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Imagistic Noninvasive Assessment of Skin Ageing and Anti-Ageing Therapies

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1. Introduction

The significant increase in life expectancy and the process of population ageing are aspects that generate important social and economic changes and influence the health and research policies throughout the world. The ageing phenomenon represents a natural, slow and irreversible process, which affects all body tissues, being determined by a multitude of factors that contribute in different proportions to the characteristic molecular, cellular, tissular and clinical changes. (Kohl et al, 2011)

The skin is a bio-membrane situated at the interface with the external environment. It reflects the state of health of the body, the human personality and has numerous psycho-social implications. Ageing is a complex process that implies external and internal factors. Chronological skin ageing comprises those changes in the skin that occur as a result of passage of time alone. Photo-ageing comprises those changes in the skin that are result of chronic sun exposure superimposed on chronological skin ageing. Several scientific theories on the ageing regulation at molecular, cellular and systemic levels have been postulated in order to define and control this process.

1.1 Ageing theories

For decades, researchers concluded that ageing comes as a consequence of both genetic and environmental influences. Among the environmental factors, solar UV radiation is the most important cause of premature aging.

1.1.1 The genetic theory

The theory of error accumulation, described by Orgel in 1963, interferes with the DNA replication, RNA transcription and translation into proteins. The risk for errors increases with age, determining at a certain moment a critical change in the genome that triggers senescence, apoptosis and cellular death.
1.1.2 The free radical theory

Postulated by Harman in 1956, this theory proved that the reactive oxygen species interfere with the cellular and subcellular systems, inducing molecular degradations.

1.1.3 The mitochondrial theory

It is based on the fact that the mitochondria are the main source of free radicals within the cell. Skin exposure to sun determines the accumulation of mutations in the mitochondrial DNA, with implications in senescence (Shy et al, 2010).

1.1.4 The telomerase theory

Telomeres are sequences of nucleic acids extending from the ends of chromosomes. Every time our cells divide, telomeres are shortened, leading to cellular damage and cellular death associated with ageing. Telomerase, the "immortalizing" enzyme, appears to repair telomeres, manipulating the "clock" mechanism that controls the life span of dividing cells. The telomerase controls the telomere length and could be involved in the prevention of the ageing process. Recent studies have shown that specific molecules, applied topically or generally can activate the telomerase, preventing thus the shortening of the telomeres. (Han et al, 2009)

1.1.5 The theory of glycation

Maillard's theory is widely recognized as a general intrinsic ageing mechanism, focused on another potentially destructive agent, glucose. Glycation is the non-enzymatic reaction of a sugar and a protein forming multiple chemicals called advanced glycation end products (AGEs) (Pageon et al, 2010). The reaction products accumulate during the ageing process, and seem to also be involved in different pathologies associated with diabetes, atherosclerosis, Alzheimers disease, arthrytis (Bos et al, 2011). Proteins with a long biological half-life (collagen, elastin) are more affected. Glycation has different side effects on extracellular matrix fibers, leading to stiffer and more brittle collagen. In addition, elastin is easily glycated. Denaturated elastin is associated with slackened skin. It is important to mention that AGEs have cellular receptors that initiate inflammatory reactions when they are activated by an AGE complex. These reactions are associated with metabolic disorders, arterial diseases, the premature ageing process and the whole associated pathology. It has been reported that glycation affects the precise aggregation of collagen monomers into fibers, aspect that may be correlated with the different amplitude of the pixels when performing high-frequency ultrasound. Literature data as well as our own studies show that the echogenicity of the pixels and their density, correlated with the classical histological aspect of the integument, offer important information regarding age, cutaneous phototype, anti-ageing therapies, cutaneous pathologies etc. The extension of glycation in the skin can be measured with an instrument that measures a fluorometric chemical named pentosidine. Pentosidine is a fluorescent crosslinker that accumulates in a linear fashion in the collagen of all tissues. The fluorescence degree is correlated with the amount of accumulated pentosidine, age, risk of developing a certain pathology etc.

According to literature, collagen may be considered the key protein that allows the noninvasive assessment (fluorimetry, ultrasonography) of the cutaneous senescence process.
as well as the efficacy of various anti-ageing therapies. The non-invasive assessment of the cutaneous structure opens a new era of skin care and anti-ageing treatment.

Photoaging occurs as a result of cumulative damage from ultraviolet (UV) radiation. The UV rays induce and accelerate the glycation process, interact with cells and extracellular matrix, induce the synthesis and release of cytokines, stimulate the metalloproteinase synthesis, especially collagenase and elastase, and represent the major aggression factor on the cellular DNA. In photoaged skin collagen fibrils are disorganized, and abnormal elastin accumulates.

Despite the progress in aging research, these have yet to be an unanimous vote on one specific theory of ageing. Most of these theories have been disputed by researchers over and over again and many of them, as Dr. Hans Kugler editor of the Journal of Longevity Research, said, "...are dying of old age." Age-related changes do not occur uniformly in individuals because they are under genetic and environmental control. What is certain is that we are all involved in a global-ageing phenomenon.

1.2 Signs of skin ageing

The skin is the only organ completely displayed at the body surface and represents the ideal system for the study of both the intrinsic and extrinsic ageing process. Changes of the skin structure, such as wrinkles, irregularities of pigmentation, in contrast to ageing of other organs are visible and provide social clues to estimate the individual age.

1.2.1 Intrinsic aged skin occurs as a result of passage of time alone

**Clinical manifestations** include xerosis, laxity, wrinkles, slackness, benign tumors (cherry angiomas, seborrheic keratoses). Chronological ageing is affected by the changes of hormones and growth factors that appear with age. **Histological features** involve the epidermis, dermis and appendages. The hallmarks of intrinsic ageing are the thinning of the epidermis, flattening of the dermo-epidermal junction and reduction of extracellular matrix components.

1.2.2 Photoaged skin occurs as a result of cumulative damage from UV radiation

**Clinical manifestations** include roughness, irregular pigmentation, wrinkles, pseudo-scars, fine nodularity (elastotic material), telangiectasia, sebaceous hyperplasia, etc. **Histological manifestations** involve irregular epidermal thickness, nodular aggregations of elastotic material in the papillary dermis. The most obvious histological aspect is solar elastosis along with an increased amount of ground substance consisting of glycoaminoglycans and proteoglycans, and a decreased number of collagen fibers. Solar elastosis may correspond to the subepidermal low echogenicity band (SLEB) a specific imagistic parameter that appears on photoagressed areas. See Figure 3

In the reticular dermis collagen fibers appear degraded, clumped and fragmented. In addition, an inflammatory infiltrate can be identified. The elastosis process, collagen degenerescence, inflammatory infiltrates (histological aspects identified in usual or special stains) represent the morphological substrate of the sonograms. The amplitude and density of the pixels, correlated with the histological aspect, quantify different molecular, cellular, biochemical and structural reactions that govern the ageing process.
The response to UV-induced damage is correlated with the individuals’ skin type. Thus, subjects with skin type II show an atrophic and dysplastic response to UV rays, present fewer wrinkles, smoother skin, actinic keratoses, and epidermal malignancies (carcinoma, melanoma). The individuals with skin type III or IV show hyperplastic responses, present thick skin with coarse wrinkles. Our observations on 140 subjects have shown that individuals belonging to phototype class II (70 subjects) have a different imagistic pattern on photoexposed and photoprotected sites, in comparison to subjects belonging to phototype class III (70 subjects). The dynamics of the pixels on the studied areas indicate significant variations according to the phototype class and are correlated to different clinical aspects of the ageing process. The specific ageing features of phototype class II and III are shown in Table I.

