



Design and evaluation of a portable optical-based biosensor for testing whole blood prothrombin time



Chin-Lung Yang^{a,e,1}, Song-Jeng Huang^{b,e,1}, Cheng-Wei Chou^{c,e}, Yan-Chao Chiou^{a,e},
Kun-Piao Lin^{d,e}, Min-Shiun Tsai^{b,e}, Kung-Chia Young^{c,e,*}

^a Department of Electrical Engineering in National Cheng Kung University, Tainan, Taiwan

^b Department of Mechanical Engineering in National Taiwan University of Science and Technology, Taipei, Taiwan

^c Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Tainan, Taiwan

^d Department of Pathology, National Cheng Kung University Hospital, Tainan, Taiwan

^e Department of Mechanical Engineering, National Chung Cheng University, Chia-Yi, Taiwan

ARTICLE INFO

Article history:

Received 14 April 2013

Received in revised form

24 July 2013

Accepted 25 July 2013

Available online 31 July 2013

Keywords:

Point-of-care diagnostics

Prothrombin time

Optical-based device

Whole blood

Biosensor

Light transmittance

ABSTRACT

Point-of-care diagnostics (POCD) for blood coagulation benefit patients on-site, but available POCD devices are too expensive to be affordable in many countries. Optically based methodologies are cheap and reliable, and have been exploited in bench-top coagulometers to monitor coagulation with plasma, but not whole blood, which contains cellular components that cause massive interference. However, the POCD testing of whole blood gives a more accurate picture of physiological conditions than does testing plasma. In this study, a portable device for performing the prothrombin time (PT) test was designed, comprising an optical sensor, an electrical processing and control circuit to monitor the optical changes that occurred during the coagulation process in whole blood. The PT was when the slope of the first-order derivative of the coagulation curve, recorded from real-time light transmittance signals, was maximal. The POCD PT testing of 167 samples revealed that 153 (91.6%) were successfully detected and the results were highly consistent with the results of whole blood international normalized ratio (INR) ($r=0.985$, $p<0.001$) by the conventional manual method and those of plasma INR ($r=0.948$, $p<0.001$) with the ACL TOP 700 bench-top coagulometer (Beckman Colter). Hematological parameters were further analyzed, revealing that fibrinogen titers ($p=0.036$), red blood cell numbers ($p=0.017$) and distribution of red cell width ($p=0.015$) affected the effectiveness of the current POCD PT determination. Furthermore, a highly positive correlation was revealed between fibrinogen titers and the maximum speed of change in transmittance (v/t) ($r=0.805$, $p<0.001$), suggesting that fibrinogen might be evaluated simultaneously in this POCD testing. In conclusion, the proposed portable optical-based device performs the highly sensitive and accurate determination of whole blood PT and has commercial potential because of its small volume and low fabrication cost.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Hemostasis testing measures the time of onset of blood clotting, which involves an activating cascade of coagulation factors that ends with the formation of a cross-linked fibrin clot. Coagulation time tests are required for patients who are receiving anticoagulant therapy [1,2]; undergoing pre-operation evaluation [3,4], or suffering from hepatic or renal disorders [5,6]. Furthermore, coagulopathy parameters are also useful in the diagnosis of severe disease progression owing to virus infection, such as dengue hemorrhagic

* Corresponding author at: Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Tainan, Taiwan. Tel.: +886 6 2353535x5787; fax: +886 6 2363956.

E-mail address: t7908077@mail.ncku.edu.tw (K.-C. Young).

¹ The authors contributed equally.

fever [7]. Accordingly, the rapid and accurate detection of prolonged coagulation using point-of-care diagnostics (POCD) might be of great help in screening or monitoring risks of bleeding tendency near where a patient is located.

Prothrombin time (PT) is one of the most frequently performed tests for monitoring hemostasis [8]. The measurement of PT depends on activation of the tissue factor, followed by coagulation factors in the order VII, X, V, thrombin and fibrin. The reference PT for healthy subjects is from 11 to 13 s (s). Therefore, a POCD device for making real-time measurements of the rapid reaction dynamics is required in PT testing.

Optical analyzers, incorporated into bench-top automatic coagulometers, have been utilized to measure PT for years. This approach is regarded as one of the most reliable and reproducible methods that can be applied to a plasma sample in the central laboratory of a hospital [9,10]. Laboratory-based instruments that

sense scattered light signals do not allow the detection of coagulation in whole blood because of which cellular components interfere massively with the signals. However, testing whole blood is not only easier and more time- and labor-effective than testing plasma samples, but also does not deplete the erythrocytes that contribute to the physiological coagulation reaction under *in vivo* conditions [11].

As compared to the POCD devices for determining PT which have been designed based on electric impedance [12,13], a piezoelectric quartz crystal [14], surface acoustic waves [15], surface plasmon resonance [16] and a fluorescent probe [17], optical modules with POCD capacity are cheaper, more versatile and less bulky; they have the potential to determine hematological parameters accurately and sensitively. Different from the central laboratory bench-top coagulometers which detect plasma, this proposed optical based POCD device detects whole blood samples for PT. In this device, the optical biosensor consists of commercial off-the-shelf components including the light source, the light detector and the bioelectric circuit, which was optimized by applying stable and precise electronics to resolve medical or biological measurement problems, so the PT measurement can be consistent and repeatable. During blood coagulation process, the proposed signals are based on changes in light transmittance, which is defined as the ratio of the intensity of the transmitted light to the intensity of the incident light at a specified wavelength that passes through a sample. The optical biosensor recorded the light transmittance signals, which was transformed into voltage and then used to plot a first-order differential curve. The PT time is then determined from the slope that is maximal. The effectiveness of this optical POCD device was verified on 167 clinical samples, yielding results that were consistent with those of standard PT assays. Additionally, a highly positive correlation was revealed between fibrinogen (FIB) titers and the maximum speed of change in transmittance, implying a potential for simultaneous evaluation of FIB in this novel POCD testing.

