An optofluidic point-of-care device for quantitative investigation of erythrocyte aggregation during coagulation

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ABSTRACT

Coagulation, the process leading to clot formation with the interplay of blood constituents, is a self-regulating mechanism, requiring attentive and periodic monitoring for numerous clinical cases. Erythrocyte aggregation (EA) is a characteristic behaviour of erythrocytes forming reversible clumps especially in vitro at low shear rates. The effect of EA during coagulation is overlooked in whole blood (WB) clotting assays, and the relationship between the two mechanisms is not well understood. We present an optofluidic point-of-care device enabling quantitative investigation of EA from 50 μ l WB during the coagulation process. Not only did we explain the coagulation mechanism considering EA, but we also demonstrated coagulation time measurement from optical EA analysis. The device consists of a disposable cartridge and a handheld analyzer containing a pinch valve for fluid motion and optics for transmitted light measurement. Following the sample introduction and cessation of the valve operation, the optical signal is the lowest due to shear-induced cell disaggregation. Then, the signal increases due to EA until reaching a peak, indicating blood clotting. The working principle was proven through clinical tests for prothrombin time measurement. In addition to revealing the relation between coagulation and aggregation, this device is promising for rapid WB coagulation time measurement.

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1. Introduction

Blood coagulation is a process involving the interaction of various plasma proteins, coagulation factors, and platelets to eventually form stable fibrin strands for the prevention of hemorrhage [1]. This process consists of two pathways, named intrinsic and extrinsic, which are triggered by different activators, but lead to a common pathway. The common pathway ends with the conversion of fibrinogens into fibrin clots [2]. In vitro measurement of coagulation time is required for individuals who are under operational evaluation [3,4]. Also, patients who are diagnosed with or have the risk for coagulation disorders, embolism, stroke, heart attack, and other cardiovascular diseases (CVD) need coagulation time measurement [5,6]. Anticoagulant therapy, the prescription of blood-thinning medication, is the common strategy to combat coagulation related disorders and to regulate clotting status of patients [7]. The traditional approach for coagulation time monitoring is the use of benchtop instruments in clinical settings. This necessitates periodic hospital visits, which is generally costly and time-consuming.

It is even potentially life-threatening if hospital visit is delayed. This is especially a concern for patients receiving anticoagulant therapy as the medications have narrow therapeutic windows [8].

The use of point-of-care (POC) technologies for coagulation time measurement aims to alleviate the drawbacks of the conventional equipment [9]. There are portable coagulation analyzers in the market that facilitate self-monitoring and reduce turnaround times [10]. These devices employ various measurement principles such as optical (e.g., detection of change of particle oscillation frequency by light reflection), mechanical (e.g., viscosity change detection by cantilever or magnet position), or electrochemical (e.g., amperometric detection using substrate molecules) [11]. From a finance point of view, the high price tag for both the main device (purchased once) and the disposable test cartridges (purchased repeatedly; generally having complicated fabrication) is a drawback. Health insurances in many countries (e.g., the authors' country of origin) do not cover the purchase of such costly equipment for home use. This places such devices beyond the reach of many people who, therefore, visit hospitals/anticoagulation clinics for periodic tests. There are also studies in the literature using microsystems technology for coagulation time measurement. Examples include paper-based [12], cantilever-based [13], impedimetric [14], quartz crystal microbalance (QCM) [15], surface plasmon resonance (SPR)

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[16], migration distance-based [17], and elastometry-based [18]. The practical issues associated with these studies are limited quantitative measurement, dependence to cleanroom fabrication, need for electronic integration, set-up complications, necessity for plasma separation, standardization, and viscosity dependency, respectively, hindering their adaptability as common techniques. The two biological samples analyzed with these platforms are whole blood (WB) and plasma. The use of WB for coagulation assays offers ease-of-use, low sample requirement, and short turnaround times in comparison to the use of plasma. On the other hand, current WB coagulation assays fall short of understanding the effect of erythrocyte aggregation (EA), which is an inherent mechanism affecting the assay kinetics. Herein, we explored this intricate relationship between coagulation and EA using a real-time optical analysis device.

