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FLUORESCENCE-BASED BLOOD COAGULATION ASSAY DEVICE FOR MEASURING ACTIVATED PARTIAL THROMBOPLASTIN TIME.

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ABSTRACT

The measurement of blood clotting time is important in a range of clinical applications such as assessing coagulation disorders and controlling the effect of various anticoagulant drug therapies. Clotting time tests essentially measure the onset of clot formation which results from the formation of fibrin fibers in the blood sample. However, such assays are inherently imprecise due to the highly variable nature of the clot formation process and the sample matrix. This work describes a clotting time measurement assay which uses a fluorescent probe to very precisely detect the onset of fibrin clot formation. It uses a microstructured surface which enhances the formation of multiple localized clot loci and which results in the abrupt redistribution of the fluorescent label at the onset of clot formation in both whole blood and plasma. This methodology was applied to the development of an activated partial thromboplastin time (aPTT) test in a lateral flow microfluidic platform and used to monitor the effect of heparin dosage where it showed linearity from 0 to 2 U/mL in spiked plasma samples (R^2=0.996, n=3), correlation against gold standard coagulometry of 0.9986 and correlation against standard hospital aPTT in 32 patient samples of 0.78.

INTRODUCTION

The activated partial thromboplastin time (aPTT) test was originally developed for screening for coagulation factor deficiencies and substituted the older partial thromboplastin time test [1]. Classically, the analysis procedure consists of two steps; the first is the incubation of phospholipids and contact activator with a citrated plasma sample to activate Factors XI and XII. The second is the recalcification of the sample to induce fibrin clot formation. Materials used as contact activators are kaolin, silica or ellagic acid. The preincubation step – usually three minutes at $+37°C$ – initiates contact activation in which factors XII and XI are activated by prekallikrein and high-molecular weight kininogen, facilitated by the phospholipids. The phospholipids were originally purified from brain tissue and made devoid of tissue factor, hence the term partial thromboplastin. By adding adsorbed plasma to the aPTT assay one may diagnose specific factor deficiencies. aPTT is dependent on the intrinsic pathway of the coagulation system, i.e. factors VIII, IX, XI, XII, prekallikrein and high-molecular weight kininogen and factors of the common pathway, i.e. fibrinogen, prothrombin, factor V and X. Factors VII and XIII have no influence at all on the aPTT. aPTT is still the most used screening test for deficiencies in coagulation factors, but is now also used to monitor therapy with unfractionated heparins and to detect lupus anticoagulant.

Heparin is a powerful blood anticoagulant essential for open heart surgery, organ transplants, bypass surgery, and in thrombosis prophylaxis such as the prevention and treatment of recurrent venous thromboembolic disease [2,3]. Heparin exerts its anticoagulant activity by reversibly binding to antithrombin (AT) naturally occurring in the blood, and accelerating its ability to neutralize thrombin, FXa, IXa, XIa, XIIa and plasmin [4], where thrombin and FXa are the most sensitive to inactivation by the heparin-AT complex [5]. Dosage requirements for full-dose heparin therapy vary widely between individuals and type of therapy. Inappropriate dosing can result in thrombosis or bleeding which are the most common side effects of heparin. Standard therapy for venous thromboembolism is based on the aPTT and the clotting time (CT) value returned should be $1.5 – 2.5$ times that of the control [6]. However, the dose of heparin varies widely depending on the local reagent-instrument combination. Another method is the calculation of heparin units per kg of body weight. However, aPTT is still monitored every six hours to maintain it within the suggested range as indicated above [7]. An

alternative assay is the anti-FXa test which is useful for patients with prolonged baseline aPTT due to the presence of lupus anticoagulants. Activated clotting time (ACT) is also an established approach [8] for monitoring of heparin therapy, but only useful at high concentrations, where the aPTT response is non-linear [9,10] (above 1-2 U/mL). Despite the excellent anticoagulation effect of heparin, accurate monitoring is critical to ensure patient safety.

