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Research Article

Influence of the Biochemical Properties of the Human Skin Fibers on its Mechanical Properties *in Vivo* According to Age for Two Body Areas: the Forearm and the Thigh

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Abstract

Human skin has a stratified and very complex structure with a dense network of collagen and elastic fibers. The skin structure induces associated biochemical and mechanical properties, which are linked and influenced by the fiber's properties. These properties change with age and depend on the body area. In this study, we propose to evaluate the influence of the biomechanical properties of the human skin fibers on its mechanical properties *in vivo* according to age for two body areas: the forearm and the thigh using two experimental tests: the Diffuse Reflectance Spectroscopy (DRS) and the non-contact impact test. Experimental tests were performed for 42 female volunteers representing two age groups: [20-30] and [45-55] years old. The diffuse reflectance spectroscopy allows measuring the fluorescence intensity of the Advanced Glycation End Products (AGEs) which are markers of aging. Non-contact impact tests were conducted using the WaveSkin® device. This test generated a Rayleigh wave that spread in the skin. The speed of this wave was measured in seven directions and values of the Young's moduli are deduced. The results of this study show that the mechanical properties of the skin decrease with age due to the degradation of the biochemical properties of skin fibers. This degradation is caused by the accumulation of the AGEs in the skin with age. The intensity of aging effect depends on the body area and on the measurement direction. Aging effect is more important on the thigh and in direction perpendicular to the direction of skin tension i.e., direction of Langer.

Keywords: Human skin *in vivo*; Non-contact impact tests; Diffuse reflectance spectroscopy; AGEs; Skin tension

Introduction

Human skin has a stratified and very complex structure undergoing constant renewal. Each layer of the skin has different components. The dermis forms the thickest layer (from 50µm to 1,2mm) with a complex and a dense network of collagen and elastin fibers [1,2]. The various components of the skin have specific optical properties, which allows them to be distinguished. These properties also provide access to the composition of the tissue and its biochemical properties. The absorption and diffusion properties of the skin

depend on the scale of observation of these properties. These properties are not the same for the skin layers as for the elements it contains at the cellular level. The skin is known for its non-homogeneous behavior due to the large number of cellular-scale elements it contains. For this reason, it is often considered that the optical properties of skin layers are those of optically homogeneous elements [3]. The epidermis has an absorption coefficient, which varies between 35 and 66cm^{-1}, and a diffusion coefficient, which varies between 450 and 800cm^{-1} for a wavelength between 415 and 633nm. The dermis is made up of a dense network of collagen and elastin fibers. Accordingly, diffusion of the dermis is described as multiple diffusion of these fibers. It varies between 187 and 320cm^{-1} for a wavelength between 415 and 633nm. However, the absorption coefficient varies between 1.9 and 4.7cm^{-1} for a wavelength between 415 and 700nm [4].

Thanks to the optical properties of the skin (absorption, refraction and diffusion), which ensure its protective function against UV rays, and which provide information on the properties of its different layers, it is possible to characterize and identify the biochemical components of the skin, to quantify and locate them. The identification of these components makes it possible to understand their contribution to the structuring of the skin tissue, as well as the modifications they may undergo with age. Consequently, the modifications of the mechanical properties, which result from it. Optical techniques and measuring instruments have been developed for this such as Raman spectroscopy, diffuse reflectance, fluorescence [5], etc. In this study, we are interested in diffuse reflectance spectroscopy, which uses the scattering of light in the skin. It allows the analysis of backscattered light in a wide spectral range extending from 200 to 1000nm, and it provides information on fluorescence. This technique makes it possible to macroscopically characterize the tissues at a depth that varies from 2 to 5mm [5]. The skin has endogenous fluorescence due to the presence of fluorophore molecules such as aromatic amino acid rings or the presence of Advanced Glycosylation End-products (AGEs) [6,7]. These AGEs are markers of skin aging. Previous studies carried out on mouse skin and human dermis ex vivo [6,8,9] show, using the technique of diffuse reflectance spectroscopy, that the amplitude of the fluorescence of the advanced glycation end products increases with age. Interestingly, the accumulation of AGEs in human skin *in vivo*, related to age and body area, has not yet been addressed in the literature.

