Bioimpedance Measurement of Knee Injuries using Bipolar Electrode Configuration

Xuanjie Ye
University of Alberta
Lexi Wu
Zhejiang University School of Medicine
Kaining Mao
University of Alberta
Yiwei Feng
University of Alberta
Xianfeng Lin
Medical College of Zhejiang University
Jie Chen (jc65@ualberta.ca)
University of Alberta

Research Article

Keywords: bioimpedance, bipolar electrode configuration, knee injuries, machine learning

Posted Date: July 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-690831/v1

Bioimpedance Measurement of Knee Injuries using Bipolar Electrode Configuration

Xuanjie Ye[1], **Lexi Wu**[2], **Kaining Mao**[1], **Yiwei Feng**[1], **Xianfeng Lin**[3,*,+], **and Jie Chen**[1,**,+]

[1]Department of Electrical & Computer Engineering, University of Alberta, Edmonton, Alberta, Canada.
[2]Zhejiang University School of Medicine, Hangzhou, Zhejiang, People's Republic of China.
[3]Department of Orthopaedic Surgery, Sir Run Run Shaw Hospital, Medical College of Zhejiang University, Hangzhou, Zhejiang, People's Republic of China
[*]xianfeng_lin@zju.edu.cn
[**]jc65@ualberta.ca
[+]Both Jie Chen and Xianfeng Lin are the corresponding authors of this paper.

ABSTRACT

Currently, there is no suitable solution for the point-of-care of knee injuries. A potential portable and low-cost technique for accessing and monitoring knee injuries is bioimpedance measurement. This study validated the feasibility of the bipolar electrode configuration for knee bioimpedance measurements with two electrodes placed on a fixed pair of knee acupuncture locations called Xiyan. Then, the study collected 82 valid samples to investigate the relationship between bioimpedance and knee injuries, among whom 45 patients, each with one healthy knee and one injured knee, and 37 individuals all with healthy knees. The self-contrast results indicated that knee injuries caused a reduction of bioimpedance of the knee by about 5% on average, which was detectable at around 100 kHz ($p \approx 0.001$). Furthermore, the results analyzed by principal component analysis and support vector machines show that the detection sensitivity can reach 91.11% using the leave-one-out cross-validation.

bioimpedance, bipolar electrode configuration, knee injuries, machine learning

Introduction

Every year, approximately 720 out of 100,000 people are diagnosed with common knee injuries, including osteoarthritis (OA), ligament ruptures, and menisci lesions[1]. OA is the most common form of arthritis, especially prevalent among elderly people[2,3]. Considering the longer life expectancy of the general population, more OA patients are anticipated in the future[4]. In addition, ligament ruptures and menisci lesions are common injuries among those who frequently engage in vigorous physical activities[5–8]. As physical exercises have gradually become a part of daily life for most people, these knee injuries are more widespread than ever before[9].

The major obstacle to point-of-care for knee injuries is the lack of a suitable solution to provide a rapid and low-cost assessment. Currently, knee injuries are primarily examined with magnetic resonance imaging (MRI)[10]. Other regular alternative methods, including X-ray, computed tomography (CT), and ultrasound scan, are also used[11,12]. Although these imaging-based methods provide accurate information about knee conditions, expensive and large infrastructures as well as professionally trained technicians are required. Clinicians also assess knee injuries by asking patients about their physical limitations and handicap conditions in some clinical practices[13–15]. However, these examination methods are difficult to be applied to monitoring long-term rehabilitation progress or self-diagnosis in the early stage. Therefore, it is urgently needed to develop a low-cost, portable, and easy-to-use assessment technology for the point-of-care of knee injuries.

Bioimpedance measurement is such a promising technique that is non-invasive and can be embedded in low-cost portable devices. The technique measures the electrical impedance of the biological tissues in a specific frequency range. In the low-frequency range (< 1 kHz), the injected current only flows in the extracellular fluid within biological tissues because the current cannot penetrate through the cell membrane. However, the membranes are no longer obstacles to current flow when the frequency is sufficiently high (> 1 MHz)[17]. Usually, the bioimpedance measured from below 1 kHz to around 1 MHz can be used to analyze the intracellular fluid and extracellular fluid, which may reflect the electrochemical changes in biological knee tissues[18]. The bioimpedance technique has been widely applied to clinical practices, such as evaluation of muscle injury severity level[19], measurement of body composition[20,21], monitoring venous ulcers[22], obstetric anal sphincter injury diagnostics[23], and diagnosis of breast tumor[24].

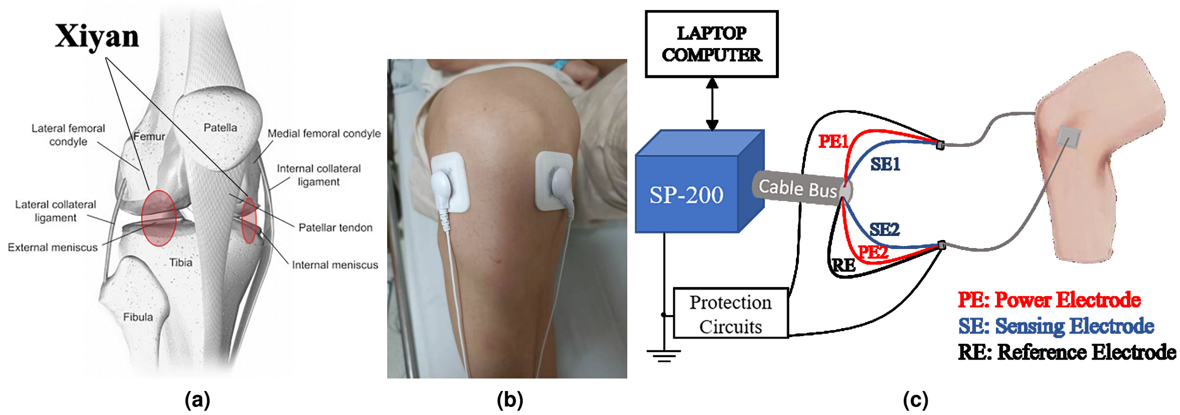


Figure 1. (a) Illustration of the positions of Xiyan (reproduced, with slight modification, from: Affatato S, editor. Surgical techniques in total knee arthroplasty and alternative procedures. Elsevier; 2014, with permission from Elsevier). (b) The placement of bipolar electrodes on Xiyan in a real test (c) Setup for the bioimpedance measurement (not to scale)

