the collagen structure.

Results: Visual assessment of LC-OCT images revealed an age-related decrease in density and reflectiveness of the collagen. Aged collagen was more fibrous and increasingly embedded in dark non reflective structures. Intraindividual differences between dorsal and volar collagen pattern gradually became larger with increasing age, as the dorsal collagen change started earlier at age and developed quicker.
In some of the literature documented methods, aging collagen presents differently from LC-OCT images. Different contrasting methods and magnifications are the main reasons for the findings. The greatest similarity of the LC-OCT results was found with confocal reflectance microscopy imaging and compared here.

Conclusion: The LC-OCT method as a non-invasive in vivo method was found to be well suited to visualize signs of aging of collagen in the papillary dermis. The results observed complement the literature documented collagen imaging and measurement. Advantageous is the fast image acquisition and the spatial resolution of about 1 µm, which is excellent for confocal systems. However, the collagen of the deeper reticular dermis cannot be evaluated with this method, since the images clearly lose sharpness and contrast from a depth of about 200 µm on. For an evaluation of the collagen in the papillary dermis, the method is well suited.

Keywords: Confocal Raman Spectroscopy; LC-OCT; collagen content; photo-aged skin; dermis; in vivo quantification

Introduction

While the epidermis is regenerative and forms the main skin barrier to water loss and the invasion of chemicals, particles and bacteria, the dermis is the layer that gives the strength to the human skin. It consists of two layers: uppermost is the papillary dermis which is in direct contact to the dermo-epidermal junction (DEJ) and contains loop like capillaries that nourish the epidermis. The deeper reticular dermis, houses larger blood vessels and large fiber bundles of structural proteins. With a share of 90 % and about 75 % of the dermal dry weight, collagen is the most abundant protein in the dermis [1-3]. The highly structured collagen bundles are largely responsible for the skin's resistance and elasticity [4].

With increasing age, signs of intrinsic ageing appear. Less collagen is produced [5], collagen degrades and fibers become cross-linked. Skin elasticity decreases and wrinkles form. A major cause of extrinsic aging, collagen degradation and loss, is long term sun exposure and to a lower degree air borne pollution [6]. While UV-B light mainly acts in the epidermis, UV-A rays and high energy visible light penetrate into the dermis and generate free radicals [7]. The skin reacts e.g. with the formation of metalloproteinases, enzymes that cut collagen fibers, and the collagen degrades [8].

Loss of skin elasticity and the resulting wrinkle formation are one of the main causes of loss of visual beauty and attractiveness [9]. Therefore the cosmetic industry is highly interested in a better understanding of dermal ageing and cosmetic treatments that prevent it or improve the skin appearance.

Representation of the collagen network with invasive methods using punch biopsies and histological staining is established for a long time [10-12]. However, in cosmetic research, noninvasive methods should be used whenever possible. A number of imaging techniques that can be applied directly to living skin are available. These include skin ultrasound imaging as first described by deRigal [13], two photon microscopy [14-15], confocal reflectance microscopy [16-17], and Line Confocal Optical Coherence Tomography (LC-OCT) [18]. Furthermore, there exists an indirect method to measure collagen density in vivo and depth-resolved with confocal Raman spectroscopy by quantification of the dermal water content [19]. Due to different resolution and different measurement principles, the age-related degradation of collagen in the papillary dermis presents differently with the different methods. The aim of this work was to compare the age-related collagen degradation as presented by LC-OCT with the methods reported in the literature. In a clinical study, images of papillary collagen were acquired in subjects of different ages using LC-OCT. The suitability of LC-OCT for in vivo collagen imaging, its advantages and disadvantages are discussed based on our own study, and the current literature on existing techniques.

Materials and Methods.

Participants

This non-medical study on healthy human subjects was executed according to the principle requirements of the declaration of Helsinki and according to the main principles of Good Clinical Practice (GCP). Volunteers were informed orally and written on the study details

including potential risks and inconveniences. They provided their written consent before they were included in the study. Forty-four healthy subjects, 7-70 years old with fair skin and no signs of pronounced photoaging were included in the study.

