MICROCANTILEVER BASED LOC SYSTEM FOR COAGULATION MEASUREMENTS

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ABSTRACT

In this paper, a microcantilever-based system enabling multiple coagulation tests on the same disposable cartridge is demonstrated. The system consists of independent cartridge and reader unit. The actuation of the nickel cantilevers is conducted remotely with an external electro-coil and remote optical read-out is utilized for sensing. Both Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) tests can be conducted on the same cartridge. The system's repeatability and accuracy is investigated with standard control plasma samples. The results are concordant with the manufacturer's datasheet. The architecture of the system and the repeatable results makes the system suitable for Point-of-Care applications.

KEYWORDS: Coagulation Time, Blood Plasma, Microcantilever, MEMS, PT, aPTT

INTRODUCTION

Monitoring coagulation time is important for patients suffering from cardiac diseases. For instance, periodical blood coagulation time measurements are necessary for patients under heparin medication to adjust the dosage.

In our previous studies we utilized our system for blood plasma viscosity measurements [1]. Also we showed the first usage of our system for blood coagulation time monitoring [2]. We now report the full validation of our system with reference control plasmas which represent both healthy and unhealthy individuals. The purpose of this work is to develop a portable system for patient self-testing. Other approaches for this goal include Quartz Crystal Microbalance (QCM) and electrical measurement methods. However, QCM based systems requires surface modification [3, 4] for coagulation measurements. Electrical measurement approaches [5] require electrical connections between the cartridge and the analyzer which makes them prone to failure in long-term. The proposed system monitors coagulation with a non-contact actuation and detection method. Having no electrical connections makes our approach suitable for multiple coagulation tests on the same disposable cartridge for Point-of-Care applications.

EXPERIMENTAL

In the proposed system, we utilized an electro-coil to excite the microcantilevers around their resonant frequency and conducted the read-out with a Laser-Doppler-Vibrometer (LDV) (Figure 1A). During coagulation the viscosity of the blood plasma increases which results in a change in resonant frequency and quality factor of the cantilever. We track this change by simultaneously monitoring the phase difference between the input of the electro-coil and the LDV output with a lock-in amplifier (ZI HF2LI). This type of phase monitoring technique enables the multiple measurements in different channels on the same cartridge. Figure 1B shows a PMMA cartridge with 5 separate microchannels.

In the experiments, we used three control plasmas (DIAGEN) with different coagulation times. For APTT tests 5µl of Micronized Silica Platelet Substitute is mixed with 5µl of plasma sample and placed on top of the MEMS sensor. After 5 minutes of incubation 5 µl of 25mM $CaCl_2$ is added to initialize the coagulation. The time where t = 0 shows this calcium addition. A similar protocol is conducted for PT measurements with Calcium Thromboplastin reagent. For PT tests additional $CaCl_2$ is not necessary since calcium is included in the reagent. All the measurements are conducted at 37±0.1°C. The temperature is stabilized with a temperature controller unit [2].

