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FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

DETERMINING OF DIFFERENCES BETWEEN TISSUES USING THEIR IMPEDANCE VALUES

İMRAN YILDIRIM

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

İSTANBUL, JANUARY/ 2015

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ASSIST. PROF. DR. ŞÜKRÜ OKKESİM

İSTANBUL, JANUARY / 2015

T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

DOKULAR ARASINDAKİ FARKLILIKLARIN EMPEDANS DEĞERLERİ KULLANILARAK AYIRT EDİLMESİ

İMRAN YILDIRIM

YÜKSEK LİSANS
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

DANIŞMAN
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İSTANBUL, OCAK / 2015

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FATIH UNIVERSITY

INSTITUTE OF BIOMEDICAL ENGINEERING

İmran Yıldırım**, a** MSc **student of Fatih University** Institute of Biomedical Engineering **student 52011117, successfully defended the** thesis/dissertation **entitled "Dokular Arasındaki Farklılıkların Empedans Değerleri Kullanılarak Ayırt Edilmesi",** which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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It is approved that this thesis has been written in compliance with the formatting rules laid down by the Institute of Biomedical Engineering.

Prof. Dr. Sadık KARA
Director

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To my lovely husband,

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Firstly, I would like to thank to my supervisor Assist. Prof. Dr. Şükrü OKKESİM for patient, receptive and motivated.

Also I am greatful to my husband for his support, patience and sacrifice. And my sweet daughter is a source of happiness during my work and forever.

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January 2015 İmran YILDIRIM

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ABBREVIATIONS

AC : Alternative Current
CT : Computerized Tomography
dB : Desibel
DC : Direct Current
ECW : Extra Cellular Water
EIS : Electrical Impedance Spectroscopy
ICW : Intra Cellular Water
kHz : Kilohertz
MHz : Megahertz
MRI : Magnetic Resonance Imaging
PET : Positron Emission Tomography

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SUMMARY

DETERMINING OF DIFFERENCES BETWEEN TISSUES USING THEIR IMPEDANCE VALUES

İmran YILDIRIM

Biomedical Engineering Programme

MSc. Thesis

Advisor: Assist. Prof. Dr. Şükrü OKKESİM

One of the most frequent cancer types in the world is breast cancer. Common diagnostic methods like mammography, ultrasound or magnetic resonance imaging are not sufficient for each medical state to determine about the precise nature of the lesions found in the breast tissue. Thus, doctors need biopsy as an invasive test. The aim of this study was to evaluate the electrical impedance of abnormal and normal breast tissue, in order to discuss the success of Electrical Impedance Spectroscopy as a new method, for reliable, non-invasive diagnoses of breast cancer. For this purpose, breast tissues taken from sixteen patients were provided Bezmialem Vakıf University Faculty of Medicine Department of General Surgery, and impedance measurements have been done at the Pathology Department of the same university. Analysis of impedance values has shown significant difference between healthy and cancerous tissues.

Keywords: Electrical Impedance Spectroscopy, Breast Cancer, Bode Plot, Breast Tissue, Biopsy.

ÖZET

DOKULAR ARASINDAKI FARKLILIKLARIN EMPEDANS DEĞERLERI KULLANILARAK AYIRT EDILMESI

İmran YILDIRIM

Biyomedikal Mühendisliği Programı

Yüksek Lisans Tezi

Danışman: Yrd. Doç. Dr. Şükrü OKKESİM

Dünyadaki en yaygın kanser türlerinden biri meme kanseridir. Mamografi, ultrason ve manyetik rezonans görüntüleme gibi teşhis ve görüntüleme yöntemleri meme dokusundaki bütün lezyonların türünü tespit etmede yetersiz kalmaktadırlar. Bu sebeple girişimsel bir yöntem olan biyopsiye ihtiyaç duyulmaktadır.

Bu çalışmada, normal ve tümörlü meme dokularının elektriksel empedans değerlerinin araştırılarak meme kanseri teşhisinde kullanılabilecek girişimsel olmayan, yeni ve güvenilir bir yöntem geliştirmek amaçlanmıştır. Bu sebeple Bezmialem Vakıf Üniversitesi Genel Cerrahi Bölümünde onaltı hastadan sağlıklı ve kanserli meme dokuları alınmıştır. Patoloji bölümünde de elektriksel empedans ölçümleri yapılmıştır. Bu ölçümlerle ilgili gerçekleştirilen analizler sağlıklı ve kanserli dokular arasında istatistikel olarak anlamlı farklılıkların olduğunu ortaya koymuştur.

Anahtar kelimeler: Elektriksel Empedans Spektroskopi, Meme Kanseri, Bode Diyagramı, Meme Dokusu, Biyopsi.

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Breast cancer is originated from breast tissue, either occurring at milk canals or at lobs [1]. If occurrence location is canal, cancer is called ductal carcinoma. If, on the other hand, occurrence canal is lobs, then cancer is classified as lobular carcinoma. Breast cancer can be seen in humans and other mammals. Although it is more common in females, males can suffer from the disease [2]

Treatments vary according to patient's age, environment, stage of the disease and other parameters. Surgery, drug treatment (hormone or chemotherapy), radiation or immunotherapy can be applied[3]. In most cases, surgery is only treatment. Sometimes chemotherapy is accompanied. Radiation is usually applied after the surgery and aims to preserve the condition and prevent the growing of new cancer tissue. In most cases, it is necessary for survival [4]. Some patients with breast cancer exhibit sensitivity to hormones like estrogen or progesterone. During the treatment procedure, such hormones are blocked.

Ratio of breast cancer to other type of cancers in World women population is 22.9% (excluding non-melanoma skin cancers). In 2008, breast cancer caused 458.503 deaths [5].

Result of the treatment and survival ratio depends on type of cancer, stage of the disease, treatment and geographical location of the patient. Survival ratio is higher in the Western World [6]. For instance, 8 patients over 10 diagnosed survived for at least 5 years in England [7].

For diagnosis of breast cancer, scanning methods and microscopic analysis by biopsy is used. Most common scanning methods used for noninvasive examination are mammography, ultrasound and MR imaging. This method usually detects lumps in breast tissue, but they are not efficient in determining whether those lumps are malign or benign. Thus, deterministic diagnosis is achieved by biopsy followed by microscopic analysis. Biopsy procedure is simply a minor operation to take a sample from cancer tissue and its pathological examination. Biopsy results are verificative, but procedure is invasive and takes time to obtain the results. Due to this situation, patient comfort and diagnosis time is influenced negatively.

In this study, Electrical Impedance Analysis methods are used to examine the electrical characteristics of breast tissues to distinguish normal and abnormal breast tissues according to their electrical impedance characteristics. By this study, a preliminary work to pave the way of a novel alternative diagnosis method which is more practical, cheaper, faster and reliable was accomplished. Applying voltages in different frequencies, tissues are compared in terms of their responses.

1.2 Arrangement of the Thesis

This thesis is set up as follows:

In the next chapter, purpose of the thesis is explained in the context of breast tissue and cancer.

In the third chapter, materials used in the thesis and methods applied during the study is explained.

In the last chapter, results are given, and conclusion is made based on these results.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer cells proliferate uncontrollably which causes malignant cancers, many varieties available is defined as a disease.

Cancer, the uncontrolled growth and to multiply and infect the body tissues are located near.

Cancer symptoms do not appear immediately, but when it starts to occur mass or ulcer symptoms occur in the formation. These findings location of Cancer and occurs according to the type of Cancer. Local symptom mass or ulceration occurs as the show itself. For example, lung cancer that occurs in the closing of a mass causes bronchial cough and pneumonia that creates [8].

Sudden weight loss, fever, fatigue, general symptoms such as changes in the skin that direct or metastasized cancer does not show that Fever of unknown cause, Hodgking's disease, leukemias, kidneys or liver cancer can be the reason [8]

Some prominent systemic symptoms, has become the phenomenon of the formation of some cancers. For example, the appearance of myasthenia gravis in thymoma and clubbing in lung cancer [8]

When metastasized cancer symptoms are more pronounced. These over- enlarged lymph nodes , enlarged liver , enlarged spleen may take the form [8]. Each cancer is not cancerous. Referred to as benign cancers do not grow unchecked, cannot be spread to tissues near, do not spread to the whole body.

Many cancer, symptom, sign, or screening tests revealed. This means undetermined cases, pathological tissue is taken as an example to examine. To people with suspected cancer are also some medical tests done. These include: endoscopic screening, CT scan,

x-rays, blood tests. Cancer is learned with great sadness of the people live. Risk of suicide in people suffer from cancer compared to normal people are more than twice.

Cancer usually chemotherapy, radiotherapy is also treated with surgery. The possibility of life, on the type of cancer, where it occurred, the span depends on the amount and when treatment is started.

Some types of cancer can be seen at any age, but for others being at an older age can increase the risk of cancer.

Ulceration, bleeding in the lungs when, bloody coughs, rectal bleeding, intestine, urinary tract, the uterus causes the blood occurs. Localizer is at an advanced stage of cancer where the pain is felt too.

Some cancers, increases fluid in the abdomen and chest[8] 90 % -95 % of cancers are caused by environmental factors, while 5 % -10 % of genetic factors. Some environmental factors are like diet and obesity (30-35 %), tobacco (25-30 %), infections (15-20 %), radiation (both ionizing and non- ionizing, up to 10 %), lack of physical activity, stress, and environmental pollutants [8]

The reason may be due to many causes cancer. Smoking, pollution, obesity, physical activity, etc.. Comprising the formation of cancer genes or genetic damage to the cell is caused by genetic defect.

Any environmental factors that cause cancer is almost impossible to prove. Because multiple reasons may cause cancer. For example, if a man use and lung cancer is cigarette smoking if you use probably because it is said to be cancer. However, air pollution radiation for reasons like, everyone, albeit small, that there is a risk of lung cancer.

Cancer development and metastasis squeezed proliferated cells can be demonstrated with the following DNA mutations.

