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Endothelium Function Assessment with Radial Pulse Wave Signals

Hsien-Tsai Wu, Chun-Ho Lee, Tsang-Chin Wu, and An-Bang Liu

***Abstract*—This study proposes a method of measuring vasodilatation via air pressure sensing to assess the function of endothelium cells. The vasodilatation index is calculated according to the change of area of waveform caused by stimulation to the blood vessels, and uses this index to reflect the function of endothelium cells; therefore, early self-monitoring of cardiovascular dysfunction and arterial stiffness can be easily and effectively achieved. Only a few minutes are needed for conducting a self endothelial function assessment.**

Furthermore, this study improves the high cost pressure sensors used in Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT) and its inconveniences. 30 test subjects with no previous cardiovascular disease record were included for testing the reproducibility of the instrument. It has been proven that the air pressure sensing method proposed in this study has higher reproducibility and practicality than the Photoplethysmography (PPG) system for assessing the function of endothelium cells.
Keywords: endothelium dysfunction, vasodilatation index, reproducibility.

I. Introduction

Endothelial dysfunction is an indication of early formation of atherosclerosis [1]. Endothelium cells are a thin layer inside the arteries, and are responsible for controlling the contraction and dilatation of blood vessel. Endothelial dysfunction causes arterial stiffness that will cause fracture to the blood vessels, one of the main causes of the formation of atherosclerosis. Endothelium cells produce nitric oxide (NO), which is called the endothelium derived relaxing factor (EDRF) [2]. When NO is diffused into the smooth muscle layer after being released from the endothelium cells, it will inhibit the contraction of the smooth muscle layer, causing the relaxation of the blood vessel. So whenever the NO limits the contraction of the smooth muscle, the blood vessel will be in relaxation status [3]. Previous stu d ies have shown that reactive hyperemia is mainly caused by the endothelium derived NO. Some studies have shown that the bioavailability of NO has a direct effect on the dilation of arteries; therefore using reactive hyperemia to assess the function of endothelium cells has become one of the most effective methods to detect the early formation of atherosclerosis [4].

Currently endothelial function assessment can be classified into two categories: invasive and non-invasive.Venous Occlusion Plethysmography(VOP) is an invasive assessing method. Many studies have found good reproducibility and some even regard the technique as the 'gold-standard' for the assessment of vascular function [5], but because it is a highly invasive method, it is not recommended for normal healthy test subjects.

Ultrasound scanners are widely used for endothelial function assessment. Flow–mediated vasodilatation (FMD)[6] uses high-resolution ultra sound scanners to observe the dilation of blood vessels after stimulation.

Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT)[7] is based on the method but does not use high resolution ultra sound scanners for endothelial function assessment. Rather it uses tonometry to sense the change of amplitude of arterial waveforms for assessing endothelial function. The correlation between coronary and peripheral microvascular endothelial function is well established, and in previous studies, using Photoplethysmography (PPG)[8] to calculate the area under the curve of the waveform for assessing the function of endothelium cell has been proven to be highly correlated with the traditional RH-PAT method. The area under the curve of finger digital volume pulse grossly represents the volume change of blood flow into this finger during each pulse [9].

In order to verify the validation of the vasodilatation index as measured by our instrument and mediated by the function of vascular endothelia, a specific NO synthesize inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), was injected intra-arterially into five adult male Wistar-Kyoto (WKY) rats aged from 10 to 12 weeks. After the injections, the dilation of each rat's vessel was decreased by 34% [10]. This proves that the PPG method can be used for endothelial function assessment.

However, the PPG assessing method did not show good reproducibility in peripheral vascular endothelial function assessment. Therefore, this study combines the advantages of previous studies [7][8][9], then proposes a non-invasive, easy to operate air-pressure sensing for endothelial function assessment system, with the high reproducibility and practicality of this system being proven through real human

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testing and static data analysis.

II. Endothelial Cell Function Assessment of the Previous Literature Review

This study focuses on two methods: RH-PAT and PPG. Both assess endothelial function through the change of waveform after stimulation. Two methods will be discussed as follows:

A. Reactive Hyperemia Peripheral Arterial Tonometry

The correlation between coronary and peripheral microvascular endothelial function is well established in previous studies. Reactive hyperemia peripheral arterial tonometry (RH-PAT) is a non-invasive technique to assess peripheral microvascular endothelial function by measuring changes in digital pulse volume during reactive hyperemia [11].