2. Material and methods

2.1. Patients and sample collection

One hundred and sixty-seven citrated whole blood samples were collected anonymously from the Central Hematological Laboratory of National Cheng Kung University Hospital for PT checking by the conventional manual method and the newly developed POCD test method. Laboratory data were collected using a bench-top instrument for comparison, by performing plasma PT (38 normal and 129 prolonged) and FIB tests using an ACL TOP 700 (Beckman Coulter, Brea, CA, USA). Routine parameters of complete cell count were measured using an LH-750 (Beckman Coulter). These included white blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts, hemoglobin (HB) concentration, hematocrit (HCT), red cell distribution width (RDW). This study was approved by the local Institutional Review Board.

2.2. Thromboplastin reagent

The thromboplastin reagent that comprised recombinant human tissue factor, synthetic phospholipids, calcium chloride and heparin-neutralizing polybrene was purchased commercially (HemosIL Recombiplastin, Beckman Colter). The thromboplastin reagent was reconstituted and stored following *per* manufacturer's protocol, and its international sensitivity index (ISI) was 0.96.

2.3. Conventional manual method for whole blood PT testing

The use of the thromboplastin reagent to sample a volume of whole blood rather than plasma followed the adjustment rule, as described elsewhere [18]. Briefly, 140 μ l of whole blood was loaded into a glass test tube (13 $\times$ 100 mm) and pre-warmed in a water bath at 37 °C for 3 min. After 200 μ l of pre-warmed thromboplastin reagent was added, the contents were manually stirred with an iron loop to identify the beginning of fibrin clot formation, at which point the time in seconds was determined as the PT. The international normalized ratio (INR) values were then calculated accordingly.

2.4. Design of optical sensor and bioelectric circuit in novel POCD whole blood PT testing device

The following two parts of this portable POCD device for testing whole blood PT were fabricated and integrated. (A) The optical sensor comprised a light emitting diode (LED) as a transmitter, a detector module and a glass slide; (B) the bioelectric circuit of processor and control comprised a signal conversion module, a microprocessor, a stable power supply, a timer and display module, and a Bluetooth transmitting device.

2.4.1. Optical sensor

The infrared LED transmitter supplied light with a wavelength at 770 nm (model # MTE1077, Marktech Optoelectronics, Latham, New York, USA) to the top of the detection area and a photodiode (SFH213) was used as a detector on the bottom of the area. Those modules are high-sensitive and low-cost and have low power consumption. The detection area was placed on a disposable glass slide with dimensions of 8 mm (width) $\times$ 18 mm (length) $\times$ 0.2 mm (height). A black acrylic shielding box was made to cover the sensor modules from the environmental visible light. The glass slide was inserted into the shielding box, and the detection area was positioned accurately between the LED and the photodiode. When the mixture of whole blood and thromboplastin was loaded onto the glass slide, the change in the transmitted light intensity in the coagulation process was recorded for 180 s and analyzed using the software OriginPro (OriginLab, Northampton, MA, USA). The coagulation curve was further processed by applying a digital filter in MATLAB® (MathWorks, Natick, MA, USA) to obtain the required data.

2.4.2. Bioelectric circuit of processor and control

A typical amplifier with a negative feedback structure [19] was used to detect the voltage across the photodiode in response to a variation of light intensity. The alternating current-induced noise at 60 Hz was reduced using a notch filter and noise above 25 Hz was reduced by a low-pass filter. The remaining analog signals were subsequently transformed to digits with an MSP430F149 (Texas Instruments, Dallas, TX, USA), a low power-consuming microprocessor that was composed of a 12-bit analog-to-digital converter (ADC), a 60KB+256B flash ROM, a 2K RAM and a 16-bit CPU. The analog data were collected by the ADC and transformed into the digital domain; the signal quality and detection performance were then improved by applying an advanced digital signal process (DSP). Meanwhile, a DS1302 time module counted the seconds which were output to MSP430F149, and the time and voltage were shown immediately on a display module (LCD1602). The collected data were transferred to a computer through a wireless Bluetooth device (HL-MD08R-C2A) at frequencies from 2.402 to 2.480 GHz. The voltage regulator circuit (Fairchild semiconductor IC 7805) supplied a stable 5 V DC.

2.5. Optical microscopic images of RBC

Citrated whole blood samples with and without added thromboplastin reagent were loaded onto glass slides and visualized under an optical microscope (Olympus, Tokyo, Japan) to which was attached a camera to record continuously RBC images.

2.6. Statistical analysis

The correlations between the PT and INR values that were obtained using two methods were analyzed using the Pearson correlation test and passing and Bablok regression analysis. The Mann–Whitney *U* test was performed to compare the routine parameters in the complete cell count for the POCD-detectable group and the POCD-non-detectable group. Univariate and multivariate regression models were implemented to evaluate the parameters that were correlated with POCD PT. The receiver operating characteristic (ROC) curve was used to determine the cut-off value. Statistical analysis was performed using SPSS (version 17.0, IBM, Armonk, New

York, USA). In all cases, a two-tailed *p*-value of less than 0.05 was regarded as statistically significant.

3. Results

3.1. Design of an optical POCD PT device

Fig. 1A presents the layout of the optical POCD PT device that consists of an infrared LED light source, a photodiode detector, a bioelectric circuit that comprises a simple current-to-voltage amplifier with a gain of 3–5 (V/μA) and both active notch and active low-pass filters. Fig. 1B presents the overall diagram of this novel optical-based POCD PT device. The transmitted light signals were detected and processed by the optical sensor and the processing and control circuit block. The experimental setup also included a black acrylic shielding box that covered the sensor modules, blocking out light from the environment. Fig. 1C presents the disposable glass slide with a detection region of