DNA mutations that cause objects to as mutagen carcinogen also known as mutagens. Of Cancer particular objects in specific ways converge. Tobacco is associated with many types of cancer, and causes 90 % of lung cancer [8]. In Western Europe, 10 % of cancers in males and 3 % of cancers in females are caused by alcohol [8].

Diet, physical inactivity and obesity are quite effective in such cases of Cancer in death. Diets that contain very low vegetables, fruits and whole grains, and high processed or

red meats are related with a number of cancers. A high-salt diet is known to cause a gastric cancer.

About 18 % of the deaths from cancer in the world are associated with infectious diseases. Virus, bacterium or parasite infestations can lead to cancer. For example, if a virus causes cancer, this virus is called oncoviruses.

Up to 10% of cancers are found to be related with radiation exposure. This includes both ionizing and non-ionizing radiation. X or gamma radiation are kind of ionizing radiation while radiowave or Infrared radiation are kind of non-ionizing radiation. A cancer caused by radiation can be formed in 5-10 years. Clinical studies also visibly demonstrated that the leukemia due to radiation is formed in 2-10 years.

Physical and chemical threats that led to the formation of cancer, ionizing radiation randomly bump into molecules within the cells. In this case, came to the abnormal chromosomes, chromosomes are composed of fractures occurring. As cell death can occur in such cases, cancer cells can also be made.

In medicine, the ionizing radiation used to treat cancer, and sometimes causes the formation of a new cancer.

Long -term ultraviolet radiation exposure in the sun, malignant melanoma and other skin occurs.

Some hormones in the development of cancer cell proliferation forefoot become. Gender linked, breast, testicular, prostate, endometrium, bone, and also the thyroid hormones are effective in cancer. For example, breast cancer in women with estrogen and progesterone hormone levels, according to a woman with breast cancer is much higher.

Other cancer is one of the reasons people become obese. This human cancer -related values are higher than certain hormones.

Cancer occurs as a result of disruptions in the orderly development of the tissues occurs. Genes in the majority of cancer cell differentiation is concerned.

2.1.1 Classification

Carcinoma: Cancer of epithelial cells. It includes many common types of cancer, namely prostate, pancreas, breast, colon, and lung.

Sarcoma: This type of cancers occurs in connective tissues. For example: Bone, fat, cartilage, tendon, nerve.

Lymphoma and leukemia: Seen in the lymph nodes and blood.

Germ cell cancer: Cancer is caused by many cells. Testis and ovary are also seen.

Blastoma: Consists of cells or undeveloped embryonic tissue. Blastoma is more common in children than adults.

2.2 Breast Cancer

Due to some kind of bad factor like genetic, radiation effect or age on inner lining of milk ducts or the lobules, normal breast cell can transform malignant type and this is called as breast cancer. Breast cancer can be classified as ductal carcinomas which originating from ducts, and lobular carcinomas which originating from lobules.

Cells normally divide as many times as needed. They form a tissue with other cells, but in cancers, including breast cancer, cells lose their property to stop dividing. They continually divide, proliferate and spoil the uniformity of tissue. They tend to spread into tissue and to other tissues.

The first evident symptom of breast cancer is typically a lump that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are revealed when the patient feels a lump. The earliest way of detecting breast cancer is called mammogram. Lumps located in the armpits may indicate breast cancer as well.

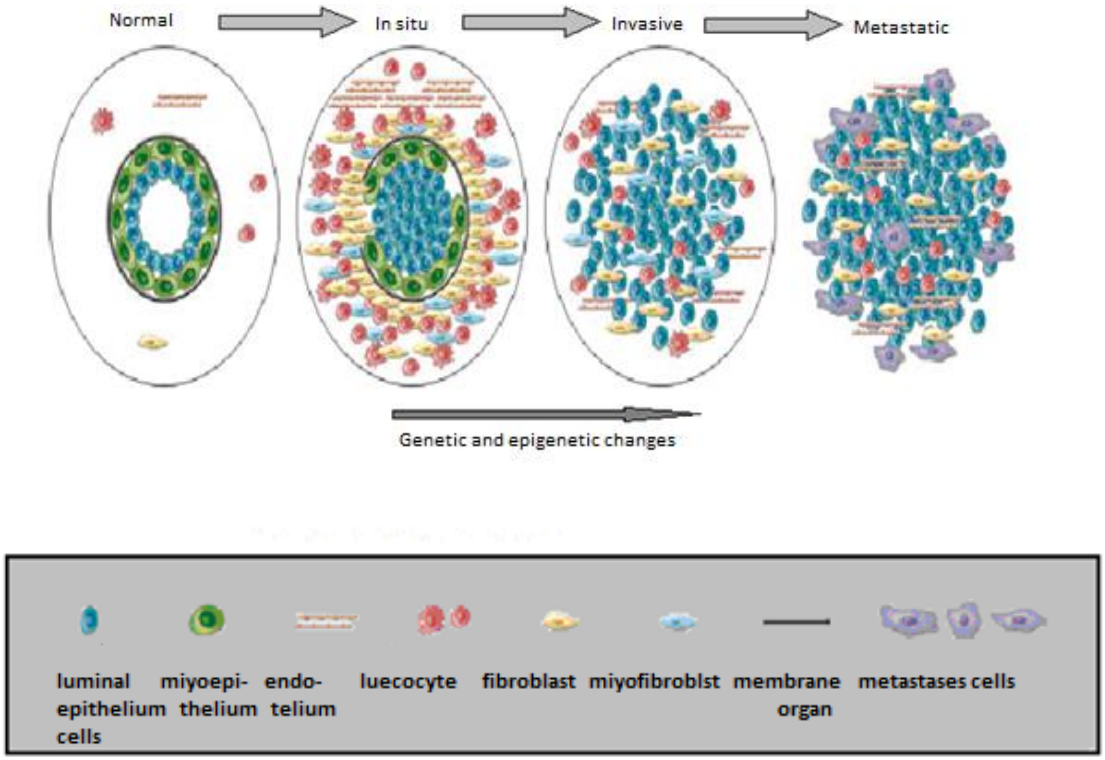

Figure 2.1 Hypothetical model development through the promotion of a breast cancer. Normally, in situ, invasive and metastatic carcinoma schematic view of the development. Normal breast ducts; basal membrane. , luminal epithelial and consist of myoepithelial cell layer. Cells which constitute the stroma; various leukocytes, fibroblasts, myofibroblasts, and endothelial cells are composed of myoepithelial carcinoma in situcells, potentially with loss of basement membrane epigenetic destroyed property and phenotypically is a decrease in number and change that . Also, stromal fibroblasts, myofibroblasts of , is increased numbers of lymphocytes and endothelial cells and myoepithelial carcinoma of duty Dbase with the loss of the membrane begins the siege of tissue and cancer cells migrate to distant organs and ultimately leads to metastasis. [9]

Indications of breast cancer other than a lump includes one breast becoming larger, thickening different from the other breast, changing in position or shape of a nipple, skin wrinkling, flow from nipple, a rash on or around a nipple, constant pain in part of the breast or armpit, and swelling in the armpit or around the collarbone.

Next to skin cancer, breast cancer is the most common cancer in American women. By the age of 80, one ninth American women develops invasive breast cancers. Breast cancer accounts for about 46 000 losses in the United States annually [10]

Prognosis and survival rate for breast cancer greatly depends on the cancer type, treatment, diagnosis stage, and location of the patient. In the Western world, survival rates are higher; like 85% of patients in England diagnosed with breast cancer live for at least 5 years [7]. In underdeveloped countries, though, survival rates are lower.

2.2.1 Breast Cancer Graduation

Several graduation standards are used in Breast cancers. Just to mention, TNM is one of them. T stands for cancer size. N stands for the case whether cancer has spread to lymph nodes. M is for the metastasis. Metastasis means of spread of cancer to a remote tissue [11]. Larger size, spreading to nodes and metastasis increases the stage number. Principal stages are as follows:

Stage 0: Cancer is seen in situ. May be told as a marker to cancer.

Stage 1-3: Cancer is either in breast tissue or lymph nodes.

Stage 4: Cancer is metastasized. This is the worst case.

2.2.2 Risk Factors

2.2.2.1 Hormonal Factor

Estrogen, and testosterone hormone, can affect the development of breast cancer. Most studies have shown that after menopause, high level of estrogen and testosterone in the blood are correlated with higher risk of breast cancer.

In premenopausal period, estrogen levels vary during the menstrual cycle and there is no clear evident how these hormones affect the risk of breast cancer in this group of women. But most risk factors for breast cancer can be explained through their effect on hormone levels.

Studies also show an increased risk of breast cancer in higher levels of insulin like growth factor 1 (IGF-1) hormone. It is not clearly understood what controls levels of IGF-1 in the bloodstream, but it is probably hereditary as well as related to body weight and how much exercise we do[12]

2.2.2.2 Family History

Some of the population may have a higher risk of developing breast cancer than the rest, since other members of their family have had certain cancers. This phenomenon is called family history. It relates to cancers seen in blood relatives. This increased risk is caused by inheritance of genes.

Having a mother, sister or daughter diagnosed with breast cancer approximately increases the risk of breast cancer by 100%. But more than 80 percent of such women will never develop it.

For identical twins, who have same genetic materials; have 3 or 4 times more risk to have breast cancer if their twin is diagnosed as breast cancer patient [12].

2.2.2.3 Radiation

High level of radiation is known to increase the risk of cancers. People exposed to the radiation caused by atomic bombs which hit Hiroshima and Nagasaki have developed many types of cancers much more frequent than normal people.

Atomic bomb is not the only source of radiation. Also Radiotherapy, which is a therapy for cancer itself, exposes you a high level radiation. Thus, it increases the risk to get a cancer, but this risk is minor regarding its therapeutic effect.

There are studies claims an increased risk of childhood leukaemia if the mother has X-rays during pregnancy. So physicians avoid X-rays for pregnant women as it is possible [12].