A number of aPTT monitoring devices have been developed and are currently available on the market, e.g., Hemochron® Jr aPTT [11]. These tests, which were developed from the original clotting time assays, are predominantly based on identifying the time at which clot formation occurs in a sample. This obviously requires detection of the clot in a reliable and reproducible manner. There are many problems associated with current aPTT testing. It is not yet possible to standardize the aPTT and introduce common units. The triggering of contact activation via the aPTT has been reported to lack reproducibility and reliability which originates from a poor onset of clotting and unpredictable patient response to surface activation [12-18]. The lack of standardized aPTT materials and measurement methods and the large variety of commercially available reagent types has been highlighted. aPTT reagents demonstrate varying responsiveness to factor deficiencies and lupus anticoagulant. The reference interval for healthy controls must thus be determined locally. [15,19,20]. To reduce the variability in aPTT results, pre-incubation of a plasma sample with surface activator and phospholipids is recommended as the standard procedure. This, in turn, introduces multiple pre-analytical steps that complicate the assay and prolongs the total test time. This also leads to more complex automation to be performed such as the requirement of pumps for mixing. Furthermore, most commercially available aPTT devices allow for clotting time monitoring in plasma, but not in whole blood samples. These limitations cause the test to be less accessible as it requires time-consuming sample preparation, trained personnel and the use of laboratory-based equipment such as centrifuges.

Coagulometry is a well-established and widely used methodology for CT determination. Coagulometers measure the ability of blood to clot by performing any of several types of tests including aPTT, prothrombin time (PT), lupus anticoagulant screens and factor assays. The onset of clotting is usually determined mechanically (rotating or vibrating metal ball) or optically (visually or via measurement of the change in light transmittance). Several other ways of determining a CT have been developed, including surface plasmon resonance [21],

surface acoustic wave device measuring changes in sample viscosity [22,23] or thickness-shear mode resonators used to characterize static rheological properties of blood [24]. Although coagulometers are relatively expensive, need to be operated by trained personnel and require large volumes of anticoagulated blood samples and activating reagents, they are still widely used in clinical practice and for research purposes. Several newly developed coagulation monitoring devices have been calibrated and validated against traditionally used coagulometers [25,26].

Simple, low cost, polymeric microfluidic devices have been shown to be very effective platforms for point-of-care coagulation monitoring applications [27]. Principally, microfluidic systems offer controlled flow conditions which additionally allow automation of many assay process steps which may require precise control of time, or benefit from changes in relative location. The simple and precise control of such processes is hugely attractive for assay design. The fabrication of polymeric substrates using rapid, high throughput production methodologies such as injection moulding and hot embossing also allows large numbers of devices to be produced at low cost and high reproducibility that is difficult to achieve with traditional natural materials such as nitrocellulose, for example. Consequently, such platforms could form the foundation for superior point of care devices for monitoring coagulation.

A novel principle of measuring clotting time via monitoring the onset of clot formation has been proposed for the development of an improved miniaturized point-of-care assay device, where the signal is detected fluorescently. It utilizes the property of clot localization using fluorescently-labeled fibrinogen. Fibrinogen is a major plasma protein and coagulation factor. It is a direct precursor of fibrin as well as an important mediator in the formation of the platelet plug where it provides support for platelet adhesion and aggregation [28,29] by binding to glycoprotein GPIIb-IIIa on activated platelets [30-33]. Due to this unique property, fluorescently-labeled fibrinogen has been widely used for the *in vivo* localization of clot formation to study platelet activation and fibrinogen binding [34]. It has been demonstrated that the fluorescently labeled fibrinogen becomes incorporated into the forming clot and has been shown to incorporate at the site of clot formation leading to localized increases in fluorescence intensity at the site of the clot. However, such a process has not previously been used in a quantitative way to show the time or extent of clot formation, either *in vivo* or *in vitro*, but might offer the potential for the more reliable detection of clot formation for clotting time determination.

MATERIALS AND METHODS

Assay platform. Assays were developed on an open lateral flow platform (4Castchip® model B2.2, Åmic BV, Uppsala, Sweden) which was fabricated from cyclic olefin polymer, Zeonor® and modified by hot embossing to form arrays of micropillars of 65-70 μm in height, top diameter ca. 50 μm, base diameter ca. 70 μm, distance between the centers of the pillars in a row of 85 μm, and distance between the centers of the pillars in a column of 185 μm (Fig. 1a). Assay platforms were modified by applying 30 μL of aPTT-SP reagent (HemosIL, Instrumentation Laboratory) supplemented with 0.75% (v/v) Triton X-100 (Sigma) on the micropillar flow channel and left to dry for 2 h.

Fig. 1. (a) Graphical representation of the B 2.2 micropillar lateral flow device (Dimensions in mm). The test channel possessed hot-embossed micropillar structure, as shown in the magnified inset. (b) Fluorescent microscope set up (Olympus IX81). (c) Sample deposition onto lateral flow platform and location of imaged area of 650 x 820 μm. (d). The imaged area is captured every 10 s and converted to an array of light intensity values and output to a video image file which is processed by a LabView interface (e) in which population mean and

standard deviation are calculated on each frame and the standard deviation is monitored with respect to time.