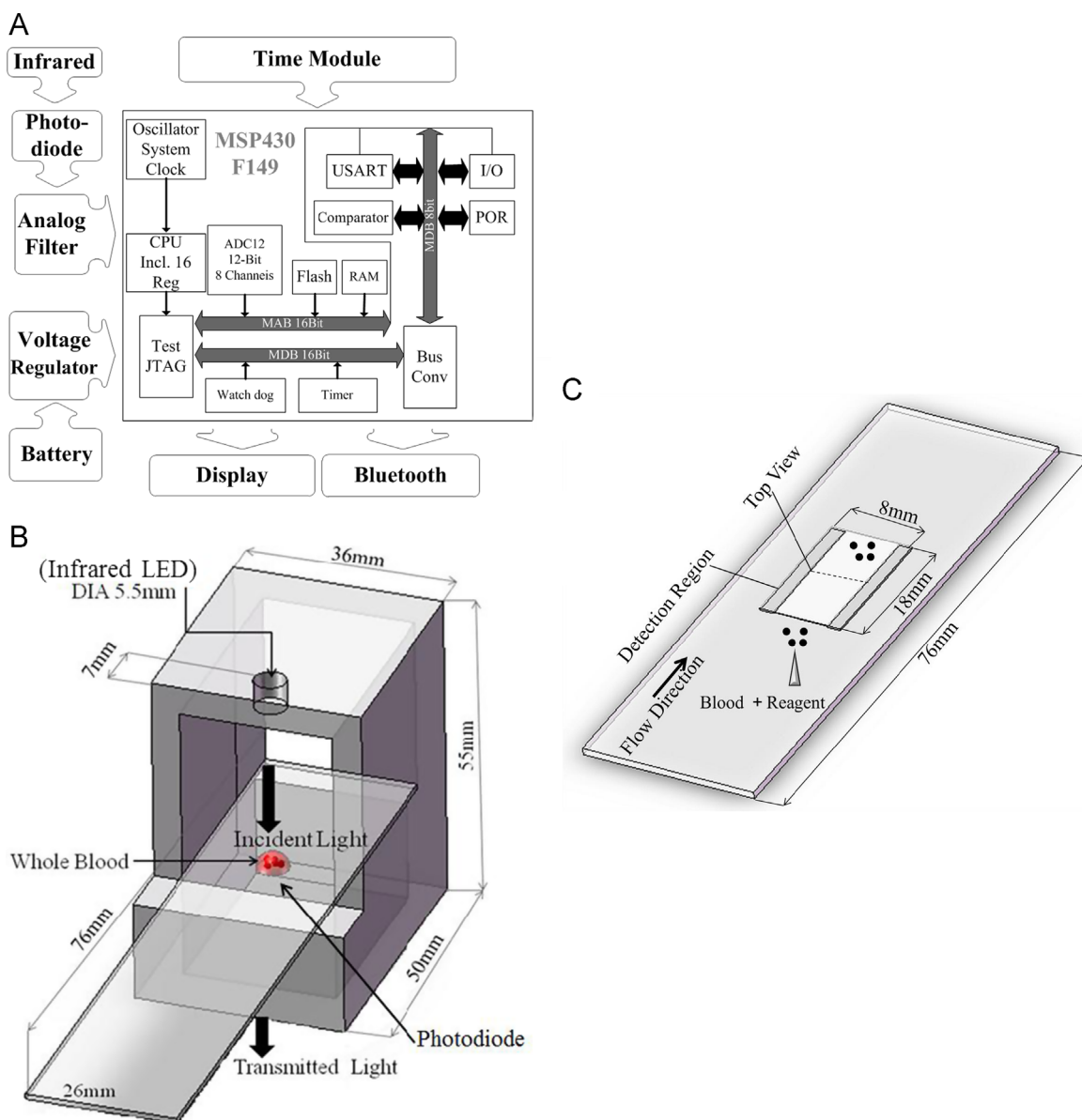


Fig. 1. (A) Layouts of infrared light source, photodiode detector and bioelectric circuit, which comprised simple current-to-voltage amplifier, and active notch and active low-pass filters. (B) Overall diagram of the novel optical-based POCD device and the black shielding acrylic box that covered sensor modules to block out light from environment. (C) Disposable glass slide with a detection area of 8 mm × 18 mm (top view) × 0.2 mm (lateral view).

8 mm × 18 mm (top view) × 0.2 mm (lateral view) which was placed between the infrared LED and the photodiode.

3.2. Eliminating noise with two active filters

To determine the cut-off frequency (F_c) and the gain of the filter, the original coagulation signal obtained using the data acquisition (DAQ, USB-6361, 2 MS/s, 16-bit) card was transformed into the frequency domain. The spectrum of transmitted light revealed that the main noise spike was at 60 Hz and corresponding harmonic frequencies, whereas the signals were concentrated at lower frequencies < 10 Hz (Fig. 2A). A twin-T notch filter was used to filter out the 60 Hz noise, and a fourth-order Butterworth with a cut-off frequency of 25 Hz was used to eliminate the remaining high-frequency noise. Both filters were active filters that were based on Sallen–Key filters [20]. A comparison of the transmittance curve

