POINT-OF-CARE BLOOD COAGULATION MONITORING USING LATERAL FLOW DEVICE

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ABSTRACT

A simple and affordable method of evaluating blood coagulation ability using microfluidic paper-based lateral flow assay (LFA) device is reported. Rabbit whole blood treated with citrate as anti-coagulant was used in experiments. Re-establishment of blood coagulation cascade and creation of different coagulation abilities were achieved by adding $CaCl_2$ solutions of varying concentrations. Since viscosity change of blood depends on its coagulation ability, travel distance of red blood cells (RBC) on nitrocellulose membrane can be used as an indicator of blood clotting time. RBC travel distance is found to decrease linearly with increasing $CaCl_2$ concentration.

KEYWORDS: Paper Microfluidics, Lateral Flow Device, Blood Coagulation, Point-of-Care

INTRODUCTION

Patients suffering from cardiovascular disease are treated with anti-coagulants, such as warfarin, to decrease the risk of stroke and heart attack [1]. To reduce the cost of care and to allow more frequent monitoring, there's a huge demand for point-of-care (POC) testing devices which can be used directly by patients and their caregivers at a reasonable price [2]. Existing portable coagulation monitoring devices are beyond the reach of a large portion of population due to their high costs ($1,000-$2,000) [3]. In this manuscript, a simple and affordable paper-based LFA device is presented for the detection of blood coagulation ability.

EXPERIMENTAL

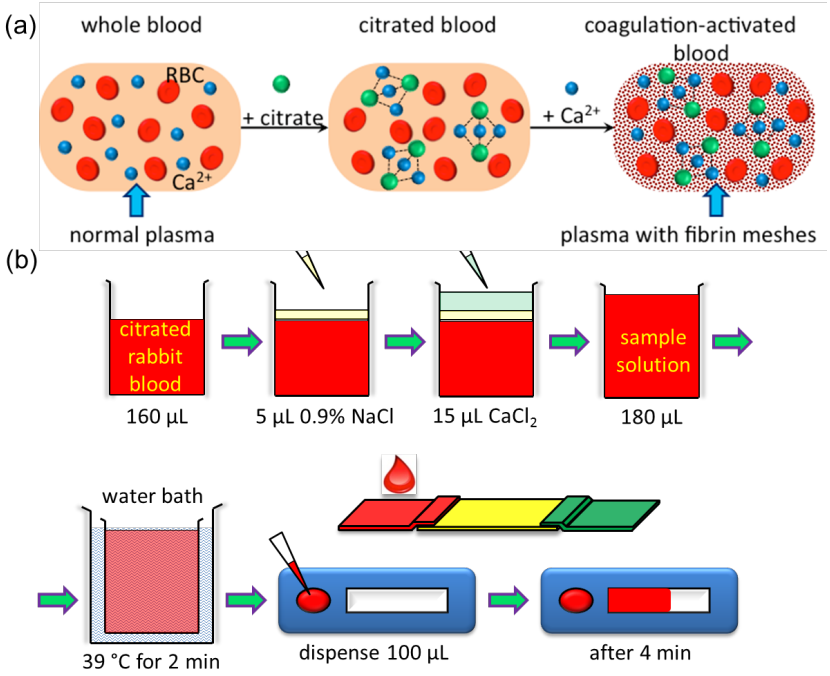

Figure 1: Experimental principle and procedure.

The experimental principle is illustrated in Fig. 1a. The starting point is citrated animal (rabbit) whole blood. The citrate disables the coagulation ability of whole blood by immobilizing free Ca^{2+} ions

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that are essential in the coagulation cascade. By adding free Ca^{2+} ions in the form of $CaCl_2$ solution, the coagulation cascade is able to resume and blood clots form in a few minutes [4].

Figure 1b shows the experimental process of preparing blood samples and their subsequent transport on the LFA device. 160 µL of citrated rabbit blood is added to 5 µL 0.9% NaCl solution, followed by adding 15 µL $CaCl_2$ solutions (50 mM to 500 mM in increments of 50 mM). After each sample is made, it is incubated in a 39°C water bath for 2 minutes to initiate the blood coagulation process by providing an environment similar to a rabbit's body. Next, 100 µL from the overall sample is dispensed immediately into the LFA inlet, which consists of sample pad, nitrocellulose membrane and wicking pad.

In order to confirm the relationship between fluid viscosity and its travel time on nitrocellulose membrane, 15 µL of citrated rabbit blood and various concentrations (40 wt.% - 70 wt.% in increments of 5 wt.%) of glycerin/water mixtures are used to perform fluid transport experiments on LFA device.

RESULTS AND DISCUSSION

The travel distance of the RBC component of blood samples in LFA devices are shown in Figure 2 for different concentrations of added $CaCl_2$ solutions. In Figure 2a, the square of the RBC travel distance is plotted versus time. It is apparent that the flow of RBCs follow fairly closely to the Washburn equation [5], $L^2 = \gamma Dt/4\eta$, where L is the travel distance, γ is the surface tension, D is the effective pore diameter, t is the travel time and η is the viscosity. The RBC travel distance decreases monotonically as more Ca^{2+} ions are added to blood sample. Clearly, as the viscosity of the blood increases with the addition of $CaCl_2$, the travel distance in the LFA is reduced.