RH-PAT places a cuff in the upper arm to stimulate endothelium cell to produce NO, and use tonometry of the finger for measuring bio-signals. Before cuff inflation, $\text{Amplitude}_{\text{Baseline}}$ ($Amp_{Basline}$) is collected. $\text{Amplitude}_{\text{Baseline}}$ is on average 3.5 minutes of the bio-signal from the finger. Then inflate the upper arm cuff for 5 minutes. The $\text{Amplitude}_{\text{RH}}$ will be collected for one minute after one minute of release of the cuff, these one minute signals will be averaged (Amp_{RH}). RH-PAT index is then taken. $\text{Amplitude}_{\text{RH}}$ divided by 3.5 minutes of $\text{Amplitude}_{\text{Baseline}}$, RH-PAT index is shown in Eq.(1) [7]:

$$RH - PAT = {Amp_{RH}} \big/ {Amp_{Baseline}} \tag{1}$$

B. Photoplethysmography Vaso-dilatation Index

Peripheral microvascular endothelial function assessment has been proven to be effective in the RH-PAT studies; previous study [8] also proposed a second method that assesses endothelial function by observing the change of waveform after the stimulation as well, that is, the PPG system. The PPG system uses an infrared sensor on the left index finger's microvascular bed; it extracts numerous digital volume pulse waveform to serve as a baseline ($A_{Basline}$), then uses a pressure cuff on the left upper arm for occlusion for 4 minutes; this will cause endothelium cells to react and produce NO for vasodilatation. When the upper arm cuff is released, the flow of blood to the lower arm will be increased, and this is called Reactive Hyperemia (RH); then we can record and calculate the area under the curve of the digital volume pulse waveform under the RH condition (A_{RH}).

Waveform analyzing method use area under the curve of waveform to reflect the change of blood flow; by comparing A_{Baseline} to A_{RH} the vasodilatation index can be extracted as shown in Eq.(2).

$$DI - Area = {A_{RH}} \big/ {A_{Baseline}} \tag{2}$$

III. New Endothelial Function Assessment via Air Pressure Sensing System

This study proposes a novel endothelial function assessment via air pressure sensing system. It combines RH-PAT's tonometry pressure sensing a PPG's area under curve algorithm. Endothelial function assessment via air pressure sensing system hardware structure, software analysis and experiment procedures and method will be explained in the following sections.

A. Hardware structure

In the hardware part, there are a radius/upper arm air pressure sensing device, analog processing unit, radius/upper arm air pump motor unit, mixed signal processing unit, peripheral unit, and memory unit. The analog signal processing unit will convert the pulse wave into DC and filters out the noises then amplifies it, with the purified analog signal converted into digital data by the mixed signal processing unit (MSP430F449) with a sampling rate of 200Hz. Then the digital data is transmitted to the PC's analyzing software via UART (RS-232); the analyzing software will plot the pulse waveform on the screen then analyze it. The Radius and Upper arm automatic inflation/deflation controlling unit's design is based on a traditional sphygmomanometer, which includes pressure calculation, air pump control, and a mixed signal unit that uses PWM to control air pump inflation and deflation using a valve. Figure 1 is the block diagram of the system.

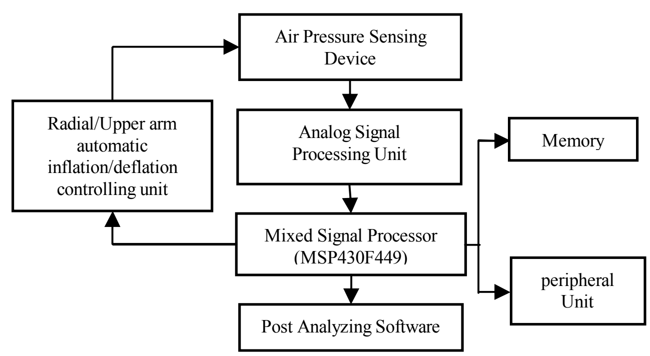

Figure 1. System Diagram

B. Software analysis

The software part is responsible for receiving the digital data from the mixed signal processing unit, then uses a pulse waveform analyzing algorithm to calculate the vasodilatation index and measure data storage. Figure 2 is the analyzing software flow chart.

