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Photoaging related protein fiber depletion in the human dermis is accompanied by a marked loss of skin elasticity and increase of water in the tissue

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Introduction

The connective tissue fibers collagen and elastin present in the dermis are of particular interest in the field of anti-aging research, as they provide stability and firmness to the skin [1]. In young subjects, these are present in optimal concentration and structure. However, with increasing age, less collagen is synthesized in the dermis and existing collagen is fragmented, resulting in a decrease in collagen density [2]. With up to 80%, chronic sun exposure causes the largest proportion of skin aging; the so-called photoaging [3]. Long-wave UVA rays penetrate particularly deep into the dermis and lead to an increased production of matrix metalloproteinases (MMP's), enzymes that cleave collagen fibers [4, 5]. Consequently, the collagen network becomes disorganized, degraded, and loses density. Abnormal and degraded material of elastotic fibers accumulates in the dermis [6]. Clearly visible skin wrinkles form by intrinsic aging. On sun exposed skin areas, these wrinkles develop earlier and become much deeper. Until now, conventional in vivo imaging techniques have had limited ability to quantify connective tissue structures in the dermis due to low optical resolution, especially in deep areas of the skin [7]. Line-field confocal optical coherence tomography (LC-OCT) is a new, non-invasive imaging technique that allows in vivo visualization of the skin in real time [8]. It has a very high optical resolution of about 1 µm in all spatial directions and a quite good penetration depth of more than 400 µm, which allows imaging of connective tissue structures down to the upper reticular dermis [7, 9].

In this work, we conducted in vivo measurements of optical dermal tissue density to assess fiber density in the human photo-exposed and photo-protected skin and compared the results with in vivo skin elasticity and dermal water content measurements. Correlations between the assessed parameters, as well as their relationships with age, were calculated.

Materials & Methods

Twelve female subjects with fair skin were included in the study, and divided equally into a younger (18-24 years; mean 20.8 ± 1.7) and an older (60-69 years; mean 62.5 ± 2.3) age group. Images were taken on the volar sun-protected and the dorsal sun-exposed forearm. Skin elasticity was assessed with a commercial suction device (parameter R7; Ur/Uf). Confocal Raman Microspectroscopy (CRM) was used to measure water content in the upper reticular dermis (see Figure 1). Water concentration profiles were taken from 130 to 150 µm depth with steps of 5 µm in the dermis. This depth was chosen based on existing literature [10]. Between 8 and 10 water profiles were recorded per subject and test area with an integration time of 2 seconds per measurement.

Line-Field Confocal Optical Coherence Tomography (LC-OCT) was used to measure fiber density in the upper dermis. Three 3D images with a depth of 300 µm were generated per test area. In the 3D images, the fiber density was determined using the optical attenuation (OA) method. The depth of the OA was determined individually for each subject so that the start of the measurement was 10 µm below the dermoepidermal junction (DEJ). From this point, the OA was measured down to a depth of 60 µm below DEJ.

Image analysis was used to assess OA in the complete measured volume. To adjust for varying z-positions of the skin surface, the whole LC-OCT 3D-block was divided into x-y-windows. From each sub-block, a z-profile of optical density was calculated by averaging the logarithmic values in each x-y plane (see Figure 2).

Figure 1: Exemplary measurement of the volar forearm of a subject using the CRM.

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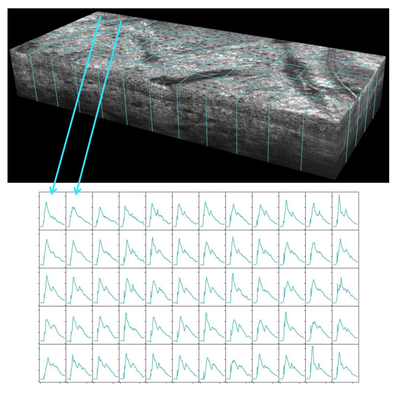


Figure 2: LC-OCT 3D-block divided into 60 x-y-windows (100 pixel x 100 pixel). From each sub-block, a z-profile of optical density was calculated by averaging the logarithmic values in each x-y plane.

Conclusions

The loss of fiber density in photoaged dermis could successfully be quantified in vivo by dermal LC-OCT measurements, as well as water and skin elasticity assessments.

These non-invasive in vivo methods confirm the existing ex vivo data and directly quantify the in vivo composition of fibers and fluids in aged and photoaged human dermis, unbiased by skin preparation.

We conclude that all three methods are promising candidates for measuring the effects of topical or systemic treatments to improve signs of ageing and photoaging in the human dermis.

