ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

MICROWAVE DIELECTRIC PROPERTY CHARACTERIZATION OF HEALTHY AND CANCEROUS BONE CELLS WITH OPEN-ENDED COAXIAL PROBE

M.Sc. THESIS

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Department of Electronics and Communication Engineering

Biomedical Engineering Programme

JUNE 2019

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SAĞLIKLI VE KANSERLİ KEMİK HÜCRELERİNİN MİKRODALGA DİELEKTRİK ÖZELLİKLERİNİN AÇIK UÇLU KOAKSİYEL PROB İLE KARAKTERİZASYONU

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HAZİRAN 2019

Zeynep MACİT, a M.Sc. student of ITU Graduate School of Science Engineering and Technology 504141419, successfully defended the thesis entitled "MICROWAVE DIELECTRIC PROPERTY CHARACTERIZATION OF HEALTHY AND CANCEROUS BONE CELLS WITH OPEN-ENDED COAXIAL PROBE", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 3 May 2019
Date of Defense : 11 June 2019

To my parents, siblings and spouse,

FOREWORD

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Zeynep Güzel Macit
Genetic Engineer

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ABBREVIATIONS

LF	**:** Low Frequencies
VHF	**:** Very High Frequencies
ANN	**:** Artificial Neural Network
SaOS	**:** Human Osteosarcoma Cell Line
hFOB	**:** Human Fetal Osteoblastic Cell Line
DMEM	**:** Dulbecco's Modified Eagle Medium
FBS	**:** Fetal Bovine Serum
PSO	**:** Particle Swarm Optimization
ANOVA	**:** Analysis of Variance

SYMBOLS

F	: Frequency
T	: Temperature
I_c	: Capacitive Current
C	: Capacity
U	: Applied Voltage
D	: Displacement
P	: Polarization
E	: Electric Field
H	: Magnetic Field
ε	: Absolute Permittivity
ε^*	: Relative Permittivity
μ^*	: Permeability
ε'	: Dielectric Constant
ε''	: Dielectric Loss Factor
ε_∞	: Relative Dielectric Constant
ε_s	: Static Dielectric Constant
τ	: Relaxation Time
α	: Distribution of Relaxation Time
σ	: Conductivity
a	: Radius of Inner Conductor
b	: Radius of Outer Conductor
μ_0	: Permeability of Free Space
ε_c	: Complex Permittivity
Y_0	: Characteristic Admittance
Y	: Input Admittance
Γ	: Reflection Coefficient
σ_i	: Ionic Conductivity
$\Delta\varepsilon$	: Relative Dielectric Constant Reduction

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MICROWAVE DIELECTRIC PROPERTY CHARACTERIZATION OF HEALTHY AND CANCEROUS BONE CELLS WITH OPEN-ENDED COAXIAL PROBE

SUMMARY

Especially in recent years, microwave-based diagnosis and treatment of cancer studies are the most frequently studied areas. These studies are based on the principle of determination of dielectric properties of normal and cancerous tissues. For this purpose, different techniques such as dielectrophoresis, waveguide, coaxial probe are used. Studies generally have been done to determine the dielectric properties of tissues by microwave techniques. However, studies performed on cells are limited. These studies have been carried out mostly at low frequencies and made on cells suspended in a growth medium. In this study, it is aimed to characterize the microwave dielectric properties of human healthy and cancerous bone cells in the frequency range of 500 MHz to 20 GHz using an open-ended coaxial probe on the pellet and suspension forms of cells.

As the amount of water contained in the cancerous tissues is higher than normal tissue, their dielectric properties are expected to be higher than dielectric properties of normal tissues. With the same principle, we expect that cancer cells have higher dielectric values than healthy cells. The cell line of hFOB 1.19 was selected as human healthy bone cell, and the SaOS-2 cell line was selected as the cancerous bone cell. After cell cultures were prepared by standard cell culture protocol, cell numbers were determined, and cell pellet was obtained. The numbers of cells used in the experiments were 12500000 cells/ml for SaOS and 22500000 cells/ml for SaOS in the second experiment and 17700000 cells/ml for hFOB. The number of cells is very high compared to similar studies in the literature, and it is aimed to increase the accuracy of the study.

A network analyzer is used in the open-ended coaxial probe technique. The network analyzer calculates and records the S-parameters, dielectric losses and dielectric constants of the substance through the software it uses. In the study, the frequency range was determined as 500 MHz-20 GHz and it was requested to measure at 79 points at 250 MHz intervals. Two different probes with diameters 0.5 and 2.2 mm were used in the experiments. Ten measurements have been performed for each sample. Using ANOVA test, their p-values were calculated, and very small p-values have been obtained between cancerous and healthy cells. According to the results, it is observed that there is statistically significant difference between cancerous bone cell and healthy bone cell and they have different dielectric values. In addition, it is revealed that repeated ten measurements are also consistent with ANOVA test. In this research, cell pellet and cell suspension were compared to determine which one gives more accurate result. It is seen that cell pellet was more convenient to measure dielectric properties of cell particles.

SAĞLIKLI VE KANSERLİ KEMİK HÜCRELERİNİN MİKRODALGA DİELEKTRİK ÖZELLİKLERİNİN AÇIK UÇLU KOAKSİYEL PROB İLE KARAKTERİZASYONU

ÖZET

Özellikle son yıllarda mikrodalgaya dayalı kanser teşhisi ve tedavisi en çok çalışma yapılan alanlardan olmaktadır. Bu araştırmalar normal ve kanserli dokuların dielektrik özelliklerin belirlenmesi prensibine dayanmaktadır. Bu amaçla dielektroforez, dalga kılavuzu, koaksiyel prob gibi çok farklı teknikler kullanılmaktadır. Dokuların mikrodalga teknikleriyle dielektrik özelliklerinin belirlenmesine yönelik çok fazla çalışma yapılmıştır. Buna rağmen hücre üzerinde yapılan çalışmalar kısıtlıdır. Hücrelerin dielektrik özelliklerinin belirlenmesine yönelik yapılmış çalışmalar daha ziyade düşük frekanslarda gerçekleştirilmiştir ve çoğunlukla besi yeri içerisinde bulunan hücre süspansiyonu üzerinde yapılmıştır. Hücre besi yerinin yalnızca hücrelerin dielektrik özelliklerini yansıtmasına engel olacağı sebebiyle hücre peleti de çalışma konusuna eklenmiştir. Bu çalışmada, insan sağlıklı ve kanserli kemik hücrelerinin pelet ve süspansiyon formları üzerinde açık uçlu koaksiyel prob kullanarak mikrodalga dielektrik özelliklerinin 500 MHz – 20 GHz frekans aralığında karakterizasyonu amaçlanmıştır.

Kanserli doku içerdiği daha fazla su miktarı dolayısıyla normal dokudan daha yüksek dielektrik özellikler göstermesi beklenmektedir. Aynı prensiple kanserli hücrelerin de sağlıklı kemik hücrelerinden daha yüksek dielektrik değerlerine sahip olmasını beklemekteyiz. İnsan sağlıklı kemik hücresi olarak hFOB 1.19 hücre hattı, kanserli kemik hücresi olarak SaOS-2 hücre hattı seçilmiştir. Hücre kültürleri standart hücre kültürü protokolü ile hazırlandıktan sonra hücre sayıları belirlenmiş ve hücre peleti elde edilmiştir. Deneylerde kullanılan hücrelerin sayıları ilk deneyde SaOS için 12500000 hücre/ml, ikinci deneyde SaOS için 22500000 hücre/ml ve hFOB için 17700000 hücre/ml'dir. Hücre sayıları literatürdeki benzer çalışmalara nazaran oldukça yüksek tutulmuştur bu verinin çalışmanın doğruluğunu artırması hedeflenmektedir.

Ölçümler öncelikle hücre peleti üzerinde başlanmış daha sonra üzerine besi yeri eklenerek süspansiyon formuna dönüştürülmüş ve ikinci ölçümler hücre süspansiyonu üzerinde yapılmıştır. En son besi yerinin dielektrik parametreleri hesaplanarak hücre süspansiyonu, besi yeri ve hücre peletinin dielektrik özellikleri karşılaştırılmıştır. Literatürde hücre süspansiyonu üzerinde yapılan çalışmalarda besi yeri ve hücre süspansiyonu ayrı ayrı ölçüldükten sonra Maxwell karışım denklemi kullanılarak yalnızca hücre parçacıklarının dielektrik değerleri hesaplanmıştır.

Açık uçlu koaksiyel prob tekniğinin kullanılması için ağ analizörü gerekmektedir. Ağ analizörü belirlenen frekans aralığında sinyal üretir ve yansıyan sinyali algılar. Prob bu sinyali ölçülecek maddeye iletip almaya yarar. Ağ analizörü, kullandığı yazılım sayesinde maddenin S-parametrelerini, dielektrik kayıp ve dielektrik sabitlerini hesaplayıp kaydeder. Çalışmada frekans aralığı 500 MHz-20 GHz olarak belirlenmiş ve 250 MHz aralıklarla 79 noktada ölçüm yapması istenmiştir.

Çalışmaya başlamadan önce ağ analizörünün yeterli bir süre boyunca ısınması gerektiği literatürde çeşitli kaynaklarda belirtilmiştir. Bu sebeple deneyden en az bir saat önce ağ analizörü başlatılmış ve deneye kadar hazır ve stabil olması sağlanmıştır. Deneylerde yarıçapları 0.5 ve 2.2 mm olmak üzere iki farklı prob kullanılmıştır. Her deneyde başlamadan önce probların kalibrasyonu hava, saf su ve üretici tarafından sağlanmış özel iletken bir madde ile tamamlanmıştır. Sırasıyla hücre peleti, hücre süspansiyonu ve besi yeri ölçümleri her iki prob kullanılarak her bir ölçümden on ayrı ölçüm alınarak tamamlanmıştır. Bu sırada hücrelerin oda sıcaklığında ve bir strese maruz kalarak yaşam sürelerinin düşeceği göz önüne alınarak deney oldukça hızlı bir biçimde, hücrelerin canlı kalabilecekleri süreye kadar gerçekleştirilmiştir. En az on kere alınan ölçümlerle hata payını en aza indirmek hedeflenmiştir.

İki farklı hücre formu, bir besiyeri ve iki farklı hücre hattında, farklı konsantrasyonlarda 79 noktada alınan değerlerle oldukça fazla veri elde edilmiştir. Dielektrik sabitleri, dielektrik kayıpları ve kompleks permitivite değerleri kaydedilmiştir. Ardından bu değerleri Debye ve Cole-Cole denklemleri uygulanmış, her bir ölçüm için Debye için 3, Cole-Cole için 5 ayrı parametre (statik dielektrik, yüksek frekanslarda dielektrik, rahatlama frekansı, dağılım değişkeni ve statik elektriksel iletkenlik) elde edilmiştir. Bulunan parametreler denklemde tekrar yerleştirilerek dielektrik sabiti ve dielektrik kayıp hesaplanmış ve ölçülerek bulunan değerlerle kıyaslanmıştır. Sonuç olarak tutarlı ve uyumlu sonuçlar elde edilmiştir.

Hücre süspansiyonu üzerinde yapılan ölçümler besi yerinin dielektrik özelliklerine yakın değerler vermişken, hücre peleti bariz bir şekilde onlardan ayrılmış ve beklendiği gibi içerdiği su oranı daha az olduğu için her ikisinden de düşük dielektrik değerler sergilemiştir. İlk deneyde hücre peleti üzerinde çalışma yapmanın hücrelerin gerçek dielektrik özelliklerini belirlemek için daha doğru bir yöntem olduğu belirlenmiş ancak deneylerin doğruluklarını artırmak amacıyla, ikinci deneyde de farklı konsantrasyon yoğunluklarında hücre peleti ve hücre süspansiyonu üzerinde deney yapılmaya devam edilmiştir.

Elde edilen onar adet ölçüm önce kendi aralarında ANOVA testi uygulanarak p değeri hesaplanmıştır. Buradaki amaç ölçümlerin kendi içinde tutarlı olup olmadıklarının belirlenmesidir. ANOVA testi sonucuna göre yapılan 10'lu ölçümlerin tutarlı ve birbirine yakın değerler olduğu ortaya konmuştur.

Ölçümlerin medyanı alınarak daha sonra t-test uygulanmıştır. T-test hem farklı hücre formlarını (pelet, süspansiyon), hem de kanserli ve sağlıklı hücre gruplarının dielektrik değerlerini karşılaştırma amaçlı yapılmıştır. Beklenildiği gibi p değeri alfa değerinden (0.05) çok küçük sonuçlar vermiştir. hFOB pelet ve hFOB süspansiyonunun 2.2 mmlik probla yapılan ölçümlerine t-test uygulandığında $1x10^{-33}$ gibi çok küçük bir değer elde edilmiştir. SaOS pelet ve SaOS süspansiyonunun 0.5 mmlik prob ölçümlerinden $3,65x10^{-23}$, 2.2 mmlik probla yapılan ölçümlerinden $2,5x10^{-7}$ p değerleri bulunmuştur. SaOS hücre süspansiyonu ile hücre besi yerinin 2.2 mmlik probla yapılan ölçümleri kıyaslandığında p değeri 1 olarak elde edilmiştir. SaOS hücre peleti ile hücre besi yerinin 2.2 mmlik prob kullanılarak elde edilen ölçümleri karşılaştırıldığında ise çok küçük ($3,06x10^{-7}$) p değeri gözlemlenmiştir. Bu sonuçlara göre hücre peleti ile hücre süspansiyonu arasında bariz ve istatiksel olarak anlamlı bir fark olduğu ortaya konarken yine hücre peleti ile besi yeri arasında da istatiksel olarak anlamlı bir fark olduğu, ancak hücre süspansiyonu ile besi yeri arasında anlamlı bir farklılık olmadığı, sonucuna varılmıştır.

