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In Vitro Evaluation of Finger's Hemodynamics For Vein Graft Surveillance Using Electrical Bio-Impedance Method

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ABSTRACT

Electrical bio-impedance measurement has great potential in many biomedical applications including vein graft surveillance. Studies have shown that thrombosis was the major cause of the vein graft failure. The meticulous skills of the surgeon and effective postoperative surveillance of vein graft remain the cornerstones of clinical success in the current surgical management of vein graft survival. Vascular blood flow is the key clinical indicators for the evaluation of patency of the vein graft and ensuring the patient's quality of life. In this work, electrical bio-impedance method has been proposed as an alternative to the existing surveillance method as it is non-invasive, portable, easy applicable in practice, fast response, radiation free, and required only low-cost instrumentation. It was employed to measure pulsatile changes in longitudinal bio-impedance to quantify arterial blood flow and blood volume. We expect that by measuring the changes in tissue bio-impedance which can be used to evaluate important peripheral hemodynamic, it allows the detection of early stage stenosis within vascular and vein graft as well as estimate its severity with predetermined normative data provided.

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INTRODUCTION

Peripheral arterial disease (PAD), a manifestation of atherosclerosis, is a critical healthcare dilemma worldwide since it is associated with significant morbidity and mortality (Bennett *et al.*, 2009). It is a condition where stenosis or occlusion of peripheral arterial vessels results from atherosclerotic plaque that restricts proper blood flow, eventually leading to ischemia and afterwards infarction of downstream tissues and organs.In the United States, it affects at least twelve millions Americans and the estimated prevalence could reach up to nineteen millions by 2050 (Criqui, 2001).

As the medical treatment for established vascular disorders is remaining unsatisfactory and controversial, surgical procedures to overcome deficient arteries in certain aspects are common. Surgical vascular reconstruction such as vein bypass or interposition vein grafting was performed in order to rehabilitating the patency of occluded arteries (Rahman *et al.*, 2012; Kryger *et al.*, 2007). These medical procedures consist of replacing or bypassing an occluded section of the arteries with reversed autogenous vein grafts. Although these interventions are very common, despite the initial success performed by surgeons, failure of the treatment within five years reaches up to 60% (Norgren *et al.*, 2007). Several reports divulged that approximately one-half applied vein grafts fail within 10 to 15 years after surgery, and it is associated with worse clinical upshot including higher rates of in-stent re-stenosis, chronic cardiovascular disorders event, and even mortality (Lee *et al.*, 2011).

Preceding studies has shown that thrombosis formation within the graft or near the inflow and outflow arteries was the major cause that diminishing the long-term patency of these grafts (Rahman *et al.*, 2012; Loscalzo, 2000). Thrombosis plays a pivotal role, both by predisposing to thrombus formation that lead to acute occlusion and by functioning as a stimulant for neointima formation which renders the arterialized vein graft susceptible to atherosclerosis and eventually lead to graft failure(Stary *et al.*, 1992). The meticulous skills and surgical techniques of the surgeon (Rahman *et al.*, 2012; David *et al.*, 2001; Joseph, 2011), graft properties i.e. type of graft (Conte, 1998; Zhao *et al.*, 1997), conduit orientation (Shah *et al.*, 1995), graft's length and diameter (Rahman *et al.*, 2012; Towne *et al.*, 1991; Wengerter *et al.*, 1990), and effective postoperative surveillance of vein

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graft (Moody *et al.*, 1990; Harris, 1992; Golledge *et al.*, 1996) remain the cornerstones of clinical success in the current surgical management of vein graft survival.

Early diagnose is the key to surviving from graft failure. An enduring improvement in graft patency rates of 15% can be achieved by the introduction of a systematic and effective postoperative graft surveillance (Moody et al., 1990). Unfortunately, the existing equipments or methods that used for graft surveillance such as Doppler ultrasound and angiography are not practical and cost-effective for early continuous monitoring of the implanted vein grafts (Divyesh et al., 2013).Not only with regard to the initial outlay for the particular equipment but also with regard to the employment of trained vascular technologist as well as funding for the additional interventions performed.

