

# EEMCO Guidance for the *in vivo* Assessment of Biomechanical Properties of the Human Skin and Its Annexes: Revisiting Instrumentation and Test Modes

Luis Monteiro Rodrigues<sup>a</sup> Joachim W. Fluhr<sup>b</sup> the EEMCO Group

<sup>a</sup>CBIOS – Universidade Lusófona Research Centre for Biosciences and Health Technologies, Lisbon, Portugal;

<sup>b</sup>Department of Dermatology and Allergology, Charité – Universitätsmedizin Berlin, Berlin, Germany

## Keywords

Mechanical properties · Elasticity · Skin · Hair · Nails · Testing methods · Testing modes · Anisotropy · Instrumental measurements · Cosmetics efficacy

## Abstract

Biomechanics of the skin is an important subject in skin research. It has been studied for many decades involving various technologies and methods to characterize and quantify mechanical properties of the skin under different *in vivo* conditions. The present EEMCO paper reviews the current relevant information, providing practical orientation to researchers dedicated to *in vivo* assessment of biomechanics of skin and its annexes. We discuss the available non-invasive instruments, including their principles and variables. A correspondence between the descriptors nomenclature proposed by Agache and the designation for the suction-based standard instruments is proposed. The addressed properties include skin softness/stiffness, firmness, elasticity, elastic and viscoelastic properties, extensibility, resilience, anisotropy, acoustical shock wave hardness, friction (in relation to topographic properties), thickness, fiber/stress mechanics (bending, cyclic, tensile, fatigue, or torsion), and hardness. We provide the relation of these properties to biomechanical descriptors and in some cases to SI units. Practi-

cal guidance for the proper use of these instruments, limitations, and possible interpretations are provided, while discussing the meaning of descriptive or “phenomenological” variables. For studies intended to quantify the effect of an intervention with regard to mechanical properties, we recommend a minimum of 30–40 participants, based on normal distribution of the data sets. Some important limitations are recognized, including the lack of standardization of procedures and calibration of instruments, which compromises the relevance and real nature of the descriptors/parameters obtained with these devices. The present work highlights an approach to a better practice and a science-supported biomechanical assessment of human skin, hair, and nails.

© 2019 S. Karger AG, Basel

## Introduction

Biomechanical properties are an important theme in skin research. It has been studied for many decades [1, 2] and has fostered a variety of related knowledge which in-

EEMCO Group (<http://www.eemco.eu/>) current composition: L. Monteiro Rodrigues (Chairman; POR), Enzo Berardesca (I), Virginie Couturaud (F), Joachim W. Fluhr (D), Marie Loden (S), P. Masson (F), Hassan Zahouani (F).

cludes diverse technologies and methods to understand, characterize, and quantify its properties under different *in vivo* conditions. Almost 20 years ago, the EEMCO group published two papers on this topic for human skin [3, 4]. These papers reviewed the knowledge and discussed the available non-invasive instruments, including their principles and variables. In addition, practical guidance for their proper use, limitations, and interpretation was provided, while discussing the meaning of so-called phenomenological variables [2].

Extensive research on different aspects of skin “anisotropy,” a term broadly used to address the different orientation patterns of mechanical stress, has since been published [5–10]. Variation with skin thickness, the specific contribution of skin layers to this property, and distinctions related to anatomy and gender have been addressed [3, 11]. This research has been approached from the skin ageing perspective and, furthermore, by aesthetic dermatology, regenerative medicine, and cosmetology. It has been suggested that skin biomechanics is a dynamic process, also influenced by other parameters, e.g., structural biomics, epidermal water and lipid content, as well as microcirculation [12, 13]. An accurate quantitative assessment of skin biomechanics is useful for characterization of some skin diseases and to monitor the efficacy of therapeutic/aesthetic/cosmetic interventions.

Additionally, the fast-growing cosmetic market of makeup and haircare products expanded research on hair and nail properties. The efficacy of cosmetics on hair characteristics, including mechanics, is a consistent issue for clinical and aesthetic dermatology. The complexity of this keratin-material, a consequence of a very particular alignment of its components, structure, and substructure, explains the different deformation modes and behaviour in different environments (humidity/water content), all directly relating to hair tensile properties. Hair anisotropy, primarily caused by the orientation of keratin fibres, has been explored as a comparator [14–16]. Recent evidence suggests that the complex mechanics involved in bending and twisting, very different from the longitudinal stretch deformation, also depends on longitudinal shear stress [17]. In the last 20 years several instruments have been developed to measure the mechanical properties of human skin, hair, and more recently, fingernails [18].

The EEMCO publication of 2001 [4] is one of the most cited papers in *in vivo* evaluation of skin biomechanics. After 20 years, a full update of instrumentation and measuring modes is justified. The choice of which equipment(s) and measurement parameter(s) are adequate to

evaluate skin mechanical properties is still under discussion. Each device generates several parameters, adding to the complexity and discrepancy between those “descriptors” and physical mechanical equivalents obtained from elastic or plastic materials [4]. However, it is not yet fully understood if and how descriptors obtained from each technique are related to each other, nor their exact clinical relevance.

The present paper revisits and extends the scope of the previous publications to recent domains including commercially available innovation such as hair and nail biomechanics. It is not a systematic review but rather a compilation of information gathered by the authors considering the journals’ relevance in the area. Characterisation of commercially available instruments (e.g., Table 1) exclusively results from information obtained at each manufacturer’s website. The search options were determined by the relative presence in PubMed (e.g., for suction methods with approx. 300 references, 90% used the Cutometer<sup>®</sup> system) while new devices and/or applications were chosen on the basis of the scientific novelty and content consistency. Ultimately, the aim is to add practical objective orientation to researchers dedicated to better understanding the *in vivo* biomechanics of skin and its annexes.

### Measuring Systems for Biomechanical Assessment

Most of the currently used systems to assess the biomechanical properties of human skin are based on imposing a variable load, vertical (positive or negative), horizontal, or linear, on the measuring surface [4]. Some of these instruments are commercially available (Table 1). The Cutometer<sup>®</sup>, the Dermal Torque Meter<sup>®</sup>, and the DermaLab<sup>®</sup> are certainly still in use. Advances in electronics and basic knowledge led to an evolution of older principles (Indentometer<sup>®</sup>, Elastimeter<sup>®</sup>, Durometer<sup>®</sup>), but also promoted new approaches such as the microconformal modulus sensor, the CutiScan<sup>®</sup>, and the Khelometer<sup>®</sup>.

Studies on mechanical properties of human hair and wool were initiated more than 90 years ago [19–21]. The main motivation, to understand the mechanisms of hair damage and to study the impact of hair treatments on hair properties, was the consequence of a fast-growing consumer market (dyes, wave lotions, bleaches, and straighteners). The first studies on human hair mechanics received a valuable contribution from the textile industry, considering the experience gathered around wool [19, 22,

**Table 1.** Summary of commercially available systems

Devices	Addressed properties	Biomechanical descriptors/units
Indentometer IDM 800	Skin softness/stiffness	Depth (mm)
Cutometer <sup>®</sup> Dual MPA 580	Skin firmness, elasticity, elastic and viscoelastic properties, extensibility, resilience	R1-R9, F and Q parameters (mm/AU/AUC)
CutiScan <sup>®</sup> CS 100	Viscoelasticity and anisotropy	V parameters (mm)
Reviscometer <sup>®</sup> RVM 600 <sup>1</sup>	Acoustical shock wave hardness	Resonance running time (RRT)
Frictiometer FR 700	Skin friction (in relation to topographic properties)	Friction (AU)
Nail StrainStress Meter NM 100	Firmness, elasticity, and thickness	Deformation (AU), resistance (AU), thickness (mm)
DermaLab <sup>®</sup> and DermaLab Combo <sup>®</sup>	Elastic properties	Young's modulus (N/m <sup>2</sup> ), retraction time (s), and viscoelasticity (AU's)
Dermal Torque Meter <sup>1</sup> Torsional Ballistomer <sup>®</sup> (BLS 780)	Skin firmness and elasticity	Indentation (mm); K as the start height of the probe tip above the skin surface (AU); Alpha as the rate of energy damping (AU) coefficient of restitution – CoR (AU), and area
Dia-Stron MTT 690 <sup>®</sup>	Fibre/stress mechanics (bending, cyclic, tensile, fatigue, or torsion)	Stress/strain curves with break detection, stress relaxation, hysteresis, and creep
Elastimeter <sup>®</sup>	Skin elasticity	Instant skin elasticity (ISE) (AU)
SkinFibroMeter <sup>®</sup>	Skin and subcutaneous induration	Force (N)
Durometer <sup>®</sup>	Skin hardness	SU (shore units)
DynaSKIN <sup>®</sup>	Skin firmness and elasticity	Deformation (AU)

Available systems to assess biomechanical properties of the skin and its annexes. Respective properties and measurement descriptors/parameters are also indicated (all information gathered from the manufacturer's websites). AU, arbitrary units; AUC, area under the curve. <sup>1</sup> Currently not available (the Dermal Torque Meter has been replaced by the Torsional Ballistomer (BLS 780)).