Based on the literature study, we hypothesize that the physical-chemical processes and tissue changes involved in knee injuries can induce a reduction of bioimpedance. When disorders occur in the knee joint, they are usually associated with the abnormal accumulation of fluid in or around a joint, i.e., joint effusion[25,26]. As a kind of extracellular fluid, the effusion is more conductive than other tissues in the knee[27]. When effusion accumulates and distributes throughout the knee joint, its bioimpedance decreases due to the "short-circuit" effect. Moreover, the breaks or damages of cartilage and other tissues may also decrease bioimpedance since they provide more current flow paths. Researchers have explored the relationship between bioimpedance and knee injuries. For example, Pichonnaz *et al.* measured the bioimpedance of legs after total knee arthroplasty surgery and then concluded a significant relationship between the bioimpedance and the knee odema volume[28,29]. Nevertheless, the electrode placement they adopted in the experiments would measure the bioimpedance of the legs, not localize to the knees. Hersek *et al.* developed wearable devices to access knee health based on bioimpedance measurement[30,31]. The devices measured the bioimpedance of the knees using a tetrapolar electrode configuration with a sinusoid injection current at 50 kHz, and the results showed 98.2% precision in detecting knee injuries.

This study aimed to validate the feasibility of the bioimpedance measurement with bipolar electrode configuration as a technique for detecting knee injuries. First, an experiment was conducted to evaluate the feasibility of the bipolar electrode configuration in the bioimpedance measurement. Then, 51 patients and 45 healthy individuals were recruited to collect the bioimpedance data of their knees. Finally, the bioimpedance data were analyzed to reveal the correlation of bioimpedance with knee injuries using IBM SPSS Statistics 25.0 software and machine learning methods, including principal component analysis (PCA) and support vector machine (SVM)[32,33].

Materials and Methods

Feasibility Study of Bipolar Electrode Configuration

Bipolar Electrode Configuration

In this study, two Xiyans of each knee were selected for the placement of the electrodes. Xiyan is an acupuncture point in traditional Chinese medical science, literally the "eye" of the knee. Xiyans are the two hollows formed when the knee is bent, immediately next to both media and lateral to the patellar ligament and below the patella, as shown in Fig. 1a[34]. There is little ligament or skeleton closely beneath the Xiyan, ensuring that the injection current of the bioimpedance measurement can easily traverse the knee joint cavity, where the effusion most commonly happens[35]. In addition, the knee joint cavity volume can be extended when the knee is flexed 90°, which may make the injection current more sensitive to the changes in the knee joint cavity. For the electrode configuration, bipolar electrode configuration (two electrodes) rather than tetrapolar electrode configuration (four electrodes) was chosen for the knee bioimpedance measurements primarily for its simplicity and easy implementation. Furthermore, due to the negative sensitivity zone formed between the power electrode and the sensing electrode[38], where the impedivity increment will result in lower measured impedance, the tetrapolar electrode configuration probably could not precisely reflect the difference among the bioimpedances of the knees. on the contrary, the bipolar electrode configuration does not have negative sensitivity problem.

Unfortunately, the bipolar electrode configuration is likely to be affected by external factors, including electrode-skin impedance, lead inductance, and parasitic capacitance. The major external factor is the electrode-skin impedance, which varies depending on different skin types, the electrode gel, the adhesion degree, and the thermal noise[39,40]. Moreover, the electrode-skin impedance is also affected by the temporal duration of sticking (DS), which is how long electrodes stick on the skin[41]. The lead inductance and parasitic capacitance mainly depend on the geometric configuration of the lead, probe, and electrodes, which are extremely difficult to keep identical for every test. Therefore, the impact of these external factors should be studied to evaluate the feasibility of bipolar electrode configuration.

Experiement Design

Three volunteers with healthy knees were selected to study the influence of external factors on bioimpedance measurement. During the measurement process, the knees were flexed by 90° to expand the knee joint volume, and two disposable gelled Ag/AgCl electrodes (model 2228 3M Inc., USA) were placed on the two Xiyans of each knee, as shown in Fig. 1b. The bioimpedance of both knees was measured once per day for four consecutive days. The bioimpedance data were collected from 100 Hz to 1 MHz using SP-200 (Biologic Inc., France) at 1 min, 5 min, 15 min, 30 min, and 60 min DS. A 1.5 V peak-to-peak sinusoidal wave signal was applied for less than 0.1 second at each frequency point. The effective current was below 0.1 mA, which complies with the IEC-60601-1 International Medical Alarm Standard[42]. The protection circuits limited the output voltage within the safe range for human beings. The whole setup is shown in Fig. 1c.

Bioimpedance Measurement of Knee Injuries

After confirming that the bipolar electrode configuration is potentially feasible for collecting the knee bioimpedance data, we studied the impact of knee injuries on the bioimpedance. The study was conducted in the Sir Run Run Shaw Hospital, Medical College of Zhejiang University, China. Moreover, The study protocol was defined in accordance with the ICH GCP guidelines and was approved by the Ethics Committee of Sir Run Run Shaw Hospital, Medical College of Zhejiang University (approval No.: Scientific Research 20210205-36). Written informed consent was obtained from each of the patients and the control healths before they were included in the study.