Results & Discussion

During the measurement with CRM, two subjects dropped out of the study as no normal Raman spectra were measurable. The skin of both subjects was pre-tanned. It is known that melanin in the skin can strongly interfere with Raman measurements due to its fluorescence [10]. This is therefore assumed to be the cause of the unusable spectra.

While optical density in the upper dermis slightly decreased with increasing age in photo-protected skin, a much stronger depletion was observed on the dorsal photo-exposed skin. In line with this depletion, the skin elasticity clearly dropped with age. Again, on photo-exposed dorsal forearm skin, the effect was more pronounced than on the sun-protected volar forearm. The water content of the upper dermis continuously increased with aging, and was even more pronounced with photoaging. There was a clear correlation between the decrease of optical attenuation as measured with LC-OCT in photoaged dermis and the increase of water content in this tissue.

Table I: Mean water content, optical attenuation (OA) and skin elasticity (R7) for A, dorsal forearm and B, volar forearm.

Table I A Dorsal forearm	Water content [%]	Optical attenuation (OA)	Skin elasticity (R7)
N	10	10	10
Mean	70.6639	0.0100	0.4299
SD	1.9678	0.0025	0.1415
CI	1.4077	0.0018	0.1012

Table I B Volar forearm	Water content [%]	Optical attenuation (OA)	Skin elasticity (R7)
N	10	10	10
Mean	72.4990	0.0092	0.5545
SD	2.3779	0.0011	0.1489
CI	1.7011	0.0008	0.1065

Figure 3: A clearly positive correlation was observed between water content and OA at the dorsal forearm (A) (R2=0.48) but no relevant relation was found on the volar forearm (B) (R2=0.16).

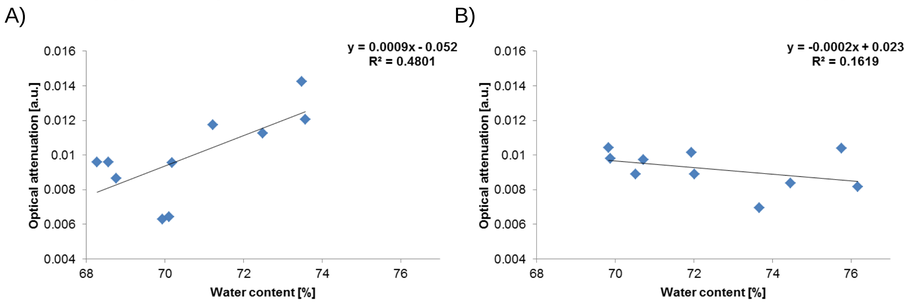
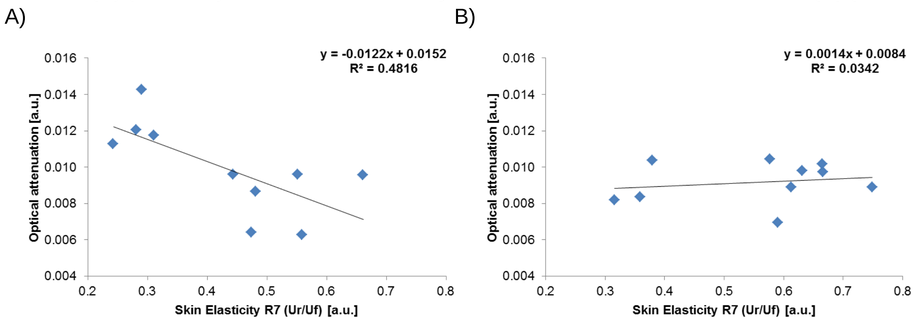


Figure 4: A clearly negative correlation was found between skin elasticity and OA at the dorsal forearm (A) (R2=0.48) and also for elasticity no correlation at the volar forearm (B) (R2=0.03).



Our results confirm that an increase of optical attenuation in the upper dermis can be interpreted as reduced reflectivity of the tissue due to a loss of fiber density. Although it is well known that elastotic material is deposited in photoaged dermis, this material seems to be much more loosely packed and less reflective than in the tissue of young and undamaged skin. We assume that the depletion of collagen is mainly responsible for the measured effects, as collagen is the most abundant protein in the dermis, responsible for approximately 90% of the layer's protein composition [11]. The correlation of the dermal water content with optical attenuation supports our hypothesis that the fiber material is less dense and partly replaced by water in photoaged dermis [10]. In addition, the marked loss of elasticity that we have measured fits well to the observed reduced fiber density and the replacement of the fibers by water.

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