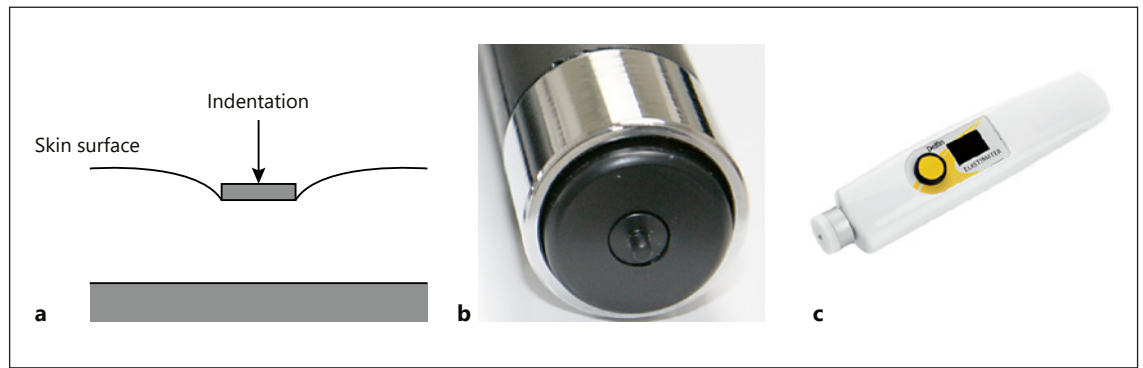
23]. It rapidly evolved to a specific area of knowledge, adapting known instruments to study the physical and mechanical properties of human hair [24]. The Dia-Stron MTT 690<sup>®</sup>, a pneumatic dynamometer, became a reference for hair fiber tensile studies, providing full assessment of hair mechanics [25] (Table 1). The main features have been addressed in recent research papers and reviews [16, 24, 26].

#### *Instruments and Descriptors for Skin Biomechanics Assessment*

In the last 15 years, several comprehensive reviews of the available equipment to measure the biomechanical behaviour and properties of the human skin have been published [12, 13, 27–29]. The growing interest in this domain also encouraged the development of new instru-

ments, novel approaches, and new applications of already established principles, but have not all reached the market.

“Indentometry” is an example of this evolution, long used to calculate the Young modulus by the deformation of skin and underlying tissues. Several systems have been developed by applying the Hertz theory of contact mechanics, quantitatively described by the Kelvin-Voigt model [30]. This technique, developed to quantify stiffness/hardness/firmness/softness, was successfully applied to in vivo skin studies [8, 29, 31, 32], especially to skin ageing, to in vitro skin models [30], and related medical applications [33]. The measurement principle of the Indentometer IDM 800<sup>®</sup> (Courage and Khazaka, Cologne, Germany) and the Elastimeter (Delfin Technologies, Finland) is based on skin deformation induced



**Fig. 1.** Illustration of the working principle of “indentometry” (a): probe head from the Indentometer IDM 800, CK Electronics (b), and a portable Elastimeter unit from Delfin Technologies (c). Measurement is based on the force (by a spring) used on the small indenter of the probe to deform the skin. The device measures how the probe indenter displaces the skin (the firmer/stiffer the skin, the lesser displacement). According to the manufacturer, the pen-

etration depth of the pin is measured in millimetres and an instant skin elasticity (ISE) can be obtained with the Elastimeter (from [www.courage-khazaka.de/en/scientific-products/all-products/probe-systems/16-wissenschaftliche-produkte/alle-produkte/173-indentometer-e](http://www.courage-khazaka.de/en/scientific-products/all-products/probe-systems/16-wissenschaftliche-produkte/alle-produkte/173-indentometer-e) and [www.delfintech.com/en/product\\_information/elastimeter/](http://www.delfintech.com/en/product_information/elastimeter/); accessed May 7, 2019).

by forces of the instrument’s probe, displacing the skin (Fig. 1). The firmer/stiffer the skin, the less deep is the registered displacement. More recently, the SkinFibroMeter<sup>®</sup> (Delfin Technologies) was introduced to measure the force of induration on skin and subcutaneous tissue [34]. Neither the Indentometer IDM 800 nor the SkinFibroMeter have been sufficiently reviewed to allow a proper evaluation of their data quality and applicability.

The Durometer (Rex Gauge, IL, USA) measures the hardness of non-metallic materials and was the first instrument to measure skin hardness [33]. The system contains a calibrated gauge by which a good reproducibility and accuracy have been reported [33, 35]. “Durometry” is, therefore, a technique with great interest, for recognized accuracy but also for its ease of use. Nevertheless, its dependence on the subcutaneous tissue, which is needed for a more precise measurement, might limit its application in normal skin and cosmetic testing [33] (Fig. 1).

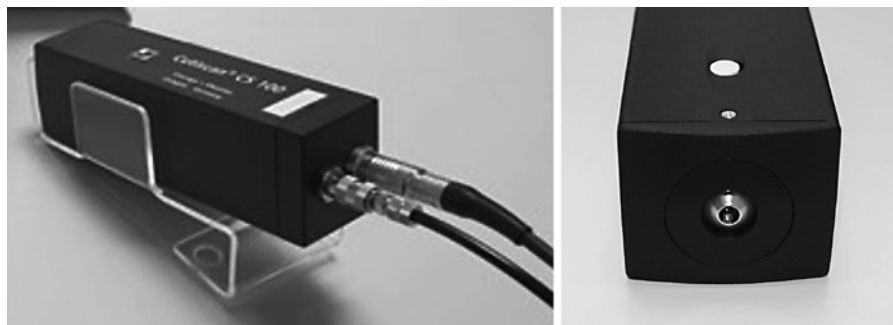
A few nano/microscale indentation techniques provided interesting information on the mechanical contribution of individual skin layers. One paper on porcine skin samples suggested that the stratum corneum elastic modulus is approximately three times higher than that of the dermis [11]. The authors concluded that for relatively shallow and deep indentations, skin elasticity is differently determined by the stratum corneum and dermis, respectively. This model, supported by microscopy observations and indentation measurements, further suggested that skin deformation could be interpreted in

the context of a layered structure model consisting of a stiff and hard surface layer on a compliant and soft substrate.

Opto-electronic sensors or strain gauges provided innovative approaches by recording the resistance of skin to deformation and its recovery with time. An alternative non-mechanical approach determines the propagation of shear waves [5]. The measured variations primarily depend upon the hardness of a given material, including skin. The Reviscometer RVM 600<sup>®</sup> (Courage and Khazaka) is based on the transmission of an acoustical shock wave across the skin and in the measurement of the resonance running time (RRT) [36]. RRT might also be measured in different orientations (0–360°) providing a semi-quantitative assessment of anisotropy, mainly as a function of the RRT<sub>max</sub>/RRT<sub>min</sub> ratio [5–7]. The measuring principle of the device is especially influenced by the pressure used on the skin. A higher RRT means the waves take more time to propagate, e.g., the less stiff is the skin [5]. However, this method is still under discussion since different researchers found both positive and negative significant correlations between RRT and skin elasticity associated to ageing [7, 8, 31]. At the time of writing, this system was not commercially available (Table 1).

The CutiScan CS 100 (Courage and Khazaka) is a device that quantifies skin elasticity over 360°, designed to assess *in vivo* skin biomechanics including anisotropy and directionality. According to the manufacturer, the probe (Fig. 2) combines mechanical force and imaging, including a built-in suction ring that draws the skin uni-

**Fig. 2.** Measuring probe of the CutiScan CS100, equipped with a 14-mm-diameter suction ring which draws the skin uniformly at a constant pressure for a defined time span and then releases the applied pressure. A high-resolution CCD camera inside the probe monitors the displacement of the skin during suction and release by an optical flow algorithm (Horn-Schunck method), generating the graphical representation of the movement from which several quantifiers are obtained [37].



formly in all directions with a constant negative pressure [37].

The system provides a high-resolution video of the skin movement during suction and release, generating an “elasticity curve” graph showing the displacement (height in a 3D graph) over the measurement time along with new mechanical descriptors [37]. The maximum displacement during suction time, called V1, relates to “firmness” and indicates the ability of the skin to resist the displacement. The less firm the skin, the higher V1. The returning rate during the relaxation time V2 represents the ability of the skin to retract to its original state. The closer V2 values are to the maximum amplitude V1, the higher the return rate and the better the viscoelasticity. The software also generates a V3 graph, showing a curve of the ratio of V2/V1 expressed as a percentage. It relates to the ability of resisting the displacement versus the ability to return to the original position. The higher the V3, the better the elasticity. The more uniform the curve, the less anisotropic the skin. The CutiScan parameters have been compared with those provided by Cutometer and Reviscometer<sup>®</sup> in delivering some relationships, while providing more detailed information about skin anisotropy through a 360° analysis [8]. Active 3D representations of in vivo skin biomechanics were proposed by the displacement analysis of time-angle-height provided by the CutiScan. Age-related and anatomically based differences in the viscoelastic profile in vivo could be identified [38, 39].

The extension assessment consists of a test with two moving pads attached to the skin surface and measurement of the force induced by the pad displacements. Recent advances in this methodology have addressed the issue related to the earlier instruments held by a stand. In the early models forces from interactions between the tested skin and the experimental device had to be kept sufficiently small to be neglected, or measured to be taken into account during analysis [40]. To solve this problem,

various prototype hand-portable devices operating directly on free skin were developed to be insensitive to small body movement disturbances [9, 41]. Recently, an ultra-light prototype extensometer device was developed to perform various uniaxial tensile tests with either effort or displacement control [40]. This traction test is complemented with full thickness measurements, by ultrasound, which allows the acquisition of stress-strain curves while a parallel imaging unit enables the image recording of the area and strain fields along with the respective digital analysis.

The development of biosensors provided solutions to deal with practical difficulties related to measuring in high mobility curved anatomical areas (e.g., joints) [42, 43]. A microconformal modulus sensor system enabling soft and reversible conformal contact with the underlying complex topography and texture of the human skin was designed to provide accurate and reproducible non-invasive measurements of the viscoelastic modulus under both quasi-static and dynamic conditions [44]. The use of ultrathin, stretchable networks of mechanical actuators and sensors constructed with nanoribbons of lead zirconate titanate and attached via soft, reversible lamination onto the skin, enables rapid, quantitative assessment of viscoelastic moduli, with the ability for spatial mapping [42].

