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BIOIMPEDANCE MEASUREMENT OF HEALTHY SKIN

Master of Science Thesis
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November 2021

ABSTRACT

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Master of Science
Tampere University
Biomedical Sciences and Engineering,
Major: Health Technology and Informatics.
November 2021

Skin which is the largest organ of the human body consists of three major layers stratum corneum, epidermis and dermis. The electrical signal flows through skin using two layers- stratum corneum known as the dielectric layer and the base membrane which is referred to as the conductive layer. Whereas bioimpedance is a frequency dependent variable which can be defined as a passive electrical characteristic that deals with the capacity of biological materials to resist electrical current. For monitoring wound status of skin bioimpedance measurement seems promising and effective. In this thesis work, however, we wanted to measure the bioimpedance of healthy or undamaged skin by utilizing a quasi-monopolar bioimpedance measuring system that includes a bioimpedance measurement device, measurement software, and a screen-printed electrode sensor array.

The Main objective of this study was to measure the bioimpedance of healthy skin to see how stable the electrode pairs impedance value remains during the measurement period. Besides, we wanted to observe the variations of bioimpedance in different skin types of different test person, which factors affect the changes and how stable variations can be achieved after analysing the data. Moreover, we also tried to find out how bio-impedance changes in different test person for different measurement frequencies. To achieve our goal, we have selected 8 different test persons for measuring their skin bioimpedance for 5 consecutive days. After selecting the test person, by using the electrode sensor array along with bioimpedance measurement device and the measurement software the bioimpedance is measured at 150 Hz, 300 Hz, 1 kHz, 3 kHz, 5 kHz and 10 kHz frequencies and for the analysis the bioimpedance obtained from 300 Hz, 3 kHz, 5 kHz and 10 kHz measurement frequencies is used. The measured data is processed and analysed by using Microsoft Excel and then the statistical analysis is performed. After analysing the impedance data, we found out that most of the electrode pairs impedance data varies during each of the measurement and hence produces unstable response. The results also suggested that, if the measurement is taken immediate right after any physical activity or labour-intensive work then the bioimpedance value will decrease while if the measurement is taken after few hours of any activity then it will cause no effect in the impedance value. Besides, we also found out that, if there is no moisture or sweat present between the skin and electrode contact during the measurement then it will cause no effect as well. On the other note from the result, we have seen that under the same measurement setting and similar frequency, different test persons average impedance is different. On the other note, from this study we came to know that the higher the frequency is the lower the bioimpedance can be achieved. This work reflects the possible use of bioimpedance measurement in the field of biomedical and dermatology research and depicts the significance of bioimpedance data for patient monitoring for future work.

Keywords: Skin, Bioimpedance, Measurement, Quasi-monopolar, Frequency, Stability.

PREFACE

This Master of Science Thesis work was conducted under the faculty of Medicine and Health Technology. I have started this thesis work on 1st March 2021.

I would like to express my gratitude to my supervisors Assoc. Prof. Jari Viik and Ph.D. Atte Kekonen for giving me the opportunity to work with such an interesting topic which requires practical implementations. Their active supervision and guidance were really helpful for completing this thesis work. Besides, whenever I was in any problem situation they provide and shares their valuable experiences and knowledge which helped me a lot during this journey.

At the beginning, because of the Covid-19 pandemic situation it has not been an easy journey to find the test person for the bioimpedance measurement. However, I want to thank all the test persons who were generous enough to volunteer and agreed to allow me to measure their skin bioimpedance. I am grateful to them for their time and effort.

At last, I want to thank my parents for their enormous support and trust in me during this journey. I would also like to thank my elder brothers for having their believe in me. They were my real inspiration and are the only one who had never doubted my abilities.

Tampere, 18 November 2021

Md Imran Hosen

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1. INTRODUCTION

Bioimpedance is a frequency dependent variable which can be defined as a passive electrical characteristic that describes the capacity of biological materials to resist electrical current. Bioelectricity relies on the electrical qualities of the tissues along with the responses of skin tissue for induced electricity.[1] The human body is a complex biological system made up of various tissues. Intracellular fluids and cell envelopes (cell membrane for animal cells) are present in all biological cells. An alternating electrical signal causes animal cells suspended in extracellular fluids to behave differently, resulting in a complicated electrical impedance known as bioelectrical impedance or electrical bioimpedance.[2]

The bioimpedance analysis is based on the electrical conductivity of different tissues. While the electrolyte-rich bodily fluids readily conduct electricity, adipose tissue acts as an insulator [3]. Bioimpedance analysis can use these qualities to discriminate tissue and determine a person's body composition. Bioimpedance has been effectively utilized to assess skin condition also. Skin erythema is a symptom of skin irritation generated by a variety of chemical or physical stressors. It is biologically derived from dilatation of local subcutaneous blood vessels, where an excess of water influences the skin's electrical impedance. In addition, bioimpedance is used in a variety of ways in healthcare institutions, including disease prognosis and monitoring vital signs. According to most studies, body structure is directly linked to fitness. Longevity and well health are connected to a healthy body fat distribution. Excess fat in relation to lean body mass: a shift in body composition raises the risk of heart disease, diabetes, and other disorders. [4] In the field of biomedical and dermatology research the use of bioimpedance is inevitable and increasing day by day. By using the skin bioimpedance skin cancer assessments[5], the estimation of the skin surface hydration [6] and wound healing status monitoring can be done [7].

Bioelectrical impedance measurement is a non-invasive method of analysing cellular architecture and function that can monitor the wound healing status. A wound is defined as a cut or opening in the skin. It could be anything as simple as a scratch or something as serious as a paper cut. A large scrape, abrasion, or cut may occur as a result of a fall, accident, or trauma. On the other hand, a chronic wound is a skin wound that does not

heal, heals slowly, or heals but recurs. Trauma, burns, skin cancers, infection, or underlying medical disorders such as diabetes are only a few of the various reasons for chronic skin wounds. If a wound fails to heal in a timely manner through normal tissue healing mechanisms despite active therapy, it is ambiguously described as chronic. It's essential to pick the right wound dressing. Because venous ulcers are usually moist, initial dressings that absorb moisture are employed. [8] Wound healing is a complex, multi-step process involving molecular, cellular, and metabolic processes. Skin impedance, which is related to the biological structure of skin, has an electrical characteristic that can be approximated by an electrical impedance, which could explain the impedance trend during wound healing [9]. Despite breakthroughs in wound healing mechanisms, the estimated annual cost of treating wounds in the United States exceeds $20 billion, with lower-extremity wounds accounting for the majority of this [10].

The degree of skin impedance, especially at lower measuring frequencies, is determined by a variety of parameters, including the skin's moisture content, thickness, and physiological state. It is generally recognized that measuring skin impedance over lengthy periods of time in a reliable and consistent manner is difficult. The precise coordination of multiple different cell types in sequential phases is required for skin repair. The epidermis is the outer, impermeable layer of the skin that protects it from the hostile external environment in healthy skin. [11] Sebaceous glands, sweat glands, and hair follicles are all found in the epidermis. Visual assessments of characteristics like the hue of the wound's surface, the amount of effusion and detritus, as well as the colour of the effusion and detritus, and the fragrance and overall health of the tissue in the area are commonly employed in clinical practice to assess and monitor wounds [12]. The size of the wound needs to be frequently measured. Constriction of the wounded blood arteries and activation of platelets to produce a fibrin clot are the first responses to a wound [11]. The integrity of healthy skin is critical for the human body's physiological equilibrium to be maintained. The skin is in a constant state of rejuvenation. Furthermore, the skin's impedance is strongly reliant on its moisture level. Even in a normal state, the impedance of healthy skin varies slightly [13]. Mathematical approaches can be used to lessen this variation. Based on the experiments, it was expected that a minimal impedance for intact and healthy skin under normal moisture conditions could be defined with fair certainty at a specific frequency.

As tissue physiology and pathology change with tissue health, the bioelectrical impedance changes from healthy to diseased. Blood is a good electrical conductor, malignant tissue with more blood has a lower impedance path to the electrical current. [14] Multifrequency impedance study provides more information about tissue properties, which

aids in improved tissue characterisation, because the response of electrical bioimpedance varies with signal frequency [15]. Because healthy skin differs from injured skin in so many ways, the result of skin impedance in this situation is important. Frequency rate of skin has an impact in the case of measuring bioimpedance. Whether the current passes through the cells or persists in the extracellular compartment is determined by the frequency. Higher frequencies permeate the cells, while lower frequencies pass through the extravascular space[16]. This enables the evaluation of resistance in both extracellular and intracellular layers.

Bioimpedance measurement can be used to assess the condition of wound skin. The main purpose of this thesis is to determine the bioimpedance of healthy or undamaged skin. For this project, bioimpedance of healthy skin is measured from 8 different test persons by using a 5x5 electrode sensor array for 5 consecutive days. Observations of variations in bioimpedance obtained from the electrode pairs in different skin kinds of different test subjects were observed for this study, and then we discovered how bioimpedance fluctuates in different individuals. For the purpose of the experiment, frequencies of 300 Hz, 1000 Hz, 3000Hz, 5000 Hz, and 10000 Hz are used. And another objective was to see how stable the bioimpedance values of the electrode pairs remain over the course of five days from different healthy skins, which factors affect the changes and how stable variations can be achieved after analysing the data. Moreover, how the bioimpedance changes with the use of different measurement frequencies is also observed. In this thesis the following research questions are addressed and investigated

Research Question 1. How stable the electrode pairs are in terms of impedance values of the healthy skin of different test person during the measurement period?

Research Question 2. How different types of physical activities and time of the measurement causes any affect in the impedance measurement? If causes, then what will be the variations like?

Research Question 3. How the healthy skin bioimpedance varies for different test person? what is the variations in impedance data for different measurement frequency and does it cause fluctuations in bioimpedance during the impedance measurement for different test person?

There are six chapters to this thesis. Section 2 discusses the study's background and related investigations involving skin, bioimpedance, and bioimpedance measuring methodologies, among other topics. Section 3 summarizes the materials and methodology

used in this thesis, as well as the analysis. The analysis' outcomes and results are presented in Section 4. The overall discussion, challenges, limitations, and future work are all discussed in Section 5. Finally, Section 6 provides the conclusion of this thesis work.

2. BACKGROUND

In the field of biomedical and dermatology research, the healthcare system is presently progressing at a rapid pace and the use of bioimpedance is inevitable and increasing as well. A lot of new techniques have been proposed and, in some cases, some methodologies are being refurbished in this healthcare system. Every day, new ideas are developed in the field of biomedical research. Bioelectrical Impedance, or Bioimpedance, is one of these techniques. This section will cover the definition of skin along with its properties and types, bioimpedance, its features, advances, challenges, different types of measurement methods etc.

2.1 Skin

The skin is the body's largest organ, with a contact area of roughly 1.9 square meters. The skin protects us from microorganisms and the surroundings, assists in body temperature regulation, and allows us to feel contact, warmth, and coldness. The skin protects the body by keeping critical proteins and minerals in place while also acting as a barrier to hazardous substances produced in the body and shielding it all from the sun's UV rays.[17] The texture and density of it differs from one section of the body to the next. The epidermis of human mouths and eyelids, for example, is very thin and fragile, whereas the skin of the human foot is thicker and firmer.

Human skin is one of the largest organs in the body, designed to protect it from external stimuli and possessing a number of electrical properties [18]. The integumentary system is made up of the skin and its derivatives (hair, nails, perspiration, and oil glands). Protection is one of the most important roles of the skin. It defends the body from infections, toxins, and extremes of temperature [19]. The skin has a complex structure, which consists of an external layer called epidermis and the inner layer named as dermis [20]. The keratinized stratified epithelium, which is the epidermis, has several layers, the stratum corneum is the outermost. The stratum corneum consists of stacked keratin plates with intercellular lipids to fill the plate gaps [21]. Besides, the thickness of this layer is not the same for all. The rest of the epidermis is composed of live cells under the stratum corneum. The dermis is a connective tissue layer that contains the hypodermis or the subcutaneous tissue, which mostly consists of loose connective tissue that is also interconnected with blood vessels. Besides, glands and hairs are also present in the skin to open the skin surface pores. Motor nerve fibers are provided by the muscular planes

which are separated by fascial layers from hypodermis and situated deep to the hypodermis [22].

