International Journal of
Cosmetic Science

International Journal of Cosmetic Science, 2018, 1–8 doi: 10.1111/ics.12484

Review Article

An updated review of clinical methods in the assessment of ageing skin – New perspectives and evaluation for claims support

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Received 19 March 2018, Accepted 23 July 2018

Keywords: claims substantiation, clinical assessment ageing skin, non-invasive instrumentation, skin ageing, skin efficacy testing, skin physiology/structure

Abstract

With the advancement of skin research, today's consumer has increased access to an informed understanding of ageing skin and its appendages, together with a plethora of targeted products to meet such needs. In recent years, increased legislative demands for quality evidential claims support have led not only to the development and validation of clinical methods to measure and quantify ageing skin, but also a clearer understanding of the skin ageing process–especially the impact of both its internal and external environments–as well as a tougher stance on clearly unjustifiable claims. Traditional testing methods used to research and evaluate anti-ageing products claim to employ sophisticated instruments. Today, however, since the term anti-ageing can be considered a misnomer, intelligent use of combined more advanced clinical methods has enabled the development of technologically improved consumer products providing enhanced efficacy and targeted performance. Non-invasive methods for the assessment and quantification of the causes of ageing skin provide tools to the clinical researcher as defined by key clinically observed ageing parameters. Where evidence requires additional support, a number of clinical procedures evaluating ageing skin and hair products are combined with invasive procedures, thus enabling an added value to product claims. As discussed herein, given the enhanced understanding of ageing, we provide an update to our previous reviews of clinical methods used in the assessment of skin ageing, to include the wider aspects of environmental exposure; skin pigmentation; microbiome disturbance; surface topography; colour, radiance, and pH; and structural integrity–all requiring a disciplined approach to their use in dermatological investigations and product claims evidence.

Résumé

Grace aux progrès de la recherche dermatologique, les consommateurs ont aujourd'hui accès à une meilleure compréhension du vieillissement de la peau et de ses corrélations, ainsi qu'à une pléthore de produits ciblés pour répondre à ces besoins. Ces dernières années, des demandes législatives accrues pour que les allégations soient étayées de preuves de qualité ont permis non seulement le développement et la validation de méthodes cliniques pour mesurer et quantifier le vieillissement de la peau, mais aussi une meilleure compréhension du processus de vieillissement de la peau - en particulier l'impact des environnements internes et externes, ainsi qu'une position plus ferme contre les allégations clairement impossibles à prouver. Les méthodes de tests traditionnelles utilisées pour rechercher et évaluer les produits anti-âges disent employer des instruments sophistiqués. Aujourd'hui cependant, le terme anti-âge étant une appellation quelque peu trompeuse, une utilisation intelligente de plusieurs méthodes cliniques plus avancées a permis le développement de meilleurs produits d'un point de vue technologique offrant une meilleure efficacité et une performance ciblée. Les méthodes non-invasives d'évaluation et de quantification des causes du vieillissement de la peau offrent des outils aux chercheurs cliniques définis par des paramètres de vieillissement clés observés cliniquement. Quand les preuves doivent être étayées, plusieurs procédures cliniques évaluant les produits pour peaux et cheveux matures sont associées, permettant ainsi d'appuyer les allégations sur le produit. Comme indiqué ici, au vu de la meilleure compréhension du vieillissement, nous fournissons une mise à jour de nos examens précédents des méthodes cliniques utilisées pour évaluer le vieillissement de la peau, pour inclure les aspects plus larges de l'exposition à l'environnement : pigmentation de la peau; dérèglement du microbiome; topographie du relief de la peau; couleur, luminosité, et pH; et intégrité structurelle — tous ces aspects doivent être utilisés avec discipline dans les enquêtes dermatologiques et les preuves des allégations sur le produit.

