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Passive electrical properties of human stratum corneum in vitro depending on time after separation

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Abstract

The passive electrical properties of human skin after separation from the body are predominated by the stratum corneum. Skin within a bath medium (150 mM phosphate buffered saline) at constant temperature (37°C) exhibits a characteristic change of the passive electrical properties with time. Independent of the time the locus in the Z-plane is a depressed semicircular arc. The angle between the lines from the center of the arc to the points where the locus reaches the real axis remains unchanged. The difference between the high and low frequency resistivity ($R_0 - R_\infty$) increases over 10 h, reaches a plateau and decays after 20 h exponentially with a time constant of about 40 h. As model for the impedance we used a 5 element electrical circuit (R_0, R_1, R_2, C_1, C_2), describing 3 pathways, (0) the dc path (appendages; R_0), (1) tortuous pathways around the cell structures (R_1, C_1) and (2) direct pathways involving the corneocytes (R_2, C_2). There are characteristic changes with time in the elements of the equivalent circuit up to about 200 h after excision. Dramatic changes in C_1 and R_2 at about this time after separation strongly suggests destruction of the lipid structures. It will be suggested that the use of separated human stratum corneum as model for in vivo yields unreliable results after this time.

Keywords: Skin; Passive electrical properties; Electrical impedance; Time dependence

1. Introduction

The electrical impedance of human skin is important for most in vivo electrical measurements on the body (EEG, ECG, whole body impedance for determination of the body composition, impedance plethysmography, electrical impedance topography, psychophysiological studies) and for some electrical therapy such as electrochemotherapy, transdermal drug delivery by iontophoresis or radiofrequency therapy. Basic research often uses in vitro models, with a wide variety of values for impedance. The values depend even for normalized measurement methods on the body location, the condition of blood circulation and the density of open sweat ducts. In addition a characteristic dependence on the time after separation was found for in vitro preparations.

Immediately after separation the passive electrical properties of the skin specimen are similar to those in vivo. But they change within hours and thus measurement without taking into account the time after separation yields misleading results.

The skin impedance was investigated under different conditions [1–3]. It is widely accepted that the main electrical impedance resides in the stratum

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corneum while the impedance of the other layers is several orders of magnitudes lower. This means that the skin impedance is dominated by the passive electrical behavior of the stratum corneum. The stratum corneum (Fig. 1) is the outermost part of the skin and consists of about 15 layers of corneocytes surrounded by some double layers of lipids (sphingolipids). The corneocytes, (diameter ca. 20–40 μm), contain randomly orientated α-keratin fibers (5–10 μm diameter), surrounded by a matrix of proteins. Between the corneocytes are layers of sphingolipids, fatty acids and cholesterols with a spatial separation of about 1 μm.

The water content of the stratum corneum is very low, not more than 20%, compared to 70% in the underlying tissue [4,5].

The low frequency pathway is dominated by the appendages such as hair follicles and sweat ducts. Lipid lamellae are borderlines between very low conductivity (lipids) and high conductivity (electrolyte) forming a capacitor. There are two distinguishable pathways involving the lipid layers: a direct pathway through the ceratinocytes and a tortuous pathway using hydrated sites around the corneocytes. Technically, we can model this as a resistor for the appendages and an *RC*-combination for each capacitive pathway in parallel. Since the parameters of the capacitive pathways are distributed, the number of *RC*-combination should be enormous. The impedance locus of a specimen such as the skin is a depressed semicircle in the fourth quadrant reaching the real axis at the frequencies $f = 0$ Hz and at lim $f \to \infty$, i.e. at a point where all the capacitances are shorted and only the parallel resistors account for the impedance. The center of the circle is above the real axis (Fig. 2). The angle ϕ is connected with the distribution of time constants. This means in the case of $\phi = 0$ only one characteristic time is present while a large ϕ is the result of a broad distribution of time constants. Here, we measured the frequency range between 10 Hz and 1 MHz. A reasonable fit was found with two characteristic times, τ_1 and τ_2 reflecting the direct and the tortuous pathway. Of course this is only an approximation. In general we have to consider distribution of time constants around τ_1 and τ_2. For better interpretation of the results an electrical equivalent circuit was introduced (Fig. 2), where R_0 represents the dc path through the appendages and both the RC combinations $\tau_1 = R_1 C_1$ and $\tau_2 = R_2 C_2$ are associated with the capacitive pathways (tortuous and direct).