Figure 1. shows the human skin including three major layers of skin: stratum corneum, epidermis and dermis

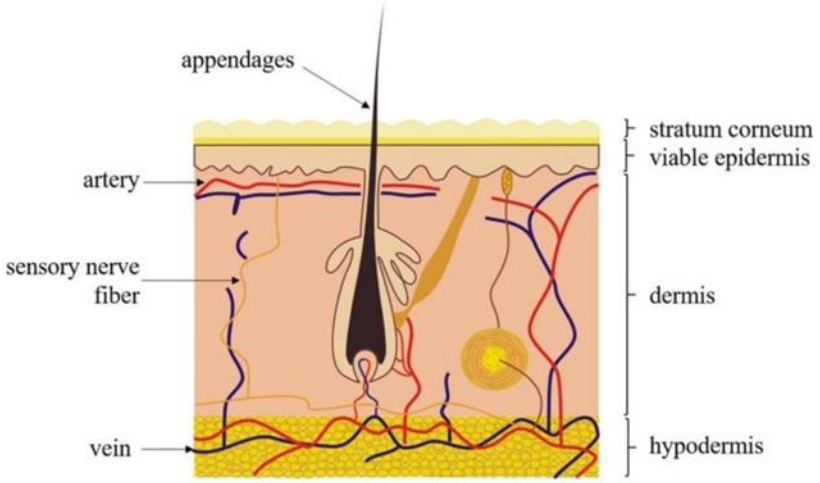

Figure 1. *Different layers of skin. The stratum corneum (outer layer), the viable epidermis, and the dermis are all visible from the outside to the inside*[23].

Keratinocytes make up most of the epidermis. The basal layer is a slender, multi-layered tissue that ties the epidermal to the dermis underneath the epithelium [24]. The hypodermis is mostly made up of fat. The skin of an individual changes as they grow older. It thins out and becomes more vulnerable to harm. The epidermis replaces dead skin more slowly, and the recovery process is delayed. Generally, a person's skin will indeed be slightly less dense. This is a component of the immune cells and guards a person from infections. Human skin aids in the regulation of the individual body's metabolism. To remove heat from one’s body, blood circulation to human skin's surface, while circulation of blood to human skin lowers to retain heat in [25]. This has an impact on how much humidity drains from human skin, which in turn has an impact on the body's warmth.

The impedance of the skin changes with time. External factors such as humidity levels have a significant impact. The electrodes have a significant impact on the measurement equipment. Skin impedance is affected by the thickness of the skin, the frequency of skin layers, and the activity of sweat glands, and it varies by individual and skin type.[26]

2.2 Electrical Properties of Skin

The electrical properties of the skin are very complex and are defined by the composition of the skin epidermis, which is made up of stratum corneum, stratum lucidum and stratum

granulosum. The first shield as an ion-impermeable coating is the lightweight stratum corneum. The second barrier may be the base membrane of the germinating layer. The multi-layered stratum granulosum and stratum germinativum structure consisted of a strong capacitance with a polarization portion.[27] Therefore, electrical signals first pass through the stratum corneum and other epidermis before it reaches towards motor nerve fibers. Electrical signal flowing path of skin consists of two layers, stratum corneum known as the dielectric layer and the conductive layers are the ones which are deep to the stratum corneum [22].

In response to the AC signals the electrical properties of human skin, it was found that the high frequency skin impedance is roughly 100Ω, compared to the impedance from 10k Ω to 1MΩ at low frequency [28]. Human skin impedance has been found to be regulated and dominated by the stratum corneum at frequency which is low and can be below 1 kHz and at higher frequencies the viable skins can be achieved. Nearly 50% of the skin impedance can be accounted for at 10 kHz stratum corneum, while at 100 kHz, the skin impedance accounts for just 10 percent [29].

The resistance and capacitance values of skin can be achieved in response to the dc signals effects on human skin. To get the DC response of human skin different formulated skin voltages have been used. For this reason, long pulse durations in the millisecond range have been used, as well as voltage amplitudes of 3.5V, 10V, or 18V[30]. The capacitance value of stratum corneum was found around 2nF (changes a lot according to e.g., moisture, the skin condition) and the skin's capacitive charging requires as many as 200μs[31]. Notably, the high resistance value of stratum corneum decreases when the potential difference and current density increases. For healthy intact skin the resistance value is 2.5kΩ whereas, for abraded or damaged skin the resistance values could be 500Ω [32].

From the permittivity and conductivity values the human skin’s electrical properties can be achieved where the permittivity and conductivity are both strong frequency and temperature dependent and vary according to them. With the increase in frequency the permittivity decreases whereas the conductivity increases with respect to if the frequency increases [33]. From assessments in the wide spectrum of (10 Hz to 100GHz) skin permittivity and conductivity was observed and found that when frequency is increased from 10Hz to 100GHz the relative permittivity decreases down from 105 Sm−1 to 10 Sm−1. On the other hand, conductivity rises from 10−4 Sm−1 to 10 Sm−1 while frequency increases from 10 Hz to 100GHz [34].

2.3 Skin Types

The thickness of the skin, the frequency of skin layers, and the activity of sweat glands all have an impact on skin impedance, which varies throughout individuals and skin types. The various types of skin are classified using several criteria. Fitzpatrick's classification, for example, was first defined in 1975 which was based on skin colour and reaction to sun exposure [35].

Skin is graded cosmetically based on many factors that affect its balance, including sebaceous secretion, hydration, and sensitivity. As a result, each skin type will have its own characteristics which will necessitate different skin care. There are several kinds of healthy skin. The three basic types of healthy skin are dry, oily, and sensitive. These are detailed more in below.

Dry Skin:

A lack of moisture in the skin barrier causes dry skin, which is a common complaint. The technological innovations have shed light on the pathogenesis of dry skin at the molecular scale. External factors such as temperature, low air humidity, and immersion in hot water can produce dry skin, which is usually only temporary. It can, however, occur more often in some people and even be a lifelong disorder for others. While dry skin can crack, exposing it to bacteria, this is generally not a serious problem, it can cause other skin diseases, such as eczema, or make a person more susceptible to infections if he/she doesn't take care of it. The signs and symptoms of dry skin might differ depending on a variety of factors, such as age, health, etc. It is distinguished by a tightness and roughness in the body. Desquamation, scratching, redness, and small cracks can appear, as well as an ashy Gray colour. [36] Recent research has expanded the rationale for using physiological lipids in a dry skin moisturiser, as they have been discovered to be necessary for an adequate formulation and organization in the cell membrane, but they are diminished in dry skin [37]. Due to the reduction of enzyme activity responsible for the formation of hydrating substances when skin is dry, a new study has confirmed the incorporation of specified antioxidants into a topical emollient for dryness.

Oily Skin:

The presence of oily skin is porous, humid, and shiny. Excess fat development by the sebaceous glands induces it, and it is normally determined by genetic and/or hormonal factors. It's common in teenagers and young adults under 30 years old, and it's usually associated with skin problems. In some studies, imaging or other approaches were used to overcome the problem of oily skin. Excessive light, enlarged pores, and acne are clinical signs of oily skin that, when paired with a hydrolipidic mantle deficiency, can have a

long-term negative influence on a person's quality of life. [38] The salivary glands produce excessive sebum, resulting in oily skin. These glands are located beneath the skin's surface. Sebum is a sticky, fat-based substance. Sebum isn't all terrible; it nourishes and protects skin, and it keeps the hair shiny and silky. Despite the fact that high sebum production has no physical effect on body function, those who suffer from chronic oily skin find it distressing.[39] Oily skin is greasy and oily, and it contributes to the development of acne. It is often associated with large glands on the face [40]. The production of sebum is stimulated by humidity and hot temperatures, resulting in more oil on the skin. In contrast, skin may become dry in arid or cold climates, causing oil glands to overwork to adjust.

Sensitive Skin:

Sensitive skin is more likely to respond to stimuli that have no impact on general skin. It is a delicate skin that is often accompanied by unpleasant sensations such as heat, stiffness, redness, or itching. This form of skin loses its protective barrier, allowing microbes and irritant compounds to easily penetrate, raising the risk of infection and adverse reactions. No agreement has been reached on the concept of sensitive skin and attempts to establish a reliable diagnostic test to objectively define the entire profile of sensitive skin have failed in some research. The complexity of signs, the subjective experience of sensory pain, and the lack of obvious clinical features all contribute to the diagnosis of sensitive skin. [41][42] It is a fragile skin that necessitates extra attention in order to combat dryness, roughness, and its typical appearance.

Apart from these three major types of skins, there are another two types, which are: normal skin and combination (both oily and dry skin) skin. Skin that is normal is hardly too dry enough or too oily. It has a normal surface, no flaws, and a clean, soft appearance, and it requires no special maintenance. On the other hand, combined skin has both dry and oily characteristics since the allocation of sebaceous and sweating glands isn't uniform. The T-zone (forehead, nose, and chin) is normally oilier, whereas the skin mostly on cheeks is natural or dry. Overconsumption of sebum causes the oilier areas of the combined skin. A shortage of sebum and a resulting lipid imbalance triggers the drier sections of the combined skin.[43] All these skin types have some good points, and some negative impacts too on the human body.

2.4 Bioimpedance

Impedance is the result of combining resistance and reactance. It's an essential part that prevents electrons from flowing freely through a circuit. Bioimpedance is a term that describes this occurrence. It is also known as Bioelectrical Impedance. The reaction of a living tissue to an externally applied electrical stimulus is known as bioimpedance. It is the reversal of electrical characteristics and increases the concentration of electric current flowing through membranes.[44] It regulates the speed with which a reduced electrical charge can move through the human without causing discomfort. Now-a-days, bioimpedance analysis is a widely used technique in healthcare assessment systems. Bioimpedance measurement has been used in a variety of areas, incorporating clinical prognosis and crucial status monitoring, and it is rapidly advancing.

The use of bioimpedance analysis employing needle electrodes to estimate water balance in the body [45]. In the case of bioimpedance tests, Nyboer et al. proposed an approach for measuring the biological body's fat-free weight using quad surface electrodes observations [46]. Lukaski et al. modified the bioimpedance study theoretical frameworks for physiological behavior and disease diagnosis evaluation in a research article [47]. Bioimpedance measurements are used to determine the structure, conductors, and insulators of electrical current in human tissues. Bioimpedance is used in electrical impedance tomography to generate clinical data of the inside of the human body. Innocuous electric currents are administered to the patient's skin using an electrode array, and the applied electrical potentials are recorded [48]. Bioimpedance technologies are found in haematocrit meters, cultured cells related equipment, and Lab-on-Chip implementations in clinical laboratories.

Bioimpedance study enables for the early identification of an unbalanced body structure, allowing for more effective intervention and prevention. Bioimpedance measurement can also be used to determine fluid and body fat, which can be a good way to figure out how healthy a person is. The bioimpedance measurement is separated into five sections of the human body that are all different. Among those, one is for the spine, two for the upper extremities, and two for the lower extremities.[49] The human body is made up of fat mass and fat-free mass , which are minerals in the bones, as well as the number of cells in the body, which are high in protein and water, and are mainly made up of extravascular, intracellular fluid and space. Bioimpedance analysis has been utilized in dermatological research to monitor skin hydration, identify skin cancer, and quantify transdermal drug distribution, among other things [6]. The bioimpedance technique could be utilized to track acute and chronic wound healing in nonlinear data. A wound is a break in a body

structure's continuity, particularly an injury to the protective covering, such as the skin. In a study, researchers revealed a novel multi-electrode sensing device model under initial wound care for long-term bioimpedance measurement for monitoring the healing status [8]. Swisher et al. used bipolar bioimpedance measurement to develop a smart dressing for timely identification of pressure-induced skin injury in a rat model [50]. Aside from these, bioimpedance method creation has been used in a lot of research for analysing skin injury, analysing the consistency of human skins, and so on.

Electrodermal activity is a measurement of the skin's behaviour, including its resistance and conductivity. It is connected to bioimpedance because when measuring the overall impedance of a section of the body with electrodes attached to the skin, the electrodermal activity is included in the total impedance. Certain relaxation activities exist because the epithelial tissue is a mosaic, with layers of laminated, isotropic cell membrane stacked on top of one another. The effect of moisturizing cream is also investigated using an electrical susceptance-based calculation on human skin.[51] As a result, bioimpedance appears to be a promising method for determining how a moisturizing cream affects human skin

2.5 Bioimpedance Measurement

Early detection of an unbalanced human body structure using bioimpedance allows for more prompt intervention and prevention. It also provides fluid and body mass data, which might help a person to figure out how healthy he/she is right now. There are various methods for measuring bioimpedance with different electrode setup. Electrical bioimpedance measurement is a non-invasive detection system like microfluidic cell detection, but it concentrates on tissue rather than individual cells. The system is used in medicine and works in conjunction with physicochemical and biochemical approaches. Bioelectrical impedance analysis, electrocardiography, electrical impedance tomography, and electrical impedance myography are only a few of the applications. The healthy tissue, which consists of resistive extracellular fluid and capacitive cell membrane, is exposed to a weak AC voltage or current transmission in these implementations. [52] The body is subjected to a low-level, waterproof electricity. Tissues that take in a lot of fluid, such as blood, have a lot of permeability, whereas lipids and bones slow the signal down [53]. Since bioimpedance measures the resistance to electric current as it circulates through the body, it involves determination of body water, which can then be used to measure body fat using specific equations.

