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Non-invasive measurement of normal skin impedance for determining the volume of the transdermally extracted interstitial fluid

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ABSTRACT

Normal skin impedance, which has a good correlation with skin permeability, can be used to calculate the volume of extracted interstitial fluid. However, it is still very difficult to determine non-invasively the normal skin impedance. In this study, a novel non-invasive method based on equipotential theory for real-time, in vivo and accurate measurements of normal skin impedance was proposed. The suggested method was based on the theory of an equipotential between the saliva and interstitial fluid of an organism, and this method was compared with the method based on an implanted electrode. The effects of humidity and pressure on the measurement accuracy of normal skin impedance were also studied. The feasibility of this method was verified by the results of the experiments. The proposed method is expected to enhance the blood glucose prediction accuracy and demonstrates a huge significance for the minimally invasive measurements of blood glucose in clinical application.

1. Introduction

Continuous glucose monitoring is highly important because diabetes has become a worldwide problem [1,2]. Additionally, the glucose concentration of interstitial fluid (ISF) is closely related to the blood glucose level. Thus, ISF is used to predict blood glucose levels because it can be transdermally extracted to continuously monitor blood glucose levels in a minimally invasive manner [3]. However, the volume of the ISF transdermally extracted for a patient in a single position fluctuates with time. And it fluctuates more widely for different positions of the same patient or for different patients. These fluctuations seriously affect the measurement accuracy of continuous glucose monitoring. Thus, it is very important to measure the volume in real time. Although the volume of the ISF transdermally extracted can be detected by a microflow meter which is integrated into a microfluidic chip [4], liquid residue remained in the microchannels makes a big influence on the measurement results. In fact, even with ultrasonic skin pretreatment, only a tiny volume of ISF (approximately 1 μL) could be obtained after 15 min vacuum extraction. But clinical application may require shorter extraction time (e.g. 5 min or less), which makes the volume of the extracted ISF much smaller. Additionally, the tiny extracted ISF scatters on the skin surface with an area of 0.785 cm^2 similar to micro-dewdrops under a microscope. Therefore, the extracted ISF is difficult to be collected and measured directly. Thus, until now it is still a challenge to accurately measure the volume of the ISF transdermally extracted in vivo.

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Modern medical research indicates that normal skin impedance is an indicator of the skin permeability coefficient [5]. The term "normal impedance" represents the skin impedance containing epidermis, dermis and subcutaneous tissues in the normal direction. The volume of ISF can be determined based on the relationship between the normal skin impedance and the skin permeability coefficient if the normal skin impedance can be accurately measured [6,7]. Therefore, the measurement of normal skin impedance is critical for continuous glucose monitoring based on transdermally extracted ISF. Several methods have been proposed to accomplish the measurement of skin impedance. Kinouchi et al. [8] proposed a fast in vivo method based on two electrodes to measure the local tissue impedance. The most significant drawback of the method is the invasion of a needle implanted subcutaneously. Martinsen et al. [9] put forward a system to detect live finger, which was based on simultaneously measuring the electrical bio-impedance of different skin layers. Ivanic et al. [10] proposed a method using a thin-film non-symmetric microelectrode array for impedance monitoring of human skin. Colbert et al. [11] proposed a single channel skin impedance system to measure the skin impedance of an acupuncture area. All of these methods lost the sight of the aeolotropy of skin, and the skin impedance achieved had no specific direction.

A novel method for measuring the normal skin impedance, in which one electrode is placed on the skin surface and the other one is kept in the mouth for complete contact with saliva, has been investigated in this paper. The proposed method is based on the theory of an equipotential between the saliva and ISF of an organism [12]. Moreover, the method can specifically obtain the normal impedance in real time non-invasively. The value of the obtained normal skin impedance can be used to calculate the volume of the ISF transdermally extracted. Animal experiments were performed to verify the feasibility of the proposed method. The effects of humidity and pressure on the accuracy of measuring the normal skin impedance were also studied in this paper.