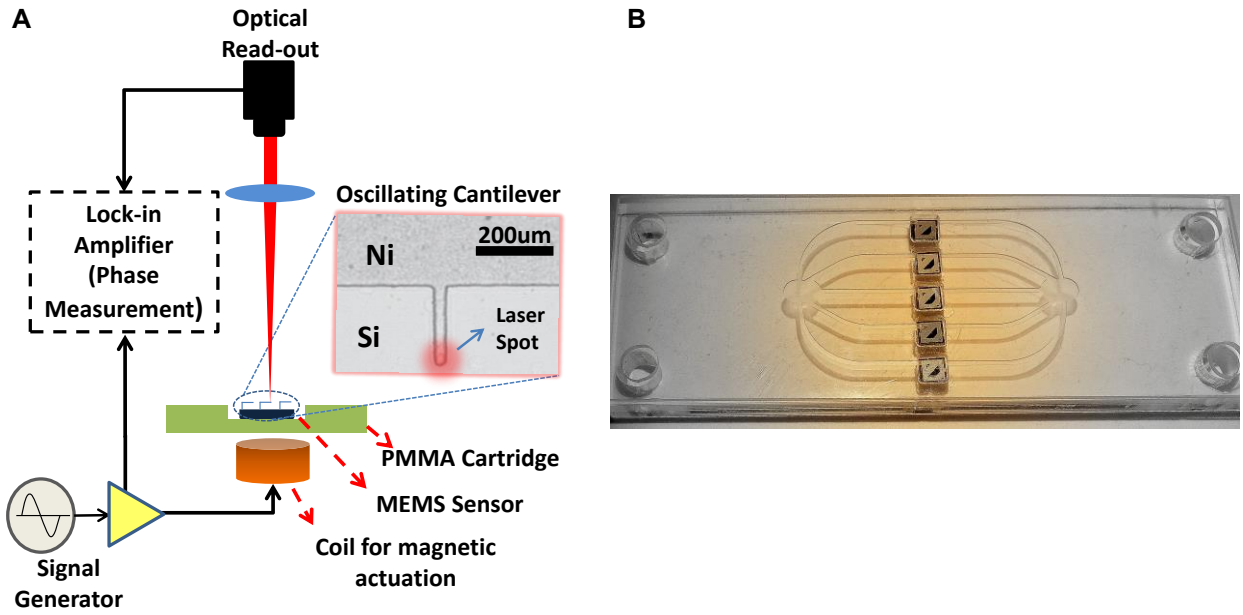

Figure 1: (A) Illustration of the measurement setup. The nickel cantilevers are actuated remotely with an electro coil and the read-out is conducted with an LDV. (B) A photo of the PMMA cartridge for multiplexed coagulation measurement. Magnetic actuation of the cantilevers in different channels is possible with only one external coil. A detector array can be utilized to monitor the coagulation simultaneously in each channel.

RESULTS AND DISCUSSION

The PT and aPTT tests are conducted with different plasma samples. Figure 2 shows the result of the APTT tests with Normal, Abnormal1 and Abnormal2 plasma samples and phase change during coagulation. Polynomial functions are fit to the real-time data. The points where the derivative of the phase change equals to zero (df/dt→0) indicates the onset of the fibrin formation which we report as coagulation time. The first The Normal, Abnormal1 and Abnormal2 samples can be easily distinguished from each other. The same procedure is also conducted for the PT tests. We found that this measurement method matches well with datasheet values. The normalized ratios for the APTT and International Normalized Ratios (INR) for the PT tests are presented in Figure 3. Total of 36 tests were performed with 36 separate MEMS chips. The results are coherent with the reference values provided by the manufacturer. The error bars show the standard deviations of the repetitive measurement made with MEMS sensor (n=6 for each). Standard value for the reference value is indicated in the product data sheet of the control plasmas.

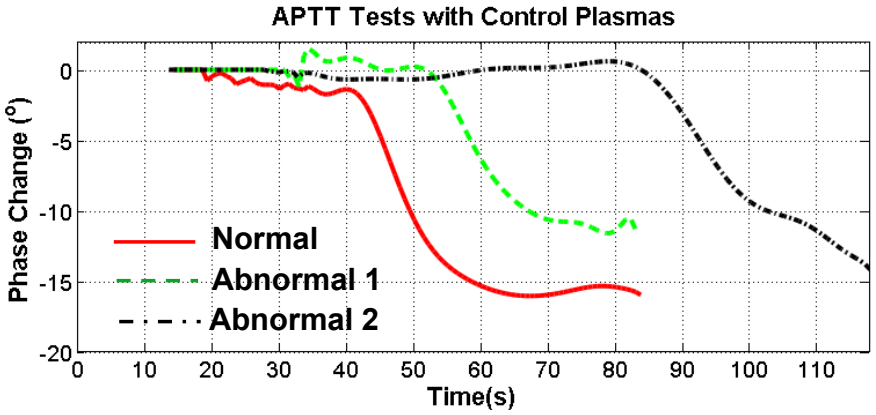

Figure 2. APTT test results for Normal, Abnormal1 and Abnormal2 plasma samples

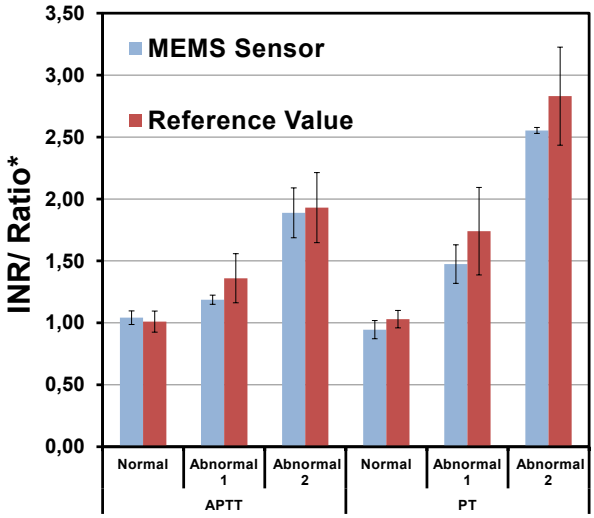

*Figure 3. The results of the APTT and PT tests in comparison with the reference values provided by the control plasma manufacturer. *Clotting time divided by mean normal clotting time*

CONCLUSION

In this paper we demonstrated, a sensor array platform enabling multiple coagulation tests on the same cartridge. System's repeatability and accuracy is investigated with control plasma samples having standard coagulation times. The proposed system gives concordant results with the manufacturer's datasheet. The independent cartridge and reader unit capability and the repeatable results makes the system suitable for point-of-care application.

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