This frequency band is also inadequate to capture the full image of tissue resistance, and it is ineffective at penetrating cell membranes or distinguishing low-frequency impulses from heartbeats or breathing. In bioimpedance experiments, the same or different electrodes transmit the excitation signal and take the reaction, converting the electronic charge to charge density and conversely.[54] When it comes to alternating current (AC), the proportion of voltage (V) over current (I) is known as electrical impedance (Z). The calculated impedance is influenced by the tissue's resistive (R), capacitive (C), and inductive (L) materials. Then the equation be like:

$$Z = V/I, \tag{1}$$

The magnitude of a circuit's impedance Z is equal to the maximum potential difference, or voltage, V (volts) across the circuit divided by the maximum current I (amperes) through the circuit.

Several electrode arrangements can be used to track the attributes of a tissue engineering application. The resistivity at the intersection in between electrode and electrolyte or perhaps the live cell is represented and influenced by two and three circuit layouts. [55] One of the advantages of four electrode layouts over two electrode arrangements is that the polarization impedance is lowered by decreasing the effect of contact impedance and tissue or electrolyte interface [56]. Electrical impedance analysis is an easy and accurate tool for determining the thickness of cultured cells, as well as the condition of cellular membranes and the condition of intra and extracellular networks [57].

It has been shown that using high-power signals to create an electric field can trigger cellular membrane collapse, resulting in changes in intracellular components and resistivity. The electrical impedance of a sample of human tissues over a frequency range can be used to determine quantitative shifts in cells, such as cell population.[58] In a variety of biological applications, impedance spectroscopy is being utilized to track stem cell metabolism. Electrical impedance spectroscopy demonstrated a rise in the projected impedance resonance of a substantial magnitude of neuronal stem cells that have been treated with osteogenic factors as compared to control samples. The use of electrical current spectrometry to track real-time regeneration of skeletal critical size defects and the efficacy of nanomaterials and skeletal synthetic biology in critical size defects detection is being investigated. Furthermore, electrical impedance spectroscopy was used to show the ability to measure changes in elastin and salicylic acid in the wounded tissue on a quantitative basis.[59] There is another method called Electric Cell-substrate Impedance Sensing. A mild alternating current is used to assess the impedance differences

between a minimal electrode surface where cells are grown with a large reference electrode. This offers information about the complicated properties of the tissues cultivated upon that counter electrode. This approach was used to investigate cell adhesion, barrier function, spreading, density, as well as cell micromotion.[60]

Bioimpedance measurements have been utilized to define biological components and organic tissues used in the detection of hypertension, diagnose skin disorders, detect cancerous tissues, control ischemia during the transplant process, and even estimate blood pressure and blood glucose levels in a non-invasive and nonobstructive manner. However, the measurement procedure must be improved, and various frequencies must be used.

2.6 Bipolar Bioimpedance Measurement Method

Bipolar measurement is thought to be the worst method for estimating subcutaneous tissue composition because sensitivity is highest near the electrode surfaces (the skin), and the skin's impedance (especially the stratum corneum) is very high in comparison to any other tissues between the two electrodes. The impedance result is essentially made up of the impedance of the skin beneath the two electrodes and the electrode impedances of the two electrodes, to put it simply.[61] The impedance generated by electrode polarization is incorporated into the measurement of the material and electrode tissue interface when the alternating current passes through the electrodes. As a result, sample impedance is overestimated because of this. With only two electrodes, the same electrodes can deliver a continuous current, known as the excitation current, as well as sense, or detect, the impedance imposed by the section on the excitation source. [62] Although it may appear that placing only two electrodes on the client is simple, the quality of the electrode to skin contact path can affect the bipolar technique.

In a bipolar arrangement, two electrodes are positioned at the needle's tip. A modified percutaneous access needle with an electrical impedance sensor at its tip detects electrical impedance every 200 milliseconds is used in the needle system [64]. Through a little bit of tissue, electricity passes from one electrode to another that separates them, helping to determine the impedance. To establish a conventional bipolar needle setup, two of the Simplex needles mentioned earlier were attached next to each other. A two-electrode design is the simplest setup for impedance measurements in perfusion-based techniques. It's also often used to measure impedance in epidermal cultured cells when the electrodes are indeed implanted in the vessel's bottom. In this approach, cells would grow on the sensor's surface, and variations in impedance between the sensing and counter electrodes would be detected [65]. The amount of cell coverage on the electrode

surface might have a massive effect on the impedance evaluated. Although the results are intriguing, they don't give a precise estimate of the surrounding tissue that has developed on the electrode's interface.

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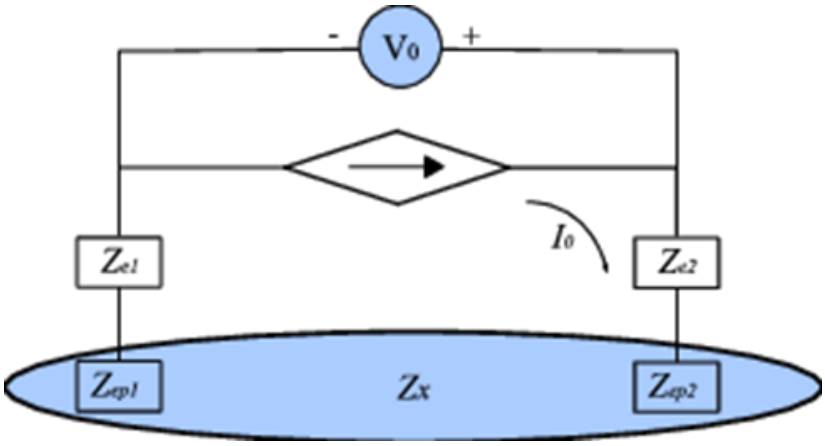


Figure 2. *Bipolar technique for measuring the bioimpedance. In this case, the impedance Zx is computed using Ohm's law to calculate the relationship between the measured voltage Vo and the current Io* [63].

The existence of electronic communications and digital impedance in the output voltage of bipolar bioimpedance systems is a major disadvantage that must be retrieved when evaluating the signal gathered. The electrode impedance detracts from the signal in an unwanted way. When a known current pass through an unknown load resistance in a bipolar setup, the unknown resistance is estimated by dividing the voltage that occurs across it by the injected current. [66] The perceived voltage is measured not only throughout the unknown resistance, but also all over the resistance of the wires and contacts when a two-electrode configuration is utilized. Because the same two electrodes are used for reactive voltage monitoring and energy insertion, the two-electrode technique's electrode connection capacitance of the electrical impedance measurement technique is heavily dependent on it [67]. As a result, in the two-electrode approach of electrical impedance spectroscopy analysis, electrode impedance must be taken into account. When skin electrodes are used to measure bipolar impedance, the measured impedance is that of the body tissues in both parallel and perpendicular directions to the longitudinal axis.

For the bipolar bioimpedance analysis, when alternating current runs through the electrodes, the impedance induced by electrode polarization is integrated into the measurement of the material and electrode tissue contact. Because bipolar includes skin impedance, it would be an equally poor choice for body composition analysis.

2.7 Quasi- monopolar Bioimpedance Measurement Method

The electrode arrangement and placement have a considerable impact on the sensitivity field. Apart from this, there is a technique for measuring bioimpedance called quasi-monopolar bioimpedance measurement. Quasi-monopolar bioimpedance measurements use a two-electrode setup with electrodes of unequal surface area. The quasi-monopolar placement is required for focusing sensitivity on the desired area. Because of its high peak current and tolerance, the component of a small electrode exceeds the entire impedance. [54]

It is feasible to keep a serious injury under basic wound coverings for a long period using the quasi-monopolar bioimpedance approach. Electrodes with a small skin contact area contribute a large impedance to the total impedance in the unipolar setting provided for the direct connection between the two-electrode bioimpedance configurations applied current and sensitivities [68]. The quasi-monopolar bioimpedance measurement-based technique appears to be a viable tool for non-continuous monitoring of venous ulcer healing. A new multi-electrode covering design for wound healing surveillance was introduced by the authors of a study. To illustrate its functionality, they utilized quasi-monopolar bioimpedance experiments. They also showed that the multi-electrode dressing may be used to observe acute wounds that take a long time to heal from beneath the primary bandages. [69] The wound was dressed with a specially designed multi-electrode dressing and remained under the conventional dressings until they're all done with re-epithelialization.

One-port measurements are possible using a two-electrode configuration. Both excitation and measurement are carried out with the same pair of electrodes. By providing a controlled potential excitation signal between the electrodes and measuring the current via the leads, the impedance of the entire system of electrodes and sample may be determined. The impedance is measured in series along the current channel through the entire setup, with the sample, electrode interfaces, electrodes, and connecting leads all contributing to it.

If the electrode size is increased towards the sample size, the relative difference in sensitivity between the corners and the construction zone will diminish if we compare with bipolar bioimpedance. The relative difference is raised by doing the opposite, reducing the size of one of the electrodes. The impedance contribution from this electrode can be made insignificantly little if the other electrode is sufficiently large.[1] A quasi-monopolar arrangement can be created with one small active measuring electrode and one large

indifferent electrode using these adjustments. Figure 1 depicted the simulation's output with a side-by-side comparison to a bipolar system.

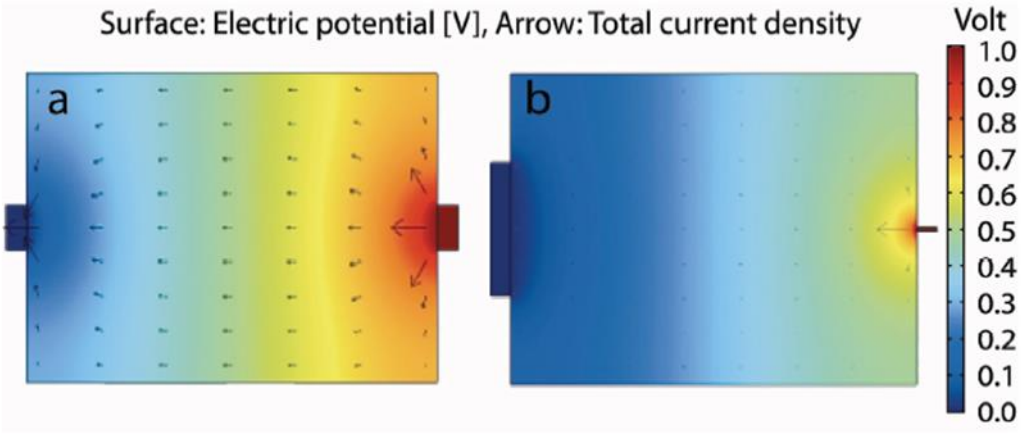


Figure 3. *Large differences in active electrode area produce bipolar and quasi-monopolar setups* [1].

The operational electrode with the closer skin contact zone contributes to numerous impedances across the impedance in the quasi-layout polarity which is helpful for a direct link between the responsiveness of the two electrode bioimpedance designs and their flow rate [68].

2.8 Monopolar Bioimpedance Measurement Method

A contact between the biological substance and the electronics is required to quantify bioimpedance. This interface is provided via electrodes. The charge carrier shift occurs at the electrode, from free-flowing electrons throughout the metal to ions in the biological substance, and vice versa [70]. Electrodes with an ion-filled gel are frequently utilized. The electrode polarization impedance is low when the measuring electrode has a large metal-electrolyte interfacial interaction.

A monopolar measurement signifies that one of the electrodes contributes most of the impedance contribution. Making one of the electrodes dominant while utilizing two or more electrodes can result in monopolar readings. In a two-electrode arrangement, this can be accomplished by raising or lowering the size of one electrode relative to the other. The slightly monopolar nature of a three-electrode arrangement can be increased by adjusting the electrode diameters. Many studies have been done for the monopolar measurement. The monopolar arrangement was proven to be capable of measuring tissue impedance in a spherical region 3–4 times of the electrode's circumference at the needle's point [54]. A monopolar configuration was found to be preferable to a bipolar

arrangement in non-inhomogeneous materials, such as human skin [71]. In a separate study, the researchers examined bipolar and monopolar measurement settings to see if one is better than the other. Experimental procedures were used to look into the technology's prospective applications, such as the differentiation of nerve tissue and spinal fluid [72]. Calculating the electrical impedance of different tissue kinds can help determine what kind of tissue is at the needle's point.

