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Lab-on-a-disc agglutination assay for protein detection by optomagnetic readout and optical imaging using nano- and micro-sized magnetic beads

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We present a biosensing platform for the detection of proteins based on agglutination of aptamer coated magnetic nano- or microbeads. The assay, from sample to answer, is integrated on an automated, low-cost microfluidic disc platform. This ensures fast and reliable results due to a minimum of manual steps involved. The detection of the target protein was achieved in two ways: (1) optomagnetic readout using magnetic nanobeads (MNBs); (2) optical imaging using magnetic microbeads (MMBs). The optomagnetic readout of agglutination is based on optical measurement of the dynamics of MNB aggregates whereas the imaging method is based on direct visualization and quantification of the average size of MMB aggregates. By enhancing magnetic particle agglutination via application of strong magnetic field pulses, we obtained identical limits of detection of 25 pM with the same sample-to-answer time (15 min 30 s) using the two differently sized beads for the two detection methods. In both cases a sample volume of only 10 μl is required. The demonstrated automation, low sample-to-answer time and portability of both detection instruments as well as integration of the assay on a low-cost disc are important steps for the implementation of these as portable tools in an out-of-lab setting.

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1. Introduction

Magnetic nano- and microbeads are being widely used for sensing of various biomolecules in different microfluidic systems (Antunes et al., 2015; Kwakye et al., 2006; Steigert et al., 2005; Wang et al., 2013; Zaytseva et al., 2005; Choi et al., 2002). Magnetic beads (MBs) can be utilized for detection of pathogens (Mezger et al., 2015; Beyor et al., 2008; El-Boubbou et al., 2007; Gu et al., 2006; Li et al., 2013; Wang and Gan, 2009; Zaytseva et al., 2005), small molecules (Yang et al., 2016; Wu et al., 2011; Zhang et al., 2013) and proteins (Nam et al., 2003; Tsai et al., 2007; Horng et al., 2006), for drug delivery (Chertok et al., 2008; Gonzales and Krishnan, 2005; Jain et al., 2008; Wilson et al., 2005), and even for testing certain characteristics of a drug (Quan et al., 2015). Due to the weak magnetic properties of biomolecules, a readout based on magnetic beads (MBs) is insensitive to chemical and biological parameters that may affect other readout techniques and is thus highly attractive (Yang et al., 2016; Bejhed et al., 2015; Hecht et al., 2013). In addition, due to the simple readout, the detection devices can be produced at a low cost, facilitating their usage in resource-limited settings (Yager et al., 2008).

Assays based on agglutination of MBs, i.e., the formation of MB clusters in order to detect biomarkers, are being widely studied due to the simplicity of the readout (Donolato et al., 2015; Göransson et al., 2010; Ranzoni et al., 2012). In these assays, the beads are coated with molecules with specific affinity to bind a target molecule. The presence of the target molecule causes the MBs to bind together to form aggregates of different sizes and shapes. Based on the physical characteristics of the aggregates as well as their rotational dynamics in response to an external oscillating magnetic field, the concentration of the target molecule can be determined. Magnetic bead-based agglutination assays are promising for point-of-care (POC) diagnostics.

However, in several studies of agglutination assays using magnetic beads, the use of complex microfluidics, sophisticated readout methods, large sample volume and lack of automation have limited their real point-of-care potential. For instance, Chen et al. (2013) developed a microfluidic chip-based assay using aptamer-functionalized magnetic beads labelled with fluorescent dye Cy3 for detecting thrombin. 50 μl of biotinylated Aptamer II

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labelled with Cy3 was incubated with 50 μl thrombin solution for 30 min. Aptamer I functionalized with magnetic beads was then loaded into the chip through external tubing. Since the chip was placed under a permanent magnet, the aptamer-functionalized beads were aggregated. Subsequently, aptamer II labelled with Cy3 and functionalized with thrombin was loaded into the chip to react with aptamer I. Finally, any unreacted aptamer II was washed out using an external syringe pump followed by fluorescent measurement with a microarray scanner. Thus, the whole process involved large assay time, multiple manual steps and external components.

Tennico et al. (2010) performed a similar study on a further developed chip, but used quantum dot nanocrystals instead of Cy3 dye and integrated a nanoport on the chip to connect with external syringe pump for washing in an automated fashion resulting in less reagent consumption and a shorter reaction time. The steps of the assay included: loading of 3 μl aptamer I functionalized with magnetic beads into reaction chamber followed by magnetic bead aggregation by on-chip magnets, rinsing of excess aptamers using external syringe pump, addition of 3 μl of thrombin solution into the reaction chamber followed by 5 min incubation period, rinsing and washing with binding buffer, addition of 6 μl aptamer II labelled with quantum dot nanocrystals into reaction chamber followed by further 5 min incubation, and finally a last washing step before measurement by a fluorescent microscope. Although the sample consumption and assay time was reduced compared to that presented in Chen et al. (2013), it still suffered from the limitations imposed by use of syringe pump as well as multiple assay steps including washing and rinsing, which makes it difficult to use it in an out-of-lab setting.