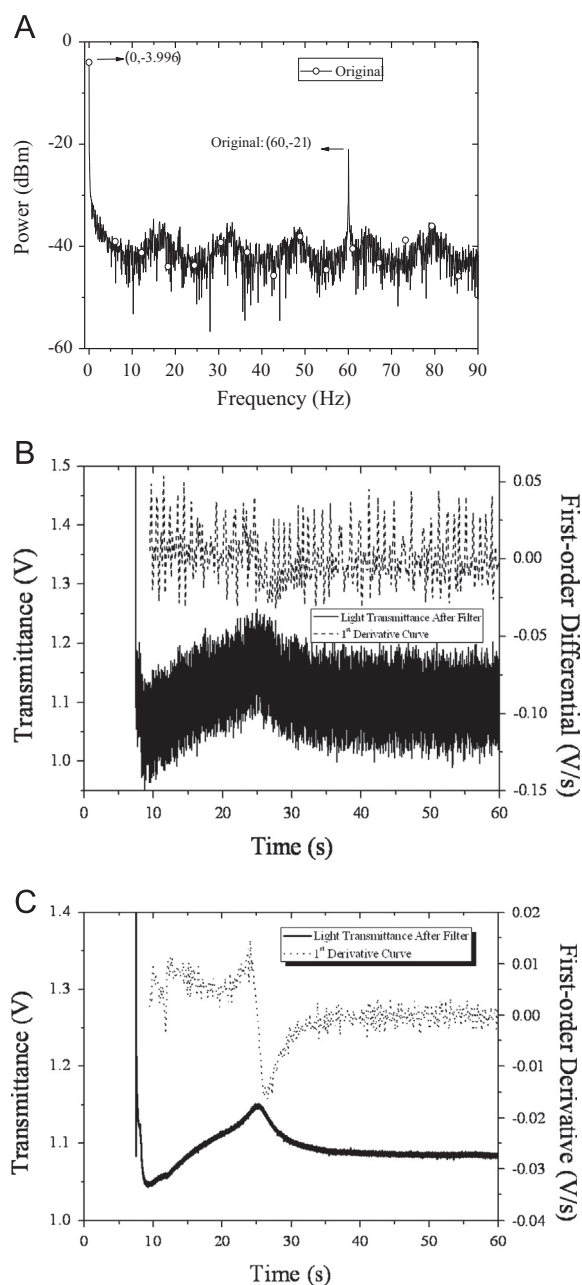


Fig. 2. (A) Spectrum of transmittance signal that was detected by the optically based POCD device by frequency and power. Transmittance curve and first-order derivative thereof before (B) and after (C) noise filtering.

before (Fig. 2B) and after (Fig. 2C) revealed that high-frequency interference was effectively eliminated by noise filtering, thus yielding a readable differentiation curve (Fig. 2C).

3.3. POCD PT testing with whole blood

Light transmittance signals from tested human samples were recorded using this optical-based POCD PT device. First, the light transmittance curves of whole blood with and without coagulation were displayed. As shown in Fig. 3A, the intensity of the observable transmittance signal from the citrated whole blood decreased during the sample loading period; then reversed, increasing to a plateau, plausibly because RBC aggregation facilitated light transmittance in the non-coagulated whole blood. Adding thromboplastin reagent to whole blood caused the light transmittance curve to exhibit a second turning point, revealing the onset of clot formation, which produced an RBC cross-linked gel-like structure, reducing the light transmittance. Optical microscope (Fig. 3B) revealed RBC aggregation in the citrated whole blood, indicated by the darkening of the speckles of RBC and the enlarging of the space from 5 to 35 s. In contrast, the addition of thromboplastin reagent weakened RBC aggregation during whole blood coagulation, potentially by inducing the homogenous crossing-link of RBC state.

3.4. Comparison of whole blood PT values measured by optical POCD device and by standard assays

The time of the maximal change in light intensity was used to determine the PT of this proposed optical-based POCD device, which implements the same algorithm as was applied in the PT test by the bench-top ACL TOP 700 instrument [9]. The transmittance falls over time, so the slope has a negative sign. The PT is determined from the minimum of the first-order differential curve, where the absolute value of the curve slope is highest. As presented in Fig. 4A, the first-order derivative curve was plotted as a function of the transmittance of light through whole blood as it undergoes the coagulation reaction. The timing of the nadir was measured as the PT of the sample. The PT was 34 s in this representative case.

The performance of the optical POCD device was compared with that of standard assays. Whole blood samples were collected from 167 patients who had been asked to undergo a PT check at the Central Hematological Laboratory of National Cheng Kung University Hospital. The results that were obtained using the ACL TOP 700 instrument comprised 38 normal and 129 plasma prolonged PT values. Following routine testing, residual whole blood samples were collected and measurements were made of them within an hour by the optical POCD PT device and by the conventional manual method.

POCD whole blood PT results were obtained from 153 samples (153/167, 91.6%). The other 14 samples failed to yield a first-order derivative curve with a readable point of maximum changing light intensity. For the 153 samples whose whole blood INR values could be measured using the novel POCD device, these values were highly correlated with those obtained using the conventional manual whole blood assay (Figs. 4B, $r=0.985$, $p<0.001$) and the plasma values obtained using the ACL TOP 700 (Figs. 4C, $r=0.948$, $p<0.001$). The comparisons were further performed with Passing and Bablok regression analysis for POCD versus manual ($p=0.19$, Fig. 4D) and for POCD versus ACL TOP ($p=0.65$, Fig. 4E), respectively, showing no significant deviation from linearity.

3.5. RBC and FIB influence the performance of optical POCD PT device

The natural properties of whole blood samples that could influence the performance of an optical POCD method for determining whole