A highly monopolar measurement can be performed by utilizing a small measurement electrode, such as an insulated needle electrode with a little exposed area on the tip. The measurement would be even more monopolar if we combined this with a three-electrode arrangement. In a monopolar configuration, the needle tip has only one electrode. The distance seen between needle's point and the referenced electrode, the current flows through the entire volume. The peak current at the tip of the needle remains much higher because the counter electrode has a substantially bigger surface area than the tip of the needle. As a result, the measured impedance represents the finite volume resistivity all the way around the needle electrodes.[1] This focuses current along channels with lower electrical resistance, such as neurons or vascular systems. As a result, the path current takes may have no relation to anatomical distance. Furthermore, current leakage from the bipolar device could make it operate like a monopolar electrical component [73]. Figure **4** shows the setup of Monopolar electrical impedance.

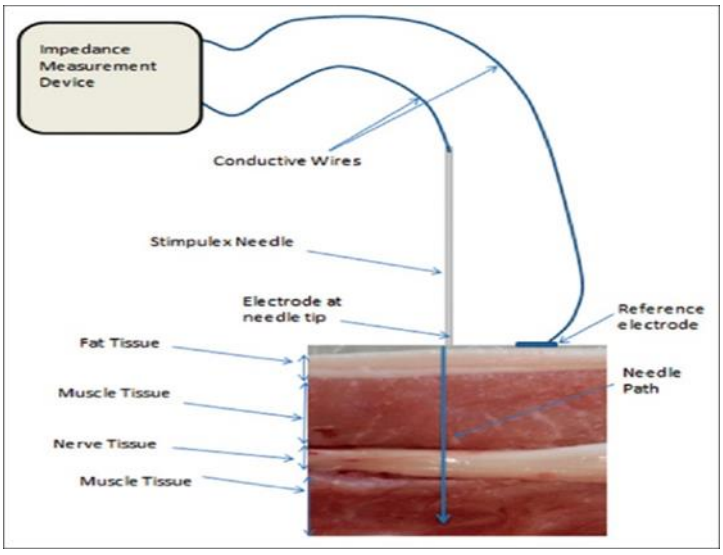


Figure 4. *Monopolar electrical impedance measurement setup. Only one electrode is present at the needle tip in this configuration. The current travels across the volume between the needle tip and the reference electrode*[72].

Two electrodes of varying diameters are used in a monopolar arrangement. The variation in size should be enough to make one of the electrodes' current density much higher than the other. In other words, one "active" electrode that dominates the results and one "neutral" electrode that doesn't matter are needed. The electrodes were linked to the input impedance in a monopolar configuration, with the blade electrode operating as the active electrode and the unbiased electrodes being a 9x9 cm aluminium, copper, and brass plate.[74] A crocodile clip was used to secure the plates, revealing 8x9 cm of the panels to the saline solution.

In this monopolar setup, the most crucial thing to know about the sensitivity field is how far into the tissue the electrode is monitoring. If the sensitivity field is too large, the spatial precision may be compromised. In contrast to the connective tissue layers' impedance, the monopolar and bipolar arrangements directly measure the tissue's impedance underneath the electrode. The electrode impedance has an impact on both setups. Sensitivity of the monopolar and the bipolar analysis is maximum under the electrode and diminishes with distance from the electrode.

2.9 Tetrapolar Bioimpedance Measurement Method

Material and device characterization, as well as sensor applications, rely heavily on tetrapolar (four-point) impedance measurements. Bio-impedance measurements with tetrapolar electrode systems are frequently utilized for tissue characterisation, including hypoxic maintenance and cancer tissue detection. Many impedance analysis methods, however, are ineffective at accomplishing tetra-polar impedance observations in circumstances if the contacting impedances are high.

Tetrapolar bioimpedance tests can undeniably diminish the impact of impedance of the electrodes but failing to recognize the potential risks could lead to inaccurate results and misreading. Tetrapolar bioimpedance measurements are widely suggested or implied to get the same benefit as Lord Kelvin's four-wire impedance measurement technique, which is to minimize the implication among the potential leads, which is incredibly beneficial in low-value resistance metrics.[75] The process of accumulating octopolar sensors or electrodes is frequently used in bioimpedance investigations on segments to evaluate segments in various parts of the body. The tetrapolar electrode strategy has become widely used for bioimpedance tests across the board due to the consistency of current transmission, especially when compared to monopolar electrodes [76].

The method for calculating body impedance is based on the imposed current state in the organism. When biological structures are subjected to a constant alternating current, it causes a frequency-dependent impedance to the current flow. According to a paper, researchers developed a tetra-polar bioelectrical impedance analyser to determine the overall amount of water in the body, body fat, and body muscle, as well as to determine the reliability of impedance measures and investigate the validity of these measurements by comparing them to commercially available instruments.[77] The voltage is detected by the electrodes under the heel and current is delivered through the electrode pads under the front of each foot. Since the goal of bioimpedance measurement is to determine the impedance of deep tissues, the tetra-polar approach is recommended because the voltage drop obtained using a separate set of electrodes will be that of the deep tissues, as the electrodes draw negligible current. The tetrapolar configuration is the most extensively utilized for evaluating cellular media because it reduces the impact of the electrodes on the testing process. This is the concept of the circular space utilized in the research of breast cancer cell lines, with spherical electrodes as potential electrodes at the endpoints and pointed electrodes.[78] The asymmetry of tissues, neuron fibers, muscle, and so on or blood arteries, might, however, impact the tetrapolar electrode design.

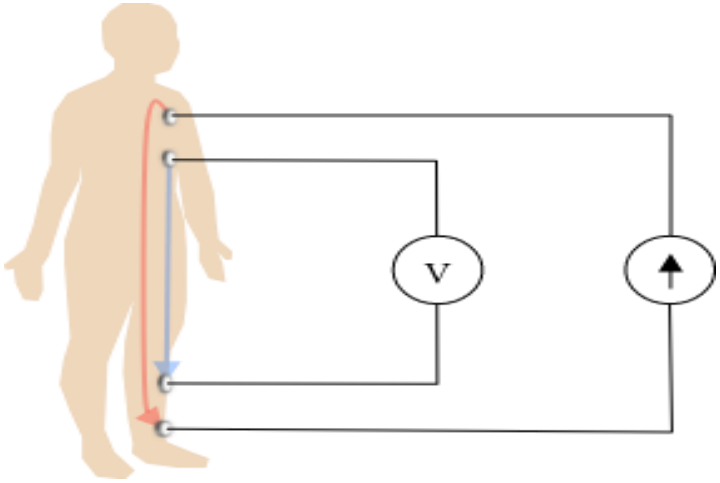


Figure 5. *Tetrapolar method for measuring the bioimpedance. The external electrodes inject the current, and an instrumentation amplifier measures the voltage in the internal electrodes*[79].

Because the interface between the electrode impedance and the medium is in collaboration with the sensing devices to be determined, significant inaccuracies are possible. The four-electrode setup (tetrapolar) is commonly used to solve this problem. The signal is passed via the electrodes on the outside, whereas the current is set track by an amplifier circuit in the interior electrodes. The voltage loss at the sensor surface can be

regarded as modest because the measurement amplifier's input voltage is exceptionally high. [80]

To reduce skin-electrode impacts, a tetrapolar electrode design is also popular. Because of the sensitivity of bronchial tissue, the major portion of impedance fluctuations are induced by electricity flowing through the chest and back. Voltage rises and falls in response to respiratory activity are linked to loading and releasing of the lungs with air, with a linear relationship between variations in resistivity. Because blood vessels, neuron fibers and muscle have inhomogeneous characteristics that affect the tetrapolar electrode configuration, which is generally used during bioimpedance measurements, new electrode combinations and methodologies must be reviewed in order to alleviate these consequences or enable their measuring system as a factor of diagnosis of diseases.

3. MATERIALS AND METHODS

The sensor array and the bioimpedance measurement system are the main elements of measurement instrumentation. The sensor array imprinted on the surface of a film-like substance, bioimpedance measurement instruments, and PC application for regulating the measurement method make up the bioimpedance measurement instrumentation system. In this chapter the materials that are used for the impedance measurement along with the measurement process and method is discussed.

3.1 Materials

Sensor Array:

The electrode array which is made up of sensors is used for monitoring the bioimpedance of the healthy skin. Electrode's array is designed in such a way that the circular electrodes are assembled in a 5 x 5 array in the patch's middle area. The electrode array is encircled by four counter electrodes named as K1, K2, K3 and K4. Each of the 25-round silk-screen-printed electrodes used to have a dimension of 2 mm and was ringed by four counter electrodes measuring 4 x 35mm. These, 25 round silk screen printed electrodes are denoted as A, B, C, D, E and F where each row has 5 electrodes from A1 to A5, B1 to B5, C1 to C5, D1 to D5, E1 to E5 and F1 to F5 respectively. The array's electrodes were spaced 12mm apart. The electrode head of the sensor array is 95x100mm while the tail of the array is about 40-50 x 400mm.[68] The sensors and connections were made of silver-ink that was electrically conducting, and a light coating of medical grade carbon ink was applied to the electrode surface. The electrode array's transparent substrate consists of 15 μm thick foil made of thermoplastic polyurethane, on which screen is printed with medical grade carbon ink electrodes. A 15μm sheet of thermoplastic polyurethane is layered on top of the first sheet containing 5mm diameter circular holes at each wound circuit point that can offer insulation between circuit wiring and also the circuit contents [81].

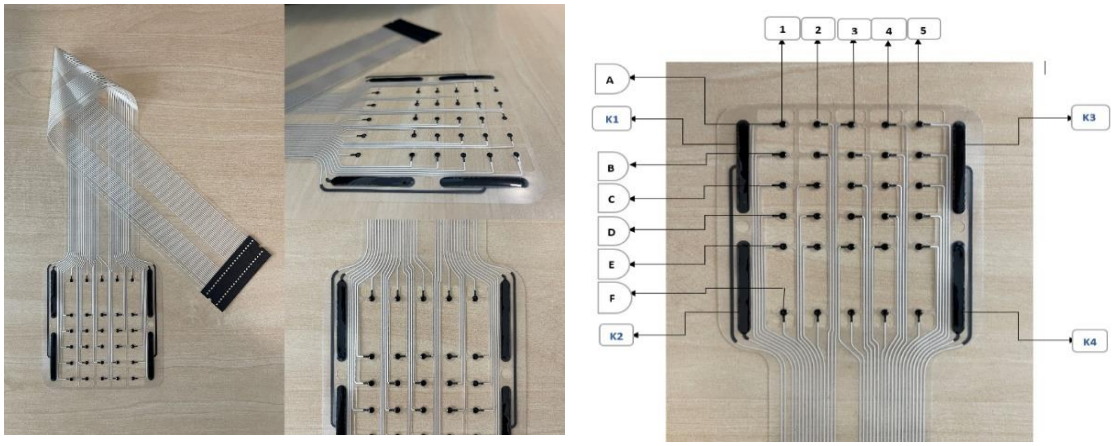


Figure 6. *Photograph of Electrode Sensor array where K1, K2, K3 and K4 are the 4 counter electrodes and A1-A5, B1-B5, C1-C5, D1-D5, E1-E5 are the 25 round silk electrodes.*

Measurement Device:

The measurement device is built in such a way that it can be attached to the wound patch with the help of the connector in order to measure the bioimpedance of the skin whether it is wound skin or healthy. A multiplexer block is used on the device so that every electrode can be selected for the separate measurements. In order to transmit the measured data to the software the device also contains a Bluetooth radio link [81].

Figure 7. *Photograph of the measurement device which is used for the impedance measurement along with the electrode dressing connected to it.*

Software:
The measurement device is controlled by the software and the primary data can also be processed by using it. In the user interface of the software first we have to select user, the Patient Id is required for starting the measurement, treatment period is also included. The measuring frequencies can also be selected individually or multiples from the software. Besides, during measurements the initial measuring data can also be checked and after the measurement the results are stored as an excel file format which can be used for the post measurement analysis.[82]

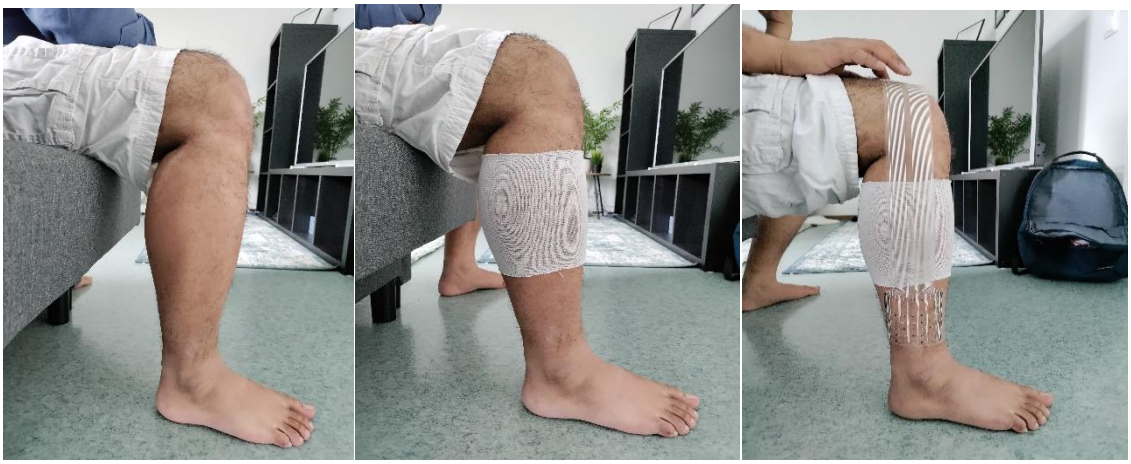
Dressing materials:
For attaching the electrode patch on top of the skin to measure the bioimpedance several medical grade dressing materials were used. Tubifast two way stretch tubular bandage were used on the shin so that the tail of the electrode array does not get to the direct contact of the skin which will prevent itching also. Mepilex foam dressing was used on top of the electrodes so that the electrodes remain on place and it helps to prevent the damage of the electrodes. This foam dressing is 10x10 cm in size. Finally, a 10cm x 7m long stretch bandage made by Elodur forte is used as the pressure dressing for covering the full electrode array from head to tail so that the electrode patch remains in position and intact.