2. The method of measuring normal skin impedance

2.1. The extraction method for interstitial fluid

The extraction method for ISF has been published, the Sonoprep was used to enhance the skin permeability [13]. The ISF was extracted by the vacuum generated from an oil-less vacuum–pressure pump (Tianjin Auto-science Instrument Ltd., China), then the variation of the normal skin impedance caused by the extraction of ISF could be detected by the measuring electrode. Thus, the volume of the extracted ISF could be determined accordingly with the impedance measurement results. In order to effectively collect the tiny volume of the transdermally extracted ISF which scatters on the skin surface, a defined volume of buffer solution was injected into the chamber before extraction. At the end of extraction, the mixture including buffer solution and the extracted ISF were collected to measure the glucose concentration using an enzyme-based biological sensing analyzer (SBA-40C, Key Laboratory of Biosensor in Shandong Province, Jinan, China). Furthermore, to miniaturize the glucose measurement system, other methods may be utilized to extract and collect ISF in the future. For example, the hollow-microneedle array used by Chua et al. [14], the interspace of which can be used as a cavity to hold buffer solution, and the measuring electrode can be coupled with the tip of the microneedle which contacts to the epidermis.

2.2. The method for measuring the volume of interstitial fluid

The transdermal permeation rate of glucose molecules is inversely proportional to the skin impedance in the normal direction [10,15]. Additionally, the volume of ISF is determined by the transdermal permeation of the skin and the extraction time. Thus, the volume of ISF can be calculated based on the acquired normal skin impedance [16].

The relationship between the volume of extracted ISF and the skin permeation rate can be expressed as [17]:

$$V = P \cdot t \cdot A \tag{2.1}$$

where V is the volume of the ISF extracted transdermally; P is the permeation rate of ISF through the skin; t is the extraction time; and A is the skin area of the ISF extraction. The following relationship between P and the skin conductivity could be demonstrated as [6]:

$$P = C\frac{\sigma}{\Delta x} \tag{2.2}$$

where C is a constant related to the size of skin pores, the permeating substance, the inherent characteristics of the electrolyte solution, and the charged ions; σ is the skin conductivity; and Δx is the thickness of the stratum corneum. Considering the following relation:

$$\frac{\sigma}{\Delta x} = \frac{G}{A} = \frac{1}{A \cdot Z} = \frac{1}{A \cdot (R + jX)} \tag{2.3}$$

where G, Z, R and X are the conductance, normal skin impedance, resistance and reactance, respectively, of the skin with an area A and thickness Δx (A is much bigger than Δx). Additionally, the reactance can be ignored as it is too small compared to the resistance when 10 Hz low-frequency signal is used in the measurement, so we can obtain the following relation from Eqs. (2.2) and (2.3):

$$P = C\frac{1}{A \cdot R} \tag{2.4}$$

Thus, the relationship between the normal skin impedance and ISF volume can be expressed as:

$$V = C \cdot \frac{1}{A \cdot R} \cdot t \cdot A = \frac{C \cdot t}{R} \tag{2.5}$$

where C is a constant related to the skin, t is the extraction time, and R is the normal skin impedance. Thus, we can determine the value of V by measuring the skin impedance as C and t are known.

2.3. The method for measuring the normal skin impedance

The most popular method to measure skin impedance directly and accurately in the normal direction is to use an electrode on the skin surface with another subcutaneously implanted electrode underneath it, as shown in Fig. 1 [17]. An AC voltage is applied to the two electrodes, and then the normal skin impedance can be obtained by measuring the current. However, the method is invasive, making it almost impossible to be applied to human body in clinic, and the method cannot be applied in the ISF extraction process because one electrode is implanted subcutaneously underneath the extraction area which may significantly affect the extraction. Thus, a novel method for measuring the normal skin impedance, in which one electrode is set on the skin surface and the other one is kept in the mouth for complete contact with saliva as a substitute for the implanted electrode, has been investigated in this paper. The proposed method avoids the invasive and can be utilized in the ISF extraction process.