Erythrocytes constitute reversible face-to-face aggregates under stationary conditions or at low shear rates in blood [19,20]. The aggregation of erythrocytes is governed by cellular surface properties (e.g., surface charge), plasma protein concentrations (e.g., fibrinogen), and shear rate that can disaggregate the cell clumps once applied above a threshold [19]. Normally, erythrocytes stay dispersed in vivo due to the shear rate created by blood flow, but inadequate shear results in aggregation related disorders such as hypertension and sepsis [21,22]. Measurement of erythrocyte aggregability is, therefore, of clinical value. The in vitro measurements are performed by aggregometers that employ a variety of mechanical means for cell disaggregation and photometric methods for light quantification [23–25]. Several attempts have previously been made to monitor coagulation through the aggregation phenomenon [26–29]. However, the fundamental mechanisms for the relationship between coagulation and aggregation have remained unclear, and the studies lacked clinical verifications.

We present a portable lab-on-a-chip (LOC) device for the optical analysis of the EA kinetics during the coagulation process. The device is employed for two main purposes: (i) measuring coagulation time from a drop of WB (50 μ l) in less than 2 min using EA and (ii) demonstrating the presence/effect of EA in a coagulating blood to understand the fundamental relationship between these two mechanisms. The device is used for prothrombin time (PT) measurement. The measurement principle is based on the optical investigation of EA during coagulation by illuminating the blood sample with near-infrared light and recording the transmitted light intensity. We explained the interrelation between these two physiological phenomena, coagulation and EA, which have long been studied independently of each other. Microscopy observations during the coagulation provided in-depth understanding of the actual physical correspondence of the optical measurement signals, supporting the results obtained from our platform. In addition, the clinical tests allowed comparison of the device with a conventional benchtop equipment, which showed the potential of the study beyond research laboratories.

2. Experimental section

2.1. Materials

Ethical approvals were attained from the Ethics Committees of both Bilkent and Yildirim Beyazit Universities. Prior informed consent was obtained from the volunteers whose health conditions were unknown to us. 21 volunteers (11 males, 10 females) were chosen, ensuring that a wide PT range was covered. 3 ml venous WB was acquired from the volunteers into sodium citrate containing vacuum tubes, and all the experiments were performed at 37 °C within the same day of blood draw. The benchtop equipment available in the hospital for the conventional measurements

was Sysmex CA-1500 System (Siemens, Germany). Thromborel S Human Thromboplastin (Siemens, Germany) reagent was used for the PT tests. 7.6 mg Thromborel S Human Thromboplastin is dissolved in 100 μ l deionized (DI) water by gentle mixing at 24 °C. 25 μ l dissolved reagent is pipetted into the cartridges which are then kept at –80 °C for 2 h. The reagents in the cartridges are then lyophilized in a freeze-dryer (Labconco, US) for 6 h. Following, the cartridges are stored at 4 °C with a desiccator and used within 6 h.

Human fibrinogen conjugate labeled with Alexa Fluor 488 (Molecular Probes, US) was prepared by reconstituting the supplied sample in 0.1 M sodium bicarbonate (pH 8.3) at room temperature. The stock solution is divided into aliquots and stored at –20 °C. Prior to use, the solution is brought to room temperature. The required amount (1 μ l, 2 μ l, 3 μ l, or 4 μ l) is then gently mixed with 100 μ l WB and kept intact at room temperature for 30 min.

70 kDa dextran is purchased from Sigma-Aldrich and stored at room temperature. Three microcentrifuge tubes are filled with 200 μ l WB. To prepare WB samples with 5 mg/ml, 10 mg/ml, and 15 mg/ml dextran concentrations, 1 mg, 2 mg, and 3 mg dextran are added to the tubes and dissolved by gentle mixing.