Signal detection and analysis. Fluorescence measurements were performed using an optical system consisting of a CCD camera (Hamamatsu Orca ER) attached to a fluorescent microscope (Olympus IX81) equipped with a climate chamber and heating block and a motorized stage (Fig. 1b). Fluorescence signals were monitored using the following settings: magnification: 10 x, excitation at 488 nm and emission at 519 nm, exposure time: 21 ms. The autofocus and the brightness auto-adjustment functions were switched off at all times. Experiments were performed in a dark room at 37 °C. An image of the centre of the lateral flow platform with an area of approx. 650 x 820 μm was taken every 10 s for up to 1500 s (Fig. 1c). Each frame was converted to an array of 1360 x 1024 pixels ($N > 1.39$ x 10^6), each one outputting a value of fluorescence intensity (Fig. 1d). Each frame was output as a .avi file to a LabView interface which automated calculation of the mean fluorescence and standard deviation for each frame (Fig. 1e). The standard deviation was then plotted against time. The recorded data was also subject to visual analysis, where necessary.

Investigation of the clot formation and localisation principle. A conjugate of human fibrinogen labeled with Alexa Fluor 488 (Molecular Probes, F-13191), was prepared in 0.1 M sodium bicarbonate (pH 8.3) and stored at -20°C. Alexa Fluor 488 has absorption and fluorescence emission maxima of 496 nm and 520 nm, respectively. Citrated, pooled, platelet poor plasma (HemosIL, Instrumentation Laboratory B.V., Netherlands) and anonymous donor whole blood were supplemented with the fluorescently-labeled fibrinogen (5% v/v, which equals 2.25 μL labeled fibrinogen in 25 μL plasma sample based on the assumption that the average normal fibrinogen level in plasma is 2.8 g/L [35]). 25 μL of 0.025 M $CaCl_2$ solution (Stago Diagnostica) was then added to reverse the effect of citrate. Immediately after addition of calcium, 25 μL of the test mixture was applied to a test chip and fluorescence measurement was initiated. Blank controls were prepared by replacing $CaCl_2$ with NH_4Cl in order to prevent clotting while keeping the dilution factor constant.

Plasma and whole blood samples were externally recalcified, supplemented with the fluorescently-labeled fibrinogen as determined above and applied to chips which were modified with aPTT assay reagents (Fig. 1c). Fluorescence signal was monitored for up to

1700 s. Clotting time values were extracted following analysis of the fluorescence responses as described above.

Calibration with heparin-spiked plasma samples. Control plasma samples spiked with a range of heparin concentrations (0 – 2 U/mL) were tested using the fluorescence aPTT assay in order to assess the effect of heparin on the CT. Results were correlated with aPTT CT values obtained from the KC4 coagulometer (Amelung) and the ACL TOP® clinical laboratory analyzer (Instrumentation Laboratory). Coagulometry was performed according to the instrument protocol in which 50 µL of plasma was incubated with aPTT reagent in a coagulometer cuvette for 3 min at 37°C. The aPTT measurement was started with the addition of 50 µL 0.025 M $CaCl_2$ (BioData). Testing using ACL TOP® was performed according to the accredited procedure at the Linköping University Hospital, Sweden by laboratory personnel.

Validation with patient samples. A cohort of 32 anonymous normal and abnormal patient plasma samples sourced at the Linköping University Hospital, Sweden, were analyzed using the fluorescent assay and with the ACL TOP® aPTT assay.

RESULTS

Investigation of the clot formation and detection principle. A conjugate of fibrinogen labeled with Alexa Fluor 488 was used in the assay. In the presence of thrombin, the soluble fibrinogen is converted into insoluble fluorescently-labeled fibrin. The labeled fibrin competes for unlabeled, endogenous fibrin for incorporation into the forming clot allowing its optical fluorescent localization. Additionally, binding of the labeled fibrin to the GPIIIa-IIb receptor on activated platelets could take place which would supplement the process of fibrin incorporation into a clot.