The baseline is defined as 20 sets of area under the curve of pulse waveform before the inflation, noted as ABaseline, ARH is defined as 20 sets of area under the curve of pulse waveform after 1 minute of cuff deflation, then Eq.(2) is used for vasodilatation index calculation[8].

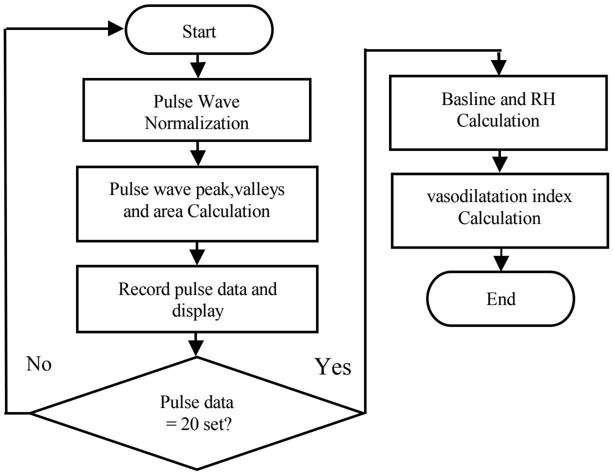

Figure 2. Software flow chart

C. Experiment method

The air pressure sensing system's experiment procedure is described as follows: The radial cuff is inflated at a constant pressure of 40±3mmHg, a low constant pressure and low pressure error, which allows the pulse wave to become stable.

The subject will be asked to fill out the personal information form and the experiment will be performed in a quiet environment at 25℃. Every subject will participate in the experiment for 6 days, only one measurement is performed per day, and no other constraint is required. The air pressure sensing system will be used for the measurement in the first 3 days, then in the last 3 days the PPG system will be used. Then the recorded data will be saved and analyzed by statistical methods such as coefficient of variation, correlation and standard deviation (via SPSS and EXCEL). The data will be plotted in the graph to show the two systems' reliability and all data is represented at mean±SD,P<0.05.

IV. RESULTS

This study tested 30 non-cardiovascular disease volunteers, among them, 18 males and 12 females. All subjects are rested in the supine position and tested on their left arm. During the 6 days of experiment, both the air pressure sensing method and PPG system are performed 3 times for DI-Area measurement. Table 1 shows the information of the testing subjects in the study.

TABLE 1.STUDY POPULATION CHARACTERISTICS

Male/Female	18/12
Age, years (yr)	23.87±4.87
Hight (cm)	168.53±8.41
Weight (kg)	64.90±11.98
BMI(kg/㎡)	22.68±2.79

A. Two systems' reproducibility analysis

Vasodilatation Index data acquired from both systems is normalized, and with correlation analysis for 3 trials data of the PPG system, the correlation coefficient of the first trial and second trial is R=0.081 (P=0.666). The second trial and the third trial is R=0.610 (P<0.01). There is no doubt that R=0.108 (P=0.561) for the first trial and the third trial. The result shows that only the first trial and the third trial have better correlation while others show poor correlation, so it is easy to see that the PPG system cannot provide stable experiment results.

For the DI-Area data of the air pressuring sensing system correlation analysis, R=0.944 (P<0.01) in the first two trails, R=0.978 (P<0.01) in the second and third trials. There is no doubt that R is equal to 0.943 (P<0.01) for the first and third trails. All correlation analysis shows all set of data have high correlation to each other. Therefore, the air pressure sensing system has a higher reproducibility than the PPG system, and also provides higher stability DI-Area measurement results.

B. Two systems' measurement error analysis

The difference between 3 times measurements is called a variation or an error in the measurements. The error in measurement is a mathematical way to show the uncertainty in the measurement.

The DI-Area data of 30 subjects are taken for measurement error analysis with coefficient of variation (CV) method. Let X be the overall mean, and S be the standard deviation of within-subject, CV is shown in Eq.(3)

$$CV = {}^{S}\!/_{X} \qquad (3)$$

Figure 3 is the measurement error percentage of two systems, and it is obvious to see that the PPG system's percentage of error is as high as 55.05±20.32(%), so there must be some unknown or unseen reason that causes this high percentage error. However, the air pressure sensing system proposed in this study has only 8.49±3.26(%) of measurement error; the lower the value of measurement error, the better the reliability of the system is, thus, better reproducibility. The measurement error of DI-Area has a noticeable difference between the two systems (P<0.01).