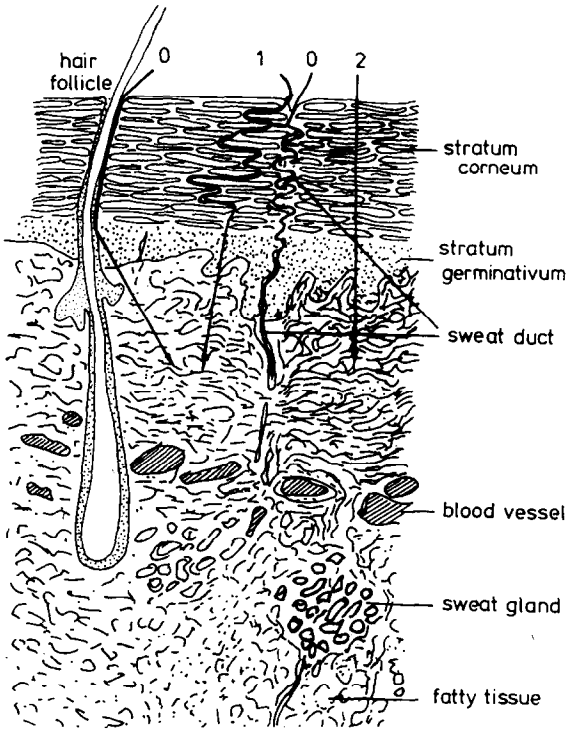

Fig. 1. Schematic overview of the human skin. The resistivity of the stratum corneum is several orders of magnitudes higher than that of the lower skin structures. 0 is the predominant dc path and 1 and 2 are the ac current pathways.

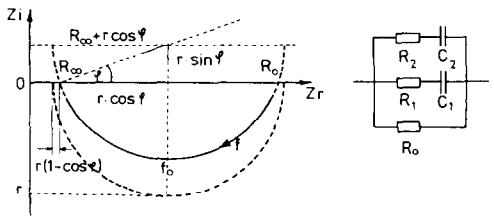

Fig. 2. The impedance locus ($Zi(\omega) = f(zr(\omega))$ (full line) between 10 Hz and 1 MHz is a depressed semi-circle with the central point above the real axis, depending on ϕ, here found constant at $\phi \approx 20°$. The arrow indicates the locus progression as the frequency increases. A suitable equivalent circuit for this behavior is a resistor R_0 parallel with two serial *RC* combinations. The high frequency resistance R_∞ is given by $R_\infty = R_0 R_1 R_2 / (R_0 R_1 + R_0 R_2 + R_1 R_2)$.

The impedance of such a circuit is

$$Zr = \frac{AC + BD}{C^2 + D^2} \text{ and } Zi = \frac{-AD + BC}{C^2 + D^2} \qquad (1,2)$$

where

$$A = R_0 - \omega^2 C_1 C_2 R_1 R_2 R_0,$$

$$B = \omega R_0 (C_1 R_1 C_2 R_2),$$

$$C = 1 - \omega^2 C_1 C_2 (R_0 R_1 + R_0 R_2 + R_1 R_2),$$

and

$$D = \omega (C_1 (R_0 + R_1) + C_2 (R_0 + R_2)).$$

2. Material and method

Human skin, obtained by surgery from a donor, was immediately placed in phosphate buffered saline (150 mM PBS, pH 7.4) and after 30 min clamped in a side-by-side permeation chamber at 37°C (Fig. 3). The chamber provides 4 electrodes, two at each side, used for a four electrode interface impedance measurement system. In the experiments described here a Solartron 1260 was employed for impedance measurements in the range 10 Hz to 1 MHz at a constant voltage of 1 V. At certain times a sweep cycle involving 200 discrete computer-controlled frequencies was performed. From these measurements the impedance locus was drawn.

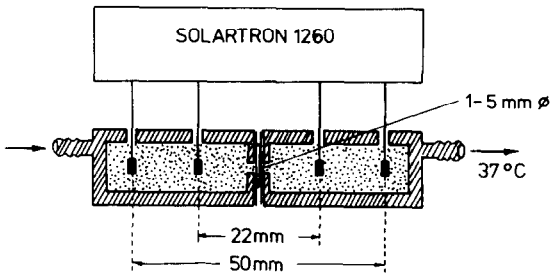

Fig. 3. Schematic overview of the equipment used here. The skin was placed in a side-by-side permeation chamber, providing 4 electrodes, two for the stimulus application, while the other two were used for sensing. A computer-controlled Solartron 1260 (Schlumberger) was used for impedance measurements.