<table>
<thead>
<tr>
<th>Clinical aspects</th>
<th>Phototype class II</th>
<th>Phototype class III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth skin, less superficial wrinkles, numerous pre-malignant lesions and cutaneous carcinomas</td>
<td>Thick, pigmented, deeply wrinkled skin</td>
<td></td>
</tr>
<tr>
<td>Imagistic aspect: Photoexposed area (zygomatic area)</td>
<td>Thicker epidermis</td>
<td>Thinner epidermis</td>
</tr>
<tr>
<td></td>
<td>Thicker dermis</td>
<td>Thinner dermis</td>
</tr>
<tr>
<td></td>
<td>Increased number of LEP, MEP, HEP</td>
<td>Lower number of LEP, MEP, HEP</td>
</tr>
<tr>
<td></td>
<td>Thinner epidermis</td>
<td>Thicker epidermis</td>
</tr>
<tr>
<td>Imagistic aspect: Photoprotected area (medial arm)</td>
<td>Thicker dermis</td>
<td>Thinner dermis</td>
</tr>
<tr>
<td></td>
<td>Higher amount of LEP</td>
<td>Lower amount of LEP</td>
</tr>
<tr>
<td></td>
<td>Lower amount of MEP, MEP, LEPs/LEPi higher</td>
<td>Higher amount of MEP and HEP LEPs/LEPi lower</td>
</tr>
</tbody>
</table>

Table 1. Clinical and imagistic characteristics of skin phototype II and III.

It is well known that phenotypical and functional skin differences of individuals belonging to different ethnic backrounds are related to genetic factors, pigmentary system, life-time UV exposure, life-time style. In contrast to the studies on the pigmentary system, we can appreciate that there are other histological, biochemical differences, which govern the different shades of color. Despite the interest in finding objective markers for phototype classification, more complex studies comparing ageing and phototype between different ethnic groups remain to be published. Our observations suggest a complex interrelationship between the histological structure and the individual pigmentary system. The identification of certain measurable objective markers for every phototype will allow an optimisation of the phototherapy protocols and will reduce the photoinduced side-effects.

1.3 Histology of the skin

From histological point of view, skin consists of two layers of different origin, structure and function.

The epidermis (0.07 to 0.12 mm) is the outermost structure, derived from ectoderm, consisting of cells organized into five layers. Stratum basale (germinativum), supported by a
basement membrane consists of a single layer of mitotically active cells. Stratum spinosum, the thickest layer of the epidermis consists of polyhedral to flattened cells, attached to each other by unstable desmosomes, conferring it a prickly appearance. Stratum granulosum consists of cells that contain lipid-rich granules that act as a waterproof barrier. Stratum lucidum is present only in thick skin. Stratum corneum is the most superficial layer, composed of numerous layers of flattened, keratinized cells.

The dermis (corium), lying directly beneath the epidermis is derived from the mesoderm and is subdivided into two layers: the superficial, loosely woven papillary layer and the deeper, much denser reticular layer. The dermis ranges in thickness from 0.6 mm to 3 mm. Histologically, the dermis is a dense, irregular collagenous connective tissue, containing mostly type I collagen fibers and networks of elastic fibers, which support the epidermis and bind the skin to the underlying hypodermis.

The papillary dermis is a loose connective tissue consisting of: type III collagen fibers, elastic fibers, fibroblasts, mast cells etc. The reticular layer is composed of dense, irregular collagenous connective tissue, displaying thick type I collagen fibers, closely packed into large bundles lying mostly parallel to the skin surface. Thick elastic fibers form networks that are more abundant around sebaceous and sweat glands. Proteoglycans fill the interstices of the reticular dermis. Cells are sparser and include fibroblasts, mast cells, macrophages, lymphocytes and fat cells. The hypodermis (subcutaneous adipose tissue) is considered a diffuse organ, normally well represented. From structural and functional point of view it is well integrated with the dermis and epidermis thru vessels and nerve structures. It lies underneath the reticular dermis, being composed of adipose cells, disposed in adipose lobules, separated by conjunctive septa that contain blood vessels, lymphatics, nerve fibres and numerous mastocytes. The architecture of the adipose tissue differs for men and women. The subcutis is not part of the cutaneous structure, but is studied together with it, due to the associated pathology.

1.3.1 Epidermis

Epidermis is a stratified squamous nonkeratinized epithelium that covers the body on its surface. It consists of cells organized into five rows. Among keratinocytes that represent the most important cellular population, other cells, such as melanocytes, Langerhans cells and Merkel cells are found.

Imagistically, the epidermis appears as a hyperechogenic band, displayed parallel to the cutaneous surface, having a thickness that can be assessed in mm. The thickness of the epidermis changes in relationship to the ageing process, applied therapy, associated pathology.

1.3.2 Dermo-epidermal junction

The junction between epidermis and dermis is a special ondulated basement membrane rich in collagen type IV filaments, collagen type III, collagen type VII, glycoproteins. The morphofunctional integrity of this barrier is essential for the skin protection function.

Imagistically, it is visualised as an extremely thin band situated at the limit of the hyperechogenic epidermis and the underlying dermis.
1.3.3 Dermis

Is a dense connective tissue, situated between the epidermis and hypodermis. The dermis consists of cells and extracellular matrix, composed of collagen and elastic fibres, proteoglycans, glycoproteins, tissular fluid. The limit with the hypodermis is a straight or sometimes ondulated line, because of the underlying adipose lobules, that are prominent in the lower dermis (visible aspect in „orange skin appearance“ cellulitis). (Crisan, 2007)

Imagistically, we can assess the thickness of the dermis in mm as well as the number of pixels with different amplitudes, each codifying different structural, physiological or pathological aspects.