Kanserli hücre ve sağlıklı hücrelerin 0.5 mm’lik prob için hücre peletlerinin permitivite değerlerinin istatistiksel olarak karşılaştırıldığında, p değeri 0.034 olarak, hücre süspansiyonlarının değerleri karşılaştırıldığında p değeri $1,42x10^{-7}$ olarak bulunmuştur. Bu değerlere bakılarak kanserli kemik hücresi ile sağlıklı kemik hücresi arasında anlamlı farklılık olduğu ve farklı dielektrik değerler açığa çıkardıkları gözlemlenebilmektedir. Sonuç olarak her iki hücre formu, besiyeri, farklı konsantrasyon ve kanserli ve kansersiz olmak üzere iki farklı hücre hattı için tüm analizler yapılmış detaylı bir şekilde çalışmanın sonuç bölümünde sunulmuştur.

.

1. INTRODUCTION

1.1 Purpose of Thesis

Dielectric properties provide significant data about biological substances (tissues, body fluids or cells). After determining the dielectric properties of healthy and non-healthy biological substances, the difference in the mentioned data can be a good sign in diagnosis of diseases. This usage of dielectric properties accelerates to use electromagnetism in development of new diagnostic and treatment methods which do not hurt the patient, save time and minimize the risk of infection.

Various studies have performed to determine the dielectric properties of several tissues in animal and human at large range of frequencies. As for cells, there are limited studies in the literature especially in performed at high frequencies [1]. Cells are used in many researches to understand the disease mechanisms in cell level and develop suitable tools. Complex permittivity data provide information for characterization, detection and identification of cell type especially for cancer cells. Although numerous studies have focused on characterizing the dielectric properties of cancer cells, only a limited number of researches have studied and compared the dielectric properties of both cancerous and healthy cells.

There are many different methods to study the dielectric properties of materials. Recently, the dielectric properties of biological substances were measured using dielectric spectroscopy techniques [1]. One of the most promising method to measure dielectric properties from high radiofrequency to microwave frequency range is open-ended coaxial probe technique, proposed by Athey et al [2]. This method will be detailed explained in the next parts.

In this study, the purpose is threefold:

- To determine the dielectric properties of cancerous and healthy bone cells and reveal their differences if exist,

- Using two forms (cell suspension and cell pellet) of cells, analyze which one gives more accurate dielectric results,
- To examine the effect of number of cells to the results of dielectric properties.

1.2 Microwave Dielectric Properties of Cells

In the literature, dielectric properties of cells have been determined mainly in low frequencies (LF) to very high frequencies (VHF) ranging from 30 kHZ to 300 MHz [3-8]. In contrast, there is limited number of studies which is focused on the dielectric measurements of cells in the microwave frequencies (300 MHz to 300 GHz). In these studies, cells have been measured in different forms; pellet (bulk form of cells formed by centrifugation process), cell suspension or embedded in a gel. In addition to this, measurements on cell suspensions take a large space in the literature.

To measure the dielectric properties of cells, several methods can be performed such as dielectrophoresis, resonant cavity perturbation technique, and open-ended coaxial probe method.

First examples of measurement of dielectric properties at microwave frequencies backs to early 1986’s. The target frequency range was between 100 MHz and 1 GHz. Webb and Church (1986), made a research to measure dielectric constant and loss tangent of minerals at microwave frequencies [9]. However, studies measuring the dielectric properties of cells were started in late 1980s.

Dielectrophoresis technique is based on the principle of the motion of the particles due to their polarization when applied a non-uniform electric field in order to research their dielectric properties. In 2013, Salmanzadeh et al. focused on the dielectric properties of mouse ovarian surface epithelial cells. In this study, contactless dielectrophoresis technique has been applied to the cells which are at different stages (early, early intermediate, intermediate, late) [6]. They have found that malignant and benign cells are differentiated in metabolism, proliferation cytoskeleton and also some other functional properties [10]. These differences cause distinct dielectric properties between these type of cells [11].

Open-ended coaxial measurement technique is most used since this method is non-destructive and provides for both ex vivo and in vivo broadband measurements [12,13].

1.2.1 Open-ended coaxial probe technique

In the literature, open-ended coaxial probe is used generally for broadband measurements of dielectric behaviors of the biological substances without destruction [2]. Main advantages of this technique:

- performing broadband measurements, capable for measuring up to 50 GHz,
- sample preparation is not required, the probes can be easily used in liquids and semi-solid materials,
- providing plenty of data.

The disadvantages of this technique are having high measurement error (5%) [14] and low repeatability [15]. It is also dependent to calibration part and warming up sufficiently the network analyzer before the measurement. Network analyzer should be set on for heating up at least for one hour before starting the experiment [1]. Some of the factors that affecting the repeatability and accuracy of the probe are:

- dehydration of the measured tissue;
- tissue fluids left on the aperture of the probe;
- pressure applied on the probe;
- placing the probe on the tissue in incorrect angle;
- alterations in temperature of the tissue;
- being not homogenous of the tissue [12].

Open ended coaxial probe is mostly used in distinguishing the tissues (healthy, malignant or benign) according to their dielectric properties. Especially, bone, skin, muscle and breast tissues have been the interest mostly in the literature [12, 15-18].

Within the probe, usually a dielectric material made of glass or teflon is used as they are resistant to temperature changes. Measurements are carried on by measuring the reflection coefficient with coaxial probe connected to a network analyzer. Then dielectric properties of the material are calculated with using reflection coefficient by a software.

The probe that is used in this study is Agilent 85070E probe kit and the network analyzer is Agilent N5230A PNA-L Network Analyzer [14]. With this probe kit, measurements can be done from 200 MHz to 50 GHz. In this study, the frequency range has been determined as from 500 MHz to 20 GHz.

The probe kit has three types of probes: high temperature, slim form and performance. All the probes shown in Figure 1.1 needs calibration. Calibration process will be explained deeply in the Chapter 4.

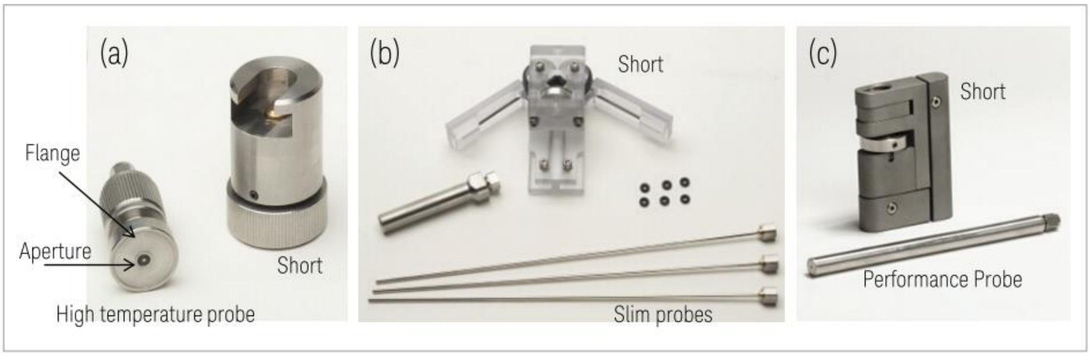


Figure 1.1: Agilent different probe types: a) high temperature probe, b) slim form probe, c) performance probe [14].

The earliest studies on biological substances were performed on animal tissues using open-ended coaxial probe method. Dielectric properties of rat tissues in the frequency range from 30 MHz to 2 GHz were measured by Joines et al in 1980, and cat tissues from 0.01 to 1 GHz4 were measured by Stuchly et al in 1982. Later on, the dielectric studies were extended to human tissues [19]. Dielectric studies performed on cells are limited as compared to studies performed on tissues.

5.2.1. Measurements performed with mammalian cell pellet

As in the situation that dielectric properties of cells have not been widely reported in microwave frequencies, open-ended coaxial probe measurements performed with pellet are likewise limited.

In the literature, using cell pellet to examine the dielectric properties of cells is rare. However, in order to minimize the effect of culture media to the measured sample, using the cell pellet is more appropriate. It is noteworthy to mention that cells without media cannot survive for as long time as a cell suspension.

5.2.2. Measurements performed with mammalian cell suspension

The dielectric properties of cell suspension (cells in a specific media solution) were measured mainly through dielectric spectroscopy techniques [1].

In a very recent research published in March 2019, Hussein et al. examined dielectric characterization (dielectric constant, dielectric loss and conductivity) of non-tumorigenic cell (MCF-10A), breast cancer cell lines (MDA-MB-231, Hs578T, T47D and MCF-7), and colon cancer cell line (HT-29) with open-ended coaxial probe technique the frequencies from 200 MHz to 13.6 GHz [19]. According to their results, breast cancer cell lines have higher values comparing to their healthy counterpart. Also, they compared colon cancer cell with breast cancer cell and they deduced that different cancer types have different dielectric signals. As a sample preparation, after seeded the cells in a 12-well plate, they have placed the cells with plate in an incubator (37C) to form the cells a homogenous monolayer. After 24 hours, the measurements have been done in 12-well plate using DAK-3.5 Dielectric Probe, the sensing depth of which is high in respect to sample measured. They have measured cells with and without media. For the second measurement (without media), the culture media has been removed from the cells by using a micropipette. Therefore, two different values have been obtained and compared the difference between cells with media and cells without media. Especially the dielectric values of the cells without media has been quite low regarding to cells with media. As the well is quite small with respect to the diameter of the probe, the sample (cells without media) volume has been relatively small with respect to sensing depth of the probe. Hence, the environment within the distance of sensing depth might have been also measured by the probe. Consequently, the differences may be due to the relation between sensing depth and the sample volume.

In 2014, Odelstad, Raman, Rydberg, and Augustine examined complex permittivity of hFOB, SaOS-2, and C2C12 cell lines in different concentrations. The measurements have been performed in the 2-50 GHz frequency range with using open-ended coaxial probe. They have measured cell suspensions and medium without cells and then calculated the permittivity of only cells by using Maxwell's mixture equation [20]:

$$\frac{\varepsilon_1-\varepsilon_s}{2\varepsilon_1+\varepsilon_s} = p\,\frac{\varepsilon_1-\varepsilon_2}{2\varepsilon_1+\varepsilon_2} \qquad (1.1)$$

where ε_1, ε_2, and ε_s permittivity of medium, permittivity of cell particles, and permittivity of cell suspension respectively. *p* represents the volumetric fraction of cell particles in the solution. Hence, permittivity of cell particles, ε_2, can be calculated with using the formula (1.1) above. Among the measurements performed on different concentrations, statistically significant difference in both real and imaginary part of the permittivity between cell suspension and medium, has been observed only in the highest concentration (1350000 cells/ml). This suggests that the more concentration increases, the more the results are accurate [20].

1.2.2 Other techniques

In order to measure microwave dielectric properties of cells, different methods have been applied including resonant cavity perturbation, waveguide penetration; and dielectrophoresis methods.

To find out the cell structure, In 1996, Asami showed a connection between structure of cells and its dielectric loss, which changes the dielectric measurements into an effective method [21].

5.2.1. Measurements performed with mammalian cell pellet

Recently, in 2017, Nerguizian, Alazzam, Stiharu and Burnier. examined the dielectric properties of several cell lines as uveal melanoma, prostate (PC3), cervical (HeLa), breast (MDA231) cancer cell lines, and white blood cells WBC-Jurkat clone E6 at microwave frequencies. They have used the resonant cavity perturbation technique and determined relative dielectric loss and relative dielectric constant of cells. This article is the first to report in respect to the measurement of complex permittivity of several cells types at high frequency ranging from 2 to 4.5 GHz. As determining complex permittivity of a single cell is not possible yet, therefore they have suggested to measure bulk form of cells (pellet) with a known volume and count, and then estimate the dielectric properties for only one single cell. Firstly, they have measured the complex permittivity of the pellet and medium, and then they have calculated the complex permittivity of a single cell by using Hanai equation. After measurements they have found the relative dielectric constant changing between 42 and 68 and the relative dielectric loss changing between 12 and 22 for a single cell [1].

5.2.2. Measurements performed with mammalian cell suspension

In 2010, Zhang, Zhong, Tan, and Xu applied the dielectric spectroscopy technique to measure the dielectric properties of red blood cell suspension. This method contains a temperature control system, low-frequency and high-frequency parts, and a liquid electrode. The system allows measuring the samples at different temperatures and at frequencies from 1 MHz to 1 GHz. This paper notes that biological substances are highly sensitive to temperature, pressure and frequency differences as they are mostly polar materials. Therefore, they experimented the sample at different temperatures, in a broad frequency range. Hence, they showed that frequency and temperatures changes are important factors that affect the relaxation process; as the temperature increases the loss tangent peak will be at higher frequency point and the relaxation time will decreased. They also observed that higher cell concentration has higher permittivity and lower dielectric loss value. Accordingly, they indicated that this influence is due to capacitive membrane of the red blood cells and revealed that there is a relation between the cell membrane and β dispersions [22].

Saito, Suzuki, Taguchi, and Taki (2009) investigated complex permittivity of Chinese hamster ovary (CHO)-K1 cells at frequencies between 1.7 and 2.6 GHz, and 50 and 65 GHz by waveguide penetration method. They found real and imaginary values of complex permittivity for CHO-K1 as shown in Table 1.1. As they claimed that measurement of dielectric values of only cell is difficult because of obtaining sufficient amount cell, they realized experiments in a mixture including cells and various liquids. Then they used linear least squared method and calculated the complex permittivity of CHO-K1 cells [23]:

Table 1.1. : Real and imaginary parts of complex permittivity values of CHO-K1 cells [23].

Frequency range	Real part of complex permittivity (ϵ')	Imaginary part of complex permittivity (ϵ'')
1.7 and 2.6 GHz	59.9	15.6
1.7 and 2.6 GHz	64.1	20.2
50 and 65 GHz	10.6	15.2
50 and 65 GHz	14.2	20.0

2. ELECTROMAGNETIC CHARACTERIZATION OF BIOLOGICAL SUBSTANCES

Investigation of how electromagnetic fields interact physically with biological systems constitutes an important part of the studies on biological effects caused by exposure. Measurement and calculation of the internal electric fields in an object irradiated by dosimetry, that is, radio frequency radiation, constitutes the basic principle of the studies. Dosimetry is a very important concept because the biological effects depend on the internal areas within the body. Whether it is a calculus or an experimental way, it is difficult to find interior spaces. In general, interior spaces; It depends on the area, the shape and size of the object, the electrical properties and the frequency of the incoming field. Therefore, it is different in the interior areas of different objects in the same outer space [24].