2.3 Method Used for Diagnosis of Breast Cancer

2.3.1 Mammography

In general, the early the diagnosis, the better the chances for survival. Today's gold standard for screening breast cancers is the mammogram. According to the Breast Cancer Detection Demonstration Project [13] rates which compare mammographic findings with the real state of the patient (confirmed by ultrasound, pathophysiological tests, clinical consequences), the sensitivity for mammography is 74% which means its false-negative rate is 26% [14].

Mammography is especially less successful to find malignancies in younger women since they have denser breast tissue. The true negative rate of mammography is typically 60%, which means false-positive rate is 40% [14]. After ten sessions of mammogram, a woman has a cumulative probability of almost 50% of undergoing an unnecessary biopsy. 70% or 80% of the women who undergo biopsies due to their mammographic findings don't have malign lesions. In addition to a high level of false positives, mammograms also cause cumulative X-ray exposure and are ineffective in young women due to denser breast tissue. Therefore, routine mammography is recommended for women older than 40 years. Many women hesitate from mammograms since they find the breast compression uncomfortable and even sometimes painful [15].

Regarding the aforementioned limitations of mammography, Electrical Impedance Spectroscopy (EIS) may be a good alternative for screening breast tissue. One of the first researchers to try Electrical Impedance Spectroscopy was Jossinet [16, 17]. In 1988, a breast examination table was described which had a circular opening with 16 pneumatically activated electrodes [17, 18]. An accompanying current mode EIS system operating at 1 MHz was produced [19]; but no in vivo breast images were reported. However, Jossinet performed many important ex vivo experiments with cut out breast tissues [17, 20].

2.3.2 Ultrasound Scan

An ultrasound scan uses ultrasonic waves to form an image of the breast tissue.

If patient has been referred to a breast clinic, an ultrasound scan is usually acquired in addition to mammograms, since they provide different information.

An ultrasound scan is noninvasive, painless and, usually takes only couple of minutes, although sometimes a confusing image may need a longer examination [21].

In spite of all advantages of ultrasound scanning, it is neither a sensitive method enough nor specific. That is why it is not usually used solely, without a complementary imaging modality.

2.3.3 MRI

Magnetic Resonance Imaging (MRI) is another imaging modality that can be used to diagnose breast cancers. In this modality, huge magnets are used to polarize hydrogen atoms. Than Radio Frequency pulses are applied, and resonance of atoms are received via Radio Frequency Antennas. This modality is known for good soft tissue discrimination. But it is a relatively expensive method, so not frequently used for breast cancers. Also it has low spatial resolution and is prone to motion artifact.

2.3.4 Biopsy

In most cases, lobular and ductal carcinomas are easy to diagnose via a microscopic analysis of suspected tissue itself. To do this analysis, a tissue sample has to be taken out. This operation is called biopsy [22].

There are three main types of biopsy as follows:

-Fine needle biopsy

-Core needle

-Surgical biopsy

In fine needle biopsy, some cells are removed from tissue. Also tissue fluids can be removed by this method. Since the needle is very thin, it almost doesn't cause bruising. Usually no anesthesia is needed. Needle is usually guided via ultrasonic imaging to reach the suspected area.

Since fine needle biopsy takes very small amount of tissue and usually tissue morphology varied, it has a non-negligible probability to miss cancer in breast.

In core needle biopsy, a thicker needle is used to remove specimen. Thus, a larger amount of tissue is removed, and in most cases, tissue morphology is not changed, so it is easier to determine whether cancerous or benign. Since thicker needles are used, it is more invasive comparing to fine needle biopsy. Ultrasound guiding is used too, but this time local anesthesia is mostly performed. Bruising is accompanied but usually recovers without scar.

In surgical biopsy, or open biopsy, an incision is done to remove a part of tissue. Local or general anesthesia can be accompanied. This type of biopsy is further invasive [23, 24].

2.3.5 Histopathological Examination

If a patient is diagnosed as breast cancer, or strongly suspected to be so, the breast is removed partially or totally. If only cancerous part is removed, surgery is called lumpectomy. If nearly quarter of the breast is removed, operation is called quadrantectomy. If breast is totally removed, it is called a mastectomy or mammectomy.

After such an operation, removed breast tissue is examined under microscope, so that it is confirmed to be cancer. Also sub types can be determined, and further treatments or operations are decided if needed. This examination is called histopathological examination [25].

Table 2.1 Characteristic of Tissues

Number of patients	18	4
measurement Posts	104	13
tissue types	breast	ovar pancreas spleen fat
date	16.04.2013-19.11.2013	03.04.2013-11.04.2013
measuring the elapsed time before	in an hour	in an hour
result of pathological tissue	ductal carsinom	benign cancer
Characterized in that the measurement tap	49 cancers, 55 healty	3 cancers, 10 healty