The proposed method is based on the theory of an equipotential between the saliva and ISF of an organism. The saliva and ISF of an organism are connected to each other, and the gradient of bio potential between saliva and ISF was found during experiments. However, the voltage drop from saliva to ISF was much smaller than that from skin surface to subcutaneous tissue. In addition, the voltage drop from saliva to ISF was more stable. Therefore, the electric potentials of saliva and ISF are approximately equal, which means that subcutaneous tissue can be taken as an equipotential material, as shown in Fig. 1. As a result, one electrode is set on the skin surface and another oral electrode is placed in the mouth for complete contact with the saliva to measure the impedance between the two electrodes. The impedance between the oral electrode and the skin surface electrode is equal to the skin impedance at the detecting area in the normal direction. Compared with the impedance in the normal direction, the transverse impedance is extremely large, which makes the current flow in the normal direction naturally; thus, the impedance between the two electrodes is the normal skin impedance. Therefore, the proposed method can specifically obtain the normal impedance, which many other methods cannot.

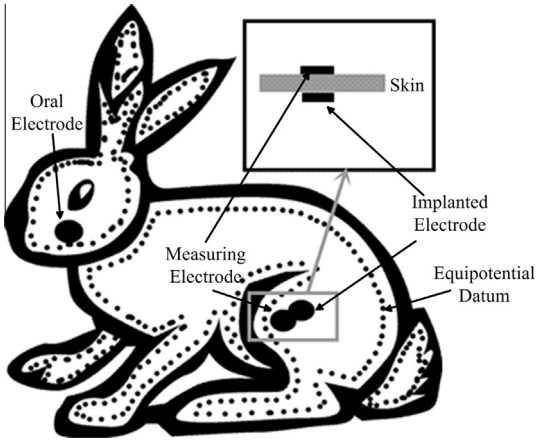

Fig. 1. Schematic diagram of the proposed method and the method based on an implanted electrode.

3. The experiments for normal skin impedance measurements

In vivo experiments were conducted on rabbits to establish the feasibility of the proposed equipotential method. Although the skin impedance measurement using a subcutaneously implanted electrode is invasive, the measurement accurately reflects the skin impedance of the testing area in the normal direction and can thus be used to evaluate the feasibility of the proposed equipotential method. Thus, comparative experiments between the proposed equipotential method and the method based on a subcutaneously implanted electrode were performed. Specifically, an oral electrode was placed in the mouth for complete contact with the saliva, a second electrode was set on the skin surface (the electrode area was 0.68 cm^2), and a third electrode was implanted subcutaneously underneath the second one. Thus, two values were measured: the impedance between the surface electrode and either the oral electrode or the subcutaneously implanted electrode. Then, the proposed method was verified by comparing the two values.

3.1. Experimental system

A schematic diagram of the experimental system is shown in Fig. 2. The system consisted of the following devices: a signal generator (AFG310, Tectronic, Ellesmere Port, England), a voltmeter (34401A6, Agilent, Santa Clara, America), a micro-current meter (6458, Keithley, Cleveland, America), an ultrasonicator (SonoprepST, Sontra Medical, Cambridge, America), and three discoid electrodes. One of the discoid electrodes was used as the oral electrode.

Two AC voltages with the same value (100 mV, 10 Hz) which was biologically compatible were applied to the two circuits. In addition, a 10 Hz low-frequency signal was chosen to remit the effect of capacitance on the skin impedance measurement.

3.2. Experimental procedure

The stratum corneum is the main obstacle in extracting ISF transdermally. Thus, a pretreatment method is necessary to enhance the skin permeability by destroying the stratum corneum, which makes it easy to transdermally extract ISF. Low-frequency ultrasound was used as a pretreatment to create micropores to increase the skin permeability to transdermally extract ISF more easily [18] in our experiments.

The experimental procedure was as follows.

(1) Anesthetize the rabbit by an ear vein injection of sodium pentobarbital (1 mL/kg).
(2) Mark the detecting area (inside the hind leg of the rabbit) as the measurement area.