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Introduction

The appearance and characteristics of the skin change with ageing. Given the rapid increase in the elderly population worldwide [1], the shift in understanding the needs of ageing skin has moved towards one of clear management strategies and healthy well-being. This in part is also driven by the knowledge that the environment of the skin externally and internally through an unhealthy lifestyle will play a major part in determining how aged the body and therefore the skin actually becomes. For society as a whole, the negative effects of ageing are unattractive for both males and females, with the majority striving for fitness for as long as possible, the older they become.

Since the historical yet still fashionable concept of eternal youth is unachievable, the term anti-ageing can be considered a

misnomer since ageing is natures' course for the human body taking it from birth to death. However, how well and how healthy we age is very important. The skin, as an organ of the body, reflects in part, how healthy we are as a whole rather than just reflecting symptoms of the external environment. With an increasing knowledge of the molecular mechanisms of skin ageing and the development of new products to target specific concerns for both men and women, more emphasis and pressure need to be placed on the legislative claims criteria [2] and thus will be demanded in order to justify so-called anti-ageing claims associated with such products. It is argued that alongside newly developed measuring devices, these claims criteria should be regularly updated not only to control unwarranted claims, but also to provide strong weighted credible support to basic and clinical dermatological research [3].

Previous reviews have described the main clinical aspects of ageing skin [4, 5] and methods used to evaluate them [6]. In addition to instrumental methods used to evaluate ageing skin and the effects of cosmetic products, clinical ageing scales are also widely employed to assess the ageing defined parameters; severity of these parameters; and reduction of these parameters post-treatment. These scales have been systematically reviewed and encompass all parts of the body, notable face, hands, arms and legs [7, 8].

In this review, and in the light of increasing knowledge of aged skin, we update our previous reviews of clinical methods used in the assessment of skin ageing, to include highlight methods for aspects of environmental exposure; skin pigmentation; microbiome disturbance; surface topography; colour, radiance, and pH; and structural integrity–all requiring a disciplined approach to their use in dermatological investigations and product claims evidence.

Environmental exposure–oxidative stress and pollution

Today, it is a given that skin changes are markedly affected by environmental exposure especially sunlight and pollution [9]. Furthermore, notwithstanding the effects of UV exposure and consequential oxidative stresses, other light wavelengths are also reported to negatively affect the skin such as infrared and blue spectrums [10]. However, there are beneficial wavelengths within the sunlight spectrum which are used in the treatment of a number of hyperproliferative skin disorders such as psoriasis [11].

Human skin is naturally exposed to ultraviolet radiation (UVR) and unnaturally to environmental air pollutants such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), oxides, particulate matter (PM), ozone (O3) and cigarette smoke [12, 13]. Although the skin shields against pollution, prolonged and repetitive exposure results in accelerated skin ageing [14]. Consequently, the continual increase in air pollution has major negative effects, including skin conditions such as atopic dermatitis and eczema [15], psoriasis [16] and even acne [17]. Skin cancer is the most obvious serious effect of exposure to pollution [18]. With more than 80% of people living in urban areas being exposed to air pollution exceeding WHO (World Health Organization) limits [19], the damaging effects on the skin in both the short- and long-term are clear.

The development of skincare regimes to tackle these deleterious effects requires solid evidential support, especially in terms of product efficacy. A number of both *in vitro* and *ex vivo* methods are available to demonstrate antioxidant activity of pollution-treated skin models and explants [20, 21]. These testing methods are helpful to select possible actives, but of limited clinical relevance and can be unreliable in correlating the antioxidant performance of a product to its efficacy in counteracting environmental impact. Furthermore, extrapolation of *in vitro* efficacy to physiological benefits is poor science and unable to capture a cosmetic formulations ability to penetrate the stratum corneum–which is necessary for an antioxidant to counteract actual free radical production in the skin.