Both theoretical and experimental studies are needed to find radio frequency fields in the radiated body. Theory of interior spaces; explain how it is connected to the absorber and the incoming areas. Theoretical studies allow the adaptation of the biological effects observed in animals related to radio frequency radiation to the possible effects on humans. This is of particular importance because experimental studies on biological effects of radio frequency radiation cannot be directly tested on humans [24].

Experimental studies are needed to validate theoretical studies, to understand other aspects of internal spaces and to obtain some data that cannot be obtained by theoretical study. Using theoretical calculations and experimental techniques together, researchers have obtained as much useful information as possible about dosimetry. Theoretical methods Maxwell's equations; swallowed, reflected and taking into consideration the incoming wave, consists of a solution in a variety of ways. The mathematical models used with these methods represent a convergence of actual physical values, with calculations that are highly limited in representing incoming and swallowed areas [24].

The experimental methods consist of measuring the internal electric field or measuring the temperature rise at various points within the body. In order to be able to measure these values, it is necessary to prepare appropriate devices and to have adequate preliminary information. In this study, recommendations and evaluations were made about an experimental setup to be established and the path to be followed and some basic information was given.

Electromagnetic radiation (non-ionizing radiation) causes the power density expressed in Watts per surface to be absorbed in the living body and damage is caused by tissue warming. This absorbed power (SAR) depends on the frequency of the incoming wave, the angle of incidence, the water content of the living tissue and the electrical properties of the biological material (conductivity, dielectric constants). This interaction results from the transfer of energy to the tissue material of the inner spaces induced by the electromagnetic wave in the living body as described below.

- Electric fields give kinetic energy to the free electrons of any atom.
- Electric fields affect the electric dipoles in atoms and molecules. Polarization is called friction due to this phenomenon in the tissue material heat occurs.
- Electric fields bring together existing dipoles in the material. However, the resulting friction transfers energy to the material [25].

2.1 Dielectric Properties of Biological Tissues

Dielectric properties are also important parameters in the development electromagnetic diagnostic and therapeutic techniques such as microwave ablation and microwave hyperthermia for cancer treatment. In the literature, the dielectric properties of several biological tissues have been widely studied and reported. The behavior of various tissues in relation to their dielectric properties has been examined in comprehensive studies. These studies contain both human and animal tissues. The most studied tissue for dielectric properties in the literature is breast tissue. However, major variations have been observed regarding the dielectric data of breast tissue in the literature. These variations arise from heterogeneous structure of the breast tissues. One of those heterogenous tissues is bone as it is composed of cortical and cancellous structure [16]. Cortical bone is ticker and outer structure of long bone which constitutes 80% of human skeleton. Cancellous bone is less dense and softer bone and occurs at the end of the long bones.

Table 2.1: Permittivity and conductivity values of various tissue samples at ultra high frequencies (500 MHz) [26].

Tissue	Permittivity	Conductivity (S/m)
Bone (cortical)	12,9	0,1
Muscle	56,4	0,82
Fat	11,5	0,085
Blood	63,3	1,38
Breast	54,9	0,036
Bone (cancellous)	21,9	0,25

Several studies have been carried out to measure the dielectric properties of bone tissues in both the low-frequency range [27,28], and in microwave frequency range [29-32]. These studies are different in terms of used measurement techniques, location of bone sample, bone type, and method of sample preparation. Each of these factors can influence the measured dielectric properties. A study has been performed by Amin et al [16] to compare and contrast differences in the studies that examines dielectric properties of bone and their relationship for the diagnosis of some bone diseases. Figure 2.1 shows the detailed results of the studies performed on dielectric properties of bone in terms of study type, frequency range and measurement technique [16].

Reference	Study type	Frequency range	Source	Measurement technique	Dielectric properties
Ivancich et al. [14]	In vitro	10 MHz–1.3 GHz	Adult bovine cortical and trabecular tibial bone	Thin cell time domain spectroscopy (TDS), HP (1815B) Sampler, HP (1801A) oscilloscope, SNA (HP8711A)	$\varepsilon = 9.64$ (demineralized bone, 238.49 MHz), $\varepsilon = 17.75$ (native bone, 414.85 MHz)
Gabriel et al. [22]	In vitro	10 Hz–20 GHz	Porcine cortical bone	OECL, IA(HP4192A), IA (HP 8753C), IA(HP8720)	$\varepsilon = 1.0E+3 - 1.0E+1$ $\sigma\ (Sm^{-1}) = 1.0E-2 - 1.0E+1$
Peyman et al. [25]	In vitro	50 MHz–20 GHz	Porcine cortical bone	OECL, Network Analyser (Agilent 8720D)	$\varepsilon = 28.1 \pm 2.0$, $\sigma\ (Sm^{-1}) = 0.34 \pm 0.04$ (10 kg, 450 Hz)
Irastorza et al. [13]	In vitro	80 MHz–1 GHz	Bovine diaphysis femur cortical bone	OECL, HP (1815B) TDR/Sampler, HP (1801A) oscilloscope, SNA (HP8711A)	$\varepsilon = 14.8$ (natural bone) $\varepsilon = 27.5$ (demineralized bone) (80 MHz)
Meaney et al. [15]	In vitro	900–1300 MHz	Porcine trabecular femoral bone	MTI	$\varepsilon = 48$, $\sigma = 1.9$ (1100 MHz)
Meaney et al. [10]	In vivo	900–1700 MHz	Human trabecular calcaneus bone	MTI	$\varepsilon = 13.6$, $\sigma = 0.84$ (1300 MHz)
Irastorza et al. [11]	In vitro	100–1300 MHz	Human trabecular femoral head bone	OECL, HP (1815B) TDR/Sampler, HP (1801A) oscilloscope, SNA (HP8711A)	$\varepsilon = 46.85$, $\sigma = 0.578$ (400 MHz)

ε relative permittivity, σ conductivity, *IA* impedance analyzer

Figure 2.1: Comparison of studies performed on dielectric properties of bone in the literature [16].

In 2012, Meaney et al. [33] has compared the dielectric properties of the human calcaneus (heel) bone from two patients across the frequency range of 900–1700 MHz.

From this study, it can be deduced that dielectric properties of normal heel bone have lower values when compared to the affected heel bone. Table 2.2 summarizes the dielectric properties of two different tissues. Dielectric properties were measured at only one frequency.

Table 2.2: Dielectric properties of heel bones of two patients [16].

Patient	Foot	Relative permittivity	% difference	Conductivity (S/m)	% difference
1	Affected	13.6	8.4	0.84	45.2
1	Normal	12.5		0.53	
2	Affected	16.7	21.1	0.92	13.9
2	Normal	13.5		0.80	

In the studies carried out until the beginning of 1920s, it was understood that the tissues conveyed electricity, the resistance changed with frequency, the conductivity was caused by the ion movement. The electrical properties of the muscles and nerve tissues, which are known as the capacitive properties of tissues, have emerged in those years. After the 1920s, the dielectric studies are concentrated in the wide frequency range and on various materials. The capacity and resistance characteristics of the blood from 266 Hz to 2 MHz were examined and for the first time both components were measured for complex tissues in the wide frequency range. In 1948, Rajewsky and Schwan found a complex dielectric constant of blood tissue around 1 GHz. Between 1950 and 1960, dielectric properties of vesicles, organelles, and DNAs of various tissues and protein containing many materials were investigated [25].

The dielectric properties of the tissues are important in the calculation of internal electric fields within the body in the case of exposure to electromagnetic waves and in the development of medical applications such as diagnostics and therapy of electromagnetic energy, the probable damages of these electromagnetic fields. Knowing the dielectric properties of biological tissues also plays an important role in the development of application areas of many electromagnetic energy such as food processing, agricultural purposes, drying of various products [25].

In a material whose conductivity is σ, the heat generated by an active value electric field in E is σ.E2. While conductivity is significantly increased due to the dielectric loss above 100 MHz, the ionic conduction passing through the extracellular and intracellular fluids at low frequencies makes the most important contribution to conductivity. Although the change of conductivity by frequency depends on the changes in the dielectric with the Kremers - Kronig equations, conducting the conductivity in a separate way as in the dielectric mixing theory provides more useful information [25].

2.2 Dielectric Properties of Biological Cells

Effect of very low frequency electric fields on tissues; The relationship between the electric field and current density can be understood by the relationship between the electric fields of different environments and the macroscopic properties of the tissues [25].

At very low frequencies, conductivity in tissues is almost independent of frequency. Electrical conductivity varies widely according to the materials used in engineering. As can be seen in Table 3.1, the complexity of the biological tissues in the power frequencies can be shown only by the real part. The actual conductivity of air is 10-13 S / m and the virtual conductivity is 3.3.10-9 S / m. In understanding the relationship between field and current density, it is important that the conductivity is virtual, real or complex [25].

Cell suspension in a medium is the oldest and simple model demonstrating the electrical conductivity of a biological substance [34].

At the microscopic level, all tissues were composed of cell and extracellular fluids. The cells are electrically composed of two different elements. These are high conductivity membrane and core, such as externally insulating membrane and internal liquid. Because of the presence of the membrane, the cells are isolated and flow between all current cells induced by the outer area of 50 Hz [25].

Macroscopically, the electrical properties of the tissues give information at three points. These:

a) The direct effects on the cell elements are very different due to the very small intracellular space.

b) Almost all of the induced current (except long cells directed to the area) passes through the extracellular fluid.

c) The only thing known about the effect of electric fields on the extracellular fluid is that they are large enough to cause heating [25].

Table 2.3: Similar studies related to dielectric properties of cells in the literature

Article	Method	Frequency Range	Cell type	Cell preparation
Dielectric Characterization of Breast Cancer Cell Lines Using Microwaves [35]	open ended coaxial cable	200 MHz-13.6 GHz	Human breast cancer cells MDA-MB-231, Hs578T, MCF-7 and T47D	Media and three replicates for each cell type have been tested.
Dielectric spectroscopy of normal and malignant human lung cells at ultra-high frequencies [36]	open-ended coaxial probe	200 MHz-2 GHz	human lung epithelial cancer cell line SK-MES, and the normal corresponding NL-20	Cells were embedded in agarose. Diameter of cells were measured. Agarose gel without cells measured first. Cells were spun down and the pellet mixed with agarose.
Characterization of several cancer cell lines at microwave frequencies [1]	cavity perturbation technique	2-4.5 GHz	Cervical (HeLa), Prostate (PC3), Breast (MDA231), Uveal melanoma (92.1 and OCM) cancer cell lines and white blood cells WBC-Jurkat Clone E6	Cells counted and verified under the microscope to evaluate the average size of the cells and their viability. Cells were suspended in a D-PBS solution and centrifuged to form a cell pellet. The bulk form (pellet) of cancer cells is measured.
Experimental Procedure for Determination of the Dielectric Properties of Biological Samples in the 2-50 GHz Range [20]	open-ended coaxial probe	2 - 50 GHz	Human osteoblast hFOB 1.19, human osteosarcoma SaOS-2, Mouse myoblast C2C12	Cells suspension and culture medium were measured.
Characterizing the dielectric properties of human mesenchymal stem cells and the effects of charged elastin-like polypeptide copolymer treatment [7]	dielectrophoresis	0.010–35MHz	Human mesenchymal stem cells (hMSCs)	Standard cell culture protocol
Investigating dielectric properties of different stages of syngeneic murine ovarian cancer cells [6]	Contactless dielectrophoresis	Low frequency	Mouse ovarian surface epithelial cells	Standard cell culture protocol
Dielectric properties of dipicrylamine-doped erythrocytes, cultured cells and lipid vesicles [4]	dielectric spectroscopy	10 Hz - 10 MHz	Horse erythrocytes, murine lymphoblasts (L5178Y) and lipid vesicles	Standard cell culture protocol

Article	Method	Frequency Range	Cell type	Cell preparation
Dielectric inspection of erythrocytes [5]	dielectric spectroscopy	40 - 100 MHz	rabbit erythrocytes	Standard cell culture protocol
Dielectrophoretic spectra of single cells determined by feedback-controlled levitation [8]	Feedback controlled levitation	1 kHz - 50 MHz	Rabbit ligament fibroblasts	Standard cell culture protocol
Breast cancer cells exhibits specific dielectric signature in vitro using the open-ended coaxial probe technique from 200 MHz to 13.6 GHz [19]	Open-ended coaxial probe	200 MHz-13.6 GHz	Healthy non-tumorigenic, MCF-10A and breast cancer, MDA-MB-231, Hs578T, T47D and MCF-7	Cells were placed to form homogenous monolayer

3. THEORY

3.1 Dielectric

Nowadays, with the technological developments, the usage areas of electrical insulators are increasing. These areas are used as main insulators in high voltage power system components and low voltage high frequency applications. Knowing how the electrical properties of these insulators change over a wide range and under different conditions is very important for safe operation. For this reason, the dielectric properties should be measured under conditions where the insulating materials used in different areas and operating conditions are operated. Dielectric constant and loss factor are the leading features; describes the interaction of the material with the electric field and the loss of energy of the material due to the effect of the electric field, respectively [1]. However, these measurements require advanced devices and take a very long time. Instead, once measured over a wide range and under different operating conditions, the determination of the dielectric properties of the same insulating material under any other operating conditions by Artificial Neural Network (ANN) will provide significant contributions in terms of time and cost. For this purpose, the dielectric constant (ε) and loss factor (tanδ) data of the polyester, which is used as the insulator, according to the temperature-frequency values, is trained in an ANN model. Later, this ANN model was tested with unused data. The dielectric constant of an insulator varies with frequency (F) and temperature (T) and decreases depending on the frequency [37].

$$\varepsilon = f(\mathrm{F},\mathrm{T}) \tag{3.1}$$

The dielectric constant is one of the factors that determine the capacity of an insulator. Furthermore, the decrease or increase of dielectric constant also affects the puncture resistance. The other important parameter of the insulator is the loss factor. In an ideal insulator, the loss factor is zero. However, because the insulator is not ideal, a leakage current (Ir) passes and is defined as the loss of dielectric losses caused by this current.

