A planar micro-sensor for bio-impedance measurements

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Abstract

The outermost layer of the human skin, the stratum corneum (SC), is comprised of structurally inter-positioned anatomical regions. This inter-positioning renders the electrical characterization of its individual region difficult. To address this difficulty, the SC’s electrical properties at the cellular level were investigated. Impedance measurements were performed using a planar microelectrode sensor, which was dimensionally comparable in diameter to individual SC corneocytes. In this study, the SC was characterized by detecting micro-heterogeneity within the tissue architecture. The magnitude of the impedance and equivalent parallel capacitance of the SC specimens were measured by applying current to a specified electrode and measuring the resultant voltage potential from its corresponding electrode. Raw data show that the SC impedance at different frequencies depicted a linear approximation which does not fit the Cole–Cole model. These data also suggest that this sensor could be used to probe the molecular structure of the skin.

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1. Introduction

Human skin is a complex, multi-functional, structurally heterogeneous medium which protects internal organs and adaptively communicates with its environment. The outermost layer of the skin, the stratum corneum (SC), provides a vital barrier to the external environment, protecting the body from infectious microorganisms and noxious substances. The SC also serves as a barrier to excess loss of hydration from the body. Structurally, this layer is composed of a hydrophobic lamellar lipid matrix containing flat polygonal dead corneocytes interconnected by molecular “rivets” called corneodesmosomes. The surface area of an individual corneocyte is approximately 900 $\mu m^2$. The SC’s ability to regenerate and respond to dynamic environmental influences suggests that this interfacial layer has many properties characteristic of a “smart” polymeric material [1].

The electrical properties of biological tissues and organs have been studied since the mid 20th century [2,3]. Skin surface electrodes are often used to measure the electrical activity of internal organs, such as the heart (electrocardiogram) or the brain (electroencephalogram). In obtaining data from these internal organs, the primary surface for interfacing the body with skin surface electrodes is the SC. However, because the SC is an anisotropic structure with significantly higher impedance than its underlying tissue, it is usually removed, disrupted by abrasion or modified by pretreatment with an alcohol wipe prior to electrode attachment [4].

The current medical focus, on long term in vivo monitoring and on transdermal drug delivery, has driven a concerted effort to study the skin with better electrical compensation within circuits [5,6]. A few commercial instruments developed to electrically sample skin in vivo include the corneometer [7,8] and the dermal phase meter [9]. These instruments have provided some bulk electrical properties of the skin due...
to their relatively large interfacing electrodes. Mathematical models that correlate the electrical behavior of the SC with its structure are absent. Existing models could presumably be improved by understanding the electrical behavior of the SC at the cellular level. The collection of this data requires that the skin-interfacing electrodes are comparable to the cellular components of the SC. The ability, to non-invasively collect and interpret this data, would give healthcare providers accurate and objective information for skin diagnoses.

In vitro and in vivo measurements of the skin require a strict testing protocol for accurate data interpretation. Factors such as skin temperature, duration of measurement, and skin hydration have a considerable impact on the measurements. It has been reported that temperature and measurement time have significant influence on passive electrical properties of excised skin even though maintained at 37 °C [10]. Also, electrical fields induced by current densities between 0.13 and 1.3 mA cm$^{-2}$ affected the skin’s overall intercellular lipid structure, capacitance, and resistance [11].

In this study, the electrical characterization of the SC is investigated using a planar micro-sensing electrode array. It is hypothesized that the SC can be electrically mapped on a cellular basis as its electrical properties are the summation of cellular (corneocyte) and inter/extra cellular regions. The varying impedance values will then be the characteristic of its constituent domains.

2. Sensor design

A planar micro-sensor interfaced with 16 miniature electrodes tracks was designed to analyze the electrical properties
Schematically illustrated in Fig. 3, this sensor’s tracks were arrayed in a circle with a diameter measuring 250 μm. The dimension of each micro-track is illustrated in Fig. 1b. Additionally, a circular post was fabricated at the tip of each track to ensure good contact with the specimen under investigation. At the end of each track, a designated area, known as the bond pad, was used to apply current or voltage to the tips (measuring electrodes).