An electro-mechanical device, the Khelometer (Asahi Techno Lab, Japan), was introduced for measuring the lateral stiffness/rigidity of the skin using a specially designed probe applicable to measure all anatomical regions [45]. After being calibrated with different stiffness elastomer substrates, the device records the force opposing to a progressive lateral constraint (slightly compressing). The small amplitude and speed of the lateral constraint can be adjusted according to the requirements of the anatomical site. Results from preliminary studies suggest that this device is adaptable to almost all anatomical skin sites, the

scalp being the most evident example of specific requirements, with higher stiffness than other body areas. The lateral skin deformation can be adjusted (length of displacement, application time) according to the specific requirements (e.g., size, stiffness, presence of hair) of the anatomical site to be studied [45]. This device measures skin stiffness in N/mm.

High-frequency ultrasound elastography has been developed to assess in vivo skin biomechanics by the shear wave velocity from intrinsic deformation induced by arterial pulsation [46]. Elastography estimates mechanical properties of the tissue. High-frequency ultrasonography provides high-resolution measurements, allowing the in vivo assessment of flexibility and retractability, which results from a combination of viscoelasticity and microstructures unevenly distributed within the dermis. According to the authors, the shear wave velocity, estimated from the measured velocity, was 0.14 m/s in the epidermis, and 0.06 m/s in the dermis. Due to the proportionality of the stiffness of the skin and the shear wave velocity, the authors concluded that the stiffness of the epidermis was higher than that of the dermis [46].

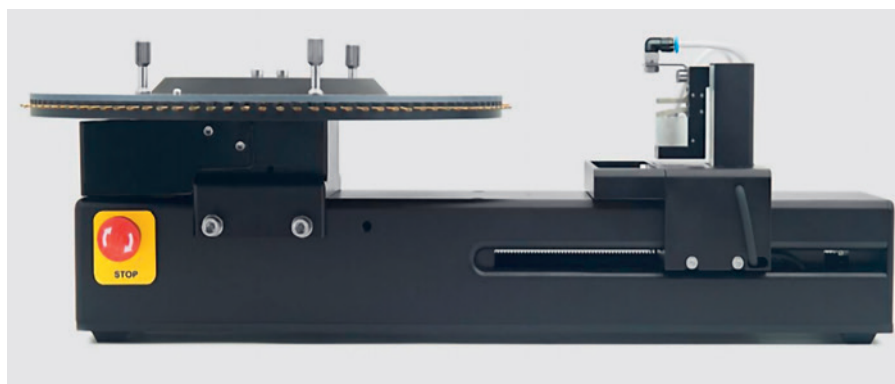
Non-contact techniques have been developed to overcome some of the restraints associated with contact devices. The weight of the probe/device in contact with the skin, or the adhesive tape that is used to keep the probe in a constant position, can change its mechanical behaviour. In fact, it has been long known that the double-sided adhesive tapes used in this type of measurements show time-dependant creep deformation [47]. Non-contact techniques may eliminate these artefacts and other potential sources of measurement errors [4, 12, 48]. Prototypes of non-contact devices using air flow pressure have been employed in vivo for skin biomechanics and in ageing studies [49, 50]. Only recently, a non-contact optical measurement technique based on three-dimensional digital image correlation (3D-DIC) was applied to skin mechanical analysis and compared with the established Cutometer [51]. According to the authors, 3D-DIC allows a visual mapping of mechanical metrics at the measurement surface. They claim that the new device provides better precision and higher accuracy measurements by being less prone to the variability due to the subject's in and out-of-plane positioning when compared to 2D imaging techniques. Moreover, measurements are preceded by the construction of a pixel-to-length scale, a calibration procedure that ensures accuracy of all image correlations of grey value variations between the target and reference images [51]. Skin mechanical properties are described in terms of major strain, minor strain, and displacement.

Significant relationships between 3D-DIC and Cutometer descriptors were calculated for  $\mu$  (displacement) and R5, R7, and R8 (see below) [51]. The 3D-DIC does not impose any mechanical displacements itself, which reduces this source of error. Moreover, it provides directional and spatial information which can be associated with the mechanical properties. This is not possible using conventional (contact) methods [38]. Another potential interest might come from those mechanical metrics not related to the Cutometer descriptors, as other biomechanical-related properties or views may be obtained [51].

A new device, the DynaSKIN, using non-contact mechanical pressure in combination with fringe projection is able to quantify and visualize the skin response in 3D [52]. The DynaSKIN, in contrast to other pressure-related devices, uses a positive pressure to deform the skin and test its response to mechanical force application. The instrument releases a force-calibrated jet of air, close to the skin surface, that indents the skin simulating, according with the authors, the consumer's tactile judgement of firmness. The deformations' 3D geometry and deformation recovery are quantifiable. These parameters correspond to the mechanical properties of the skin described by its stiffness or firmness. The larger the deformation, the less firm the skin [52]. The interest of this new instrument is indisputable, but more studies are needed for a proper evaluation of its usefulness.

#### *Hair Biomechanics Assessment*

Tensile strength analysis of single hair fibres is a regular requirement for haircare industry development to provide a measure for efficacy and exposure tests for specific haircare products. Hair mechanical characterization essentially involves resistance to stretch (strain rate), elasticity, and hydrophilic power [16, 24]. Hair behaves both elastically and plastically within the elastic and transformation regions [16]. Hair shows different strain-rate behaviours in different ambient humidity and temperature ranges. Stretching is a hair attribute under the action of a distal force (length), returning to the initial dimension when force stops. When dry, the hair thread may stretch 20–30% of its length, and when wetted this may reach up to 50% [15, 16, 24]. Exaggerated exposure to these variants or other physical and chemical elements, including UV radiation, hairdryers, and heated plates or barrels, modify these properties. Assessment of resistance to stretching (hair rupture tension), elasticity (stress-strain), hydrophilic power, combability, and detangling are of interest for the haircare industry.



**Fig. 3.** The Dia-Stron MTT 690 is a cassette-based automated tensile tester designed for single hair fibre measurements. The system is based on a circular sample cassette, which allows the automatic measurement of up to 100 pre-mounted fibre samples. Hair fibre samples are mounted using brass crimps and placed onto a 100-slot rotary cassette. A pneumatically operated sample gripper,

mounted on a moving bridge, picks up the sample. The gripper is mounted onto a load cell, which measures the force applied to the sample (from the Dia-Stron Manual; <https://www.diastron.com/app/uploads/2018/06/Dia-Stron-MTT690-Brochure-V2.pdf>; accessed May 7, 2019).

Several prototypes, inspired by experience from the textile industry, were progressively applied to human hair mechanical analysis [53, 54], leading to the Dia-Stron<sup>®</sup>, currently the best-known commercially available system. The Dia-Stron MTT 690 system (Fig. 3) offers a broad variety of fully automated tensile hair mechanical testing, providing consistent testing parameters such as strain/stress curve analysis, stress relaxation, and hysteresis. Furthermore, the system allows both wet and dry measurements. A recent publication with this device underlined the importance of torsional evaluations, in addition to tensile deformation, to assess the shear stiffness changes associated with cuticle damage [55]. The authors proposed the Dia-Stron FTT 950 system (Dia-Stron Ltd, Andover, UK) to measure the torsional modulus with adequate consistency and reproducibility. The cuticle layers seem to play a relevant role in the torsional deformation [55].

#### *Nail Biomechanics Assessment*

Nails have been analysed for multiple diagnostic purposes, from toxicological detection of specific components to nutritional imbalances and pathology [56], as metabolic processes, detectable in the blood and bone system, influence the nail bed content [57, 58]. Nail disorders are a common concern, with onychomycosis being one of the most prevalent expressions in the general population, in particular in high-risk patients [59, 60]. There is growing evidence of the importance of nail mechanics in nail physiology and pathology as in other keratin-

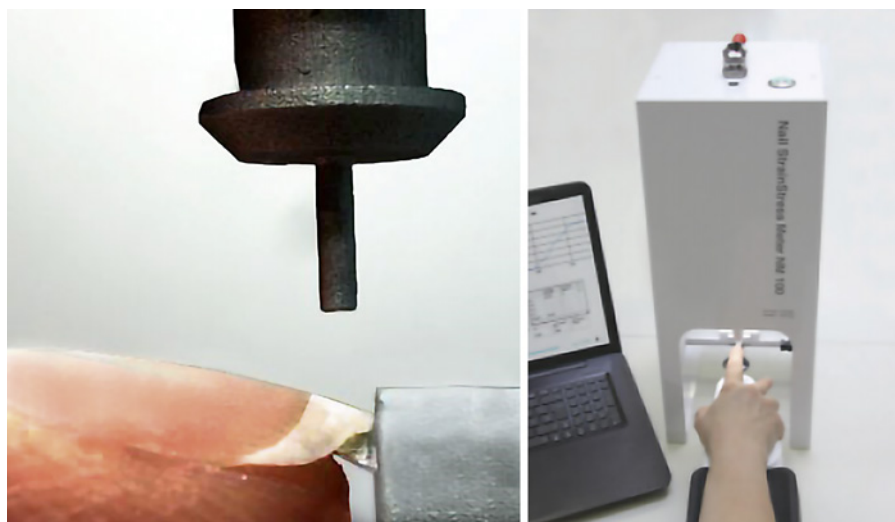
based materials such as skin and hair. When pressed, fingertip haemodynamics change due to mechanical interactions (force/shear force) between the fingernail and bone [61]. It was suggested that nails have an automatic curvature function that keeps the necessary rigidity to adapt to gripping [23, 61]. Conversely, mechanical impairment seems to modify the normal nail configuration, inducing deformations [61]. A typical example is seen in onychomycosis, where disruption of the nail matrix alters the plate rigidity and the physiological nail morphology [60].