These losses act as a thermal source at high voltage or high frequency. This situation causes more thermal stress of this insulator during operation and affects its properties. For this reason, it is very important to know how the loss factor changes as much as possible while selecting the insulating material. The loss factor is defined by tanδ and is defined as the ratio of the leakage current (Ir) to the capacitive current (Ic) as follows:

$$Tan\delta = \frac{I_r}{I_c} \tag{3.2}$$

Here, the capacitive current varies depending on the frequency. Dielectric loss,

$$W_d = 2\pi f C U^2 \tan\delta \quad \text{(w/m)} \tag{3.3}$$

Here; f shows the frequency (Hz), C capacity (F) and U applied voltage (V).

The most extensively used models to explain the electrical behavior of both tissues and liquid solutions are Cole-Cole, Debye, and Cole-Davidson models [38].

3.2 Dielectric Relaxation

Dielectric relaxation is the adjustment of dielectric displacement (D) or polarization (P) to the time-dependent electrical field (E).

The relation between a material and electromagnetic wave is based on electronic, atomic or charge polarizations and their reflection and transmission waves. This relation is described with Maxwell Equation:

$$\nabla x E = i\omega\mu^* H \tag{3.4}$$

$$\nabla(\varepsilon^* E) = 0 \tag{3.5}$$

$$\nabla x H = i\omega\varepsilon^* E \tag{3.6}$$

$$\nabla . H = 0, \tag{3.7}$$

where H represents magnetic field, E shows electric field, ε* represents permittivity and μ* is permeability [39].

Complex permittivity can be defined as

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega) \tag{3.8}$$

where ε’ is the real part and ε” is the imaginary part which are described as:

$$\boldsymbol{\varepsilon}' = \boldsymbol{\varepsilon}_\infty + \frac{\boldsymbol{\varepsilon}_s - \boldsymbol{\varepsilon}_\infty}{1 + \boldsymbol{w}^2\boldsymbol{\tau}^2} \tag{3.9}$$

$$\boldsymbol{\varepsilon}'' = \frac{(\boldsymbol{\varepsilon}_s - \boldsymbol{\varepsilon}_\infty)\boldsymbol{w}\boldsymbol{\tau}}{1 + \boldsymbol{w}^2\boldsymbol{\tau}^2} \tag{3.10}$$

where $\boldsymbol{\varepsilon}_\infty$ is permitivity at infinite frequency, $\boldsymbol{\varepsilon}_s$ is static permittivity measured at lower frequencies.

The equations (3.9) and (3.10) are commonly known as Debye Dispersion formula which are referred to the situation where equilibrium is attained with time when a constant external electric field is imposed on a dielectric.

Relaxation time (τ) is the time required for dipoles to become oriented in an electric field. When an electric field is applied to the material, molecules turn slowly and reach the final state of orientation polarization with relaxation time constant τ.

$$\boldsymbol{\tau} = \frac{1}{\boldsymbol{W}_c} = \frac{1}{2\boldsymbol{\pi}\boldsymbol{f}_c} \tag{3.11}$$

3.3 Debye Equation

Debye relaxation equation is used to model materials which exhibit a single relaxation time:

$$\boldsymbol{\varepsilon}(\boldsymbol{w}) = \boldsymbol{\varepsilon}_\infty + \frac{\boldsymbol{\varepsilon}_s - \boldsymbol{\varepsilon}_\infty}{1 + \boldsymbol{j}\boldsymbol{w}\boldsymbol{\tau}} \tag{3.12}$$

where $\boldsymbol{\varepsilon}_s$ (static) and $\boldsymbol{\varepsilon}_\infty$ (infinite) are constants measured at lower and higher frequencies respectively, and w is angular frequency.

3.4 Cole-Cole Equation

To describe relaxation process of dielectric materials, Cole-Cole equation is used since there is a distribution of relaxation times. Cole-Cole equation is expressed as follows:

$$\boldsymbol{\varepsilon} * (\boldsymbol{w}) = \boldsymbol{\varepsilon}_\infty + \frac{\boldsymbol{\varepsilon}_s - \boldsymbol{\varepsilon}_\infty}{(1 + \boldsymbol{i}\boldsymbol{w}\boldsymbol{\tau})^{(1-\alpha)}} \tag{3.13}$$

where α is the distribution of relaxation time.

Results of fitting Cole-Cole equation have been presented in Chapter 5.

3.5 Dielectric Dispersions

When conductivity or permittivity was measured, it is shown that conductivity increases, and permittivity decreases as the frequency increased, which is seen as dispersion regions. Generally, three dispersion region(α, β, and γ) is seen [40]:

- Alpha dispersion is seen from 10 Hz to few kHz,
- Beta dispersion is seen from 1 kHz to several MHz,
- Gamma dispersion is seen higher than 10 GHz [41].

4. MATERIALS AND METHOD

4.1 Experimental Procedure

4.1.1 Samples

Cells were obtained from Prof. Dr. Fatma Neşe Kök İstanbul Technical University and the all laboratory works were done in Istanbul Technical University Molecular Biology and Genetics Department laboratories.

To examine the cancerous and healthy bone cells, two cell line samples have been used in experimental investigation. Human osteosarcoma cell line (SaOS-2) and human fetal osteoblastic cell line (hFOB 1.19) have been investigated. The use of bone cancer cell line and healthy bone tissue cell line is for cancer research is trustworthy since cells maintain all the genomic specifications of tissues [42].

As a first experiment, dielectric properties of only cancer cell line SaOS has been measured. Two types of cell forms (cell pellet and suspension) have been prepared. Cell suspension is collection of cells in a liquid medium. Cell pellet is solid mass of cells that is obtained from cell suspension. These two forms and medium have been measured by using open-ended coaxial probe, Agilent 85070E software and network analyzer.

Based on the information that presence of medium will alter the dielectric behavior of the cells, to measure the cells only in bulk form without medium -cell pellet-, is reliable as it purified from medium as much as possible. It is supposed that cell pellet reflects the dielectric properties of cells much more than cell suspension. Medium without cells is also measured to compare with the dielectric properties of cell suspension. As it is mentioned early, some studies measured the complex permittivity of cell suspensions and liquid medium without cells, and then difference between two samples has been calculated by using Maxwell mixture equation [20]. Dielectric permittivity of cells can also be evaluated with this equation.

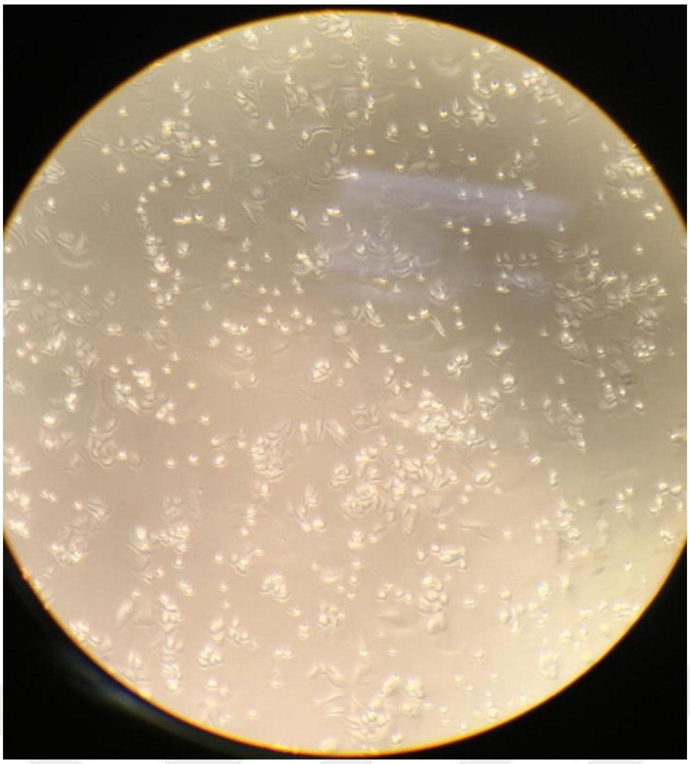


Figure 4.1: Image of SaOS cells under microscope.

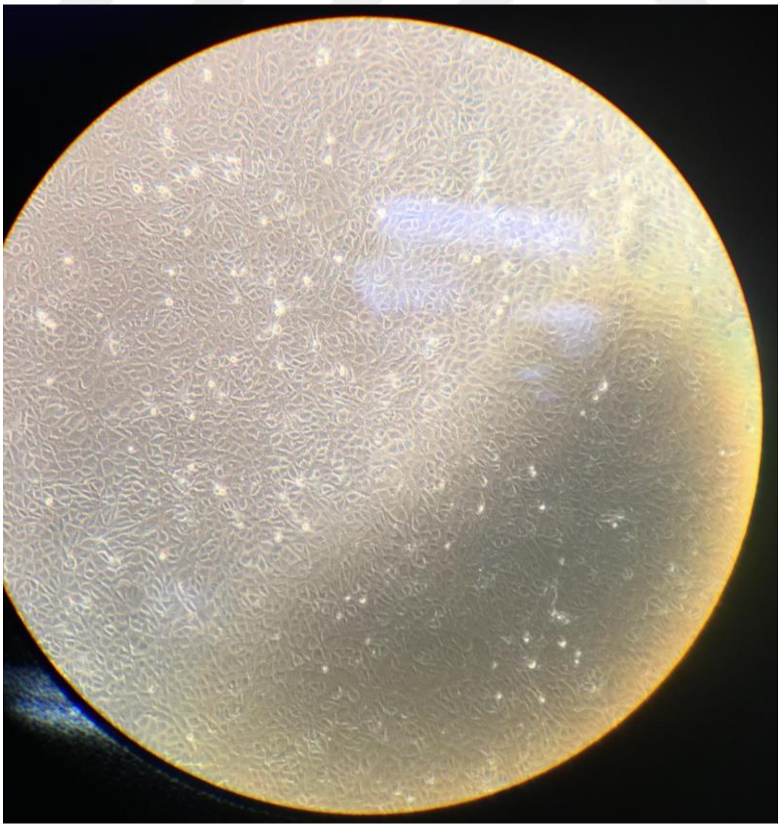


Figure 4.2: Image of hFOB cells under microscope.

4.1.2 Cell preparation

To prepare the cells, standard adherent cell culture protocol has been applied. They were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 1% Penicillin-Streptomycin and maintained in a 37°C incubator. When the confluency reached approximately 80% of the flask, cells were passaged with using trypsin. SaOS and hFOB cells were plated in 25 cm^2 cell culture flask firstly, then they were transferred into one 75 cm^2 flask and after a few days, they were passaged into two 75 cm^2 flasks. Media was changed every two days.

Before the experiment, cells were removed from the flask by trypsinization. 2 ml trypsin were added to the flasks and removed. They were incubated at 37°C incubator for 5 minutes. The flask was observed under microscope to confirm the cells were detached from the flask. After 90% of cells have detached, 4 ml of complete growth medium were added to flask and cells were collected into 15 ml falcon tube and centrifuged for 5 minutes at 1000 rpm. After centrifugation, cell pellet and supernatant are formed. Supernatant was discarded, and pellet was resuspended with a minimal volume of medium without serum. A sample was taken and dyed with equal volume of Trypan Blue for counting. The sample placed into hemocytometer and observed under microscope. Cell counting is based on the principle that living cells exclude the Trypan Blue dye and appear white while dead cells take up the dye and appear blue. After counting with hemocytometer, number of cells have been calculated with the following formula:

$$\boldsymbol{Total\ cells\ counted\ x\ \frac{dilution\ factor}{number\ of\ squares}\ x\ 10^4} \tag{4.1}$$

After calculation, number of cells was found as shown in the following table:

Table 4.1: Number of cells in measured samples

	Cell	Number of cells (cells/ml)
1st Experiment	SaOS	12500000
2nd Experiment	SaOS	22500000
2nd Experiment	hFOB	17800000

After cells were counted, they were centrifuged again at 1000 rpm for 1 minutes to collect the cells. Supernatant was discarded, and measurements have been done on cell pellet firstly.

Secondly, cell pellet was suspended with 3 ml medium without serum. Then the measurements were repeated on cell suspension.

Lastly, medium without serum has been measured with the open-ended coaxial probe same as cell pellet and cell suspension.

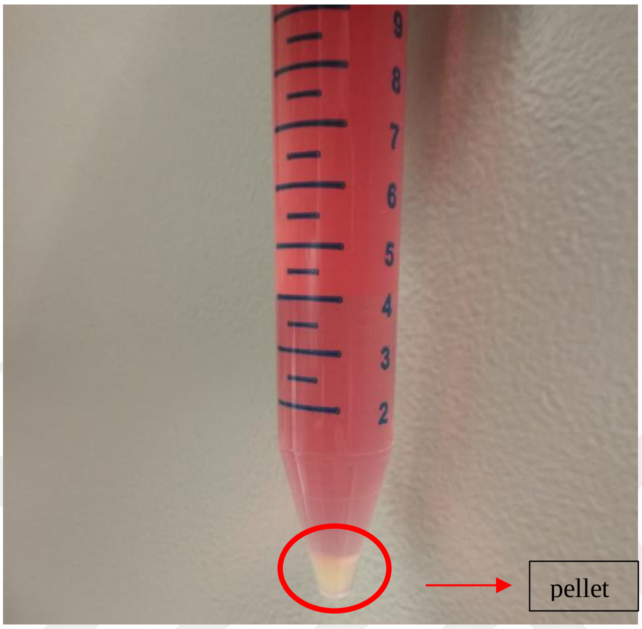


Figure 4.3: Cell pellet (bottom) and supernatant (top).

4.2 Measurement Setup

The device used in this study is Agilent N5230A PNA-L Network Analyzer. The probes used in the study Agilent N1501A Dielectric Slim Form Probe. The other materials that are used in the study are Agilent N1501A Dielectric Slim Form Probe, a laptop and Agilent 85070E software.