2.1. Fabrication

An array of 16 micro-sensors was fabricated on a 2-in. boro-float substrate. Glass was chosen as a substrate over silicon (Si) because it eliminated any current leakage through the substrate. Each micro-sensor die measured 4 mm × 4 mm. Fig. 2a through d schematically illustrate the fabrication process of the sensor. As illustrated in Fig. 2a, a cleaned substrate was metallized with evaporated chromium (Cr)/gold (Au) (0.04/0.1 μm) to form the seed layer for electroplating. Au was the material of choice because it is inert to the environment. AZ 4620 Photoresist was spun on the substrate and lithography was done to expose the tracks. Next, in Fig. 2b, the tracks were electroplated to a thickness of 2 μm using Technic 25E gold plating solution. The resist was then stripped. The resist was spun and patterned to form the electrode tips. The tips were plated to a thickness of 4 μm as shown in Fig. 2c. The resist was stripped and the seed layer was removed with a timed etch. Finally, Shipley 1813 Photoresist was used to expose the tips and the bond pads as shown in Fig. 2d. This resist was left on the device and cured. It acted both as an insulator between each micro-track and an insulator between the tissue specimen being tested and the track.

A 38 μm diameter contact tip was fabricated. Due to its size on the track, the 38 μm tips were placed at an offset, approximately 134 μm from the tip of the track, to form a circular diameter of 500 μm (see Fig. 3). The tip size was chosen to approximate the surface area of an individual corneocyte within the SC.

3. Measurement protocol

Cryo-preserved split-thickness skin (epidermis plus partial dermis) was obtained from US Cell and Tissue (Cincinnati, Ohio, USA). Pieces of split-thickness skin, isolated SC and isolated delipidated SC were cut into 3 mm × 3 mm specimens. The SC was isolated from the split-thickness skin by enzymatically treating the tissue with 0.05% trypsin overnight at room temperature and partially delipidated by washing the tissue with acetone for 10 min at room temperature. This acetone wash left the intercorneocyte lamellar lipids intact and removed the SC surface lipids.

Room temperature (21 °C) and humidity (40%) were kept constant during measurement. Each specimen was placed on the top of the micro-electrodes with the surface to be measured against the sensor. Next, the micro-sensor was placed on a Cascade Summit 11562 (AttoGuard) Probe Station. The manipulators/probes were connected to the bond pad and impedance measurements were obtained using a 4294A Precision Impedance Analyzer. From these measurements, the absolute impedance with its equivalent parallel capacitance was derived from the signals’ resistive and phase components. To minimize inter-track capacitance between the electrodes, eight diagonal positions were designed to obtain the measurements (see Fig. 1a). Measurements were taken on two diagonal tracks at 2 min intervals until all positions were recorded.

At any given position, one probe contained the high current (Hc) and high voltage (Hp) terminal while another probe contained the low current (Lc) and low voltage (Lp) terminal. The measurement procedure involved applying a voltage potential to a specified electrode and recording the resultant current from its corresponding electrode. The impedance spectroscopy ranged from 10 kHz to 1 MHz. The oscillating voltage was set at 250 mV to give a 2 μA maximum output.

4. Results and discussion

Impedance (Z) refers to the “measure of the total resistance to electrical current flow” [12]. It is comprised of a real component, which is resistive, and the imaginary component, which contains the capacitive or inductive phase of the signal. It has been reported that plotting the real (Re) and imaginary (Im) components of impedance on typical skin tissue, measured at different frequencies, with macro-electrode contacts, ranging from centimeters to millimeters, depict a bell-shaped curve [13]. This relationship is known as the Cole–Cole plot. Most electrical impedance data (obtained with macro electrode) on biological tissues are represented by the Cole–Cole plot.
To assess the effect of the split-thickness skin’s orientation on the sensor, data was collected both on the stratum corneum (SC) side and on the dermis side of the specimen (see Fig. 4). For these measurements, the probes were placed at position 2. Data were collected at 5-min intervals for 55 min at this position. This test was conducted on the sensor at 10 kHz. Each measurement used a new specimen to ensure minimal starting variation. Hence, the repeatability of this experiment on every specimen tested (10 specimens of each set) gave different values but similar impedance frequency curves due to the skin’s anisotropic nature. The results shown here are for one specimen set.

As seen in Fig. 4, unlike the SC surface, the impedance and equivalent parallel capacitance of the dermal surface of the split-thickness skin did not dramatically vary in magnitude. The data show that the dermal surface does efficiently retain water even though it has been excised from the body. To reduce the effects of dehydration during testing, a 2.5 μL pipette drop of de-ionized water was placed on the dermis side of the isolated and delipidated SC specimens.