The clotting of plasma samples supplemented with the fluorescently-labeled fibrinogen was monitored using fluorescence microscopy on aPTT-coated chips. The middle of the lateral flow channel was monitored. Representative images captured at 30, 720 and 1650 s are shown in Fig. 2. Initially, there was an even distribution of fluorophore with relatively low average background intensity (Fig. 2a). However, brightly fluorescing formations could be observed at 720 s (Fig. 2b). At 1650 s, the fluorescence intensity in the monitored area increased further with the signal mostly confined to areas around the pillars (Fig. 2c).

Fig. 2. Images of micropillar test channels containing a normal clotting plasma sample supplemented with fluorescently-labeled fibrinogen captured using fluorescence microscopy with attached video camera at (a) 30 s, (b) 720 s and (c) 1650 s after sample application.

The change in the average fluorescence of the test zone was measured over time (data not shown). The sample showed some initial increase in fluorescence intensity, due to the influx of label to the area as it passed down the channel. At some point, however, there was an actual decrease in fluorescence intensity, followed once again by a gradual rise. The point at which this decrease occurred appeared to correlate with clot formation. However, the changes in fluorescence intensity generated were not very well defined and the change in the profile was difficult to identify. Thus, alternative methods of correlating clotting with changes in fluorescence were investigated.

Although it was not reliable to extract the CT values from the total change in fluorescence intensity, visual observation had suggested that, as well as changes in the average fluorescence, the localized redistribution of the label within a specific area might also be changing in a time-dependent manner. Therefore, the fluorescence standard deviation of the test zone for citrated plasma with the addition of $CaCl_2$ (clotting) and citrated plasma with addition of NH_4Cl (non-clotting) was assessed (Fig. 3). For the clotting sample, within the first 300 s only small changes in SD were observed with values of 3–5. This phase corresponded to the period of uniform, low fluorescence signal illustrated in Fig. 2a with the distribution of fluorescence being derived from the difference between the darker pillars and the brighter fluidic regions between the pillars. This period of minimal change was equated with the lag time typical of other clotting time assays. After this period, a rapid increase in SD to approx. 14–15 was observed. Following this abrupt change in SD, no further change was observed for nearly 500 s. This period was associated with the redistribution of the fluorescent label as illustrated in Fig. 2b and which, we suggest, corresponds to the onset of clotting and was subsequently taken to be the CT. During this period, intensely fluorescent areas between the pillars could be seen which conform to patterns of sample flow between the pillars, with adjacent areas with visibly reduced fluorescence. This was supported by visual and white light microscopy observation which indicated the formation of adherent fibrin fibers at this time. Further intensification is likely as sample continuing to flow through is captured by the forming clot loci. It is likely that the detection phenomenon can be further optimized by alteration of the chip pillar geometry to generate further enhancements in the change in fluorescence distribution. However, such alterations in chip design were not possible in this work. A further significant change in SD was observed at around 810 s followed by a gradual and continuous decrease starting at around 1050 s. Visual analysis revealed that after around 800 s the fluorescent label began to become concentrated around

the micropillars (Fig. 2c). This phenomenon is likely due to evaporation. As the sample evaporates, it concentrates label around the base of the pillars. Eventually, when all the liquid is evaporated, the fluorescent signal is quenched and then decreases. These processes correlated with visual observations of evaporation.

A gradual and steady increase in the SD was observed for the plasma with NH_4Cl. No clot formation could be observed visually and the insignificant increase in the SD value may have occurred due to label clumping, accumulation and settling over time, rather than clotting. The rapid increase in the SD of fluorescence signal was observed only for the clotting sample regardless of the fact that the same concentration of fluorescently-labeled fibrinogen was present in both clotting and non-clotting samples.

Fig. 3. Change in SD of the fluorescence intensity over time for a normal clotting plasma (circles) and citrated plasma with addition of NH_4Cl (triangles) supplemented with fluorescently-labeled fibrinogen in the micropillar channel modified with aPTT-SP reagent. The filled black symbol indicates the time point for the appearance of highly fluorescent regions. Signal measurement interval: 30 s.

Comparison of whole blood and plasma. Fig. 4 illustrates the clotting profiles obtained for a clotting plasma sample and a clotting whole blood sample monitored at equal brightness settings as optimized earlier for plasma and additionally for whole blood after compensating for brightness. The fluorescence emission from the whole blood sample was significantly lower than for plasma. This is likely due to the presence of red blood cells which result in absorption and scatter [36]. Whether uncompensated or not, the observed changes in SD were similar for plasma and whole blood. Following brightness adjustment, no significant difference in the SD profiles was evident between the two samples, with both showing a clotting time for this sample of approx. 190 s. This would suggest that the assay would have the potential for clotting time determination in both plasma and whole blood.

