INTEGRATION OF AGGLUTINATION ASSAY FOR PROTEIN DETECTION IN MICROFLUIDIC DISC USING BLU-RAY OPTICAL PICKUP UNIT AND OPTICAL FLUID SCANNING

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ABSTRACT

We present a novel strategy for thrombin detection by combining a magnetic bead based agglutination assay and **low-cost microfluidic disc**. The detection method is based on an optomagnetic readout system implemented using a Blu-ray optical pickup unit (OPU) as main optoelectronic component. The assay, from sample to answer, is fully integrated on a microfluidic disc which embeds **on-disc mixing** ensuring full automation of the assay along with less sample-to-answer time compared to similar methods. Moreover, we compare the optomagnetic readout to the cluster size distribution determined using a commercial optical scanning imaging instrument.

KEYWORDS: Agglutination assay, Aptamer, Magnetic beads, Centrifugal Microfluidics, Thrombin, Microfluidic disc

INTRODUCTION

In recent years, readouts based on magnetic beads have been implemented in several microfluidic systems for biodetection [1]. Moreover, agglutination assays based on the formation of magnetic bead clusters and using the detection of these to quantify the presence of the target biomarker are being developed [2], [3]. However, in many different cases, the use of complex microfluidics, sophisticated readout methods or long sample-to-answer time has limited their real point-of-care potential.

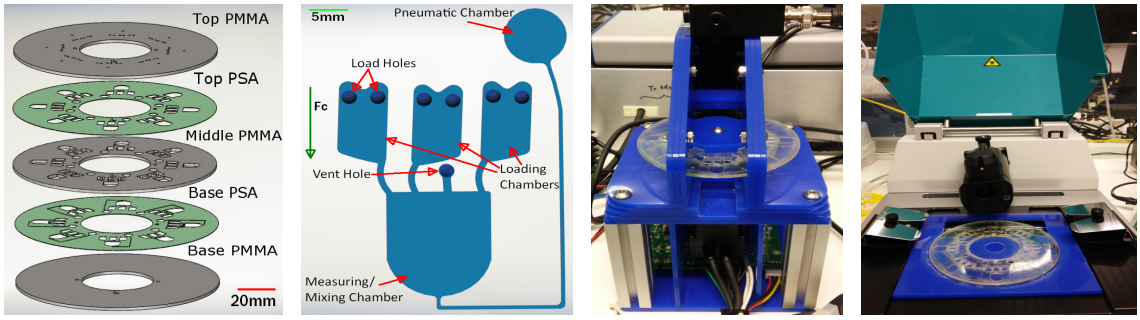
A magnetic microbead-based agglutination assay using aptamers for detecting thrombin in buffer solution was reported in [3] where the sample-to-answer time was about 40 min involving several manual steps. In this work, we use a similar agglutination scheme for thrombin detection and further integrate sample preparation and detection on a single automatized and low-cost platform. As a significant achievement, by using magnetic nanobeads (MNBs) and on-disc mixing, we reduce the sample-to-result time from 40 min to 15 min involving fully-automatized steps. The applied sensing technique is based on an optomagnetic readout system, which is sensitive to the formation of nanobead clusters [4]. We compare the size-sensitive optomagnetic spectra with the size distribution obtained using a commercial optical scanning imaging instrument (oCelloScope, Philips-Biocell). Having an optical resolution of 1.3 µm, the instrument has the unique property to scan all the objects of interest in a sample and quantify them as well as their physical characteristics using the 'Uniexplorer' instrument software.

EXPERIMENTAL

The assay platform in this work is a low-cost microfluidic disc. A disc was fabricated from three layers of Poly(methylmethacrylate) (PMMA) bonded by pressure sensitive adhesive (PSA) in less than 20 min (Fig. 1a). Each disc contained eight microfluidic units each with three inlet chambers, a mixing/measuring chamber, vent holes and a structure for pneumatic mixing (Fig. 1b).

The assay was performed using two batches of 100 nm MNBs (#10-19-102, Micromod), labeled MNB1 and MNB2, functionalized with two widely used anti-thrombin aptamers to form a MNB1-thrombin-MNB2 sandwich. One of the aptamers consists of 15-mer (*GGT TGG TGT GGT TGG*) and other one 29-mer (*AGT CCG TGG TAG GGC AGG TTG GGG TGA CT)*. Aptamer-functionalized MNBs (1.5 µl of each type with beads concentration of 0.1mg/ml) and thrombin solution (27 µl) were injected into separate loading chambers of a microfluidic unit. Spinning of the disc transferred the liquids to the measuring chamber. Liquids were mixed by a pneumatic mixing routine for 30 s [5] and incubated for

10 min. Subsequently, a 5 min incubation in a strong uniform magnetic field was performed to enhance MNB cluster formation. Finally, the optomagnetic spectrum was measured in the setup incorporating a stepper motor, the Blu-ray OPU (λ = 405 nm) and a photodetector (Fig. 1c). This setup measured the in-phase component of the 2nd harmonic of the light transmitted through the sample chamber (V_2') vs. frequency f of a sinusoidal magnetic field excitation (2 mT amplitude) applied along the light propagation as described in [4].