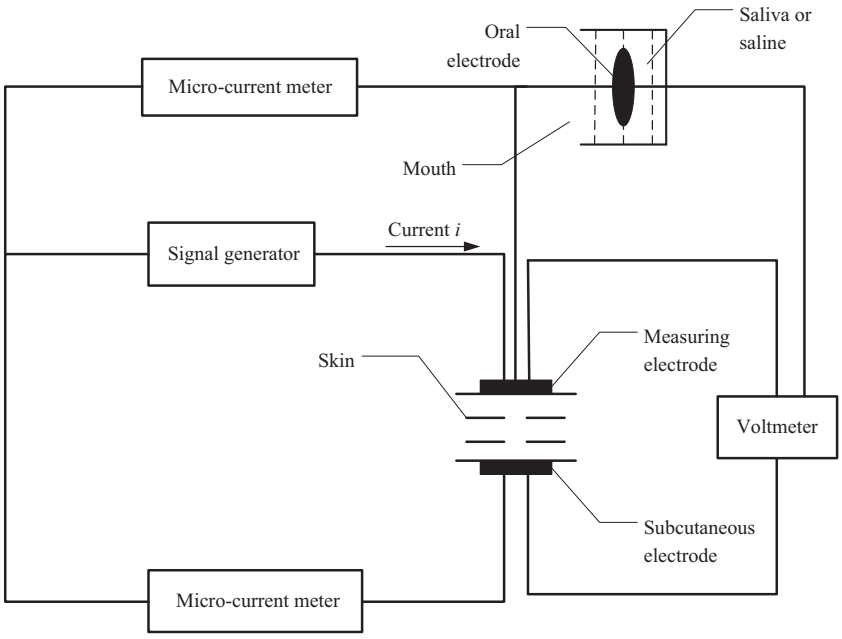

Fig. 2. Schematic diagram of the experimental system.

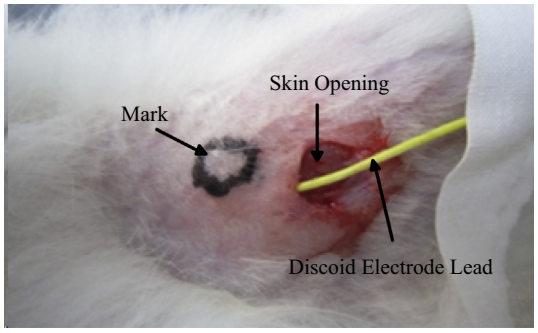

Fig. 3. Photograph of implanting electrode.

(3) Create a skin incision at a certain distance from the detecting area and implant an electrode in the detecting area underneath the skin, as shown in Fig. 3.
(4) Set another discoid electrode on the skin surface at the testing area with pressure-sensitive adhesive. Then, as shown in Fig. 4, fix the electrode by the adhesive tape. Additionally, keep the measurement conditions constant during the experiments.
(5) Afterward, place the third discoid electrode into the mouth of the rabbit for complete contact with the saliva, as shown in Fig. 5. Saline may be used to fill the mouth to ensure full contact between the oral electrode and tissue if there is not sufficient saliva.
(6) Connect the circuits when the rabbit becomes stable, turn on the power, and wait for the stabilization of the current through the body. Then, the data can be collected from the voltmeters and micro-current meter.

Fig. 4. Fixation the subcutaneously implanted electrode and the surface electrode.

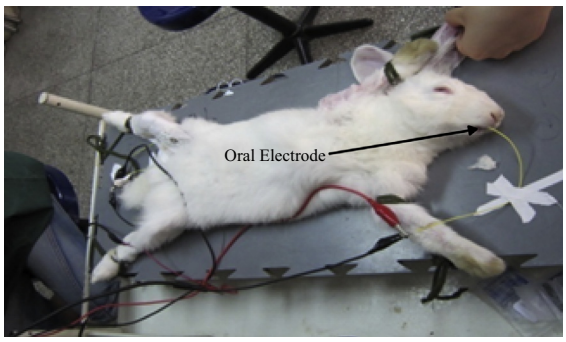

Fig. 5. Photograph of the skin impedance measurement using an oral electrode.

(7) Next, treat the skin testing area with low-frequency ultrasound. Collect a new set of data after the rabbit stabilizes (approximately 1 h after the low-frequency ultrasound treatment).