The present work proposes an experimental characterization of the biochemical and mechanical properties of the human skin *in vivo* for two body areas (the forearm and the thigh) with statistical analysis. The biochemical test is diffuse reflectance spectroscopy that allows measuring the fluorescence intensity of the AGEs. The mechanical experimental tests are non-contact impact tests performed to measure the Rayleigh wave propagation speed and to calculate the Young's modulus. These methods are described and detailed in the section 2. The experimental results are presented in section 3, discussed, and analyzed in section 4. We finally conclude on the results obtained.

Materials and Methods

Volunteers

The tests were performed on the right forearms (12 cm above the wrist) and the left thighs (12cm above the knee) of 42 Caucasian French women volunteers divided into two groups: a young group [20-30] years old (21 volunteers) and an elderly group [45-55] years old (21 volunteers). Volunteers were non-smokers, in good health and they had healthy skin in the zones studied of the forearm and thigh without scars and tattoos. The volunteers had: a Body Mass Index (BMI) between 18.5 and 27 kg/m^2, the cellulite index < 2 for the thigh, and the phototype between I and III.

The volunteers did not apply any cosmetic products on the body on the test day. After an acclimatization period of at least 10 minutes in an air-conditioned room (T = 21 ± 2 °C and H = 50 ± 10%), the tests were carried out in another room under the same conditions. The volunteer sat in an armchair (dentist type), her legs extended, uncrossed, and slightly bent, and her right arm resting on an armrest, the palm of her hand upwards. The volunteer was asked not to move for the duration of the measurements to ensure the homogeneity of the records and to reduce the level of noise in the data recorded as much as possible. All the volunteers participated after giving informed consent and all the procedures adhered to the latest revision of the Declaration of Helsinki.

Biochemical Properties of Skin: Diffuse Reflectance Spectroscopy (DRS)

In this study, we are interested to evaluate the biochemical modifications that collagen and elastin fibers can undergo with age due to the accumulation of AGEs in the fibers. In particular, the AGEs used as markers of skin aging studied here are Pepsin-Digestible Collagen Cross-Links (PDCCL), Collagenase-Digestible Collagen Cross-Links (CDCCL), and Elastin Cross-Links (ECL). We therefore use the fluorescence of the accumulation of these AGEs as a marker of the rate of aging and the rate of degradation of the fibers properties.

The study of the fluorescence of AGEs is carried out by diffuse reflectance spectroscopy, which is a quantitative method using the scattering of light in the skin. The spectrofluorometric used in this study is Jobin Yvon Fluorolog® (17-FRA-QOT1389G-FL3-LR) from HORIBA Scientific, France. It makes it possible to analyze backscattered light in a wide spectral range extending from 200 to 1000nm and to provide information on endogenous fluorescence *in vivo* on human skin. It is made up of three essential elements: An excitation source: 450 W continuous Xenon lamp

white light (ozone-free), a distribution system: multimode optical fiber, and a signal acquisition and processing system: the R928 photomultiplier. A computer and SynerJY software controls the whole system. *In vivo* diffuse reflectance spectroscopy is performed by placing the fiber optic probe in contact with the skin area of interest (Figure 1). The acquisition of fluorescence intensity spectra of the skin can be done by recording the excitation spectrum or by recording the emission spectrum. The excitation spectrum is made by fixing the fluorescence emission length and sweeping the excitation wavelength. Whereas for the emission spectrum, it is made by fixing the excitation wavelength and sweeping the fluorescence emission wavelength. In this study, the excitation spectrum is the chosen method to measure skin fluorescence *in vivo*. Indeed, the excitation spectra are like the absorption spectra and the bands tend to be narrower than in the acquisition of emissions. This facilitates the identification of individual fluorophores in a complex spectrum as shown by Stamatas et al. [6]. We referred to the literature to identify the emission and excitation parameters suitable for carrying out our measurements [6,9]. The parameters used are shown in Table 1.

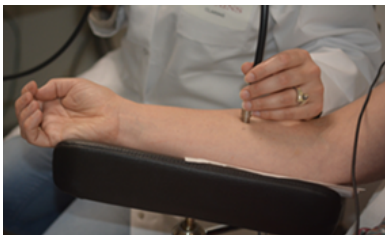

Figure 1: DRS measurement probe.