Figure 2.2 Cancer infected breast tissue

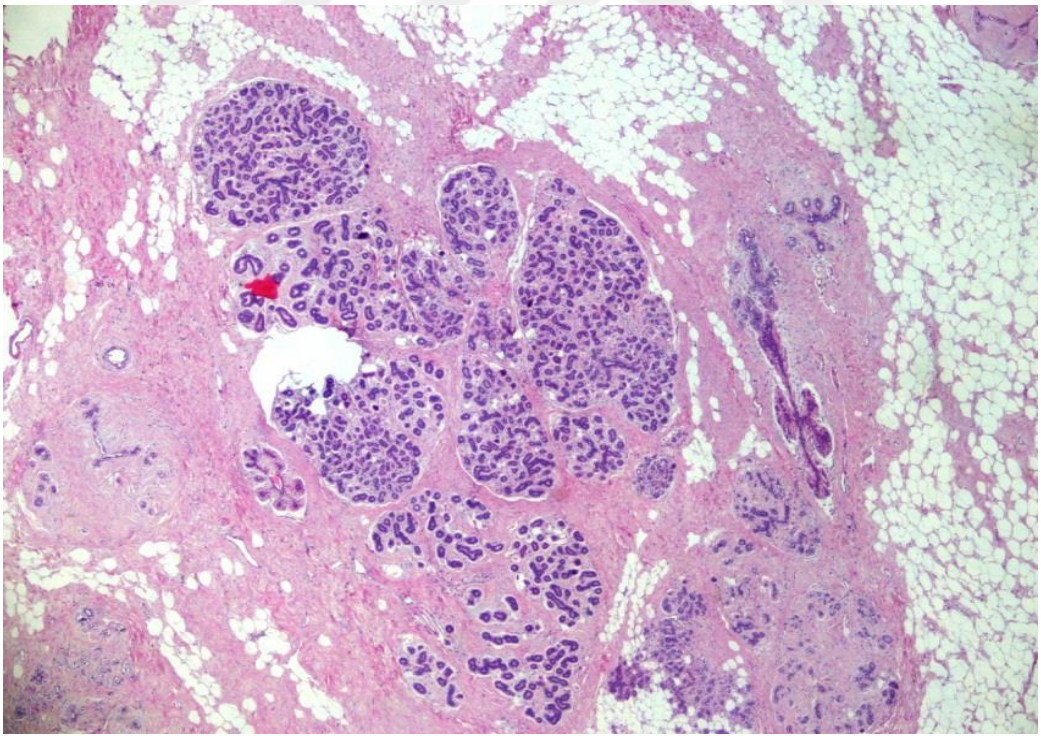

Figure 2.3 Normal breast tissue

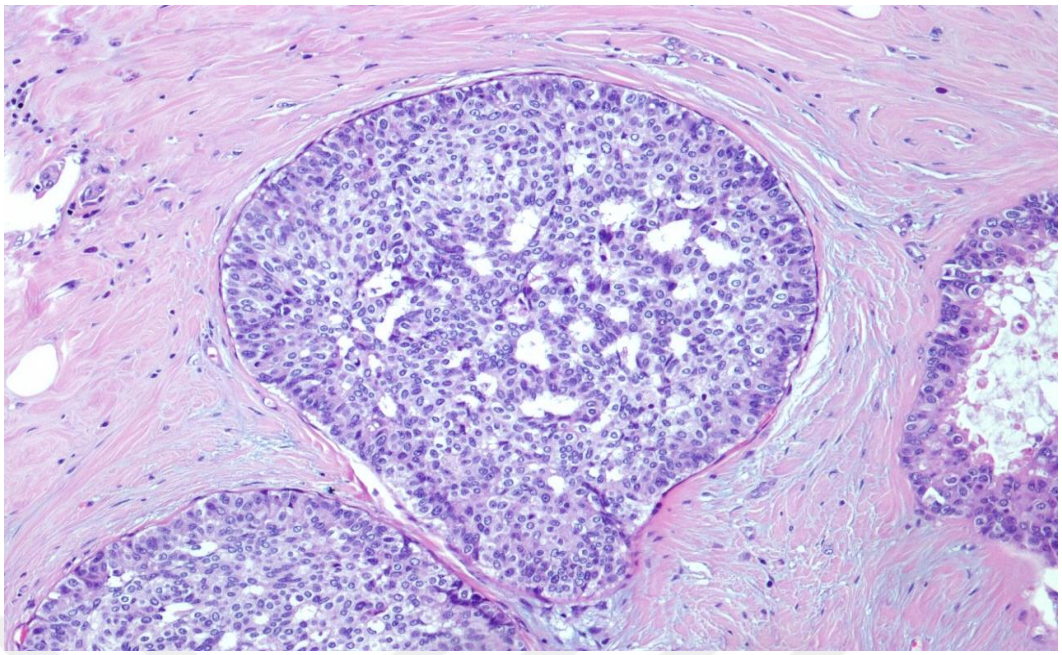

Figure 2.4 Insitu ductal carcinoma

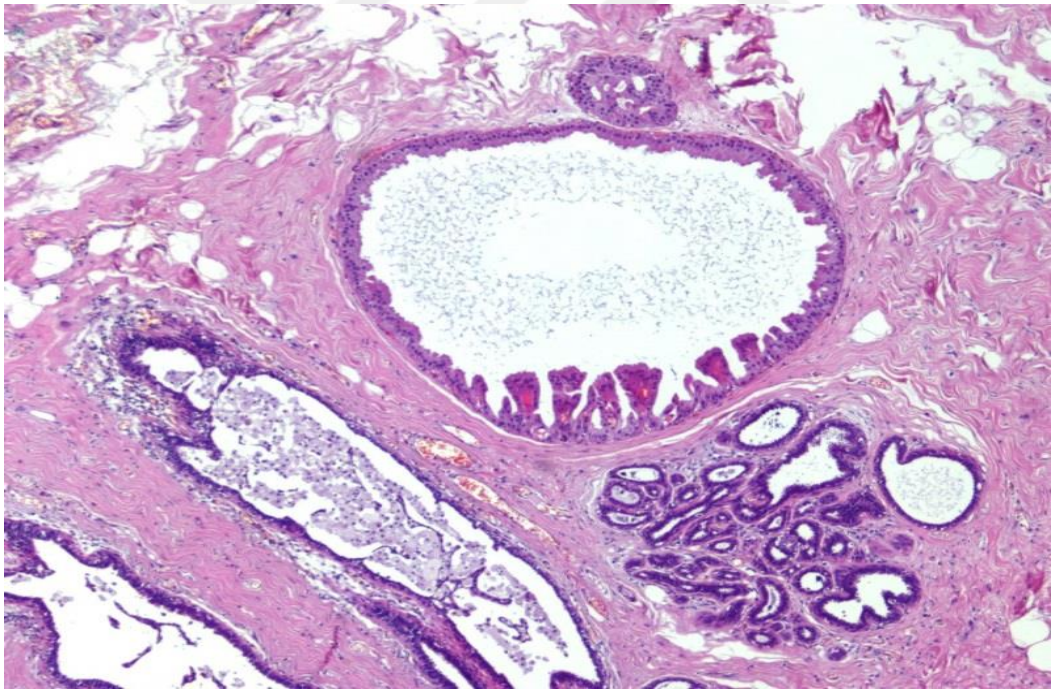

Figure 2.5 Fibrocistic changes

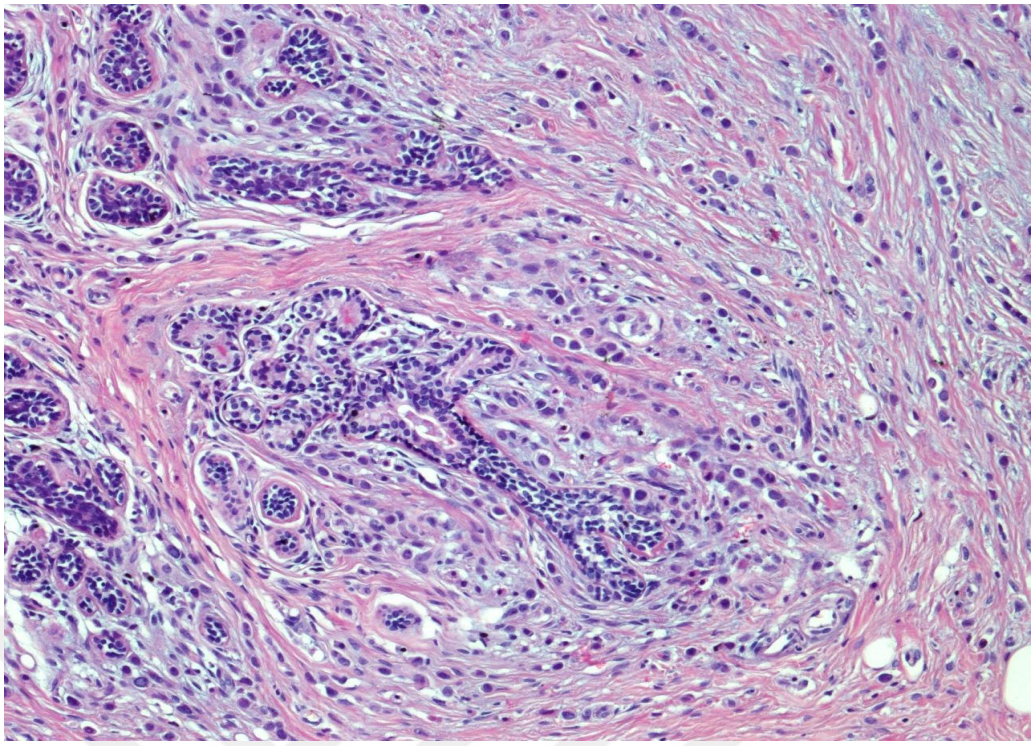

Figure 2.6 Invasive lobular carcinoma

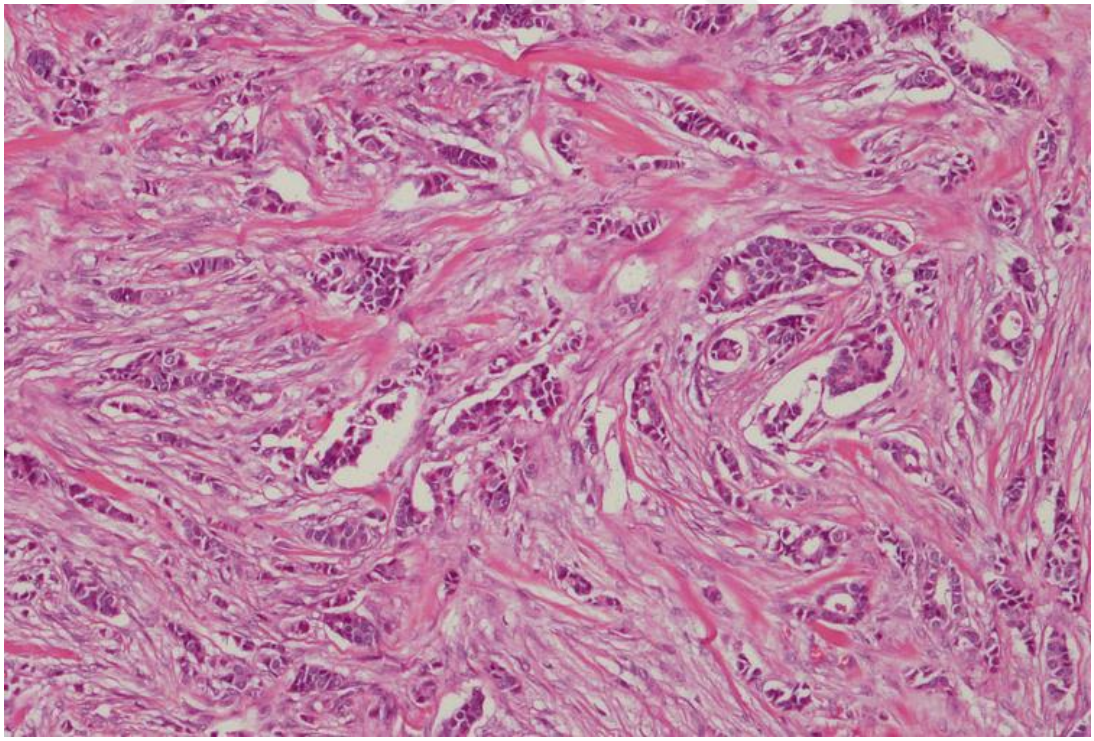

Figure 2.7 Invasive ductal carcinoma

2.4 Summary of research on EIS & Breast Cancer in the last decade

There are a lot of paper that focused on EIS & Breast Cancer in the last ten years, However, Just five of them are summarized here. These five research are enough to give opinion to the readers about the range of the research on EIS & Breast Cancer.

Y.Zou and Z. Gou summarized some papers about electrical impedance techniques for breast cancer detection with an emphasis on noninvasive impedance imaging techniques. They suggest that for improved breast cancer detection, an invasive impedance technique may be used by combination with other cancer indicators.

M. Lazebnik et. Al studied on 354 tissues samples to find out ultrawideband microwave dielectric properties of normal breast tissues. They used 0.5 to 20 GHz frequency range to compute the impedance values using a precision open ended coaxial probe and cole-cole model were used to get features. They observed no statistically difference between the within-patient and between –patient variability in the dielectric features.

J.-L. Hong et. Al presented a method for differentiating four kinds of cell using impedance measurements at various voltages and frequencies. Their results were revealing that different kinds of cell have different tolerances to an electric field.

Halter et. Al investigated the possibility of different normal and malignant prostate tissue using electrical impedance values. They designed a probe which its signal-to-noise ratio (SNR) is below the 84 dB to measure impedance spectra over the range of 10 kHz 1 MHz. Their results indicate that conductivity and permittivity were both higher in normal prostate tissues than they were in malignant tissue making them suitable parameters for tissue differentiation.

P.Aberg, I.Nicander and S. Ollmar described and investigated a new type of skin impedance probe with a dedicated surface structure that penetrates through stratum corneum but not into dermis, called the minimally invasive probe. Their results indicate that the effect of the stratum corneum was significantly reduced compared to regular noninvasive skin impedance.

Dijana Popovic et. al studied precision open-ended coaxial probes for in vivo and ex vivo dielectric spectroscopy of biological tissues at microwave frequencies. Their results showed that the precision probes, used with the prescribed measurements

protocols and data-processing techniques, provide highly accurate and reliable in vivo and ex vivo biological tissue measurements.

Liju Yang et al studied on oral cancer cells to show the cellular activities of oral squamous cell carcinoma cells using the electric cell-substrate impedance sensing system. They suggested the impedance-based method to provide a useful analytical approach for cancer research.

Peter Aberg et. al aimed to distinguish skin cancer from benign nevi using multifrequency impedance spectra. They proved that the power of skin cancer detection using electrical impedance is as good as usual visual screening made by clinicians.

J.C. Marquez, Student, F. Seoane, and K. Lindecrantz, worked to figure out the influence of increasing the skin-electrode area on the estimation of body compisition parameters using EIS. The results indicate that an increment on the area of the skin-

electrode interface produced noticeable changes in the bioimpedance spectra as well as in the body composition parameters.

