Validation of Multiple Frequency Bioimpedance Analyzer using Biological Phantom

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Abstract— Quantification of internal body composition such as fat mass and body fluid volume recently relies on expensive and complex modalities. The aim of this study is to validate the ability of a multiple frequency bioimpedance analyzer (MFBIA) to estimate the change of internal fat and fluid content in a phantom model. Biological phantom was structured using fresh cucumbers filled with mixture of different percentages of oil and saline fluid seeds being removed and. Surface impedance was measured in four frequencies using commercial and designed MFBIA. The correlation between internal composition and impedance was evaluated using linear regression model. The regression between impedance obtained in different frequencies and both for fat mass and saline fluid ranges from 0.94 and 0.99 for the commercial and the designed MFBIA respectively. The main achievement of this study is that a MFBIA can detect changes in fat mass and saline fluid contents within a biological phantom.

Keywords—Bioimpedance analysis; Body composition; Biological phantom.

I. INTRODUCTION

Bioimpedance analysis is noninvasive, low cost, portable and commonly used method for body composition estimation and evaluation of clinical conditions. The electrical properties of biological tissues such as bioimpedance have been studied since the late 18th century [1]. The electrical properties of biological tissues are classified into active and passive response based on the source of the electricity. Active response or bioelectricity is the response when the biological tissue produces electricity from cellular ionic activities, as in electromyography (EMG) signal. Passive response as bioimpedance is the reaction when biological tissues are simulated through an external electrical current source [2]. Bioimpedance or biological impedance defined as the ability of biological tissue to impede electric current [3].

Electrical impedance (Z) is the expression of obstruction to the flow of an alternating current through media. Impedance is frequency dependent and defined in resistance (R) and reactance (Xc) in Cartesian domain; and impedance magnitude (|Z|) and phase angle (φ) in polar domain as shown in Equations (1)–(3) [1].

\[
Z = R + jX_c \tag{1}
\]

\[
|Z| = \sqrt{R^2 + X_c^2} \tag{2}
\]

\[
\phi = \tan^{-1}\left(\frac{X_c}{R}\right) \tag{3}
\]

Resistance (ohm) of an object is determined by a shape, that is described as length (L) in meter and surface area (A) in meter square, and material type, that is described by resistivity (ρ), as shown in Equation (4) [1]. Reactance (Xc) of an object is resistance to voltage variation across the object, and is inversely related with signal frequency (f) in hertz, and capacitance (C) in farad, as shown in Equation (5) [1].

\[
R = \frac{\rho L}{A} \tag{4}
\]

\[
X_c = \frac{1}{2\pi f C} \tag{5}
\]

In biological systems, bioimpedance is a complex quantity composed of resistance (R) which is caused by total body water and reactance (Xc) that is caused by the capacitance of the cell membrane [2, 3].

Estimation of body composition using basic means of bioimpedance measurements is based on determination of body volume (V) in meter cube, where (R) is resistance in ohm, (L) is length in meter and (ρ) is resistivity in (ohm. Meter), as shown in Equation (6):

\[
V = \frac{\rho L^2}{R} \tag{6}
\]

Human body volume is composed of fat mass (FM) and fat free mass (FFM). FM is considered as a non-conductor volume of electric charge and is equal to the...
difference between body weight and FFM. FFM is considered as the conducting volume due to conductivity of electrolytes dissolved in body water and composed of bone minerals, body cell mass (BCM), protein and total body water (TBW) that consists of extracellular fluid (ECF) and intracellular fluid (ICF). Figure 1 explore the main components of human body.

![Image](https://via.placeholder.com/150)

Figure 1. The main components of human body.

Bioimpedance measurements obtained from human body change according to several factors such as anthropometric measurements, age, gender, ethnic group and bioimpedance analyser [2]. There are two main approach of bioimpedance analysis based on frequency used. Single-frequency bioimpedance analysis (SF-BIA) is the analysis of bioimpedance information obtained at 50 KHz electric current. SF-BIA is the earliest proposed methods for the estimation of body compartments, based on the inverse proportion between assessed impedance and TBW[2, 4]. Analysis of bioimpedance that is obtained at more than two frequencies is known as multiple frequency bioimpedance analysis (MFBIA). MF-BIA is based on the finding that the ECF and TBW can be assessed by exposing it to low and high frequency electric currents, respectively.