Plotting the Re and Im components of impedance, using micro-electrode contacts on the SC, revealed a linear relationship, as shown in Fig. 5, which does not conform to the Cole-Cole plot [13]. The slight askew shape of the curve in Fig. 6a may be attributed to changes in the corneocyte or lipid hydration. The observed variability in the data between specimens may also be the result of the variability in the positioning of the contact tips with respect to the specimen under test. The contact tips may be measuring lipids, coral-
cytes, or corneodesmosomes or some combination of these components.

Figs. 6 through 8 show the absolute impedance and equivalent parallel capacitance, respectively, for each specimen at 10 kHz, 100 kHz, and 1 MHz. Partially delipidating the SC (surface lipids removed) reduced its overall impedance and increased its capacitance as expected. This result is expected because the skin surface lipids are mostly non-polar. Therefore, these lipids do not readily conduct an electrical charge. The delipidated surface reveals hydrated corneocytes that are more conductive. The discrepancies that are observed between the different electrode positions may be due to a cell polarization response to the applied field, to a distinct intercorneocyte lipid interface, or to differences in tissue hydration. Coupled optical/electrical measurements are planned to resolve the issue of electrode placement.

5. Conclusions

Electrical impedance measurements were made on the SC and partial dermis. The results show that the absolute impedance of the specimens is inversely proportional to the parallel capacitance. The magnitude of the impedance was affected by hydration, the heterogeneity of the specimens and the location of the micro-electrodes contact. Impedance measurements using micro-electrodes on various specimens of human SC confirmed that the SC frequency response curve, relating to impedance, does not fit the Cole–Cole plot as previously reported [13,14]. Because the area of the electrode tissue contact (∼1104 μm²) approximates the surface area of an individual corneocyte (∼900 μm²), the results suggest that this MEMS sensor would be a useful probing device to electrically map the molecular structure of the SC. The observed variations with respect to the circumferential contact positions deserve further investigation using an optical and tetra-polar measurement system.

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References

Biographies

Helen Benjamin is currently a graduate student continuing PhD research on the analysis of the stratum corneum’s impedance properties using micro-sensors. She received her BS (2001) and MS (2004) degrees in Electrical Engineering at the University of South Florida. She is the first recipient of the NSF Graduate Fellowship Award in the Electrical Engineering Department.

Shekhar Bhanwali is currently an Associate Professor in the Department of Electrical Engineering and Nanomaterials and Nanomanufacturing Research Center at the University of South Florida. His interests are in the areas of Bio-MEMS, sensors, and microsystems. He is the recipient of the NSF CAREER award.

Steven Hauth is a Medical Doctor and Professor of Pediatrics (Neonatology) at the University of Cincinnati and Medical Director of the Skin Sciences Institute at the Cincinnati Children’s Hospital Medical Center. He is an expert in newborn intensive care and is particularly knowledgeable regarding the biology of vernix caseosa, epidermal barrier development, and the application of skin-based sensing systems for noninvasive biomedical monitoring and measurement. Dr Hauth was among eight international researchers in the first NIH-NASA project to assess the effects of space flight and zero gravity on mammalian pregnancy and fetal development.

William L. Pickens is a Senior Research Scientist in the Department of Pediatrics at the University of Cincinnati and the Skin Sciences Institute at the Children’s Hospital Medical Center in Cincinnati Ohio. He is an expert in epidermal barrier development and is one of the seminal researchers studying the properties of the neonatal biofilm, vernix caseosa and its role in extraterrestrial transition at birth. Along with Dr Steven Hauth, he was a co-investigator on NASA/NIH-R1, through which they investigated the effects of microgravity on stratum corneum development during gestation.

Rod Smallwood is the Director of Research for Engineering and Professor of Computational Biology at the University of Sheffield, a Fellow of the Royal Academy of Engineering and an Honorary Fellow of the Royal College of Physicians of London (an unusual combination!). He is a past president of the Institute of Physics and Engineering in Medicine. He was employed by the National Health Service for 25 years doing everything from designing instrumentation for measuring blood flow to planning new imaging departments in hospitals, before moving to the University of Sheffield. During the past 8 years as an academic he has set up undergraduate and postgraduate courses in medical physics, and pursued research into electrical impedance tomography for lung imaging, electrical impedance spectroscopy for screening for epithelial cancers, virtual reality systems for surgical training, thermal microscopy, and, most recently, computational modeling of cellular interaction.