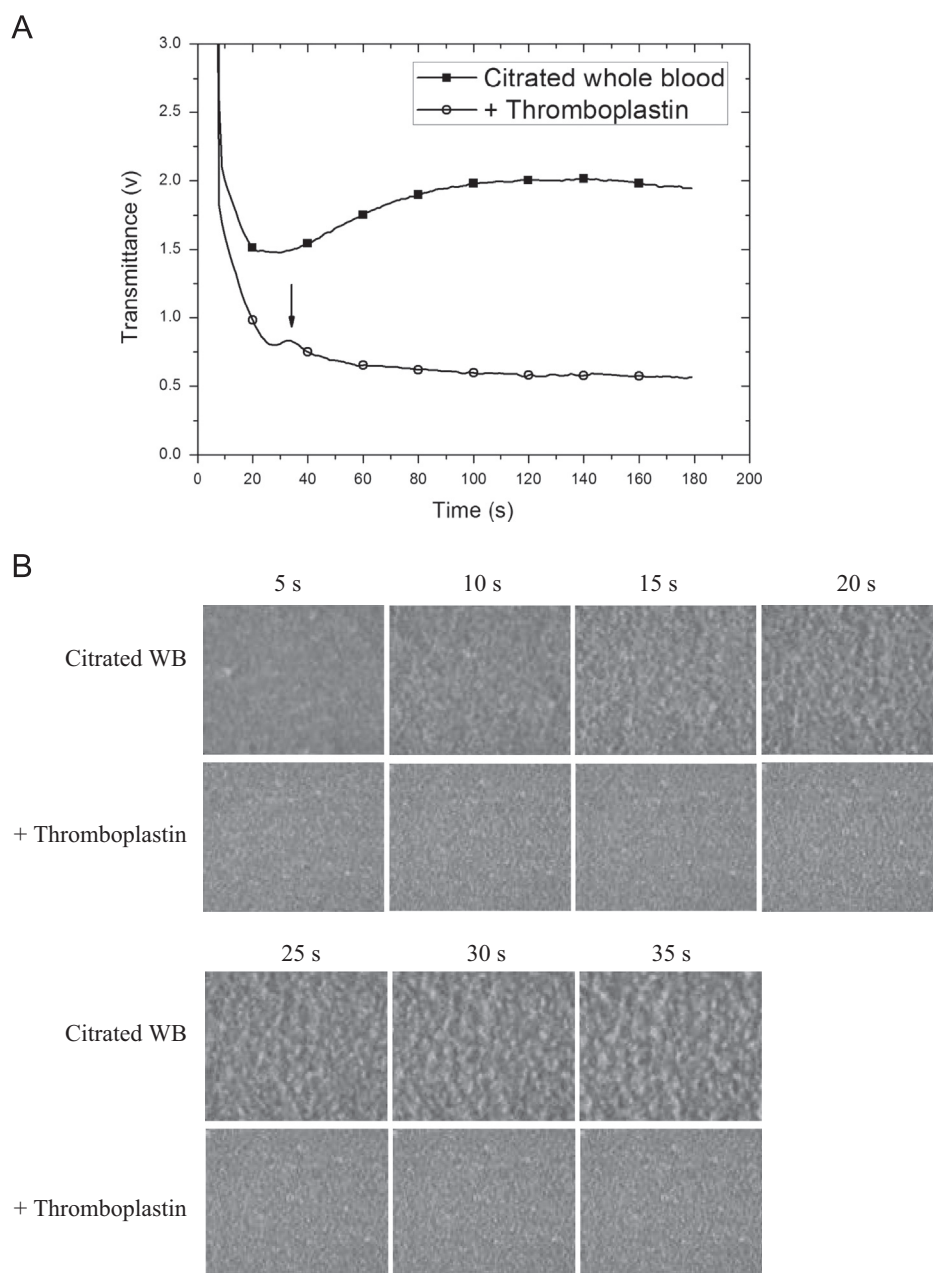


Fig. 3. Features of whole blood with and without coagulation were characterized. (A) Light transmittance signals were recorded using optically based POCD PT device from citrated whole blood and from same with added thromboplastin reagent to trigger coagulation reaction. Arrow indicated turning point of transmittance curve, associated with occurrence of clot formation, which causes decline of light transmittance. (B) RBC images of citrated whole blood and same with added thromboplastin reagent to trigger coagulation reaction captured in parallel under an optical microscope.

blood PT were further studied by analyzing the differences between the hematological parameters of POCD-detectable ($n=153$) and POCD-non-detectable ($n=14$) groups. Table 1 summarizes the results. FIB, RBC, HB, HCT and PLT were significantly higher, and RDW was significantly lower in the detectable group than in the non-detectable group, according to both nonparametric (Mann-Whitney test) and univariate binary regression statistics. The results of multivariate regression analysis further verified the association of higher FIB (odds=0.980, 95% confidence interval (CI)=0.962–0.999, $p=0.036$) and RBC (odds=0.005, 95% CI= <0.001–0.388, $p=0.017$) with a lower non-detectable incidence, and that of a higher RDW (odds=1.443, 95% CI=1.075–1.936, $p=0.015$) with a higher non-detectable incidence. The cut-off value as determined by ROC between detectable and non-detectable groups was 236.1 mg/dl for FIB ($p<0.001$), $2.89 \times 10^6/\mu\text{l}$ for RBC ($p<0.001$) and 16.8% for RDW

($p<0.001$). Since FIB is an acute phase protein that can promote RBC aggregation [21] and fibrin formation may cause the cross-linking of RBC, the relationship between FIB concentration and the maximum speed of change in transmittance (v/t), which was determined from the first derivative at the PT point, was further analyzed. The results reveal a highly positive correlation between the concentration of FIB and the maximum speed of change in transmittance (v/t) ($r=0.805$, $p<0.001$) in the POCD-detectable samples ($n=153$) (Fig. 5).

4. Discussion

The detection module, comprising an infrared LED with a wavelength of 770 nm and a photodiode (SFH213), was used to detect the change in light transmittance during blood coagulation.