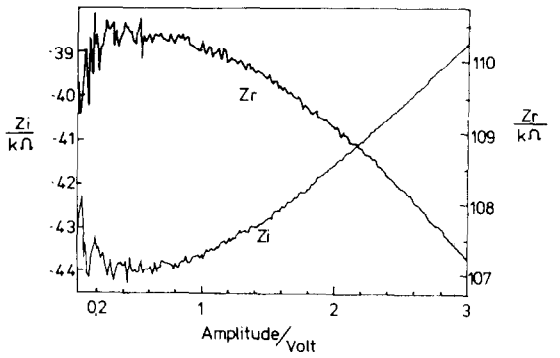

Fig. 4. The skin impedance, $Z = Zr + jZi$, at $f = 100$ Hz vs. the measuring voltage. Up to about 1 V the skin impedance is about linear, while the further increasing of the voltage yields a nonlinearity of more than 3%.

2.1. Influence of measuring conditions

2.1.1. Voltage

Like other biological tissue the skin shows a nonlinear impedance behavior, e.g. the impedance changes with higher voltage or current density. The voltage where the skin impedance is still linear depends on the frequency and increases with higher frequency. A change in the impedance of more than 1% due to the measuring voltage was found for 10 Hz at more than 1 V, where this level was for 1 kHz at 3 V. In Fig. 4 the real and the imaginary part of the impedance at 100 Hz is shown. The uncertainty of the measurement associated with noise increases with smaller measurement voltage. That is why the highest voltage where the skin impedance is still linear was chosen for our experiments.

2.1.2. Bias

If a bias between -1.66 V and $+1.66$ V is applied, the dynamic resistance $Z_{dy} = du(t)/di(t)$, where u and i are the complex voltage and current, changes reversibly. The relative changes due to a bias below 1.6 V are about 20% for frequencies below 1 kHz and vanish for higher frequencies. The influence of a bias at a frequency of 100 Hz is shown in Fig. 5.

2.1.3. Temperature

An electrolyte has a temperature coefficient of about 2%/K. As seen in Fig. 6 a temperature rise

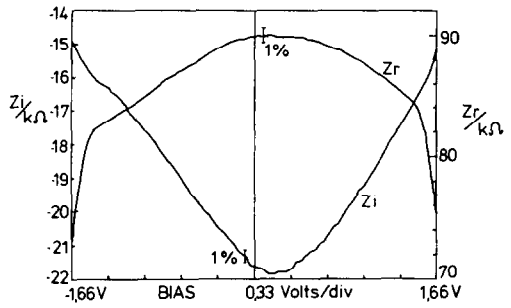

Fig. 5. The skin impedance, $Z = Zr - jZi$, at $f = 100$ Hz vs. the bias voltage. The impedance measured here is the small signal impedance or dynamic impedance. A bias of more than 0.2 V yields a significant alteration of the skin impedance.

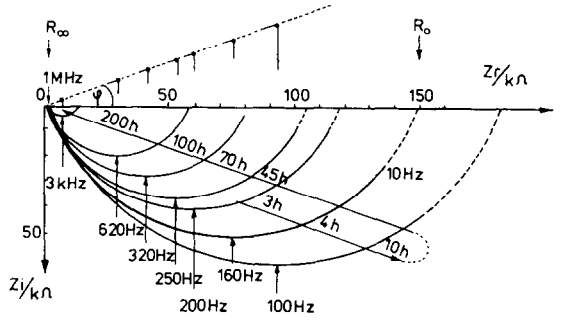

Fig. 7. Impedance loci at different times after separation from the body in constant conditions ($\vartheta = 37°C$, no bias and 1 V measuring voltage). The curves are parts of a semi-circle. The radius increases for the first 10 h and decreases than with time. The angle ϕ was found to be time invariant.

from 25°C to 37°C results in a change of the dc resistance from 8.6 kΩ to 6.8 kΩ, i.e. 2.7%/K, while the magnitude of the impedance at 1 MHz changes only about 1.5%. The frequency, f_0, where the imaginary part of the impedance reaches its minimum rises from 2.2 kHz to 4.5 kHz or 8.7%/K, while the angle ϕ remains constant at $\phi = 20°$. In order to avoid uncertainties, associated with temperature effects, the temperature during our experiments was held constant at 37°C.

