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Simultaneous measurement of erythrocyte aggregarion and oxygen saturation under *in vitro* pulsatile blood flow by high-frequency photoacoustics

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***Abstract*—In this paper, high-frequency photoacoustic (PA) imaging is proposed to simultaneously measure erythrocyte aggregation (EA) and oxygen saturation (SO_2). EA is a reversible phenomenon where red blood cells aggregate under flowing conditions and it becomes pathological when enhanced in a number of circulatory disorders. Here we investigate the feasibility of PA imaging in detecting EA-induced changes in SO_2 in a simulated circulatory flow system. For all optical wavelengths of illumination (750 and 850 nm), the mean PA amplitude inside the region of interest cyclically varied at intervals corresponding to the beat rate (30, 60, and 90 bpm). The vessel diameter also cyclically varied at the same time interval, but the phase of its variation was reversed compared to the PA amplitude variations. This was expected: as the blood velocity decreased, the shear rate in the radial direction also decreased, resulting in increased EA thus enhancing the PA amplitude due to the increased effective absorber size. When the velocity is increased, the aforementioned process is reversed, resulting in decreased EA and PA amplitude. The cyclic variation in SO_2 was evident for the highest beat rate, and differences in the mean PA amplitude at 750 and 850 nm were detected for all beat rates. This indicates that the SO_2 was varying while blood was flowing with the different beat rates. The temporal variation in SO_2 can be correlated to EA, since it has been reported that oxygen release is inhibited by EA.**

Keywords*—erythrocyte aggregation, oxygen saturation, high-frequency photoacoustics, pulsatile blood flow

I. Introduction

The main role of erythrocytes is oxygen delivery to tissues via blood flow, and the delivery of oxygen is governed by the oxygen saturation (SO_2) [1]. Erythrocytes become aggregated when flowing blood is exposed to stasis or very low shear rate conditions, and erythrocyte aggregation (EA) is a phenomenon present at enhanced rates in various pathologies such as deep vein thrombosis [2], diabetes [3] or a stoke [4]. In addition, EA is known to alter blood viscosity which affects blood flow dynamics, vascular resistance and tissue perfusion [5]. The relation between oxygen delivery and EA, hence, may provide a new biomarker.

The SO_2 can be measured through conventional optical spectroscopy [6] but it is limited by the penetration depth and lack of spatial resolution. Over the past decade, SO_2 measurement methods have been significantly improved through advances in photoacoustic (PA) imaging [7, 8]. However, these studies have focused on the PA measurement of the SO_2 in a micron-diameter vessels without considering hemodydnamic and hemorheological properties that control EA. Ultrasound imaging of EA is promising noninvasive tool, and has been widely studied [9-11]. However, ultrasound doesn't provide information about the functional aspects related to oxygen delivery in flowing blood.

In this paper, high-frequency PA imaging is proposed to simultaneously assess the SO_2 and EA under blood flow. In the previous studies by our group [12, 13], the PA measurement of SO_2 and EA was preliminarily conducted under the static conditions. In order to further advance the clinical applicability of PA imaging, the SO_2 and EA should be investigated simultaneously under flowing conditions.

II. Materials and Methods

A. Blood Source

Ethics approval was granted by the Research Ethics Boards of Ryerson University and the Canadian Blood Services. All experiments were conducted using whole blood units donated by healthy volunteers recruited by the Canadian Blood Services' Network Center for Applied Development (Vancouver, BC). Whole blood units from three volunteers were used in order to ensure the repeatability of experimental results.