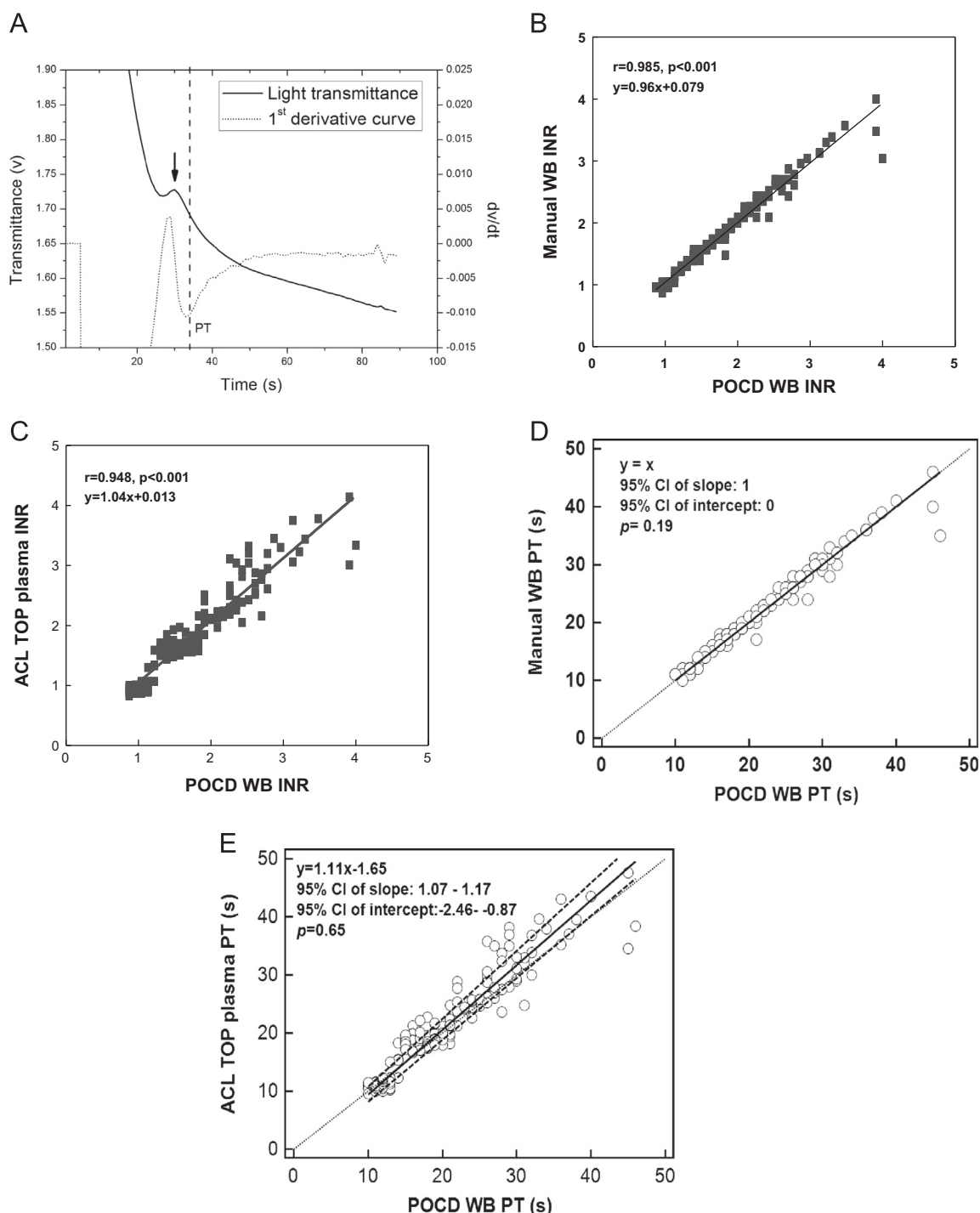


Fig. 4. (A) Whole blood PT values were determined using newly developed optical-based POCD device. First-order derivative was plotted versus a representative light transmittance of whole blood during coagulation reaction. Arrow indicates turning point of transmittance curve. Time at nadir indicated maximum rate of change of light intensity and was defined as PT (dash line). (B) Correlation of whole blood INR values obtained using POCD device with those obtained using manual method ($n=153$, $r=0.985$, $p<0.001$). (C) Correlation of whole blood INR values obtained using POCD device with plasma values obtained using the ACL TOP 700 ($n=153$, $r=0.948$, $p<0.001$). Passing and Bablok regression analysis (D) between POCD and manually obtained whole blood PT values, with a p -value of 0.19; (E) between POCD whole blood PT and ACL TOP plasma PT, with a p -value of 0.65, indicating no significant differences between each paired methods.

The POCD PT device could record very small changes in light intensity when the combination of emitting diode and sensor elements was optimized. The PT was determined from the time of the maximal rate of change in the slope of the recorded signals, and that PT information could be obtained by performing a differentiation operation. However, the basic differentiation operation whose response function $H(\omega)=j\omega$ revealed that the high-frequency components of signals and interference had strong

effects on the differentiating outcomes. Most of the interference and noise occurring at high frequencies might inhibit the determination of PT more seriously after the differentiation operation (Fig. 2B). Therefore, a high-order filter had to be implemented to suppress this unwanted interference (Fig. 2C). Additionally, as for the post-processing after microcontroller MSP430F149, FIR-type filters can further be designed with some fixed-point simulation to estimate its induced errors from the signal processing. Other

Table 1
Comparisons of hematological parameters between POCD detectable ($n=153$) and non-detectable ($n=14$) groups

	PT (s)	FIB (mg/dl)	WBC ($10^3/\mu\text{l}$)	RBC ($10^6/\mu\text{l}$)	HB (g/dl)	HCT (%)	RDW (%)	PLT ($10^3/\mu\text{l}$)
Detectable ($n=153$)								
Median (range)	19.4 (9.6–47.6)	363.3 (119.6–907.7)	7.3 (0.2–62.9)	4.15 (1.97–6.49)	12.6 (6.5–17.4)	37.2 (18.2–51.5)	14.6 (12.2–34.8)	167 (5–468)
Mean	21.3	384.9	8.3	3.99	12.3	36.2	15.6	178
Standard deviation	8.7	147.8	6.4	0.80	2.4	6.9	3.4	95
Non-detectable ($n=14$)								
Median (range)	24.1 (15.3–52.6)	162.7 (107.1–358)	9 (2.3–20)	2.63 (1.3–3.91)	9.2 (5.1–12.3)	26.8 (14.3–36)	21.5 (13.6–25.9)	74 (17–284)
Mean	26.5	176.3	9.6	2.55	9.0	26.0	20.5	92
Standard deviation	10.5	65.3	5.9	0.70	2.0	6.2	3.6	84
Mann–Whitney test								
P-value	0.067	< 0.001	no significance	< 0.001	< 0.001	< 0.001	< 0.001	0.001
Univariate binary regression								
P-value	–	< 0.001	no significance	< 0.001	< 0.001	< 0.001	< 0.001	0.003
Coefficient	–	–0.025	–	–2.371	–0.614	–0.212	0.251	–0.014
Odds	–	0.975	–	0.093	0.541	0.809	1.285	0.986
95% CI	–	0.964–0.986	–	0.031–0.280	0.397–0.736	0.729–0.898	1.121–1.473	0.977–0.995
Multivariate binary regression								
P-value	–	0.036	–	0.017	0.928	0.554	0.015	0.145
Coefficient	–	–0.020	–	–5.320	–0.211	0.519	0.367	0.012
Odds	–	0.980	–	0.005	0.810	1.680	1.443	1.012
95% CI	–	0.962–0.999	–	< 0.001–0.388	0.009–76.746	0.302–9.358	1.075–1.936	0.996–1.029
ROC curve								
P-value	–	< 0.001	–	< 0.001	–	–	< 0.001	–
Area	–	0.934	–	0.913	–	–	0.851	–
Cut-off	–	236.1	–	2.89	–	–	16.8	–