Congo Tak-Shing Ching et. al studied the electrical properties of cancerous tongue tissue and normal tongue tissue to obtain a new approach for low-cost, noninvasive, and real-time screening of oral cancer. They found significantly differences results especially for 50 kHz

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

Ethics approval was obtained for the study from Bezmialem University Clinical Research Ethics Committee- Decision No: 71306642/050-01-04/ 59.

Normal fibroglandular and cancerous breast tissues taken from sixteen patients. Pathologic evaluation and preparation for impedance measurements were done simultaneously just after the excision. Cancerous tissues declared as invasive breast carcinoma and normal healthy tissues were determined. Impedance measurements are done in a specially prepared laboratory at most one hour after the surgery. Laboratory temperature was kept at 24 C°. The measurements were evaluated by drawing Bode diagrams. This type of diagrams are commonly used for tissue impedance analysis [20].

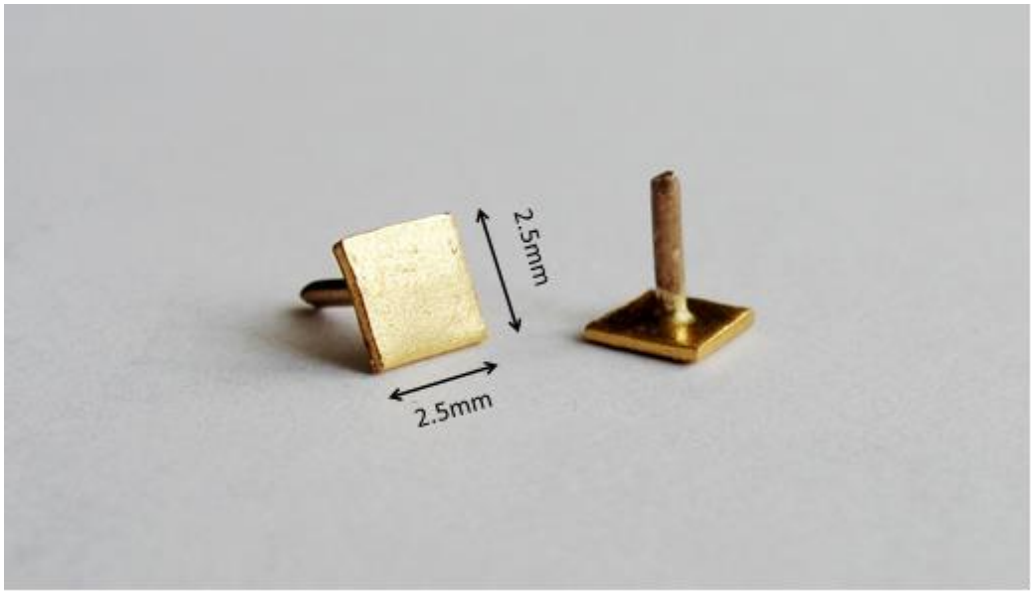

Figure 3.1 The gold electrodes

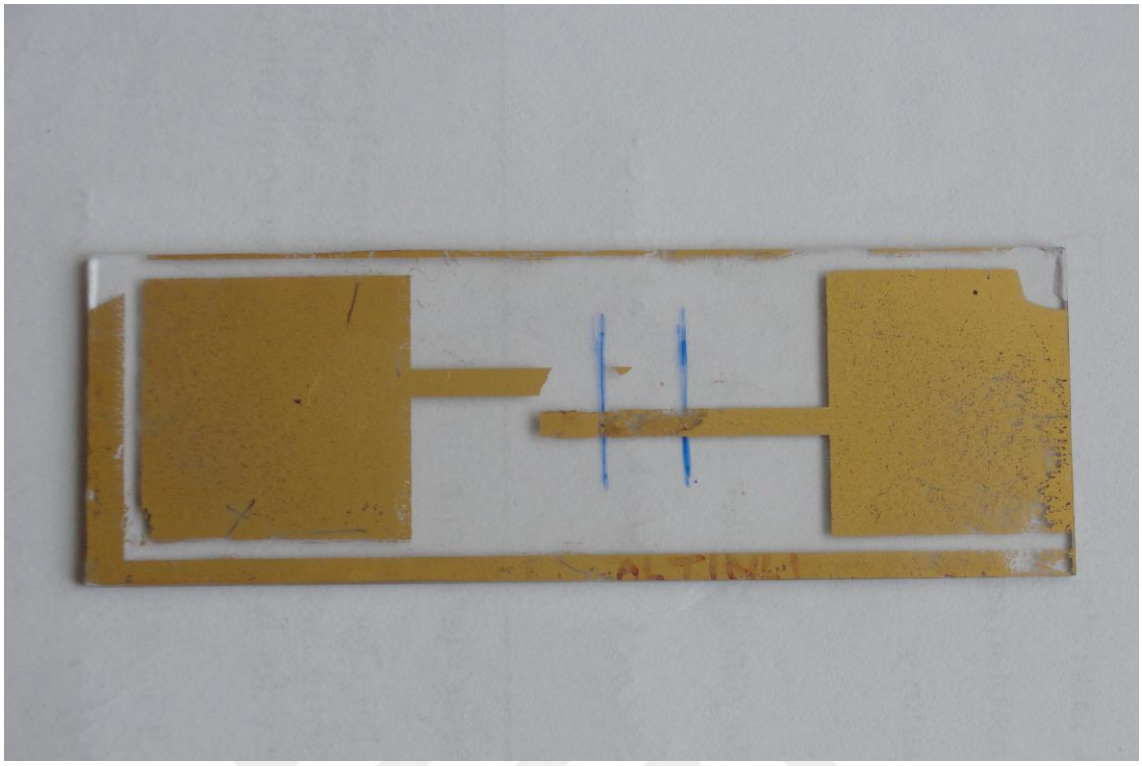

Figure 3.2 On-glass electrode system that we designed and produced for best fixed distance measurements. However, gold bonding was not strong enough and it was detached after cleaning with solution. Thus we couldn't use this electrode practically.

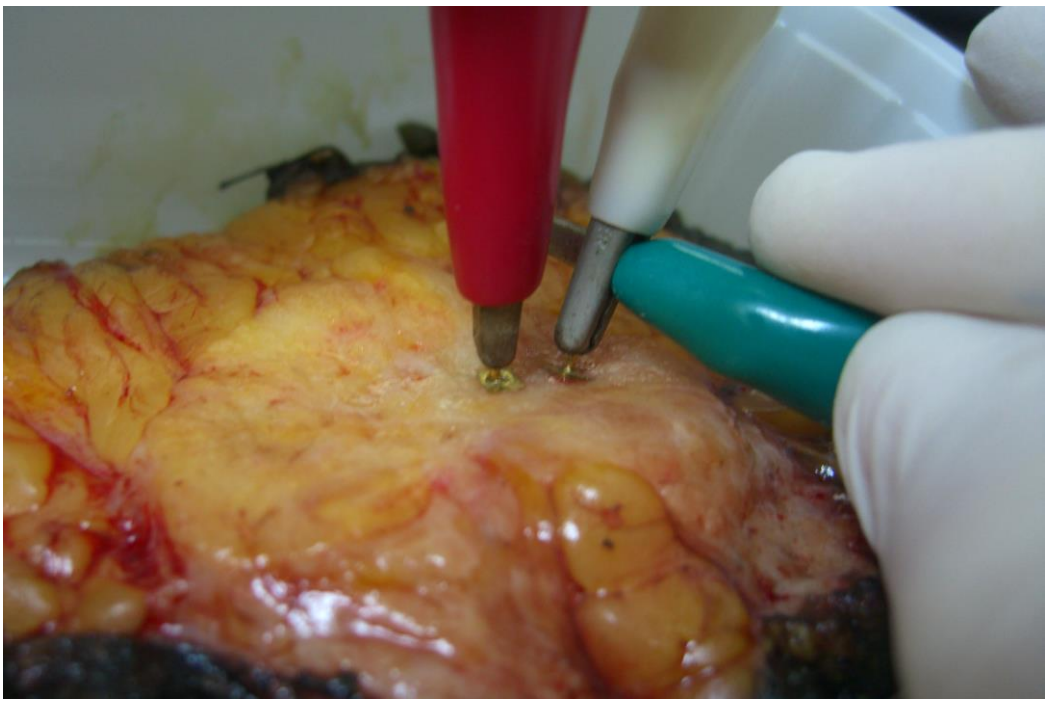

Figure 3.3 A photograph from experimental procedure

The data were analyzed using the SPSS (v.13.0) statistical package (SPSS Inc., Chicago, IL).[26].

3.2 Method

3.2.1 Electrical Impedance

Electrical Impedance based techniques for tissue identification have been used for long time. One of the most well-known example is impede-cardiography [27]. These sort of techniques have also made impedance mapping possible [27], [28]. Electrical impedance is a phenomenon in Alternative Current reciprocal to resistance in Direct Current. It is denoted by Z, and consists of Resistance (R), a reel part and Reactance (X), an imaginary part, as shown below.

$Z= R + jX$

Impedance of a certain tissue is determined by its both dielectric and electric properties. These properties depend on many things including but not limited to cell concentration, membrane capacitance, electric conductivity in interstitial space and the intracellular medium [27]. Being easy to perform, low cost and noninvasive or minimally invasive are features making impedance based techniques popular.

In recent decades, electrical impedance based measurements in breast tissues, either in-vivo or ex-vivo setups, have been performed for research purposes [27]. In the range of 488Hz-1 MHz, noteworthy differences in the impedance magnitude and phase from among six groups of breast tissue were found in a study [27].

Each cell consists of intracellular fluid and surrounding cell membrane. There are ion channels on this membrane that provides ion balance of intracellular and extracellular fluids. These fluids comprising from water and electrolytes are resistive. As can be seen electrically equivalent model of a single cell in Fig. 3.4, ion channels on the membrane shows capacitive and resistive characteristics [29]. Therefore, a living tissue reveals electrical impedance under AC current [30]. In the 10 Hz-10 kHz frequency range, impedance is mainly affected by the ionic structure around the cell. However, in the 10 kHz-10 MHz frequency range, impedance is affected by membranes as well [31].