Fluorophore	$\lambda_{Excitation}$ (nm)	$\lambda_{Emission}$ (nm)
Pepsin-digestible collagen cross-links	335	360-600
Collagenase-digestible collagen cross-links	380	400-520
Elastin cross-links	260-470	500

Table 1: Acquisition parameters of the fluorescence spectra where λ Excitation and λ Emission are respectively the excitation and emission wavelengths.

Mechanical Properties of Skin: Non-Contact Impact Test

The mechanical properties of skin were determined by the non-contact impact test. This test was performed using the WaveSkin® device developed in the Laboratory of Tribology and Dynamics of Systems (LTDS, Lyon, France) and described in Ayadh et al. [10]. Its principle is to mechanically generate an air flow and apply it onto the skin surface, then to measure the resulting displacement of the skin using a laser profilometer (l = 7 mm, 800 sensors, wavelength = 405nm). The pressure applied generates the propagation of a Rayleigh wave in the skin. The propagation speed of the wave is calculated by the method described in Ayadh et al. [10] and synthetized here. The minima of the displacement curve for each sensor and the time at which they are reached are identified. Therefore, it is possible to calculate the propagation speed of wave V such that: $V = \Delta x / \Delta t$, with Δx being the distance between two sensors and Δt the time taken by the wave to propagate between the two sensors. Knowing the wave propagation speed in the skin, an approximation of the Young's modulus E of the skin can be assessed based on the following assumptions: the skin is assumed to be made of homogeneous, linear elastic and isotropic material. In this case, the propagation speed of wave V and Young's modulus are linked by the following relation [11].

$$V = (0{,}87+1{,}12\nu)/(1+\nu)\ \sqrt{(E/(2\rho(1+\nu)))} \qquad (1)$$

Where ρ is the volumetric mass density and ν is the Poisson's ratio of the skin.

Young's modulus E of the skin is deduced from formula (1) calculated with a Poisson's ratio taken as equal to 0.45 and with a density equal to 1.02 $g.cm^{-3}$ [12].

In this study, the non-contact impact tests were performed on the forearm and the thigh with an impact pressure of 2 bar and an impact time of 10ms. These tests were recorded in seven measurement directions: from 0° to 180° with steps of 30° (Figure 2a). The tests were repeated three times in each direction. (Figure 2b) shows typical 3D images of the skin surface obtained during a non-contact impact test carried out on the forearm of an elderly volunteer in the direction 90°.

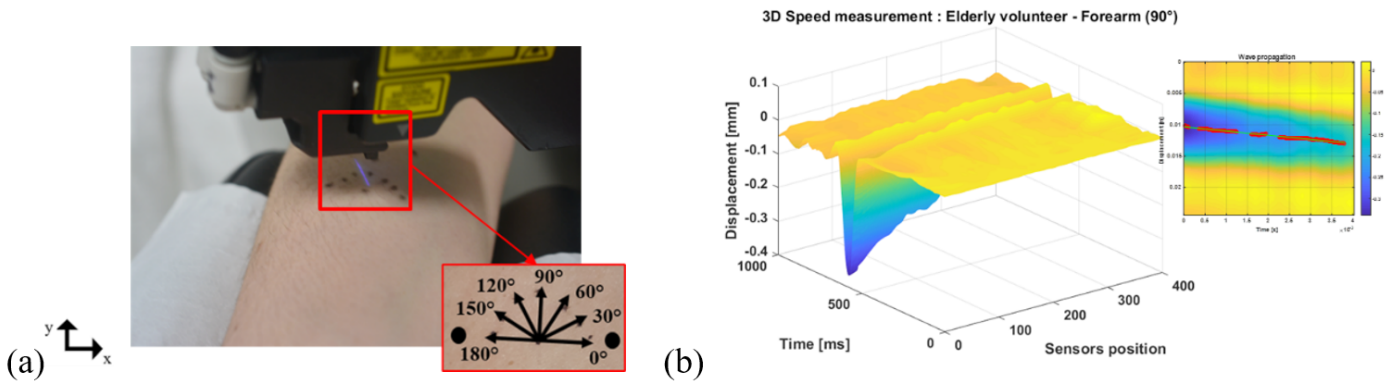

Figure 2a: Forearm under the WaveSkin® device. **Figure 2b:** Example of 3D speed measurement for the forearm of an elderly volunteer at 90°. The in-plane axis represents the time and the sensor positions of the profilometer laser, i.e., the spatial axis. Position 0 corresponds to the sensor closest to the load. The vertical axis corresponds to the amplitudes of the displacements of the skin surface.