3.2 Measurement Process and Methods

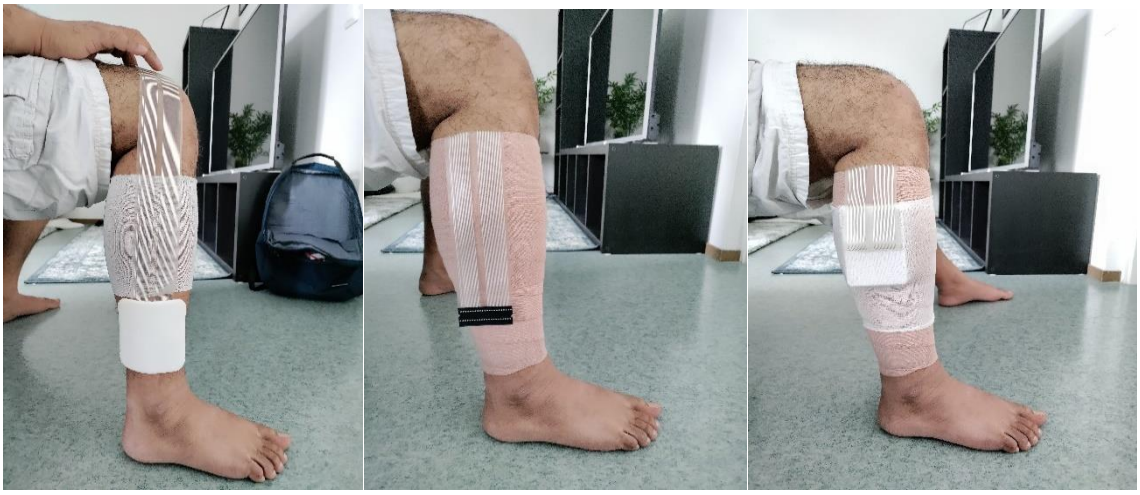
Measurement procedure and Steps:

After selecting test person based on the predefined criteria, the measurement process started. Measurement data was taken one time in each day for five consecutive days. In the beginning based on the test person's wish the skin area was selected from the leg shin. Then, a two way stretch tubular bandage is placed up to knee from the upper of the measurement area so that the tail of the electrode patch does not get the direct contact of the skin. After that, the electrode array patch is placed on the skin in such a way that the tail of the patch remains on top of the tubular bandage and the sensor electrodes are on the bottom of the bandage. After placing the electrodes, the electrodes are covered with a Mepilex foam dressing so that the electrodes can remain on the same place besides it will prevent the electrodes from being damaged. Finally, by using long stretch compressor bandage the electrode patch from head to tail was covered with moderate pressure. This pressure bandage helped the electrode patch to remain in place during walking, sleeping or any other activities. After that, another two way stretch tubular band-

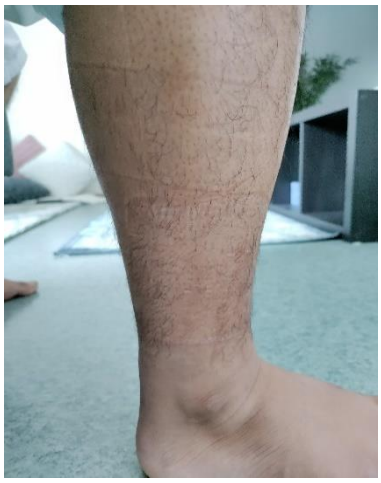
age was used to cover the pressure dressing which helps to hold the measurement device during measurements. After those steps the first measurement was taken. On the next following day, before measurement I checked the condition of the skin by removing all the dressings except the foam dressing and electrode patch to ensure that there are no side effects or any kind of rashes or allergic reactions. Besides, from previous measurement to till the next measurement time any activities or any physical work done by the test person also recorded in each day. After each measurement the presence of any moisture or sweat is also observed. The similar measurement steps were followed till the fifth measurement day. In figure 8 all the measurement steps are shown.



a) b) c)



d) e) f)



g)

Figure 8. *The materials used for the impedance measurement and the measurement process. (a, b, c) The electrode sensor array attached on the right leg shin. (d) A foam dressing is placed on the top of the sensor array to cover the electrodes. (e) Using a compression pressure bandage, the sensor array is covered by leaving the connector outside. (f) The measurement device is attached with the connector of the sensor array. (g) The condition of the skin after the measurement period.*

Method for removing outliers:

During the data analysis, from the obtained impedance data from all the test persons several unreliable impedance data were found which differ from all the other impedance data. Sometimes they are extremely high or sometimes extremely low from the other impedance data therefore we referred them as the outliers. Those, outliers are causing the large deviation during the calculation of average and standard deviation even because of these outliers the stability response graph does not seems reliable therefore decided to remove those outliers. For removing the outliers, I have set the upper boundary and lower boundary for the outliers.

The Lower Boundary Limit = 1^{st} Quartile value – (Interquartile Range x 1.5)

The Upper Boundary Limit = 3^{rd} Quartile value + (Interquartile Range x 1.5)

By using these two boundary limits[83] I have removed the strange implausible impedance data by identifying the lower impedance data along with the data which are less than the lower boundary limit selecting the. Similarly, the highest impedance data and the data which are greater than upper boundary limits are omitted from the dataset as outliers. Moreover, because of the broken electrodes sometimes very extremely large

data were found which we also considered as outliers and removed them from the data set during the analysis.

3.3 Study Population

For this project work 8 test person were selected based on their prior consent to measure their skin's bioimpedance for five consecutive days. For selecting the test person, several criteria were set which the test person should meet. First of all, the age of the test person should be less than 45years. The condition of the skin is important for this project, so for this project the test subject who has healthy undamaged skin is preferred for the measurement. Moreover, the test person also ensured that they do not have any epoxy allergy. For this project all the selected test persons are male, and the skin type is nearly similar of all the test person which is dry skin. All the test persons weight is between 65kg to 97kg, and the height of the test persons varies from 160cm to 190cm.

Table 1 shows the information of all the test person. In this table, the gender, age, height, weight and skin types of all the test persons are recorded. From this table we can see that test person 1 is the oldest among all the other test person while test person 8 is the youngest. Similarly, test person 1 has the highest height than all the other test person whereas test person 8 has the lowest height.

Table 1. *Test person History containing Gender, age, height, weight and skin type.*

Test Person	Gender	Age	Height	Weight	Skin Type
1	Male	40	190 cm	97 Kg	Dry Skin
2	Male	28	175 cm	72 Kg	Dry Skin
3	Male	29	172 cm	66 Kg	Dry Skin
4	Male	30	178 cm	90 Kg	Dry Skin
5	Male	35	170 cm	65 Kg	Dry Skin
6	Male	32	168 cm	84 Kg	Dry Skin
7	Male	31	173 cm	65 Kg	Dry Skin
8	Male	26	166 cm	75 Kg	Dry Skin

4. RESULTS

The obtained results and graphical representation of my thesis work can be found from the below figures and tables. The initial goal of my project was to observe whether the measurement of bioimpedance of intact healthy skin remains stable during the observation time. The impedance of intact skin was measured at 150 Hz, 300 Hz, 3 kHz, 1 kHz, 5 kHz, and 10 kHz frequencies for five consecutive days. But, for the results and analysis of my project I have considered impedance data which were measured at 300 Hz, 3 kHz, 5 kHz, and 10 kHz frequencies. To see the stability responses of the measured bioimpedance data I have plotted the impedance data over hour. For getting the required output I have considered all the four counter electrode which are K1, K2, K3 and K4 along with 25 round silk printed electrode they are from A1 to A5, B1 to B5, C1 to C5, D1 to D4 and E1 to E5. For the graphical presentation, the bioimpedance data were placed at y-axis of the plot while x-axis represents the measurement hour from when the measurement was taken. For showing the stability of the measured bioimpedance the response graphs obtained from test person 4, test person 8, test person 1 and test person 7 at 3000Hz is shown here.

Figure 9 [a, b, c, d] represents the stability response of the measured impedance from the 2nd day of measurement to till fifth day of the measurement period at 3KHz for test person 4, test person 8, test person 1 and test person 7 respectively. During the 1st day of measurement, the obtained impedance data was higher due to the new electrode contact and therefore the contact between electrode leads and skin was not stabilized. That is why, in the stability response plots the measurement data of day 1 is not included. In figure 9 [a, b, c, d], each of the plots contains 25 different colored line graphs, where each of the line graph represents one electrode pair.

From figure 9 [a] for test person 4, during the 2nd measurement at 24th hour, the difference between the highest and lowest electrode pairs impedance data is around 48% while at 46th hour the difference is about 46%. In 3rd day at 46th hours of measurement, most of the electrode pairs impedance data was stable and nearly identical with the 2nd days of measurement data except the impedance data of K1E2, K1D1, K1E1, K1A3 and K1D2 electrode pairs. During the 3rd measurement the impedance data of K1E1 and K1E2 slightly decreased while K1D1, K1A3 and K1D2 showed some increase in impedance value. At 59th hour when the 4th measurement was taken the difference between the highest and lowest impedance value is around 54%. During the 4th measurement most

of the electrode pairs fluctuates noticeably in their impedance values. On the 5th measurement day 55% difference was seen between the highest and lowest impedance values.

In figure 9 [b] most of the electrode pairs fluctuates in values in each day of the measurements where the difference between the highest and lowest impedance values is 45%, 36%, 40% and 44% respectively from 2nd measurement to till 5th measurement. From this figure it is easy to depict that K1A5 is the electrode pair which fluctuates the most for the test person 4 at 3KHz measurement. Similarly, from figure 9 [c] for test person 1 at 3Khz frequency, almost all the electrode pairs provide different values in each day of the measurement which can be seen from the figure that all the line graphs obtained from the electrode pairs fluctuates from day 2 to till day 5 but K1E1, K1E5, K1C2 and K1A2 are the electrode pairs that shows the larger variations in the impedance values within the measurement period.

On the other note, in figure 9 [d] for test person 7 at 3KHz frequency measurement, it clearly seen that none of the electrode pair was stable as their impedance values changes during each of the measurements till day 5. From this figure, we can see that during the 3rd measurement at 44th hour all the electrode pairs decreased 40% in their impedance values. As during this 3rd measurement time, the measurement is taken right after the test person returned home by cycling which might cause the large decrease in the impedance values as the measurement is taken within immediate period of some physical activity. Whereas during the 4th measurement an increase of about 30% in impedance can be seen. Besides, during the 5th measurement the impedance values of the electrode pairs also shoes some increase in values.

However, by analyzing all these plots obtained from the different test persons under the same measurement setting at same frequency, we can easily observe high number of fluctuations in impedance value of the electrode pairs at different days of measurement. Besides, the other test persons except these four also followed the same kind of pattern in their impedance response plots of the electrode pairs at rest of the frequencies.