Fig. 4. Change in SD value over time for clotting plasma (open circles), for whole blood observed at the same settings as plasma (triangles) and for whole blood after adjustment of the image brightness (filled circles).

The effect of heparin on standard deviation profiles. Control plasmas were spiked with concentrations of heparin from 0 to 2 U/mL and these samples were subject to the fluorescent

assay (Fig. 5). In order to emphasize the prolongation in clotting time due to increased heparin concentration, only the initial parts of the profiles are shown.

Fig. 5. Profiles of change in SD over time obtained for plasma samples spiked with heparin at 0 (filled circles), 0.25 (open circles), 0.5 (filled reversed triangles), 0.75 (open reversed triangles), 1 (filled squares), 1.5 (open squares) and 2 (filled diamonds) U/mL.

The change in the detected SD signal was sudden and well-defined. CT values could be easily extracted on the basis of the generated profiles. A significant prolongation in the onset of clotting with increased heparin concentration was obvious. A non-anticoagulated sample (0 U/mL heparin) returned a rapid CT of 170 s. CTs were between 177 − 483 s for 0.25 − 2 U/mL. As illustrated in Fig. 6, the correlation between the extracted CTs and heparin dose was close to linear ($R^2 = 0.996$) in the tested range of heparin concentration. A slope value of 156 s.mL.U^{-1} and a difference of at least 36 s between adjacent heparin doses indicated a relatively high heparin sensitivity of the assay with RSD ≤ 8.4%. Results obtained for samples at 0.75 U/mL heparin or more were less reproducible than CTs in the lower heparin range. In

these tests, the clotting occurs both later and more slowly. This is known to lead to increased variation in the onset of coagulation [37] with resulting increases in CV.

Fig. 6. Calibration curve of heparin concentration vs. extracted clotting time in platelet poor pooled plasma samples spiked with 0 – 2 U/mL of heparin (n=3). $y = 156.29x + 181.28$; $R^2 = 0.996$.

Correlation with coagulometry. Typically, aPTT assays are performed in two steps. The first is contact activation which is initiated in the absence of clotting in the presence of citrate. This typically takes three minutes and is designed to reduce the variability inherent in the contact activation step. However, in the test developed here, the assay was performed in a single step to eradicate manual processing. Thus, the clotting time returned is the sum of all the assay steps which would otherwise be performed separately in a traditional test. Consequently, the absolute CTs obtained by this method were considerably longer than the standard laboratory aPTT. The test was correlated with standard coagulometry using the Amelung KC4® (Trinity Biotech, Ireland), which has mechanical clot detection (Fig. 7).

CTs for plasma spiked with 0 – 1 U/mL of heparin were 71.7 – 196.8 s and 146.7 – 276.7 s for coagulometer and the new technique, respectively. Given that the laboratory method required 3 min incubation, this would extend the total assay time to 251.7 – 376.8 s. Thus, the total test times were, on average, 110 s faster for the new assay. In spite of the differences in the absolute CTs obtained, the correlation between the fluorescence-based method and coagulometer was found to be close to linear with $R^2 = 0.9986$ and a slope of 1.05.

Fig. 7. Correlation between aPTT values for heparin-spiked plasma (0 – 1 U/mL) by the developed aPTT assay device (field method) and coagulometer Amelung[®] KC-4(n=3). Trend line parameters were as follows: y = 1.0462x + 69.49; $R^2 = 0.9986$.

Correlation with routine hospital aPTT. CTs obtained from the fluorescence-based method for heparin-spiked plasma were also compared using an automated coagulation analyzer, the ACL TOP[®] coagulation system (Fig. 8), which detects clot optically. CT values derived by the hospital method were lower than the fluorescence assay results. Again, this was due to the introduction of plasma and aPTT reagent in a pre-incubation step (as was the case with the

coagulometer). Taking this into account, the methods differed by approx. 10 s. Given that the fluorescent assay only takes a reading every 10 s, these could be considered equivalent. In addition, the hospital method was incapable of determining CT values for high heparin concentrations $\geq$ 1 U/mL. Therefore, correlation was only established on the basis of CT values obtained for 0 – 0.75 U/mL where it was close to linear (R^2 = 0.9365, n=3). The new method appeared to be a reliable tool for heparin dose monitoring in spiked samples at least. In addition, higher drug doses (up to 2 U/mL) could be detected using the fluorescence-based method, which was not possible with the standard hospital technique. This higher range typically requires use of the ACT assay. The new assay was also more sensitive to low heparin dosage as plasma CT was prolonged by 43.3 s upon addition of 0.25 U/mL heparin in comparison to only 18.9 s in the ACL TOP[®].