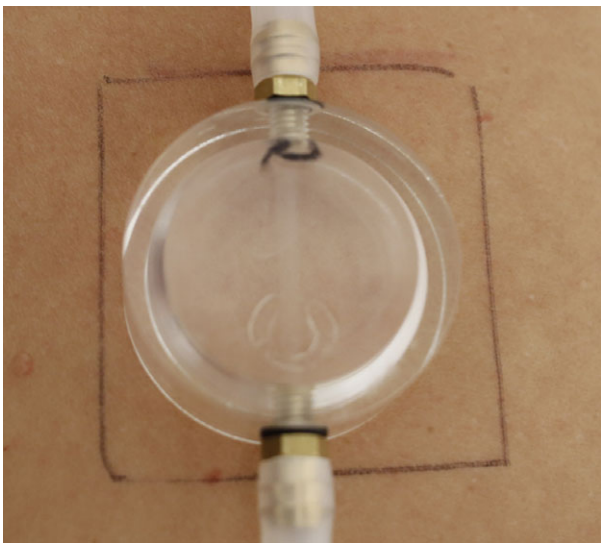

Figure 1 Cigarette smoke can be applied to defined areas on the skin by use of a smoke chamber. In the chamber displayed, the exposure is limited to an area of 3 cm in diameter. By regulating both time and vacuum of a suction pump, a defined smoke exposure can be obtained.

Gaseous combustion model

In order to prove, figuratively and legislatively, real consumer benefit(s) of skin barrier protection and antioxidative damage, human studies are paramount. Since environmental pollution is variable depending on its source and distribution, relevant pollutant models need to be chosen in studies in order to obtain meaningful data while also providing more general conclusions. This is easily achieved by exposing skin with combustion smoke, for example, from cigarettes and measuring the degree of lipid and/or protein peroxidation in the stratum corneum when the skin is treated with test products [22]. The skin of human volunteers is treated with the product under test, exposed to cigarette smoke, and then, peroxidation of human sebum or skin barrier lipids is assessed using GC-MS/LC-MS (Fig. 1). Key advantages of this method are the small number of volunteers required, and the reproducibility and sensitivity of the method to detect protective and antioxidative properties of cosmetic active ingredients and cosmetic formulations. Pollutant cigarette smoke when applied only once on small test areas is a suitable substance containing all key pollution components that can be used in human volunteers without the moral and ethical issues as compared to other pollution sources and exposures. Herein lies a slight disadvantage of this method; however, this controlled clinical method also enables a pollution stress on 'living skin'.

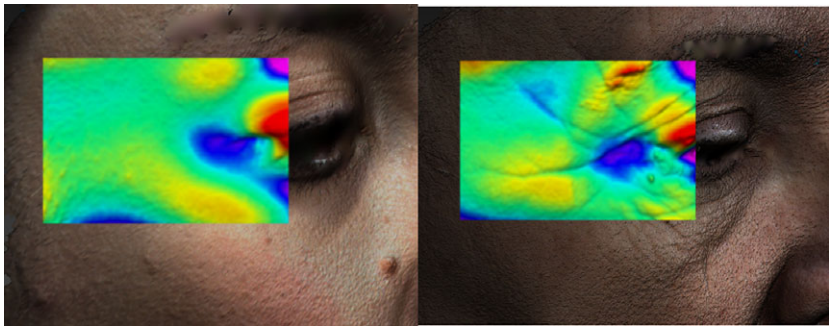

Figure 2 Skin surface profile can be measured *in vivo* by use of the fringe projection technology. A three-dimensional profile is shown by including colour-coded images (blue and green= low regions; yellow and red = elevated areas). The image shows periorbital wrinkles of young (left image) and aged skin (right image).