Test Schedule and Methods

The study was performed on one study day. Images of papillary collagen were obtained with LC-OCT. Images were taken on the inner, sunlight-protected forearm and on the sun-exposed dorsal forearm respectively.

Equipment

LC-OCT 800 3D (DAMAE Medical, Paris, France): Line-Field Confocal Optical Coherence Tomography is a technique that combines the principles of time-domain optical coherence tomography and confocal microscopy. With this technique skin can be imaged in vivo in its native state without further preparation. This method enables an in vivo mapping of the skin through measuring the echo-time delay and amplitude of light backscattered from cutaneous microstructures through low-coherence interferometry associated with confocal spatial filtering. The different layers of the epidermis, the dermis and the dermal-epidermal junction can be clearly imaged using a live vertical, live horizontal and 3D mode. For the 3D mode a 3D stack of size of 1.2 mm x 0.5 mm x 0.5 mm is generated with a resolution of 1.3 µm x 1.3 µm x 1.1 µm.

Evaluation

The optical attenuation is calculated as the slope of the average logarithmic values of the LC-OCT data in z direction between specified depths. We chose the depth between 140 and 170 µm from the skin surface to calculate the optical attenuation in the dermis (Figure 1).

To adjust for varying z-positions of the skin surface, the whole LC-OCT stack is divided into x-y-windows (100x100pixel). From each block, the z-profile is calculated by averaging the logarithmic values in each x-y plane. These 60 z-profiles are aligned and averaged for the combined z-profile. From the resulting average profile the optical attenuation is calculated as the difference between the values at depth 140 and 170 µm.

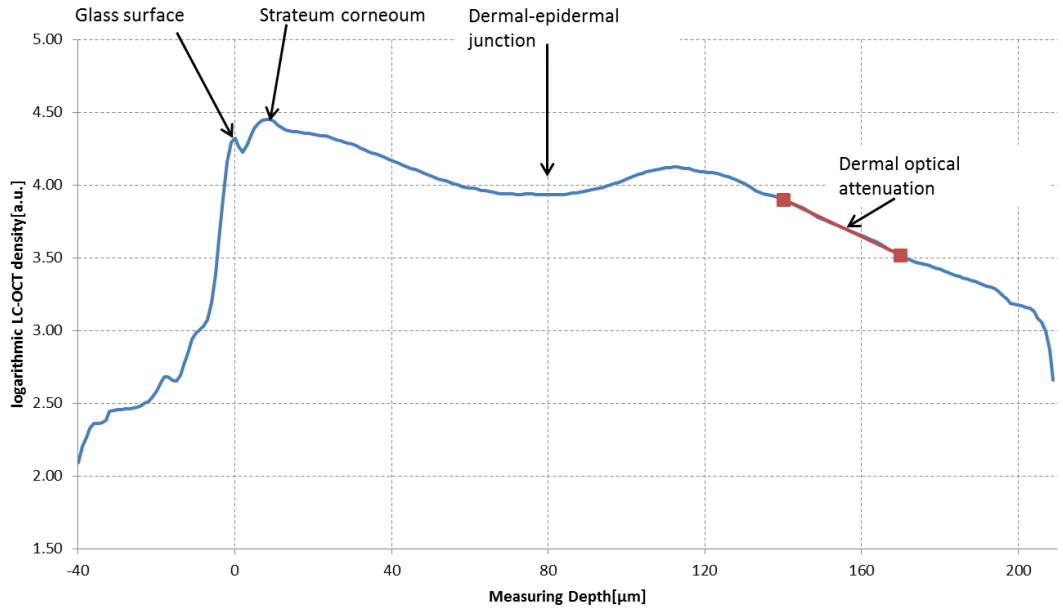


Figure 1: Example showing the calculation of the dermal optical attenuation.

