Objective Assessment Techniques: Physiological Parameters in Scar Assessment

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18.1 Background

With advances in medicine, we are able to modulate wound healing and scarring. In order to assess new and often costly treatments, the need for objective scar measurement tools has become increasingly important. A combination of subjective and objective measures should be the aim of every researcher. In daily practice, time is a limiting factor, and the choice for objective measurements should always incorporate feasibility and cost. Objective assessments provide a quantitative measurement of the scar. Quantitative assessment of scars requires devices to measure their physical and physiological properties. These properties include, but are not limited to, the following:

Physical parameters:

- Color
- Thickness
- Elasticity or pliability
- Texture
- Surface
- Tissue anisotropy

Physiological parameters:

- Skin hydration
- Transcutaneous oxygen level
- Tactile sensitivity

In this chapter, an overview of the assessment tools to evaluate physiological scar parameters will be given. Physiological scar parameters are scar characteristics relevant to pathological scarring which cannot be seen with the bare eye. This also means that they can only be assessed with objective assessment tools. This overview lacks skin characteristics which are measured with digital imaging systems. These assessment tools will be discussed in a separate chapter.

18.2 Skin Hydration

Skin hydration is defined as the water content of the epidermis and the dermis. Approximately 20% of water present in our body is accumulated in the skin. Of this amount, 60–70% is located in the dermis, and 15–30% is retained in the stratum corneum (SC). Functionally, the amount of water in the skin can be divided into free and bound water. In healthy skin, most of the water are bound to macromolecules. The ability of the skin to retain water is primarily related to the SC, which plays the role of the outer skin barrier, protecting the skin from water loss. The SC consists of cells called corneocytes and various lipids (fats) between them. The retention of water in the SC is dependent mainly on the

presence of natural hygroscopic agents within the corneocytes and the SC intercellular lipids orderly arranged to form a barrier to trans-epidermal water loss [1]. The corneocytes are often compared to bricks and the intercellular lipids to mortar, an appropriate metaphor for a layer of skin that serves as a barrier. The corneocytes are dead cells without nuclei. They contain various substances that hold water. For our skin to feel smooth and supple, the SC has to be at least 10% water; ideally, it is 20–30%. The SC can absorb as much as five to six times its own weight and increase its volume threefold when soaked in water. But it is not simply the water content that matters. Water has an effect on the enzymes that control orderly shedding of corneocytes, a process dermatologists call desquamation. Without water, the corneocytes accumulate, so the skin becomes flaky instead of peeling off nicely, and the SC gets disorganized and full of cracks instead of being tightly packed.

The glycosaminoglycan (GAG) polymer hyaluronan (HA, hyaluronic acid) forms a scaffold on which proteoglycans and matrix proteins are organized. These supramolecular structures are able to entrap water and ions to provide the skin with hydration. HA occurs in both the dermis and epidermis, with the dermis containing the greater proportion. HA present in the epidermis may play a role in an epidermal barrier function and SC hydration [1]. The lack of interaction between water and surrounding molecules contributes to dry appearance of the skin. Water content can vary depending on varying factors as skin site, skin depth, body mass index, age, sex, diurnal hour, seasons, and climates.

18.2.1 Skin Hydration in the Epidermis

The epidermal thickness is variable. It had been reported to be between 40 and 240 μm thick, depending on the measuring area and method used. Water originates in the deeper epidermal layers and moves upward to hydrate cells in the outermost skin layer, the stratum corneum (SC). Aquaporins (AQPs), cell membrane-bound water channels present in the epidermis, are essential hydration-regulating elements controlling cellular water and glycerol transport. Glycerol, thus present in the outer epidermal layers, binds and holds water, important for maintaining optimal skin hydration. The epidermis contains two different levels of water, separated by the interface between the stratum granulosum (SG) and the SC. The largest gradient of water in the epidermis occurs in the underlying layers of the SG (viable epidermis), while the SC water content is 4–5 times lower [2]. This gradient isolates the SC from the body, helping to conserve important solutes and water within the viable epidermis. The presence of a water gradient at

the deeper part of the SC triggers important keratinocyte functions such as the production of natural moisturizing factors (NMF). Dehydration of the upper skin layers increases when the SC water is lost more quickly than that which is received from the lower layers of the skin (viable epidermis and dermis), thus affecting the natural flow of water. Water originates in the deeper epidermal layers and moves upward to hydrate cells in the SC, eventually being lost to evaporation. Then, an evaporation barrier is needed to maintain body water homeostasis. The SC functions as the main evaporation barrier [2, 3].