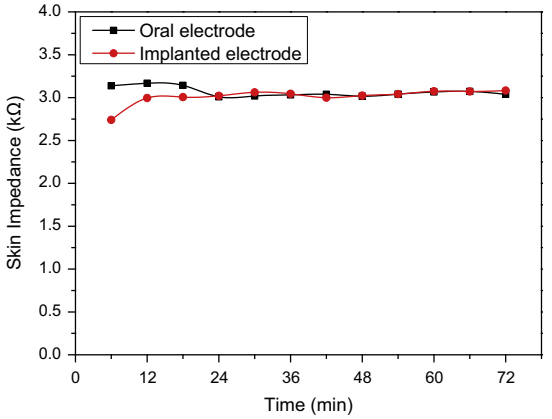

Fig. 6. The normal skin impedance before the low-frequency ultrasound treatment.

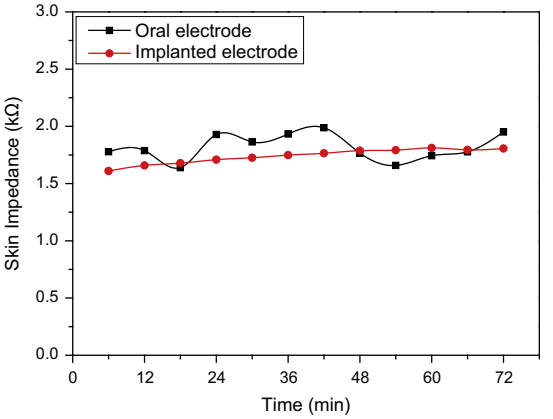

Fig. 7. The normal skin impedance after the low-frequency ultrasound treatment.

4. Results and discussion

4.1. Results of the normal skin impedance measurement of a rabbit hind leg before ultrasound

A rabbit hind leg was chosen as a measurement area that is similar to human wrists, which are frequently used for transdermal ISF extraction. The 12 measurement values of the normal skin impedance before the treatment with the low-frequency ultrasound are shown in Fig. 6. The black and red[1] curves show the normal skin impedances measured by the proposed equipotential method based on an oral electrode and the method based on an implanted electrode, respectively. The early difference in Fig. 6 may be caused by the instability of initial measurement. After that, the measured values using the two methods are both approximately 3 kΩ, which shows the good consistency of the two measurement results.

4.2. Results of the normal skin impedance measurement of a rabbit hind leg after ultrasound

The 12 measurement values of the normal skin impedance after the low-frequency ultrasound treatment are shown in Fig. 7. The data were obtained approximately 1 h after the low-frequency ultrasound treatment when physical stability had been achieved. As shown in Fig. 7, the value of the normal skin impedance acquired from the oral electrode varies slightly at approximately 1.81 kΩ, while the implanted electrode shows values of approximately 1.73 kΩ. The fluctuations of the values may be caused by the quantify effects of ultrasound on the skin. Fig. 8 also shows that the results measured with the equipotential method are consistent with those measured using the method based on a subcutaneously implanted electrode.

The values obtained by an oral electrode may be a little bigger than that from an implanted electrode in general.

[1] For interpretation of color in Fig. 6, the reader is referred to the web version of this article.

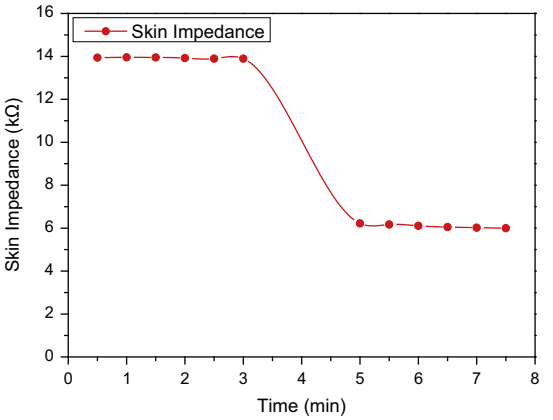

Fig. 8. Effect of humidity on the measurement result.