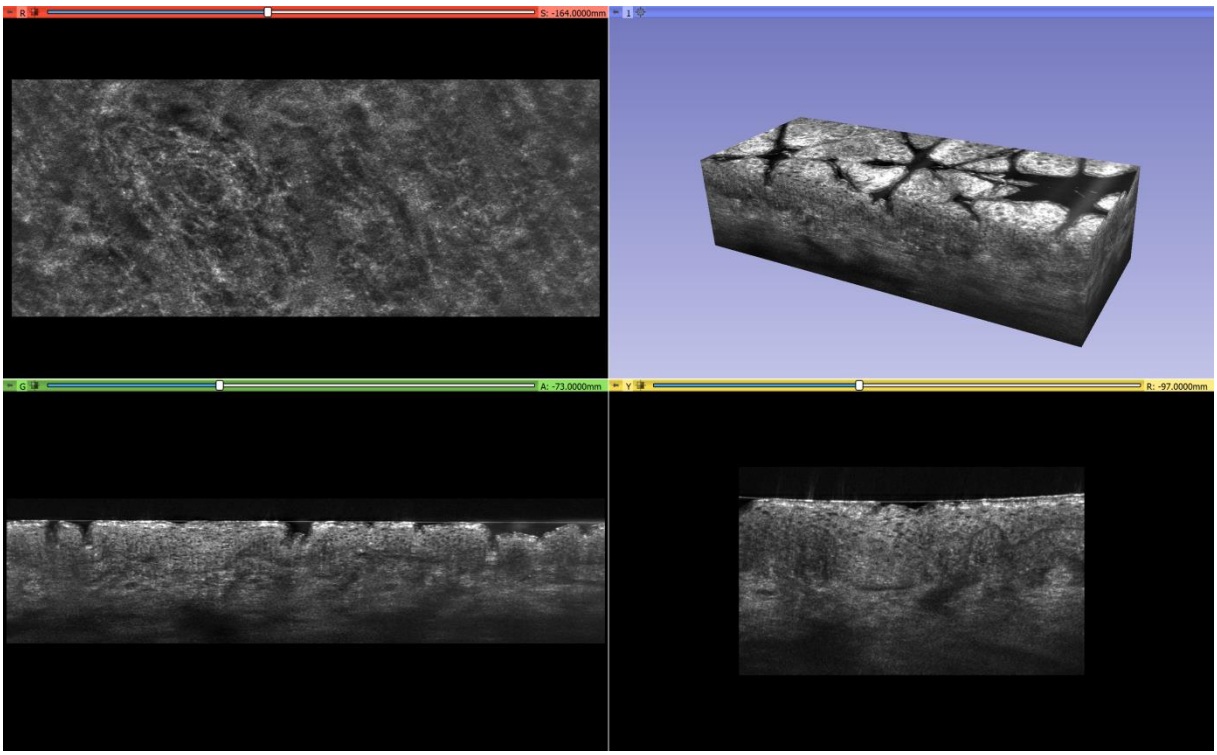


Figure 2: Example LC-OCT Stack of dorsal arm of 20 year old female

Raman Spectroscopy

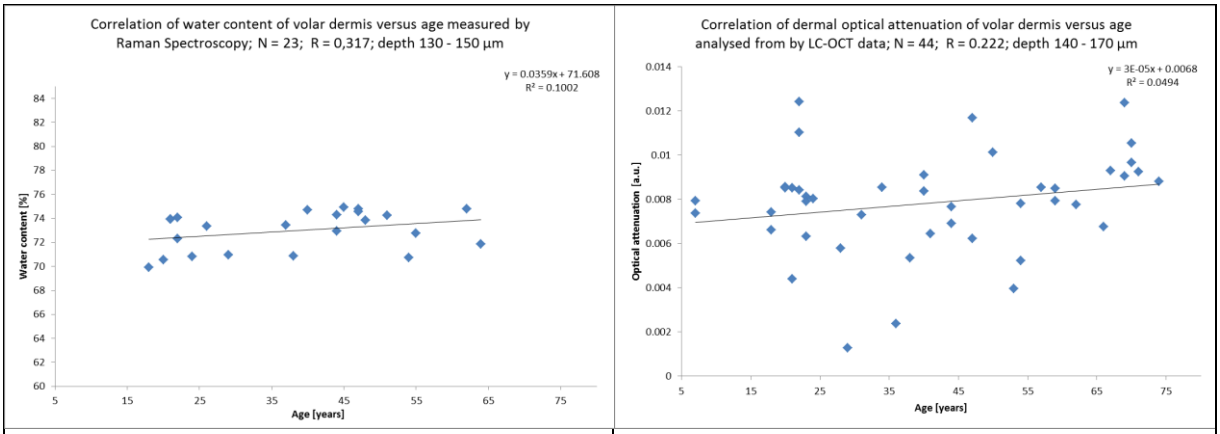
Data from Confocal Raman microspectroscopy (CRM) are taken from a previous publication [19] for comparison. The Raman instrument used was a gen2-SCA Ultimate (RiverD International B. V.). It is specifically designed for in vivo skin analysis. Water concentration profiles were taken from 100 to 150 ± 5 µm depth with steps of 5 µm in the dermis.