18.2.2 Dermal Water Content

The dermis is between 1 and 4 mm thick, and it consists mainly of connective tissue. Dermis thickens as it binds more water. In the dermis, the collagen fibers, the interstitial space GAGs, and the proteoglycans can absorb large quantities of water, which leads to youthful skin. With skin fibrosis, the collagen fiber network is stretched by external mechanical forces, reduces its absorption capacity and water retention, and can lead to prolonged inflammation. Dermal hydration is highly related to the content and distribution of GAGs. The GAGs most often present in the human skin are hyaluronic acid (not attached to a protein core) and the proteoglycan family of chondroitin sulfates (GAGs attached to a protein core). GAGs bind up to 1000 times their volume in water [4]. GAGs in photodamaged or scarred skin are abnormally deposited in the papillary dermis, rather than diffusely scattered as in young skin. This aberrant localization interferes with normal water binding by GAGs, despite their increased quantity [4].

18.2.3 How to Measure Skin Hydration?

Many approaches exist to measure skin water content. One single method is often not enough to capture all the relevant information. Trans-epidermal water loss, stratum corneum water content, and dermal water content are equally important and related to each other.

The skin acts as a barrier against the water evaporation from the internal tissue. The water content in the skin preserves the softness and the smoothness of the skin surface. Diffusion of condensed water through the stratum corneum (SC) occurs and is highly elevated in pathological situations such as scarring; this can be measured by trans-epidermal water loss (TEWL). The results of these measurements are a good indicator of the recovery of the skin barrier and are a useful indicator of the scar maturation process [5]. TEWL is strongly

related with the moisture content of the skin and can be measured with open- or closed-chamber systems [6]. The open chambers are the oldest but still most widely used.

18.2.3.1 Trans-Epidermal Water Loss: Measuring Principle

Trans-epidermal water loss represents the outward permeation of condensed water through the stratum corneum by means of diffusion [7]. TEWL can be measured by using an open-chamber method or closed-chamber method. Open chambers are open to the surrounding atmosphere and are therefore easily influenced by external air convection and turbulence. Closed-chamber methods have been designed more recently, the measuring chamber is enclosed from the surrounding atmosphere, and measurements are therefore not influenced by external air convection and turbulence.

TEWL can be calculated by measuring the water vapor pressure (VP) gradient at the skin surface. In the open-chamber method, the VP gradient is calculated by measuring the difference in VP between two distinct points aligned perpendicularly to the skin surface (see ■ Fig. 18.1). VP is calculated as the product of relative humidity (RH) and saturated VP; this is dependent on temperature. RH is measured using capacitive sensors; temperature is measured with fast thermistors located in the cylindrical measuring chamber with open ends [7–11].

■ Fig. 18.1 TEWL measured by using an open-chamber method. Reused with permission from Courage + Khazaka electronic GmbH. © All rights reserved

18.2.3.2 Open-Chamber Method

To date, the open-chamber method is still considered the most reliable method to measure water evaporation in scarred skin. Open-chamber systems measure water evaporation rate based on diffusion principles [9, 11]. The vapor density gradient is measured indirectly by two pairs of sensors (temperature and relative humidity) inside a hollow cylinder [7, 11]. The resulting data are analyzed by a microprocessor. Measurement values are given in $\text{g/m}^2/\text{h}$. The physiological water vapor mantle surrounding the human skin is a gradient of water with a thickness of about 10 mm. The two sensors of the evaporimeter probe are typically placed 3 and 6 mm above the skin surface. Open-chamber evaporimeters have limitations in practical use and should ideally be used in a climatized room with control of convection, temperature, and humidity [9, 10]. Used outside a climatized room, special attention should be given to air convection, room temperature, and ambient humidity [9].

The most widely used systems are the Tewameter® TM300 (Courage+Khazaka, Cologne, Germany) and the DermaLab® (Cortex, Hadsund, Denmark). Both are available as stand-alone devices or as a probe attached to a Multi Probe Adapter System like the Scarbase Duo™ (Courage+Khazaka, Cologne, Germany) and the DermaLab Combo® (Cortex, Hadsund, Denmark).

Both the Tewameter® (■ Fig. 18.2) and the DermaLab USB® TEWL (■ Fig. 18.3) showed good to excellent reliability and validity with rather high minimal clinically important difference (MCID) [12, 13]. The MCID is the smallest change in an outcome that a patient or a clinician would identify as important. An overview of the results is set out in ■ Table 18.1.