Statistical Analysis

Statistical analysis was performed using the XLStat software (version 2019.4.2, Addinsoft, France). Shapiro-Wilk normality tests were performed. In this study, the data did not follow a normal distribution. Hence for these non-normal distributions, the Mann-Whitney non-parametric test was performed with a significance level of 5% to assess the significance of the differences observed between the two age groups (young and elderly) and the two body areas (forearm and thigh). To evaluate the links between the data, a correlation test was carried out using the Spearman correlation test. This test provides a correlation coefficient (r) that makes it possible to characterize linear and non-linear monotonic bonds. The Spearman coefficient (r) lies between -1 and 1: if $0.3 < r < 0.5$ (or $-0.5 < r < -0.3$) then the relationship is weak, if $0.5 < r < 0.8$ (or $-0.8 < r < -0.5$) then the relationship is of medium intensity, and if $r > 0.8$ (or $r < -0.8$) then the relation is strong. In this study, only coefficients of which the associated p-value is significant (< 5 %) are interpreted.

Results

Results of the Fluorescence Measurements of the Advanced Glycation End Products

The measurements carried out by diffuse reflectance spectroscopy give the fluorescence intensity of the accumulation of the three advanced glycation end products in which we are interested in this study: (PDCCL, CDCCL and ECL). Figure 3 presents an example of the acquisition of the excitation spectra of each of the AGEs for a young volunteer and for an elderly volunteer. It is the fluorescence intensity of these products according to the excitation wavelength. The higher the peak fluorescence intensity, the greater the amount of the AGEs present in the skin. In the analysis, only the peaks of the fluorescence intensity of the accumulation of the AGEs will be used. These peaks represent the maximum fluorescence of the AGEs. Figure 4 shows the average peak fluorescence intensity of the AGEs as a function of age and body area.

The results (Figure 4) show that the fluorescence intensity of the PDCCL increases significantly with age for the thigh ($I_{thigh, young}$ = 1.02 ± 0.40 µA, $I_{thigh, elderly}$ = 1.37 ± 0.51 µA). This intensity is significantly higher for the forearm ($I_{forearm, young}$ = 1.60 ± 0.46 µA, $I_{forearm, elderly}$ = 1.53 ± 0.49 µA) than that for thigh for the young group (p-value < 0.0001), while no difference is observed for the older group (p-value > 0.05) between both areas.

The fluorescence intensity of CDCCL ($I_{forearm, young}$ = 1.66 ± 0.37 µA, $I_{forearm, elderly}$ = 2.05 ± 0.54 µA, $I_{thigh, young}$ = 1.24 ± 0.41 µA, $I_{thigh, elderly}$ = 2.03 ± 0.66 µA) and ECL ($I_{forearm, young}$ = 1.30 ± 0.31 µA, $I_{forearm, elderly}$ = 1.64 ± 0.37 µA, $I_{thigh, young}$ = 1.03 ± 0.30 µA, $I_{thigh, elderly}$ = 1.71 ± 0.51 µA) increases significantly with age regardless of body area. The fluorescence intensity of the latter is significantly higher in the forearm for the young group (0.003 < p-value < 0.011), while no difference is observed for the elderly group (p-value > 0.05).