(a) (b) (c) (d)

Figure 1: Illustrations of (a) assembly of the PMMA and PSA layers of the disc, (b) one of the repeating units of the disc indicating different chambers involved in pneumatic mixing; the green arrow tagged with F_c indicates the direction of centrifugal force. Photos of (c) experimental setup with Blu-ray OPU [4] and (d) the oCelloScope with a disc mounted.

To validate the agglutination based assay the disc was transferred to the oCelloScope (Fig. 1d) for scanning and recording of image data along with quantifying the size distribution of the formed bead clusters when thrombin is present in the sample.

RESULTS AND DISCUSSION

Figure 2 shows optomagnetic spectra measured vs. frequency for the indicated thrombin concentrations. The field causes the rotation of the MNBs and consequently a modulation of the transmitted light intensity. The in-phase component of the second harmonic optomagnetic spectra, V_2' vs. f, reflects the distribution of hydrodynamic sizes of MNBs and MNB clusters [4]. The peak at around 100 Hz is due to the rotation response of individual free MNBs, whereas the peak at low frequency is due to MNB clusters.

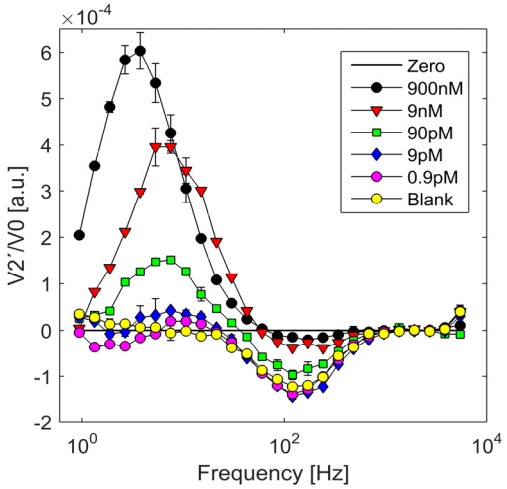
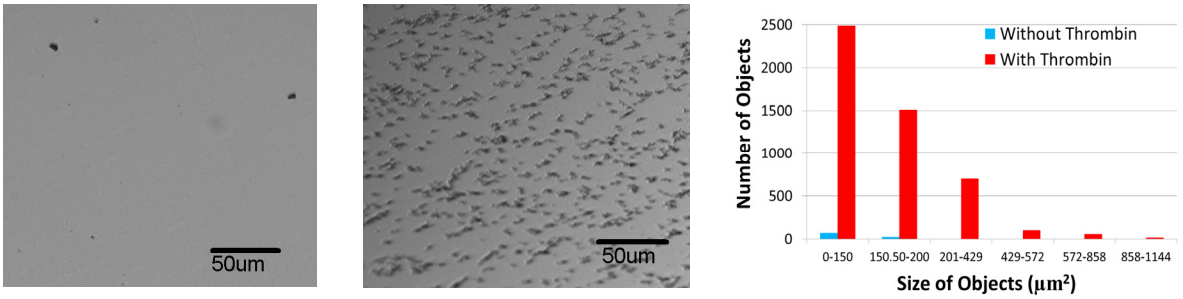


Figure 2: Comparison of V_2' optomagnetic spectra measured for the indicated thrombin concentrations. Error bars are derived from triplicate measurements

The presence of thrombin causes the two types of MNBs to link via the two aptamers and the number and size of MNB clusters are linked to the thrombin concentration. These effects cause the observed differences in the V_2' spectra in Fig. 2 recorded for thrombin samples with concentrations down to 9 pM.

Figure 3 shows images obtained using the oCelloScope without thrombin (Fig. 3a) and with 900 nM of thrombin (Fig. 3b). MNB clusters are only observed in the presence of thrombin. The scanned images were processed by the instrument software to obtain a histogram of the number of objects vs. their size (area). This histogram (Fig. 3c) shows a substantially higher number of large clusters for the investigated thrombin sample.



(a) (b) (c)

Figure 3: Scanned images of the samples with oCelloScope (a) without thrombin (no clustering) and (b) with 900 nM thrombin (high degree of clustering). (c) Histogram of the number of scanned objects vs. their size (area). The solution with 900 nM thrombin indicates a large number of objects with much higher area value - indicating numerous clusters.

CONCLUSION

We have presented a novel approach to an integrated, disc-based biosensing technology based on agglutination assay involving MNBs and aptamers. The technique holds great potential toward the realization of low-cost diagnostics using simple optomagnetic readout system. In addition, visualizing and quantifying the formed clusters along with analyzing their various physical characteristics (e.g. size) using the oCelloScope opens a wide scope for optimizing the technology as well as performing more in-depth analysis. The reason for choosing thrombin in our work as the target analyte is to facilitate the evaluation of our proof-of-principle in the context of other methods. This approach can be used as well for the detection of clinically significant small molecules.

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