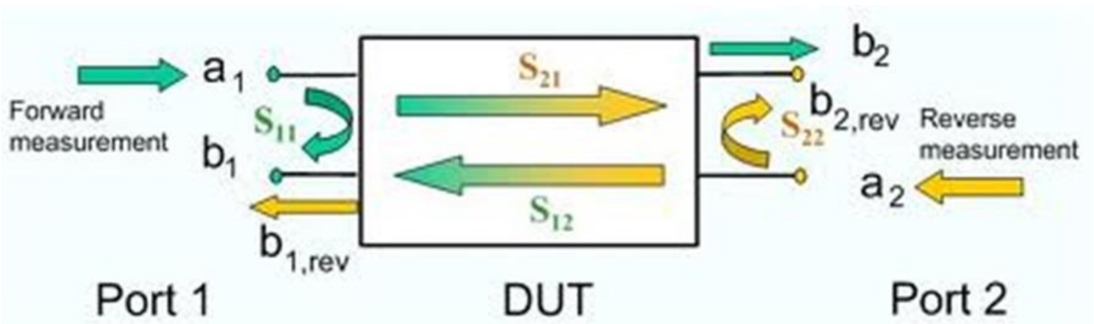


Figure 4.4: Schematic representation of a 2-port network analyzer.

Agilent N5230A PNA-L Network Analyzer measures magnitude and phase of scattering waves. There are two ports in the device which send the generated signal and receive the signals reflected. As shown in Figure 4.5, 2-port network analyzer measures four S-parameters; S11, S12, S21, S22. S-parameters describes reflection/transmission characteristics in frequency domain and represented in matrix form. The numbering of S parameters show that port numbers (first number symbolizes emerged signal, and the second number symbolizes where the signal is applied) [43].

S11 parameter - forward reflection: Signal is emerged from port 1 and reflected back to the port 1.

S12 parameter - reverse transmission: Signal is emerged from port 1, transmitted to port 2.

S21 parameter - forward transmission: Signal is emerged from port 2, transmitted to port 1.

S22 parameter - reverse reflection: Signal is emerged from port 2 and reflected back to the port 2.

Figure 4.5: Network analyzer, probes and working environment.

4.2.1 Calibration

Before the measurements, the device needs a calibration to obtain accurate results. It should be done carefully as it is suggested in software instruction.

In general, open-ended coaxial probe use three common standards for calibration: Open air, distilled water (or any liquid, dielectric properties of which is known) and conductive textile (short).

Since environmental changes may affect measurements results, temperature, humidity and pressure should be monitored [38]. Probe tip should be cleaned, and cable should be fixed before starting the calibration process. Measurement settings should be selected in the network analyzer. Temperature of distilled water is measured using thermometer and entered in the software.

Start frequency, stop frequency and number of points were entered as 500 MHz, 20 GHz and 79, respectively. Measurements were performed with 250 MHz intervals.

4.2.2 Experiment protocol

The outer diameters of the used probes are 2.2 mm and 0.5 mm. The width and depth of the sample should be at least two times bigger than outer diameter of the probe [14].

Firstly, slim form 2.2 mm probe was used. Before the measurement, probe was cleaned with ethanol and distilled water. Calibration was performed starting from the open air. The probe has measured the dielectric values of the air. Then shorting block and distilled water measurements have been performed. It is important that no air bubble was in the aperture of the probe. This will affect both calibration and the measurements. Before measuring the sample, water and methanol was measured to find out whether the calibration was good.

As shown in Figure 4.6, the probe was placed into the falcon tube and cell pellet, cell suspension and medium were measured from 500 MHz to 20 GHz, and ε' and ε'' results were saved. Ten measurements were carried out for each form of cells and medium.

4.3 Data Processing

It is necessary to transform the measured scattering parameters into the permittivity values. A software or an algorithm should be used for this reason which converts S-parameters taken from the network analyzer into permittivity and permeability values. The following explanations describe how the software performs these calculations.

The probe's normalized admittance is given as follows:

$$\frac{Y}{Y_0} = \frac{ik^2}{\pi k_c \ln\frac{b}{a}} \int_a^b \int_a^b \int_0^\pi \cos\phi' \, d\phi' \, d\rho' \, d\rho \tag{4.2}$$

where

$$r = \sqrt{\rho^2 \rho'^2 - 2\rho\rho' \cos(\phi)} \tag{4.3}$$

$$k_c = w\sqrt{\varepsilon_c \varepsilon_0 \mu_0} \tag{4.4}$$

$$k = w\sqrt{\varepsilon^* \varepsilon_0 \mu_0} \tag{4.5}$$

'a' represents radius of inner conductor and 'b' represents the radius of the outer conductor, permeability of free space and permittivity of the material that is inside the probe, and complex permittivity of the sample have been shown as μ_0, ε_c and ε^* in the equations above.

Figure 4.6: Measurement of cell pellet (left) and cell suspension (right).

The equation numbered 4.3 can be expressed with the Taylor series as follows [44]:

$$\frac{Y}{Y_0} = \frac{ik^2}{\pi k_c \ln\frac{b}{a}} \int_a^b \int_a^b \int_0^\pi [\frac{cos\phi'}{r} - \text{ikcos}\,\phi' - \frac{k^2 r}{2} cos\phi' + i\frac{k^3 r^2}{6} cos\phi']d\phi' d\rho' d\rho \tag{4.6}$$

The relationship between characteristic admittance Y_0, and input admittance, Y is used and expressed as:

$$Y_0 = \frac{2\pi}{\sqrt{\frac{\mu_0}{\varepsilon_c \varepsilon_0}} \ln(\frac{b}{a})} \tag{4.7}$$

$$Y = i\frac{2w\varepsilon^*}{[\ln(\frac{b}{a})]^2}\left[I_1 - \frac{k^2 I_3}{2}\right] + \frac{k^3 \pi w \varepsilon^*}{12}[\frac{b^2 - a^2}{\ln(\frac{b}{a})}]^2 \tag{4.8}$$

where

$$I_1 = \int_a^b \int_a^b \int_0^\pi \frac{cos\phi'}{r}\, d\phi' d\rho' d\rho \tag{4.9}$$

and

$$I_3 = \int_a^b \int_a^b \int_0^\pi cos\,\phi' d\phi' d\rho' d\rho \tag{4.10}$$

As shown in the equations above, I_1 and I_3 depend on only the physical specifications of the probe. In addition, input admittance can be expressed as:

$$Y = i\frac{2w\varepsilon^*}{[\ln(\frac{b}{a})]^2}[I_1 - \frac{k^2 I_3}{2}] \tag{4.11}$$

$$\frac{Y}{Y_0} = \frac{1+\Gamma}{1-\Gamma} \tag{4.12}$$

Reflection coefficient (Γ) can be calculated with this formula:

$$\Gamma = \frac{\rho - S_{11}}{\rho S_{22} + S_{12}S_{21} - S_{11}S_{22}} \tag{4.13}$$

Consequently, by using S-parameters obtained from network analyzer, the admittance of the probe can be calculated. As I_1 and I_3 can be calculated with using equations 4.10 and 4.11, permittivity ($\boldsymbol{\varepsilon}^*$) can be inferred from the equation 4.12.

4.4 Data Analysis

The values ε' and ε'' of cell pellet, cell suspension and medium were fitted to Cole-Cole model. These values were placed in the Cole-Cole equation:

$$\boldsymbol{\varepsilon}^*(\boldsymbol{w}) = \boldsymbol{\varepsilon}_\infty + \frac{\boldsymbol{\varepsilon}_s - \boldsymbol{\varepsilon}_\infty}{1 + (\boldsymbol{i w \tau})^{(1-\boldsymbol{\alpha})}} \tag{4.14}$$

A particle swarm optimization (PSO) algorithm is used to estimate the Cole-Cole parameters for SaOS-2 cells, hFOB cells and growth medium. Therefore, ε_∞, ε_s, τ, α, and σ values were determined for each measurement.

Analysis of variance (ANOVA) and t-test were used to analyze the measured data. The results were presented in Chapter 5.

4.5 Measurement Uncertainty

The parameter that determines of a measurement quality is accuracy. Accuracy should be supported with uncertainty analysis. Uncertainty provides a probability value between the measured and true values [45]. The difference between these two values is resulted from errors. Error can be classified into two groups: random and systematic errors. Systematic errors can be originated from source or load match, directivity, tracking the reflection and transmission frequency or isolation; while random errors are generally caused from test cable stability, noise, drift, or connector repeatability [46].

5. RESULTS

The dielectric properties of osteosarcoma cell line (SaOS), osteoblast cell line (hFOB), and growth media were measured. The data obtained from the network analyzer was compared with each other and the literature.

Firstly, median of permittivity and conductivity values of each ten measurements was taken.

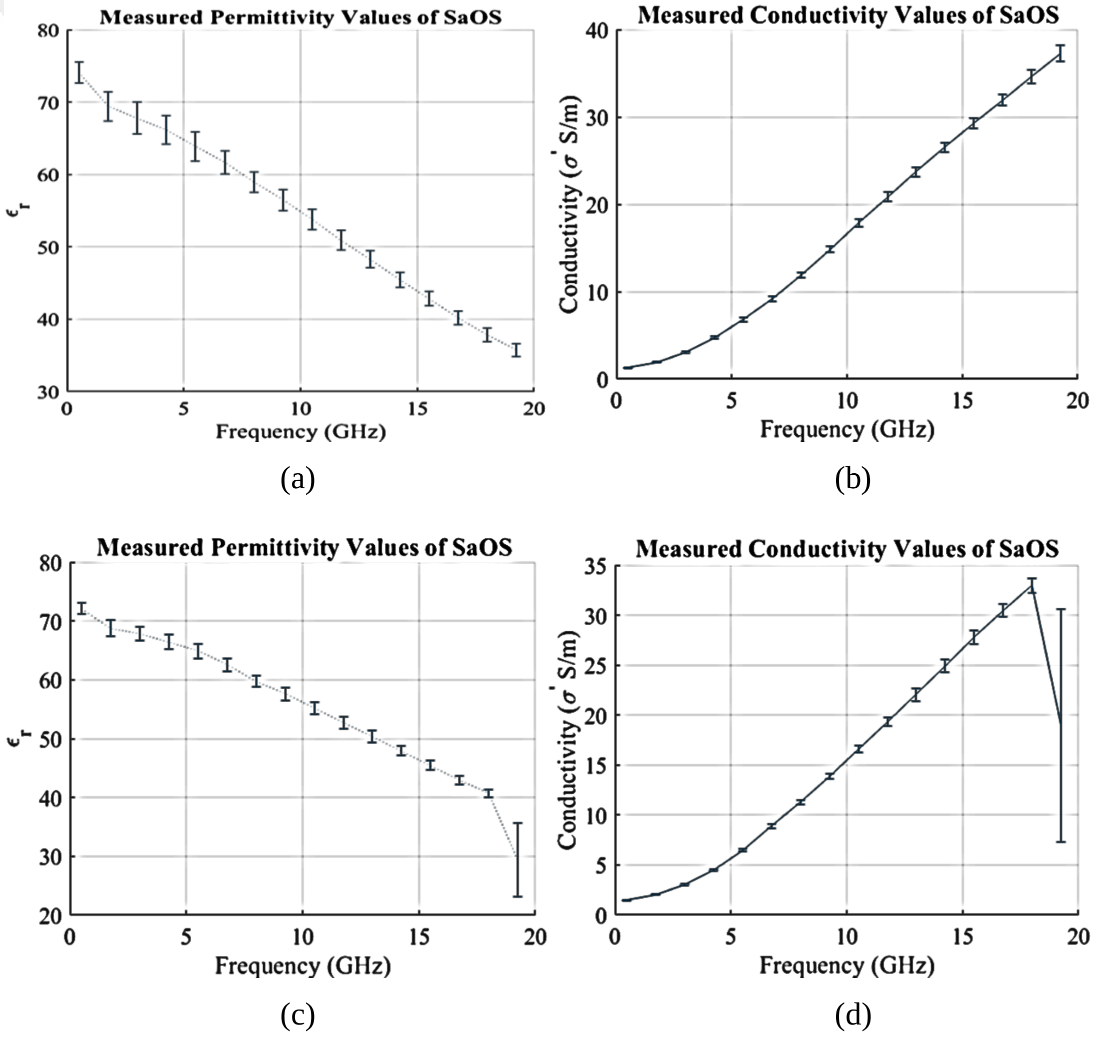


Figure 5.1: Measurement results of SaOS cell pellet (12500000 cells/ml) in the first experiment: (a) Permittivity (ε_r) of SaOS with 0.5 mm probe, (b) Conductivity (σ) of SaOS with 0.5 mm probe, (c) Permittivity of SaOS with 2.2 mm probe, (d) Conductivity of SaOS with 2.2 mm probe.

5.1 Results of the First Experiment (SaOS)

The number of cells was 12500000 cells/ml in the first experiment.

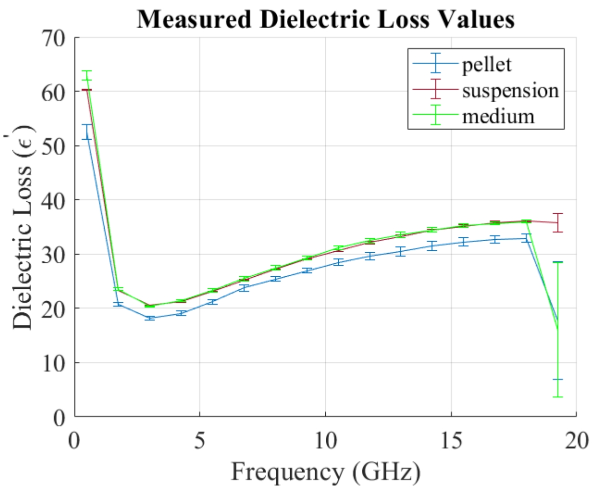
Median values of the cell pellet in each probe (0.5 mm and 2.2 mm) were shown in the Figure 5.1. Standard deviations were shown in the figure with error bars. It is seen that standard deviations were low, so that the measurements are consistent. Also, there is a sharp drop around 18 GHz in the graphs of 2.2 mm probe. It was resulted from the probe's features. Therefore, in the analysis part, we will include the data only up to the frequency of 18 GHz.

Figure 5.2 shows the comparisons of 2.2 mm probe measurements of permittivity, conductivity and dielectric constant results of SaOS cell pellet, cell suspension and medium without cell. According to the all graphs, a clear distinction is obviously seen between SaOS cell pellet and suspension and medium. Cell suspension and medium results nearly overlap in all the three graphs. It can be said that the medium inside the cell suspension affects the dielectric properties of cells and therefore it can be suggested that measuring the cell pellet gives more accurate result regarding the dielectric properties of cell particles.