Correlation analysis

Correlations between optical attenuation and age were evaluated. A good correlation of data was assumed for Pearson's r = 0.5 or higher.

Results.

To investigate and confirm the effects of aging and chronic exposure to sunlight on the collagen content in the volar and dorsal dermis, correlation of the dermal optical attenuation as a measure of collagen content was performed for both sides (volar and dorsal) of the forearm. For comparison the effects of aging and chronic sun exposure on the water content measured by confocal Raman Spectroscopy on the volar and dorsal dermis, were studies by correlation of water content with age.

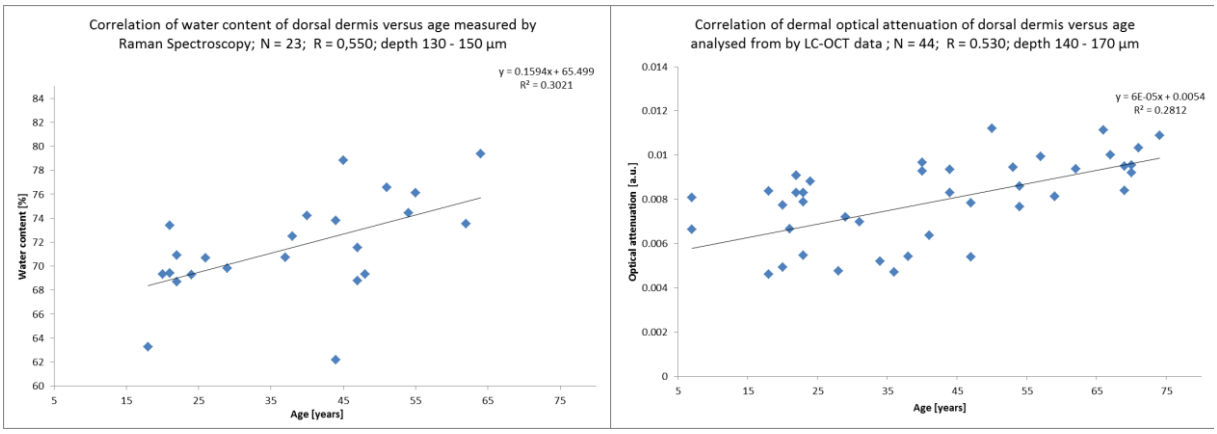


(a) (b)

Figure 3: a) Correlation of water content of volar dermis at a depth of 130 to 150 µm vs age for all valid subjects as measured by Raman Spectroscopy, (n = 23), Pearson's r = 0.317. b) Correlation of dermal optical attenuation of volar dermis at a depth of 140-170 µm vs age for all valid subjects as measured by LC-OCT (n = 44), Pearson's r = 0.222.

Correlation of optical attenuation and dermal water in the volar dermis with age

A weak positive correlation (Pearson's r = 0.222) of the dermal optical attenuation of volar dermis with increased aging was observed when measured at a skin depth of 140-170 µm (Figure 3b). Also a weak positive correlation (Pearson's r = 0.317) of the water content of volar dermis with increased aging was observed when measured the water contant at a skin depth of 130-150 µm (Figure 3a).