In this work, attempt now is on developing electrical bio-impedance spectroscopy as an alternative to the existing surveillance method as it has the advantages of non-invasive, easy applicable in practice, harmless to the human body, portable, radiation free, fast response, enable on-line monitoring and it requires only low-cost instrumentation (Tronstad et al., 2008). These are all desirable characteristics in a clinical setting.

Principle of Electrical Bio-Impedance:

The human body is an electrically conducting medium with an anisotropic conductivity distribution that determined by the electrical characteristics of various biological tissues (Grimnes and Martinsen, 2000). The electrical characteristics, especially conductivity and permittivity, of a biological tissue changes with the concentration of ions in extra and intracellular fluids, cellular structure and density, membrane characteristic, molecular composition, and many other factors (Schwan, 1957). Consequently, they reflect structural, functional and pathological status of the tissues which allows differentiating between the various tissues and the level of degeneration caused by the pathological process such as cancer and tumors development (Aberg *et al.*, 2004), lymphedema (Warren *et al.*, 2007), skin irritation (Grimnes and Martinsen, 2000), dengue (Faisal *et al.*, 2008), meningitis (Van Kreel, 2001), brain cellular oedema (Seoane *et al.*, 2005), rejection of transplanted organs (Bogonez and Riu, 2007) and grafts (Wyatt *et al.*, 1991).

Electrical bio-impedance (EBI) approach bestows a physical measurement of the small changes in tissue's electrical bio-impedance of a particular body segment by the passage of a low amplitude, high frequency, alternating current through the target segment via electrodes. The resistivity to current flow, also known as "bio-impedance" is influenced by the integrity and characteristics of the population's cell membrane, cell volume, intra and extracellular conductivity, the body's fluid and tissue composition (Baker, 1989). As a result, electrical bio-impedance of the biological tissue can be used as prognostic information for diagnostic and treatment purpose.

According to the Kirchhoff's Circuit Law, it states that electric current stream flow through lowest resistivity or highest conductance pathway. Plasma and blood which have the lowest resistivity in the body as shown in Table 1 would be the primary distributed pathway of the injected alternating current. EBI approach first procured the response voltage drop to measure the tissue's impedance to the flow of electric current based on Ohm's Law. The measured tissue's impedance represents a function of plasma, blood and extracellular fluid volume, or regards as baseline impedance, Z_b (Alfred *et al.*, 2007).

Table 1: Electrical properties of various biological fluids and tissues (Geddes and Baker, 1989).

Type of tissue	Resistivity (Ω/cm)
Blood	150
Blood plasma	63
Muscle	300-1600
Skeletal Muscle (longitudinal)	300
Skeletal Muscle (transverse)	700
Lung	1275
Fat	2500

The cyclic dynamic alteration of blood volume because of the cardiac contraction transiently changes the response impedance to the current flow (ΔZ). Transient and static values of EBI are associated with dynamic and balanced conditions of vascular blood volume discrepancies within a particular segment respectively. The blood volume could be calculated from the impedance-related volume conduction equation (Kubicek's formula):

$$BV = -\rho_b \times \left(\frac{L}{Z_b}\right)^2 \times \left(\frac{dz}{dt}\right)_{...} \quad (1)$$

where BV is the blood volume, ρ_b is the blood resistivity, L is the length of the target segment, Z_b is the baseline impedance, $(dz/dt)_{max}$ is the maximum of the first derivative bio-impedance trace, and T is the blood ejection time.The minus sign denotes that impedance indirectly proportional to the blood volume. Since ρ_b is a proportional factor in Kubicek's equation, its unbiased determination is important for assessment of blood volume. There are two general approaches to the problem in the literatures: assume that $\rho_b$is constant and within

the range of 130-150 Ω/cm, or setting ρ_b as a second order or exponential function of hematocrit (Cybulski, 2011). In this work, we assume that the blood has a constant ρ_b of 150 Ω/cm. However, in fact, ρ_b slightly decreases with velocity because of alignment of the cells with flow streamlines and movement of cells toward the axis (Webster, 2010).