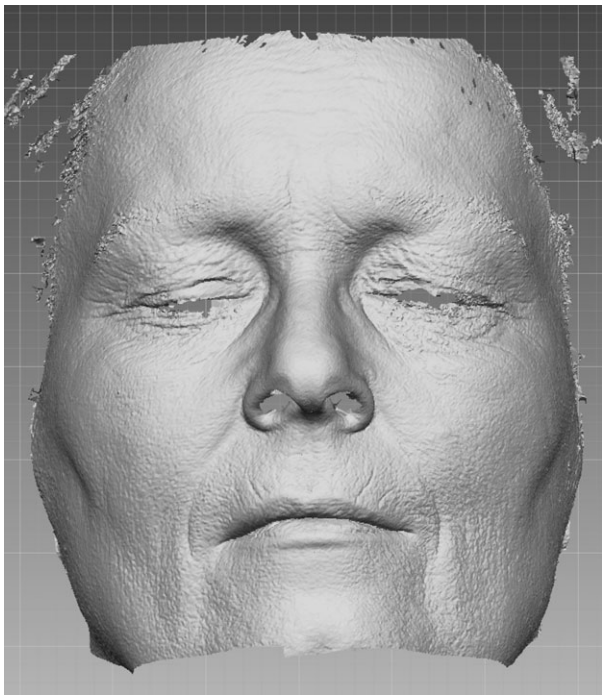

Figure 3 Fringe projection devices have improved dramatically in the size of test area and resolution. Here, the three-dimensional representation of a whole face is displayed. The advantage is that despite the large area even fine lines are visible.

Skin surface topography

As the skin ages, the surface topography of the skin also changes (Fig. 2), with an observed increase in skin roughness and dryness as a result of diminished barrier integrity and alterations of tissue matrix structures as a result of water loss [23]. Methods to evaluate these changes are well described [24, 25] with transepidermal water loss (TEWL) and corneometry and corneosurfometry being common.

More advanced techniques include *Confocal Raman* spectroscopy providing quantitative data on the amount of water in different depths of the stratum corneum and in the epidermis [26, 27]. Furthermore, the effect of moisturizers on the water mobility in the stratum corneum can be determined [26].

Profilometric parameters such as fringe projection devices are now widely employed as a standard method for wrinkle assessment and other skin topology [28, 29].

Advances in fringe projection include large field systems dedicated to contactless measurements of surface topology for cosmetics and dermatology treatments. Based on current fringe projection units using light combined with stereometry such systems offer high-resolution 3-D digital imaging of the full face as well as other body areas (Fig. 3). Advantages over conventional fringe projection are that this technique enables assessment of different or combinations of parameters from just one single acquisition, for example, large areas such as whole face; specific lines and wrinkles' skin colour parameters; and ptosis/sagging eye bags.

Pigmentation

Changes in the distribution of skin pigment increase with age, and pigment spots (age spots), age-associated yellowing, and melasma (age-related hormonal changes in females) are common occurrences [30, 31].

Melasma severity is visually evaluated using the *Melasma Area and Severity Index* (MASI) [32]. Chromametry and digital image analysis can be performed on selected facial areas. Skin colour measurements with handheld chromameters for both spots and melasma are assessed with the L*a*b* system in which L* (white/black axis) is the quantity of reflected light or skin brightness ranging from total black (L* = 0) to total white (L* = 100), whereas b* (blue/yellow axis) is indicative of pigmentation (yellowness) and a* (red/green axis) reflects the degree of skin redness [32].

In order to quantify skin pigmentation, the *Skin Tan Value* or ITA° [ITA° = arc tangent (L* − 50/b*) × 180/π] is calculated [33] and is shown to be inversely correlated to skin pigmentation. Digital macrodermatoscopic photographs together with image analysis are used to assess the pigment densities. The papillary structure in pigmented age spots (and even melasma) can also be assessed with image analysis. The advantage is that it can analyse the exact size, colour and surrounding contrast of a single spot from colour consistent images. Pigment spot changes over time can also be easily assessed (Fig. 4). Skin colour homogeneity as in melasma and colour cosmetics can also be measured–the variation in the colour is