B. Simulated Circulatory System

A simulated circulatory system was configured by a peristaltic pump (MasterFlex, Cole-Parmer, Montreal, QC), a silicon tube, a triangle beaker, and a 2-mm-diameter flow phantom made from porcine skin gelatin (Sigma Aldrich, Oakville, ON) at a concentration of 15% wt/vol in degassed water (Fig. 1). The silicone tube and beaker were used for

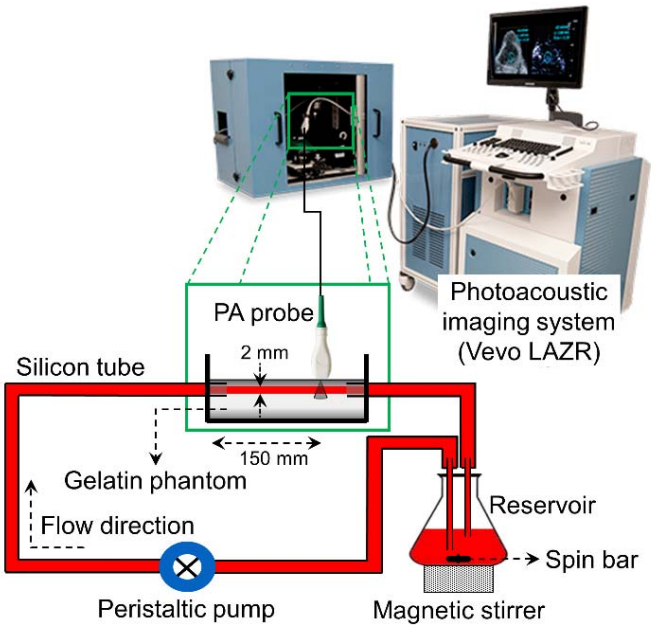

Fig. 1. Experimental setup consisting of a simulated circulatory and the PA imaging system.

circulating blood and as a reservoir, respectively. The peristaltic pump generated pulsatile flow within the phantom at beat rates of 30, 60, and 90 bpm. The end-diastolic to peak-systolic blood flow velocities corresponding to the beat rates were 3 to 20, 7 to 32, and 8 to 39 cm/s, respectively.

C. PA Imaging of Flowing Blood

PA images of flowing blood were acquired by a VevoLAZR (FUJIFILM VisualSonics, Canada) system equipped with a 40 MHz linear-array probe. An Nd:YAG pulsed laser (20 Hz, 6ns pulse width, 30 mJ/pulse) was operated at two optical wavelengths (750 and 850 nm) in order to estimate the SO_2 by the computing the ratio of oxygenated hemoglobin to the total hemoglobin assessed. PA measurements were performed for three blood samples, three beat rates, and two wavelengths. For each measurement, 200 PA B-mode frames were recorded corresponding to a measurement time of 10 seconds per laser wavelength.

D. Simultaneous Assessment of Erythrocyte Aggregation, Oxygen Saturation, and Blood Flow Velocity

Erythrocytes become aggregated when flowing blood is exposed very low shear rates, and as the aggregate size increases (i.e. the optical absorber), it results in the enhancement of the PA signal amplitude. Hence, EA formation can be assessed by the changes in PA amplitude.

The SO_2 was estimated by assuming that oxygenated (HbO_2) and deoxygenated (Hb) hemoglobin are the dominant absorbers at the two wavelengths λ_1 and λ_2, and was computed by [7]

$$SO_2 = \frac{[HbO_2]}{[HbO_2]+[Hb]} = \frac{\mu_a^{\lambda_2}\varepsilon_{Hb}^{\lambda_1} - \mu_a^{\lambda_1}\varepsilon_{Hb}^{\lambda_2}}{\mu_a^{\lambda_1}(\varepsilon_{HbO_2}^{\lambda_2} - \varepsilon_{Hb}^{\lambda_2}) - \mu_a^{\lambda_2}(\varepsilon_{HbO_2}^{\lambda_1} - \varepsilon_{Hb}^{\lambda_1})} \quad (1)$$

where $[HbO_2]$ and $[Hb]$ are the molar concentrations of the two forms of hemoglobin, respectively; μ_a is the absorption coefficient (in cm^{-1}) and is equal to $P/(\Gamma \cdot F)$ where P is the detected PA pressure, Γ is the Gruneisen parameter, F is the optical fluence of the excitation light; ε_{Hb} and ε_{HbO2} are the known molar extinction coefficients (in $cm^{-1}M^{-1}$) of the two forms of hemoglobin, respectively.