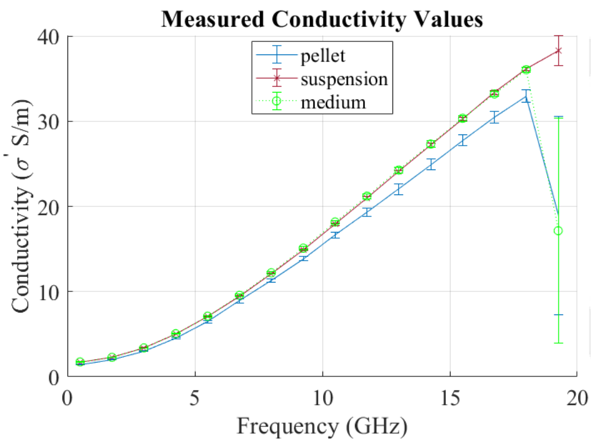
For the results of the first experiment, Cole-Cole fitting was carried out to the dielectric measurement results of the cell pellet, suspension and medium. Cole-Cole parameters (ε_∞, ε_s, τ, α, σ, and error) for the first experiment was shown in the Table 5.1. While $\Delta\varepsilon$ for SaOS pellet measured with 0.5 mm probe is 63.46, SaOS suspension measured with 0.5 mm probe is 71.26. There is a distinct difference as seen in graphs. The same result is seen also in the measurements performed with the 2.2 mm probe. There is also 5.88 difference between the two form of cells. In addition, the slope values of the lines are considerable close to each other. This means they are very similar biological substances but the main parameter that determines the difference is permittivity ($\Delta\varepsilon$) values. Their α and σ values are exactly same.

5.2 Results of the Second Experiment (SaOS and hFOB)

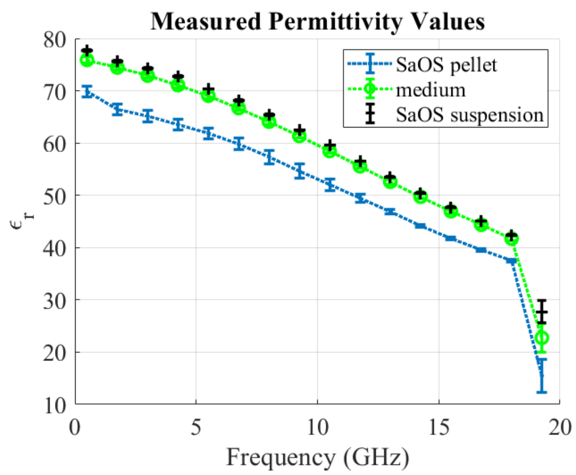
Second experiment was carried out on bone cancer cell line (SaOS) and healthy bone cell line (hFOB) to compare their dielectric properties. The experiment was performed with 0.5 mm and 2.2 mm probes. The number of cells were 22500000 cells/ml and 17700000 cells/ml for SaOS and hFOB respectively.



(a)



(b)



(c)

Figure 5.2: Comparisons of results of SaOS cell pellet (12500000 cells/ml), suspension and medium measured with 2.2 mm probe in the first experiment: (a) Dielectric loss (ε'') of SaOS pellet, suspension and medium with 2.2 mm probe, (b) Conductivity (σ) results of SaOS pellet, suspension and medium with 2.2 mm probe, (c) Relative permittivity (ε_r) of SaOS pellet, suspension and medium with 2.2 mm probe.

The aims of the experiment were to determine the most appropriate probe for a cell-based experiment; to examine the effect of number of cells to the dielectric properties by comparison with the results of the first experiment; to figure out whether a difference exists between cell pellet and suspension; and most importantly to compare the cancerous and non-cancerous cell lines regarding their dielectric properties.

5.2.1. Comparison of probes

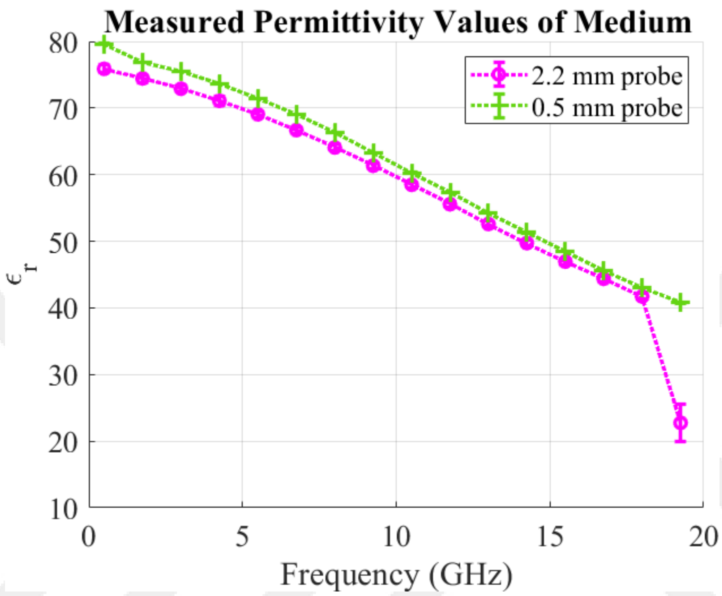


Figure 5.3: Permittivity values of medium without cells measured with 0.5, and 2.2 mm probes.

In this study, two different probes which their outer diameters are 0.5 and 2.2 mm, have been used. All experiments have been performed with two repeats with these probes to reveal which probe is more convenient for such experiment. The width and depth of the sample should be at least two times bigger than outer diameter of the probe, which is called as sensing depth. We have taken into consideration the sample volume and radius of the falcon tube to be greater than the sensing depth of the probe. Otherwise, it is inevitable to measure also the environment within the distance of the sensing depth by the probe. In parallel to this, the probe, which its outer diameter is 0.5 mm, have provided more accurate and precise data compared to 2.2 mm probe for the cell pellet (Figure 5.4 and Table 5.3). It is because of the pellets have inevitably small volume, thus the smaller probe is more appropriate for measurements. The difference between the measurements performed by two probes was decreased in measurements of suspension and medium. For the liquid samples, suspension and medium, 2.2 mm probe can be a good selection as the volume is sufficiently big.

5.2.2. Comparison of number of cells

Two experiments have been done with cancer cell line (SaOS-2). In the first experiment, samples were prepared as containing 12500000 cells/ml, and in the second experiment sample contained 22500000 cells/ml.

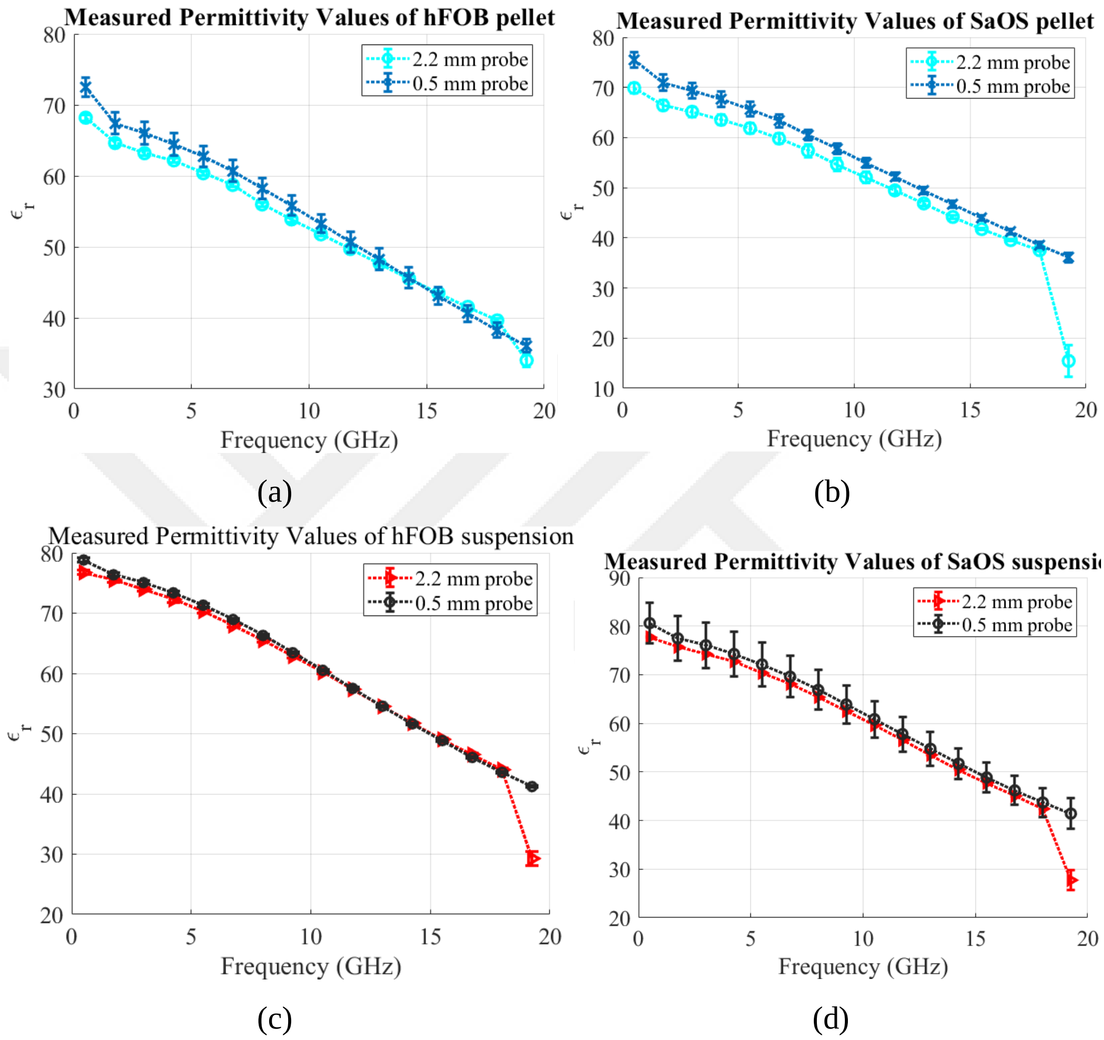
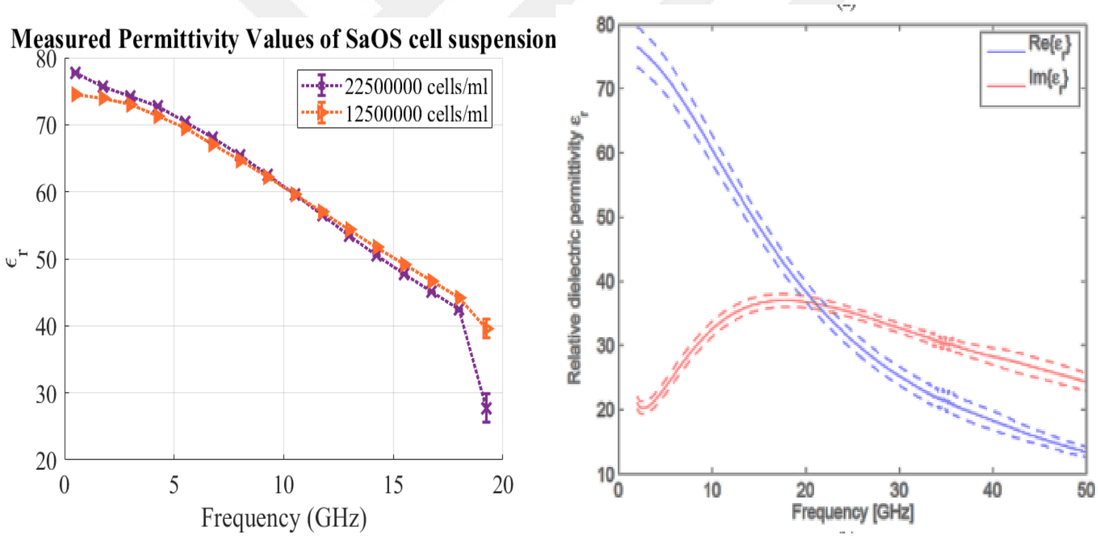


Figure 5.4: Comparison of probe to the results of dielectric permittivity. (a) permittivity values of hFOB pellet measured with 0.5 and 2.2 mm probes, (b) permittivity values of SaOS pellet measured with 0.5 and 2.2 mm probes, (c) permittivity values of hFOB suspension measured with 0.5 and 2.2 mm probes, (d) permittivity values of SaOS suspension measured with 0.5 and 2.2 mm probes.

The aim was to compare the understand the effect of the number of cells in the solution to the dielectric properties. We compared the obtained results with the data in the literature. As seen in Figure 5.8, the larger volume of sample exhibited higher dielectric values. When it is compared to the published data in [20], the results of the larger volume (22500000 cells/ml) are also consistent with the literature. The more volume of the sample increases, the more accuracy of the results increased.

5.2.3. Comparison between cell pellet and cell suspension

Cells were prepared in two forms; cell suspension and cell pellet. Cell pellet was obtained after centrifugation process, and first measurements were performed on cell pellet. After it is complete, 3 ml of medium was added to cell pellet and obtained a cell suspension. In the literature, many studies were carried out on cell suspension, while studies done on cell pellet is rare. We also measured the dielectric properties of medium to compare with the cell suspension since it also contains medium. As we can clearly see in Figure 5.5, pellet results were obviously distinguished from both suspension and medium. In addition, dielectric properties of cell suspensions are quite close to the dielectric properties of medium. It indicates that medium and suspension do not reveal distinguishable results, therefore we can say that to measure cell suspension may not give reliable results as it also includes medium. However, cell pellet provided noticeable results and it is a cue for measuring only cell particles.



(a) **(b)**

Figure 5.5: Effect of number of cells to the permittivity values. (a) comparison of permittivity of SaOS cell pellet including 12500000 cells/ml and 22500000 cells/ml measured with 2.2 mm probe, (b) SaOS-2 2700000cells /ml suspension arithmetic means of the measurements results published in [20].