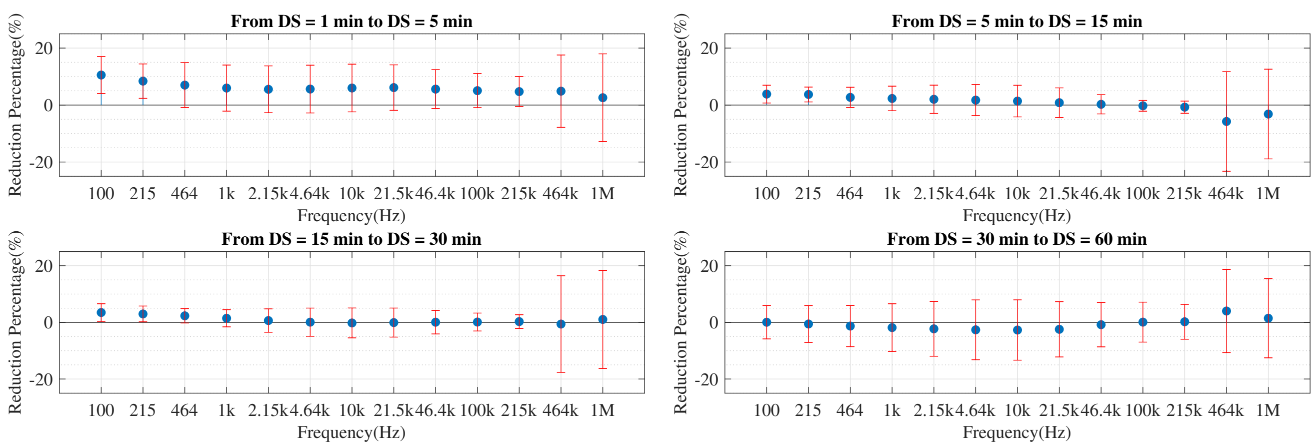


Figure 2. The averages and the standard deviations of reduction percentages of the measured bioimpedance in different DS segments over a frequency range of 100 Hz to 1 MHz

Participants

Participants were recruited from the visitors to the Sir Run Run Shaw Hospital, among whom there were 45 healthy controls and 51 patients. The 51 patients each had one healthy knee and one injured knee, which was clinically diagnosed with the following knee injuries by guidelines: osteoarthritis (OA), menisci lesion, anterior cruciate ligament (ACL) injury, and posterior cruciate ligament (PCL) injury[43,44]. One noticeable common pathological feature in these injured knees is that they all had moderate to large amount of effusion. Patients would be excluded for the following situations: 1. they could not bend their knees; 2. their knees had unhealed skin traumas that interfere with electrode placement; 3. their knees had visible deformity or swelling due to injuries. The 45 healthy controls were recruited from other visitors to the hospital. Researchers confirmed that their knees were all healthy before being included in the control group.

Study Process Design

For each patient or healthy control, the knee bioimpedance measurement was conducted after their consent was obtained. The setup and process of bioimpedance measurements followed what was proposed in the feasibility study, except that the

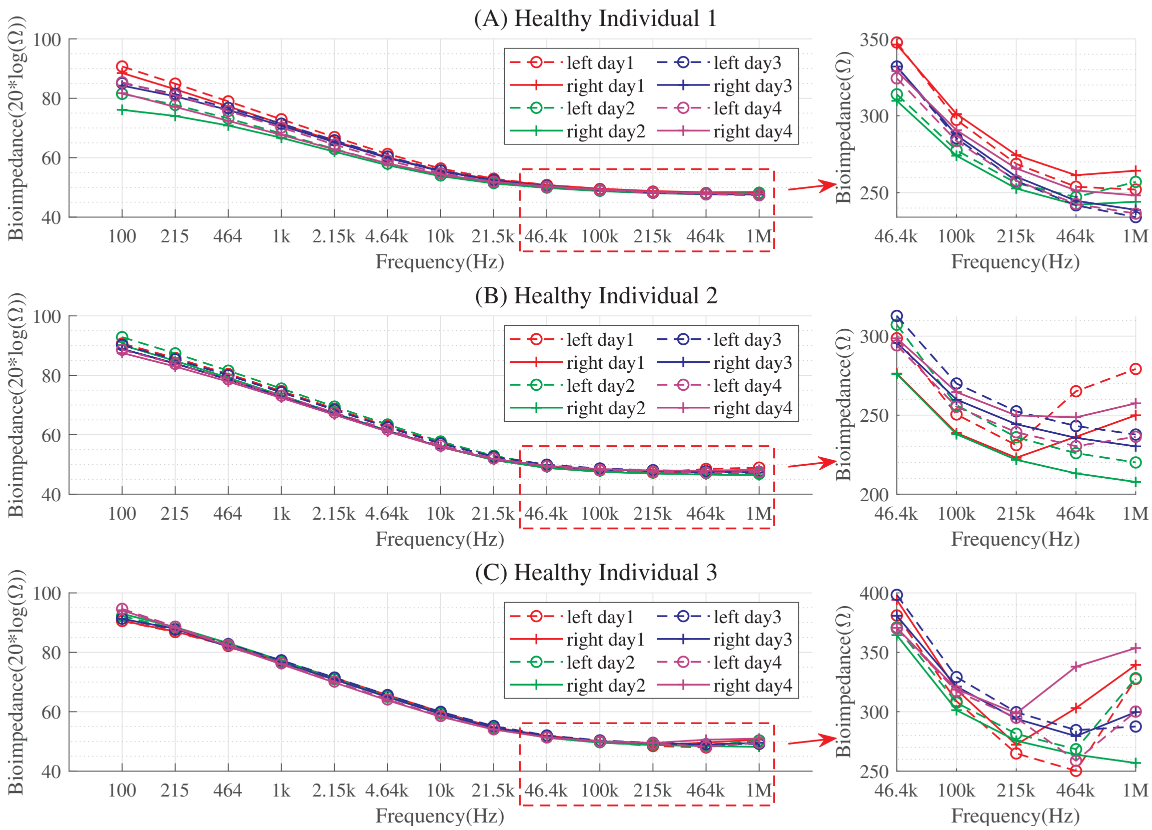


Figure 3. Bioimpedances of left and right knees of 3 healthy individuals measured at 5 min DS on four consecutive days and displayed over a frequency range of 100 Hz to 1 MHz

bioimpedance data of each knee were collected only once at 5 min DS. The reason for "5 min DS" is that the measured impedance values would be relatively stable when DS was more than 5 min, which will be illustrated in detail in the Results and Discussion sections. During the measurements, participants were required to lie on beds with their knees flexed to 90° and stayed still. None of them reported any discomfort or pain associated with the measurements.