18.2.3.3 Semi-Open-Chamber Method

Recordings with open-chamber evaporimeters are not intrusive to the 10 mm physiological water vapor mantle over the skin and due to its specificity sensitive to ambient airflow. Open-chamber instruments perform best under standardized ambient conditions with an air protection shield or box controlling the ambient air conditions, as described in standards [9, 10]. Closed-chamber methods are insensitive to ambient air but, unfortunately, directly intrusive to the water vapor mantle. Accumulation of water vapor in the chamber during the measurement instantly and directly influences the diffusion of water out of the skin. Thus, closed chambers interfere with the physiological skin barrier and thus cannot record physiological TEWL directly. For the same reason, measurements may only be made as an initial estimate and not continuously. In the DermaLab system (Cortex Technology, Hadsund, Denmark), a special grid serving as a semi-open windshield has been incorporated directly in the top of the open probe of

this instrument. This grid is open and allows evaporation of water out of the probe chamber and at the same time protects the sensors in the probe against ambient

■ Fig. 18.2 The probe of the Tewameter® TM300. Reused with permission from Oscare. © All rights reserved

■ Fig. 18.3 DermaLab® USB TEWL-probe. Reused with permission from Oscare. © All rights reserved

Table 18.1 Comparison of reliability, validity, and MCID for trans-epidermal water loss assessment on scars

Device	Intrarater reliability (ICC)	Interrater reliability (ICC)	Validity	MCID
Tewameter® [13]	0.95	0.96	Good correlation with DermaLab® TEWL ($r = 0.93$) ^b	12%
DermaLab® TEWL [12]	0.86–0.88	0.78–0.93	DermaLab® TEWL is able to distinguish normal skin from spontaneously healed scars ($p = 0.036$)	17%

air convections. The system can be considered respectful to the water vapor mantle.

18.2.3.4 Closed-Chamber Method

Two types of closed-chamber methods are available – a condenser chamber method and an unventilated-closed-chamber method. With the unventilated-chamber method, the measuring cylinder is closed off at the top. When placed on the skin, water vapor from the skin collects in the chamber, and with time, the humidity in the chamber increases slowly at first, and thereafter linearly. Flux density (amount of water diffusing through the SC per unit distance and time) is calculated from the change in RH and temperature over time. Due to the accumulation of water vapor and humidity in the chamber, these instruments cannot be used for continuous measurements. Overall, the measurement time of unventilated-closed-chamber instruments is very short (<10 seconds). Various skin barrier impairment studies revealed that the open-chamber devices are more sensitive or discriminative and are able to detect significantly smaller differences than the closed-chamber devices [14]. The VapoMeter (Delfin Technologies Ltd., Kuopio, Finland) is an example of a unventilated-closed-chamber system [15]. The VapoMeter is simple in principle; a humidity sensor in the closed chamber measures the gradual buildup of humidity. On its commercialization, it was postulated that TEWL measurements were not affected by ambient or body-induced airflows.

18.2.3.5 Stratum Corneum Hydration Level: Measuring Principle

Hydration of the skin surface is a good indicator of epidermal function. Hydration is directly proportional to retention of electrical charge and is therefore often measured by dielectric capacitance or the conductance of the superficial skin layers [11]. Technical aspects such as type of probe surface, degree of direct galvanic contact with the skin, distance between the electrodes, and depth of measurement all vary when comparing the different technologies. Due to the higher TEWL, scar sites are dryer than control sites and seem to become dryer as they mature. Water content is very important in the evaluation of biophysical properties of scars. Evidence sug-

Fig. 18.4 The measurement principle of the Corneometer® CM825 is based on the capacitance method. Reused with permission from Courage + Khazaka electronic GmbH. © All rights reserved

gests that a sufficient amount of water in the stratum corneum (SC) keeps the skin soft and flexible and ensures that the skin appears smooth and healthy. The water holding capacity of the SC also influences its barrier function and mechanical properties [16]. Measurements of hydration are often influenced by environmental factors [7].

The Corneometer CM 825® (Courage+Khazaka, Cologne, Germany) is based on the capacitance method. It is a well-known and efficient instrument in measuring hydration of the SC. In the past, the measuring probe of the Corneometer used an analog signal, but now, digital technology is used, resulting in higher stability and less interferences [17].

The Skicon instruments (I.B.S. Co., Hamama-T), based on the conductance method, are also widely used. A modern version of the Skicon (Skicon-200EX®) has been developed with a new concentric interdigital probe.

