Abstract—In vivo and in vitro studies of differential continuous wave photoacoustic spectroscopy (DCW-PAS) for non-invasive blood glucose monitoring were performed. The DCW-PAS technique utilizes amplitude modulation of dual wavelengths of light to determine changes in glucose concentration. The study compared DCW-PAS measurements with results from invasive blood glucose sensor measurements during oral glucose tolerance tests (OGTTs) of healthy people. The trends in blood glucose levels (BGLs) obtained from invasive sensors and from the photoacoustic signal have good agreement, with the standard error and correlation coefficient against BGLs of 48 mg/dL or less and 0.80, respectively. Our proposed photoacoustic spectroscopy (PAS) method shows high potential for use in a non-invasive BGL sensor.

Index Terms—Photoacoustic, continuous-wave, non-invasive, blood glucose levels, in vivo measurement.

I. INTRODUCTION

The number of diabetic patients is increasing rapidly worldwide [1], and patients are required to measure their blood glucose levels (BGLs) for proper treatment. Current techniques utilize needle pricks or an electrochemical sensor placed in tissue under the skin—methods which are not only painful and stressful but also carry the risk of infection. The need for a painless and convenient non-invasive BGL measurement method is growing. The various techniques proposed and investigated over the past few decades include optical, electromagnetic, ultrasound, and chemical ones [2], [3]. Among them, optical techniques have the advantage of non-invasive selective detection of glucose molecules through light absorption based on excitation of vibrational and/or rotational modes. To date, optical techniques that have been explored for non-invasive glucose measurement include optical transmission spectroscopy, diffuse reflectance spectroscopy, optical coherence tomography, thermal radiometry, and photoacoustic spectroscopy (PAS) [3]–[17].

There are two major issues in glucose sensing. One is strong optical scattering and decay. Techniques based on PAS are useful for mitigating this issue because they have the advantages of optical techniques with the added advantages of the high sensitivity to light and robustness of acoustic waves versus the scattering and absorbing properties of tissue [4]–[7]. The other major issue is that optical absorption by glucose molecules overlaps with absorption by water and other substances in both the near-infrared (NIR) and mid-infrared (MIR) regions [4], [5], [14]–[18]. For glucose monitoring, NIR light is the better choice due to its ability to deeply penetrate tissue; it can reach the dermis, subcutaneous tissue, or microvascular depth to detect glucose molecules in interstitial fluid or blood [5]. To remove the overlap with the background from water, we employ a differential method using two wavelengths that have almost the same optical absorbance for water but a different absorbance for glucose.

PAS measurements have been performed with a variety of systems that can be classified based on light source control, either pulsed PAS or intensity-modulated CW-PAS [6]–[13]. Pulsed PAS generates large PA signals but requires a high-power, nanosecond pulse light source, making the system complicated and large. CW-PAS has advantages over pulsed
PAS in the signal-to-noise ratio (SNR), despite using a small light source, thanks to lock-in detection.

Differential continuous-wave photoacoustic spectroscopy (DCW-PAS) is a technique we have recently developed by combining CW-PAS with dual wavelength modulation. DCW-PAS provides a linear relationship to glucose concentration in aqueous solution, with sensitivity as good as ±50 mg/dL—the level of physiologically relevant concentrations [19]. In this work, the first to investigate DCW-PAS in the human body, we performed both in vitro measurements and an in vivo pilot study with three healthy volunteers in which we compared DCW-PAS and invasive BGL sensors during oral glucose tolerance tests (OGTTs).