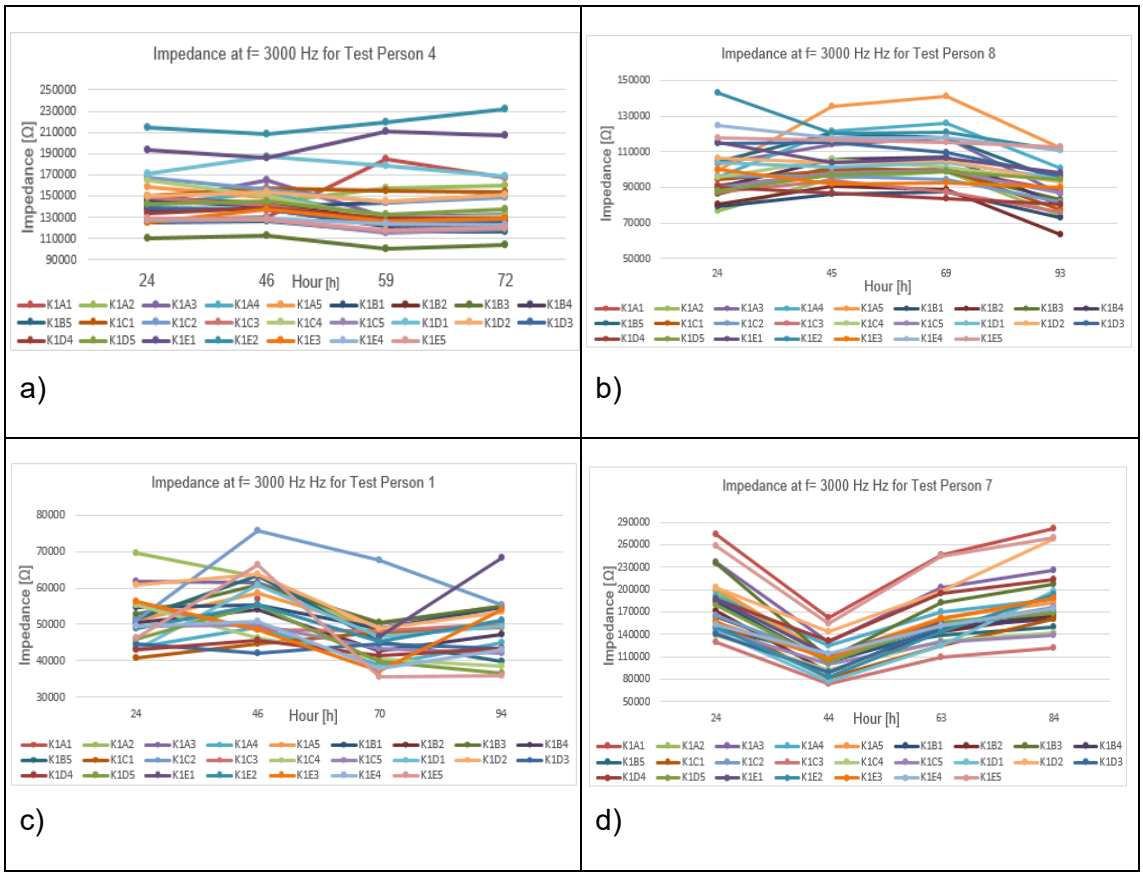


Figure 9. *[a, b, c, d]: The impedance measurement results of healthy skin for 3 kHz frequency. The measurement period was between 0 to 100 hours where, K1A1, K2A2, K3A3, K4A4, K4A5 K1B1, K2B2, K3B3, K4B4, K1B5, K1C1, K2C2, K3C3, K4C4, K1C5, K1D1, K2D2, K3D3, K4D4, K1D5 and K1E1, K1E2, K1E3, K1E4, K1E5 electrode pairs are used for the measurement.*

In Table.2 we can see the impedance data of 2 test person at 3000Hz where the measurement setting was same in both the cases. The initial data which was measured at day 1 for both the test persons seemed higher as the electrode leads are newly attached with the skin therefore the electrode contacts have not stabilized with the skin and hence gave high impedance values. From the second measurement to till fifth measurement day before the measurement was taken the information regarding all type of activities like labor intensive work, normal work, daily activities like shower, walking, running cycling, driving, sports and gym session and so on are recorded. Besides, after each of the measurement I have also checked by removing the pressure dressing that whether there is any moisture or sweat was present within the skin and electrode contact. Most importantly, all the measurement are taken after few hours from any activity done which means no measurement is taken right after any activity or physical work.

Therefore, by comparing the activity records with the impedance values recorded from the electrode pair we can see that for the test person 4 at day 2 the average impedance is 147027Ω while he did some regular activities like walking for about 3 to 4km, had shower and went for gym after the 1st measurement to till the 2nd measurement time. After the 2nd measurement to till 3rd measurement period he did some labor-intensive work and during the 3rd measurement the average impedance value is 147542Ω. However, on the 4th measurement 140388 Ω average impedance was recorded where, the test person did not perform any labor-intensive work or sport or gym session except shower after the 3rd measurement to till 4th measurement. After the 4th to till 5th measurement the test person had participated on sports and normal work and during the measurement the average impedance was 141549Ω.

Here, we can see that during this measurement period the test person performed different activities in different days and hence the average impedance values are nearly similar and varies within 140388Ω to 147542Ω. On the other hand, as after each measurement the presence of moisture and sweat also checked and found no effect on the average impedance values as no moisture or sweat is found during the measurement. On the other note, despite performing different activities in different days during this measurement time the test person 8 also provides nearly identical average impedance during the measurement period where the average impedance remains in between 89974Ω to 99901Ω.

The, rest of the test person at all the other frequencies followed the same pattern. Therefore, based on these observations we can say that bioimpedance does not depend activities like labor intensive work, normal work, daily activities like shower, walking, running cycling, driving, sports and gym session if the measurement is taken after few hours from any activity or work. Therefore, we can say that such activities may not causes any change or variations in the impedance data if immediate measurement is not taken.

Table 2. *Impedance measurement data of 2 different test person with same measurement setting at frequency 3000Hz.*

Test Person	4						8				
Days	1	2	3	4	5		1	2	3	4	5
	474203	128226	130498	184606	167189		176852	91250	96785	99764	87905
	523202	148874	144071	157256	159552		170307	76921	95037	98607	74227
	426007	144075	165241	129940	132993		189241	102874	114077	118001	86175
	404268	141290	154148	126632	123446		165566	95502	91594	86144	100518
	412577	158295	149212	128192	130052		191190	99112	85473	91032	111970
	501493	146294	140369	144009	151844		158214	78957	86270	87670	72720
	622626	148346	143143	128361	128499		138658	80365	90650	88700	63111
	383408	110923	112948	100445	104238		159368	86029	101497	98985	82721
	479647	138194	137198	121534	123028		183356	89865	105981	106800	79585
	410414	124950	126423	116081	116104		203329	104333	120001	87677	94003
	577399	150427	157646	154697	154377		150672	94630	99235	99826	77196
	566866	166896	156605	143794	148849		173958	95970	96365	94017	81928
	425569	148298	141669	124699	124759		161633	87477	93816	87473	76113
	462442	165170	149705	128604	131732		156977	95308	105819	101513	93100
	442923	126551	127899	114793	118877		148077	89966	98351	99404	86904
	526937	171373	187395	178252	168856		217629	103727	101416	103700	94708
	535894	150677	153415	145487	151697		215879	106637	104008	104885	93062
	470319	139125	137070	122527	125464		274062	114551	114910	109385	95975
	456742	133812	138524	129129	129187		159655	90091	86838	83285	80426
	468133	142736	145709	132761	137346		153236	87986	96920	98449	94184
	602641	193901	185909	210842	206597		252739	115225	104023	106416	98316
	629864	215100	208993	219289	231953		334024	142883	120148	120711	110480
	391981	125241	138101	125885	129143		206657	99792	92074	92255	90147
	396656	127710	128598	123896	122384		204355	124360	97983	117778	110978
	414143	129194	128052	117999	120557		316058	117860	96440	115043	112908
Mean		147027	147542	140388	141549			98867	99828	99901	89974

Table 3 represents the average results of the calculated mean of each day measured impedance data from day 2 to till day 5 while Table 4 shows the average of the calculated standard deviation of each day measured impedance data from day 2 to till day 5. From both of these tables we can see that at 300Hz the average impedance data is very higher for all the test person with very large deviation. Whereas, at 3000Hz the average impedance data is close to 100000 Ω for test person 2, 3, 4, 6, 7 and 8 except test persons 1 and 5 and the standard deviation is also nearly identical for test persons 2, 3, 4, 6, 7 and 8 while test person 1 and 5 shows large deviations. The average impedance and standard deviation followed the same trend at frequencies 5000Hz and 10000Hz as well.

By comparing these tables, we can see that test person 1 has half of the average impedance data than rest of the test person except test person 5. Where, test person 5 provides 174214 Ω average impedance from day 2 to till day 5 which is around 3 times higher than the average impedance of test person 1 and 1.5 times compared to the rest of the test persons. The standard deviation of these test person 1 and 5 also showed the similar pattern. As a result, a large amount of variations can be seen between test person 1 and 5 while the variations among the other test persons are less and closer to each other. Base on this analysis of average impedance and standard deviation we assumed that the physiological state of the skin might affects the bioimpedance measurement. From these, analysis it suggests that individual person's skins physiological state causes changes in the bioimpedance analysis where the fundamental factor of skin like skin type, thickness and barrier function of skin might also be the reason of the variation among different test persons.

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Table 3. *Average results of the calculated mean of each day measured bioimpedance data from 2nd day of measurement to till 5th day of measurement.*

Frequencies	Average results of the calculated mean of each day measured bioimpedance data							
	Test person 1	Test person 2	Test person s3	Test person 4	Test person 5	Test person 6	Test person 7	Test person 8
300Hz	411974	898632	1309457	1489764	2030152	1041966	1698696	973717
3000Hz	49895	88902	107625	144127	174214	93351	132835	97143
5000Hz	32187	56987	66821	86166	109142	59186	100967	63634
10000Hz	17585	31533	36809	49073	60674	32507	54238	35308

Table 4. *Average results of the calculated standard deviation of each day measured bioimpedance data from 2nd day of measurement to till 5th day of measurement.*

Frequencies	Average results of the calculated standard deviation of each day measured bioimpedance data							
	Test person 1	Test person 2	Test person 3	Test person 4	Test person 5	Test person 6	Test person 7	Test person 8
300Hz	82202	106026	175344	158150	398521	173145	293601	171980
3000Hz	5790	7160	13076	12253	28527	14972	22863	13611
5000Hz	3713	4569	6812	6467	16820	9760	16362	8746
10000Hz	2072	3014	4137	4310	8954	5612	6927	4934

In order to, see the variations among the average impedance values obtained from all the electrode pairs of all test persons at 3000Hz, 5000Hz and 10000Hz frequencies we get the variation plot in figure 10 where the fluctuations among the impedance values of

each test persons at different frequencies can easily be seen. From the figure we can easily see that, at 3000Hz all the 8-test person produces different average impedance where the deviations are also very high. Similarly, at 5000Hz and 10000Hz frequencies, the average impedance values obtained from each test persons are highly deviated from each other.

By analyzing the average impedance values from table 3 and figure 10, we observed that despite measuring all the bioimpedance from the healthy skin of all the healthy people at same frequencies where all the test person performed similar kind of activities within the measurement period under the same measurement setting does not provide nearly close average impedance data which is not reliable based on our hypothesis where we expected all the test person with healthy skin will produce nearly similar average impedance data if the physiological difference, environmental factors are similar. Therefore, we can say that, despite having the healthy skin different people will produce different average impedance. On the other hand, from figure 10 it is clearly seen that, at 3000Hz measurement frequency the impedance value is much higher than the impedance value obtained at frequencies 5000Hz and 10000Hz respectively. Whereas, at 10000Hz the measured impedance value is lower than all the other measurement frequencies for all the test person. Therefore, we can conclude that the higher the frequency is the lower the bioimpedance can be obtain during the measurement.