However, the voltage drop from saliva to ISF is much smaller than that from skin surface to subcutaneous tissue as demonstrated in part 2. The similar resistances obtained by the two methods shown in Figs. 6 and 7 confirm the feasibility of the proposed method, indicating that the oral electrode can replace the subcutaneously implanted electrode for non-invasive measurements of the normal skin impedance whether before or after low-frequency ultrasound treatment. Thus, the proposed equipotential method can be used to measure the normal skin impedance to determine the volume of ISF non-invasively during extraction. Furthermore, the proposed equipotential method can be used to measure the normal skin impedance through any other body fluids, such as tears, which are equipotential to ISF.

4.3. Factors that affect the measurement accuracy

During the experiments, the factors of humidity and pressure seriously affected the normal skin impedance measurement results.

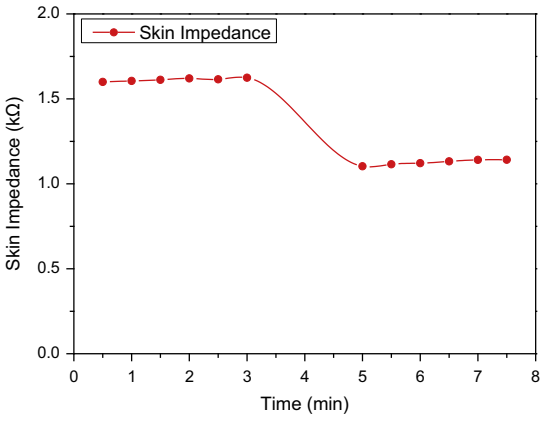

Fig. 9. Effect of pressure on the measurement result.

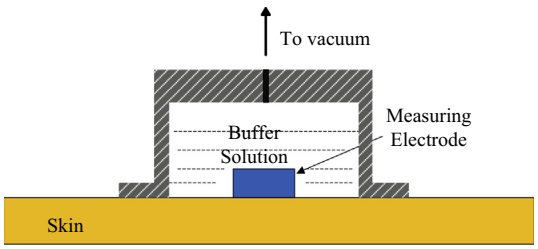

Fig. 10. Interstitial fluid transdermal extraction using a vacuum.

4.3.1. The factor of humidity

The skin impedance of the drying hind leg surface was first measured using the proposed equipotential method in a general humidity condition; then, normal saline solution was dropped onto the skin surface of the detecting area, measure the skin impedance again to determine the effect of humidity. As shown in Fig. 8, the impedance decreases significantly from approximately 13.9 kΩ to 6.2 kΩ when the skin becomes wet. Therefore, a good result may be obtained when the humidity is kept constant during the experiments.

4.3.2. The factor of pressure

To characterize the effect of pressure, the normal skin impedance was first measured in a general pressure condition; then, a constant pressure (by setting a mass on the surface electrode) was placed on the surface electrode at the detecting area, and the normal skin impedance was measured again to determine the effect of pressure. As shown in Fig. 9, the impedance decreases significantly from approximately 1.61 kΩ to 1.11 kΩ when the pressure is added. The results demonstrate the pressure between the surface electrode and the skin must be stable in the whole experiments.

The humidity and pressure were kept in constant when the proposed method was used in our glucose measurement experiments. As shown in Fig. 10, the measuring electrode is placed in buffer solution, which means it works in a liquid environment, thus the humidity can be kept in constant. On the other hand, the measuring electrode is placed on the skin surface surrounded by buffer solution in the extraction chamber, thus no force could be applied to the electrode which avoids the effect of variational pressure. However, if the proposed method is used to measure the skin impedance in other applications, how to keep the humidity and the pressure in constant should be taken into consideration as the experimental results have shown their big effects to impedance measurement.

5. Conclusions

A novel non-invasive method based on equipotential theory for real-time, in vivo and accurate measurements of normal skin impedance was proposed to determine the volume of the ISF extracted transdermally. The measurement results using the equipotential method were consistent with those using the method based on a subcutaneous electrode in animal experiments, which demonstrated the feasibility of the proposed method. Factors that affected the skin impedance measurement, such as humidity and pressure, were evaluated using experiments based on the equipotential method. The results show that the humidity and pressure should be kept constant during the experiments. The feasibility of the proposed method has been verified using rabbit experiments, and research on how the method works on humans is underway. The next work is to address the inconvenient wire-connected oral electrode to a human's mouth.

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