Once the blood volume is known, the blood flow rate can be easily calculated by using the following equation:

$$BF = BV \times HR \tag{2}$$

where *BF* is the blood flow and *HR* is the heart rate.The hemodynamic parameters, blood flow and blood volume, which has a predictive value for the vein graft survival, subject to change when there is any pathologic or functional vascular change that affects the blood circulation (Rahman *et al.*, 2012). The EBI measurement reflects the volumetric changes of the blood and its flow rate which indirectly indicates the presence or absence of occlusion at the target site.

MATERIAL AND METHODS

A. Current Excitation:

Bio-impedance measurement of a given human body segment could be performed using two methods. Either applying a constant alternating current (AC) to the target site and measuring the induced voltage response to find its impedance, or uses voltage source instead of current source and measuring the induced current response. Based on paper review, noise due to spatial variation in applied current or voltage can be greatly reduced if current is applied and voltage is detected rather than voltage is applied and current is detected (Issacson, 1986). In addition, the use of current excitation instead of voltage excitation has the advantage of reducing the possible nonlinearlities and applies an intrinsically safe current-limiting mechanism (Morucci, 1996). Therefore, a constant current source is implemented instead of a constant voltage source.

Due to the heterogeneous characteristic of the biological tissue which has both resistive and capacitive elements, high frequency AC excitation is preferred rather than direct current (DC) excitation on the human body, as DC can create charge deposition on the biological tissue and causing polarization. A current at which human can just detect a slight tingling sensation is defined as threshold current. Once a current exceed the threshold level, there is a possibility of causing different sort of damage and undesirable effects such as severe burns and heating, ventricular fibrillation and neuromuscular excitation that may threaten the human life. The human's threshold of perception can be improved with increasing in frequency of the excitation current. The higher the frequency of current, even higher the current can be tolerated.

According to the IEC 60601-1 electrical safety regulations, the maximum allowable current for DC (0 Hz) is less than 10 μA_{rms} and less than 10 mA_{rms} at 1 kHz. The maximum allowable current for frequencies above 1 kHz could be estimated by employing the following equation (IEC 60601-1, 2005):

$$I_{\max}(f) = 10^{-7} \times f \tag{3}$$

where fis the frequency of excitation current. The expression above is not valid in circumstance of excitation current exceeds 10 mA. It is desirable to apply excitation current of at least 1 mA to enlarge the resulting voltage drop as the bio-impedance of biological tissue is extremely small in order to achieve sufficient signal-to-noise ratio (SNR) (Webster, 2010).

B. Single Frequency Bio-impedance Measurement:

Single frequency bio-impedance measurement (SF-BIM) approach is relatively simple, easy to perform and provide the necessary information to diagnose the pathological processes. Simplicityis the main advantage of this approach where only a single frequency AC from frequency range corresponding to the β-dispersion in Fig. 1 (few kHz up to 1 MHz) is applied depending on the type of tissue being analyzed. Therefore, it has been promoted by some researchers as the basis for a clinical parameter to evaluate the tissue's condition instead of complex multi-frequency approach which coupled with the subsequent characterization that provide additional valuable information(York et al., 2009).For clinical applications, β-dispersion bio-impedance measurement is typically implemented (Corciova et al., 2011; Hornero et al., 2013; Bouchaala et al., 2013), as it reflects structural changes and indicates pathological status.

In principle, the threshold current required for perception augments with frequency. Therefore, frequencies above 20 kHz could refrain from perception of the current with current intensity up to few mA. This lower limit also assists in removing other common bio-potential signals (EEG, ECG, EMG, etc.). When taking the measurement of underlying tissue bio-impedance by electrodes placement on the skin's surface, it is well known that the impedance between interface of stratum corneum layer (skin) and electrode has extremely large effect on the resulting bio-impedance. This skin-electrode impedance could be decreased by a factor of about 100 as the frequency is increased from low values up to 100 kHz (Webster, 2010). Previous research also reported that the

stratum corneum layer accounted for about 50% of the measured impedance at 10 Hz, but only about 10% at 100 kHz (Rosell et al., 1988). Therefore, application of high frequency current (>100 kHz) used to eliminate or minimize both the skin-electrode impedance and the undesirable changes in the bio-impedance that result from motion artifacts. However, if the frequency higher than 100 kHz is applied, the low impedances of the stray capacitances could make the design of the instrument difficult.