3. Results

3.1. Time course of the impedance

A characteristic set of data is shown in Fig. 7. The impedance locus was recorded at different times after separation from the body. The loci fit in the frequency range from 10 Hz to 1 MHz surprisingly well parts of a circle, where all the central points aligned to a line which is in 20° angle to the real axis and can be described as

$$Zi = 0.34r + \sqrt{r^2 - \left(Zr - R_\infty - 0.94r^2\right)}. \qquad (3)$$

The loci were fitted to Eq. 3 and the elements R_0, R_1, R_2, C_1 and C_2 determined. The time course of these elements is shown in Fig. 8a–c.

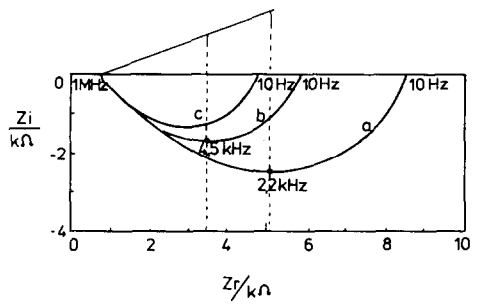

Fig. 6. The influence of the temperature on the skin impedance. The impedance locus at 25°C (a), 37°C (b) immediately after warming and at 37°C after 4.5 h (c) are shown.

4. Discussion

Fig. 7, shows the impedance loci ($Zi = f(Zr)$). It exhibits a frequency dispersion ranging from dc to about 1 MHz. In the case of a simple characteristic frequency the curve should be a semicircle with the central point at the real axis, but these loci are depressed semicircles. An angle between the real axis and the central point of the arc and $Z(f \rightarrow \infty)$ of 20° was found, i.e. more than one characteristic time is evident. Here, we fit the curve to an equivalent circuit, containing two time constants. The mean characteristic frequency f_0, i.e. the frequency where the Z_i is a minimum, decreases from 200 Hz to 100 Hz during the first hours and increases up to 120 kHz within 2 weeks. Referring to Fig. 8a, the dc-component of the impedance (R_0) increases initially and decreases during the next two weeks. A transformation onset was found after about 20 h. This result is similar to that found in other tissues [6]. A candi-

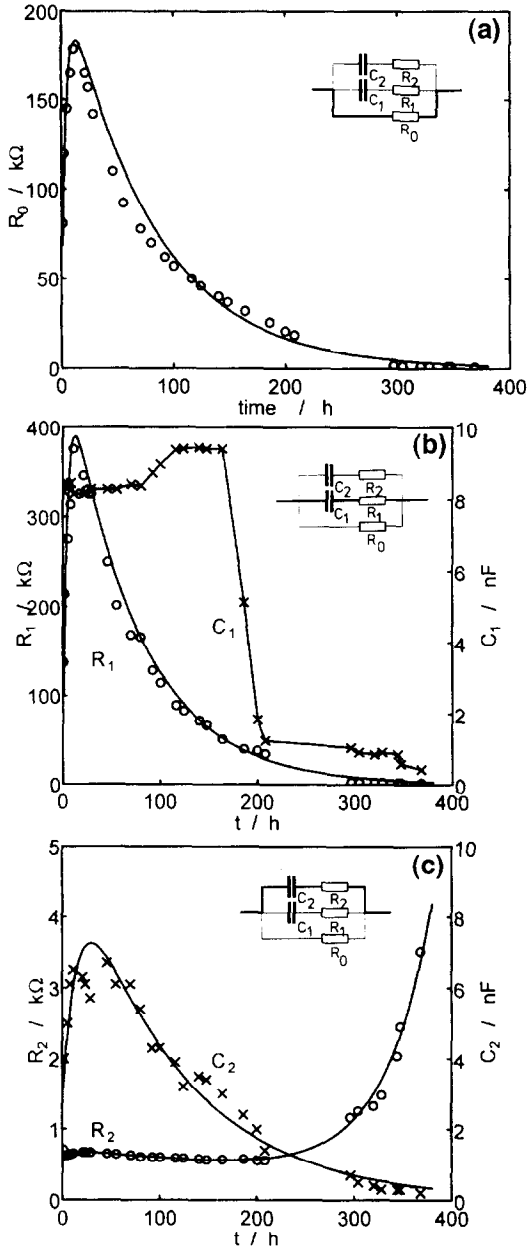

Fig. 8. (a–c) Time course of the elements R_0 (a), R_1 C_1 (b), and R_2 C_2 (c) of the electrical equivalent circuit, modeling the impedance of the skin. These elements were fitted from the impedance loci using the least complex departure from the locus as criteria [8–10]. The time tracks of R_0, R_1 and C_2 can be fitted to $y = ae^{-t/\tau 1} - be^{-t/\tau 2}$ pretty well (solid curves). C_1 and R_2 show dramatic changes after 200 h. The function for fitting R_2 was $y = ae^{-t/\tau 1} - be^{-t/\tau 2} + e^{(t-180\sim\ \mathrm{min})/\tau 3}$, while C_1 could not be fitted to a suitable function. Note that the fitting to functions is only for better clearance and has no mechanistic background.