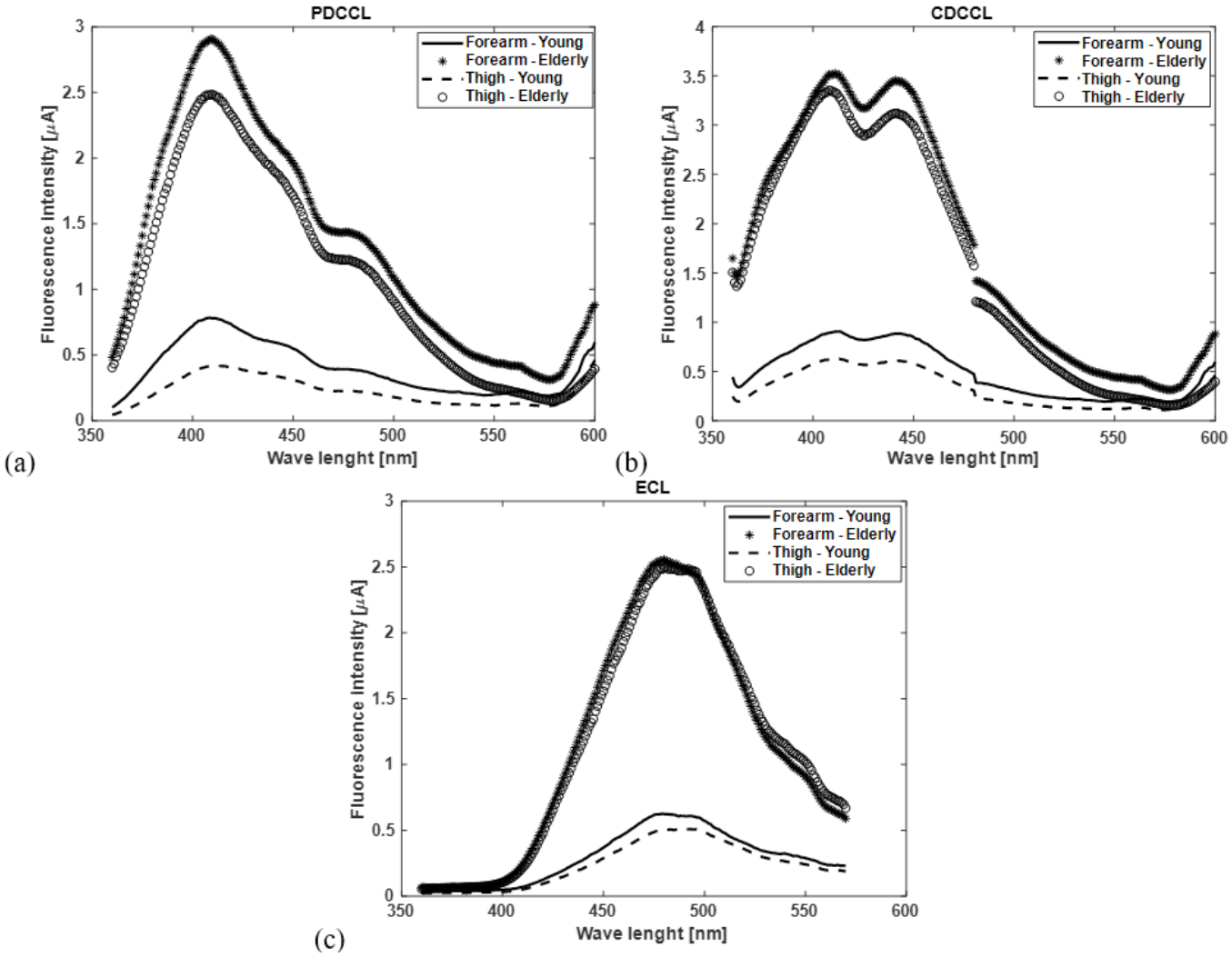

Figure 3: Example of excitation spectra of: **(a)** Pepsin-Digestible Collagen Cross-Links (PDCCL), **(b)** Collagenase-Digestible Collagen Cross-Links (CDCCL), and **(c)** Elastin Cross-Links (ECL) for a young volunteer and an elderly volunteer according to the body areas.