II. METHODOLOGY OF DCW-PAS

In the DCW-PAS method, the sample is irradiated by two lasers that are on/off amplitude modulated at the same frequency (380 kHz) but 180 degrees out of phase with each other. To remove the background from water, we use wavelengths (1382 and 1610 nm) that have identical optical absorption for water but different absorption for glucose. Absorption by other substances such as albumin and lipids also occurs at 1610 nm, but their levels change very slowly compared with glucose changes, and levels vary from individual to individual [4]. Since the duration of this study was three hours, we ignored effects from concentration changes in the other substances. The PA wave is generated by local thermal expansion in the sample induced by the light absorption. The resulting pressure of the PA wave, \( p(\tau) \), is simply given by

\[
p(\tau) = K \Gamma \mu (\Delta C) I_0
\]

(1)

where \( \mu \) is the absorption coefficient, \( \Delta C \) is the change in glucose concentration, \( I_0 \) is the intensity of the light, \( \Gamma \) is a Gruneisen parameter depending on the material of the sample, and \( K \) is an empirical coefficient which relies on the conditions of acoustic wave generation and detection. \( K \) is eliminated by the dual-wavelength protocol. PA waves deriving from each wavelength are generated. The two light sources are on/off modulated at the same frequency but 180° out of phase with each other. Let the light intensities at the two wavelengths be \( I_{\lambda 1} \) and \( I_{\lambda 2} \). The total amplitude of the acoustic waves is given by a linear superposition of Eq. (1) for each light as shown in

\[
S_{\Delta C=0} = K \Gamma \mu_0 I_{\lambda 1} - K \Gamma \mu_0 I_{\lambda 2}
\]

(2)

where \( \mu_0 \) is the initial absorption coefficient. The amplitude of the PA waves depends on the difference between the absorbed energy at the two wavelengths. The two wavelengths were both on/off modulated; the light intensity when on stays constant for \( I_{\lambda 1} \) but varies with time for \( I_{\lambda 2} \). The amplitudes of the PA wave have a linear relationship as shown in Fig. 1. The optical absorbance is changed by \( \beta \Delta C \) and the line shifts by \( \Delta I_{\lambda 2} \) as shown in

\[
S_{\Delta C} = K \Gamma \{(\mu_0 + \beta_{\lambda 1} \Delta C) I_{\lambda 1} - (\mu_0 + \beta_{\lambda 2} \Delta C) (I_{\lambda 2} + \Delta 2)\}
\]

(3)

where \( \Delta C \) is the change in glucose concentration, \( \beta \) is the absorption coefficient per molecular concentration of glucose, with the suffix showing the wavelength of the light. \( \Delta C \) causes the shift of the line as shown in Fig. 1. From Eq. (3),

\[
\Delta C \propto \frac{I_{\lambda 2} |_{l=1} - I_{\lambda 2} |_{l=0}}{I_{\lambda 2} |_{l=0}} = \frac{\Delta I_{\lambda 2}}{I_{\lambda 2} |_{l=0}}
\]

(4)

is derived, in which the ratio of the shift is proportional to \( \Delta C \). Then, the change in the concentration can be determined using the ratio of the shift of the line. The ratio in Eq. (4) is defined as the DCW-PAS signal.

III. EXPERIMENTAL

Schematics of the setups for the in vitro study of DCW-PAS are shown in Figs. 2 and 3. The sample was a glucose aqueous solution containing albumin. The glucose (D-glucose, Sigma-Aldrich Co., USA) concentration ranged from 0 to 400 mg/dL, and the concentration of albumin (Albumin bovine, Cosmobic Co., Japan) was 0, 16.5, or 33 g/dL. Albumin, a kind of protein, is a typical component of blood, and its typical physiological level in blood is 33 g/L. A function generator (WF4986, NF Co., Japan) generated a 380 kHz square wave voltage signal that was converted into current by a laser driver directly driving the two distributed feedback (DFB) lasers. The DFB laser wavelengths were 1382 and 1610 nm. Then, the two laser signals were combined with a coupler and delivered to a cylindrical chamber containing the sample solution through a single-mode optical fiber. The brass chamber was immersed in an isothermal bath kept at 27 °C ±0.02 °C. The lasers irradiated the sample through a collimator. The PA wave was detected by a sensor (M204A, Fuji Ceramics, Japan), and the
amplitude of the acoustic wave was measured by a lock-in amplifier (SR844, Stanford Research Systems, Co., US) using the signal from the function generator as a reference. The time constant of the lock-in amplifier was 3 s to reduce noise. Five percent of the laser light was split by beam splitters and constantly monitored by a power meter (AQ211, Yokogawa Meters & Instruments, Japan).