2.2. Cartridge fabrication

The cartridge design was drawn using a CAD software (Autodesk, AutoCAD 2016). For initial prototyping, the cartridge was fabricated out of poly methyl methacrylate (PMMA) using CO₂ ablation [30]. Following successful tests, a metal mold was made for the final design, and cartridges were mass-produced out of polycarbonate (PC) using injection molding to minimize chip-to-chip variations. We have two cartridge designs. For the optical measurements, the cartridge has a single channel of 1 mm depth (d), 1 mm width (w), and 50 mm length (l) with an inlet and outlet. For simultaneous optical measurements and microscopic observations, the cartridge has two adjacent channels (a deep and a shallow) sharing a single inlet and outlet. Here, optical measurements are performed in the deep channel (1 mm depth). Since small channel depths are required for clear top-view microscopic observation of individual cells due to the high population of erythrocytes in WB (around 5 million/ μ l) [31], the shallow channel (150 μ m depth) is employed to visualize the erythrocytes during the optical measurements. To apply the same shear rate on the cells in both channel segments during the disaggregation phase, the following equation should be satisfied based on the Hagen-Poiseuille law: $w_{\text{deep}}d_{\text{deep}}^2 = w_{\text{shallow}}d_{\text{shallow}}^2$. Since $d_{\text{deep}} = 1$ mm and $d_{\text{shallow}} = 150$ μ m, we let $w_{\text{deep}} = 1$ mm and $w_{\text{shallow}} = 40$ mm. Also, considering the dimensions of the device, we let $l_{\text{deep}} = 50$ mm and $l_{\text{shallow}} = 10$ mm.

2.3. Measurement platform

The platform is illustrated in Fig. 1. It consists of a single-use transparent cartridge and a portable analyzer. The cartridge contains a single channel with a depth and width of 1 mm. This deep channel allows intense interaction between the erythrocytes and the incident light. The analyzer consists of four modules: (I) cartridge fixation module, (II) shear force exertion module, (III) optical measurement module, and (IV) electronic control module. The first module is a black PMMA based cartridge holder employed to tightly fix the cartridge once inserted into the analyzer. This holder also blocks the transmission of the background light to the photodetector, caused by ambient lighting. The second module is a solenoid pinch valve (EW-98302-06, Cole-Parmer, US) that is programmed to generate 10 s-long back and forth motion of the blood sample in the cartridge. The movements of the sample with a stroke distance of 5 mm exert a shear rate on the erythrocytes above the threshold for complete erythrocyte disaggregation. In addition to the disaggregation of the cell clusters, these motions facilitate

Fig. 1. a. Schematic illustration and b. photograph of the measurement platform. The coagulation activator is lyophilized in the cartridge. The pinch valve disaggregates the erythrocyte clusters and mixes whole blood with the activator reagent. The LED illuminates the sample, and the photodetector records the transmitted light.

homogenous mixing of the blood sample with the coagulation activator reagent lyophilized in the cartridge. The measurement principle is based on the optical investigation of the erythrocyte aggregation during the coagulation onset. The optical measurement module, therefore, consists of a light-emitting diode (LED) (VSMG2700, Vishay, US) and a photodetector (VENT4700, Vishay, US). The LED illuminates the blood sample in the near-infrared band with 830 nm wavelength, at which hemoglobin and therefore WB absorption is minimum, whereas transmission of light is enhanced [31,32]. The photodetector collects the light transmitted through the blood sample. The electronic control module consists of a printed circuit and a microprocessor (IOIO-OTG, SparkFun, US). The circuit comprises a transimpedance functioning as a switch to control the pinch valve, a temperature controller to fix the test temperature at 37 °C, and a transimpedance amplifier – lowpass filter pair for the current-to-voltage conversion of the photodetector output. The microprocessor controls the assay parameters through a custom-developed Java-based software to set the LED intensity, shear force exertion time, temperature, and the total test duration.

3. Results and discussion

3.1. Optical analysis of erythrocyte aggregation

A cartridge takes 50 μ l citrated WB that starts to coagulate only when mixed with an activator reagent. The initial experiment was carried out using citrated WB without using any reagent in the cartridge. Following the introduction of the blood sample to the channel, the solenoid pinch valve was operated for 10 s to completely disaggregate the erythrocyte clusters. Then, the valve operation was terminated to let the erythrocytes re-aggregate.

Fig. 2. a. Measurement of the transmitted signal intensity of the whole blood sample using the coagulation platform when the cartridge was tested without an activator reagent and with PT reagent. The inset shows the schematic cartridge and a photograph of the lyophilized reagent within the channel. b. The transmitted signal intensity when human thromboplastin was lyophilized prior to the test. The signal profile is divided into four phases, and the maximum intensity level corresponds to the coagulation time.

During this re-aggregation process, the LED illuminated the blood sample while the photodetector measured the intensity of the transmitted light. The black signal profile in Fig. 2a shows the measured signal intensity for this case. At the end of the pinch valve operation (time: 0th s), the erythrocytes are all dispersed. Since each individual cell serves as a scattering source, the intensity is at the lowest. As time progresses, erythrocytes aggregate, and, therefore, the signal intensity increases until complete aggregation of the erythrocytes. With the complete aggregation, the signal intensity stays constant.