Thomasset [5] proposed using MFBIA to estimate ECF and TBW. Simpson et al.[6] reported that the range of MFBIA generally between 20 KHz and 50KHz. Jaffrin et al.[21] concluded that technically a MFBIA analyzer should use frequency range between 5–1000 kHz. Hamman et al.[7] stated that measured bioimpedance parameters using a frequency of less than 5 KHz and more than 200 KHz fluctuate around the real value. In general, the MFBIA method predicts ECF more precisely than the SFBIA method and estimate TBW more accurate than the bioimpedance spectroscopy method with the same predicted values of ECF for both methods[8].

Commonly most of bioimpedance analysis validation studies have being conducted using real human trials [9, 10]. Due to design and malfunction artifacts of bioimpedance analyzers, direct measurement of bioimpedance from human have high risk and considered unsafe for patients. High cost of using real human trails limit the studies of bioimpedance analysis in body composition assessment. Since the alternative solutions were needed to validate the ability of bioimpedance analysis in body composition assessments. This study introduce novel approach for validity assessment of body composition using the basic means of bioimpedance analysis. The aim of this study is to validate the ability of MFBIA to assess internal composition change in the core of a biological phantom. To simulate the human peritoneal volume, cucumber was used as biological container and filled with mixtures of corn oil and saline with various concentrations. We determine the surface impedance was using surface disposable electrodes to assess the effect on of differing internal fat and saline content.

II. METHODOLOGY

A. Materials

Cucumber with length 21 cm and circumference 6 cm was been used as biological phantom. The middle hole of the cucumbers were cylindrical in shape after seeds were taked out. The outer skin of the cucumbers were chosen to be smooth in surfaces to simplify contact with the electrodes. Each cucumber was set up to perform one experiment. All experiments were conducted in the series and replicated on a second set of cucumbers.

Pure corn oil (Mazola, Codaa Inc., Switzerland) was used to represent different level of fat mass inside the human body. Saline with concentration of 90 % sodium chloride (Pharmasafe Laboratories Sdn. Bhd, Perak Darul Ridzuan, Malaysia) was used to represent the fluid media of human body.

Ag/AgCl disposable ECG electrodes with diameter of 32 mm was used to inject the current and detect the voltage across the biological phantom. A set of four equal-length disposable electrodes was used. Two electrodes used as current electrodes and the other two used as voltage detecting electrodes. Electrodes were placed horizontally and aligned vertically as shown in figure 2. The distance between the current injecting electrodes and the voltage detecting electrodes at the same end was 0.25 of phantom length. The distance between the two source electrodes was determined by the length of the cucumber, so that the detection range covered area of the cucumber cavity.

Two bioimpedance analyzers were used in this study to verify the bioimpedance measurements from the biological phantom. Commercially available MFBIA analyzer (QuadScan 4000, Bodystat Ltd ®, Douglas, Isle of Man, UK) and locally designed MFBIA analyzer were used in this study. Both analyzers have impedance measurement sensitivity (0.1 Ω) and inject alternating current less than 1mA with frequencies 5, 50, 100 and 200 KHz. Figure 2, illustrate the schematic drawing of a cucumber phantom filled with mixture and electrodes placing.
B. Experimental setup

All cucumbers were washed with alcohol to insure the surface cleanness, bathed with water, and dried before electrode placement. The length and circumferences of all cucumber were measured using tape measure.

The length and diameter of central hole of cucumber was measured to be equal to 16 cm and 2 cm respectively. Internal volume of cucumber was calculated to be equal to 23 mL and measurement was verified graduated cylinder. All cucumbers were hold in upright position, as shown in Figure 2, and new cucumbers were used for each experiment.

MFBIA analyzers were calibrated using 680 ohm resistor before proceed with bioimpedance measurements and the results validated using pair \( t \) test. Bioimpedance measurements performed on empty phantom first to insure that the readings after filling the cucumber will reflect the internal composition.

Ten mixture samples of volume (23 mL) for each sample were prepared by mixing of oil and saline to fit the internal cavity of biological phantom. The samples arranged and distributed in sample bottles according to oil and saline percentages.

Electrodes were placed on phantom surface two electrodes for current injection and two electrodes for voltage drop measurements. Conductivity test was performed to insure the stable contact of electrodes to phantom surface. Connection pads were connected from MFBIA analyzers to electrodes to transmit the excitation current and detect the signal from the phantom to analyzer.

Biological phantom was filled by samples from containers starting from maximum saline percentage and minimum oil percentage to maximum oil and minimum saline percentages. Bioimpedance measurements were performed using commercial and designed MFBIA analyzers for each trail and each measurement performed three times in order to average the results. Figure 3 shows the experimental setup of biological phantom during bioimpedance measurements.