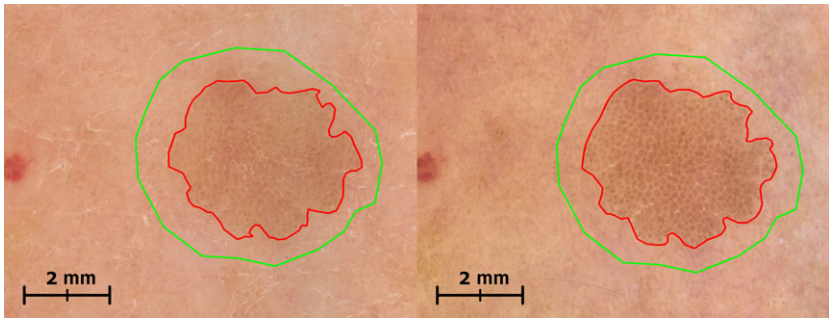

Figure 4 An age spot (lentigo senilis) is displayed in winter (left image) and summer (right image). After conversion of colours to CIE L*a*b*, detection of the spot area (red line) and demarcation of the surrounding area (between red and green line) the contrast of the spot can be measured by image analysis. Since the spot is more prominent in summer, this effect must be taken into account when assessing the efficacy of cosmetic spot treatments in studies extended over several months.

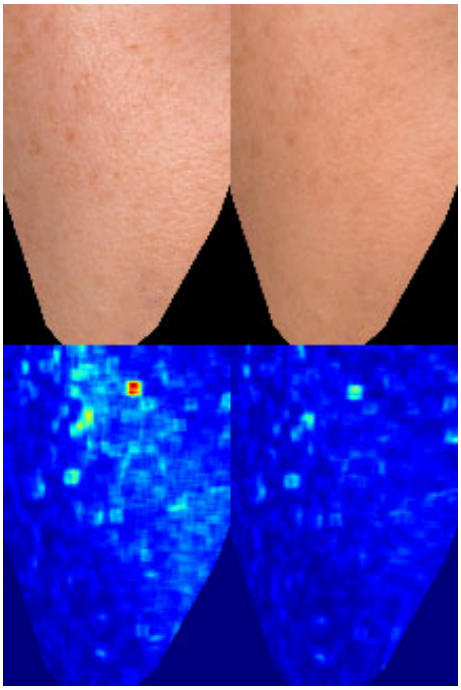

Figure 5 Assessment of skin colour homogeneity by image analysis using the moving windows technique. A region of a volunteers cheek is displayed before (left image) and after (right image) a cosmetic treatment to produce even skin tone. After transforming image colours to CIE L*a*b* a squared window chosen slightly larger than the typical colour irregularities is moved pixel by pixel across the image. After each movement step, the colour variation inside of the window is calculated as the standard deviation of b*. The colour-coded images (lower left and lower right image) display the result. High irregularity in colour is shown as yellow and red colours, whereas blue shows regions with almost good colour homogeneity. The improvement on treated skin (right images) can be seen more clearly in the colour-coded images. From the colour-coded images, an overall inhomogeneity parameter can then be calculated.

assessed as the parameter for homogeneity using a moving window approach (Fig. 5) and the feature calculated for each small window as the inverse of the standard deviation of all neighbouring windows [34].

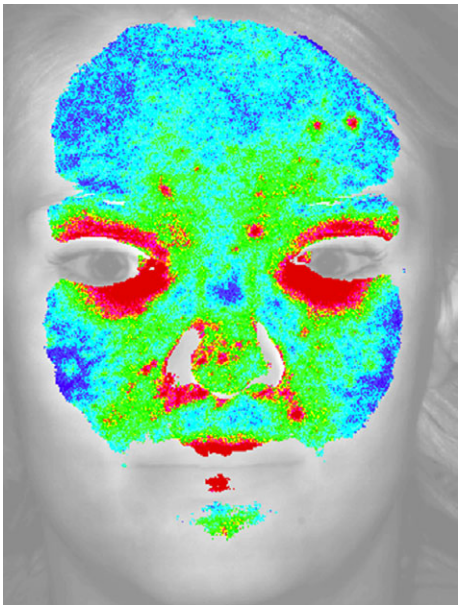

Figure 6 Blood perfusion imager showing the quantified blood flow by colour coding. Blue means low blood flow, and red means high blood flow. The background video image allows detailed allocation of the blood flow to the body area. As the laser beam is spread and not focused on a point, the device is eye save.