Ideally, the blood flow velocity should be simultaneously measured with the PA images in order to interpret the relation between EA, SO_2 and the shear rate. The PA system was limited in the ability to measure PA signals and blood velocities simultaneously. In lieu of that, blood velocity was estimated by measuring the variation in blood vessel diameter simultaneously with the PA signal since the vessel expands and contracts during systole and diastole, respectively [14]. In the PA image, the vessel diameter was calculated for all beat rates, and the relative trend of variation in blood flow velocity was estimated.

The aforementioned measurement parameters could then be simultaneously obtained since the PA image provided the PA amplitude (related to EA in the vessel lumen), the SO_2 (from two optical wavelengths), and the vessel diameter variations. For EA and the vessel diameter, the variation index (VI) was computed by

$$VI = \frac{\text{Max.} - \text{Min.}}{\text{Min.}} \quad (2)$$

in order to make it clear how much the measurement parameters varied.

III. Results and Discussion

A. Erythrocyte Aggregation vs. Blood Flow Velocity

The PA signal amplitudes at two wavelengths 750 and 850 nm and the vessel diameter varied periodically depending on the beat rate, as shown in Fig. 2. For 30 bpm, the VI_{PA} were 0.104 and 0.102 at 750 and 850 nm, respectively, and the $VI_{diameter}$ was 0.168 (Fig. 2a). For 60 bpm, the VI_{PA} were 0.125 and 0.117 at 750 and 850 nm, respectively, and the $VI_{diameter}$ was 0.244 (Fig. 2b). For 90 bpm, the VI_{PA} were 0.138 and 0.178 at 750 and 850 nm, respectively, and the $VI_{diameter}$ was 0.286 (Fig. 2c). As the beat rate increased, the magnitude of a fast Fourier transform at the beat frequencies, 0.5, 1, and 1.5 Hz increased as shown by the arrows in Figs. 2d, 2e, and 2f. This is because the VI_{PA} and $VI_{diameter}$ increased, as the beat rate increased.

The cyclic variation in both PA amplitude and vessel diameter suggested that EA and blood flow velocity also varied periodically. In addition, the phase of the PA variation was opposite to that of the vessel diameter. This result can be interpreted as erythrocytes that aggregated and disaggregated during diastole and systole, respectively since the radial shear rate decreased and increase during diastole and systole, respectively. The out-of-phase relationship between EA and blood flow velocity was consistent with a previous study using high-frequency ultrasound [15].