Human nail mechanics has not been as thoroughly studied as skin and hair, but several prototypes were produced with that purpose [15]. There is a growing interest from basic research as well as from the pharmaceutical and cosmetic industry in relation to the search for better and safer nailcare products. Recently marketed, the Nail StrainStress Meter<sup>®</sup> NM 100 (Courage and Khazaka) offers, according to the manufacturer, accurate assessment of the nail mechanics in vivo, in terms of firmness, elasticity, and thickness [61] (Fig. 4). Published data are not yet available for a balanced evaluation of this instrument.

#### **Comparing Instruments and Finding the Best Descriptors**

The number of available instruments to measure human skin, hair, and nail biomechanics is increasing, mostly motivated by the need to obtain better defined pa-

**Fig. 4.** Operation with the Nail StrainStress Meter NM 100. The nail is placed on the unit's support and a high precision load cell constantly measures the pressure required to clamp down the applicator. The force needed for the deflection of the nail is displayed in real time. When the head touches the surface of the nail, the pressure increases. A force deflection diagram curve is generated from which transversal deformation, slope, resistance to compression, and longitudinal deformation are calculated ([https://www.courage-khazaka.de/images/Downloads/Brochures/Wissenschaftlich/Brochure\\_NailStrainStressMeter.pdf](https://www.courage-khazaka.de/images/Downloads/Brochures/Wissenschaftlich/Brochure_NailStrainStressMeter.pdf); accessed May 7, 2019).



rameters, closer (for skin in particular) to tensile properties and to the physiological functions of the skin [4]. Hair mechanical analysis (originating from the basic tensile principles applied to wool textiles, as previously discussed) offers no major concerns, but nail mechanics, although far from fully explored, follows most of the considerations and concerns discussed for skin. In fact, it is still difficult to relate those “phenomenological” variables to physical parameters used in the tensile analysis of pure materials. Furthermore, the relation to particular anatomical components of skin (or nail), both at the macroscopic or microscopic level, are not established. A comprehensive study on age-related changes in skin mechanical properties was performed in 120 healthy women over the age range of 18–65 years [62]. A good correlation was detected for the ratio of elastic recovery to distensibility [ $U(r)/U(f)$ ] and gross elasticity [ $U(a)/U(f)$ ] with age on sun-exposed areas.

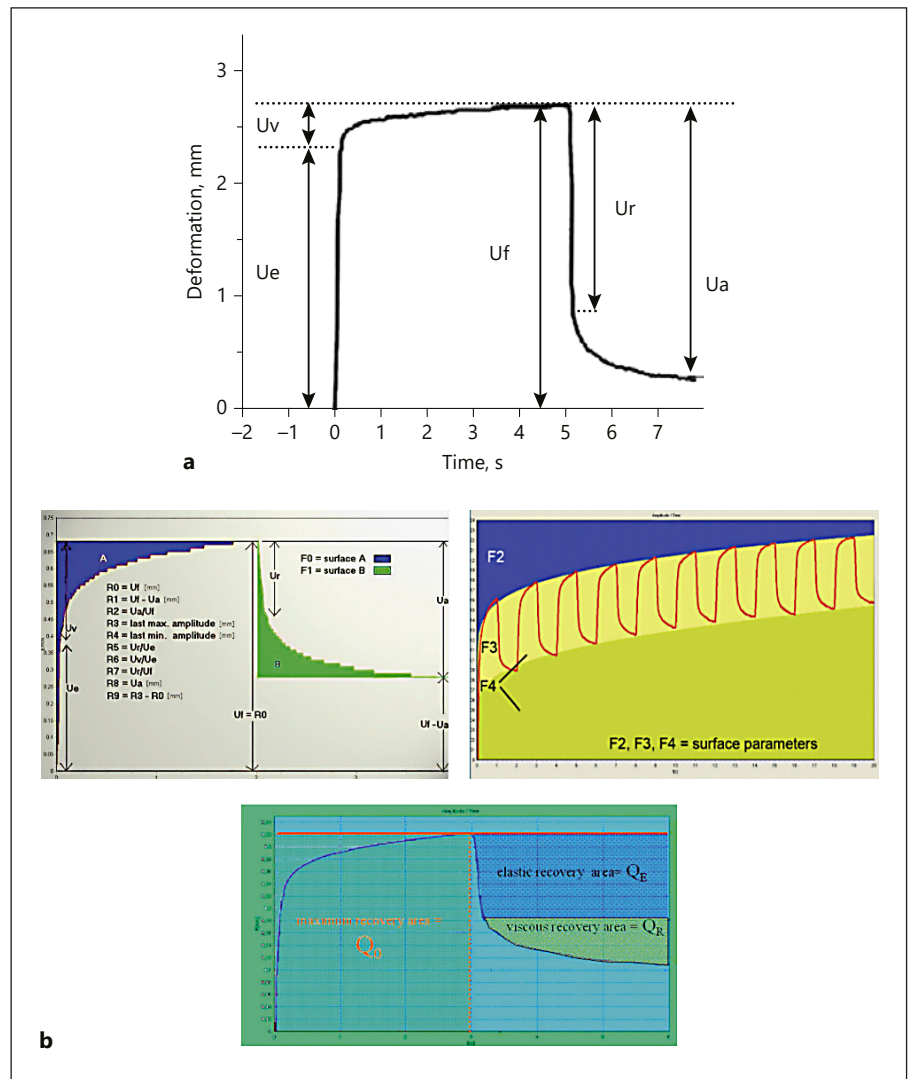
Suction and torsion methods, the most frequently used in basic and in applied skin research [8], produce a typical angular deformation under constant pressure as a function of time (Fig. 5). The deformation curve involves a purely elastic component followed by a viscoelastic and a purely viscous component [63]. From the analogies with the Young modulus, Agache et al. [63] proposed a specific nomenclature to this analysis where  $U_e$  describes the immediate deformation, or skin extensibility,  $U_v$  the delayed distension,  $U_f$  the final deformation, and  $U_r$  and  $U_a$  the immediate and late retraction, respectively (Fig. 5; Table 2). These descriptors, “inspired” by the mechanical behaviour of pure (elastic and plastic) materials, are

meant to facilitate measurements in a complex, multi-layered environment such as human skin. However, in many cases, manufacturers introduced their own descriptors which are difficult to relate to the original definitions. Table 2 summarizes the possible correspondence between the classical and the currently used descriptors from the Cutometer MPA 580 [64].

Several of these denominators, not only the R series but also the F and the Q series, combine the classical parameters in different ways. However, no demonstration of their correlation or correspondence with morphological components or physiological functions has been demonstrated, making the choice of the proper descriptors difficult.

Surprisingly, only a few studies addressed these difficulties and compared the different instruments and parameters. A recent study intending to identify the in vivo relationships in several physiological indicators including elasticity between two commercially available instruments based on the same principle, the Cutometer and the DermaLab, found interesting correlations with good repeatability [65]. The skin firmness measured by the two instruments were negatively correlated, while only the viscoelasticity showed a strong positive correlation in the two instruments. It is important to note that, although based on the same principle, probes are different in shape, weight, and operation. The DermaLab probe is attached to the skin by a double adhesive sticker, which reduces variability caused by the handling pressure. More recently, a correction procedure was proposed to compensate this influence in Cutometer measurements [66].





**Fig. 5.** A typical deformation-recovery sequence obtained by applying a vertical/parallel pressure pulse/torque on in vivo skin and respective descriptors. **a** Nomenclature proposed by Agache et al. [63]. **b** Cutometer's R, F, and Q series from the Cutometer Dual MPA580 (adapted from www.courage-khazaka.de/images/Downloads/Brochures/Wissenschaftlich/Brochure\_Cutometer.pdf; accessed May 7, 2019).

Measurements in the abdominal region with three different devices (Cutometer, Reviscometer, and Frictiometer<sup>®</sup>) were correlated for the different descriptors by factorial analysis [10]. The goal was to simplify the in vivo skin elasticity assessment. No correlations were found between the Frictiometer and Reviscometer; however, significant correlations were detected for the Frictiometer and Cutometer elasticity parameters. There were also some minor correlations between Cutometer and Reviscometer descriptors. According to the authors, different probes can measure different aspects of mechanical properties and the related micro-morphology. Nevertheless, one of the main conclusions was that the number of parameters could be reduced [10]. The Reviscometer was suggested to be used for measurements in selected per-

pendicular directions, instead of measuring in all possible directions. Results seemed to be comparable along the longitudinal axis and the transversal axis. Regarding suction-based devices, such as the Cutometer and the DermaLab, the assessment of skin elasticity can also be reduced to a minimum, selecting the first parameter to analyse from  $U_f$ ,  $U_a$ , and  $U_e$ , and selecting the second parameter from  $U_r$ ,  $U_v$ , and  $U_r/U_f$  [8, 39].