Colour and radiance

The age-dependent changes in facial skin imperfections such as wrinkles continue to be a cosmetic priority in many anti-ageing skin developments. In recent years however, the age-dependent changes in the optical-reflection characteristics that create a perception of shine or glow of the skin have only recently received a new focus. Reports have indicated that skin surface and subsurface reflection characteristics will show age-dependent changes [35–37] with younger skin having greater subsurface reflectivity and a more even surface reflectivity. Such characteristics might relate to a consumer perception that younger skin is brighter and more radiant with an internal glow, whereas aged skin is duller, shinier and glossier. Colour can be analysed by standardized image analysis

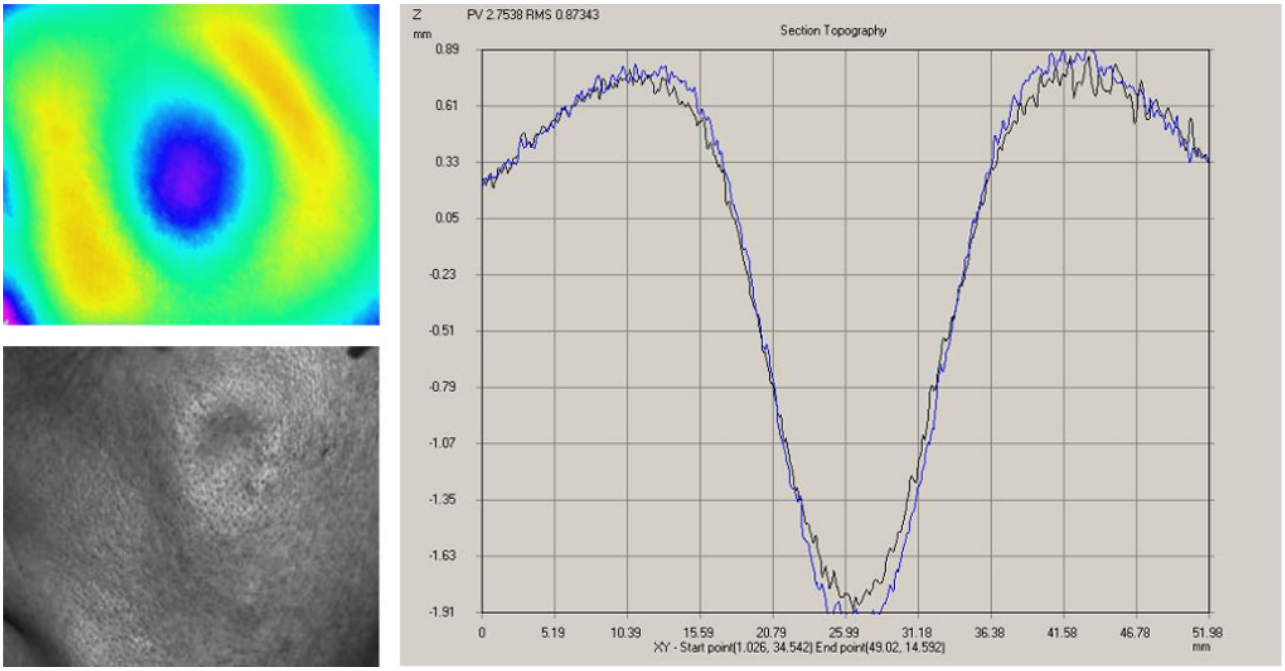

Figure 7 Measurement of skin firmness producing a standardized skin deformation/indentation using an air blow device onto a specific facial area. The 3D shape of the indentation is measured and from that skin firmness parameters are calculated.