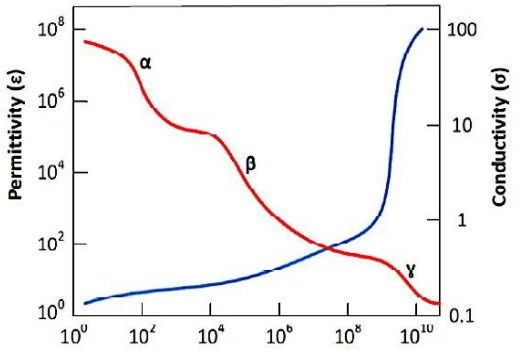

Fig. 1: Frequency dispersion of the conductivity and permittivity of biological tissue.

C. Prototype Design:

An electrical bio-impedance measurement system for vein graft surveillance consists of a sine-wave generator followed by a voltage-controlled current source (VCCS). The high frequency (100 kHz) current (1-mA) is passed through the body segment of interest (finger) via two surface band electrodes, E1 and E2 as shown in Fig. 2. The injected excitation current yields a voltage potential difference modulated in amplitude by the bio-impedance changes of the finger. This voltage signal developed along the current path is sensed by another pair of inner band electrodes, E3 and E4. The tetra-polar electrode arrangement was usedinstead of other electrode arrangement as it minimizes the electrode-electrolyte interface polarization impedance, which regarded as the main error source of EBI measurements.

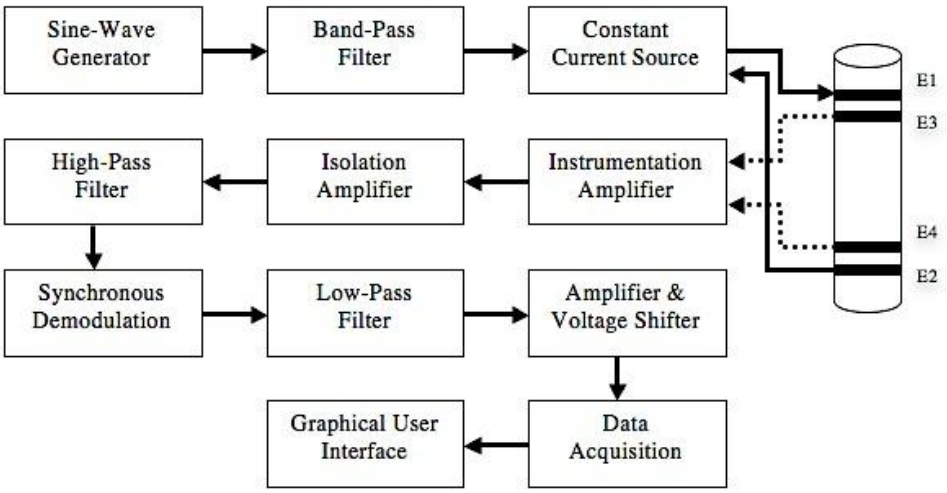

Fig. 2: Block diagram of prototype design.

Since the excitation current is constant, according to Ohm's Law, the amplitude of the signal sensed is directly proportional to the electrical bio-impedance of the body segment between the electrodes E3 and E4. After going through some signal conditioning circuit, i.e. signal acquisition, amplification, noise filtering, demodulation, isolation, and voltage shifting. The signal which is proportional to the instantaneous impedance of the body segment then sampled at a rate of 1 kHz by a programmable microcontroller PIC18F4580 for analog-to-digital conversion. After digitization, it was fed to an UART-to-USB converter to convert the bio-impedance data to an USB formatted bit-stream data.