In an extensive study conducted on a panel of volunteers with a wide age range and on different anatomical sites (neck, upper inner arm, ventral forearm, and dorsal forearm) [6], anisotropy was found to be site and age dependent. Interestingly, measurements of the anisotropy ratio at different body sites showed that the age dependence was the most pronounced change occurring on

**Table 2.** Tentative correspondence between the nomenclature by Agache and the Cutometer®

Agache's nomenclature and descriptor abbreviations [63]	Cutometer Dual MPA 580 nomenclature and descriptor abbreviations [61]		
Significance	Designations	Significance	
Immediate, elastic deformation of skin as a consequence of stress (suction or torsion)	Ue	n.e.	–
Total extensibility (maximum deformation)	Uf	R0	Total elongation
Delayed distension	Uv	n.e.	Partially R6
Deformation recovery at the end of the stress-off period	Ua	R8	Total recovery
–	ne	R1 (Uf-Ua)	Residual plasticity
Immediate retraction	Ur	n.e.	Partially R5 and R7
Biological elasticity	Ua/Uf	R2	Biological elasticity
Elastic function	Ur/Ue	R5	Net elasticity
Viscoelastic index or viscoelastic ratio or extension phase during retraction	Uv/Ue	R6	Viscoelasticity
Elastic recovery	Ur/Uf	R7	Firmness
–	ne	R3	Repeated suction: last maximum amplitude
–	ne	R4	Repeated suction: last minimum amplitude
–	ne	F series (F0 to F4)	Areas within the curve
–	ne	Q series (Q0 to Q3)	Other

Tentative correspondence between the descriptors nomenclature proposed by Agache and those proposed for the Cutometer. The descriptors abbreviations are taken from the literature. ne, no equivalent found.

body sites where the skin is considered to be “looser” or “softer,” such as the neck and the upper inner arm. Finally, a correlation of the angular anisotropy with the orientation of dermatoglyphics was demonstrated, a parameter that is related to the orientation of Langer's lines [6]. An additional study compared Cutometer, Reviscometer, and CutiScan, exploring correlations between all different descriptors [8]. This analysis was focused on age- and site-based differences in the biomechanical properties of human skin related to its viscoelasticity and anisotropy. Uf and Ua from Cutometer and RRT from Reviscometer were consistent descriptors, providing robust correlations with age in most of the experimental settings. Furthermore, the CutiScan descriptors/parameters showed an excellent relationship with those from Cutometer and Reviscometer and was the only instrument to provide information about skin anisotropy, ensuring a full 360° analysis. This system also provides 3D representations of the skin, which helps to visualize the acquired differences [8].

A recent publication addressed mechanical properties in patients with systemic sclerosis (SSc) with the Cutometer [67]. The primary objective of the study was to describe and compare the intra-day and 7-day reproducibil-

ity of elastic skin properties in healthy volunteers and SSc patients. R3 was the only parameter with a good intra-day and inter-day reproducibility in the SSc group. Other parameters exhibited good reproducibility, but not at all anatomical sites. The authors concluded that the lack of standardization in data expression, the large number of parameters provided by the device, and reproducibility concerns discourages the use of these tests in routine practice in patients with SSc. Recent publications have focused on the correlation of fibre organization and dermal matrix in terms of structural composition, sun exposure, and biomechanical properties during infancy as well as pregnancy [68–73].

### Standardization, Validation, and Practical Guidance

The absence of harmonisation of experimental procedures and instrumentation is a major difficulty in the assessment of mechanical properties of the skin. Experimental protocol, number of volunteers, metric descriptors, and data analysis can hardly be compared. The absence of a standardized calibration compromises instrumental validation as well as the meaning/interpreta-

tion and validity and relevance of the results. However, some of the systems do offer calibration procedures [33, 51, 55], promoting the necessary precision, reproducibility, and validity to the measured variables. Most of the devices offer only a pre-calibration warranty.

In vivo measurements offer several challenges, particularly in comparison with ex vivo assessments. Hair analysis, in contrast, is performed in a more standardized and reproducible way. Nevertheless, most of the experimental concerns we identify here might be applied to skin and its annexes in mechanical assessment in general.

### *The Experimental Procedure*

#### Ethics

Within the European Union (EU), the Clinical Trial Regulation EU No. 536/2014 [74] is followed by some authorities as the “gold standard” for any study involving human volunteers or even data from human origin. It is clear that this regulation favours safety for participants and transparency regarding related information. Exceptions are considered, especially in terms of compensation or insurance, in the absence of additional risks or if risks are negligible. Nevertheless, drug-similar requirements for cosmetics or other non-drug health products are not covered by such regulation. Each member state is responsible for the definition of their own health policies, explaining the different levels of regulatory rigor among EU members. Therefore, the observation of respective regulations and following the reference principles of clinical studies are recommended to avoid potential difficulties [74, 75].

#### Number of Volunteers/Dimension and Strength of the Study

There is a growing concern in health research regarding the reproducibility of results, and sample size is a critical issue, generating an intense debate [76]. The number of volunteers (sample size) for experimental studies should be determined by the purpose of the study. The sample size will impact the strength of the study and respective statistical choices and interpretations [74]. At the usual significance level of  $\alpha = 0.05$ , the sample size should be estimated so that power (that is, the probability to reject the null hypothesis when it is false) approaches 0.90, thus reducing the probability of type I or type II errors from occurring [77, 78]. To reduce the probability of false positives (type I errors), it is necessary to reduce  $\alpha$ , meaning larger sample sizes [79]. However, large samples are not always feasible in biomedical research. Instead, researchers could aim to increase the precision of measure-

ments [79]. However, this is also often not possible, especially when a study intends to test a methodological approach, to develop a study protocol, or to test new equipment. In these cases, assuming an effect size of 1 (the standardized mean difference between the test and control groups) and at a usual significance level of  $\alpha = 0.05$ , a minimum of 30 participants will be required [78]. As we know, this value has been used as a reference, also because as the Student *t* distribution increases or approaches 30 degrees of freedom it is considered as being normally distributed [80]. Normality is essential for the use of parametric tests (e.g., Student *t* test; ANOVA), that are usually more robust in finding differences between groups. Nevertheless, independently of sample size, normality of data distribution should always be assessed, either by visual inspection, or formal normality tests such as the Shapiro-Wilk test or Kolmogorov-Smirnov test [81]. If the data are not normally distributed, researchers should choose either non-parametric tests or transforming the data (e.g., by logarithms).

To correlate a large number of variables or observe the result of a short-term exposure, a larger number of participants might be required. Thus, each situation should be previously calculated on a case-by-case basis, taking into account concepts such as target population, variance, and the desired confidence interval in a pre-defined significance level (usually  $\alpha = 0.05$ ) [82, 83].

#### Other Operational Requirements

Most of the previously published [4] practical guidance and recommendations regarding volunteers are still relevant and applicable. The “critical” determinants to harmonize procedures when assessing biomechanical properties of human skin and its annexes are summarized in Table 3. Related critical aspects regarding standard operating procedures and methods are universal and widely documented [8, 9, 29, 39, 84–91], referring to:

- the controlled laboratory environment, and the influence of seasonal variations on measurements
- the influence of age, gender, phototype/recent sun exposure, circadian rhythms, and anatomical region, positioning of the extremities for measurement (supination/pronation), and site marking. Of special interest are the particularities involved with joints (especially large joints)
- the importance of concomitant medication

Recent knowledge has drawn attention to other determinants of inter- and intra-individual variability, as outlined below.

**Table 3.** Summary of “critical” determinants

Critical determinant	To observe	To check
<i>Volunteer related</i>		
Ethics	Applicable regulation (EU and state member’s) [74, 75]	Informed consent, volunteer’s insurance, study online register (if applicable), data protection form (applicable in some countries)
Dimension and “power” of the study	Statistical basis [82, 83]	Adequate definition of significance and comparison tests considering the number of variables involved and the number of volunteers
<i>Other</i>		
	Controlled laboratory environment and influence of seasonal variations [101]	Temperature and humidity control; registration charts should evidence this control
	Age, gender, phototype/recent sun exposure and anatomical region to test, positioning, and site marking, and concomitant medication [1, 8, 9, 11, 26, 32, 53]	Strict selection of volunteers, having in mind the purpose of the study For (inter- or intra-) comparison purposes always use the same referential for region, position, and anatomical site Do not include volunteers taking medication that might affect skin functions
	Biological rhythms [91, 89, 100]	Be aware of this evidence; for some studies a dietary equivalence (Frequency Food Questionnaire) may be required If applicable, control of the cycle in fertile-age women might be recommendable
	Skin anisotropy [10, 12, 17, 38]	Register and use the same orientation of the measuring probe (specially for suction and torsional methods)
	BMI [93, 95]	Included volunteers should belong to the same BMI class defined by the WHO
<i>Instrument related</i>		
Calibration	Calibration procedure [102]	If the system does not include a calibration, confirm pre-calibration with the manufacturer, and schedule a regular verification of the system
Descriptors and variables	If mechanical analysis is based on the Young modulus; if not, confirm the manufacturer’s proposal to measure biomechanics (in vivo or ex vivo) [12, 63]	Compare the proposed descriptors with the Agache nomenclature Select the minimum appropriate descriptors
<i>Claim-demonstration related</i>		
Supported claim allegations to include in the PIF (Product Information File)	Capacity to fulfill the defined sophistication level of experimentation [103]	Fulfillment of all technical and methodological requirements
<p>Summary of the previously referred to “critical” determinants to harmonise procedures when assessing biomechanical properties of human skin and annexes. These are particularly important to the protocol design preceding experimentation (see text). BMI, body mass index; WHO, World Health Organisation.</p>		

Skin anisotropy has been identified as a major factor influencing mechanical properties of in vivo skin, as well as single hair fibres. Anisotropy (i.e., directional variations in tissue organization) has been consistently reported to affect skin viscoelasticity [6, 8–10]. These properties have been studied more intensively since the commercial availability of devices such as the Reviscometer and the CutiScan for in vivo skin and the Dia-Stron FTT 950 for hair fibre assessment. No significant differences between young and middle-aged individuals were detectable for

elasticity and for the modulus of Young when assessed parallel and perpendicular to the primary lines [90]. The initial skin tension has been reported as an important parameter strongly affecting the anisotropic properties of the skin [92]; however, studies on anisotropy and viscoelasticity in a porcine model could not demonstrate a significant correlation between these variables [37]. Further studies are required to better understand the meaning and relevance of the anisotropy-related descriptors provided by these instruments.