(a) (b)

Figure 4: a) Correlation of water content of dorsal dermis at a depth of 130 to 150 µm vs age for all valid subjects as measured by Raman Spectroscopy, (n = 23), Pearson's r = 0.550. B, Correlation of dermal optical attenuation of dorsal dermis at a depth of 140-170 µm vs age for all valid subjects as measured by LC-OCT (n = 44), Pearson's r = 0.530.

Correlation of optical attenuation and dermal water in the dorsal dermis with age

The correlation between dermal optical attenuation of the dorsal dermis and age was much more pronounced (Pearson's r = 0.530) (Figure 4b). Also the correlation between water content of the dorsal dermis and age was much more pronounced (Pearson's r = 0.550) (Figure 4a).

As the volar forearm was chosen as the sun protected not photodamaged test area, this result was confirming the expectation. Apparently the collagen content was decreased and the protein (mainly collagen) had been replaced by water.

Discussion.

In recent decades in vivo imaging methods for visualizing the connective tissue of the human dermis have improved significantly. While in the last decade of the 20th century in vivo imaging could reveal depletion of collagen due to aging by 25 MHz ultrasound as a sub-epidermal non echogenic band with an axial resolution of only 80 µm [13], LC-OCT now allows the assessment of dermal structures with a resolution of approximately 1 µm [18]. As in ultrasound measurements the contrasting dermal structure is mainly the collagen network, though LC-OCT is not an acoustic but an optical method, based on laser light at 800 nm.

The strongly improved technology allows quantification of the collagen at the border of papillary and reticular dermis at a depth of approximately 120 µm where the uppermost thicker bundles of collagen fibers occur, which are formed at infancy age [20]. This collagen

structures form a shoulder in the gray scale intensity curve [20] referred to here as optical attenuation curve.

We have shown for the first time, that there is a positive age correlation with the optical attenuation in this dermal region, which is different for intrinsically aged skin and photo-aged skin. This can be concluded from the fact that the correlation was more pronounced on the sunlight exposed dorsal forearm (Figure 4b) compared to the photo-protected volar forearm (Figure 3b).

This means a slight depletion of collagen with ageing and a more pronounced depletion with photo-ageing was observed, as the shoulder in the curve formed from the collagen bundles was slightly diminished on sun protected skin and clearly diminished on sun exposed skin.

The dermis is mainly composed of water and collagen [19]. It is a good support for the LC-OCT results that the correlation curves of optical attenuation fit very well to the Raman water measurements in the same dermal layer. The water measurements are the counterpart of the optical attenuation measurements, as an increase of dermal water content correlates with a decrease in collagen [19] (Figures 3a and 4a).

Conclusion.

Optical attenuation measurements were found to be a promising technology to measure non-invasively and in vivo age dependent depletion of fiber network in the upper human dermis. Further investigations, when supported by Raman water measurements, will prove that this method is suitable to measure anti-ageing/anti photo-ageing effects of cosmetic treatments on the level of the dermis.

Conflict of Interest Statement. NONE.

References.

1. Uitto J. Connective tissue biochemistry of the aging dermis. Age-related alterations in collagen and elastin. Dermatol Clin. 1986;4(3):433-446.
2. Oikarinen A. Aging of the skin connective tissue: how to measure the biochemical and mechanical properties of aging dermis. Photodermatol Photoimmunol Photomed. 1994;10(2):47-52.
3. Caspers P. In vivo skin characterization by confocal Raman microspectroscopy. Thesis, Erasmus Universiteit Rotterdam; 2003. Article No: ISBN978-90-6734-366-4.