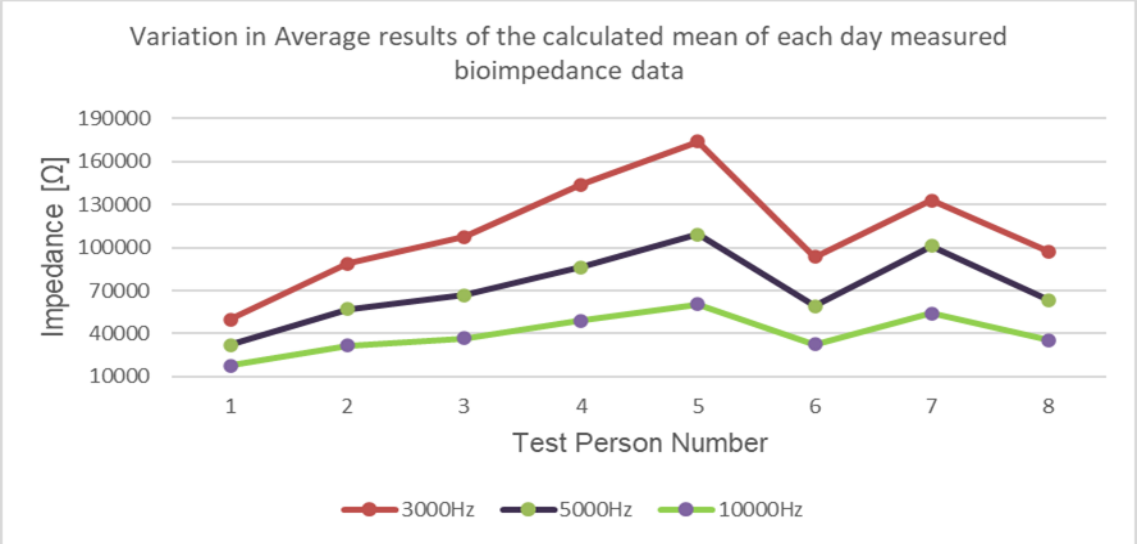

Figure 10. *The Variations among the average impedance data of different test persons under the same measurement setting at 3000Hz, 5000Hz and 10000Hz.*

The table 5 shows Coefficient of Variation of the Measured Impedance data which were measured from the day 2 to till day 5 Here, the coefficient of variation is calculated by dividing the standard deviation bioimpedance values by the average/mean values. From

this table we can see that, at 300Hz test person 1 provides the highest coefficient of variation value which is 0.20 while test person 4 shows the lowest 0.11 coefficient of variation. Besides, from this table we can understand that at 300Hz, the standard deviation of the impedance values is more spread out relative to the mean of the impedance data that's why at 300Hz relatively high coefficient of variation is achieved for each of the test person. So, the fluctuations among the impedance data are higher with higher coefficient of variation value. On the other hand, at 3000Hz, 5000Hz and 10000Hz frequency the standard deviation of the impedance values is less spread out relative to the mean of the impedance data that's why within these frequencies relatively less coefficient of variation is achieved for each of the test person. 0.08 is the minimum coefficient of variation can be seen from test person 2 at 3000Hz and at 5000Hz test person 2 and 4 shows the lowest coefficient of variation. While, at 1000Hz 0.09 is the lowest coefficient of variation which was obtained from the test person 4.

Table 5. *Average results of the calculated Coefficient of Variation of each day measured bioimpedance data from 2nd day of measurement to till 5th day of measurement.*

Frequencies	Average results of the calculated Coefficient of Variation of each day measured bioimpedance data							
	Test person 1	Test person 2	Test person 3	Test person 4	Test person 5	Test person 6	Test person 7	Test person 8
300Hz	0,20	0,12	0,13	0,11	0,20	0,17	0,17	0,18
3000Hz	0,12	0,08	0,12	0,09	0,17	0,16	0,17	0,14
5000Hz	0,12	0,08	0,10	0,08	0,16	0,16	0,16	0,14
10000Hz	0,12	0,10	0,11	0,09	0,15	0,17	0,13	0,14

5. DISCUSSION

The bioimpedance of healthy skin is measured and observed the variations and factors affecting the measurement process is also noticed in this thesis work. Healthy skin differs from injured skin in so many ways, the result of skin impedance in this circumstance is also crucial and that is why bioelectrical impedance changes from healthy to damaged skin. In the case of measuring bioimpedance, the measurement frequency of the skin has an impact. The measurement of bioimpedance in undamaged or healthy skin is the emphasis of this thesis.

At the beginning of this thesis research, we set three different research questions. The research question 1 concerns about the bioimpedance of healthy skin remains stable or not based on their electrode pairs impedance values during the measurement period, the research questions 2 concerns about how activities cause effects in the bioimpedance measurement and the research questions 3 concerns about effects of using different frequencies during the measurement and how the measurement values vary in different test persons. After collecting the bioimpedance data from 8 different test persons I have done the analysis and based on the results obtained from the analysis we have tried to answer the three research questions.

Research Question 1. How stable the electrode pairs are in terms of impedance values of the healthy skin of different test person during the measurement period?

This question is consisted of different sub question like does all the electrode pairs gave similar bioimpedance during each day of the measurement? Were there variations in the impedance response during the measurement period? How the stability response curve obtained from the electrode pairs impedance data looks and does those looks identical?

In order to determine this initial goal, I have plotted the impedance response from the obtained each day's electrode pairs impedance value. For this purpose, the impedance value obtained from the test person 4,8,1 and 7 at 3000Hz were used for creating the stability response curve from the second day of the measurement to till fifth day. From the response curve, we have seen that most of the electrode pairs provide different values in different days of the measurement beside the variations can easily be seen from the stability response curves. According to the initial goal, all the test person under the same measurement setting at similar measurement frequency should provide electrode pair impedance value within similar range during each day of the measurement. However, from the findings from the result suggested that most of the electrode pairs varies

in their bioimpedance values during each of the measurement. Where the physiological difference of each test person and environmental factors might be the reasons for variations of the electrode pairs impedance value. Hence, impedance value of 8 test person is used for the measurement and analysis of this project, but in order to get more evidence regarding the stability response of the electrode pairs bioimpedance of different test person is remain stable or not further measurement and observations with larger number of test person is required.

Research Question 2. How different types of physical activities and time of the measurement causes any affect in the impedance measurement? If causes, then what will be the variations like?

This research question can also be divided into few several questions, such as what types of physical activities can cause any effect during the measurement? what type of variations can be seen in the stability response curves? what are the factures can be responsible for any kind of variations? How significant the variations can be seen?

From the previous research [84] [85] we came to know that physical work like labor intensive, non-intensive work and daily activities like shower, walking, running cycling, driving, sports and gym session plays a significant role in the bioimpedance measurement, if the measurement is taken right after the activity done. Besides, temperature and sweating also causes fluctuations in the impedance values during measurements.

After going through all the background of related research, we tried to get into more specific details and found out that if the measurement is taken right after any physical activity, sports etc. then it will decrease the amount of electrode pair impedance. Similarly, if moisture and sweating is present during the measurement it will cause the similar effect. However, in this thesis work most of the measurements were taken after few hours during of any physical activities or work there from our finding it suggests that if the measure is taken after a few hours of gap or resting position of the test person then there will be no change in the bioimpedance measurement. On the other hand, from the result we also found out that there was significant decrease in the impedance data during the second day the measurement that's because right before the measurement the test person returned home by cycling. Which results in large decrease in the impedance data during the second day of the measurement. Whereas the rest of the days the measurements seems usual with not that much of variations. On the other note, during each measurement as I have not found any presence of moisture or sweating therefore, there was not any variations seen in the impedance data because of these factors.

Research Question 3. How the healthy skin bioimpedance varies for different test person? what is the variations in impedance data for different measurement frequency and does it cause fluctuations in bioimpedance during the impedance measurement for different test person?

This research question can be divided into some more specific question like does the average bioimpedance is same for all the test person? Is there any effect of different frequencies during the measurement? What is the effect of using different frequencies in the measurement process?

For this thesis work, bioimpedance is measured from 8 different test persons. All the test person were healthy, and their skin is healthy as well. However, based on the obtained results we observed that despite taking all the measurements under the same measurement settings along with the same measurement frequency different test persons shows different average impedance data. Where almost all the test persons have similar kind of skin types. However, their, age, height, weight and body masses were different which might be causes of different average impedance data of different test persons.

From previous study [86] suggests that during bioimpedance measurement different measurement frequency has a significant effect in the impedance output. However, from our findings we also get clear idea about how frequency plays a significant role in impedance measurement. from the variation response of average impedance data for different test person at different frequencies we can see that; at lower frequency the measured impedance is higher as during the measurement at lower frequency it covers more surface area of the skin therefore the resulted impedance output is very higher [87]. On the other at higher frequency, the measurement surface area is less as compared to the lower impedance that is why during the measurement at higher frequency, we got the lowest amount of bioimpedance value. Therefore, we can say the higher the measurement frequency the lower the bioimpedance result can be obtained from the impedance measurement.

Challenges and Limitations

For this experiment, 8 test subjects were chosen based on their prior consent to have their skin's bioimpedance measured for five days in a row. However, finding test person to get real time impedance data was challenging at the beginning of the work because of the Covid-19 situation. As all the classes and course works of university went on online and there were no contact restrictions as well, therefore it was a hurdle to find volunteer for the measurement process. Besides, the measurement process is bit lengthy, and the

test person must wear the all the dressings for continuous five days which also causes difficulties to find test person. Hence, due to those challenges I managed to get consent from few volunteers to perform the measurement test on their skin. Initially, I managed to get 11 test persons for the measurement process. After starting the measurement process, I found some challenges as well and had to stop measurement for 3 of the test persons among the 11. Two of the measurement were stopped because of the damaged electrode array dressing and another one I had to stop as the skin of the test person get swollen after two days of measurement because of the high compression of the pressure dressing. However, finally I managed to get 8 test persons skin impedance data for five days successfully.

The measurement device has some limitations as well. The measurement device instrumentation is optimized for 3000Hz frequency measurement and above. Therefore, the impedance results obtained from the 300Hz frequency is varies a lot and more fluctuations can be seen due to the instrumentation of the device. Therefore, the impedance value obtained from 300Hz cannot be considered so accurate and reliable. Hence, the rest of the frequencies provide considerably reliable and accurate impedance values during the measurement.

For this study, we have worked with only 8 test person's impedance data which is very limited to make the clear judgment that the electrode pairs impedance value remains stable or not. However, in future if the measurement can be done with increasing number of test person then we will get definite streamline reliable data to make clear judgement of electrode pair impedance value remain stable or not

6. CONCLUSIONS

Bioimpedance measurements have several benefits that have made a difference in their quick advancement: the equipment is transportable, the procedure is non-invasive, harmless, and easy to do, findings are available right away, and is necessary to replicate observations as many times as desired with high interobserver repeatability. In this qualitative study we wanted to measure the bioimpedance of healthy skin to observe the bioimpedance obtained from the electrode pairs during each of the measurement remains stable or not for different test person along with how healthy skin bioimpedance varies among different test persons and what are the factors that are responsible for the changes is also studied.

In this research, bioimpedance is measured from 8 different test persons for 5 consecutive days by using an instrumentation system consists of electrode sensor array, bioimpedance measurement device, a mobile application for managing the measurement procedure. Based on the obtained data statistical analysis is performed and based on the analysis the result shows that, electrode pairs impedance value changes noticeably during each of the measurement which indicates that the measured bioimpedance is not stable during the measurement. We also found out that, if the measurement is taken immediate right after any physical activity then the bioimpedance value will decrease while if the measurement is taken after few hours of any activity then it will cause no effect in the impedance value. The result also suggested that, if there is no moisture or sweat present between the skin and electrode contact then it will cause no effect as well. On the other note from the result, we have seen that under the same measurement setting and similar frequency, different test persons average impedance is different. Besides, from this study we came to know that the higher the frequency is the lower the bioimpedance can be obtained. However, to reach the conclusion regarding the electrode pair bioimpedance stability for different test person further study with increasing number of test person and more streamline reliable data is required.

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APPENDIX:

1. Stability Response graphs of test person 2, 3, 5 and 6

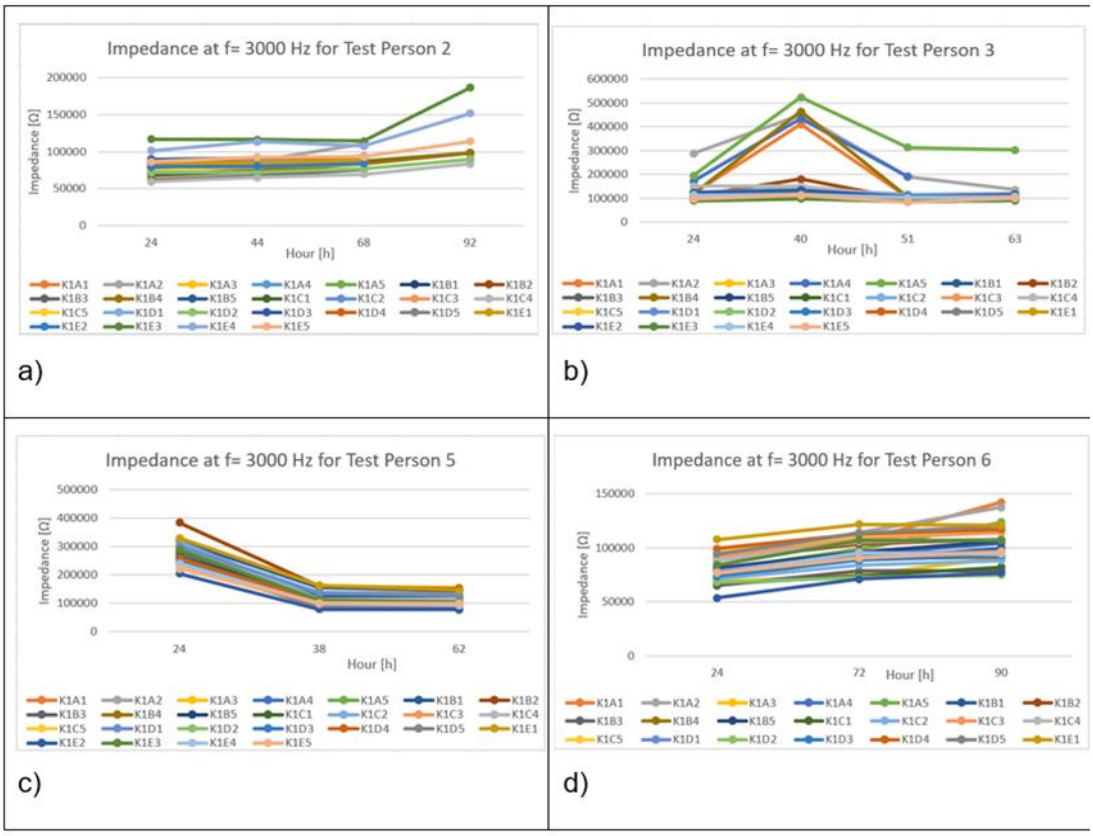

Figure 11. *[a, b, c, d]: The impedance measurement results of healthy skin at 3 kHz frequency for test person 2, 3, 5 and 6. For test person 5 impedance values of day 2, 3 and 4 is considered and for test person 6 impedance values of day 2, 4 and 5 is considered for the stability response curves.*

2. Test persons History

Table 6. *Test person activity history record from previous measurement to till next measurement.*

Test Person	**Measurement Days**	**Activities Done**	**Post Measurement Observations**
1	Day-1	1.Played Golf for 2 hours. 2.Had Shower ½ times.	No Skin reactions were Observed.
	Day-2	1.Shower. 2. No other activities.	