This relationship is shown quantitatively in Figure 2b, where the normalized RBC travel distance during a time period of 240 s is plotted directly versus the $CaCl_2$ solution concentration for three independent sets of experiments. A good linear relationship between normalized RBC travel distance and added $CaCl_2$ solution concentration is observed. Agreements with ideal linearity for the 3 sets of data are 0.978, 0.985 and 0.984 respectively, thus showing good reproducibility of the experiments. Data bars are included in the figure to indicate the data range within the 3 sets of experiments.

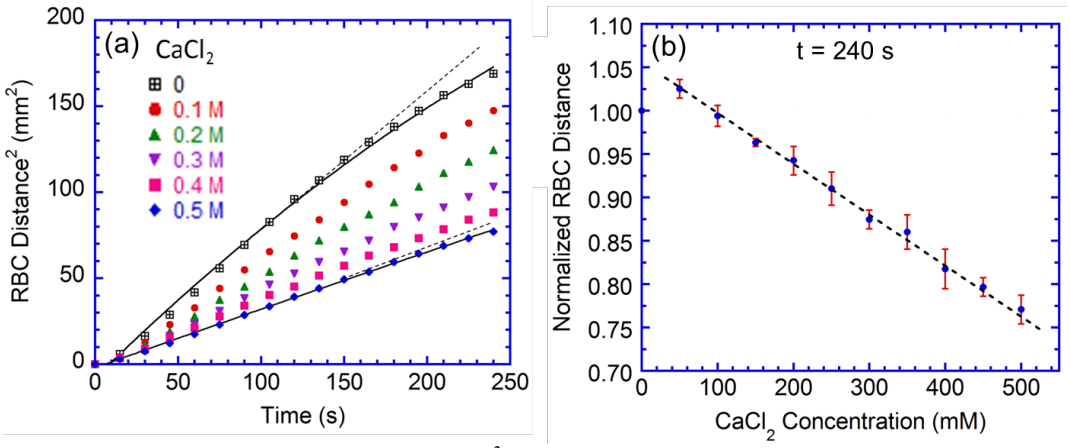

Figure 2: Experimental results. (a) RBC distance^2 vs time for different $CaCl_2$ solution concentrations. (b) Normalized RBC distance vs $CaCl_2$ concentration.

Figure 3 shows the travel time of RBCs in citrated rabbit blood and of various concentrations of glycerin/water mixtures in LFAs for a distance of 8 mm. Different glycerin/water mixtures are utilized to achieve fluids with well known viscosity values. Figure 3 supports our assumption that the travel distance of biological fluids in LFA devices is strongly and predictably affected by their viscosity by showing a linear relationship between fluid viscosity and travel time. Once the relationship between the viscosity and the travel time of fluids is established in a particular LFA device, one can determine the viscosity of sample fluids based on their travel time in the LFA. Based on Figure 3, the effective viscosity of RBCs in citrated rabbit blood can be obtained using its respective LFA travel time, with a value of ~20.1 cP. This

high viscosity value is due to the increasing separation with time/distance between RBC and plasma components of the whole blood sample, which results in an increasing RBC concentration during flow. This is consistent with the well-known sharp increase in whole blood viscosity with increasing hematocrit (RBC fraction) percentage [6].

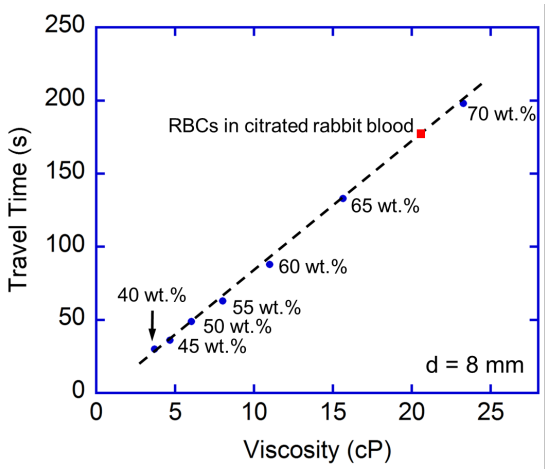

Figure 3: LFA travel time for a fixed distance of 8 mm vs viscosity of glycerin/water mixtures of different concentrations. The travel time of RBCs in citrated rabbit blood indicates an equivalent viscosity of ~20 cP.

CONCLUSION

A microfluidic paper-based LFA device for blood coagulation monitoring has been demonstrated. This simple and affordable device has been shown to detect a wide sensitivity range of Ca^{2+} ion concentration. Combined with very low inherent cost, it enables the broad use of these LFA devices for individuals as single-use evaluation tools.

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