The data stream was then transmitted to a personal computer (PC) and a state-of-the-art GUI system stores it automatically in a temporary data file specified by the user for future reference and further analysis. A proprietary computing algorithm is developed to compute the calculation of hemodynamic parameters from bio-impedance

signals originating from the peripheral circulations. The GUI system provides a real time analysis platform for the user to access the bio-impedance, blood flow and blood volume to evaluate the patency of the vein graft.

D. Study Population and Protocol:

To validate the prototype, it was tested on finger of 6 male subjects with mean age of 24 years old. No subject had a history of relevant cardiovascular disease, diabetes, hypertension, or any chronic disease that affects vascular function. During the experiment, the subject's temperature was around 36.5 to 36.8 degree Celsius. Temperature control is important since it might affect the orientation of the blood (Richelle, 2010). The room temperature was kept constant at 25 degree Celsius throughout the experimental study.

Finger blood flow was measured by the EBI method in six subjects with clinically normal finger in order to derive a "normal range" of finger blood flow associated with EBI. The obtained resultwas compared to a commercial duplex ultrasound machine in order to validate the accuracy of the developed prototype. Second measurements were conducted to evaluate the occluded finger that simulated using finger cuff. The purpose of the finger cuff was to restrict arterial blood flow in the finger area to simulate a stenosis condition. By means of applying this constriction facilitated a determination of whether the prototype was detecting the stenosis within the finger, which would be identified by a change of finger impedance.

E. Duplex Ultrasound Examination:

Each subject underwent a standard duplex ultrasound examination in the right middle finger positions, using a high-end Ultrasound machine (Siemens, ACUSON, Model S2000) with a 5.5 MHz 18L6HDtransducer probe. The examination took place at Hospital Universiti Sains Malaysia (HUSM) in Kelantan by Dr. Ahmad Helmy Abdul Karim from Radiology department of HUSM. Pulsed wave, continuous wave and digital artery's diameter analysis were conducted on all subjects. Immediately thereafter, an EBI examination was performed.

4. Electrical Bio-impedance Examination:

Before measurement procedure, the subjects first will sit quietly and relax in a chair for 5 minutes in order to establish a homeostatic condition before experiment conducted. Prior to testing, the target segment, finger was cleaned with an alcohol swab for cleanliness and the ECG electrode gel was placed on the finger in order to ameliorate conductivity between the skin and the electrodes.

The EBI measurements were recorded from 1 cm segment of right middle-finger (inter-electrode distance E3 and E4 was 1 cm). The subject's finger was put in the developed finger holder in the situation that the inner electrodes (voltage detection electrodes) are at the proximal and distal margins of the second segment of the middle finger while the outer electrodes (current injecting electrodes) were placed 0.3 cm proximal and 0.3 cm distally, as shown in Fig. 3.

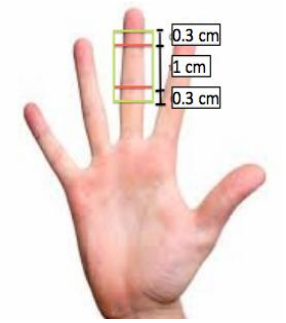

Fig. 3: Target segment of measurement.

Every examination was carried out within 5 minutes. During the recording section, the subject's arm lay on the arm-rest, with the arm comfortably supported in order to avoid any motion artifacts as muscular contraction was found to affect the measurement result. The subjects were asked to hold their breath in order to avoid movement artifacts as well.

During examination, a low amplitude (1-mA) and high frequency (100 kHz) alternating current is transmitted between current injecting electrodes and the resulting voltage is measured from voltage electrode pairs. The EBI data was recorded at least for 30 seconds but long recording to a maximum of 150 seconds was taken in order to reduce the noise interference in the average bio-impedance signal and neutralize the effect of respiration (Geddes and Baker, 1967). Subsequently, the EBI is processed in a blinded manner via proprietary computing algorithm. An example of measurement result is illustrated in Fig. 4.