1.3.3.1 Extracellular matrix

Collagen is an important protein for the skin as it is essential for the structure and function of the extracellular matrix in the dermis. Thinner and wrinkled skin are typical signs of normal ageing and are consequences of reduced collagen. Collagen may be considered a “gold protein” for the assessment of the ageing process (fluorometrical, imagistical, clinical) and risk prediction of associated pathology. Collagen type I and II are the main types of collagen in the skin. In young subjects, there is a prominence of collagen type III or reticular fibres that are organised in fibrills and disposed at the level of papillary demis. In adults there is a prominence of type I collagen, organised in fibres disposed in parallel bands in the reticular or profound dermis. Collagen type IV forms filaments and is situated at the dermo-epidermal junction. In elderly subjects all types of collagen are diminished. Collagen type I is the most common form (80%) in the dermis and is responsible for the cutaneous resistance. It is continuously produced and recycled throughout lifetime. In young subjects the synthesis process is prominent whereas in subjects over the age of 40, degradation processes are more common. (Uitto et al, 2008)

The key cell that forms and maintains the extracellular matrix is the fibroblast. The synthesis of the connective tissue fibres is initiated intracellularly, whereas the formation of filaments, fibrills or fibres are extracellular processes, controlled by several factors of the extracellular matrix. The main source of dermal echogenicity is represented by collagen fibres, disposed in an organized manner. Collagen and elastin are important proteins in maintaining the cutaneous architecture and ensuring the biostructural qualities of the integumentary system. Proteoglycans beside glycoproteins and fibers are important components of the extracellular matrix. They consist of a protein core to which different glycoaminoglycans are linked. Hyaluronic acid binds uncovalently the proteoglycans, forming macromolecules that attract water, resulting in a true hydrating capsule with great importance for the hydration of the skin. With age, the amount of proteoglycans decreases and consequently the cutaneous hydration degree as well. The degree of hydration can be assessed ultrasonographically by establishing the amount of low echogenic pixels in the skin.

1.4 Ultrasonography in dermatology

The imaging techniques have imposed themselves as useful non-invasive methods for skin examination and diagnostic tools for skin conditions. During the past years conventional and high resolution ultrasonography (US) have extended their utility in the field of clinical dermatology (Schmid-Wendtner & Burgdorf W, 2005). The procedure involving ultrasound
is a non invasive method allowing “in vivo” and “in real time” histological assessment of the cutaneous structure as well as its specific conditions. Several studies have prooven the similarities between sonograms and histological sections. (Jasaitiene et al, 2011)

The inclusion of this method among the procedures used for the diagnosis of skin diseases is an attempt to replace as much as possible the invasive procedures, especially biopsy, with non invasive ones. The motivation for the extensive use of US derives from its ability to reveal in detail the skin components, up to 1.5 cm in depth, to assess the axial and lateral tumoral extension, the inflammatory and degenerative processes, as well as the efficacy of different topical and general therapies.

1.4.1 High-frequency ultrasound

High-frequency ultrasound is a new, noninvasive method that allows an “in vivo assessment” of the physiological and pathological aspects of the integumentary system. It represents a more desirable and less emotionally-involving alternative to skin biopsy that is routinely used in the dermatological field. It also represents an important research tool for the characterization of skin properties on different intervals of age, allowing the establishment of an imagistic ageing model of the integumentary system. (Badea et al, 2010)

The use of high-frequency ultrasound in dermatology allows a clear identification of the skin layers and thus tissue assessment. At frequencies above 10 MHz, it was prooven that the technology provides enough resolution to characterize microstructures. High-frequency ultrasound allows, as the senescence process progresses, the identification of variations both in skin thickness and echogenicity, offering specific, ultrasonographic markers that allow an objective assessment of the skin ageing process. The changes of the extracellular matrix, consisting in variations of the dermal density and echogenicity throughout the physiological senescence process can be easily identified with the use of high-frequency ultrasound.

The ultrasonographic assessment of the integument can be performed with a 20 MHz high-frequency Dermascan device (Dermascan C, Cortex Technology, Denmark), as seen in Figure 1, that allows the “in vivo” acquirement of cross-sectional images of the skin (B mode) up to 2.5 cm in depth.

Fig. 1. Ultrasonographic equipment (Dermascan C, Cortex Technology, Denmark).
The device consists of three major parts: a transducer, an elaboration system and a data storing system. The ultrasonic wave is partially reflected at the boundary between adjacent structures and generates echoes of different amplitudes. The intensity of the reflected echoes is evaluated by a microprocessor and visualized as a colored two-dimensional image. The color scale of echogenicity is: white - yellow - red - green - blue - black. On a normal cutaneous image, the epidermal echogenicity appears as a white band, the dermis is expressed as a 2 color composition: yellow and/or red, and the subcutaneous layer appears either green or black, as displayed in Figure 2.

![Ultrasound image of the skin: epidermis, dermis, hypodermis](image)

The ultrasonographic images are saved and processed with a specific image analysis software (Dermavision, Cortex Technology), that has a certain property: the amplitudes of the echoes of the pixels are given as a value on a numerical scale that ranges from 0-255. On this scale, the low echogenicity pixel area corresponds to the 0-30 interval, the medium echogenicity pixels to 50-150, and the high echogenicity pixels to the 200-255 interval.

During the study, we can adjust the gain curve as well as the speed of ultrasound at tissular level. Ultrasonographic gel is applied on the aperture of the ring of the transducer, which is then placed perpendicularly to the skin surface for the acquirement of the cross-sectional image. There are several parameters that can be assessed by using Dermascan device and Dermavision analysis software as illustrated in Table 2.

The thickness of the epidermis can be obtained by establishing the mean of three measurements performed in A-mode at three different sites of each image (the 2 extremities and the center of the analyzed image). The thickness of the dermis is obtained in B-mode, by measuring the distance between the dermo-epidermal and the dermo-hypodermic junction at the same three different sites and by establishing the mean of the three values. By selecting a certain interval from the 0-255 pixel scale, we obtain values corresponding to the low, medium and high echogenic pixels, present in the analyzed image.

Additionally, the LEP can be quantified separately in the upper (LEPs) and lower (LEPi) dermis. To separate the 2 areas, we draw a parallel line to the epidermal entrance echo, dividing the dermis into 2 equally thick parts. The ratio of LEP number in the upper and lower dermis (LEPs/LEPi) can be calculated.
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of the epidermis</td>
<td>Given in mm</td>
</tr>
<tr>
<td>Thickness of dermis</td>
<td></td>
</tr>
<tr>
<td>The number of LEP (low echogenic pixels):</td>
<td>Quantify the degree of cutaneous hydration, inflammatory processes, solar elastosis, collagen degeneration</td>
</tr>
<tr>
<td>The number of MEP (medium echogenic pixels):</td>
<td>Quantify the protein structures, the collagen and elastin precursors (different assembly degrees)</td>
</tr>
<tr>
<td>The number of HEP (high echogenic pixels):</td>
<td>Quantify mature collagen assembled in fibres and disposed in parallel bands – marker of intrinsic ageing</td>
</tr>
<tr>
<td>SLEB - subepidermal low echogenicity band</td>
<td>A well delimited, subepidermal low echogenicity band (0-30), situated in the upper dermis, mainly present on photoexposed sites – marker of extrinsic ageing, shown in Figure 3</td>
</tr>
<tr>
<td>LEPs / LEPi ratio: number of low echogenic pixels in the upper dermis/number of low echogenic pixels in the lower dermis</td>
<td>Allows an appreciation of the density and integrity of the extracellular matrix, both from the upper and lower dermis, which varies according to age, UV-rays exposures, therapy – photoageing marker</td>
</tr>
</tbody>
</table>

Table 2. Ultrasonographic parameters

Fig. 3. Subepidermal low echogenicity band (SLEB)

The ultrasonographic skin examination with a high frequency transducer offers a 80 micrometer axial resolution, a 200 micrometer lateral resolution and a 1-2,5 cm depth [8,9]. According to the literature data and our experience, high frequency US is a non invasive instrument for skin examination having multiple applications both in the clinical and the research setting. We mention some of the most important contributions of the method in Table 3:
Applications of high-frequency ultrasound

1. Histologic skin evaluation and identification of each skin component (epidermis, dermis, dermo-epidermic and dermo-hypodermic junction, hypodermis); it is worth mentioning that the skin thickness is measured in “mm”, while the density of the dermis is measured in number of pixels of different amplitude.