A simple linear regression model was performed to analyze the relationship between impedance and phantom internal contents. Student’s \( t \) test was used to determine the probability value of \( \beta \) of regression equations. Coefficients of determination \( (R^2) \) were calculated. \( P < 0.05 \) was used to determine significance of all results.

III. RESULTS AND DISCUSSIONS

The results obtained based on linear regression module show that the impedance increase gradually following the increase in oil contents in biological phantom. The regression lines of impedance measurements using commercial and designed MFBIA show close slope fraction between commercial and designed MFBIA.

Coefficient of determination \( (R^2) \) between impedance measurements at 5 KHz and oil contents in biological phantom is 0.96 and 0.95 for commercial and designed MFBIA respectively. Figure 4a (i) show the regression line between impedance measurements at 5 KHz and oil percentage in biological phantom. For the impedance measurements at 50 KHz the coefficient of determination \( (R^2) \) between obtained impedance and oil contents in biological phantom is 0.95 for both commercial and designed MFBIA. Figure 4a (ii) shows the regression line between impedance measurements at 50 KHz and oil percentage in biological phantom. Coefficient of determination \( (R^2) \) between impedance measurements at 100 KHz and oil contents in biological phantom is 0.95 for both commercial and designed MFBIA. Figure 4a (iii) show the regression line between impedance measurements at 100 KHz and oil percentage in biological phantom. For the impedance measurements at 200 KHz the coefficient of determination \( (R^2) \) between obtained impedance and oil contents in biological phantom is 0.95 and 0.99 for commercial and designed MFBIA respectively. Figure 4a (iv) show the regression line between impedance measurements at 200 KHz and oil percentage in biological phantom.

The obtained results show that there is good correlation \( (R > 0.94) \) between bioimpedance parameters and the variation in fat mass contents using the biological phantom. Obtained impedance data were fitted using the linear regression module.
Figure 4. Relation between oil percentage of mixture (total internal volume was 23 mL) and surface impedance at (a(i)) 5 KHz, (a(ii)) 50 KHz, (a(iii)) 100 KHz and (a(iv)) 200 KHz.

Figure 5. Relation between Saline percentage of mixture (total internal volume was 23 mL) and surface impedance at (b(i)) 5 KHz, (b(ii)) 50 KHz, (b(iii)) 100 KHz and (c(iv)) 200 KHz.
For the saline fluid contents, the results obtained based on linear regression module show that the impedance decrease gradually following the increase in saline fluid contents in biological phantom.

The regression lines of impedance measurements using commercial and designed MFBIA show close slope fraction between commercial and designed MFBIA.

Coefficient of determination (R^2) between impedance measurements at 5 KHz and saline fluid contents in biological phantom is 0.99 and 0.96 for commercial and designed MFBIA respectively. Figure 5b (i) show the regression line between impedance measurements at 5 KHz and saline fluid percentage in biological phantom. For the impedance measurements at 50 KHz the coefficient of determination (R^2) between obtained impedance and saline fluid contents in biological phantom is 0.95 and 0.97 for commercial and designed MFBIA respectively. Figure 5b (ii) shows the regression line between impedance measurements at 50 KHz and saline fluid percentage in biological phantom. Coefficient of determination (R^2) between impedance measurements at 100 KHz and saline fluid contents in biological phantom is 0.95 and 0.98 for commercial and designed MFBIA respectively. Figure 5b (iii) show the regression line between impedance measurements at 100 KHz and saline fluid percentage in biological phantom. For the impedance measurements at 200 KHz the coefficient of determination (R^2) between obtained impedance and saline fluid contents in biological phantom is 0.99 and 0.99 for commercial and designed MFBIA respectively. Figure 5b (iv) show the regression line between impedance measurements at 200 KHz and saline fluid percentage in biological phantom.

The obtained results show that also there is good correlation (R > 0.94) between bioimpedance parameters and the variation in saline fluid contents using the biological phantom. Obtained impedance data were fitted using the linear regression module.

The regression between impedance obtained in different frequencies and both of fat mass and saline fluid ranges from 0.94 and 0.99 for both commercial and designed MFBIA. Table 1 list the determination coefficients (R^2) for impedance measurement using multiple frequencies and both of fat and saline fluid contents in biological phantom.