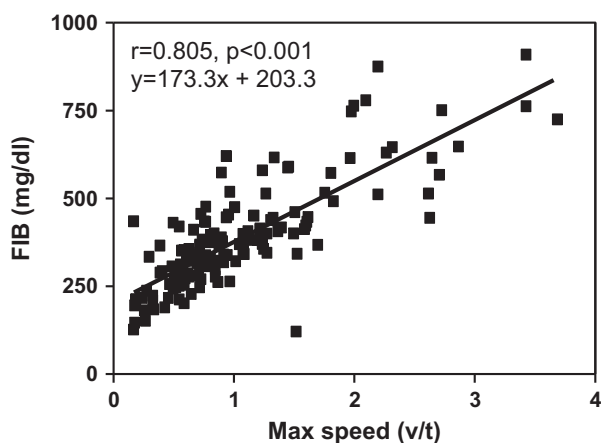


Fig. 5. Correlation of concentration of FIB with maximum speed of change in transmittance (v/t), which was calculated from first derivative plotted from results for 153 detectable samples ($r=0.805$, $p<0.001$).

feasible DSP chip can also be included to perform this task and integrated with the current POCD bioelectronic platform.

Optically based devices for measuring blood coagulation may be potentially simpler and more cost-effective than the biosensors that are based on other analytical principles. However, the presence of many RBC in a whole blood sample, which generates intense red light interference, is a major challenge to overcome. An optically based instrument, such as the ACL TOP 700, can only detect changes in scattered light during blood coagulation in plasma. In this work, a novel coagulation detector with an infrared LED as a light source was developed to enable light transmittance analyses of whole blood coagulation to be used in PT assay. Also, thermal effects are negligible, because in a temperature verification test in our lab, with an extremely low input power to an infrared LED, the temperature at the surface where the thermal sensor was attached increased by less than 0.01°C over 5 min. In fact, the detection area of the POCD device was 10 mm below the

infrared LED, and the duration of coagulation process was less than 180 s. Optimization of bioelectronic components enables that this optical biosensor can measure PT in whole blood samples, with results that are highly consistent with results obtained using the conventional manual assay for whole blood PT or the ACL TOP 700 instrument for plasma PT. Since erythrocytes exhibit pro-coagulant activity [11,22], the whole blood PT in the method herein was reasonably assumed to be slightly shorter than the plasma PT as measured using the ACL TOP 700 instrument.

Although the other optical effects due to whole blood components could not be entirely excluded, this POCD PT device exploits the dependence of the LED light transmittance on the number of RBCs in whole blood for analytical purposes, which works on the light beam passed through the sample area which is passed through the filter to the detector. A few assumptions are made to simplify the complicated heterogeneous whole blood components, including that the incident flux does not interact or affect the bio-molecules; the absorbing medium does not scatter the radiation, that is no turbidity; and the incident radiation consists of parallel rays of same wavelength. In practice of this work, in a static whole blood sample that contains anti-coagulants, individual erythrocytes reacted and aggregated, increasing light transmittance. As the sample responded to a thromboplastin reagent, blood coagulation, which formed fibrin clots, linked erythrocytes into a gel-like structure, which blocked the light transmittance. The analytic mechanism of the proposed POCD device in an RBC-dependent mode, which reconciles the finding that the fact that whole blood samples with a small amount of FIB, few RBCs and a high RDW (Table 1) do not allow the formation of a clot-induced RBC, may contribute to the failure to determine PT using the optically based POCD biosensor. Notably, the commercially available thromboplastin reagent that was used in the assay was HemosIL Recombiplastin, in which the constituent polybrene not only counteracted heparin but also increased RBC aggregation by neutralizing the zeta potential of the cell membrane, boosting the optical signal [23,24]. Notably, Dade Innovin (Siemens Healthcare, Germany) was tested in the presented system and found to

perform poorly. The Dade Innovin thromboplastin reagent contained a considerable amount of bovine serum albumin as a stabilizer, which reduced RBC aggregation, weakening the light transmittance signal. Therefore, HemosIL Recombiplastin was used as the reagent to trigger blood coagulation in the system herein.

This study echoes the two recent studies of RBC aggregation and whole blood coagulation in which transmittance or scattered intensity during coagulation was detected [25,26]. The light sources in those studies were laser diodes, which had the advantages of a high output power and narrow spectral width. However, an infrared LED (770 nm) was used in the present study because of its lower power consumption, lower cost and longer life time than the laser diode. The light output by the infrared LED nevertheless is very sensitive to the voltage across it. These features make LEDs more favorable for portable devices. Since the signal that was generated by the infrared LED herein was weak, an amplifier and two analog filters were required to block out noise and strengthen the signal, as was demonstrated above.

This POCD device directly responded to the formation of fibrin during coagulation. A previous study showed that the formation of fibrin during coagulation contributed to the turbidity of plasma, increasing scattered light intensity [27]. In that study, a standard curve of the degree of intensity change *versus* FIB was plotted to calculate the concentration of functional FIB in samples. The results in Fig. 5 that the maximum speed of change in transmittance (v/t) obtained using the POCD device herein were highly correlated with the FIB concentrations, suggesting that FIB titers could also be quantified in whole blood samples.