2. Assessment and description of pigmented and non-pigmentary tumoral structures: pigmented nevi, melanomas, carcinomas, dermoid cysts, scleroderma, etc.

3. Non invasive monitoring, both qualitative and quantitative, of the cutaneous alterations induced by senescence.


5. Monitoring the efficacy of various therapies.

6. Objective markers for determining the phototype.

Table 3. Applications of high-frequency ultrasonography in Dermatology

1.4.2 Conventional ultrasound

The use of conventional US has gained greatly in importance in clinical dermatology starting with the 70’s. It proved to be a valuable diagnostic method and to have several indications, such as: a. identification and description of visible and palpable tumors, including melanoma; b. preoperative and postoperative assessment of peripheral lymph nodes in all patients with malignant skin tumors; c. monitoring of metastases, especially during chemotherapy. (Wortsman et al, 2010) The main applications of US in dermatology in the present times are conventional cutaneous US and examination with the high frequency transducer.

2. High-frequency ultrasound study of the skin

2.1 High frequency ultrasound study of the skin aging process

High frequency ultrasound allows the “in vivo” appreciation of certain histological parameters and offers new characteristic markers, which may quantify the severity of the cutaneous senescence process. Moreover, it may differentiate between the chronological aging process and photoaging. It evaluates the physio-chemical properties of the integument, epidermis, dermis and subcutis that induce acoustical variations, expressed through certain changes of tissue echogenicity. Our study focused on measuring the changes in skin thickness and dermis echogenicity, as part of the complex ageing process, on different intervals of age.

The study was performed on 40 Caucasian patients, 12 men, 28 women, aged 4-75 years and divided into four age categories: 4-20, 21-40, 41-60, >60. For each subject, cutaneous ultrasound images were taken from 3 different sites: dorsal forearm (DF), medial arm (MA) and zygomatic area (ZA). The data we obtained was statistically assessed, based on the ANOVA and Student T test, using the EPIINFO program. We evaluated the differences between values referring to different intervals of age at the 3 examined sites. A p value < 0.05 was considered significant.

The thickness of the epidermis remains at approximately similar values on all examined sites, for all age intervals, with no statistically significant differences. The thickness of the
dermis shows certain variations. A growth of the dermal thickness at facial level can be noticed with aging. From a mean of approximately 1,320 mm on the 4-20 age interval, the dermis reached a value of 1,614 mm for the subjects taking part of the >60 age interval.

At dorsal forearm and medial arm level, we noticed that the dermis thickness varied in the same way: a decrease of the dermis thickness for the 20-40 age interval, followed by an increase for the 41-60 age interval. The 20-40 age interval, corresponding to the maturity period, is characterized by active synthesis processes, which lead to the thickening of the extracellular matrix. The degenerative processes that lead to the thinning of the dermis appear slowly after the age of 60. The variation pattern of the dermal and epidermal thickness with age can be observed in Table 4.

<table>
<thead>
<tr>
<th>Interval of age</th>
<th>Epidermis DF (mm)</th>
<th>Epidermis MA (mm)</th>
<th>Epidermis ZA (mm)</th>
<th>Dermal DF (mm)</th>
<th>Dermal MA (mm)</th>
<th>Dermal ZA (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-20</td>
<td>0,19675</td>
<td>0,165375</td>
<td>0,175875</td>
<td>1,211875</td>
<td>0,856375</td>
<td>1,320375</td>
</tr>
<tr>
<td>21-40</td>
<td>0,182333</td>
<td>0,163778</td>
<td>0,173333</td>
<td>1,191222</td>
<td>0,772889</td>
<td>1,45</td>
</tr>
<tr>
<td>41-60</td>
<td>0,182667</td>
<td>0,184889</td>
<td>0,155222</td>
<td>1,311556</td>
<td>0,838333</td>
<td>1,448289</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0,186444</td>
<td>0,169333</td>
<td>0,162333</td>
<td>1,168889</td>
<td>0,861</td>
<td>1,614</td>
</tr>
</tbody>
</table>

Table 4. Mean of dermis and epidermis thickness on 4 age intervals, at dorsal forearm (DF), medium arm (MA) and zygomatic area (ZA) level

Generally, considering the total thickness of the integument (dermis and epidermis), a significant increase may be noticed especially at the facial site, which proves that the integument thickness increase is dependant on the severity of UV photoexposure. Also, comparing young subjects (aged 4-20) with elderly ones (>60), it is noticeable that the integument is thinner in the second group at dorsal forearm level, has similar values on the medial arm and increases at facial level.

The number of hypoechogenic pixels shows a significant variation in case of the dorsal forearm and medial arm of the patients taken into study, as follows: hypoechogenic pixels significantly decrease on the dorsal forearm in the 20-40 age interval compared to the 4-20 interval (p= 0.038018, p<0.05) and increase significantly in the >60 age interval in comparison to the 41-60 interval (p= 0.00777, p<0.05); on the medial arm, hypoechogenic pixels increase significantly in the 41-60 age interval, compared to 20-40 interval (p= 0.018056, p<0.05). The significant increase of hypoechogenic pixels after the age of 40, both on photoexposed and photoprotected sites, is correlated with the degenerative changes which are typical for the ageing process in general. Initially, elastic and reticular fibres from the papillary dermis are altered. Generally, we noticed that hypoechogenic pixels are more numerous in the upper dermis in elderly subjects on all studied areas, being correlated with the elastosis and cutaneous degenerescence processes.
Intermediate echogenic (50-100, 100-150) pixels increase significantly (p<0.05) on photoexposed sites in the 20-40 age interval (synthesis processes; assembly to filaments, microfibrils) and decrease after the age of 40 (decreased synthesis and degenerative processes). The repartition dynamics of the intermediate echogenic pixels in case of the 20-40 age interval indicates the presence of intense metabolic processes that continue on to the next intervals of age, but in a much slower rhythm. We consider this interval as a”critical age interval” that represents the optimal timing to initiate the prophylaxis of the senescence process and associated pathology.