From the DI-Area's reproducibility and error analysis above, the proposed air pressure sensing system has better reproducibility and practicability for endothelial function assessment.

V. DISCUSSION AND CONCLUSIONS

The air pressure sensing system proposed in this study has proven, after the trials, with every test subject's 3-days measurement data evenly distributed, and with correlation analysis, that the proposed system has higher reproducibility than the previous PPG system. The causes that may affect the reproducibility of the system are discussed below.

For the consideration of sensors, this study proposes an air pressure sensing method that integrated a piezoresistive sensor in the radial cuff for pulse wave extraction. Since the cuff is firmly placed above the radial arteries, this can reduce the unwanted movement that may cause error during the measurement, and it also has a low cost advantage over the traditional tonometry.

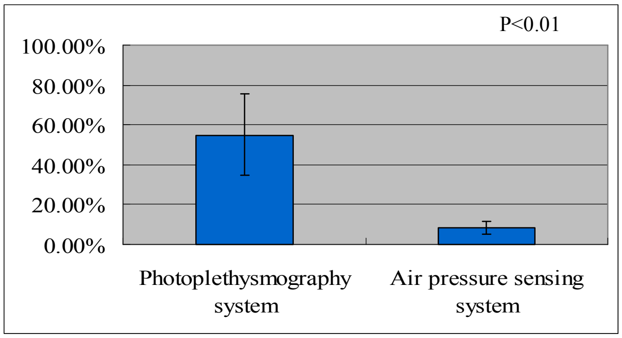

Figure 3. Measurement error percentage of the two systems

In consideration of the measuring site, this study changed the measuring site to radial arteries instead of peripheral arteries that were measured in previous studies [7]. The radial artery is classified as a mid-size artery and has a larger smooth muscle layer that results in better vessel contraction and dilatation than peripheral arteries. More importantly, peripheral arteries can adjust the blood flow automatically. In that way, the wave signals [8][9] from the PPG system's sensors will not reflect the vasodilatation index exactly. That is the primary reason why we used the proposed air pressure sensing system in this study.

After discussion of the physiological mechanism, it is known that using peripheral arteries or micro-vascular endothelial function to evaluate the function of endothelium cells may result poor reproducibility because of arteriole flow auto-regulation function[2]. For the PPG system, its error is 55.05±20.32 (%) for measuring fingertip peripheral arteries; by measuring the radial arteries, the error is as low as 8.49±3.26(%) for the proposed system; the difference of the measuring error of the two systems agrees with the physiological mechanism. Although the measuring of peripheral endothelial function can be similar to cardiovascular endothelial function assessment, because of the micro-vascular before arteriole flow auto-regulation function impact, it may eventually cause error to the measurement. If the peripheral artery is set to be the measuring site, then the variability of flow auto-regulation function needs to be occluded.

After the discussions above, this study developed a method for assessing endothelial function in radial artery by irritating upper arm endothelium cells to release NO by cuff inflation. The blood flow will increase after the vasodilatation caused by the diffusion of NO into the smooth muscle layer [2][4][6]. Then using the calculation of the area under the curve of pulse wave form to reflect the change of blood flow [9], the blood flow change before and after the inflation can then derive the vasodilatation index. This vasodilatation index can reflect the health of endothelium cells, and can also be used as a reference for early detection of atherosclerosis. This study has proven that the air pressure sensing system has good reproducibility and reliability through a series of human experiments. In the future, the proposed system will perform large scale experiment of the clinical application on the human body, with static analyzing method, to search for the relationship between hyperemia vasodilatation and atherosclerosis related diseases.

In short, a pressure cuff is placed on the subject's wrist and maintains a stable pressure. Then an analog signal processing unit extracts the subject's radial pulse wave and another pressure cuff is placed on the upper arm to stimulate endothelium cells to generate Nitric Oxide (NO). A standard pulse wave status is taken first, then the upper arm pressure cuff is inflated to a supra systolic blood pressure level that will occlude the blood flow from the heart to the radial artery which causes stimulation to the endothelium cell derived NO to dilate the blood vessels. After the release of upper arm pressure cuff, the pulse wave signals are captured to compare with the standard pulse wave; with post-processing, the response of blood vessel after the stimulation reveals the functionality of endothelium cells, thus can be used as a reference for early detection of arterial stiffness.

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