Data Analysis Process

The Pearson Chi-square test was adopted to calculate the difference in sex ratio between the patient and control groups. The Kolmogorov-Smirnov test was used to calculate the differences in age, weight, height, and body mass index (BMI). The patient group was not further divided into subgroups by different knee symptoms because our objective is to correlate the bioimpedance with knee injuries as a whole in this study.

Because body conditions vary significantly between different individuals, comparing both knees of the same person is more meaningful than comparing the knees of different individuals. Hence, the relative differences of bioimpedance between the healthy knee and the injured knee of the same person were calculated to explore the relationship between bioimpedance and injuries. The relative bioimpedance differences were calculated using the following formulas:

1. The patient group: $\Delta_{Z_p} = \frac{Z_i - Z_h}{Z_h}$
2. The healthy control group: $\Delta_{Z_h} = \frac{Z_l - Z_r}{Z_r}$

where Δ_{Z_p} and Δ_{Z_h} are the relative differences of bioimpedance between the two knees of patients and healthy controls, and Z_i Z_h Z_l Z_r are the bioimpedances measured from injured, healthy, left, and right knee, respectively. The t-test was performed using IBM SPSS Statistics 25.0 software to compare the distributions of Δ_{Z_p} and Δ_{Z_h}. The principal component analysis (PCA) and support vector machine (SVM) algorithms were used to classify these two groups, and the classification results are evaluated using the leave-one-out cross-validation (LOOCV).

Results

Feasibility Study of the Bipolar Electrode Configuration

With the bioimpedances measured from different DS, it was found that the declining trends of the measured bioimpedance values were less significant after 5 min DS. Fig. 2 displays the reduction percentages of the measured bioimpedance in different DS segments. The reduction percentage is defined as a reduction in the bioimpedance measured at a specific DS segment. For example, the reduction percentage from 1 min DS to 5 min DS is expressed as

$$ReductionPercentage_{1\sim5min} = 100\% \times \frac{Z_{1min} - Z_{5min}}{Z_{1min}}$$

where Z_{1min} and Z_{5min} are the bioimpedances measured at 1 min and 5 min DS, respectively. From 1 min DS to 5 min DS, the measured bioimpedance would reduce about 5% to 10% on average. By contrast, the reduction percentages in the rest of the DS segments were within 5%, especially for the frequency range from 46.4 kHz to 215 kHz, whose reduction percentages were near zero with relatively small standard deviations. Thus, the results implicate that the measured bioimpedances were likely to be relatively stable when the DS reached 5 mins. In addition, the bioimpedances measured at 464 kHz and 1 MHz appeared probably irrelevant to different DS. The reduction percentages at 464 kHz and 1 MHz had notably large standard deviations ($\approx$ 20%) at all DS segments, while their averages were up and down around zero.

Similar phenomena can also be observed in the bioimpedances of the knees of 3 healthy individuals measured at 5 min DS on four days, as shown in Fig. 3. The measured eight sets of bioimpedance data of the same individual varied vastly in the frequency range below 21.5 kHz. For the healthy individual 1, the bioimpedance of the left knee measured on day 1 was about five times as the bioimpedance of the right knee measured on day 2 at 100 Hz. However, such kind of variation was much less in the mid-high frequency range. From 46.4 kHz to 215 kHz, most of the differences in the bioimpedance of two knees of the same individual measured on the same day were less than 10 Ohm. In addition, the bioimpedances in some data sets became irregular and tended to increase from 464 kHz to 1 MHz, which should only happen when the inductive reactance dominates the overall change of bioimpedance.

Bioimpedance Measurement of Knee Injuries

Anthropometric Characteristics of Study Subjects

The patient and control groups do not differ significantly in age, weight, height, body mass index (BMI), and sex ratio, as shown in Table. 1. Both groups cover the population with ages from 20 to 75 years old, and most of them are middle-aged. Additionally, the BMIs of both groups are mainly in the range of normal to slightly overweight, with no underweight or extremely obese individuals.

Table 1. Anthropometric characteristics of the patient group and the control group

	Patient Group (n=45)	Control Group (n=37)	Asymptotic Significance[a]
	Means(SD)	Means(SD)	
Age (years)	48.56(17.46)	44.24(14.62)	0.311
Height (cm)	166.43(8.88)	168.81(8.83)	0.395
Weight (kg)	64.93(11.61)	68.67(13.78)	0.384
BMI (kg/m^2)	23.44(3.94)	23.91(2.94)	0.445
Sex(male/female)	22/23	24/13	0.147

[a]Asymptotic significances of age, height, weight, and BMI were calculated using the Kolmogorov-Smirnov test; The asymptotic significance of sex was calculated using the Pearson Chi-square test.

Relative difference of bioimpedance

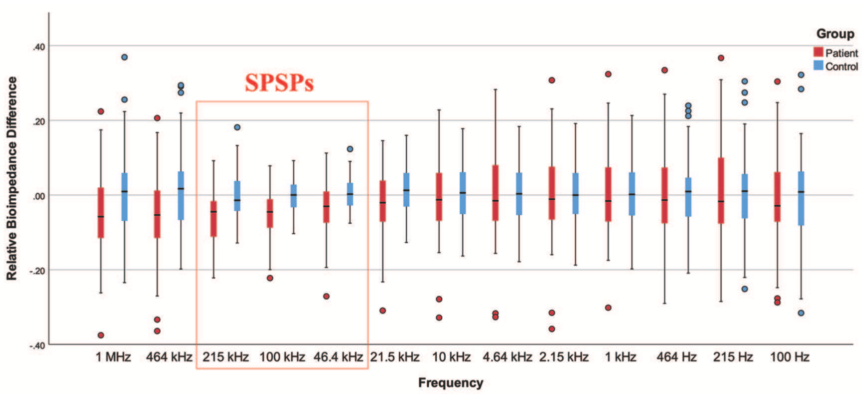
The t-test results of Δ_{Z_p} and Δ_{Z_h} at different frequencies are summarized in Table. 2. Fig. 4a shows the distribution of Δ_{Z_p} and Δ_{Z_h} versus frequency using boxplots. Samples were rejected if there was any missing data or the measured bioimpedance of one knee is more than 2 times as the other one at >400 kHz or <10 kHz, and the discussion section will explain the reason. The remaining valid samples used for the analysis consisted of 37 healthy controls and 45 patients. The most valuable results are the statistically significant differences between Δ_{Z_p} and Δ_{Z_h} at 46.4 kHz, 100 kHz, and 215 kHz, whose p-values are around 0.001.