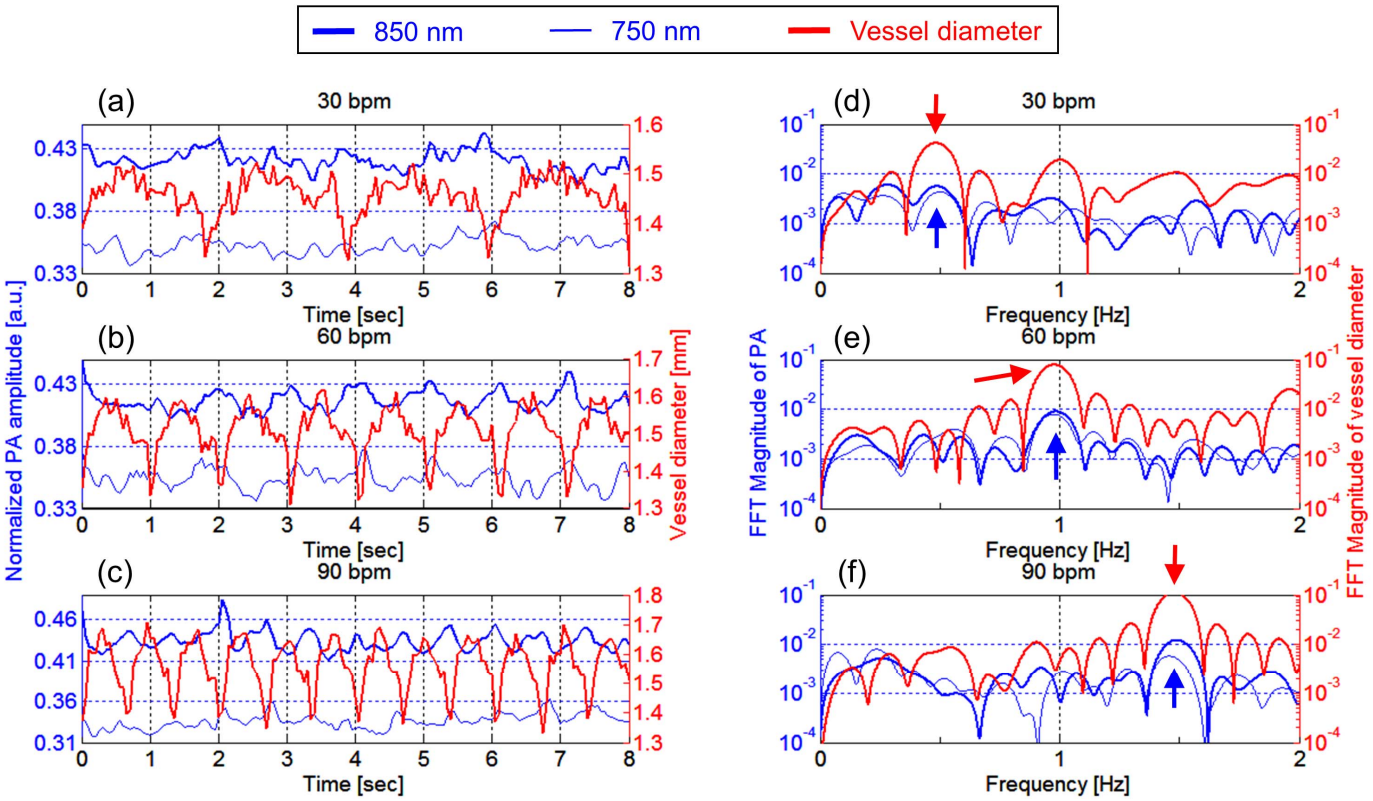

Fig. 2. Cyclic variation in photoacoustic (PA) amplitude in time (left) and frequency domain (right) at 30, 60, and 90 bpm of beat rates. The thick and thin blue lines indicate 850 and 750 nm of optical wavelength, respectively, and the thin red line indicates the vessel diameter.