RESULT AND DISCUSSION

A. Normal Finger:

To evaluate the accuracy of EBI in measuring peripheral blood flow in the finger, paired values of peak-systolic and end-diastolic blood flow by EBI and duplex ultrasound were compared. The comparison result was demonstrated in Fig. 5, 6 and 7. Disregarding the subject 6's data due to the drawback of the duplex ultrasound in the digital artery assessment, it shows that the prototype has an overall accuracy of 75.65% for the ten data being amassed from five subjects. The maximum accuracy can reach up to 98.72% and minimum accuracy as low as 43.33% for finger blood flow measurement. From the peak-systolic blood flow measurements, an average accuracy of 84% can be achieved whereas only 67.29% for accuracy for the end-diastolic blood flow measurements. This shows the limitation of the developed prototype. Further improvement was required.

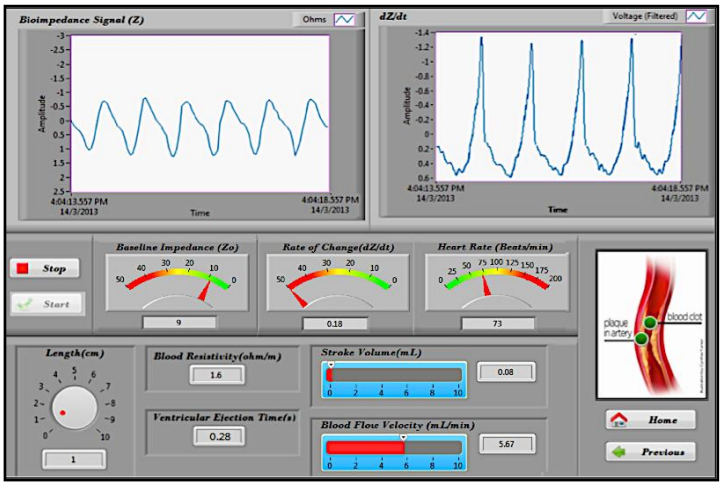

Fig. 4: Electrical bio-impedance measurement panel.

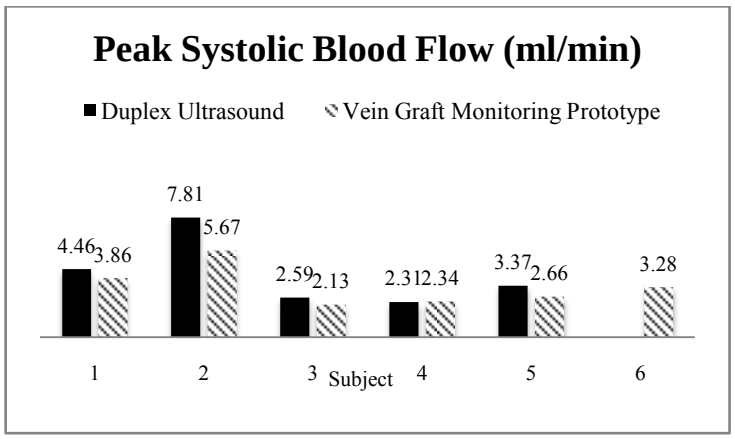

Fig. 5: Comparison of peak systolic blood flow measurement.

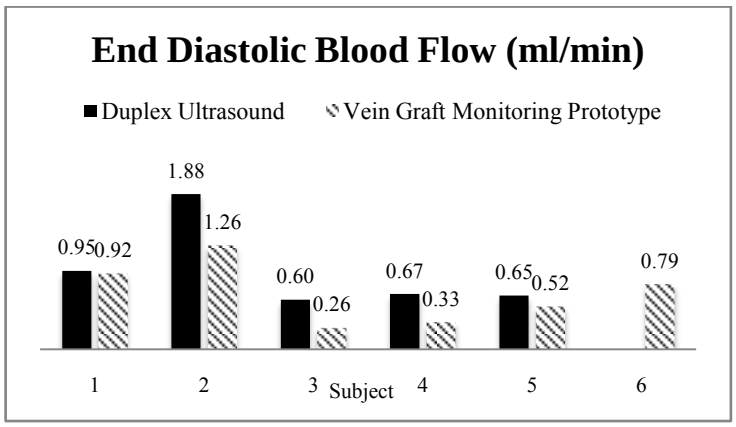

Fig. 6: Comparison of end diastolic blood flow measurement.