The coagulation cascade consists of an intrinsic and an extrinsic pathway directing to a common pathway that ends with the formation of fibrin clots. The latter is triggered by tissue factor, and prothrombin time (PT) is used for the evaluation of the extrinsic pathway. Human thromboplastin was lyophilized in a cartridge to trigger the extrinsic pathway for PT measurement. Fig. 2a also shows the measured signal intensity for this case (red signal). Here, the signal intensity increases until a maximum value and decreases back. On the other hand, for the cartridge without any activator reagent, the signal intensity continuously increases due

Fig. 3. The role of fibrinogen in coagulation and aggregation. Four whole blood samples with varying fluorescence labeled fibrinogen concentrations were prepared and introduced to cartridges with human thromboplastin. a. The sample-filled cartridges were observed using fluorescence microscopy at 40 $\times$. The illuminating regions correspond to fibrin networks formed because of coagulation. b. The sample-filled cartridges were tested using the platform. The inset shows the initial 10 s for each case. The rate of aggregation increases with the increasing fibrinogen concentration.

to the aggregation of erythrocytes (black signal). Both cases were repeated three times, and only one test was shown here for each case since the others had the same trend. It should also be noted that the absorption/transmission spectra of both the coagulating and non-coagulating WB are determined by the erythrocytes in the near-infrared band [33–35]. It, therefore, implies that the coagulation process affects the aggregation of the erythrocytes, which can optically be observed from the recorded signal profile.

Fig. 2b shows the signal profile obtained when human thromboplastin is lyophilized in the cartridge. This signal is divided into four distinct phases. The initial phase corresponds to the 10 s-long valve operation that induces shear rate on the cells for disaggregation. At the end of this phase, the signal intensity is at its lowest, confirming the complete disaggregation. Following this phase, phase-1 witnesses an increase in the signal intensity for around 30 s. Like the no coagulation case, this increase is due to the aggregation of the erythrocytes. No more increase beyond the maximum intensity is then observed. This is caused by the formation of fibrin clots preventing further clumping of the erythrocytes and, therefore, ends

Fig. 4. The employment of dextran to facilitate the aggregation process in phase-1 for the coagulating blood sample. a. Four whole blood samples with varying dextran concentrations were prepared and introduced to human thromboplastin lyophilized cartridges. Also, whole blood sample with no dextran was introduced to an empty cartridge. Optical measurements were performed with the platform. b. Initial 10 s of the optical signals given in part a.

the aggregation process. The time corresponding to the maximum intensity is named as T_{max} . The signal intensity decreases in phase-2 due to the clot contraction, a process that takes place right after the clot formation [36,37]. Following the end of phase-2, the signal intensity shows a constant trend in phase-3, suggesting that the clot is completely stabilized.

3.2. Role of fibrinogen in coagulation and aggregation

When no activator reagent was used in the cartridge, the blood sample does not coagulate but only experiences erythrocyte aggregation. The clumping of the erythrocytes progressively decreases the surface area of the cells, resulting in an ongoing increase in the transmitted signal intensity. When human thromboplastin was used in the cartridge to trigger the coagulation, we observe a similar increasing trend in the optical signal intensity until reaching a peak point.

Referring to the initial ~30 s of the red and black signals in Fig. 2a, at the start, the signal intensities are the same (at the lowest) due to the prior valve operation. Then, during phase-1, there is a dif-

Fig. 5. Comparison of the platform with the conventional equipment for PT measurement. a. Comparison with $n = 21$ whole blood samples. Goodness of the fit is 0.97 using linear regression. b. Repeatability analysis with $n = 6$ samples. Each sample is tested three times. Error bars indicate standard deviations.

ference in the intensity increase, therefore in the aggregation rate, between these two cases. When the cartridge is free of any activator, the aggregation rate of the sample (black) is higher than that of the coagulating sample (red). This is mainly due to the fibrinogen activity in the samples.

Fibrinogen, a glycoprotein in plasma, plays an integral part in the coagulation process [2]. It is converted to a fibrous protein named fibrin to form stable clots. Fibrinogen also takes role during EA and is the most essential plasma protein for this process [38,39]. According to the bridging model, fibrinogen proteins adsorb on the cell surfaces bringing adjacent erythrocytes in contact [40].