Table 2. T-test results of the patient group Δ_{Z_p} and the control group Δ_{Z_h} at different frequencies

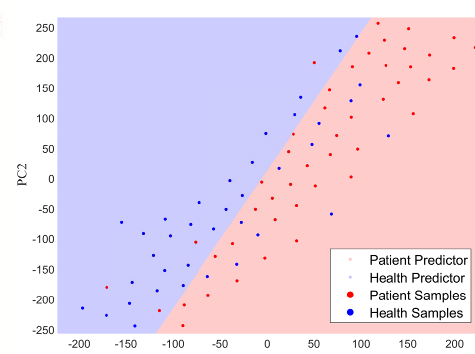
Frequency	Patient Group Δ_{Z_p}	Control Group Δ_{Z_h}	t-test for Equality of Means (Equal variances not assumed)				Test for Equality of Variances
	n=45	n=37	Sig.		95% CI		
	Means(SD)	Means(SD)	(2-tailed)	Mean Diff	Lower	Upper	Sig.
1 MHz	-6.17%(16.23%)	1.37%(13.23%)	0.0231	-7.54%	-14.01%	-1.06%	0.620
464 kHz	-4.67%(13.30%)	2.17%(12.87%)	0.0208	-6.84%	-12.62%	-1.07%	0.770
215 kHz	-5.51%(6.92%)	-0.55%(6.49%)	***0.0013***	-4.96%	-7.92%	-2.01%	0.630
100 kHz	-4.86%(6.30%)	-0.22%(4.66%)	***0.0003***	-4.64%	-7.05%	-2.22%	0.072
46.4 kHz	-3.92%(7.20%)	0.32%(4.64%)	***0.0019***	-4.24%	-6.86%	-1.61%	0.028
21.5 kHz	-2.50%(9.12%)	0.92%(6.18%)	0.0474	-3.42%	-6.80%	-0.04%	0.110
10 kHz	-1.11%(10.88%)	1.33%(7.58%)	0.2366	-2.44%	-6.51%	1.63%	0.125
4.64 kHz	-0.55%(11.91%)	1.50%(8.36%)	0.3644	-2.05%	-6.52%	2.42%	0.136
2.15 kHz	-0.16%(12.64%)	1.47%(8.91%)	0.4949	-1.64%	-6.39%	3.11%	0.135
1 kHz	0.02%(13.52%)	1.25%(9.59%)	0.6319	-1.23%	-6.32%	3.86%	0.127
464 Hz	0.25%(14.84%)	0.91%(10.69%)	0.8175	-0.65%	-6.28%	4.97%	0.133
215 Hz	0.44%(16.76%)	0.52%(12.64%)	0.9821	-0.07%	-6.54%	6.40%	0.186
100 Hz	0.54%(19.96%)	0.10%(16.29%)	0.9118	0.44%	-7.52%	8.41%	0.352

At these three frequencies, the average Δ_{Z_p} is about 5%, whereas the average of Δ_{Z_h} is nearly zero. Hence, 46.4 kHz, 100 kHz, and 215 kHz are used as the significant frequency sampling points (SFSPs) in the following discussion. On the contrary, there is no statistically significant difference between the two groups at 100 Hz to 10 kHz, where both Δ_{Z_p} and Δ_{Z_h} have means around zero. Furthermore, the statistical significance between the two groups becomes higher as the frequency increases, from 100 Hz to 100 kHz, whereas it goes lower when frequency further increases, from 100 kHz to 1 MHz. In addition, the standard deviations are relatively large at $\leq$10 kHz and $\geq$464 kHz. The tests for equality of variances indicate that the standard deviation of the patient group are probably larger than that of the control group at 46.4 kHz ($p = 0.028$).

In the machine learning process, we used Δ_{Z_p} and Δ_{Z_h} at the three SFSPs as the features. Two principal components (PC) were obtained from the 3-dimensional features using the principal component analysis (PCA); then, the support vector machine (SVM) classified the patient and healthy control groups based on the two PCs. Fig. 4b displays the visualized results of the PCA and the SVM predictor trained with all 82 valid samples. In the leave-one-out cross-validation (LOOCV), which evaluated the SVM classification results, 41 out of 45 patients and 26 out of 37 healthy controls were correctly classified. Therefore, the SVM classification results achieved a detection sensitivity of 91.11% and a specificity of 70.27%.



(a)



(b)

Figure 4. (a) Box plots of relative bioimpedance differences Δ_{Z_p} and Δ_{Z_h} at all frequency nodes. SFSP(s) is an abbreviation of significant frequency sampling point(s) (b) Principal components of the patient and control groups' SFSPs generated by PCA and the predictor generated using SVM in the 2D plane.

Discussion

Implications from the Feasibility Study of Bipolar Electrode Configuration

The influence of primary external factors that affect the measured bioimpedance can be described by a simplified four-element equivalent circuit, as shown in Fig. 5a. Increasing DS would lower R_{esp} and C_{es} by allowing the electrode gel and the skin cuticle to touch more tightly, but this effect on the bioimpedance measured at high-frequency would be weakened drastically after 5 mins DS. The significant decrease of bioimpedance over time, which eventually levels off, can be explained by closer and more stable contact between the electrode gel and the skin. At around 100 kHz, C_{es} could be treated approximately as a short-circuit, which minimizes the total effect of electrode-skin impedance. Therefore, we could observe in the results that the bioimpedances measured at around 100 kHz varied much less on different days or at different DS. However, the inductance of leads and electrode-skin cannot be neglected when the frequency reaches the MHz level[27]. This probably can explain why the measured bioimpedance sometimes was higher at 1MHz than at 464 kHz, and the bioimpedance measured at 464 kHz and 1MHz was irregular to the increasing DS. Supplement to the two-resistor model proposed by Searle and Kirup[45], this model further explains the effects of skin-electrode in the high-frequency range. The simplified models in Fig. 5b briefly illustrate how the effective components of the external factors change with frequency. This model suggests that the variations caused by the external factors can be minimized at around 100 kHz with DS greater than 5 min.