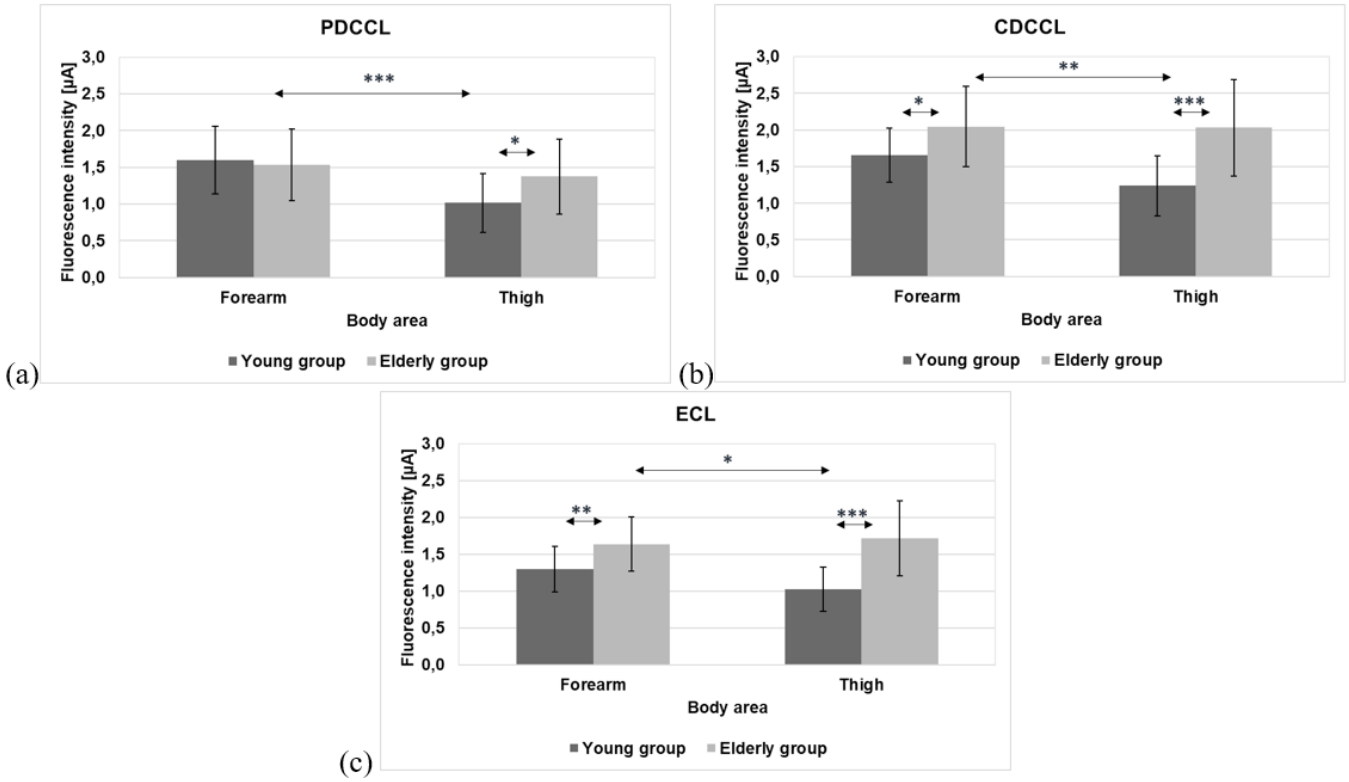

Figure 4: Fluorescence intensity peaks of accumulation of the AGEs (PDCCL, CDCCL and ECL) measured by diffuse reflectance spectroscopy according to age groups and body areas (* for 0.01 < p-value < 0.05, ** for 0.001 < p-value < 0.01, *** for p-value < 0.0001, and -- for p-value > 0.05).

Results of Non-Contact Impact Tests: Young's Modulus

The non-contact impact test results are presented in Table 2 as calculated Young's moduli in measurement directions (from 0° to180° with a step of 30°), age groups (young and elderly), and body areas (forearm and thigh).

Table 2 show that, irrespective of the body area, the Young's moduli in the skin of the young group ($3.54 < E_{Forearm} < 6.79$ kPa, $4.74 < E_{Thigh} < 6.51$ kPa) are significantly higher than those of the elderly group ($2.02 < E_{Forearm} < 5.08$ kPa, $3.12 < E_{Thigh} < 5.98$ kPa). We noted that the Young's moduli decreases with age for both body areas. However, it does not decrease with the same intensity in all directions. The decrease in directions 0°, 30°, 120°, 150° and 180° for the forearm and in directions 60°, 90° and 120° for the thigh is greater than in directions 60° and 90° for the forearm and 0°, 30°, 150° and 180° for the thigh. The Young's modulus remains high in these directions. These results show also that the skin of the forearm and the skin of the thigh do not have the same elastic mechanical properties or the same anisotropic behavior for either young or elderly volunteers. The aging effect depends on the body area and on the measurement direction.