18.2.3.6 Corneometer CM825®

The measuring principle is based on the capacitance method (see Fig. 18.4). Technical description of this type of instrument and its use has been published by

■ Fig. 18.5 Probe of the Corneometer® CM825 device. (©Oscare)

many authors. Both probes (analog and digital) contain an interdigital grid of gold electrodes, covered by a low dielectric vitrified material (see ■ Fig. 18.5). A resonating system in the instrument detects the frequency shift of the oscillating system related to the capacitance (and hence hydration) of the biomaterial in contact with the probe. Unlike the Skicon instruments, there is no direct galvanic contact between the electrodes of the Corneometer and the skin surface. To enable a constant pressure of the probe on the skin surface, a spring system is incorporated. According to the manufacturer, the pressure of the old analog probe is in the range of 1.1–1.8 N; the new digital probe operates at a lower pressure (about 1 N or less). The Corneometer is factory calibrated using an *in vitro* method. Corneometer® results obtained with different instruments in different laboratories can be pooled if a pretest validation demonstrates concordance between instrument.

Anthonissen et al. investigated the reliability of the Corneometer and concluded that the instrument can be used in clinical trials but only under very strict conditions with standardized test protocol, preferably in combination with the evaluation of other physiological parameters [18]. The results revealed excellent ICC values (ICC_{intra} = 0.985; ICC_{inter} = 0.984) with relatively low within-subject coefficient of variation (WSCV) (WSCV_{intra} = 6.3%; WSCV_{inter} = 10.6%) for, respectively, intra- and inter-observer reliability. However, the Bland–Altman plot showed that more than 5% of differences were expected to exceed 4 a.u., the limit of what has been defined as a clinically acceptable difference. Results for day-by-day variability showed good ICC

value (ICC_{day-by-day} = 0.849) and higher WSCV (WSCV_{day-by-day} = 20.5%).

18.2.3.7 Skicon-200EX

The Skicon instruments manufactured by I.B.S. Co. are based on the conductance principle. These instruments measure the conductance (μS) of a single high-frequency current at 3.5 MHz. The measuring probe consists of 75 μm large concentric interdigital electrodes with a gap of 200 μm between the electrodes. The total probe surface is 0.8 cm^2 . The probe makes use of a spring system ensuring a constant pressure force of 0.78 N when applied to the skin. The electrode makes direct galvanic contact with the skin surface. The device is calibrated *in vitro* using various external calibration standards in the range of 2–2000 μS conductance [19].

18.2.3.8 General Recommendations for Skin Hydration Measurements

The applied probe pressure has a large influence on hydration measurements. Despite the existence of a spring to ensure constant probe pressure, Clarys et al. have demonstrated that the measured hydration levels increase with higher probe pressures. This phenomenon is exacerbated on dryer skin (e.g., scar tissue). It is therefore advisable to use complete compression of the spring when applying the probe on the skin surface. Use a precision balance to practice obtaining more or less identical values. Finally, try to transpose this to the *in vivo* application of the probe on the skin surface [20].

Digital capacitance probes are very sensitive for evaluating dry/very dry skin conditions (e.g., scars), while digital conductance probes are more suited for evaluating very high levels of hydration [17].

Skin hydration has a positive correlation with ambient temperature and humidity; it is strongly affected by these two environmental parameters [7].

Results of TEWL or skin hydration measurements should be reported as differences or percent change rather than absolute values. This approach partially eliminates influencing factors.

In order to obtain reliable results for skin hydration, it is advisable to minimize, as far as possible, the influences of endogenous, exogenous, environmental, and instrument or measurement-related factors [7].

For the Corneometer®, the MCID is 7% on healthy skin and 4% on scars [18].

18.2.4 Dermal Water Content Measurement

18.2.4.1 Confocal Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique used to observe vibrational, rotational, and other low-frequency modes in a system. Raman spectroscopy is

commonly used in chemistry to provide a structural fingerprint by which molecules can be identified. Confocal Raman spectroscopy can directly measure the water content from the skin surface down to the upper epidermis with high depth resolution (5 μm) [21]. Moreover, it produces a more absolute measurement value than other methods. Using confocal Raman spectroscopy, the water content is calculated from the ratio of integrated Raman signals for water and protein. The proportionality constant is estimated from the Raman spectra of various solutions of proteins in the water. Nakagawa et al. concluded that *in vivo* measurement of the dermal water content with confocal Raman spectroscopy was highly reliable [22]. The benefit of using confocal Raman spectroscopy is that the depth increment between measurement points can be freely adjusted. The most widely used depth resolution of the confocal Raman spectroscopy is 5 μm . With confocal Raman spectroscopy, the smaller the region of interest, the higher the depth resolution.