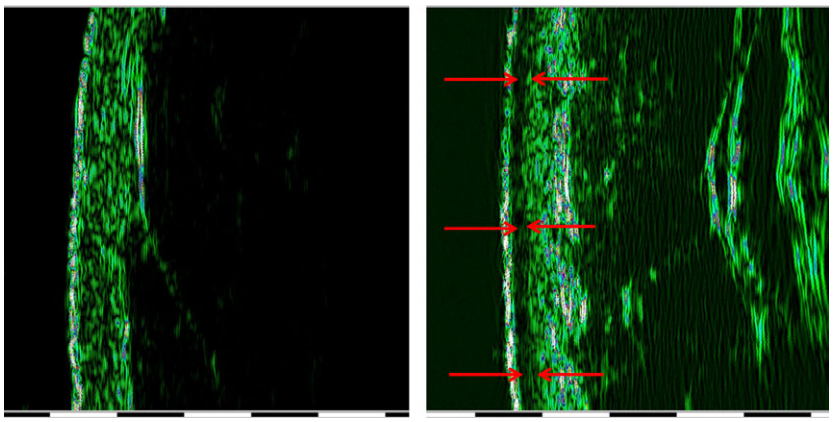

Figure 8 Photo-aged skin on the outer forearm shows the dermal collagen damage (red arrows right side image) as an echo-poor region, when assessed by 22 MHz ultrasound. The sun protected volar forearm does not show the echo-poor region (image left).

algorithms and radiance and attractiveness rated from digital photography including wrinkles [34, 38].

With age, the degree of natural 'colour' decreases with the loss of 'glow' and radiance. More in-depth approaches can evaluate the skin 'colour' in terms of vascular parameters such as blood flow. For example, the use of a blood perfusion imager–a non-contact and non-invasive technique which images blood flow in skin capillaries by use of laser speckle analysis [39]–enables a visual and quantitative process (using image analysis) of following the effects of a product (Fig. 6). Blood flow changes upon application of a cosmetic product can be quantified with image analysis of high definition colour photographs.

Multiple Spatially Resolved Reflection Spectroscopy (MSRRS) a sensor to measure carotenoids, uses several differently positioned light emitters and light detectors, exhibiting different distances between emitter and detector and different angles for irradiation and detection [40].

Human skin contains various antioxidants [41] in order to counteract free radical production caused by both internal and external stress factors. Protection mechanisms via topical or oral

application such as carotenoids are known to be highly concentrated in the stratum corneum [42] and can serve as marker substances for the complete antioxidant status of the human epidermis [43]. There is a general consensus that antioxidants help fight free radical damage and can help maintain healthy skin, by affecting intracellular signalling pathways involved in skin damage and helping to protect against photo-damage, as well as preventing wrinkles and inflammation [44]. There are also some epidemiology findings that suggest that dietary intake plays an important role in skin wrinkling [45]. Protection mechanisms via topical or oral application such as carotenoids are known to be highly concentrated in the stratum corneum [42] and can serve as marker substances for the complete antioxidant status of the human epidermis [43]. MSRRS is a sound-validated method for assessing the content of carotenoids in the skin as a marker of the skins antioxidant integrity. It is known for example that the skins antioxidant system (carotenoids) improves with the addition of such actives in topical products, and in the presence of UVA, these levels decrease [44]. This method is useful for topical products such as sunscreens as well as the evaluation of antioxidants in oral supplements [45].

Skin surface pH

The discovery of the acid mantle of the skin and its importance was confirmed with a gas chain bell electrode [46]. Potentiometric measurements and glass electrodes were then subsequently developed and validated [47]. Gradual changes in the skins surface pH as we age have been reported [48, 49]. The implications for these changes suggest that the increased pH especially in females could lead to a more sensitive skin [50] especially on the cheeks.

Methods used for evaluating skin surface pH changes include colorimetric methods, skin buffering capacity and potentiometry. As the understanding of the skins pH widens, there is a potential role for Raman spectroscopy [51] (and electron spin microscopy imaging) as further research tools. For example, a Raman spectrum can be measured inside the skin at a selected depth from its outer surface. The pH value is computed using a function that assigns this value as a function of the measured Raman spectrum. The computation may involve calculation of a number representing a ratio of concentrations of a protonated and a deprotonated version of a chemical (e.g., urocanic acid) from the Raman spectrum and generating pH information on the basis of this number [52].