date explanation can be the hydration of the stratum corneum and subsequent degradation of the corneocytes after 20 h. Compared to other tissues there are two great differences: the dc resistance is considerably higher and the time related events are of the order of one magnitude slower. This is not surprising since the behavior measured here is dominated by the stratum corneum, which is a dead layer with very little water content.

The most dramatic changes were found in the low frequency behavior, depending on the large structures, mainly on the lipid layers and appendages. Smaller structures, such as corneocytes and keratin filaments are responsible for the behavior in the high frequency range. The time courses of the elements of the equivalent circuit are shown in Fig. 8a–c. A suitable assignment of these elements to the histology of the skin can be: R_0 is the dc-path (path 0), dominated by appendages such as hair follicles and sweat ducts. Path 1 (R_1C_1) is a tortuous pathway involving the path around the corneocytes, but crossing the lamellae structure of the lipids, while path 2 (R_2C_2) is the direct path through the corneocytes which results in the remarkably low resistance found initially of two to three orders of magnitude less than R_0 and R_1. Initially the characteristic frequency for path 1 is between 80 Hz and 300 Hz, whereby path 2 exhibits a characteristic frequency between 10 Hz and 100 kHz.

The time courses of the circuit elements can be divided into three phases. During about 10 h the corneocytes are hydrated and filled with water. The rise of the resistor R_0 strongly suggests a closing of the appendages (phase 1) by swelling of the surrounding tissue. During the next 200 h no dramatic changes are evident (phase 2). The resistances are changing moderately, as well as the capacitances. A transition from a intact barrier-function of the stratum corneum to an highly conductive state was found around 200 h for skin incubated at 37°C. After this time, it seems probable that the lipid structures destroy and the capacitances vanish (phase 3). After about 300 h the magnitude of the skin impedance reached about 1–2% of the value immediately after separation. This is consistent with the behavior found for heat-stripped stratum corneum stored at high humidity ($\approx$ 100%) at 4°C. Here a stable impedance was found up to two weeks, where for longer times

already a degradation followed. Note that the experiments here were performed at 37°C. These results confirm the suitability of heat-stripped skin, not stored longer than one week, as a model for experiments where the barrier function of the stratum corneum is essential [7]. The degradation of the cell membranes fits an exponential decay well, suggesting that only one event, the permeabilization of the membrane structures is involved. The characteristic time of the decay of R_0 and R_1 is about 40 h.

One of the most striking results from these experiments is the constancy of ϕ. Even after 400 h it is still 20°. A candidate explanation may be that there are only changes in the membrane structures but no changes within the electrolytes after fully hydration, which implies that the real part of the impedance at high frequencies is always constant.

The chosen equivalent circuit is a rough approximation, but reflects the important electrical behavior of the stratum corneum. The model of the stratum corneum is sufficient since the main electrical resistance resides here and the influence of the resistance of the underlying tissue is negligible. Here we could show that the impedance of the skin is strongly time dependent. This means a description of the electrical behavior of skin should not only contain the post separation treatment, but also the exact time after separation. The incubation temperature of 37°C is too low to alter the skin thermally and the change in the passive electrical properties proceeds faster compared to lower temperatures. The changes in phase 1 and 2 (< 200 h) did not show evidence of alteration of the skin, while the skin in phase 3 was irreversibly altered.

5. Conclusions

The passive electrical properties of skin depend on time after separation from the body. This means, that in vitro studies should take into account the time after separation. For a time after separation of less than 200 h in the bath medium at 37°C, qualitative alterations of the skin were not found and thus it can be a reasonable model for the properties of skin in vivo. The change in the impedance during the first 10 h is a result of hydration of the skin and the conditions of the appendages.

The skin exhibits a strong nonlinearity in impedance if an applied bias voltage exceeds about 1 V. The temperature-dependent shift in the impedance is similar to that of electrolytes, between 2 and 3%/K.

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