To show the equally key role of fibrinogen in coagulation and aggregation, fluorescent labeled human fibrinogen was employed. Four WB samples with different fibrinogen concentrations were prepared (see Materials). Each sample was introduced to a cartridge with human thromboplastin, and the optical measurements were performed. Following the measurements, the cartridges were observed under an upright fluorescence microscope (Zeiss, Germany) at $40\times$ magnification. Fig. 3a shows the microscopic images for the four samples. The fluorescence regions in each image are the fibrin networks lighting up due to the polymerized dye-labeled fibrinogens. As the fibrinogen concentration in WB increased from 1% up to 4%, the fluorescence illumination area also proportionally increased. It suggests that with the increasing fibrinogen concentration, more fibrinogens took part in the coagulation process, and, therefore, more fibrin strands were formed. Fig. 3b shows the optical signal intensities of the four blood samples measured with the analyzer. The inset shows the initial 10 s following the complete disaggregation. As the fibrinogen concentration increases, the rate of increase in intensity, namely the rate of aggregation, also increases. It implies that the addition of more fibrinogens facilitates the aggregation of erythrocytes.

When there is no activator in the cartridge, the blood sample does not coagulate, and we observe only the aggregation of the erythrocytes (black signal, Fig. 2a). Here, fibrinogens only take role during the aggregation process. On the other hand, the presence of the activator in the cartridge triggers the coagulation cascade (red signal, Fig. 2a). In this case, fibrinogens are simultaneously appealed to take part in the coagulation and the aggregation processes. This splits their energy to two distinct roles that would otherwise play role only in EA. Since fibrinogen concentrations for both cases are the same at the start, the number of fibrinogens taking part in the

aggregation process and/or the fibrinogens' aggregation efficiency decrease for the coagulating blood sample in phase-1.

To further confirm these findings, we employed an EA inducer molecule, dextran [41], to enhance the aggregation rate that is affected by the fibrinogens engaged in the coagulation process. Firstly, citrated WB with no dextran was introduced to an empty cartridge, and optical measurement was performed with the analyzer. As shown in Fig. 4a (black signal), there is always an increase in intensity due to uninterrupted EA. Secondly, we prepared four WB samples with dextran concentrations of 0 mg/ml, 5 mg/ml, 10 mg/ml, and 15 mg/ml (see Materials). Citrated WB with no dextran was introduced to a cartridge having human thromboplastin. In this case (red signal, Fig. 4a), the intensity increase was observed with a much lower rate compared to the first case. Then, citrated WB samples with different dextran concentrations were introduced to human thromboplastin lyophilized cartridges. Fig. 4a shows the signal intensities of the measurements for all the cases, and Fig. 4b illustrates the first 10 s of each signal for ease of comparison. These results show that the presence of dextran supports the aggregation process and increases the aggregation rate in phase-1. As shown in Fig. 4b, higher dextran concentration increases the aggregation rate and, therefore, compensates for the fibrinogen deficiency of the coagulating samples.

3.3. Comparative coagulation time measurements

For the coagulating blood sample, the transmitted signal intensity initially increases due to EA and reaches a maximum intensity value. The time when the signal is at the peak is named as T_{max} . The observation that the intensity of the non-coagulating sample increases but that of the coagulating sample does not beyond T_{max} proposes that the EA in the coagulating sample stops at T_{max} . In other words, no further aggregation at and beyond T_{max} stops further intensity increase. We suggest that the reason preventing further aggregation is the fibrin strands that wrap up the erythrocytes and form the clotting at T_{max} . Therefore, T_{max} is the time when the blood coagulation occurs.

To verify that T_{max} is indeed an indication of the coagulation time, specifically prothrombin time (PT), comparative tests were performed at a hospital. First, the PT values of 21 volunteer WB samples were measured using the conventional gold standard method. Afterwards, 50 μ l from each WB sample was introduced into a