18.2.4.2 Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (from 780 nm to 2500 nm). NIRS is based on molecular overtone and combination vibrations. One advantage is that NIRS can typically penetrate much further into a sample than mid-infrared radiation. NIRS is not a particularly sensitive technique, but it can be very useful in probing bulk material with little or no sample preparation [23].

The molecular overtone and combination bands seen in the near-IR are typically very broad, leading to complex spectra; it can be difficult to assign specific features to specific chemical components. Multivariate calibration techniques (e.g., principal components analysis, partial least squares, or artificial neural networks) are often employed to extract the desired chemical information. Careful development of a set of calibration samples and application of multivariate calibration techniques is essential for near-infrared analytical methods. NIRS can measure overall skin water content as well as of the various components constituting the skin, i.e., the stratum corneum, epidermis, and dermis. This technique has the ability to directly determine the changes in the various types of water (free, bulk, and protein-bound water), which are present in the various skin layers.

Another interesting advantage is that this technique does not require direct contact with the skin and can easily be integrated in smartphones. This technique also allows to determine the thickness of separate skin layers [24]. However, its lack of sensitivity and frequent integration of algorithms raises doubts about its reliability, which still needs to be investigated in scar tissue.

18.3 Transcutaneous Oxygen Tension

Scar maturation has been related to transcutaneous oxygen tension that can be measured with electrodes on the skin. Heat is induced to the electrode and causes oxygen and CO_2 to diffuse through the skin. In hypertrophic scar, PO_2 is lower than in healthy skin. An increase over time was correlated with clinical improvement. Upregulated levels of transcutaneous oxygen tension in treated scars correlated well with a downregulation of scar thickness assessed both clinically and by ultrasound. It is hypothesized that low levels of transcutaneous oxygen pressure (tcpO₂) in evolving scars result from low oxygen diffusibility through scar tissue rather than from rapid metabolic consumption of oxygen by scar tissue [25].

18.4 Tactile Sensitivity

Tactile sensitivity of the skin can be divided in two parts. Discriminative touch is a sensory modality that allows a subject to sense and localize touch. Non-discriminative touch is a sensory modality that allows the subject to sense that something has touched them, without being able to localize where they were touched. Scars are frequently accompanied with sensory deficits often remaining present months or even years after injury.

Tactile sensitivity of the skin can be measured by esthesiometers. There are different types of esthesiometers depending on their particular function. The simplest is a manual tool with adjustable points similar to a caliper. It can determine how short a distance between two impressions on the skin can be distinguished.

Another type of manual esthesiometer is used to test lower thresholds of touch or pain. The tool uses nylon monofilaments with varying calibrated diameters (see ■ Fig. 18.6). The force needed to cause the monofilament to “buckle” determines the tactile reading (see ■ Fig. 18.7). The filaments are calibrated by force applied, rather than by gram/mm^2 pressure ratings because sensation follows force (when the stimulated area is small).

The most commonly used is the Semmes-Weinstein Aesthesiometer and its variant the Weinstein Enhanced Sensory Test (WEST) that determines the touch perception threshold (TPT). Meirte et al. concluded that the Semmes-Weinstein monofilament test is a feasible and reliable outcome measure to evaluate TPT in burn scars, showing excellent intrarater and interrater reliability (ICC >0.90) [26].

Fig. 18.6 The Semmes-Weinstein Aesthesiometer® uses nylon monofilaments. (©Oscare)

Fig. 18.7 The force needed to cause the monofilament to “buckle” determines the tactile reading. (©Oscare)

Take-Home Messages

- The stratum corneum acts as an evaporation barrier to maintain body water homeostasis.
- In the epidermis, water originates from the deeper layers.
- In scars, the absorption capacity and water retention are reduced and may contribute to prolonged inflammation.
- In scars, TEWL is higher than in normal skin.
- One single method to measure skin water content is not enough to capture all the relevant information.

- The open-chamber method is the most widely used to measure TEWL.
- The dielectric capacitance method is the most reliable method to measure SC hydration level in scars.
- Confocal Raman spectroscopy is a reliable technique to measure dermal water content.
- The Semmes-Weinstein Aesthesiometer is a reliable tool to assess tactile sensitivity in scars.

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