In addition, the model also implicates the impedance measured at low (< 10 kHz) and high (> 400 kHz) frequencies could indicate whether the influences of external factors are similar between measurements, which provides an approach to find and reject invalid data. In the data analysis process, the relative differences of bioimpedance between both knees of the same person were calculated to explore the relationship between bioimpedance and injuries, which is also known as self-contrast. To further enhance the significance of self-contrast, it is necessary to ensure that the influences of external factors are similar in the bioimpedance measurements of the same individual's two knees. Therefore, the samples were rejected if the measured bioimpedance of one knee is more than 2 times of the other one at low (< 10 kHz) and high (> 400 kHz) frequencies.

Detection of Knee Injuries Based on the Bioimpedance

The self-contrast results of the patient group and the healthy control group are consistent with the theoretical prediction for the influence of knee injuries on the bioimpedance. The common knee injuries, including OA, ligament ruptures, and menisci lesions, have effusion accumulation and damages of soft or hard tissues. The effusion helps bridge a conductive path for the current, and the damages of soft or hard tissues reduce their resistance[31]. These changes in the tissue level inside the knee could be represented by the differences in its electrical properties. In the data analysis results, the bioimpedance of the injured knee was usually around 5% lower than that of the healthy knee in the patient group at the significant frequency sampling points (SFSPs), where Δ_{Z_h} was nearly zero. The knees of the same person are genetically identical and grow under the same environment, the healthy knee can be regarded as a healthy duplicate of the injured knee. Therefore, the 5% bioimpedance reduction found in the Δ_{Z_p} was mainly due to knee injuries.

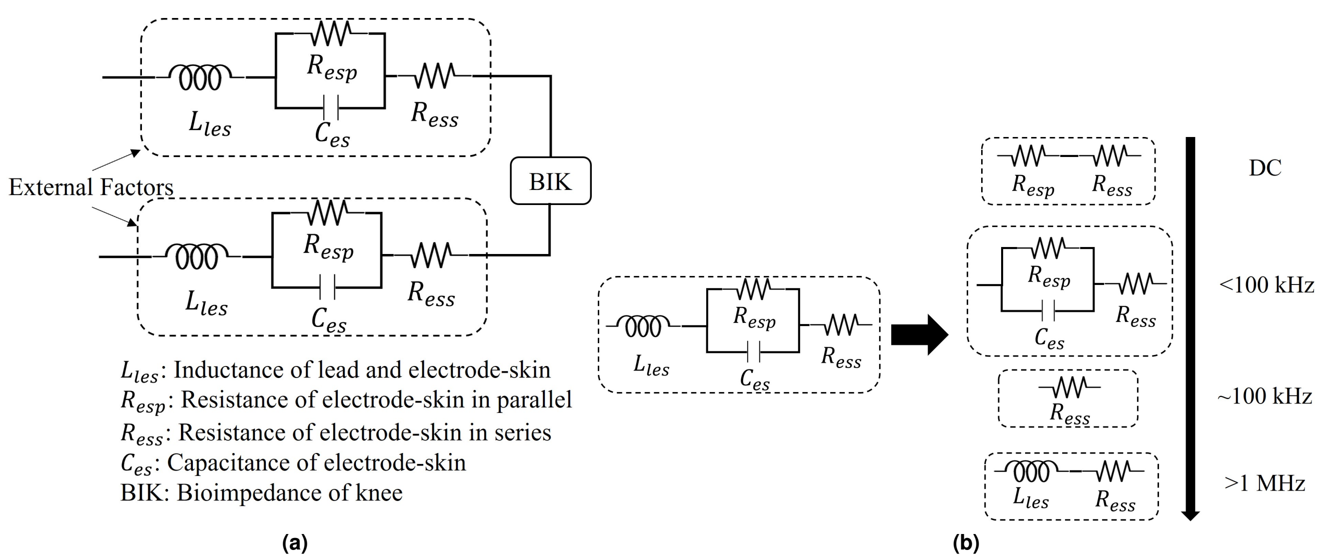


Figure 5. Equivalent circuit diagrams in the bioimpedance measurement (a) The primary equivalent circuit elements, and (b) The evolution of primary equivalent circuit elements of external factors from low (DC) to high (> 1MHz) frequency.

The results also comply with the implications obtained from the feasibility study. The bioimpedances measured at three

SFSPs presented relatively small variances, corresponding to the frequency range which minimizes the influence of external factors in the proposed model. In addition, the variance in the patient group is unlikely to equal that in the control group at 46.4 kHz. For the control group, the two healthy knees of the same individual were anatomically similar, meaning that their intrinsic bioimpedances should be almost identical. Therefore, the variance of Δ_{Z_h} resulted mainly from external factors. In contrast, the measured bioimpedances of the patient group would also be affected by internal factors, including the severity and types of the knee injuries. Consequently, the variances of Δ_{Z_p} are larger than that of Δ_{Z_h} at 46.4 kHz.

The knee injury classifier achieved a sensitivity of 91.11%, but its specificity is only 70.27%. The distributions of the two groups' PCs were distinguishable in the 2D plane, but the notable mix-up of the two clusters made it challenging to separate the two groups. The mix-up was attributed to the considerable variance resulting from external factors. The means of the Δ_{Z_p} are almost equal to the standard deviations of Δ_{Z_h} at the SFSPs. In other words, the average reduction degree of the bioimpedance due to knee injuries was similar to the measured bioimpedance error resulting from external factors. Therefore, the influence of external factors should be further minimized in the future to obtain better sensitivity and specificity.