	Forearm			Thigh		
E [kPa]	**Young**	**Elderly**	**p-value**	**Young**	**Eldely**	**p-value**
$E_{0°}$	3.95 ± 0.58	2.46 ± 1.71	***	6.51 ± 1.15	5.98 ± 1.99	0.227
$E_{30°}$	4.61 ± 0.91	3.23 ± 1.14	***	6.41 ± 1.54	5.47 ± 2.03	0.05
$E_{60°}$	6.79 ± 1.54	5.08 ± 2.12	**	5.24 ± 1.21	4.28 ± 3.15	***
$E_{90°}$	6.01 ± 1.61	4.52 ± 1.51	**	4.74 ± 0.62	3.12 ± 0.71	***
$E_{120°}$	4.23 ± 0.75	2.67 ± 1.11	***	4.80 ± 0.59	3.22 ± 0.72	***
$E_{150°}$	3.54 ± 0.48	2.02 ± 0.70	***	5.23 ± 0.54	4.35 ± 1.26	**
$E_{180°}$	3.74 ± 0.86	2.15 ± 0.74	***	6.04 ± 1.06	5.82 ± 2.01	0.821

Table 2: Young's modulus of the skin, (*for 0.01 < p-value < 0.05, **for 0.001 < p-value < 0.01, *** for p-value < 0.0001).

Results of Correlation Test

AGEs fluorescence shows positive correlations with age: correlation coefficient $r_{ECL} = 0.39$ in the forearm, and $r_{CDCCL} = 0.54$ and $r_{ECL} = 0.64$ in the thigh (Figure 5). This shows that elastin fibers and collagen fibers undergo age-related alterations related to the accumulation of the AGEs, particularly the accumulation of CDCCL and ECL. These alterations occur slightly differently in the forearm than in the thigh. While for the PDCCL, it is more present in the thigh and remains unchanged with age in the forearm. These alterations occur in both areas of the body, they seem to be more important in the thigh. Non-contact impact test results negatively correlate with age ($-0.73 < r_{Spearman} < -0.52$ for the forearm, $-0.84 < r_{Spearman} < -0.46$ for the thigh) (Figure 5). These measurements show that the elasticity of the skin decreases significantly with age regardless of the body area.

The correlations show that the elasticity of the skin decreases when the AGEs quantity increases ($-0.48 < r_{Spearman} < -0.31$ for the forearm, $-0.55 < r_{Spearman} < -0.35$ for the thigh). These results of mechanical properties are consistent with the results of the biochemical characterization of the skin. The biochemical degradation of fibers with age results in the degradation of the mechanical properties of the skin.

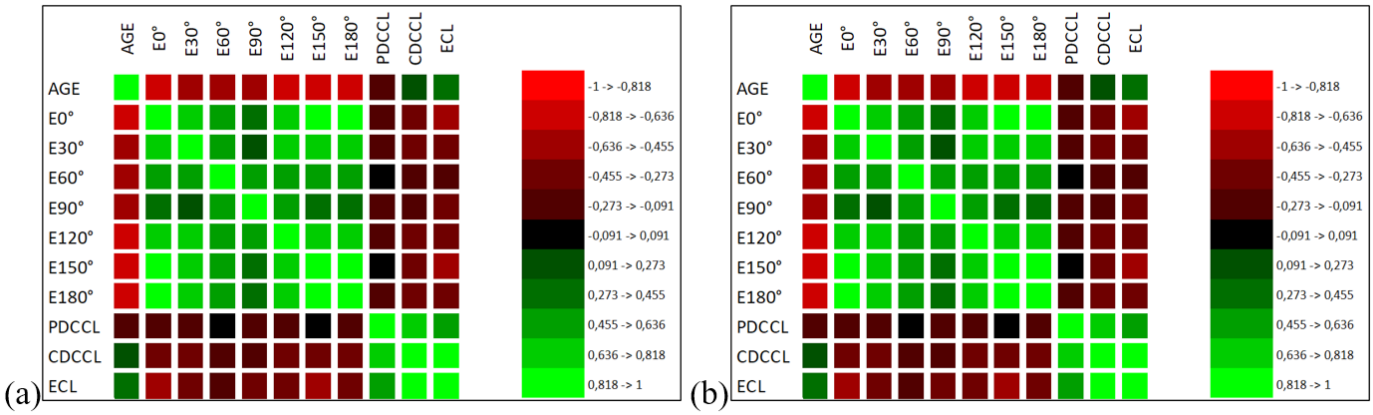

Figure 5: Correlation matrix for (a) the forearm and (b) the thigh.

Discussion and Conclusion

In this study, we were interested in two body areas: the forearm and the thigh, to characterize the mechanical and biochemical properties of the human skin *in vivo* by experimental tests, which are the diffuse reflectance spectroscopy, and the non-contact impact tests.