The volunteer's body mass index (BMI) is an additional variable that should be noted. The worldwide increase in overweight and obesity is a recognised fact, but its impact on normal skin physiology has not been thoroughly studied. BMI did not influence the RRT measured on the volar forearm of 110 volunteers [93]. A recent study with a more precise experimental design detected a negative correlation between obesity and skin biomechanical behaviour using a suction method [94]. Significant differences between obese and non-obese volunteers were observed in total elasticity, elasticity index, viscoelastic ratio, and skin total recovery, especially on the forehead, breast, and abdomen. However, when a sub-group analysis was performed with different degrees of BMI, in the morbidly obese group (BMI >40) all biomechanical indexes were close to those obtained in the normal group (19.9 < BMI < 24.9). This could be seen as an additional aspect of the so-called obesity paradox [95].

The water content of the stratum corneum is known to play an important role in different skin functions, such as the epidermal "barrier" function, and various dermatological diseases e.g., atopic dermatitis [87, 96, 97]. In addition, a direct relationship between these properties and regular dietary water consumption has not been clearly demonstrated, and only very few publications have addressed this subject [98, 99]. The water balance (including treatment with diuretics) and the diurnal variation of water accumulation in the dependant parts of the body were identified as important variables when measuring skin mechanical properties by suction methods [91]. Another study on the impact of dietary water intake and biomechanical properties of the skin found significant changes in maximum extensibility, the ability to return to the original state, total elasticity, elastic function, and the viscoelastic ratio as a function of variation of the daily water intake [100]. A 12-week study on the effects of oral *Aloe* sterol supplementation on skin elasticity, hydration, and the collagen score in healthy women ( $n = 64$ ) reported an increase of epidermal hydration and elasticity [101]. According to the authors, this could result from the increase of the dermal collagen content following the *Aloe* sterol supplementation.

### *The Instrumentation*

#### Calibration

Calibration is based in the comparison between a known measurement, adopted as the standard, and the measurement resulting from the testing instrument. It allows a confirmation of the accuracy of the in-use instrument. At the same time, calibration ensures that regular

use does not modify the device's precision as erroneous measurements can occur. In fact, the performance of any instrument changes with time, frequent use, and many other factors, thus calibration is required at frequent intervals. Standardized calibration constitutes a major determinant of the instrument's reliability and accuracy. Moreover, calibration allows a reference to a known set of parameters (ideally SI units) which represent the properties being measured [102].

The lack of calibration is a major limitation for many of the above-referred systems and devices. Thus, some of the parameters and related results do not have a direct correspondence to a physiological and/or relevant variable. Even so, manufacturers normally ensure a pre-calibration, but when acquiring a system it is important to perform an error control and a re-calibration by the manufacturer on a regular basis [102].

#### Descriptors and Variables

Suction methods are currently the most frequently used systems to assess skin biomechanical properties. As stated above, Agache's nomenclature, inspired by the Young modulus assessment, continues to represent an established and recognized way to describe the different components of the deformation curve regarding the particular composition and biomechanical behaviour of the human skin in vivo. However, as shown in Table 1, there is a wide variety of denominators of the addressed (mechanical related) properties, as well as the descriptors/parameters chosen to describe these properties. More descriptors do not guarantee more and better information. Keeping this selection as "simple" as possible makes data assessment and data analysis much more relevant and precise, since more variables imply a larger number of participants and an adapted power calculation [82].

#### *Proof of Efficacy of Cosmetic Products*

The proof of efficacy to support cosmetics' claims should be in accordance with the current Cosmetic Regulation in the EU (EC No. 1223/2009), as part of the Product Information File (PIF). The EU regulation involves and integrates the information and guidance gathered to a specific, product-related, claim. The previous EEMCO guidance indirectly approached this issue, in a very different regulatory context, by drawing the attention to the qualitative terms found in the marketed products to describe the mechanical properties of human skin [4].

A clear evolution on the subject can be observed, resulting from a demanding regulatory framework that requires a more rigorous communication between all stakeholders (authorities, industry, and the consumer). The experimental design and technical choices are critical requirements for claim substantiations. It determines the experimental level of sophistication chosen by the company to demonstrate and support the allegations (claims) that the PIF will hold in this specific frame.

A recent publication [103] suggests organising claims from a functional perspective, having in mind the intention of use. Three categories (Types) are proposed to facilitate decisions. In accordance with this publication:

- Type I claims are based on one or more properties or characteristics which are directly related to one or more measurable variables with physiological meaning (e.g., “elasticity”)
- Type II claims are based on one or more properties or characteristics only partially related to one or more measurable variables with physiological meaning (e.g., “suppleness”)
- Finally, the most complex are Type III claims – bearing no relation to physiologically measurable variables (e.g., “revitalizer”)

As in all applied research, claim substantiation must also be supported by a solid science-based framework.

## References

- 1 Dupuytren G, Grafe CF, Kalisch M. Theoretisch-praktische Vorlesungen über die Verletzungen durch Kriegswaffen. Veit; 1836.
- 2 Yang W, Sherman VR, Gludovatz B, Schaible E, Stewart P, Ritchie RO, et al. On the tear resistance of skin. *Nat Commun*. 2015 Mar; 6(1):6649.
- 3 Piérard GE; EEMCO Group. EEMCO Guidance to the in vivo assessment of tensile functions of the skin. Part 1: relevance of the structures and ageing of the skin and subcutaneous tissues. *Skin Pharmacol Appl Skin Physiol*. 1999;12(6):352–62.
- 4 Rodrigues L; EEMCO Group. EEMCO Guidance to the in vivo assessment of tensile functions of the skin. Part 2: instrumentation and test modes. *Skin Pharmacol Appl Skin Physiol*. 2001;14:52–67.
- 5 Paye M, Mac-Mary S, Elkhyat A, Tarrit C, Mermet P, Humbert PH. Use of the Reviscometer for measuring cosmetics-induced skin surface effects. *Skin Res Technol*. 2007; 13:343–9.
- 6 Ruvolo EC, Stamatias GN, Kollias N. Skin viscoelasticity displays site- and age-dependent angular anisotropy. *Skin Pharmacol Physiol*. 2007;20:313–21.
- 7 Verhaegen PD, Res EM, van Engelen A, Middelkoop E, van Zuijlen PP. (2010) A reliable, non-invasive measurement tool for anisotropy in normal skin and scar tissue. *Skin Res Technol*. 2010;16:325–31.
- 8 Rosado C, Antunes F, Barbosa R, Fernando R, Estudante M, Silva HN, et al. About the in vivo quantitation of skin anisotropy. *Skin Res Technol*. 2017;23:429–36.
- 9 Boyer G, Molimard J, Ben Tkaya M, Zahouani H, Pericoi M, Avril S. Assessment of the in-plane biomechanical properties of human skin using a finite element model updating approach combined with an optical full-field measurement on a new tensile device. *J Mech Behav Biomed Mater*. 2013;27:273–82.
- 10 Neto P, Ferreira M, Bahia F, Costa P. Improvement of the methods for skin mechanical properties evaluation through correlation between different techniques and factor analysis. *Skin Res Technol*. 2013;19:405–16.
- 11 Jee T, Komvopoulos K. In vitro measurement of the mechanical properties of skin by nano/microindentation methods. *J Biomech*. 2014; 47:1186–92.
- 12 Agache P, Varchon D. Skin Mechanical Function. In: Humbert P, Fanian F, Maibach HI, Agache P, editors. *Agache’s Measuring the Skin: Non-invasive Investigations, Physiology, Normal Constants*. 2nd ed. Paris: Springer; 2017. p. 945–62.
- 13 Aziz J, Shezali H, Radzi Z, Yahya NA, Abu Kassim NH, Czernuszka J, Rahman MT. Molecular mechanisms of stress-responsive changes in collagen and elastin networks in skin. *Skin Pharmacol Physiol*. 2016;29:190–203.
- 14 Mehta SU, Ramamoorthi R, Meyer M, Hery C. Analytic tangent irradiance environment maps for anisotropic surfaces. *Eurographics Symp Rend*. 2012;31:N4.
- 15 McKittrick J, Chen PY, Bodde SG, Yang W, Novitskaya EE, Meyers MA. The structure, functions, and mechanical properties of keratin. *JOM*. 2012;64(4):449–68.

## Conclusion

The biomechanical assessment of skin and its annexes is a complex theme motivating a growing research and knowledge development. On that basis, significant advances have been achieved not only in technological terms but also regarding its physiology and pathophysiology. Some important limitations are recognized, including the lack of standardization of procedures and calibration of instruments, leading to an ongoing discussion regarding the relevance and real nature of the descriptors/parameters obtained with these devices. The present work highlights what can be done to contribute to a better practice, and a science-supported biomechanical assessment of human skin, hair, and nails.

## Statement of Ethics

No ethical considerations are relevant to this work.

## Disclosure Statement

L.M.R. declares no conflicts of interest related to this publication. J.W.F. is a consultant for Courage and Khazaka. No funding was provided.