However, considering that it is low-cost, portable, and easy-to-access, the bioimpedance measurement is still a promising technique to be implemented in the early diagnosis of knee injuries and the real-time monitoring of postoperative rehabilitation. For example,[46] developed a portable system that can complete the bioimpedance measurement from 100 Hz to 500kHz within 1 second with an error of less than 2.5%, whose total cost is only $45. In addition to the low-cost bioimpedance measurement system, many research groups also tried to develop reusable electrodes made of metal, carbonized rubber, or textile[47–50]. These reusable electrodes do not use electrolytes or adhesive, so the skin irritation problems caused by sticking electrodes can be avoided[51]. With the help of inexpensive portable bioimpedance measurement systems and reusable non-stick electrodes, the bioimpedance measurement technique will become more affordable and convenient in the future. Moreover, by developing it into a wearable device, the bioimpedance data of the knee can be dynamically collected and then sent to the physicians for further consulting.

Conclusions

This study validated the feasibility of the bipolar electrode configuration for knee bioimpedance measurements and investigated the relationship between bioimpedance and knee injuries. The feasibility study revealed that the measurement error induced by external factors could be minimized at around 100 kHz with > 5 min DS. Furthermore, the bioimpedances measured at other frequencies can indicate whether the influences of external factors are similar between measurements. The self-contrast results indicated that knee injuries could reduce bioimpedance by an average of 5%. Using the principal component analysis (PCA) and support vector machine (SVM) for the sample binary classification based on the knee bioimpedances at the significant frequency sampling points (SFSPs), the classifier achieved a sensitivity of 91.11%. In conclusion, our results indicate that the portable and inexpensive bioimpedance measurement technique is promising for detecting and monitoring knee injuries.

Acknowledgements

The scholarship support from China Scholarship Council for Xuanjie Ye is gratefully acknowledged (No. 201806320348). We would like to thank Bing Wang, Wei Zhang, Shiang Qi, and Xiaoxue Jiang for their help with this project.

Author contributions statement

X.Y., X.L and J.C. conceived the experiment(s), X.Y. and L.W. conducted the experiment(s), X.Y., K.M. and Y.F. analyzed the results. X.Y. drafted the original manuscript, and all authors reviewed the manuscript.

References

1. Peat, G., Bergknut, C., Frobell, R., Jöud, A. & Englund, M. Population-wide incidence estimates for soft tissue knee injuries presenting to healthcare in southern sweden: data from the skåne healthcare register. *Arthritis research & therapy* **16**, R162 (2014).

2. Felson, D. T. *et al.* The prevalence of knee osteoarthritis in the elderly. the framingham osteoarthritis study. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* **30**, 914–918 (1987).

3. Woolf, A. D. & Pfleger, B. Burden of major musculoskeletal conditions. *Bulletin of the world health organization* **81**, 646–656 (2003).

4. Sun, B. H., Wu, C. W. & Kalunian, K. C. New developments in osteoarthritis. *Rheumatic Disease Clinics of North America* **33**, 135–148 (2007).

5. Dehaven, K. E. Diagnosis of acute knee injuries with hemarthrosis. *The American journal of sports medicine* **8**, 9–14 (1980).

6. Margheritini, F., Rihn, J., Musahl, V., Mariani, P. P. & Harner, C. Posterior cruciate ligament injuries in the athlete. *Sports Medicine* **32**, 393–408 (2002).

7. Majewski, M., Susanne, H. & Klaus, S. Epidemiology of athletic knee injuries: A 10-year study. *The knee* **13**, 184–188 (2006).

8. Beaufils, P. & Verdonk, R. *The meniscus* (Springer Science & Business Media, 2010).

9. Hopper, M. A. & Grainger, A. J. Knee injuries. In *Essential Radiology for Sports Medicine*, 1–28 (Springer, 2010).

10. Tiderius, C. J., Olsson, L. E., Leander, P., Ekberg, O. & Dahlberg, L. Delayed gadolinium-enhanced mri of cartilage (dgemric) in early knee osteoarthritis. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* **49**, 488–492 (2003).

11. Woods, G. W., Stanley JR, R. F. & Tullos, H. S. Lateral capsular sign: x-ray clue to a significant knee instability. *The American journal of sports medicine* **7**, 27–33 (1979).

12. Friedman, L., Finlay, K. & Jurriaans, E. Ultrasound of the knee. *Skeletal radiology* **30**, 361–377 (2001).

13. Lequesne, M., Mery, C., Samson, M. & Gerard, P. Indexes of severity for osteoarthritis of the hip and knee: validation–value in comparison with other assessment tests. *Scandinavian Journal of Rheumatology* **16**, 85–89 (1987).

14. Bellamy, N., Buchanan, W. W., Goldsmith, C. H., Campbell, J. & Stitt, L. W. Validation study of womac: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *Journal of rheumatology* (1988).

15. Lysholm, J. & Gillquist, J. Evaluation of knee ligament surgery results with special emphasis on use of a scoring scale. *The American journal of sports medicine* **10**, 150–154 (1982).

16. Neves, E. B., Pino, A. V., De Almeida, R. M. V. R. & De Souza, M. N. Knee bioelectric impedance assessment in healthy/with osteoarthritis subjects. *Physiological measurement* **31**, 207 (2009).

17. Ivorra, A. Bioimpedance monitoring for physicians: an overview. *Centre Nacional de Microelectrònica Biomedical Applications Group* **11**, 17 (2003).

18. Grimnes, S. & Martinsen, O. G. *Bioimpedance and bioelectricity basics* (Academic press, 2011).

19. Nescolarde, L. *et al.* Effects of muscle injury severity on localized bioimpedance measurements. *Physiological measurement* **36**, 27 (2014).

20. Gudivaka, R., Schoeller, D., Kushner, R. & Bolt, M. Single-and multifrequency models for bioelectrical impedance analysis of body water compartments. *Journal of Applied Physiology* **87**, 1087–1096 (1999).

21. Jebb, S. A., Cole, T. J., Doman, D., Murgatroyd, P. R. & Prentice, A. M. Evaluation of the novel tanita body-fat analyser to measure body composition by comparison with a four-compartment model. *British Journal of Nutrition* **83**, 115–122 (2000).

22. Kekonen, A. *et al.* Bioimpedance method for monitoring venous ulcers: Clinical proof-of-concept study. *Biosensors and Bioelectronics* **178**, 112974 (2021). URL https://www.sciencedirect.com/science/article/pii/S0956566321000105.