The relationship between the DCW-PAS signal and the glucose concentration is shown in Fig. 4. In the range of glucose concentrations from 0 to 400 mg/dL, the relationship between the PA signal and the glucose concentration is linear. As the albumin concentration increases, the slope increases. The sensitivities of the PA signal for glucose concentration are 0.1, 0.2, and 0.3% per 100 mg/dL at the albumin concentrations of 0, 16.5, and 33 g/L, respectively. The albumin concentration may affect the characteristics of the DCW-PAS, depending on individual differences in albumin levels, which requires individual calibrations. However, this effect can be ignored in obtaining the time sequence of the BGL, since albumin levels produce smaller changes and vary much more slowly than BGL. The standard error and correlation coefficient were 15 mg/dL and 0.99, respectively.

A. In Vivo Study

A photograph and schematic of the sensor interface for the in vivo study are shown in Fig. 5. The two light signals are combined with a coupler (Custom order, Optoquest Co., Japan) and sent to the measured part, namely the earlobe. Five percent of the laser light is split off by beam splitters in the coupler and constantly monitored by a power meter (Custom order, Optoquest Co., Japan). The light is collimated at the sensor interface and radiated to the measured part. Ultrasonic gel (TOWA gel, TOWA Tech Co., Japan) was used for acoustic impedance coupling between the sensor interface and skin of the earlobe. The skin temperature was monitored with a platinum resistance thermometer (NX-1200, NETSUSHIN Co., Japan) installed at the sensor interface (see Fig. 5), since the intensity of the PA wave was also affected by the temperature at the laser-irradiated part (i.e., the skin temperature of the earlobe).

Ethical approval was obtained from the University of Tokyo Hospital Ethics Committee. All experiments were performed in accordance with relevant guidelines and regulations. Volunteers were healthy people, and informed consent was obtained from the all volunteers by the University of Tokyo Hospital.
We compared the DCW-PAS with commercially available invasive BGL sensors during OGTTs. (OGTTs induce an increase in blood glucose levels (BGLs) by ingesting glucose.) The BGLs were monitored with these sensors and by drawing venous blood. A flash glucose monitoring tool (FGM) (FreeStyle Libre™, Abbott Co., UK) inserted into the left upper arm measured the BGLs every 5 min, and a self-monitoring blood glucose tool (SMBG) (Medisafe Fit™, TERUMO Co., Japan) measured them at the fingers of the right hand every 30 min. Venous blood was drawn from the right arm every 30 min. The sensor interface of the DCW-PAS was attached to the left earlobe. While the skin temperature changed for physiological reasons and due to the effect of contact with the sensor, the BGLs stayed approximately constant in the first 30 minutes, and we evaluated the effect of skin temperature during that time. For the OGTTs, liquid glucose containing 75 g of glucose (TRELAN G75, AY PHARMACEUTICALS Co., Japan) was used to induce an increase in BGLs. It was ingested by the volunteer 30 min after the start of the DCW-PAS measurement. The total duration of the test was 3 h.

### IV. RESULTS AND DISCUSSION

The typical temperature profile at the measured part is shown in Fig. 6. For each volunteer, the temperature started to rise from the room temperature level of 26°C right after the sensor interface had been attached, and it reached a stable level of 30°C within 30 min. Liquid glucose was taken 30 min after sensor attachment. The glucose concentration change is given by

$$
\Delta C = \alpha (P - \zeta T)
$$

where $P$ is the DCW-PAS signal, $T$ is the change in temperature, $\alpha$ is the sensitivity between the reference BGLs and the DCW-PAS signal, and $\zeta$ is the coefficient to compensate for the effect of temperature change. Coefficient $\zeta$ had been determined before the glucose liquid was taken by comparing the DCW-PAS signal and the temperature of the measured part for each volunteer. It varied from 0.01 to 0.02 among the volunteers, as shown in Table I. Figure 7 shows a typical comparison with and without temperature correction.