Hyperechogenic pixels also display statistically significant variations on the three analyzed regions: on the dorsal forearm, high echogenic pixels increase significantly in the 20-40 interval of age, compared to the 4-20 interval (p=0.025154, p<0.05, and slightly decrease after the age of 40; on the medial arm, they decrease in the 40-60 age interval compared to the 20-40 age interval (p= 0.038523, p<0.05) and at facial site, high echogenic pixels increase in the 21-40 interval (p= 0.025405, p< 0.05) and decrease between 41-60 (p= 0.048694, p<0.05). The highest amount of hypoechogenic pixels was identified at facial level, an intensely photoaggressed site, whereas the highest amount of hyperechogenic pixels was found at the medial arm site, a less photoexposed area. High echogenic pixels (200-255) are poorly expressed in patients belonging to the 4-20 age interval, and much better expressed in the 20-40 age interval on all studied areas. According to Table 5, the mean of hyperechogenic pixels is higher on photoprotected areas compared to the photoaggressed ones for all intervals of age. Thus, we may consider hyperechogenic pixels as ultrasonographic markers of the chronological ageing process.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>0-20</th>
<th>50-100</th>
<th>100-150</th>
<th>200-255</th>
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<tbody>
<tr>
<td>0-20</td>
<td>11656.50</td>
<td>2184.12</td>
<td>627.62</td>
<td>470.50</td>
<td></td>
</tr>
<tr>
<td>20-40</td>
<td>7657.00</td>
<td>3581.55</td>
<td>1314.11</td>
<td>1221.55</td>
<td></td>
</tr>
<tr>
<td>40-60</td>
<td>12613.11</td>
<td>2413.44</td>
<td>740.555</td>
<td>701.33</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>11007.44</td>
<td>2093.00</td>
<td>708.444</td>
<td>737.55</td>
<td></td>
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<tr>
<td></td>
<td>MA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1036.50</td>
<td>1960.00</td>
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<tr>
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<td>916.11</td>
<td>1644.88</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>5584.88</td>
<td>2204.66</td>
<td>822.22</td>
<td>1450.66</td>
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<tr>
<td></td>
<td>ZA</td>
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<td></td>
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<td></td>
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<tr>
<td>0-20</td>
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<td>1568.87</td>
<td>348.25</td>
<td>120.75</td>
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<tr>
<td>20-40</td>
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<td>821.77</td>
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<td>1561.88</td>
<td>394.77</td>
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<tr>
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<td>19055.56</td>
<td>1823.44</td>
<td>504.77</td>
<td>301.22</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Mean of 0-30, 50-100, 100-150, 200-255 pixels measured on subjects divided into 4 age intervals, at the examined sites: dorsal forearm (DF), medial arm (MA) and face level (ZA)
Subepidermal low echogenic band (SLEB) was identified in case of the subjects part of the 41-60 and >60 age intervals, and appeared especially on photoexposed sites (dorsal forearm, face) [10]. In some patients though, especially the younger ones, we were able to identify SLEB at medial arm level as well. On photo-aggressed sites, it may be noticed that the echogenicity of the upper dermis decreases with age.

SLEB may be considered a specific ultrasonographic parameter that allows a noninvasive quantification of the elastosis degree and actinic collagen degeneration. (Lacarrubba et al, 2008) SLEB varies in thickness and localization according to age and UV exposure. In young subjects, SLEB is present in the lower dermis and quantifies the degree of cutaneous hydration, since the extracellular matrix is rich in proteoglycans and hyaluronic acid.

Hyaluronic acid binds uncovalently the proteoglycans, forming macromolecules that attract water, forming a true hydrating capsule. In elderly subjects, SLEB quantifies the elastosis process and basophilic degenerescence of collagen, common aspects of the senescence process, but increased by UV. Thus, we may consider SLEB as a qualitative marker of the photoagression process.

The ultrasound study shows different echogenicity degrees for the upper (LEPs) and lower (LEPi) dermis. For the upper dermis, the study revealed an increase of hypoechogenic pixels (0-30), in comparison to the lower dermis, for all 4 age intervals studied. According to Figure 4, the hypoechogenicity degree is higher on photoexposed sites, both for the upper and the lower dermis. LEPs/LEPi ratio showed a statistically significant increase (p<0.05) for the 20-40 and 40-60 age intervals on photoexposed sites, especially at facial level (p= 0.000999, p<0.05).

On the medial arm, a progressive decrease was noticed till the age of 60, followed by a light increase in people >60 years. This aspect may be explained by the increase of hypoechogenic pixels in the upper dermis. Unlike the upper dermis, in the lower dermis, an increase of echogenicity may be noticed with ageing as visible in Figure 4. The ratio between the echogenicity of the upper and lower dermis represents an objective marker of the photoageing process.

Fig. 4. Ultrasonographic aspect of the dorsal arm, medial arm and facial site on the 4 studied age intervals
The thickness of the integument, SLEB, as well as the dermal echogenicity are parameters that evaluate, with high accuracy the cutaneous senescence process at a microscopical level. The ratio between the echogenicity of the upper and lower dermis represents an objective marker of the photoaging process. SLEB is an ultrasonographic marker of the collagen degenerescence process and photoinduced cutaneous elastosis.

2.2 Non-invasive imagistic assessment by “in vivo” histological sections, of the efficacy of anti-ageing therapies

Taking into consideration that nowadays we assist a general ageing tendency of the world population, the antiaging therapy is a priority and a continuous challenge for researchers. The identification of the mechanisms involved in the cutaneous aging process and their impact on certain age categories, correlated with the hormonal and neurogenetic constellation of the subject would be highly desirable since it is estimated that about 31% of the population is over the age of 60 (US Census Bureau, online database, www.census.gov).

The increase of life expectancy, the psychosocial impact of the cutaneous aspect justifies the high amount of research studies of the ageing mechanisms as well as of the efficacy of certain anti-aging therapies. (Vaupel, 2010) The purpose of the 2 studies to be presented was the assessment with the help of high-frequency ultrasound of the cutaneous changes induced by topical use of Viniferol-containing products as well as by topical anti-ageing product (Interactive P63).

2.2.1 Efficacy of Viniferol as anti-ageing therapy

The first study assessed, with the help of high-frequency ultrasound, the cutaneous changes induced by topical use of products containing Viniferol. As far as the anti-ageing therapy is concerned, Viniferol ® (Resveratrol), an extract from Bordeaux vine stalks is one of the newest and more efficient anti-wrinkle and anti-ageing agents. Having a direct action upon the protein expression of the genes involved in the proliferation and differentiation of integumentary cells, Viniferol profoundly restructures and regenerates the skin. Due to its antioxidant properties, it reestablishes the metabolic balance of the cutaneous cells, slowing down the tissular degeneration and disorganization process (Vranesic-Bender, 2010). Even though the general anti-ageing effects of the flavonoids are well known, until now no scientific studies investigated the action of Viniferol at cutaneous level by using high-frequency ultrasound. Eighty female subjects, aged 22-75, who presented themselves to the practice for prophylaxis and anti-ageing therapy with flavonoids, were prospectively included in the study. 50% of the subjects belonged to Fitzpatrick phototype class II and 50% to phototype class III. The study excluded patients with known allergies to topical flavonoids, cutaneous facial lesions, resurfacing or other anti-ageing therapies in the last 2 months, or those who used phototherapy or oral contraception. The subjects taken into the study were divided into 2 categories: a study group and a control group.