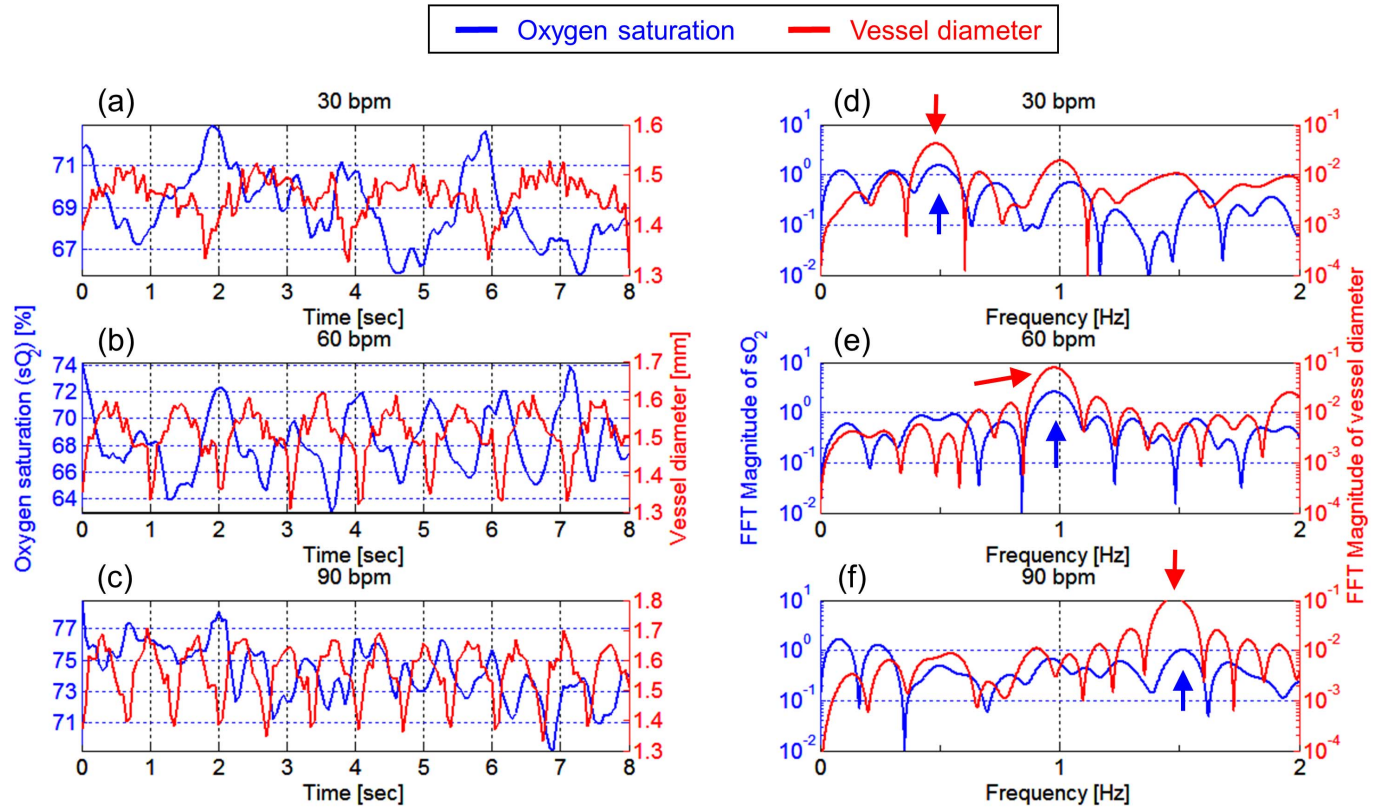

Fig. 3. Cyclic variation in oxygen saturation (SO_2) in time (left) and frequency domain (right) at 30, 60, and 90 bpm of beat rates. The blue and red lines indicate SO_2 and vessel diameter, respectively.

B. Oxygen Saturation vs. Blood Flow Velocity

The SO_2 also varied periodically depending on the beat rate as shown in Fig. 3. The minimum to maximum mean SO_2 values were 66 to 73%, 63 to 74%, and 69 to 79% at 30, 60, and 90 bpm, respectively (Figs. 3a. 3b, and 3c). As the beat rate increased, the corresponding frequencies, 0.5, 1, and 1.5 Hz were more pronounced as shown by the arrows in Figs. 3d, 3e, and 3f. According to Tateish et al. [16], the oxygen release is inhibited by erythrocyte aggregation, so that the SO_2 increases and decreases during aggregation and disaggregation, respectively.

IV. Conclusion

In this paper, the SO_2 and EA were simultaneously measured by high-frequency PA imaging under pulsatile blood flow, and their relationship was studied. The SO_2 and EA varied periodically depending on the beat rate, and these parameters were out of phase with each other, which can be interpreted as oxygen release being inhibited by EA. These findings suggest that PA assessment of EA in various circulatory disorders could be achieved through imaging of accessible human blood vessels such as the radial artery or vein. This study is a preliminary investigation that has opened new avenues towards understanding the relationship between hemodynamics and PA imaging.

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