| Volunteer | Coefficient to Compensate for Temperature, $\zeta$/°C |
|-----------|-----------------------------------------------|
| a         | 0.010                                         |
| b         | 0.020                                         |
| c         | 0.017                                         |

| TABLE II  | CORRELATION COEFFICIENTS |
|-----------|---------------------------|
| Volunteer | Sensitivity against glucose concentration, $\alpha$ /[100 (mg/dL)] | Correlation coefficient | Standard error [mg/dL] |
| a         | 0.098                      | 0.80                       | 19                      |
| b         | 0.035                      | 0.79                       | 21                      |
| c         | 0.067                      | 0.58                       | 48                      |

The typical time-sequence relationships between the BGLs from SMBG, FGM, drawn blood, and the DCW-PAS signal are shown in Fig. 8, where closed, open circles, triangles, crosses are the results from the DCW-PAS, FGM, SMBG, drawn blood, respectively. The values of coefficient $\zeta$ were 0.02, 0.017, and 0.01 as shown in Table II. The BGLs of every volunteer varied from 70 to 160 mg/dL. The time sequence of DCW-PAS signals shows good agreement with the invasively measured BGLs. However, in the case of the third volunteer, some lag of peak time compared with the references can be seen, and the standard error reached 48 mg/dL. There were some differences among BGLs measured by SMBG, FGM, and drawn blood. The BGLs of SMBG show the glucose level in a mixture of arterial and venous blood, while that of FGM shows it in interstitial fluid. It has been reported that the time delay in reflecting BGLs in interstitial fluid is up to 10 min due to perfusions [21], [22]. Using this sensitivity, we drew the consensus error grid shown in Fig. 9, where the A+B zone contains more than 90% of the plots. Table II shows the sensitivity, correlation coefficients, and standard errors for each volunteer compared with FGM on the left arm. Sensitivity $\alpha$ was determined under the condition that...
the signal of the DCW-PAS was calibrated by the reference BGLs of the FGM in the left arm. Compared with the sensitivity in the in vitro study, the sensitivity was larger in all volunteers. The DCW-PAS measures glucose in interstitial fluid in tissue under the skin (epidermis, dermis, subcutaneous tissue). This tissue is composed of fat (15%), protein (30%), and other substances (approximately 60%). Fat has much less ability to absorb light in the wavelength range used in this experiment [4], and protein increases the sensitivity of the DCW-PAS, as mentioned in the section describing the in vitro study. We believe that the increased sensitivity relative to the in vitro results was caused by the increase in light permeability due to the existence of fat and by protein’s effect on DCW-PAS.

V. CONCLUSION

A study of our proposed DCW-PAS was performed both in vitro with glucose aqueous solution and in vivo by OGTTs with three healthy volunteers. The duration of the tests was 3 h, where the DCW-PAS measurement started 30 min before glucose was ingested. BGLs were also monitored with three kinds of invasive sensing methods (SMBG, FGM, and drawing venous blood) as references. The time sequences of blood glucose levels from the invasive methods and DCW-PAS have good agreement for two cases. The correlation coefficients ranged from 0.58 to 0.80. As the DCW-PAS signal was calibrated by blood glucose levels obtained from FGM, the standard errors ranged from 21 to 48 mg/dL. The sensitivity in the in vivo study was larger than in the in vitro study, probably because the effective concentration of glucose in tissue under the skin was more than ten times larger. There were some differences in the time sequence of glucose concentration among the results for the invasive sensing methods. These differences are probably due to differences in perfusion, which depends on the part of body used for the invasive monitoring. This should be investigated in a study with a larger number of volunteers and that includes diabetes patients to cover the range from 50 to > 300 mg/dL. Additionally, these differences should also be taken into account when discussing the applicability or actual usage of a non-invasive blood glucose monitor. Although there is still some room for improvement, DCW-PAS has potential as a healthcare tool for monitoring daily BGL time sequences during exercise or when eating.

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