The study group followed the proposed antiaging therapy for 12 weeks, according to a standard protocol. In the morning, a hydrating emollient cream, based on occlusive hydrating agents, was applied at facial level (including zygomatic area), lightly massaging the area for 2 minutes. In the evening, an anti-ageing product containing Viniferol, extracted from grapevine, was applied in the same manner. No other cosmetic products were used by the subjects during the 12 weeks of the study. The control group followed a placebo therapy.
for 12 weeks, using only moisturising cream in the morning and evening, applied at facial level. For every subject, ultrasonographic images were taken from zygomatic level initially and 12 weeks after local application of the emollient, hydrating product and anti-ageing, Viniferol-based cream. The data we obtained was analyzed, calculating the mean and standard deviation for the quantitative variables of every group and the proportions for the qualitative variables. The difference of means before and after treatment was tested using T-test for paired samples and the relationship between different parameters was assessed thru Spearmann correlation coefficients. A p-value <0.05 was considered significant.

All subjects involved in the study tolerated well the therapy, without evoking adverse effects (erythema, pruritus, ocular disturbance). Subjectively, post flavonoid-therapy a significant hydration of the skin throughout the day and an increase of the cutaneous tonicity was noticed.

After therapy, an increase of the mean thickness of the epidermis (0.129 ± 0.237 mm vs 0.150 ± 0.323 mm, p<0.000), and of the dermis (1.434 ± 0.241 mm vs 1.569 ± 0.219 mm, p<0.0001) was observed. (fig 3, 4) The thickness of the dermis increased mainly in the 40-60 age interval (1.413±0.280 mm vs 1.569±0.279 mm, p=0.001), and less, but still significantly < 40 years (1.416±0.266 mm vs 1.585±0.150 mm, p=0.015), while >60 years the increase was not statistically significant (1.480±0.157 mm vs 1.554±0.204 mm, p=0.097). At the same time, at dermal level, the number of low echogenic pixels decreased (15153.53 ± 3589.86 vs. 12958.48 ± 3628.35, p<0.0001), but this aspect was only noticed in the lower dermis (6949.75 ± 1966.93 vs 6257.62 ± 2224.88, p=0.016), not in the upper dermis (7290.55 ± 1794.60 vs 6940.65 ± 2150.30, p=0.168). Overall, the LEPs/LEPi ratio increased significantly after flavonoid therapy (1.092 ± 0.330 vs 1.259 ± 0.631, p=0.011). We also noticed an increase of medium echogenic pixels (3359.72 ± 1457.36 vs 3983.47 ± 1401.24, p=0.013) and high echogenic pixels (460.27 ± 323.93 vs 750.90 ± 493.82, p<0.0001) after therapy. The general variation pattern of the quantifiable ultrasonographic parameters after flavonoid therapy is illustrated in Table 6.

<table>
<thead>
<tr>
<th>parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of epidermis (mm)</td>
<td>0.129 ± 0.237</td>
<td>0.150 ± 0.323</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Thickness of dermis (mm)</td>
<td>1.434 ± 0.241</td>
<td>1.569 ± 0.219</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LEP</td>
<td>15153.53 ± 3589.86</td>
<td>12958.48 ± 3628.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MEP</td>
<td>3359.72 ± 1457.36</td>
<td>3983.47 ± 1401.24</td>
<td>0.013</td>
</tr>
<tr>
<td>HEP</td>
<td>460.27 ± 323.93</td>
<td>750.90 ± 493.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LEPs</td>
<td>7290.55 ± 1794.60</td>
<td>6940.65 ± 2150.30</td>
<td>0.168</td>
</tr>
<tr>
<td>LEPI</td>
<td>6949.75 ± 1966.93</td>
<td>6257.62 ± 2224.88</td>
<td>0.016</td>
</tr>
<tr>
<td>LEPs/LEPi</td>
<td>1.092 ± 0.330</td>
<td>1.259 ± 0.631</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Table 6. Cutaneous parameters quantified by high-frequency ultrasound before and after treatment.
If we consider the variation of the ultrasonographic parameters after topical flavonoid therapy according to the phototype class of the subjects, it can be noticed that after therapy, there is a significant increase of the LEPs/LEPi ratio in the subjects belonging to phototype class II, not III, as shown in Table 7.

<table>
<thead>
<tr>
<th></th>
<th>Phototype 3</th>
<th>Phototype 2</th>
<th>P</th>
<th>Phototype 3</th>
<th>Phototype 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis (mm)</td>
<td>Before treatment 0.1288±0.026</td>
<td>After treatment 0.151±0.027</td>
<td>&lt;0.0001</td>
<td>Before treatment 0.129±0.021</td>
<td>After treatment 0.148±0.037</td>
<td>0.020</td>
</tr>
<tr>
<td>Dermis (mm)</td>
<td>1.441±0.270</td>
<td>1.570±0.263</td>
<td>0.001</td>
<td>1.427±0.214</td>
<td>1.568±0.172</td>
<td>0.003</td>
</tr>
<tr>
<td>LEP</td>
<td>15059.25±4063.97</td>
<td>13864.75±3824.33</td>
<td>0.015</td>
<td>15247.8±3162.14</td>
<td>12052.2±730.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MEP</td>
<td>3120.10±1725.95</td>
<td>3850.95±1487.32</td>
<td>0.046</td>
<td>3599.35±1122.42</td>
<td>4116.0±1334.62</td>
<td>0.151</td>
</tr>
<tr>
<td>HEP</td>
<td>379.35±280.94</td>
<td>645.50±373.59</td>
<td>&lt;0.0001</td>
<td>541.20±350.25</td>
<td>856.3±581.03</td>
<td>0.004</td>
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<tr>
<td>LEPs</td>
<td>7071.65±1754.22</td>
<td>6961.1±2236.66</td>
<td>0.725</td>
<td>7509.45±1852.70</td>
<td>6920.20±2118.53</td>
<td>0.148</td>
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<td>LEPi</td>
<td>7007.65±2150.80</td>
<td>6737.15±2205.91</td>
<td>0.480</td>
<td>6891.85±1818.86</td>
<td>5778.10±2193.29</td>
<td>0.010</td>
</tr>
<tr>
<td>LEPs/LEPi</td>
<td>1.035±0.262</td>
<td>1.096±0.300</td>
<td>0.200</td>
<td>1.149±0.384</td>
<td>1.4227±0.820</td>
<td><strong>0.026</strong></td>
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</tbody>
</table>

Table 7. Variation of the cutaneous parameters quantified by high-frequency ultrasound before and after treatment, according to phototype

In the placebo group, we noticed no significant increase of the epidermis and a slight increase of the dermis after therapy (1.433 ± 0.34 mm vs. 1.486 ± 0.14 mm). The number of low echogenic pixels at dermal level also show a slight increase (13213 ± 1284 vs. 15374 ±2318 , p=0.1) due to an optimal hydration of the skin and a discrete decrease of high echogenity pixels (421.8 ± 121.18 vs 368. 3 ±104.03, p=0.07). The LEPs/LEPi ratio showed no particular display according to the age or phototype of the subjects.