- 16 Yu Y, Yang W, Wang B, Meyers MA. Structure and mechanical behavior of human hair. *Mater Sci Eng C*. 2017 Apr;73:152–63.
- 17 Breakspear S, Noecker B, Popescu C. Hair Mechanical Anisotropy-What Does It Tell Us? *J Cosmet Sci*. 2018 Sep/Oct;69(5):305–14.
- 18 Bracchi M, Musitelli G, Capra P, Bleva M, Perugini P. Gold standard “in vitro” procedure to evaluate safety and efficacy of nail care products. Proceedings of the 4th International Summit on Nail Diseases; 2017; Athens.
- 19 Beyak R, Meyer CF, Kass GS. Elasticity and tensile properties of human hair. I. Single fiber test method. *J Soc Cosmet Chem*. 1969; 20(16):615–26.
- 20 Speakman JB. The plasticity of wool. *Proc R Soc Lond B*. 1928;103(725):377–96.
- 21 Reese CE, Eyring H. Mechanical properties and the structure of hair. *Text Res J*. 1950; 20(11):743–53.
- 22 Hearle JW. A critical review of the structural mechanics of wool and hair fibres. *Int J Biol Macromol*. 2000 Apr;27(2):123–38.
- 23 Chapman BM. A mechanical model, for wool and other keratin fibers. *Text Res J*. 1969; 39(12):1102–9.
- 24 Velasco MV, Sá Dias TC, Freitas AZ, Júnior ND, Oliveira Pinto CA, Kaneko TM, et al. Hair fiber characteristics and methods to evaluate hair physical and mechanical properties. *Braz J Pharm Sci*. 2009;45(1):153–62.
- 25 Dia-Stron [Internet]. <https://www.diastron.com/personal-care/>.
- 26 Sayahi E, Harizi T, Msahli S, Sakli F. Physical and mechanical properties of Tunisian women hair. *Int J Cosmet Sci*. 2016 Oct;38(5):470–5.
- 27 Agache P, Varchon D. Mechanical behaviour assessment of the skin. In: Humbert P, Fanian F, Maibach HI, Agache P, editors. *Agache’s measuring the skin: non-invasive investigations, physiology, normal constants*. 2nd ed. Paris: Springer; 2017. p. 963–1010.
- 28 Piérard GE, Hermans-Lê T, Piérard-Franchimont C. Skin tensile strength in scleroderma. In: Humbert P, Fanian F, Maibach HI, Agache P, editors. *Agache’s measuring the skin: non-invasive investigations, physiology, normal constants*. 2nd ed. Paris: Springer; 2017. p. 1011–37.
- 29 Serup J, Jemec G, Grove G (Serup J, Jemec G, Grove G, editors). *Handbook of Non-Invasive Methods and the Skin*. 2nd ed. Boca Raton: CRC Press; 2006.
- 30 Jachowicz J, McMullen R, Prettypaul D. Indentometric analysis of in vivo skin and comparison with artificial skin models. *Skin Res Technol*. 2007 Aug;13(3):299–309.
- 31 Xin S, Man W, Fluhr JW, Song S, Elias PM, Man MQ. Cutaneous resonance running time varies with age, body site and gender in a normal Chinese population. *Skin Res Technol*. 2010 Nov;16(4):413–21.
- 32 Dikstein S, Hartzshtark A, Bercovici P. The dependence of low-pressure indentation, slackness, and surface pH on age in forehead skin of women. *J Soc Cosmet Chem*. 1984;35:221.
- 33 Panduri S, Dini V, Romanelli M. The durometer measurement of the skin: hardware and measuring principles. In: Humbert P, Fanian F, Maibach H, Agache P, editors. *Agache’s measuring the skin*. Paris: Springer; 2017. p. 985–91.
- 34 Kim MA, Kim EJ, Lee HK. Use of SkinFibrometer® to measure skin elasticity and its correlation with Cutometer® and DUB® Skinscanner. *Skin Res Technol*. 2018 Aug; 24(3):466–71.
- 35 Falanga V, Bucalo B. Use of a durometer to assess skin hardness. *J Am Acad Dermatol*. 1993 Jul;29(1):47–51.
- 36 Courage+Khazaka electronic GmbH. Information and operating instruction for the Reviscometer® RVM 600. 2014. p. 1–21.
- 37 Courage+Khazaka electronic GmbH. Information and operating instruction for the CutiScan CS 100. 2014. p. 1–21.
- 38 Silva H, Rego F, Rosado C, Rodrigues LM. Novel 3D “active” representations of skin biomechanics. *Biomed Biopharm Res*. 2016; 13(2):219–27.
- 39 Rosado C, Antunes F, Barbosa R, Fernando R, Rodrigues LM. Cutiscan® – a new system of biomechanical evaluation of the skin in vivo – comparative study of use depending on the anatomical site. *Biomed Biopharm Res*. 2015;12(1):49–57.
- 40 Jacquet E, Joly S, Chambert J, Rekik K, Sandoz P. Ultra-light extensometer for the assessment of the mechanical properties of the human skin in vivo. *Skin Res Technol*. 2017 Nov; 23(4):531–8.
- 41 Jacquet E, Josse G, Khatyr F, Garcin C. A new experimental method for measuring skin’s natural tension. *Skin Res Technol*. 2008 Feb; 14(1):1–7.
- 42 Huang X, Yeo WH, Liu Y, Rogers JA. Epidermal differential impedance sensor for conformal skin hydration monitoring. *Biointerphases*. 2012 Dec;7(1-4):52.
- 43 Dagdeviren C, Shi Y, Joe P, Ghaffari R, Balooch G, Usgaonkar K, et al. Conformal piezoelectric systems for clinical and experimental characterization of soft tissue biomechanics. *Nat Mater*. 2015 Jul;14(7):728–36.
- 44 Yuan J, Dagdeviren C, Shi Y, Ma Y, Feng X, Rogers JA, et al. Computational models for the determination of depth-dependent mechanical properties of skin with a soft, flexible measurement device. *Proc Math Phys Eng Sci*. 2016 Oct;472(2194):20160225.
- 45 Nomura M, Velleman D, Pierre J, Flament F. Quantitating the lateral skin stiffness by a new and versatile electro-mechanical instrument. Preliminary studies. *Skin Res Technol*. 2017 Aug;23(3):272–82.
- 46 Nagaoka R, Kobayashi K, Saijo Y. Measurement of Skin Elasticity Using High Frequency Ultrasound Elastography with Intrinsic Deformation Induced by Arterial Pulsation. In: Sasaki K, Suzuki O, Takahashi N, editors. *Interface Oral Health Science 2014*. Tokyo: Springer Japan; 2015. p. 245–55.
- 47 Finlay B. Dynamic mechanical testing of human skin “in vivo”. *J Biomech*. 1970;3:557–68.
- 48 Boyer G, Pailler Mattei C, Molimard J, Pericci M, Laquieze S, Zahouani H. Non contact method for in vivo assessment of skin mechanical properties for assessing effect of ageing. *Med Eng Phys*. 2012 Mar;34(2):172–8.
- 49 Fleury V, Al-Kilani A, Boryskina OP, Cornelissen AJM, Nguyen T-H, Unbekandt M, et al. Introducing the scanning air puff tonometer for biological studies. *Phys Rev E*. 2010;81: 021920.
- 50 Fujimura T, Osanai O, Moriwaki S, Akazaki S, Takema Y. Development of a novel method to measure the elastic properties of skin including subcutaneous tissue: new age-related parameters and scope of application. *Skin Res Technol*. 2008;14:504–11.
- 51 Xu Z, Dela Cruz J, Fthenakis C, Saliou C. A novel method to measure skin mechanical properties with three-dimensional digital image correlation. *Skin Res Technol*. 2019 Jan; 25(1):60–7.
- 52 Kearney EM, Messaraa C, Grennan G, Koeller G, Mavon A, Merinville E. Evaluation of skin firmness by the DynaSKIN, a novel non-contact compression device, and its use in revealing the efficacy of a skincare regimen featuring a novel anti-ageing ingredient, acetyl aspartic acid. *Skin Res Technol*. 2017 May; 23(2):155–68.
- 53 Zahuani H, Pailler-Mattei C, Vargiolu R, Abellan MA. (2002) Assessment of the elasticity and tactile properties of the human skin surface by tribological tests. Proceedings of the 22nd IFSCC Congress in Edinburgh, podium 33.
- 54 Benzarti M, Tkaya MB, Mattei CP, Zahouani H. Hair mechanical properties depending on age and origin. *World Acad Sci Eng Technol*. 2011;74:471–7.
- 55 Lunn RJ, Leray Y, Bucknell S, Stringer DM. Quasi-Static Torsional Deformation of Single Hair Fibers: Application of a Modeling Approach and Results from Cosmetic Treatments. *J Cosmet Sci*. 2018 Sep/Oct;69(5):383–96.
- 56 Saeedi P, Shavandi A, Meredith-Jones K. Nail Properties and Bone Health: A Review. *J Funct Biomater*. 2018 Apr;9(2):31.
- 57 Brzozka P, Kolodziejki W. Sex-related chemical differences in keratin from fingernail plates: a solid-state carbon-13 NMR study. *RSC Advances*. 2017;7(45):28213–23.
- 58 Ohgitani S, Fujita T, Fujii Y, Hayashi C, Nishio H. Nail calcium and magnesium content in relation to age and bone mineral density. *J Bone Miner Metab*. 2005;23(4):318–22.
- 59 Wollina U, Nenoff P, Haroske G, Haenssle HA. The Diagnosis and Treatment of Nail Disorders. *Dtsch Arztebl Int*. 2016 Jul;113(29-30):509–18.
- 60 Baraldi A, Jones SA, Guesné S, Traynor MJ, McAuley WJ, Brown MB, et al. Human nail plate modifications induced by onychomycosis: implications for topical therapy. *Pharm Res*. 2015 May;32(5):1626–33.