cartridge, and optical measurements were performed using our analyzer. $T_{\max}$ values were measured from the transmitted signal intensities using a peak detection algorithm in MATLAB. Fig. 5a shows the regression analysis for the comparison of the conventionally measured PT values (x-axis) and the $T_{\max}$ values measured by our system (y-axis). A linear fit was used to find the linear equation for the $T_{\max}$, and the correlation coefficient (R^2) of the regression fit was calculated as 97%, demonstrating the high correlation between the measurement platforms. Then, 6 WB samples with PTs in between 10–15, 15–20, 20–25, 25–30, 30–35, 35–40 s ranges were chosen. The samples were tested three times with the analyzer for repeatability analysis, and three $T_{\max}$ values for each volunteer sample were averaged. Fig. 5b shows the mean and standard deviation (SD) of the averaged $T_{\max}$ values measured by our system (y-axis). The SD for the sample in the healthy range was around 2.5 s, whereas it was around 4 s for the other samples. These regression and repeatability analyses were promising for initial point-of-care PT measurements, and the platforms has a potential for commercialization after clinical trials for both venipuncture (venous) and fingerprick (capillary) samples.

3.4. Simultaneous optical measurement and microscopic observation

We also aimed to observe a blood sample under an optical microscope while measuring the optical transmission signal to demonstrate the actual physical correspondence of the optical signals. Two cartridges were employed for the observation of erythrocytes for coagulating and non-coagulating blood samples as schematically shown in Fig. 6a inset. These cartridges were uniquely fabricated to monitor the erythrocytes in real-time under a microscope from the shallow channel segment as well as simultaneously obtaining optical transmission signal from the deep channel segment. Since the erythrocyte population in WB is high, diluted WB (hematocrit: 5%) was used to distinguish individual cells within the sample. We preferred lower concentration human thromboplastin (19 g/l) as an activator reagent for a slower coagulation process to have more detailed cell visualization. 4 μ l reagent solution was added into the inlet of one of the cartridges. To ensure the same dilution ratio, 4 μ l PBS was added into the inlet of the other cartridge. Following the introduction of the reagent solution or PBS, the blood samples were gently mixed with the reagents for 3 s at the inlets and pipetted into the cartridges for real-time observation under a microscope at 40x magnification during the optical measurements.

The “no coagulation” case in Fig. 6b represents the non-coagulating blood sample, whereas the “coagulation” case in Fig. 6b represents the coagulating blood sample. At time: 0 min, the cells were all disaggregated in both cases. At the “no coagulation” case, the erythrocytes started to partially aggregate as time passed, and they completely aggregated at time: 6 min due to no internal (e.g., coagulation) or external (e.g., shear rate) intervention on them. At the “coagulation” case, the erythrocytes started to aggregate with a lower rate, compared to the “no coagulation” case, due to the onset of the coagulation. The aggregation process was clearly observed from the 0th min (b_1) to the 3rd min (b_2). Then, aggregation hardly progressed, and, finally, the cells were all packed at the 6th min (b_3). This demonstrates that the coagulating blood sample coagulated at the 6th min. This was also verified in Fig. 6a showing the transmitted signal intensity for the coagulating blood sample that reaches its maximum in around 350 s ($T_{\max} = 350$ s).

Fig. 6. a. Measurement of the transmitted signal intensity for a coagulating whole blood using the point-of-care platform. The maximum signal intensity is reached in 6 min. The inset is the schematic representation of the simultaneous optical measurement and microscopic observation. b. Real-time top-view observation of the non-coagulating (a_1 – a_3) and coagulating (b_1 – b_3) blood samples under an optical microscope. Erythrocytes completely aggregate in the former sample at the end of the 6th min without any interruption. Erythrocytes partially aggregate in the latter sample, followed by the prevention of the aggregation due to the coagulation of the sample.

4. Conclusion

We presented a portable lab-on-a-chip device that enables optical investigation of EA during coagulation for demonstrating the interrelation between aggregation and coagulation processes as well as measuring WB coagulation time using a novel methodology employing EA mechanism. The device optically investigates the aggregation of the erythrocytes and extracts the time for the formation of fibrin networks hence the time of coagulation. The assay was shown to be responsive to the evaluation of the extrinsic pathway of the coagulation cascade (prothrombin time). It requires only 50 μ l WB and is completed in less than 5 min. The test results demonstrated the high correlation of the platform with a conventional benchtop equipment. The role of fibrinogen proteins in the aggregation and coagulation processes and therefore on the measured optical signals was shown through fluorescence imaging of labeled fibrin strands as well as using dextran as an aggregation inducer in the blood samples. This sample-in-result-out platform is a potential candidate for periodic, facile, low-cost, rapid, and low sample

volume measurement of coagulation time for both lab-based and point-of-care use.

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