Previous studies have shown that the thickness of the epidermis and dermis, as well as the dermal density are important parameters that assess the cutaneous regeneration process (Crisan M et al, 2009). The neosynthesis of the proteic structures induces an increase of the dermal echogenicity and density, local cell architecture changes and implicitly there is an increase of the dermis and epidermis thickness. It has been proved that certain ultrasonographic markers, such as SLEB (subepidermal low echogenity band) or the LEPs/LEPi ratio can quantify the cutaneous senescence process, as well as the efficacy of various antiaging therapies.

The obtained results are in accordance with the data published in literature. Thus, locally applied flavonoids induce the neosynthesis of the fibrillary structures, but also of glicosaminoglycans, intense hydrophil molecules, favouring the cutaneous hydration. It is well known that flavonoids have important antiaging properties not only at cutaneous level, but at the level of the entire organism. Viniferol, a molecule with proven anti-ageing and antioxidant properties, exhibits a complex action at cutaneous level: it interacts with fibroblastic receptors, amplifies the interrelation fibrocyte-extracellular matrix, modulates the adhesivity molecules and interferes with the oxidative stress process and non-enzymatic glycation, with regenerative effect at cutaneous level.
After therapy, a significant increase of the mean thickness of the epidermis and dermis was noticed, fact that once again confirms the presence of a complex, regenerative dermal process, induced by flavonoids. The dermal thickness increased the most in the 40-60 age interval, to a lesser extent, but still significant under the age of 40, and insignificantly over 60 years. We can affirm that topical flavonoid products have the best efficacy on mature integument, with specific structural and hormonal characteristics. In young subjects (<40) the thickness of the dermis increases discretely as the dermis is a young connective tissue, rich in glicosaminoglycans and thus, properly hydrated. After the age of 60, interval characterized by the presence of degenerative changes of the extracellular matrix the flavonoid-based anti-ageing therapy induces less intense regenerative changes that could be amplified by the association of products able to interfere the characteristic age-related aging mechanisms. At the same time, concomitantly with the change in dermal thickness, the number of low echogenic pixels (LEP) decreased in the lower dermis, not in the upper part. The LEPs/LEPi ratio also increased significantly after therapy. The decrease of the number of low echogenic pixels in the lower dermis is proportionate with the significant increase of medium and high echogenic pixels that quantify proteic neosynthesis, as well as cytoarchitectural reorganizations of the extracellular matrix.

Our data shows important ultrasonographic changes at cutaneous level after anti-ageing therapy, as visible in Figure 5. Flavonoids have a complex action at the dermal level, interfering with several mechanisms involved in the senescence process. They act at the level of fibrocytes, on specific receptors, turning inactive mature cells into young, metabolically active ones.

<table>
<thead>
<tr>
<th>Age interval</th>
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<td>&lt;40</td>
<td>![Before Therapy Image]</td>
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<tr>
<td>41-60</td>
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</tr>
<tr>
<td>&gt;60</td>
<td>![Before Therapy Image]</td>
<td>![After Therapy Image]</td>
</tr>
</tbody>
</table>

Fig. 5. Ultrasonographic skin aspect before and after topical therapy, on different age intervals
The synthesis of the proteic structures is initiated at intracellular level. The trophocollagen molecules, elastin, the glicosaminoglycans are extracellularly assembled into microfibrills, fibers or proteoglycans. Depending on the biochemical structure, the level of organisation, architectural orientation and quantity, the proteins show a certain cutaneous echogenity degree. The low echogenity pixels that quantify the hydration degree of the extracellular matrix especially in the lower dermis are replaced by medium and high echogenity pixels, quantifying proteic synthesis. We can consider that MEP codify elastic and collagen precursors that are to be assembled into mature connective tissue fibers, codified by HEP.

The increase of the LEPs/LEPi ratio quantifies the replacement of the hypoechogenic pixels from the lower dermis with medium and high echogenic pixels as a result of protein neosynthesis. Type I collagen, that is prominent at the dermal level (punctiform hyperechogenic pixels) is organised in fibers, visible as hyperechogenic bands, having a parallel display in the lower dermis. These hyperechogenic bands, visible especially on photoprotected sites represent an ultrasonographic marker of the intrinsic aging process.

If we consider the significant changes of the ultrasonographic parameters after anti-ageing therapy depending on the phototype of the subject, a significant increase of the LEPs/LEPi ratio is present in the subjects in phototype II class, but not class III. This observation would justify the correlation of the anti-ageing therapy with the cutaneous phototype. Further studies are necessary to confirm the different reactivity of the phototype classes to local therapies.

Flavonoids, through complex mechanisms, interfere with the reactions involved in the senescence process, and induce the synthesis of the extracellular matrix. According to our data, Viniferol-based products are more efficient in the 40-60 age interval, characterized by complex biological changes at cutaneous level. Viniferol shows real and important anti-ageing properties, since it interferes concomitantly with the genetic, oxidative, immunologic, metabolic mechanisms that are involved in the cutaneous aging process. The prophylaxis of the ageing process should start before the age of 40, preferable in the “critical age interval” (20-40 years), that is characterized by important changes at tissular, cellular and molecular levels, (Crisan et al, 2010)

The optimization of the anti-ageing therapy, according to special studies requires targeted, personalized therapies, adapted to the hormonal, genetic, oxidative, immunologic and metabolic status of the subject, capable of interfering with deficient mechanisms on certain age intervals. Viniferol-based products have a higher efficacy in phototype II subjects compared to phototype III ones.

2.2.2 Efficacy of INTERACTIVE PEEL P63 as anti-ageing therapy

Interactive P63 is a metabolical dynamiser, capable of interacting simultaneously at different cutaneous levels, both on anabolic and catabolic mechanisms. It contains 8 active principles, among which: alphahidroxiacids, retinoids, a complex derived from growth factors, gluconolactone encapsulated in liposomes etc. This anti-ageing complex has a simulataneous action on three levels, epidermis, dermis and dermoepidermic junction. It has been tested in vitro on human fibroblasts cell cultures (Line Hs27) for cytotoxicity, apoptosis, proliferation index, collagen synthesys, matrix metaloproteinases activity.

www.intechopen.com
This study included fifty female subjects aged 40-75, who addressed themselves to the practice for anti-ageing therapy. The subjects were divided into 2 groups of 30 and 20 patients. From the study group (30 subjects), 16 subjects belonged to Phototype class II, 14 subjects to phototype class III, whereas from the placebo group, 10 subjects were phototype II and the rest of 10 phototype III. The subjects were divided into 3 age categories: 40-50, 51-60, >60. The subjects from the study group underwent topical therapy with Interactive P63 product, whereas the rest of 20 subjects from the control group used a placebo product. The subjects taken into the study followed the proposed antiaging therapy for 12 weeks, according to a standard protocol. The Interactive P63 and placebo product were applied twice a week for 30 minutes at facial level for 12 weeks. During this period, no other treatments apart from moisturising cream were applied. For every subject, ultrasonographic images were taken from zygomatic area, initially and after 12 weeks of treatment. The data we obtained was analyzed, calculating the mean and standard deviation for all quantitative variables. The difference of means before and after treatment was tested using T test for paired samples. A p-value <0.05 was considered significant.