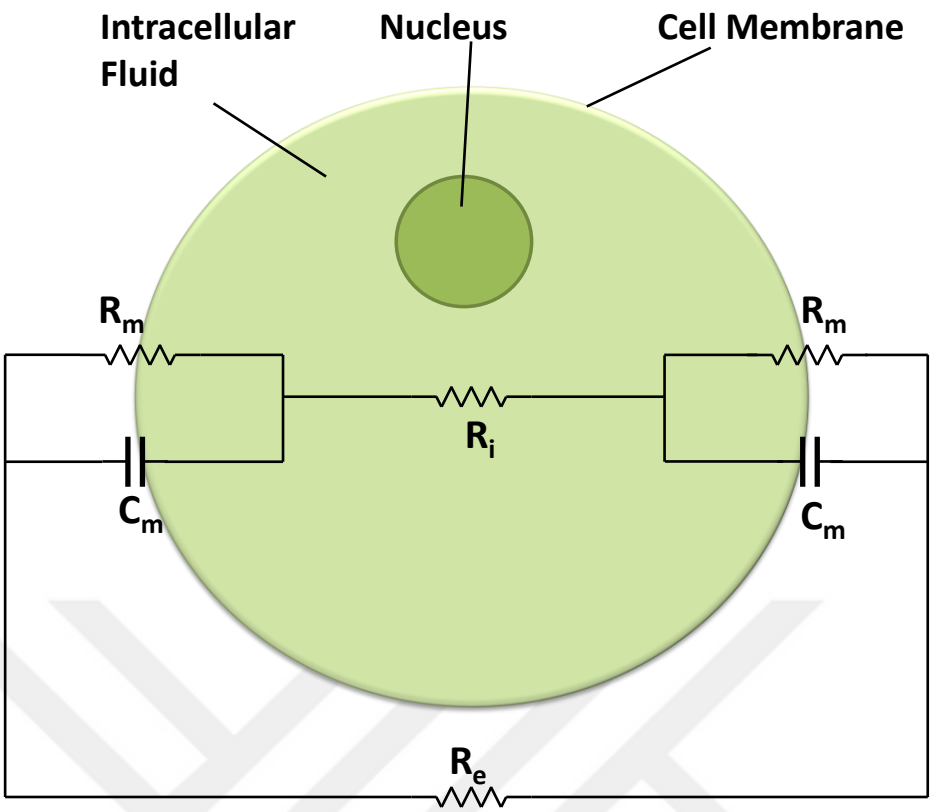

Figure 3.4 Electrically equivalent model of a single cell. Re: Exracellular fluid resistance, Ri: Intracellular fluid resistance, Rm: Cell membrane resistance, Cm: Cell membrane capacitance[29]

It is shown for the first time that cancerous and normal tissues show different impedance characteristic for the 20 kHz - 100 MHz frequency range [32]. Morimoto et al. found important changes between malignant and benign tissue at 200 kHz in vivo test [33]. In another study conducted by Stelter, [34] sufficient differences of electrical impedance values between cancerous and normal tissue for 100 Hz - 10 MHz frequency range was found.

In this study, electrical impedance values of the breast at frequencies between 10 Hz and 100 kHz were measured and bode plot differences of normal fibroglandular and cancerous tissues were analyzed.

When an alternating current with a low frequency (typically <5 kHz) is applied to the body, most of the current will pass through the extracellular space without penetrating the cellular membrane (Figure 3.4) At higher frequencies (typically>50 kHz) on the

other hand, the current will do penetrate the cell membrane, and both the extracellular and intracellular fluid spaces are accounted the conductance (Figure 3.4).

3.2.2 Impedance Measurements

Measurements were performed with CH Instruments 6005D impedance analysis device at frequencies ranging from 10 to 100,000 Hz, at 48 different frequency points increasing geometrically. Applied voltage was 5 mV during the test. Two seconds of quiet time is applied between different frequency measurements.

Two Au electrodes configuration was used to measure the electrical impedance (Fig.3.2). Inter-electrode distance was manually set as 2 mm.

Figure 3.5 Electrical Impedance Measurement Device

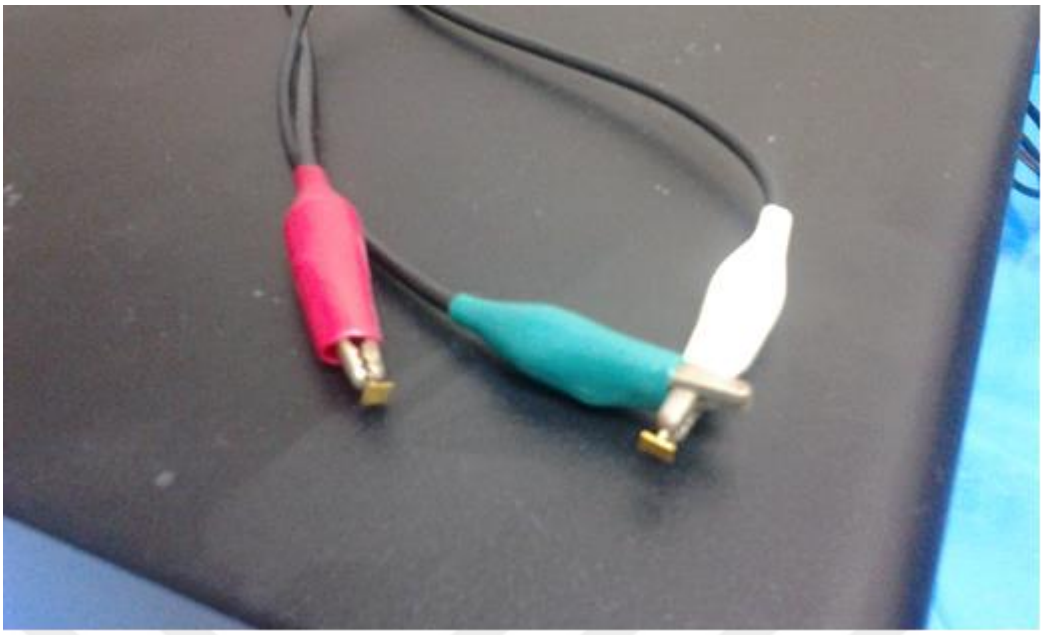

Figure 3.6 Electrical Impedance Electrode Connection

3.2.3 Bode Plots

Bode Plot is an approximate visualization tool for a certain Linear Time Invariant System. It shows a system's frequency response. Usually it is a set consisting of magnitude and phase plots. Magnitude plot shows change of response magnitude according to different frequencies, on the other hand, phase plot shows how system's phase shift is affected by different frequencies.

Magnitude plots have two axes. Y axis shows magnitude, and its unit is decibels. X axis shows frequencies in a logarithmic manner, so unit is Hertz.

Phase plots have two axes as well. Y axis shows phase shift in degrees, or sometimes radians. Scale of y axis is linear, which is logarithmic in magnitude. X axis shows frequencies in a logarithmic scale again. Usually multiples of ten is selected such as (0.1, 1, 10, 100, 1000) and this is called decade.

3.2.4 Physical Principles

Differences part in tissues cause interfaces and dividing regions of properties, to electrical charge as a stimulus potential is changed. These interfaces creates a frequency(f) dependent Z. This dispersion found in the low-frequency (LF) radio range (1kHz to 100 MHz) is known as B-dispersion. It is produced by cell membrane capacitance(Cm). The B-dispersion is minimal conduction through the cells because of

the high Z of the Cm, and conductivity is directed primarily by the properties of the ECW. As frequency increases into alternating current (AC), the Z of the Cm decreases, allowing more current to flow into the ICW compartment. Hence the change in opposition that occurs with AC current, the cell membrane charges and discharges the current at the rate of the f. The Z decreases with f, because the amount of conducting volume is increasing. At higher frequencies (HF), the rate of charge and discharge becomes such that the effect of the Cm diminishes to insignificant proportions, and the current flows through both the ECW and ICW compartments in proportions depent on their relative conductivity and volumes. Thus, at both very low and very high frequencies, the overall Z is essentially independent of the Cm, while at the mid- or characteristic frequency(fc) the dependence on the value of the Cm is at a maximum.

These points is called an impedance locale, and is character is a result of the electrical and structural characteristics of the tissue.

CHAPTER 4

RESULTS AND CONCLUSION

Results and Discussion

In this study, electrical impedance measurements are performed and differences between breast cancer and healthy tissues are investigated. Measurements are shown as Bode plots after processing with MATLAB.