- 61 Sano H, Ogawa R. Clinical Evidence for the Relationship between Nail Configuration and Mechanical Forces. *Plast Reconstr Surg Glob Open*. 2014 Apr;2(3):e115.
- 62 Krueger N, Luebbarding S, Oltmer M, Streker M, Kerscher M. Age-related changes in skin mechanical properties: a quantitative evaluation of 120 female subjects. *Skin Res Technol*. 2011 May;17(2):141–8.
- 63 Agache PG, Monneur C, Leveque JL, De Rigal J. Mechanical properties and Young's modulus of human skin in vivo. *Arch Dermatol Res*. 1980;269(3):221–32.
- 64 Courage and Khazaka Electronic GmbH [Internet]. <https://www.courage-khazaka.de/en/scientific-products/all-products/probe-systems/>.
- 65 Hua W, Fan LM, Dai R, Luan M, Xie H, Li AQ, et al. Comparison of two series of non-invasive instruments used for the skin physiological properties measurements: the DermaLab<sup>®</sup> from Cortex Technology vs. the series of detectors from Courage & Khazaka. *Skin Res Technol*. 2017 Feb;23(1):70–8.
- 66 Muller B, Elrod J, Pensalfini M, Hopf R, Distler O, Schiestl C, Mazza E. (2018) A novel ultra-light suction device for mechanical characterization of skin. *PLoS One*. 2018; 13(8):e.0201440.
- 67 Blaise S, Roustit M, Cracowski JL. Skin biomechanical properties in patients with systemic sclerosis: what parameter should be used? *J Eur Acad Dermatology Venereol*. 2017;12:3218–3221.
- 68 Langton AK, Graham HK, Griffiths CE, Watson RE. Ageing significantly impacts the biomechanical function and structural composition of skin. *Exp Dermatol*. 2019 Aug;28(8): 981–4.
- 69 Langton AK, Alessi S, Hann M, Chien AL, Kang S, Griffiths CE, et al. Aging in skin of color: disruption to elastic fiber organization is detrimental to skin's biomechanical function. *J Invest Dermatol*. 2019 Apr;139(4):779–88.
- 70 Berkey C, Oguchi N, Miyazawa K, Dauskardt R. Role of sunscreen formulation and photostability to protect the biomechanical barrier function of skin. *Biochem Biophys Rep*. 2019 Jun;19:100657.
- 71 Langton AK, Graham HK, McConnell JC, Sherratt MJ, Griffiths CE, Watson RE. Organization of the dermal matrix impacts the biomechanical properties of skin. *Br J Dermatol*. 2017 Sep;177(3):818–27.
- 72 Visscher MO, Burkes SA, Adams DM, Hammill AM, Wickett RR. Infant skin maturation: preliminary outcomes for color and biomechanical properties. *Skin Res Technol*. 2017 Nov;23(4):545–51.
- 73 Boyer G, Lachmann N, Bellemère G, De Belilovsky C, Baudouin C. Effects of pregnancy on skin properties: A biomechanical approach. *Skin Res Technol*. 2018 Nov;24(4): 551–6.
- 74 The European Parliament and the Council of the European Union. Regulations. [https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg\\_2014\\_536/reg\\_2014\\_536\\_en.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2014_536/reg_2014_536_en.pdf).
- 75 Tenti E, Simonetti G, Bochicchio MT, Martinielli G. Main changes in European Clinical Trials Regulation (No. 536/2014). *Contemp Clin Trials Commun*. 2018 May;11:99–101.
- 76 Wagner PD. Cores of Reproducibility in Physiology (CORP): advancing the corpus of physiological knowledge. *J Appl Physiol* (1985). 2017 Jan;122(1):89–90.
- 77 Curran-Everett D. Explorations in statistics: standard deviations and standard errors. *Adv Physiol Educ*. 2008 Sep;32(3):203–8.
- 78 Curran-Everett D. CORP: minimizing the chances of false positives and false negatives. *J Appl Physiol* (1985). 2017 Jan;122(1):91–5.
- 79 Sterne JA, Davey Smith G. Sifting the evidence – what's wrong with significance tests? *BMJ*. 2001 Jan;322(7280):226–31.
- 80 Kwak SG, Kim JH. Central limit theorem: the cornerstone of modern statistics. *Korean J Anesthesiol*. 2017 Apr;70(2):144–56.
- 81 Kim HY. Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restor Dent Endod*. 2013 Feb;38(1):52–4.
- 82 Jones SR, Carley S, Harrison M. An introduction to power and sample size estimation. *Emerg Med J*. 2003 Sep;20(5):453–8.
- 83 Martínez-Mesa J, González-Chica DA, Bastos JL, Bonamigo RR, Duquia RP. Sample size: how many participants do I need in my research? *An Bras Dermatol*. 2014 Jul-Aug; 89(4):609–15.
- 84 Jacquet E, Chambert J, Pauchot J, Sandoz P. Intra- and inter-individual variability in the mechanical properties of the human skin from in vivo measurements on 20 volunteers. *Skin Res Technol*. 2017;23(4):491–99.
- 85 Ryu HS, Joo YH, Kim SO, Park KC, Youn SW. Influence of age and regional differences on skin elasticity as measured by the Cutometer. *Skin Res Technol*. 2008;14:354–8.
- 86 Flynn C, Taberner A, Nielsen P. Measurement of the force–displacement response of in vivo human skin under a rich set of deformations. *Med Eng Phys*. 2011;33:610–19.
- 87 Darlenski R, Kazandjieva J, Tsankov N, Fluhr JW. Acute irritant threshold correlates with barrier function, skin hydration and contact hypersensitivity in atopic dermatitis and rosacea. *Exp Dermatol*. 2013;22:752.
- 88 Hassan AA, Carter G, Tooke JE. Postural vasoconstriction in women during the normal menstrual cycle. *Clin Sci*. 1990 Jan;78(1):39–47.
- 89 Silva H, Ferreira HA, da Silva HP, Monteiro Rodrigues L. The venoarteriolar reflex significantly reduces contralateral perfusion as part of the lower limb circulatory homeostasis in vivo. *Front Physiol*. 2018 Aug;9:1123.
- 90 Barel A, Courage W, Clarys P. Suction chamber method for measurement of skin mechanics: The new digital version of the Cutometer. In: Serup J, Jemec G, Grove G, editors. *Handbook of Non-invasive Methods and the Skin*. 2nd ed. Boca Raton: CRC Press; 2006. p. 583–91.
- 91 Gniadecka M, Serup J. Suction chamber method for measurement of skin mechanical properties: The Dermaflex. In: Serup J, Jemec G, Grove G, editors. *Handbook of Non-invasive Methods and the Skin*. 2nd ed. Boca Raton: CRC Press; 2006. p. 571–7.
- 92 Gahagnon S, Mofid Y, Josse G, Ossant F. Skin anisotropy in vivo and initial natural stress effect: a quantitative study using high-frequency static elastography. *J Biomech*. 2012;45:2860–5.
- 93 Hermanns-Lè T, Jonlet F, Scheen A, Pièrard GE. Age- and body mass index-related changes in cutaneous shear wave velocity. *Exp Gerontol*. 2001;36(2):363–72.
- 94 Tavares L, Palma L, Santos O, Almeida MA, Bujan MJ, Rodrigues LM. Impact of overweight on the normal physiology of human in vivo skin. *Biomed Biopharm Res*. 2013; 10(1):55–63.
- 95 Monteiro Rodrigues LM, Palma L, Santos O, Almeida MA, Bujan J, Tavares L. Excessive weight favours skin physiology - Up to a point: another expression of the obesity paradox. *Skin Pharmacol Physiol*. 2017;30(2):94–101.
- 96 Rosado C, Rodrigues LM. In vivo study of the physiological impact of stratum corneum sampling methods. *Int J Cosmet Sci*. 2003 Apr;25(1-2):37–44.
- 97 Russel M, Walters P, Khanna M, Chu M, Mack C. Developmental changes in skin barrier and structure during the first 5 years of life. *Skin Pharmacol Physiol*. 2016;29:111–8.
- 98 Williams S, Krueger N, Davids M, Kraus D, Kerscher M. Effect of fluid intake on skin physiology: distinct differences between drinking mineral water and tap water. *Int J Cosmet Sci*. 2007;29:131–8.
- 99 Wolf R, Wolf D, Rudikoff D, Parish LC. Nutrition and water: drinking eight glasses of water a day ensures proper skin hydration – myth or reality? *Clin Dermatol*. 2010;28:380–3.
- 100 Palma L, Marques LT, Bujan J, Rodrigues LM. Dietary water affects human skin hydration and biomechanics. *Clin Cosmet Investig Dermatol*. 2015 Aug;8:413–21.
- 101 Tanaka M, Yamamoto Y, Misawa E, Nabeshima K, Saito M, Yamauchi K, Abe F, Furukawa F. Effects of Aloe sterol supplementation on skin elasticity, hydration, and collagen score: a 12-week double-blind, randomized, controlled trial. *Skin Pharmacol Physiol*. 2016;29:309–317.
- 102 Dietrich C. *Uncertainty, Calibration and Probability*. New York: Routledge; 1991.
- 103 Rodrigues LM. Discussing cosmetics through a functional scope. In: Brain KR, Chilcott RP, editors. *Advances in dermatological sciences*. London: RSC; 2014. p. 240–55.