Firmness, elasticity and echo-density

Of the changes apparent in skin ageing, the loss of skin resilience and resultant skin sagging as a consequence of both intrinsic and extrinsic factors is clearly visible [53, 54]. A number of clinical methods based on skin deformation are available to assess these parameters–one method uses torque deformation created by angular rotation of a probe fixed to the skin; a second method uses a mechanical indentation technique; and a third approach uses a device which creates skin distortion via suction (Figs. 5 and 6). Claims relating to skin firmness and tone can be supported through these methods.

A more recent non-contact method uses air to provoke skin deformation. An air-blowing device is an add-on instrument to a fringe projection system. It provides firmness assessments by blowing air perpendicular to the skin area of interest (Fig. 7) and then measuring the indentation with a 3D fringe projection sensor.

Confocal laser microscopy for epidermal and dermal histopathology – Age-related epidermal and dermal changes can be quantitatively assessed by means of *in vivo* confocal microscopy and has been validated as having good potential for skin ageing 'assessment' scoring [55–57].

Ultrasound–The echo-density of the dermis can be evaluated using an ultrasound or echography technique. Here, echo-poor regions are indicative of a damaged dermis due to degradation of the tissue matrix (Fig 8). This technique remains valuable in the assessment of ageing skin [58].

Microbiome balance

The skin is naturally covered with its own microbiome [59], and these are unique to each individual, with no two persons microbiome being exactly identical. Each skin's microbiome plays a key role in the maturation and homeostatic regulation of keratinocytes and immune system [60] with disfunction of both implicated in ageing processes and disease [61]. As our primary connection with the external environment, the skins' microbiome biodiversity is heavily influenced by many external factors, including the biodiversity of our intimate external and internal environments, lifestyle habits and exposures. These include poor diet, disease, hygiene, smoking, cosmetics, pollution, UV, drugs, etc [62]. It is the consequences of microbiome disturbances and composition that will also lead to increased skin (oxidative) stress, etc., thus an increase in certain defined ageing parameters [63]. These can be assessed and evaluated using many of the methods described herein. A suggested reason that internal ageing changes the skin microbiome could be the age-related increase in skin pH [64]. Less acidic pH promotes bacterial growth, especially Gram-negative bacteria and propionibacteria, whereas acidic pH boosts the activity of antibacterial lipids and peptides, facilitates production of natural antimicrobial peptides, wound healing, and regulating keratinization and desquamation processes [65].

The use of cosmetics for aged skin with the capacity to reduce skin pH and rebalance the skin microbiome may well be beneficial in preventing pathological skin conditions in older age. Measurement of skin pH and assessment of skin microbiome are relevant tools to help prove efficacy of these types of products.

Concluding remarks

An ever-widening variety of so-called anti-ageing products and treatments are available to the consumer, and many of these are overtly advertised with little evidential support. Despite legislative demands for credible supportive evidence, it is still disconcerting that most of these products only rely on *in vitro*/supplier data or small user trials. Few anti-ageing products have efficacy proven by evidence-based clinical studies. With the ability of many clinical methods able to be combined with *in vitro* and *ex vivo* procedures, they can add further value to consumer perception and ageing research knowledge. Furthermore, the development and technological advances in clinical methods for both dermatology research and product claims support should also aim to drive legislation.

Since most clinical measurement methods assess endpoints that cannot be perceived by the consumer, it is vital prior to any study commencement, to establish clear sound study objectives with the sponsor and clinical development team – taking into account both regulatory requirements as regards potential claims, as well as consumer perception. When designing a study protocol, these points

must be taken into consideration. To encourage equivalence between clinical and consumer significance, involvement of the study volunteers throughout the study execution through simultaneous self-assessments, for example, is important in relation to consumer relevance and acceptance.

Moreover, with both scientists and consumer groups constantly maintaining that anti-ageing advertising claims of the cosmetic industry are frequently misleading, a more ethical approach to anti-ageing should be considered and focus more on health and well-being rather than unachievable eternal youth.

Acknowledgements

This work was fully funded by proDERM GmbH.

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