23. Borycka-Kiciak, K., Młyńczak, M., Kiciak, A., Pietrzak, P. & Dziki, A. Non-invasive obstetric anal sphincter injury diagnostics using impedance spectroscopy. *Scientific reports* **9**, 7097 (2019).

24. Mahdavi, R. *et al.* Bioelectrical pathology of the breast; real-time diagnosis of malignancy by clinically calibrated impedance spectroscopy of freshly dissected tissue. *Biosensors and Bioelectronics* **165**, 112421 (2020).

25. Sutton, S. *et al.* The contribution of the synovium, synovial derived inflammatory cytokines and neuropeptides to the pathogenesis of osteoarthritis. *The veterinary journal* **179**, 10–24 (2009).

26. Pezeshki, S., Vogl, T. J., Pezeshki, M. Z., Daghighi, M. H. & Pourisa, M. Association of the type of trauma, occurrence of bone bruise, fracture and joint effusion with the injury to the menisci and ligaments in mri of knee trauma. *Muscles, ligaments and tendons journal* **6**, 161 (2016).

27. Gabriel, S., Lau, R. & Gabriel, C. The dielectric properties of biological tissues: Ii. measurements in the frequency range 10 hz to 20 ghz. *Physics in medicine & biology* **41**, 2251 (1996).

28. Pichonnaz, C., Bassin, J.-P., Currat, D., Martin, E. & Jolles, B. M. Bioimpedance for oedema evaluation after total knee arthroplasty. *Physiotherapy Research International* **18**, 140–147 (2013).

29. Pichonnaz, C., Bassin, J.-P., Lécureux, E., Currat, D. & Jolles, B. M. Bioimpedance spectroscopy for swelling evaluation following total knee arthroplasty: a validation study. *BMC Musculoskeletal Disorders* **16**, 100 (2015).

30. Hersek, S., Töreyin, H. & Inan, O. T. A robust system for longitudinal knee joint edema and blood flow assessment based on vector bioimpedance measurements. *IEEE Transactions on biomedical circuits and systems* **10**, 545–555 (2015).

31. Hersek, S. *et al.* Wearable vector electrical bioimpedance system to assess knee joint health. *IEEE Transactions on Biomedical Engineering* **64**, 2353–2360 (2016).

32. Wold, S., Esbensen, K. & Geladi, P. Principal component analysis. *Chemometrics and intelligent laboratory systems* **2**, 37–52 (1987).

33. Chang, C.-C. & Lin, C.-J. LIBSVM: A library for support vector machines. *ACM Transactions on Intelligent Systems and Technology* **2**, 27:1–27:27 (2011). Software available at http://www.csie.ntu.edu.tw/~cjlin/libsvm.

34. Deadman, P., Al-Khafaji, M. & Baker, K. *A manual of acupuncture* (Journal of Chinese Medicine Publications East Sussex, UK, 1998).

35. Fenn, S., Datir, A. & Saifuddin, A. Synovial recesses of the knee: Mr imaging review of anatomical and pathological features. *Skeletal radiology* **38**, 317–328 (2009).

36. Areny, R. P. Tetrapolar bioimpedance measurements compared to four-wire resistance measurements. *Journal of Electrical Bioimpedance* **9**, 1–2 (2018).

37. Brown, B., Wilson, A. & Bertemes-Filho, P. Bipolar and tetrapolar transfer impedance measurements from volume conductor. *Electronics Letters* **36**, 2060–2062 (2000).

38. Grimnes, S. & Martinsen, Ø. G. Sources of error in tetrapolar impedance measurements on biomaterials and other ionic conductors. *Journal of Physics D: Applied Physics* **40**, 9 (2006).

39. Fernandez, M. & Pallas-Areny, R. Ag-agcl electrode noise in high-resolution ecg measurements. *Biomedical instrumentation & technology* **34**, 125–130 (2000).

40. Chi, Y. M., Jung, T.-P. & Cauwenberghs, G. Dry-contact and noncontact biopotential electrodes: Methodological review. *IEEE reviews in biomedical engineering* **3**, 106–119 (2010).

41. Hewson, D. J., Hogrel, J.-Y., Langeron, Y. & Duchêne, J. Evolution in impedance at the electrode-skin interface of two types of surface emg electrodes during long-term recordings. *Journal of Electromyography and Kinesiology* **13**, 273–279 (2003).

42. Sidebottom, C. & Harvey Rudolph, M. Iec 60601-1–the third edition. *Journal of Medical Device Regulation-May* 9 (2006).

43. Jevsevar, D. S. *et al.* The american academy of orthopaedic surgeons evidence-based guideline on: treatment of osteoarthritis of the knee. *JBJS* **95**, 1885–1886 (2013).

44. Shea, K. G. *et al.* Management of anterior cruciate ligament injuries. *J Bone Joint Surg Am* **97**, 672–676 (2015).

45. Searle, A. & Kirkup, L. Real time impedance plots with arbitrary frequency components. *Physiological measurement* **20**, 103 (1999).

46. Jiang, Z. *et al.* Development of a portable electrochemical impedance spectroscopy system for bio-detection. *IEEE Sensors Journal* **19**, 5979–5987 (2019).

47. Meziane, N., Webster, J., Attari, M. & Nimunkar, A. Dry electrodes for electrocardiography. *Physiological measurement* **34**, R47 (2013).

48. Kusche, R., Kaufmann, S. & Ryschka, M. Dry electrodes for bioimpedance measurements—design, characterization and comparison. *Biomedical Physics & Engineering Express* **5**, 015001 (2018).

49. Colyer, S. L. & McGuigan, P. M. Textile electrodes embedded in clothing: A practical alternative to traditional surface electromyography when assessing muscle excitation during functional movements. *Journal of sports science & medicine* **17**, 101 (2018).

50. Ankhili, A., Tao, X., Cochrane, C., Coulon, D. & Koncar, V. Washable and reliable textile electrodes embedded into underwear fabric for electrocardiography (ecg) monitoring. *Materials* **11**, 256 (2018).

51. Baek, J.-Y., An, J.-H., Choi, J.-M., Park, K.-S. & Lee, S.-H. Flexible polymeric dry electrodes for the long-term monitoring of ecg. *Sensors and Actuators A: Physical* **143**, 423–429 (2008).