5.2.4. Comparison between cancerous and non-cancerous bone cell lines

The main aim of the study was to compare dielectric properties of normal and cancer bone cells. We measured their real and imaginary parts of relative permittivity values (ε', ε''). Figure 5.7 and Figure 5.8 illustrates permittivity and conductivity values of

SaOS (cancer cell line) and hFOB (normal cell line) results measured with 0.5 and 2.2 mm probes respectively. For comparison, we used only pellet measurements data as we previously decided to cell pellet gives more accurate results respecting to cell suspension.

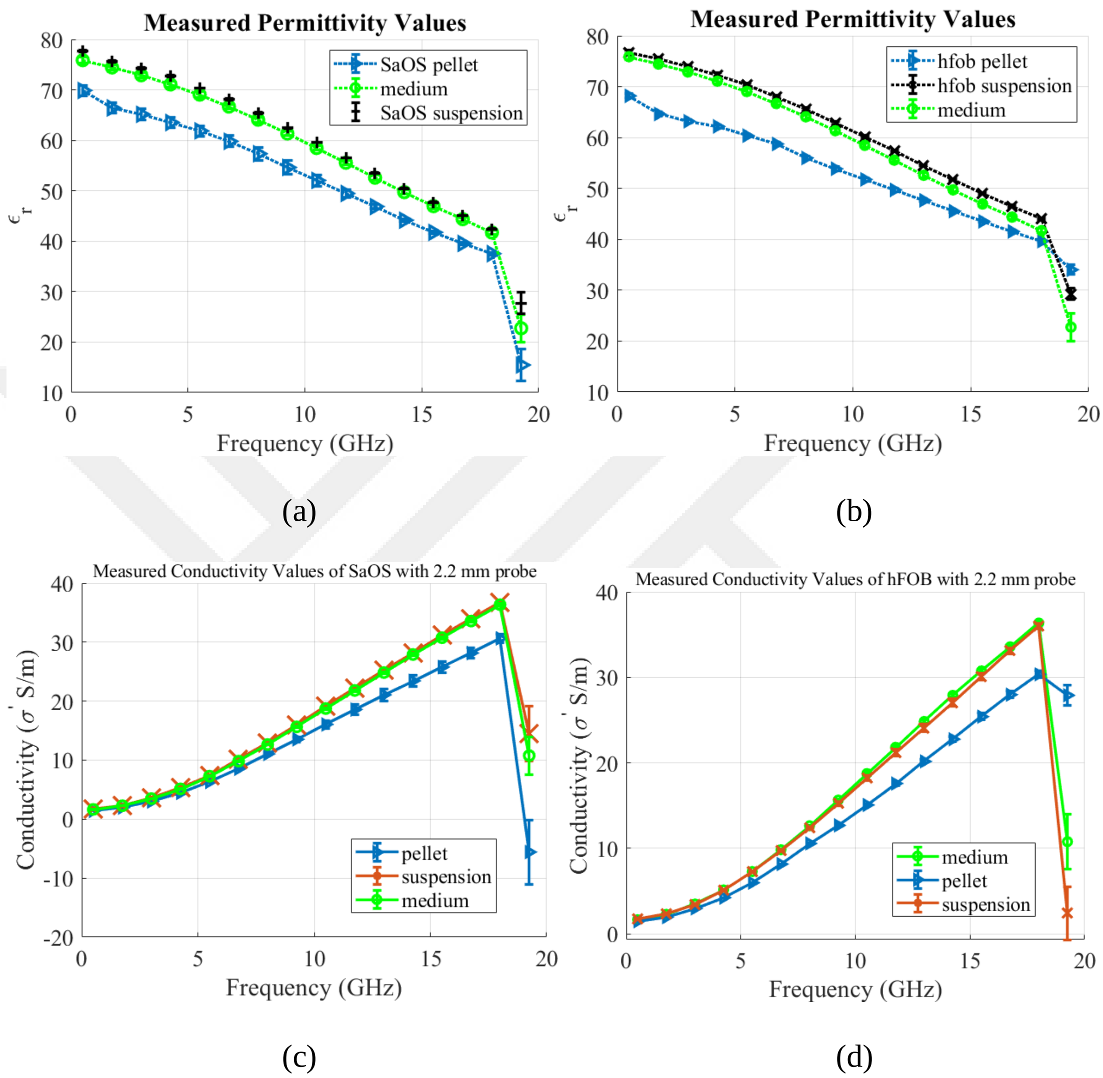


Figure 5.6: (a) Permittivity comparisons of SaOS pellet, suspension and medium measured with 2.2 mm probe, (b) Permittivity comparisons of hFOB pellet, suspension and medium measured with 2.2 mm probe, (c) Conductivity comparisons of SaOS pellet, suspension and medium measured with 2.2 mm probe, (d) Conductivity comparisons of hFOB pellet, suspension and medium measured with 2.2 mm probe.

As seen in Figure 5.7, Figure 5.8 and Table 5.1, dielectric properties of bone cancer cells are higher than normal bone cells. In tissues, cancerous tissues have larger dielectric properties than the normal tissues as cancerous tissues have more water content. As so in tissues, it is expected to see larger dielectric properties in cancer cells because of the water content. Likewise, SaOS cells exhibited larger dielectric properties than the hFOB cells. To interpret the measured difference is statistically significant, ANOVA tests and t-tests have been applied to the measured data. In the section 5.2.3, ANOVA and t-tests results are presented. According to statistical analysis, the obtained difference is statistically significant. Therefore, it can be said that the results are successful, and the techniques used in this study can be followed to distinguish normal and cancer cells.

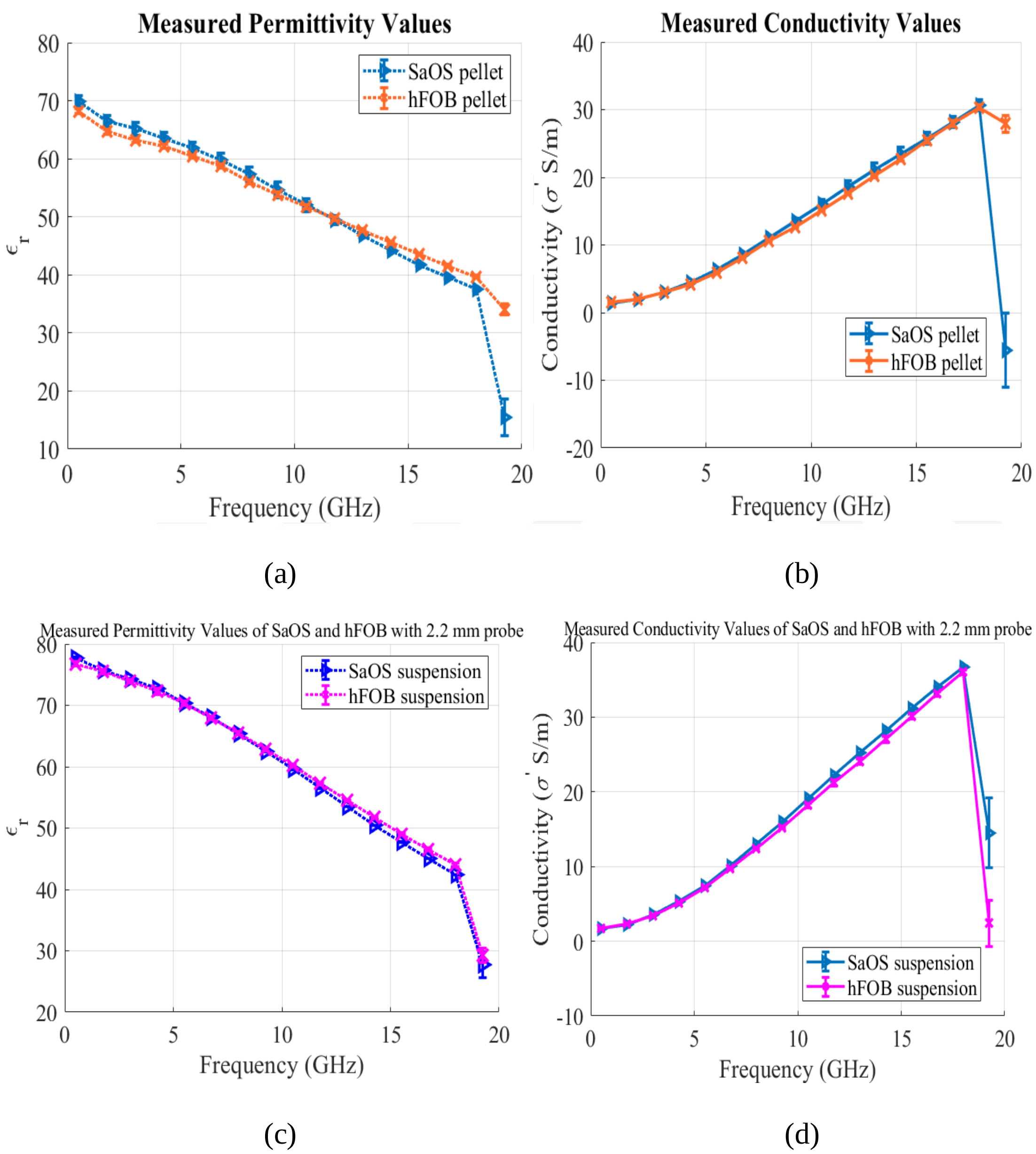


Figure 5.7: (a) Permittivity and conductivity results of cancerous (SaOS) and healthy (hFOB) cell pellet measured with 2.2 mm probe, (a) permittivity of SaOS vs hFOB cell pellet (b) conductivity of SaOS vs hFOB cell pellet (c) permittivity of SaOS vs hFOB cell suspension, (d) conductivity of SaOS vs. hFOB cell.

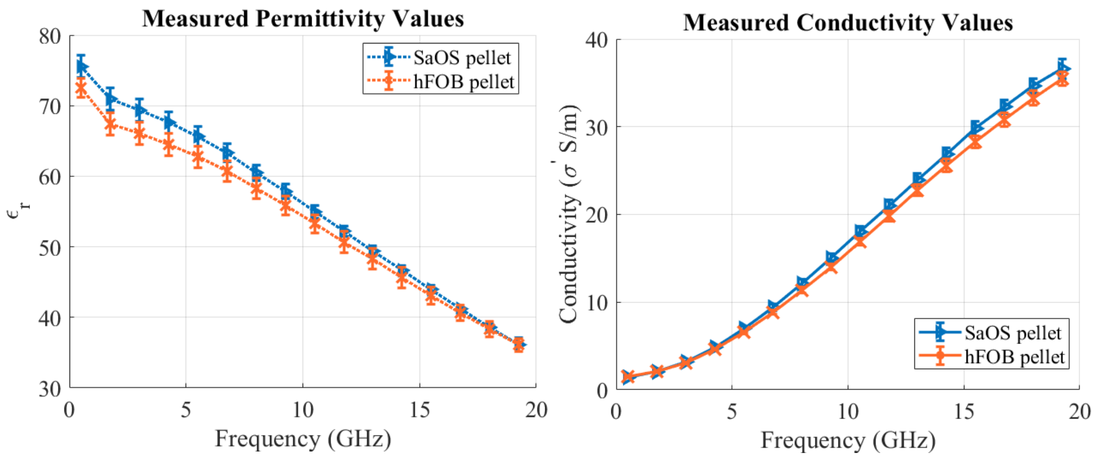


Figure 5.8: Permittivity and conductivity results of cancerous (SaOS) and healthy (hFOB) bone cell lines measured with 0.5 mm probe.

Table 5.1: Medians dielectric property measurements of cancer (SaOS-2) and normal (hFOB 1-19) cell lines with 0.5 and 2.2 mm probes.

	Cell Pellet							
	0.5 mm probe				2.2 mm probe			
	SaOS-2		hFOB 1.19		SaOS-2		hFOB 1.19	
Frequency (GHz)	ε′	σ (S m^{-1})	ε′	σ (S m^{-1})	ε′	σ (S m^{-1})	ε′	σ (S m^{-1})
0,5	74,84	1,42	73,02	1,47	70,56	1,42	68,59	1,45
1	72,22	1,60	70,21	1,65	68,78	1,59	66,46	1,60
2	70,43	2,22	68,18	2,27	67,21	2,17	65,19	2,09
5	66,49	5,98	63,38	5,69	63,16	5,55	61,27	5,28
10	56,04	16,52	54,32	15,76	53,98	15,56	52,73	14,11
15	45,12	28,19	44,32	27,29	42,87	26,19	44,43	24,43
20	35,84	38,03	35,19	36,95	36,21	34,35	36,69	34,11
	Cell Suspension							
	0.5 mm probe				2.2 mm probe			
	SaOS-2		hFOB 1.19		SaOS-2		hFOB 1.19	
Frequency (GHz)	ε′	σ (S m^{-1})	ε′	σ (S m^{-1})	ε′	σ (S m^{-1})	ε′	σ (S m^{-1})
0,5	76,82	1,49	78,79	1,67	77,64	1,68	77,13	1,71
1	74,82	1,67	77,83	1,84	75,99	1,84	76,56	1,87
2	73,12	2,29	76,20	2,50	75,99	2,47	75,21	2,50
5	69,03	6,19	72,17	6,46	71,22	6,53	71,51	6,39
10	47,19	29,31	49,90	30,28	48,84	30,19	50,07	29,80
15	37,59	39,50	39,83	40,70	39,65	40,39	41,58	40,13
20	76,82	1,49	78,79	1,67	77,64	1,68	77,13	1,71

5.3 Cole-Cole Fitting Results

After the measurements, Cole-Cole equation was fitted to the obtained data. Five Cole-Cole parameters; relaxation time constant **α**, relaxation time for a dispersion region **τ**, ionic conductivity **σi**, relative dielectric constant reduction **Δε (εs − ε∞)** and relative dielectric constant $\varepsilon\infty$ were obtained by using particle swarm optimization (PSO) algorithm. PSO is an heuristic algorithm and works for minimizing or maximizing the user-defined single or multiple objective functions. Thus, Cole-Cole parameters for medium, cell suspension and cell pellet of cancerous and healthy bone cell line with 0.5 and 2.2 mm probes were obtained. Table 5.2 shows the Cole-Cole parameters for the first experiment, Table 5.3 shows the Cole-Cole parameters for the second experiment.