Finally, a magnetic microbead-based fluorescent-independent agglutination assay using the same aptamers as used in the above-mentioned studies for detecting thrombin in buffer solution was reported by Hecht et al. (2013). The rotational period of the formed aggregates as well as their shape, size and fractal dimension were measured to quantify the concentration of thrombin. There, the sample-to-answer time was more than 40 min and the assay required multiple manual steps.

Here, we present a conventional sandwich agglutination assay for detection of the model protein thrombin in buffer (Hecht et al., 2013), but the assay steps are integrated and automated on a low-cost microfluidic disc. As a significant achievement, thanks to the introduction of an on-disc magnetic field assisted incubation protocol (Antunes et al., 2015), we reduce the sample-to-result time from 40 min to 15 min 30 s while utilizing only 10 μl of sample. We verify that an optimum incubation of the samples with magnetic beads under magnetic field, hereafter termed 'magnetic incubation', effectively enhances the MB agglutination (Antunes et al., 2015; Ranzoni et al., 2012; Baudry et al., 2006) and results in fast detection. By utilizing the magnetic incubation protocol, we detect different concentrations of thrombin based only on the size distribution of MB aggregates.

We validate the lab-on-a-disc agglutination assay for both micro (1 μm) and nano (100 nm)-sized magnetic beads. We use two different detection methods for the MNB and MMB-based assays. The detection method used for MNB-based assay is the previously presented optomagnetic readout method (Mezger et al., 2015; Bejhed et al., 2015; Donolato et al., 2015). The optomagnetic setup (Donolato et al., 2015) uses a Blu-ray optical pickup unit (OPU) as the excitation element and a photodetector as the sensing element. An AC magnetic field excitation is provided by an off-chip electromagnet. The frequency-dependent modulation of the optical transmission signal correlates with the hydrodynamic size of the MNB aggregates and enables a sensitive and quantitative detection of a target molecule based on the aggregate size (Donolato et al., 2015). The detection method used for the MMB-based assay is an optical imaging method that detects the area of particle aggregates formed due to the presence of thrombin by fast direct imaging. We use a commercial imaging and scanning instrument 'oCelloScope' (Philips BioCell), which combines the optical sectioning principle of confocal microscopy with an automated scanning principle. A detailed description of the oCelloScope setup can be found in (Fredborg et al., 2013) and in the Supplementary material Section S2. The automation, user-friendliness, fast detection and portability of both detection instruments along with the integration of the assay steps on the disc make both systems strong candidates for a simple sample-to-answer device for use in an out-of-lab setting.

2. Experimental

2.1. Materials and chemicals

The assay platform in this work is a microfluidic disc. Each disc (thickness: 2 mm) contains eight microfluidic units each with three inlet chambers, a mixing/measuring chamber and a pneumatic chamber for ensuring pneumatic mixing (Fig. 1a and b). The disc was fabricated from three layers of Polymethylmethacrylate (PMMA) bonded by pressure sensitive adhesive (PSA) in less than 20 min. The detailed fabrication procedure of the disc is presented in (Donolato et al., 2015) and in the Supplementary material Section S1.

The MBs used in this study are streptavidin coated beads with diameters of 100 nm and 1 μm, respectively. The particular bead size was chosen as previous studies showed that the optomagnetic signal is more sensitive to formation of aggregates when the MNB size is about 100 nm (Donolato et al., 2015; Yang et al., 2016); and the 1 μm bead size was reported as optimum for MMBs based on sedimentation rate and available binding sites (Hecht et al., 2013), making it an ideal candidate for the optical imaging study. The MNBs were purchased from Micromod (Micromod Partikeltechnologie GmbH, Rostock, Germany). The MMBs (T1 Dynabeads) as well as the human alpha thrombin (product code T6884) were purchased from Sigma-Aldrich. Three different buffer solutions were prepared for washing, aptamer binding and thrombin binding, the details of which are provided in Supplementary material Section S1. Two widely used anti-thrombin aptamers functionalized with 5′ biotin and consisting of 29-mer and 15-mer with 20 base poly-T tails (Table 1) were purchased from DNA technology (Denmark). We have used these two particular aptamers because of their well-proven specificity to bind with thrombin molecule (Bock et al., 1992; Tasset et al., 1997).

2.2. Magnetic bead functionalization

We prepared two sets of aptamer-functionalized MBs, one for nano-sized beads and the other for micro-sized beads. An aliquot of 10 μl of streptavidin-coated MNBs (10 mg/ml) was washed three times with the washing buffer, resuspended in 100 μl of aptamer-binding buffer and subsequently split into two equal aliquots (Hecht et al., 2013). 4 μl of 50 μM biotinylated aptamer solution (each of 29-mer or 15-mer) was added to each aliquot and incubated for 45 min at room temperature to functionalize the beads with the specific aptamers by forming the biotin-streptavidin bonds. The MNB solution was then washed three times with thrombin-binding buffer and finally resuspended at a bead concentration of 0.1 mg/ml (Antunes et al., 2015). The same protocol was followed for functionalizing the MMBs.

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