	Day-3	1.Shower. 2. No other activities.	
	Day-4	1.Shower. 2. No other activities.	
	Day-5	1.Shower. 2. No other activities.	
2	Day-1	1.Shower 2.Driving for 4 hours.	No Skin reactions were Observed.
	Day-2	1.Walked for about 4/5km. 2. Shower.	
	Day-3	1.Shower. 2. No other activities.	
	Day-4	1.Shower. 2. Driving for 3/4 hours.	
	Day-5	1.Shower. 2. No other activities.	
3	Day-1	1.walked 3/4 km. 2. Went gym for 1 hour.	No Skin reactions were Observed.
	Day-2	1.Shower. 2. No other activities.	
	Day-3	1. Shower. 2. Went gym for 1 hour.	
	Day-4	1.Shower. 2. No other activities.	
	Day-5	1. Shower. 2. Went gym for 1 hour.	
4	Day-1	1.Shower. 2. No other activities.	No Skin reactions were Observed.
	Day-2	1.walked 3/4 km.	

		2. Shower. 3. Went Gym for 1 hour.	
	Day-3	1.Shower. 2. Worked for 6 hours.	
	Day-4	1.Shower. 2. No other activities.	
	Day-5	1.Played Badminton for 1 hour. 2. Shower.	
5	Day-1	1.Shower. 2. No other activities.	No Skin reactions were Observed.
	Day-2	1. Walked for about 4/5km. 2. Shower.	
	Day-3	1. Walked for about 4/5km. 2. Shower. 3. Went Gym for 1 hour.	
	Day-4	1.Shower. 2. No other activities.	
	Day-5	1.Shower. 2. Walked for about 4/5km.	
6	Day-1	1.Shower. 2. Performed physical work for 3 hours.	No Skin reactions were Observed.
	Day-2	1.Shower. 2. Went gym for 1 hour.	
	Day-3	1.Shower. 2. No other activities.	
	Day-4	1.Shower. 2.Walked about 3/ 4 km	
	Day-5	1.Shower. 2. No other activities.	

7	Day-1	1.Shower. 2. No other activities.	No Skin reactions were Observed.
	Day-2	1.Shower. 2. Worked for 4 hours. 3. Did cycling for 20mins before the measurement.	
	Day-3	1.Shower. 2. No other activities.	
	Day-4	1.Shower. 2. worked for 4 hours shift. 3. cycling	
	Day-5	1.Shower. 2. No other activities.	
8	Day-1	1.Shower. 2. No other activities. 3. Walked for 3/4 km	No Skin reactions were Observed.
	Day-2	1.Shower. 2. Office work.	
	Day-3	1.Shower. 2. Office work.	
	Day-4	1.Shower. 2. Office work.	
	Day-5	1.Shower. 2. Office work.	

3. Average Impedance data

Table 7. *Results of average calculated impedance data of each day for all the test person at frequency 300Hz, 3000Hz, 5000Hz and 10000Hz.*

Frequency	Measurement Days	Average of Measured Impedance data							
		Test Person 1	Test Person 2	Test Person 3	Test Person 4	Test Person 5	Test Person 6	Test Person 7	Test Person 8
300Hz	Day-1	992513	1318510	1988228	4589127	2653236	3231377	5918195	1739290
	Day-2	429991	753767	1278579	1525984	3516498	821157	1940832	940554
	Day-3	469336	814807	1523523	1606802	1287940	N/A	1251130	1066775
	Day-4	379679	892944	1164377	1385649	1286017	1099072	1689984	1103348
	Day-5	368890	N/A	1271347	1440619	N/A	1205669	1912837	784190
3000Hz	Day-1	119173	145373	172245	465134	217116	309347	568397	180900
	Day-2	50991	79827	108600	142113	277711	80714	168078	98867
	Day-3	56000	82626	120216	142113	126330	N/A	117629	104628
	Day-4	44797	86912	96432	130814	118602	95839	154369	104701
	Day-5	47793	106242	105253	133762	N/A	103501	173078	89974
5000Hz	Day-1	78961	95910	119115	292906	137736	198695	359708	120331
	Day-2	32588	52067	68515	88738	178971	50926	120813	63407
	Day-3	35354	52755	71887	88765	75049	N/A	65864	66938
	Day-4	29261	54867	60836	82687	73407	60838	100847	66952
	Day-5	31545	68257	66044	84473	N/A	65796	116347	57239
10000Hz	Day-1	44321	58516	65197	169564	77939	112455	209001	69678
	Day-2	17718	28168	37738	50382	100922	28315	62905	35194
	Day-3	19146	29305	40105	50927	40435	N/A	36810	37211
	Day-4	15736	31235	33296	47164	40665	33387	53631	36969
	Day-5	17741	37424	36099	47819	N/A	35817	63606	31859

4. Standard Deviation of Impedance data

Table 8. *Results of average Standard Deviation of the impedance data of each day for all the test person at frequency 300Hz, 3000Hz, 5000Hz and 10000Hz.*

Frequency	Measurement Days	Standard deviation of Measured Impedance data							
		Test Person 1	Test Person 2	Test Person 3	Test Person 4	Test Person 5	Test Person 6	Test Person 7	Test Person 8
300Hz	Day-1	153729	163426	335455	899136	418132	586600	1023486	324488
	Day-2	80823	97879	184874	203450	692637	136609	336435	165803
	Day-3	92132	87962	208041	145246	249744	N/A	183476	179275
	Day-4	79083	82866	155911	135245	253182	165175	288461	187985
	Day-5	76771	155396	152551	148658	N/A	217651	366032	154858
3000Hz	Day-1	15413	12383	23831	55934	25347	56223	59181	27544
	Day-2	6491	7561	11530	12604	41320	12460	26123	15236
	Day-3	6539	8101	21282	9698	22721	N/A	18657	12461
	Day-4	4191	5218	8018	12080	21539	14608	21947	13625
	Day-5	5938	7760	11474	14630	N/A	17849	24725	13120
5000Hz	Day-1	9543	6493	18068	30434	15059	32967	36745	18383
	Day-2	3950	4274	7221	6398	24800	8111	21072	10095
	Day-3	4155	5145	6921	6914	12927	N/A	8796	7841
	Day-4	3080	3466	5406	7009	12732	9541	14361	8753
	Day-5	3668	5391	7697	5548	N/A	11628	21218	8294
10000Hz	Day-1	5185	4236	9978	12772	8600	18497	20060	11460
	Day-2	2232	2799	4219	4031	13656	4815	9547	5780
	Day-3	2389	2875	4443	4021	6245	N/A	5279	4483
	Day-4	1211	3138	3385	4887	6961	5523	5002	4891
	Day-5	2456	3243	4501	4299	N/A	6497	7879	4584

5. Co-efficient of Variation of Impedance data

Table 9. *Results of average Co-efficient of Variation of the impedance data of each day for all the test person at frequency 300Hz, 3000Hz, 5000Hz and 10000Hz.*

Frequency	Measurement Days	Coefficient of Variation of Measured Impedance data							
		Test Person 1	Test Person 2	Test Person 3	Test Person 4	Test Person 5	Test Person 6	Test Person 7	Test Person 8
300Hz	Day-1	0,15	0,12	0,17	0,20	0,16	0,18	0,17	0,19
	Day-2	0,19	0,13	0,14	0,13	0,20	0,17	0,17	0,18
	Day-3	0,20	0,11	0,14	0,09	0,19	N/A	0,15	0,17
	Day-4	0,21	0,09	0,13	0,10	0,20	0,15	0,17	0,17
	Day-5	0,21	0,14	0,12	0,10	N/A	0,18	0,19	0,20
3000Hz	Day-1	0,13	0,09	0,14	0,12	0,12	0,18	0,10	0,15
	Day-2	0,13	0,09	0,11	0,09	0,15	0,15	0,16	0,15
	Day-3	0,12	0,10	0,18	0,07	0,18	N/A	0,16	0,12
	Day-4	0,09	0,06	0,08	0,09	0,18	0,15	0,14	0,13
	Day-5	0,12	0,07	0,11	0,11	N/A	0,17	0,14	0,15
5000Hz	Day-1	0,12	0,07	0,15	0,10	0,11	0,17	0,10	0,15
	Day-2	0,12	0,08	0,11	0,07	0,14	0,16	0,17	0,16
	Day-3	0,12	0,10	0,10	0,08	0,17	N/A	0,13	0,12
	Day-4	0,11	0,06	0,09	0,08	0,17	0,16	0,14	0,13
	Day-5	0,12	0,08	0,12	0,07	N/A	0,18	0,18	0,14
10000Hz	Day-1	0,12	0,07	0,15	0,08	0,11	0,16	0,10	0,16
	Day-2	0,13	0,10	0,11	0,08	0,14	0,17	0,15	0,16
	Day-3	0,12	0,10	0,11	0,08	0,15	N/A	0,14	0,12
	Day-4	0,08	0,10	0,10	0,10	0,17	0,17	0,09	0,13
	Day-5	0,14	0,09	0,12	0,09	N/A	0,18	0,12	0,14

6. Inter-quartile range of impedance data

Table 10. *Results of average Inter-quartile range of the impedance data of each day for all the test person at frequency 300Hz, 3000Hz, 5000Hz and 10000Hz.*

Frequency	Measurement Days	Interquartile Range of Measured Impedance data							
		Test Person 1	Test Person 2	Test Person 3	Test Person 4	Test Person 5	Test Person 6	Test Person 7	Test Person 8
300Hz	Day-1	305064	201530	538525	1023568	611270	1048256	1566699	518012
	Day-2	112402	125048	213286	351095	1438826	201101	416646	169608
	Day-3	157633	147870	299259	212739	354643	N/A	137718	274464
	Day-4	147610	145427	222140	242805	482814	330012	474697	315788
	Day-5	105219	230674	239308	267725	N/A	258868	341253	269173
3000Hz	Day-1	21103	17307	34437	98988	31832	63980	93486	45051
	Day-2	9050	11789	21312	20941	64808	19319	38864	16560
	Day-3	11793	12792	19140	12635	39128	N/A	23149	19974
	Day-4	7444	7426	14562	18167	41206	21503	20956	20127
	Day-5	10767	10688	16604	22841	483941	25963	28189	17140
5000Hz	Day-1	14027	9828	28662	43986	19151	39024	59226	30738
	Day-2	5666	6036	14610	12634	38353	12727	26545	11419
	Day-3	7869	9317	7742	13387	20639	N/A	13310	12906
	Day-4	3975	4257	9965	6786	20459	13819	11896	13495
	Day-5	6736	9203	12544	7389	N/A	16947	22871	11637
10000Hz	Day-1	8027	6342	14040	22746	11204	18102	33092	17437
	Day-2	2907	4091	8138	7718	21266	7278	15516	6782
	Day-3	3966	4797	5376	7294	6933	N/A	7835	7296
	Day-4	1780	2848	5761	7622	10763	7873	9068	7228
	Day-5	3688	5201	7224	3986	N/A	9318	9886	6662