Table 5.2: Cole-Cole fitting results of the first experiment.

	Cole-Cole parameters					
	$\varepsilon\infty$	$\Delta\varepsilon$	τ	A	σ	Error
SaOS Pellet - 0.5 mm Probe	1,09	63,46	5,75	0,01	0,01	0,071982
SaOS Suspension - 0.5 mm Probe	1,01	71,26	5,16	0,01	0,01	0,063323
SaOS Pellet - 2.2 mm Probe	1,01	63,03	5,15	0,01	0,01	0,059838
SaOS Suspension - 2.2 mm Probe	1	68,91	5,2	0,01	0,01	0,068052
Medium - 0.5 mm Probe	2,85	70,64	5,32	0,01	0,01	0,066882
Medium - 2.2 mm Probe	1,02	68,65	5,08	0,01	0,01	0,066219

Shown in Table 5.2, as seen in the previous dielectric measurements, Cole-Cole fitting results are also parallel to the previous results. Relative dielectric constant reduction ($\Delta\varepsilon$) value of pellet (64,46 and 63,03 with 0.5 mm and 2.2 mm probes, respectively) was lower and distinguishable from $\Delta\varepsilon$ value of suspension (71,26 and 68,91 with 0.5 mm and 2.2 mm probes, respectively) and medium (70,64 and 68,95 with 0.5 mm and 2.2 mm probes, respectively).

As seen in Table 5.3, while relative dielectric constant reduction ($\Delta\varepsilon$) value of SaOS pellet (65,64) measured with 0.5 mm probe was higher than hFOB pellet (62,94) measured with 0.5 mm probe as it is expected; Cole-Cole fitting results of the measurements performed with 2.2 mm probe was opposite (57,85 and 59,8 for SaOS and hFOB pellets, respectively). It can be explained by the sensing depth of 2.2 mm probe may be larger than the sample volume (0.3 ml). Suspension measurements of cancer cell (SaOS) ($\Delta\varepsilon$=67,47) was a little higher than suspension of normal cell (hFOB) ($\Delta\varepsilon$=67,17) performed with 2.2 mm probe as it is expected. It is also evidence there is a measurement issue between sample volume and sensing depth of the probe.

Slopes of the measurements of SaOS and hFOB pellet are same (τ=4,97), therefore this means the samples are highly similar, but they are different in permittivity (Δε).

Table 5.3: Cole-Cole fitting results of the second experiment.

	Cole-Cole parameters					
	ε∞	Δε	τ	α	σ	Error
SaOS Pellet - 2.2 mm Probe	1,01	57,85	4,97	0,01	0,01	0,156359
SaOS Suspension - 2.2 mm Probe	1,1	67,47	5,39	0,01	0,01	0,07163
hFOB Pellet - 2.2 mm Probe	1,01	59,8	4,97	0,01	0,01	0,058834
hFOB Suspension - 2.2 mm Probe	1,03	67,14	4,98	0,01	0,01	0,141605
Medium - 2.2 mm Probe	2,75	65,38	5,51	0,01	0,01	0,08074
SaOS Pellet - 0.5 mm Probe	1,01	65,64	5,48	0,01	0,01	0,067142
SaOS Suspension - 0.5 mm Probe	1,03	71,86	5,38	0,01	0,01	0,063693
hFOB Pellet -0.5 mm Probe	1,01	62,94	5,39	0,01	0,01	0,069697
hFOB Suspension - 0.5 mm Probe	2,26	69,84	5,44	0,01	0,01	0,065009
Medium - 0.5 mm Probe	1,01	71,1	5,47	0,01	0,01	0,063753

5.4 Statistical Analysis Performed with ANOVA and T-test

Measured dielectric properties was analyzed with using analysis of variance (ANOVA) to compare within the repeated measurement and sample-to-sample variability. ANOVA gives us these values: Sum of squares, degrees of freedom, mean squares between groups and within groups; F-statistic, p-value and F-critical value. If the p-value is greater than alpha value (α=0.05) we accept the null hypothesis (H_0) or do not reject the null hypothesis. Also, if calculated F value (F critical) is larger than the F-statistic, it is also a requirement to accept the null hypothesis. If p-value is less than alpha (0.05) or F statistic is larger than the F-critical value, both situation is a reason to reject the null hypothesis. According to these rules, we can evaluate the results as seen in Table 5.4, p-values are greater than alpha value and f-statistic valueas are smaller than the f-critical; therefore it is necessary to accept the null hypothesis. So it can be said that measured data are from same sample or they are quite similar. These comparisons seen in Table 5.4 were performed to ensure that the repeated measurements are consistent gave nearly same result in each repeat.

The second ANOVA test in Table 5.5 was performed for finding out whether there is statistically significant difference between pellet, suspension and medium. This test was also performed on the data in the first experiment. According to the results in Table 5.5, it can be inferred that there is statistically significant difference between cell pellet and cell suspension of SaOS cell line. This might be arisen from the dielectric properties of medium contained in cell suspension. Secondly, as seen in Table 5.5, p-value of the ANOVA test between SaOS suspension and medium was obtained as 1, which is greater than alpha value (0.05). This means there is not statistically significant difference between these two measurement. Thus, we can say that the dielectric properties of cell suspension and medium are quite similar which reveals no significant difference. Lastly, p-values of ANOVA test between SaOS pellet and medium exhibits quite smaller values than alpha value as seen in the test between pellet and suspension. It clearly shows that cell pellet is quite different sample from the cell growth medium.

Table 5.4: One-way ANOVA test result between the ten repeated measurements for the first experiment (only cancer cell).

			F-statistic	P-value	F-critical
SaOS Pellet	0.5 mm probe	permittivity	1,08951	0,367911	1,891867
		conductivity	0,089551	0,999766	1,891867
	2.2 mm probe	permittivity	0,554342	0,834703	1,891867
		conductivity	0,168221	0,997001	1,891867
SaOS Suspension	0.5 mm probe	permittivity	0,004623	1	1,891867
		conductivity	0,000629	1	1,891867
	2.2 mm probe	permittivity	0,021619	0,999999	1,891867
		conductivity	0,008216	1	1,891867

The other and the most significant statistical analysis was performed between the dielectric data of cancerous and normal bone cells to figure out whether there is statistically significant difference between these two type of cells. For this aim, t-test was applied into two measurement results (median of the each ten measurement for both cell line). The results in Table 5.6 demonstrates us all p-values are quite smaller than the alpha (α=0.05), therefore in all tests, permittivity and conductivity values obtained with different probes (0.5 and 2.2 mm), it is seen that cancer cells and normal cells have statistically different dielectric properties.

Table 5.5: One-way ANOVA test results performed for permittivity values of SaOS cell pellet, suspension and medium.

		F-statistic	P-value	F-critical
SaOS pellet vs SaOS suspension	0.5 mm probe	8,47232849	3,65E-23	1,593187
	2.2 mm probe	3,60380977	2,5E-07	1,593187
SaOS suspension vs medium	2.2 mm probe	0,0221595	1	1,593187
SaOS Pellet vs medium	0.5 mm probe	12,6405156	4,14E-37	1,593187
	2.2 mm probe	3,57425592	3,06E-07	1,593187

Table 5.6: T-test results performed for permittivity and conductivity values of SaOS cell pellet, suspension and hfOB pellet and hFOB suspension.

			t-statistic	P-value	t-critical
SaOS pellet vs hFOB pellet	permittivity	2.2 mm probe	18,301092	4,76E-23	1,67866
	conductivity		7,06906447	3,59E-09	1,67866
	permittivity	0.5 mm probe	30,6689788	1,43E-32	1,67866
	conductivity		8,16594144	8,43E-11	1,67866
SaOS suspension vs hFOB suspension	permittivity	2.2 mm probe	-6,8219626	8,43E-09	1,67866
	conductivity		8,26436909	6,05E-11	1,67866
	permittivity	0.5 mm probe	-109,2973	1,57E-57	1,67866
	conductivity		-15,125934	8,94E-20	1,67866

6. CONCLUSION

In this study, microwave dielectric property characterization of cancerous and healthy bone cells with open-ended coaxial probe was aimed. Dielectric property measurements was performed with using Agilent 85070E probe kit and Agilent N5230A PNA-L Network Analyzer. Two cell forms (cell suspension and cell pellet) for each cell line (SaOS-2 and hFOB 1.19) have been prepared to determine which form will give more accurate results. As the cell pellet is more concentrated form of cells and do not include cell culture medium, it is expected to get more accurate dielectric data with respect to cell suspension. After cells were seeded and grown in cell growth medium, they were collected to measure their dielectric properties.

Firstly, cell pellet was obtained using centrifuge and S-parameters were measured for the cell pellet with 0.5 mm and 2.2 mm probes. Then, medium without serum was added to cell pellet and obtained cell suspension that contains cells and medium. Secondly, cell suspension was measured with the same probes. Lastly, dielectric properties of only medium were measured to compare the data with cell suspension to understand the effect of medium to the dielectric properties of cell suspension. Each measurement was taken ten times to obtain precise results. The measurement frequency is linearly increased from 500 MHz to 20 GHz at 79 points and 250 MHz intervals. This protocol was applied for both cancerous cell line SaOS-2 and healthy cell line hFOB 1.19. The dielectric constant and dielectric loss factor of each sample were obtained at seventy-nine frequency points and then, Cole-Cole equation was fitted to the measured properties. Five Cole-Cole parameters; relaxation time constant $\boldsymbol{\alpha}$, relaxation time for a dispersion region $\boldsymbol{\tau}$, ionic conductivity $\boldsymbol{\sigma_i}$, relative dielectric constant reduction $\boldsymbol{\Delta_\varepsilon}$ ($\varepsilon_s - \varepsilon_\infty$) and relative dielectric constant $\boldsymbol{\varepsilon_\infty}$ were obtained by using particle swarm optimization (PSO) algorithm. PSO is an heuristic algorithm and works for minimizing or maximizing the user-defined single or multiple objective functions. Thus, Cole-Cole parameters for medium, cell suspension and cell pellet of cancerous and healthy bone cell line with 0.5 and 2.2 mm probes were obtained.

To ensure the measurements are statistically significant, ANOVA and t-test were applied to the obtained data. ANOVA was applied to understand whether repeated results are consistent; t-test was applied to the median of the measured ten data of the two samples to understand whether they are statistically different. For the first test, we expected to have p value to be bigger than alpha (0.05) to accept the null hypothesis. For the second test, we expected to have smaller p value than alpha to reject the null hypothesis and explain that they are different (cancer and normal cells). In the first ANOVA results, these p-values were obtained; 0,367911, 0,999766, 0,834703, 0,997001, 1, 1, 0,999999, 1 which they are all greater than the alpha value. This result indicates us the repeated measurements are consistent. Secondly, ANOVA was performed to see the difference between pellet, suspension and medium. The obtained p-values between the measured groups ($3{,}65 \times 10^{-23}$ and $2{,}5 \times 10^{-07}$ between SaOS pellet vs SaOS suspension with 0.5 mm probe and 2.2 mm probe, respectively) are quite smaller than the alpha. This also confirms that there is statistically significant difference between cell pellet and cell suspension of SaOS cell line. This is probably caused from the dielectric properties of medium contained in cell suspension. Also, according to statistical results, it can be inferred from the Table 5.5, the diectric properties of cell suspension and medium are quite similar which reveals no significant difference and cell pellet is quite different sample from both suspension and medium and represents the only dielectric properties of cell particles. Finally, t-test was performed to compare the median of the permittivity and conductivity values of cancer and normal bone cells. Obtained p-values shown in Table 5.6 are quite smaller than the alpha value (α=0.05), therefore it is clearly revealed that we obtained statistically different dielectric properties between bone cancer cells and normal bone cells.

In conclusion, the first result that can be drawn from this study according to measured data is the comparison of dielectric properties of bone cell lines revealed a significant dielectric contrast between healthy and cancer cells. Permittivity and conductivity values of bone cancer cells were higher than normal bone cells. The reason of the higher dielectric properties of cancerous cells mainly arise from its increased water content with respect to normal cells. This can contribute to the increased scattering of microwaves.

Secondly, it was inferred that cell pellet provided more accurate result rather than cell suspension. The data of cell pellet and cell suspension were compared to data in the literature, and dielectric properties of cell pellet were consistent with the literature.

Finally, the number of cells is an important parameter that affects the dielectric properties. Two sample that contains different number (12500000 cells/ml and 22500000 cells/ml SaOS) of cells were subjected to the measurements. It was observed that as the number of cells increases the accuracy of the results increased.

Cole-Cole fitting results are also consistent with the previous results. For the first experiment performed on only SaOS (cancer cell), permittivity ($\Delta\varepsilon$) value of pellet (64,46 and 63,03 with 0.5 mm and 2.2 mm probes, respectively) was lower and distinguishable from $\Delta\varepsilon$ value of suspension (71,26 and 68,91 with 0.5 mm and 2.2 mm probes, respectively) and medium (70,64 and 68,95 with 0.5 mm and 2.2 mm probes, respectively). For the second experiment performed on both cancer cell (SaOS) and normal cell (hFOB), permittivity of SaOS pellet (65,64) measured with 0.5 mm probe was higher than hFOB pellet (62,94) measured with 0.5 mm probe as it is expected; however, measurements performed with 2.2 mm probe was not expected (57,85 and 59,8 for SaOS and hFOB pellets, respectively). It can be explained by the sensing depth of 2.2 mm probe may be larger than the sample volume (0.3 ml), therefore it also shows that 0.5 mm prob is more convenient for the small size samples such as cell pellets. Suspension measurements performed with 2.2 mm probe also confirm the situation ($\Delta\varepsilon$ of SaOS=67,47; $\Delta\varepsilon$ of hFOB=67,17) as the volume of suspension was appropriate for the measurement in regard to sensing depth. It should be noted that volume of sample must be taken into consideration along with outer diameter and sensing depth of the probe.

Conseqently, the results of the study gave reliable data in all steps of the research and clearly show that dielectric properties of cell lines can be easily determined with using pellet form of cells. Also, our results indicate that type of cell (cancer or normal) can be identified with the help of dielectric properties and this information can be used in developing biomedical applications.

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