TABLE 4.1

MEAN VALUES AND STANDARD DEVIATIONS OF ELECTRICAL IMPEDANCES AT FREQUENCY BANDS (H:HEALTHY T: CANCEROUS)

Frequency Range	Tissue type	Patient 1	Patient 2	Patient 3	Patient 4
10-100 Hz	Healthy	0.576±0.129	2.46±0.171	7.52±0.428	0.247±0.00196
	Tumorous	0.239±0.00363	0.616±0.0202	0.261±0.00961	0.199±0.00314
100-1000 Hz	Healthy	0.175±0.000338	0.201±0.00242	8.17±2.1	0.21±0.0011
	Tumorous	0.208±0.001	0.198±0.000589	0.216±0.00135	0.209±0.00197
1-10 KHz	Healthy	0.217±0.0141	0.224±0.0142	0.854±0.628	0.207±0.0112
	Tumorous	0.214±0.0152	0.205±0.0127	0.205±0.011	0.203±0.0112
10-100 KHz	Healthy	0.6±0.386	0.986±0.863	10.2±6.62	0.412±0.209
	Tumorous	0.434±0.231	0.467±0.28	0.403±0.206	0.402±0.193
		Patient 5	Patient 6	Patient 7	Patient 8
10-100 Hz	Healthy	0.208±0.00538	0.203±0.00302	1.19±0.0656	0.2±0.00309
	Tumorous	0.204±0.00366	0.257±0.00267	0.306±0.0056	0.239±0.00324
100-1000 Hz	Healthy	0.206±0.00106	0.223±0.0033	0.212±0.00171	0.203±0.0012
	Tumorous	0.21±0.0016	0.267±0.00228	0.219±0.00152	0.213±0.00149
1-10 KHz	Healthy	0.207±0.0119	0.211±0.0138	0.201±0.0115	0.213±0.0146
	Tumorous	0.208±0.0113	0.265±0.0105	0.202±0.0129	0.204±0.0128
10-100 KHz	Healthy	0.419±0.211	0.467±0.242	0.591±0.453	0.45±0.229
	Tumorous	0.398±0.194	0.468±0.2	0.382±0.231	0.448±0.242
		Patient 9	Patient 10	Patient 11	Patient 12
10-100 Hz	Healthy	0.222±0.00397	0.205±0.00341	0.202±0.00338	0.441±0.0119
	Tumorous	0.213±0.00343	0.247±0.00364	0.228±0.00292	0.228±0.00255
100-1000 Hz	Healthy	0.238±0.00336	0.214±0.00194	0.202±0.000771	0.213±0.000505
	Tumorous	0.21±0.00132	0.213±0.000804	0.205±0.000879	0.228±0.000774
1-10 KHz	Healthy	0.224±0.0111	0.218±0.0172	0.214±0.0137	0.222±0.0156
	Tumorous	0.209±0.0117	0.218±0.0165	0.206±0.0121	0.256±0.0128
10-100 KHz	Healthy	0.444±0.209	0.529±0.288	0.438±0.224	0.529±0.308
	Tumorous	0.416±0.209	0.522±0.29	0.399±0.203	0.512±0.25
		Patient 13	Patient 14	Patient 15	
10-100 Hz	Healthy	0.333±0.0062	0.206±0.00321	0.302±0.0702	
	Tumorous	0.228±0.00445	0.241±0.00352	0.213±0.00243	
100-1000 Hz	Healthy	0.208±0.000495	0.225±0.00316	0.467±0.0838	
	Tumorous	0.245±0.00364	0.204±0.00122	0.315±0.038	
1-10 KHz	Healthy	0.234±0.0138	0.215±0.0121	0.214±0.0123	
	Tumorous	0.315±0.0215	0.206±0.0124	0.215±0.0133	
10-100 KHz	Healthy	0.515±0.286	0.419±0.206	0.448±0.23	
	Tumorous	0.453±0.207	0.443±0.228	0.467±0.249	

In table 1, impedance values are shown in terms of frequency bands. Mean and standard deviation values of each measurement from each patient are written in the table. Because of tissue size, some measurements are done single time compulsorily, and this affects standard deviation. Otherwise, standard deviations are quite small. This shows that measurements are consistent. As seen in table, 10-100 Hz and 10-100 KHz frequency bands are most distinctive between healthy and cancerous tissues. In other frequency bands, electrical impedance values are usually similar.

Related figures are seen in the following line to analyze the results in detail. In the figures, green line is used for healthy tissue and red line is used for cancerous tissue. Each point in figures are used to show the measured value that made by devices for each 2 second. And results are given in log axes.

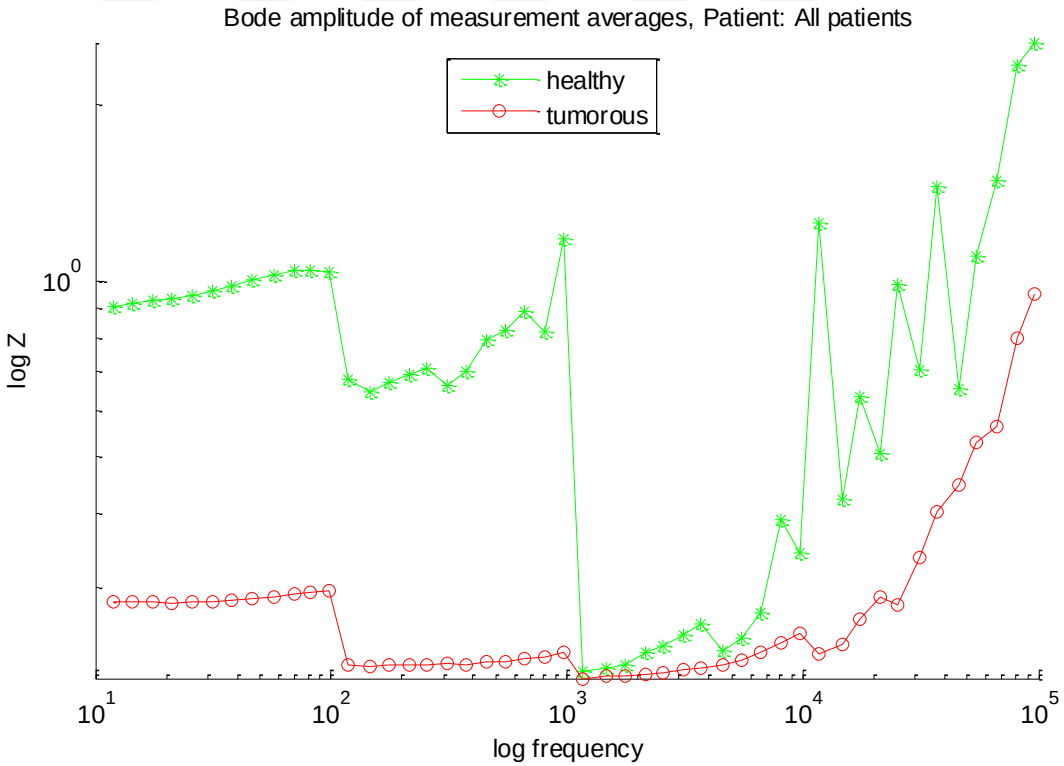

Figure 4.1: Average Bode Amplitude of all patients

In average Bode plot (see Fig. 4.1), y axis shows impedance values while x axis shows frequency values. Mean impedance graph of the 16 norrmal and 16 cancerous tissues are shown. As seen in Figure 4.1, there is a significant difference between healty and cancerous breast tissues.

Especially below the 1 kHz difference is clearer. In the range of, the 1 kHz – 10 kHz the difference between the healthy and cancerous tissue is decreased. As discussed in the introduction section, over the 10 kHz impedance is affected by membranes and intracellular liquids. Thus, when the cancerous plot is examined in Fig. 4.1, it can be seen that cancerous tissue has different impedance values below the 1 kHz and over the 10 kHz due to the abnormalities of the cancerous cell's membranes and different ion concentrations. After the 10 kHz level, a common increase in both cancerous and healthy tissue is seen. This may be due to a limitation of the device itself in higher frequencies.

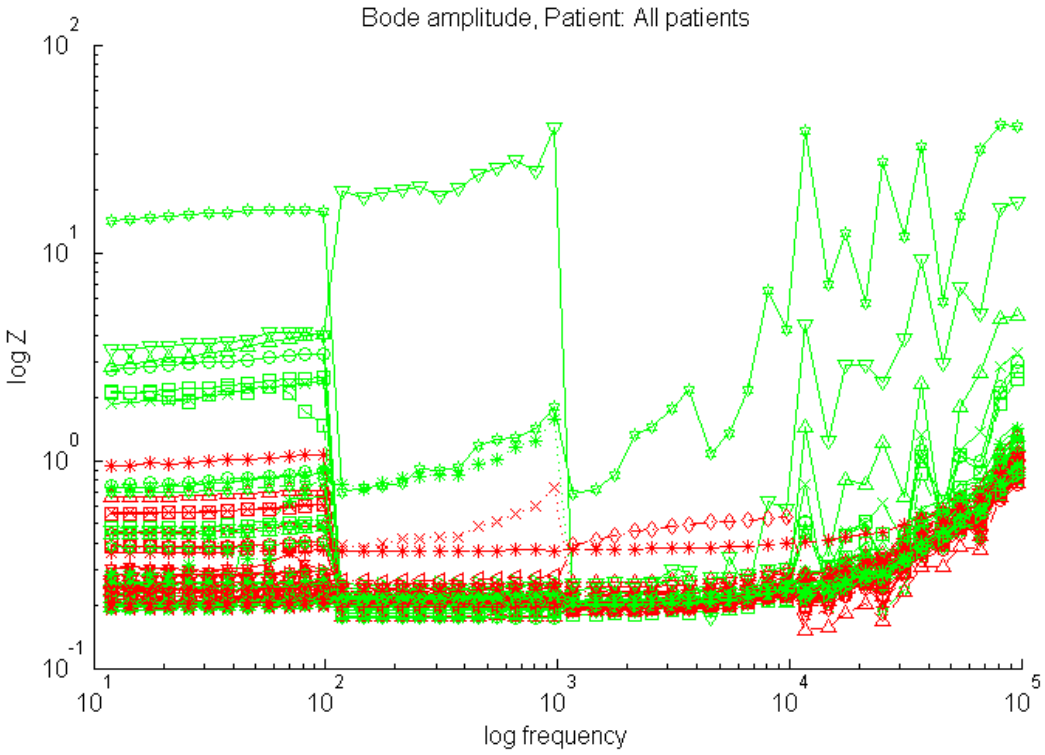

Figure 4.2: Bode Amplitude of all patients

This study are results of all the measurements taken during to show in figure 4.2 is shown logarithmically.All of the healty tissues are defined green lines and all of the the tumorous tissues are defined red lines. Each of the tissues are measured three times. Then this measurements are use for the figure .

Figure 4.3: Bode amplitude of the patient 7

Although all of the analysis results obtained in this study is able to visualize wherein some examples are mentioned. For example, patient 7 that in the study tissue impedance analysis results are shown in figure 4.3. This chart 3 healthy and 3 tumor tissues are show separately. Green lines belong to healthy tissue impedance values are greater than watch. The red lines belong to the tumor tissue is much lower than the impedance value. The measurements are observed close together.