Increasing demands on point of care systems in healthcare facilities, fasten the research in advance and new method for disease prognosis and monitoring of body vital status [11]. Bioimpedance analysis as body composition measurement tools is an effective point of care method for the quantitative measurement of tissues characteristic and evaluation of clinical condition.

The main finding of this study is that a MFBIA can detect changes in fat mass and saline fluid contents within a biological phantom.

The obtained results imply that the multiple frequency electrical current pass across both of external (FFM) and internal (FM and fluid) layers. Estimation of correlation between internal masses and bioimpedance using biological phantom, mimic the process using real human model.

This study explore the association between the multiple frequency bioimpedance and segmental ratio of FFM to total volume. Many studies address the utilization of MFBIA in estimation of FFM which relay on same basic concept [4, 12].

<table>
<thead>
<tr>
<th>Freq KHz</th>
<th>Oil contents</th>
<th>SEE (%)</th>
<th>Saline contents</th>
<th>SEE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient of Determination (R^2)</td>
<td>Commercial MFBIA</td>
<td>Design MFBIA</td>
<td>Commercial MFBIA</td>
</tr>
<tr>
<td>5</td>
<td>0.96</td>
<td>0.95</td>
<td>6.5</td>
<td>0.99</td>
</tr>
<tr>
<td>50</td>
<td>0.95</td>
<td>0.95</td>
<td>17</td>
<td>0.95</td>
</tr>
<tr>
<td>100</td>
<td>0.95</td>
<td>0.95</td>
<td>6.5</td>
<td>0.95</td>
</tr>
<tr>
<td>200</td>
<td>0.94</td>
<td>0.99</td>
<td>12.2</td>
<td>0.94</td>
</tr>
</tbody>
</table>

The assessment of conducting and non-conducting volume based on determination of electrical resistance of compartments referenced to volume. In biological phantom the multiple frequency current pass through fluid and phantom volume which is conducting volume, and the oil mass that considered non-conducting.

The biological phantom was been proved as valid module for MFBIA through the experiments. The measured multiple frequency bioimpedance parameters using applied phantom achieved almost similar respond in study done by Lukaski and Henry [13]. The results suggested that the total values of resistance and reactance decrease with increasing in frequency of applied current.

This is a novel study on validation of MFBIA using biological phantom. The results achieved show that there is proportional variation in bioimpedance obtained in multiple frequencies that follow the changes in internal contents. The results duplicated using different sets of cucumbers with different size shapes and two types of electrodes. The linear changes in bioimpedance parameters corresponding to changes in FM can be explained as linear change in non-conducting mass to total volume.

This study does not report that the variations of FM and body fluid using biological phantom, have same variation in human body. The aim of this study is to introduce low cost and acceptable validation method to assess fat and fluid mass using MFBIA compare to expensive and unsafe human trial method. It was not proven that the variations in phantom volume will effect on the obtained results, if so, this will help to determine the segmental fat content of human body using commonly used methods such as skinfold measurement. Estimation of body composition based on dividing the human body into five cylindrical compartments is commonly applied and validated method[14]. There is no significant difference between the determinations coefficients (R^2) calculated in this study compared to other validation study[15]. The slightly different in linear regression equation coefficients between designed and commercial MFBIA is due to electronic components artefacts for each designs. Moreover, the influence of anthropometric, gender and other predefined
data in commercial MFBIA, effect on estimated bioimpedance parameters.

The proposed biological phantom was designed to support the hypothesis that the multiple frequency bioimpedance measurements in association with volume parameters can be used to estimate the internal segmental fat and fluid contents. However, utilizing of our phantom to determine the fat and fluid contents in human, need more studies and investigations on volume and electrodes parameters on the readings. Nonetheless, this study shows encouraging outcomes to use the MFBIA in estimating the fat and fluid masses and variations over time.

IV. CONCLUSIONS AND RECOMMENDATIONS

Body composition measurement tools has been considered a promising point of care method for the quantitative measurement of tissues characteristic over time, in addition to direct relativity between fluctuations in body composition equivalences and survival rate, clinical condition, illness and quality of life. Fat mass and fluid volume considered as main contributors in body compartment prediction systems. Bioimpedance analysis is low cost, noninvasive and portable method for fat mass and fluid volume estimation. Significant correlation between multiple frequency bioimpedance parameters and both of fat mass and fluid contents was validated using biological phantom. Further studies are needed to evaluate the correlations between variations in bioimpedance parameters, and different concentrations of internal fluid.

REFERENCES