The results of diffuse reflectance spectroscopy show that the advanced glycation products increase in the extracellular matrix of the dermis with age, and they accumulate on collagen and elastic bonds. This leads to the modification of the mechanical properties of the fibers. These results are consistent with the *in vitro* studies available in the literature [6-9], [13]. The collagen and elastic fibers affected by glycation undergo a modification of their mechanical properties, which results in the stiffening of the fibers due to the presence of the AGEs on their bonds. In this study, the collagen and elastic fibers seem to be altered by this phenomenon. Particularly, the fluorescence intensity of CDCCL ($I_{forearm,\ young}$ = 1.66 ± 0.37 µA, $I_{forearm,\ elderly}$ = 2.05 ± 0.54 µA, $I_{thigh,\ young}$ = 1.24 ± 0.41 µA, $I_{thigh,\ elderly}$ = 2.03 ± 0.66 µA) and ECL ($I_{forearm,\ young}$ = 1.30 ± 0.31 µA, $I_{forearm,\ elderly}$ = 1.64 ± 0.37 µA, $I_{thigh,\ young}$ = 1.03 ± 0.30 µA, $I_{thigh,\ elderly}$ = 1.71 ± 0.51 µA). The AGEs are present in the skin of the aged group. These results show also that the skin on the thigh seems to be more impacted by biochemical changes than the skin on the forearm.

The results of the mechanical tests (non-contact impact tests) show that the elasticity of the skin, in both the forearm and the thigh, decreases with age. This result and the calculated Young's moduli (from 2.02 ± 0.70 to 6.79 ± 1.54 kPa) are consistent with the results and the Young's moduli obtained in the literature by other mechanical tests such as indentation or wave propagation tests [14-18]. In addition, the results showed that the elastic properties of the skin depend strongly on the direction of measurements and on the area of the body. The decrease of the skin elasticity in directions 0°, 30°, 120°, 150° and 180° for the forearm and in directions 60°, 90° and 120° for the thigh is greater than in directions 60° and 90° for the forearm and 0°, 30°, 150° and 180° for the thigh. The Young's modulus remains high in these directions. In the previous study [10], the authors show that theses directions correspond with two families of lines that are perpendicular one to the other. The first directions presented are the directions of skin tension (direction of Langer lines) and the second directions presented are the directions perpendicular to the directions of skin tension. So, the directions of skin tension for the forearm are 60° and 90°, and for the thigh they are 0°, 30°, 150° and 180°. The response of the skin to the external load applied by the non-contact impact test appears to depend strongly on the directions of the skin lines. These observations denote an anisotropic behavior of the skin. This result is coherent with the literature [1,2,10,19].

The aging effect is very remarkable on the biochemical and mechanical properties of the skin. It depends on the body areas and the direction of measurement. The influence of the biochemical properties on the mechanical properties of skin depends also on body areas and measurement directions. For the forearm, this influence is more important in the directions 0°, 30°, 120°, 150° and 180° (which are the directions perpendicular to the directions of the skin tension of the forearm) than the directions 60° and 90° (which are directions of skin tension of the forearm) [10]. For the thigh, the influence of biochemical properties is more important in the directions from 60° to 120° (which are the directions perpendicular to the directions of the skin tension of the thigh) than the directions 0°, 30°, 150° and 180° (which are the directions of skin tension of the thigh) [10,19]. These results are confirmed by the correlation tests that show the link between the mechanical and the biochemical properties of the skin ($-0.48 < r_{Spearman} < -0.31$ for the forearm, $-0.55 < r_{Spearman} < -0.35$ for the thigh). The accumulation of AGEs on the fibers network with age causes the stiffening of the elastic and collagen fibers of the dermis. This change in fiber properties contributes to the loss of skin elasticity with age.

This study shows that the degradation of the biochemical properties of the collagen and elastic fibers contributes greatly to the alteration of the mechanical properties of the whole skin. The accumulation of the advanced glycation ends products on the fibers network with age amplifies this degradation of skin properties and accelerates its aging. The aging effect depends on the body areas and on the measurement directions. It is less marked in directions of skin tension than the other directions whatever the body area. This unequal aging effect in all directions of the skin is at the origin of its anisotropic behaviour, which becomes more marked with age.

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