4. Fritsch P. Dermatologie & Venerologie für das Studium, 1st edn. Heidelberg: Springer publisher; 2009.
5. Varani, J., Dame, M. K., Rittie, L., Fligiel, S. E., Kang, S., Fisher, G. J., & Voorhees, J. J. (2006). Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. The American journal of pathology, 168(6), 1861-1868.
6. Quan T, Fisher G. Role of age-associated alterations of the dermal extracellular matrix microenvironment in human skin aging: a mini-review. Gerontology. 2015;61(5):427-434.
7. Zastrow, L., Groth, N., Klein, F., Kockott, D., Lademann, J., Renneberg, R., & Ferrero, L. (2009). The missing link–light-induced (280–1,600 nm) free radical formation in human skin. Skin pharmacology and physiology, 22(1), 31-44.
8. Rittié L, Fisher G. Natural and sun-induced aging of human skin. Cold Spring Harb Perspect Med. 2015;5(1):a015370.
9. Bielfeldt, S., Henss, R., Koop, U., Degwert, J., Heinrich, U., Jassoy, C., ... & Blume, G. (2013). Internet- based lay person rating of facial photographs to assess effects of a cleansing product and a decent cosmetic foundation on the attractiveness of female faces. International journal of cosmetic science, 35(1), 94-98.
10. Fligiel, S. E., Varani, J., Datta, S. C., Kang, S., Fisher, G. J., & Voorhees, J. J. (2003). Collagen degradation in aged/photodamaged skin in vivo and after exposure to matrix metalloproteinase-1 in vitro. Journal of Investigative Dermatology, 120(5), 842-848.
11. Lavker, R. M. (1979). Structural alterations in exposed and unexposed aged skin. Journal of Investigative Dermatology, 73(1), 59-66.
12. Griffiths, C., Russman, A. N., Majmudar, G., Singer, R. S., Hamilton, T. A., & Voorhees, J. J. (1993). Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). New England Journal of Medicine, 329(8), 530-535.
13. De Rigal, J., Escoffier, C., Querleux, B., Faivre, B., Agache, P., & Lévêque, J. L. (1989). Assessment of aging of the human skin by in vivo ultrasonic imaging. Journal of investigative dermatology, 93(5), 621-625.
14. Czekalla, C., Schönborn, K. H., Döge, N., Jung, S., Darvin, M. E., Lademann, J., & Meinke, M. C. (2017). Body regions have an impact on the collagen/elastin index of the skin measured by non- invasive in vivo vertical two- photon microscopy. Experimental Dermatology, 26(9), 822-824.

15. Czekalla, C., Schönborn, K. H., Döge, N., Jung, S., Darvin, M. E., Lademann, J., & Meinke, M. C. (2017). Impact of body site, age, and gender on the collagen/elastin index by noninvasive in vivo vertical two-photon microscopy. Skin Pharmacology and Physiology, 30(5), 260-267.
16. Wurm, E. M. T., Longo, C., Curchin, C., Soyer, H. P., Prow, T. W., & Pellacani, G. (2012). In vivo assessment of chronological ageing and photoageing in forearm skin using reflectance confocal microscopy. British Journal of Dermatology, 167(2), 270-279.
17. Longo, C., Casari, A., De Pace, B., Simonazzi, S., Mazzaglia, G., & Pellacani, G. (2013). Proposal for an in vivo histopathologic scoring system for skin aging by means of confocal microscopy. Skin Research and Technology, 19(1), e167-e173.
18. Ogien, J., Levecq, O., Azimani, H., & Dubois, A. (2020). Dual-mode line-field confocal optical coherence tomography for ultrahigh-resolution vertical and horizontal section imaging of human skin in vivo. Biomedical optics express, 11(3), 1327-1335.
19. Kourbaj, G., Bielfeldt, S., Seise, M., & Wilhelm, K. P. (2021). Measurement of dermal water content by confocal RAMAN spectroscopy to investigate intrinsic aging and photoaging of human skin in vivo. Skin Research and Technology, 27(3), 404-413.
20. Stamatas, G. N., Nikolovski, J., Luedtke, M. A., Kollias, N., & Wiegand, B. C. (2010). Infant skin microstructure assessed in vivo differs from adult skin in organization and at the cellular level. Pediatric dermatology, 27(2), 125-131.