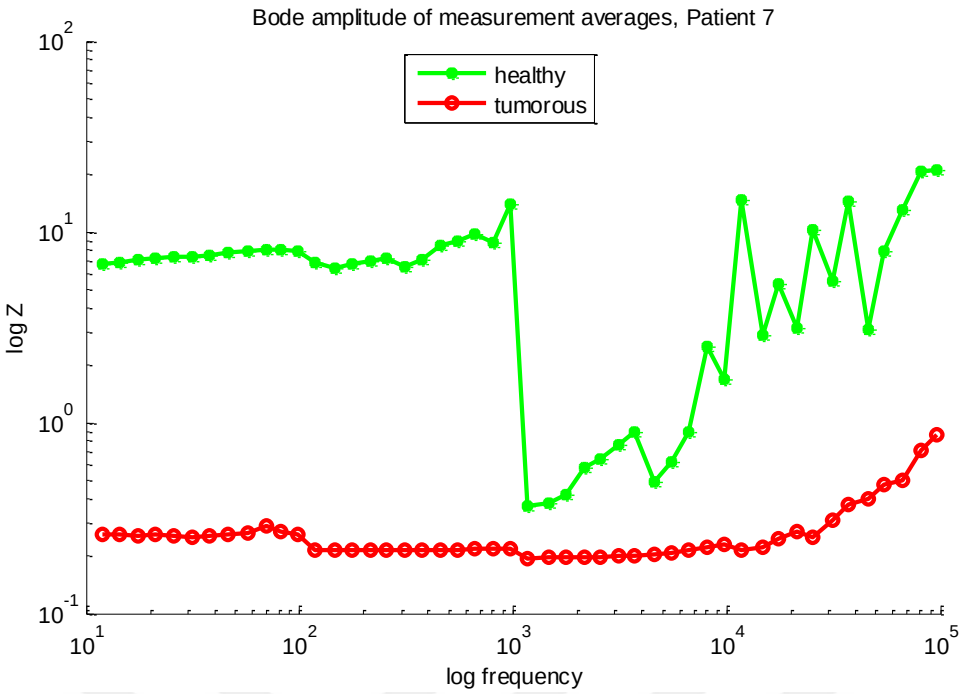

Figure 4.4: Averages Bode amplitude of the patient 7

In figure 4.4, measurement averages of healthy and cancerous tissues from 7th patient is given. Average impedance of healthy tissues lays above 1Ω level while average impedance of cancerous tissue lays below 1Ω level.

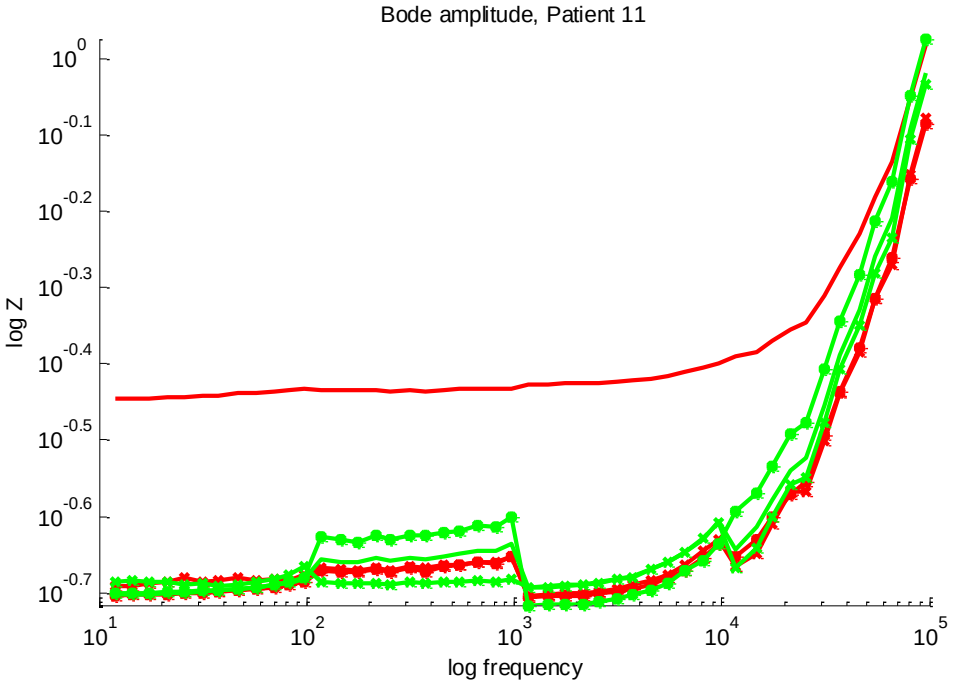

Figure 4.5: Bode amplitude of the patient 11

Figure 4.5 show measurements for 11. Patient. One of the measurements performed in the tumor tissue is seen that at a different level than any other measure.

A possible reason for this is a high impedance portion coincides found in the tumor tissue of these measurements. This also means that the average impedance value of the average of the measurements in tumor tissue affect drew up (Figure 4.6).

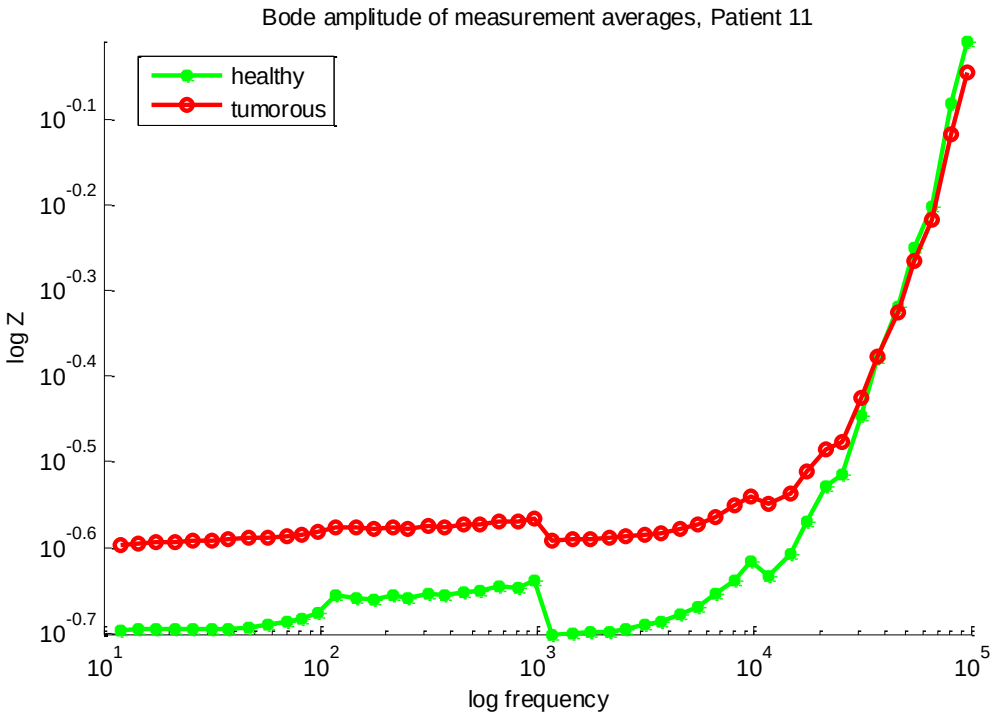

Figure 4.6: Averages Bode amplitude of the patient 11

Conclusions

M. Lazebnik et. Al studied on 354 tissues samples to find out ultrawideband microwave dielectric properties of normal breast tissues. They used 0.5 to 20 GHz frequency range to compute the impedance values using a precision open ended coaxial probe and cole-cole model were used to get features.. There are likeness to our study that we studied on 118 tissues samples and we used 10 Hz to 100 MHz. They observed no statistically difference between the within-patient and between –patient variability in the dielectric features. After all we have significantly differences between healty and morbid tissue.

Halter et. Al investigated the possibility of different normal and malignant prostate tissue using electrical impedance values. Similarly we study breast tissues. They designed a probe which its signal-to-noise ratio (SNR) is below the 84 dB to measure impedance spectra over the range of 10 kHz 1 MHz. We didn't have a special probe. Their results indicate that conductivity and permittivity were both higher in normal prostate tissues than they were in malignant tissue making them suitable parameters for tissue differentiation. This study results seem like our study.

Liju Yang et al studied on oral cancer cells to show the cellular activities of oral squamous cell carcinoma cells using the electric cell-substrate impedance sensing system. We studied on breast cancer cells of ductal carcinoma. They suggested the impedance-based method to provide a useful analytical approach for cancer research. Also we sugessted impedance-based method.

Congo Tak-Shing Ching et. al studied the electrical properties of cancerous tongue tissue and normal tongue tissue to obtain a new approach for low-cost, noninvasive, and real-time screening of oral cancer. They found significantly differences results especially for 50 kHz. Similarity we studied breast tissues. And we found differences for 100 kHz.

Significant differences found between normal and pathological tissue with most of the studied parameters confirmed that impedance spectroscopy can be considered potentially suitable for breast cancer detection [35]. However biopsy procedure is done not just to detect the cancer tissue but also distinguish the subtype of cancer and to decide the treatment procedure. Therefore investigations should go on to add these properties.

Electrical impedance has been used for living tissue characterization for over 70 years and it has advantages like having low cost, ease of use and being minimally invasive. As a preliminary study, our aim was to measure the electrical impedance value of the cancerous and normal tissue in the range of 10 Hz – 100 kHz.

In this study, the results are consistent with the literature. However, more accurate results will be obtained by getting more data. To get more accurate result the recruitment of the data is going on. We are also acquiring new data from non-cancer patients who have gone under reduction operations.

The device used in this study cannot measure the impedance value for over the 100 KHz. Another limitation of the device is that the probes cannot be fixed. We are working on a mechanism to fix the probes with a material that is resistant to our cleaning solvents.

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