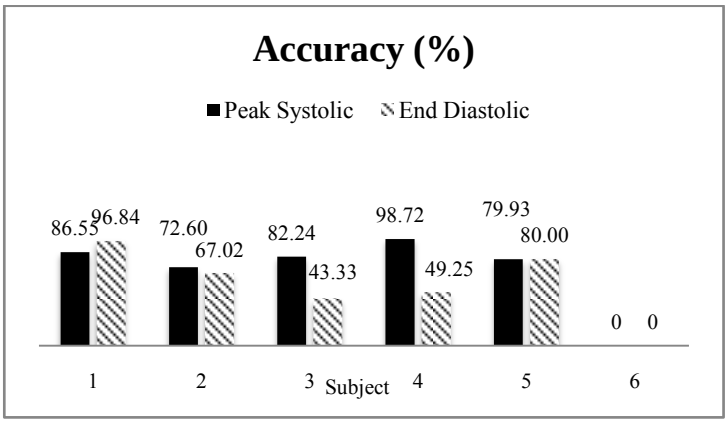

Fig. 7: Comparison of end diastolic blood flow measurement.

The accurate measurement of bio-impedance is limited by the effects of stray capacitance, noise interference, signal instability, and the electrode impedance. Errors are extremely large at high frequency current excitation application. Small tetra-polar electrodes that used for current injection and response voltage detection suffer from several problems. The electrode impedance is inversely proportional to the electrode area. Smaller electrodes may have a higher electrode impedance. The position and separation between electrodes also have to take into consideration. Electrode separation is the main factor that determines the sensitivity of the electrode with tissue depth. In order to assess more deeply, a larger electrode separation is required.

Due to the fact that it is difficult to dominate and estimate the electrode interface impedance, therefore the current source and voltage measurement circuits must have high output impedance and input impedance respectively. In practice, the measured bio-impedance from the inner electrode is a combination of the biological impedance, the electrode/tissue interface, and the impedance offered by the instrumentation.

B. Occluded Finger:

Fig. 8 shows the data acquired when the finger cuff that used to simulate a stenosis condition in the finger, was deflated at initial, inflated to 50 mmHg for approximately 15 seconds and then released. Obviously, the result in Fig. 8 reveals some important factors. Prior to finger cuff was inflated, normal bio-impedance signal with pulses was detected from the developed prototype. As the cuff was inflated to 50 mmHg, the signal amplitude slowly decreased and eventually shows no significant pulse once the finger cuff is fully inflated, which is expected due to cessation or blockage of blood flow.

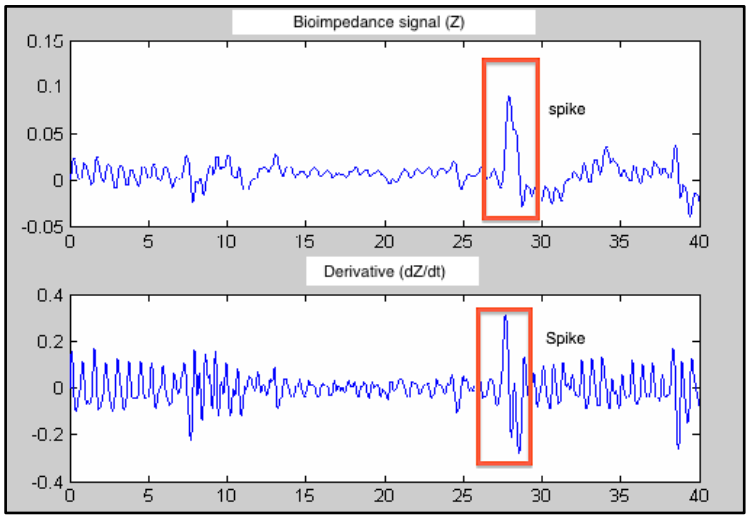

Fig. 8: Occluded finger examination result analyzed in Matlab.