All subjects involved in the study tolerated well the therapy, without evoking adverse effects (erythema, pruritus, ocular disturbance) after 30 minutes of contact. In the Interactive P63 group, after therapy, an increase of the mean thickness of the epidermis (0.117 ± 0.021 mm vs. 0.135 ± 0.023 mm, p=0.0024), and of the dermis (1.537 ± 0.23 mm vs. 1.710 ± 0.244 mm, p=0.0076) was observed. At dermal level, the number of low echogenicity pixels decreased in a significant manner after topical therapy (18484.4 ± 4666.5 mm vs. 14138.97 ± 3779.5 mm, p=0.00021) whereas the number of the medium (3118.63 ± 974.4 mm vs. 4608.93 ± 1105.6 mm, p=0.001) and high echogenic pixels (379.6 ± 274.17 mm vs. 1004.9 ± 458.78 mm, p<0.0001 ) increased significantly as displayed in Figure 6.

<table>
<thead>
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<th>Subject</th>
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<th>After</th>
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<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Nr.2</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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</tbody>
</table>

Fig. 6. Ultrasonographic evaluation of the zygomatic area, before and after topical P63 therapy
The LEPs/LEPi ratio increased significantly after therapy (1.149 ± 0.251 mm vs 1.574 ± 0.317 mm) especially due to the significant decrease of the number of low echogenic pixels (LEPi) in the lower dermis (8740.4±2711.01 vs 4921±2373.6, p=0.016).

If we consider the variation of the ultrasonographic parameters after topical therapy according to the phototype class of the subjects, it can be noticed in Figure 7 that after therapy, there is a significant increase of the LEPs/LEPi ratio in the subjects belonging to phototype class III, not II.

Considering the LEPs/LEPi parameter, we noticed a significant increase of the ratio in all subjects part of the study, especially in the 51-60 age interval (40-50 age interval: p=0.01, 51-60 age interval: p=0.009, >60 age interval: p=0.02) as visible in Figure 8:

INTERACTIVE P63 complex interacts concomitantly different mechanisms involved in the cutaneous aging process, conferring from imagistical point of view, a characteristic display of the pixels at cutaneous level. (Rouabhia et al, 2002) The increase of the epidermal/dermal thickness represents the morphological expression of the changes induced by INTERACTIVE P63 complex at fibroblastic and extracellular matrix level. The activation of the fibroblasts as well as the inductive effect upon stem cells, associated with the inhibition of the mechanisms responsible for the distruction of the fibrillary structures, induce an increase of the dermal density. Thus, we noticed a general, significant decrease of the mean number of low echogenic pixels (LEP) at dermal level, more pronounced in the lower dermis (LEPi) than the upper one (LEPs), suggesting important structural, biochemical, mollecular and architectural changes that vary according to certain particular properties of the upper and lower dermis. Parallel to the decrease of LEP after therapy, a statistically significant increase of the mean number of medium (MEP) and high ecogenic pixels (HEP) was noticed, quantifying the increase of dermal density and thus, collagen neosynthesis.

The LEPs/LEPi ratio, an essential imagistic marker that quantifies the dermal density, increased in a significant manner, due to the important decrease of the number of low echogenity pixels from the lower dermis (LEPi). Considering the LEPs/LEPi ratio on the three age categories: 40-50,51-60, >60 , a significant increase was noticed in all three age intervals. The fact that the most significant increase of the dermal density occurred in the 51-60 age interval, may be correlated with the post-menopausal status as well as with the estrogen-like activity of INTERACTIVE P63 complex (El-Alfy et al, 2010). During menopause, due to a decrease of estrogen and cutaneous estrogen receptors, a progressive decrease of dermal collagen occurs, with a loss of collagen content of 1-2% every menopausal year. Several studies certify the fact that topical estrogen therapy in menopausal women induces an increase of almost 5.1% of dermal collagen. It is also a fact that the efficacy of hormone therapy is dependant on the basal collagen status at the beginning of the therapy. The initiation of a precocious therapy in menopause has a prophylactic role, while a delayed therapy has a therapeutic purpose.
Fig. 7. Variation of the LEPs/LEPi ratio before and after topical therapy, according to phototype.

Fig. 8. The variation of the mean of the LEPs/LEPi ratio on different age interval before and after therapy.
INTERACTIVE P63 complex acts at cellular level, interfering the cutaneous estrogenic receptors (α and β) that, even though with structural and functional similarities, have different expression conditions and act differently in menopause, explaining the changes regarding dermal density on age categories. Regarding the phototype of the subjects, a certain, particular reactivity is to be mentioned: a more significant growth of the dermal density in subjects belonging to phototype class III, compared to phototype class II. The LEPs/LEPi ratio showed no significant variation neither with the age or the phototype of the subjects. Interactive P63 product acts on specific sensitive receptors may interfere with estrogen-like receptors, activating the fibroblast “key cell” and increases the synthesis of collagen. It has real and important anti-ageing properties on large age intervals, since it interferes concomitantly the oxidative, genetic, immunologic, hormonal and metabolic mechanisms. It is highly efficient especially in the 51-60 age interval and in phototype III patients.

In the past years, many advances in the diagnosis of skin ageing have become available for an earlier and more specific diagnosis. Our studies show the importance of high-frequency ultrasound as a noninvasive method for the assessment of the cutaneous senescence process. The correlations between the histological and imagistic parameters allow the establishment of noninvasive diagnosis and treatment protocols. Our observations require further development and review to determine the diagnostic accuracy. Some show great promise in assessing, with less invasive methods, histological features required for an earlier diagnosis, or for establishing the efficacy of various therapies. The need to develop new strategies on how to prevent and how to accommodate the ageing society requires the elaboration of mathematical models in order to predict the evolution of the ageing phenomenon. (Crisan et al, 2010) The anti-ageing medicine may discover there is no limit to human life span.

3. Conclusions

High-frequency ultrasound is a non-invasive histological tool that allows the visualisation of “in vivo” histological sections, offering information with microscopical correspondence and also characteristic ultrasonographic markers. The efficacy of anti-ageing therapies varies with the age interval, according both to the applied product but also the cutaneous reactivity, phototype, hormonal and metabolic status. The prevention of the cutaneous ageing process should begin in the “critical age interval” and the improvement of the clinical aspect requires precocious, personalized therapies, using efficient substances, previously tested on cell-cultures.

4. Acknowledgment

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5. References


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The book “Senescence” is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

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