Fig. 8. Correlation between aPTT values obtained for plasma samples spiked with heparin at 0 – 0.75 U/mL tested using the fluorescence-based technique (field method, n=3) and using the ACL TOP[®] (hospital method, n=1). y = 0.8065x + 169.12; R^2 = 0.9365.

Assay validation in patient samples. The viability and reliability of the fluorescence-based lateral flow assay device for heparin determination was further investigated by testing patient samples with known aPTT. A cohort of 32 normal and abnormal plasma samples were obtained from patients with clotting abnormalities, or who were receiving anticoagulant therapy (exact dosage and type of treatment was unknown). These were examined using the fluorescence-based aPTT method developed here and the ACL TOP® (Fig. 9). Total assay times obtained from the fluorescence-based assay were, again predictably greater than the hospital method. However, taking into account the 3 minute incubation, the new method was, on average 110 s quicker to return a CT. Again, samples with significantly prolonged CTs showed poorer reproducibility than those of low or normal aPTT. Nevertheless, the intra-test variability was satisfactory. The slowest clotting sample (78.4 s by hospital technique, 676.7 s by fluorescence-based method) yielded the highest %CV of 15.9%, while 24 out of 32 samples returned CVs of less than 10%, which could be considered very reproducible taking into account the inherent variability of this type of assay, particularly when testing heparinized patients where deviations of up to 200% have been documented [38].

Fig. 9. Correlation of patient aPTT values between the hospital method (ACL TOP®, n=1) and using the fluorescence-based assay (field method, n=3). $y = 8.7395x + 0.6393$; $R^2 = 0.7834$.

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Due to the wide variability in aPTT testing and individual patient responses, the relationship between the heparin dosage and the CT expressed as aPTT is very difficult to establish. Therefore, new point-of-care methods are correlated with conventional automated aPTT hospital laboratory coagulometers [39]. Herein, the obtained coefficient correlation of 0.7834 between the newly developed and standard, reference method was considered acceptable when taking into account the fact that aPTT test is still an un-standardized assay and several factors including factor deficiencies can influence the responses of both methods [40,41].

A significant disparity between the calibration curves for spiked (y = 0.6656x + 137.46; $R^2 =$ 0.959) and patient samples (y = 8.7395x + 0.6393; $R^2 = 0.7834$) by the two methods could be seen. The correlations between aPTT by the newly developed assay and the routine method for both heparin-spiked and patient samples were found to be close to linear. However, the slope values were significantly different, even though both types of sample were tested using exactly the same protocols. The laboratory method was seen to be far more sensitive to the spiked samples than the fluorescence method. However, the new test was far more sensitive to actual patient samples. The use of heparin-spiked samples for the purpose of the aPTT reference and therapeutic range establishment has been shown to be misleading [19,42]. It has previously been shown that calibrations based on *in vitro* heparin addition to pooled plasmas yield a much lower aPTT therapeutic range in comparison to calibrations based on heparin-treated patient samples [43]. Therefore, the final device evaluation should be performed using reference samples obtained from patients on heparin therapy.

Although unfractionated heparin is a widely used anticoagulant drug, several new anticoagulants are now available such as as low molecular weight heparins, heparinoids and the direct thrombin inhibitors. Further studies should be performed to ascertain the usefulness of the developed assay to monitor the anticoagulant effect of such treatments.

CONCLUSION

This work demonstrates the development of a technique that allows precise detection of the onset of coagulation in plasma and whole blood by monitoring clot formation using a fluorescent probe and its enhanced accumulation in microscopic loci which are enhanced by the flow of the sample and label between micropillar structures in a disposable microfluidic device. The method was shown to be useful for determining the effect of the anticoagulant drug, heparin on the clotting time values of both plasma and whole blood following their activation using immobilized aPTT assay chemistry. The assay was shown to correlate with both gold standard laboratory coagulometry as well as routine hospital aPTT analyzers, and was also shown to correlate with laboratory aPTT in actual patient plasma samples. The assay could be performed in a single step and was shown to have good precision and extended dynamic range over current techniques. The complex optical measurement set up can also be miniaturized to allow development of the system as a point of care test.

Acknowledgements

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