Several POCD PT/INR devices, including CoaguChek (F. Hoffman-LaRoche, Basel, Switzerland), INRatio Monitor (Alere, Reno, Nevada, USA) and ProTime Microcoagulation System (International Technidyne Corporation, Edison, New Jersey, USA), were developed to provide rapid self-testing results in the last decade [28]. The review of cases found that PT/INR self-testing and warfarin self-dosing could either lower risk of death or lower rate of blood clotting [28,29]. The current commercialized portable PT devices are expensive ranging from 1500 to 2000 USD [28]. This optical-based PT device was estimated in reducing costs of the currently available POCD coagulometers to 30–50%, holding a great promise to enter the market. Furthermore, this optical-based PT might provide the additional benefit of FIB concentration test since other POCD coagulometers on the market do not offer this measurement.

5. Conclusion

The novel portable optical coagulation detector that was presented herein can measure PT in whole blood, yielding results that are highly consistent with those obtained using two well-established methods, suggesting that the optically based methodology has potential when exploited a POCD blood coagulation device.

Competing interests

There are no competing interests.

Funding

This study was supported by the National Science Council of Taiwan under grant number NSC99-2622-B-006-002-CC1 and NSC100-2622-B-006-005-CC1. The funding source had no involvement in study design; in collection, analysis and interpretation of data; in writing of the report; and in the decision to submit the article for publication.

Acknowledgments

The authors would like to thank the Hematological Laboratory of National Cheng Kung University Hospital for the collection of blood samples and the National Science Council of Taiwan for the financial supports (NSC99-2622-B-006-002-CC1 and NSC100-2622-B-006-005-CC1). Ted Knoy is appreciated for his editorial assistance.

References

- [1] S. Lison, M. Spannagl, *Wien. Med. Wochenschr.* 161 (2011) 58–62.
- [2] G.J. Despotis, L.T. Goodnough, *Ann. Thorac. Surg.* 70 (2000) S20–S32.
- [3] W. Korte, K. Gabi, M. Rohner, A. Gahler, C. Szadkowski, T.W. Schnider, J. Lange, W. Riesen, *Thromb. Haemost.* 94 (2005) 211–215.
- [4] J.B. MacLeod, M. Lynn, M.G. McKenney, S.M. Cohn, M. Murtha, *J. Trauma* 55 (2003) 39–44.
- [5] V.L. Ng, *Clin. Lab. Med.* 29 (2009) 265–282.
- [6] M. Peck-Radosavljevic, *Alimentary Pharmacol. Ther.* 26 (Suppl 1) (2007) 21–28.
- [7] S.W. Lin, Y.C. Chuang, Y.S. Lin, H.Y. Lei, H.S. Liu, T.M. Yeh, *J. Infect.* 64 (2012) 325–334.
- [8] C.P. Hayward, K.A. Moffat, E. Plumhoff, E.M. Van Cott, *Am. J. Hematol.* 87 (Suppl 1) (2012) S45–S50.
- [9] C. Solano, P. Zerafa, R. Bird, *Int. J. Lab. Hematol.* 33 (2011) 67–78.
- [10] F. Fischer, A. Appert-Flory, D. Jambou, P. Toulon, *Thromb. Res.* 117 (2006) 721–729.
- [11] H. Iwata, M. Kaibara, *Blood Coagulation Fibrinolysis* 13 (2002) 489–496.
- [12] B. Ramaswamy, Y.-T.T. Yeh, S.-Y. Zheng, *Sensors Actuators B: Chem.* (2011).
- [13] A. Ur, *Nature* 226 (1970) 269–270.
- [14] L. Muller, S. Sinn, H. Drechsel, C. Ziegler, H.P. Wendel, H. Northoff, F.K. Gehring, *Anal. Chem.* 82 (2010) 658–663.
- [15] G. Guhr, R. Kunze, G. Martin, H. Schmidt, M. Weihnacht, S. Gehrlich, G. Siegert, in: *Proceedings of IEEE Ultrasonics Symposium*, 2006, pp. 544–547.
- [16] T.P. Vikiing, K.M. Hansson, J. Benesch, K. Johansen, M. Ranby, T.L. Lindahl, B. Liedberg, I. Lundstrom, P. Tengvall, *J. Biomed. Opt.* 5 (2000) 51–55.
- [17] A.X. Zheng, J.R. Wang, J. Li, X.R. Song, G.N. Chen, H.H. Yang, *Biosens. Bioelectron.* 36 (2012) 217–221.
- [18] T.K. Amukele, C. Ferrell, W.L. Chandler, *Am. J. Clin. Pathol.* 133 (2010) 550–556.
- [19] S. Franco, *Design with Operational Amplifiers and Analog Integrated Circuits*, third ed., McGraw-Hill, New York, USA, 2001.
- [20] S. Franco, *Electric Circuits Fundamentals*, Oxford University Press, UK, 1995.
- [21] M. Singh, S. Shin, *Indian J. Exp. Biol.* 47 (2009) 7–15.
- [22] H. Iwata, M. Kaibara, N. Dohmae, K. Takio, R. Himeno, S. Kawakami, *Biochem. Biophys. Res. Commun.* 316 (2004) 65–70.
- [23] H.E. Davis, M. Rosinski, J.R. Morgan, M.L. Yarmush, *Biophys. J.* 86 (2004) 1234–1242.
- [24] M.W. Rampling, H.J. Meiselman, B. Neu, O.K. Baskurt, *Biorheology* 41 (2004) 91–112.
- [25] H. Lim, J. Nam, S. Xue, S. Shin, *Clin. Hemorheology Microcirc.* 47 (2011) 211–218.
- [26] M. Faivre, P. Peltie, A. Planat-Chretien, M.L. Cosnier, M. Cubizolles, C. Nougier, C. Negrier, P. Pouteau, *J. Biomed. Opt.* 16 (2011) 057001.
- [27] E. Rossi, P. Mondonico, A. Lombardi, L. Preda, *Thromb. Res.* 52 (1988) 453–468.
- [28] H.E. Bloomfield, A. Krause, N. Greer, B.C. Taylor, R. MacDonald, I. Rutks, P. Reddy, T.J. Wilt, *Ann. Intern. Med.* 154 (2011) 472–482.
- [29] H.I. Bussey, M. Bussey, *Circulation* 126 (2012) e52–e54.