Once the finger cuff was released after 15 seconds, a spike was detected in both the bio-impedance signal and its derivative. Subsequently, the signal progressively back to normal. Physiologically, the spike is most probably arisen from the dilation of the arteries in response to local signals from the temporary occluded or obstructed

condition. This result clearly shows that the bio-impedance data retrieved from the prototype correspond to the impedance changes of the target segment between voltage electrode pairs when there is a fluctuation of blood flow. Overall examination result on both normal and simulated occluded fingers of peak systolic blood flow was documented in Table 2.

Table 2: Examination result on normal and occluded finger.

Subject	Heart Rate (bpm)	Normal Finger					Occluded Finger				
		VET (s)	dz/dt (ohm/sec)	Zo (ohm)	Stroke Volume (ml/beat)	PS Blood Flow (ml/min)	VET (s)	dz/dt (ohm/sec)	Zo (ohm)	Stroke Volume (ml/beat)	PS Blood Flow (ml/min)
1	69	0.23	0.21	11.30	0.06	3.86	0.22	0.13	16.90	0.015	1.03
2	72	0.28	0.18	9.80	0.08	5.67	0.26	0.09	14.70	0.016	1.17
3	66	0.24	0.33	19.20	0.03	2.13	0.23	0.15	23.20	0.010	0.63
4	67	0.25	0.35	19.40	0.03	2.34	0.23	0.13	21.40	0.010	0.66
5	67	0.25	0.31	17.10	0.04	2.66	0.24	0.14	19.90	0.013	0.85
6	70	0.30	0.29	16.70	0.05	3.28	0.27	0.16	20.10	0.016	1.12

From the result, it showed that occlusion in the finger shows a significant effect to the blood flow and the baseline impedance (Z_b). The peak systolic blood flow indices in the normal finger varied from 0.26 ml/min to 5.67 ml/min while in the occluded finger varied from 0.63 ml/min to 1.17 ml/min. The baseline impedance for normal finger varied from 9.80 Ω to 19.40 Ω while for occluded finger varied from 14.70 Ω to 23.20 Ω.

The result shows that there were considerable discrepancies between normal finger and occluded finger. The occluded finger has a higher baseline impedance, lower blood volume, and lower blood flow velocity than clinically normal finger that without any occlusion. This is most probably resulting from the stenosis site within the finger which gives rise to a reduction in vascular diameter restricted the blood flows through the segment. As a result, the red blood cells (RBCs) of the blood tend to clump together or misaligned. In this situation, the injected current has a more difficult pathway to pass through the blood and eventually results in the impedance increments. The bio-impedance depends on the local properties of vascular and blood within it. The higher the blood volume and blood flow in the target site higher conductivity and eventually results in low impedance. This shows that the bio-impedance is inversely proportional to the blood flow and blood volume.

In the case for vein graft surveillance, when there is a stenosis in the graft, the measured longitudinal bio-impedance will be higher compared to normal vein graft which indirectly indicates that there is a reducing of blood volume and blood flow. Therefore, by the measuring of these three main parameters (bio-impedance, blood flow and blood volume), the vein graft patency could be prognosticate with predetermined normative data provided. However, the normal range for finger's impedance is still under study because this method is a new approach to monitoring and diagnosing the unsuccessfulness of vein graft tapering and for postoperative surveillance. Extensive clinical evaluations have to be performed in order to creation of a database that may assist future surveillance systems development as well as help clinician to identify the grafts at risk.

Conclusion:

The preliminary results of this prototype are quite promising with its low cost instrumentation, but there is still some key issues such as the effect of stray capacitance, electrode impedance, noise interference, signal instability, and optimum hardware designs that should be take into consideration for extensive research and further improvements. A new trend of graft surveillance and graft failure diagnosis may be derive from further analysis of these preliminary results and extensive evaluation of the developed prototype. Albeit the developed prototype was preliminary evaluated only in fingers, it has shown to be capable of recording temporal changes of bio-impedance of other parts of the human body such as arms, lower extremities, and heart. Nevertheless, to prove it, those assessments are indispensable in the near future.

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