A LOW COST AND HIGH THROUGHPUT MAGNETIC BEAD-BASED IMMUNO-AGGLUTINATION ASSAY IN CONFINED DROPLETS

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ABSTRACT

Here we demonstrate the feasibility of performing a low cost, multimodal and high throughput active immuno-agglutination assay by combining magnetics beads (MB), droplets microfluidics and magnetic tweezers. Water in oil droplets containing antibody coated MB and the specific target were generated and transported in tubing. When passing in between magnetic tweezers, the MB were magnetically confined in order to enhance agglutination rate and kinetics. After confinement, the internal recirculation flows in the droplet induce shear forces that favor MB redispersion and limit non-specific interactions. The assay limit of detection (LOD) was estimated at 100 pM.

KEYWORDS

Immuno-agglutination, droplets, magnetic beads, microfluidics.

INTRODUCTION

Although immunoassay diagnostic based on ELISA technique offers high throughput and sensitivity, it suffers from laborious procedures and is time consuming. [1, 2] In that way the development of one step immunoassays were of great interest since they prevent separation and washing steps thus simplifying and shortening the whole analytical procedure. Many groups have demonstrated the potential of one step immunoassays through different approaches such as magnetic relaxation, fluorescence resonance energy transfer, enzyme multiplied immunoassay technique, etc.

Among one step immunoassays, immuno-agglutination is the most widespread method in research and clinical applications since this approach is well adapted for point of care applications. The procedure relies on aggregates formation from antibody coated particles in presence of the specific analyte. The detection is based on optical measurement or naked eye evaluation. The main limitation of this approach result from the slow beads aggregation kinetics since beads collision and bridging occurred in passive diffusion. In order to enhance the bead aggregation efficiency, magnetic enhanced agglutination using MB has been developed. [3] Pearl chains like structures of MB were formed under homogeneous magnetic field thus increasing collision probability and contact time. The assay time was reduced to 5 minutes with pM sensitivity. Nevertheless this approach was not adapted for high throughput nor miniaturization. Gij's *et al.*[4] transposed magnetic agglutination in microsystem in which magnetic actuation system was fully integrated and sample consumption was significantly reduced. This system offers a pM sensitivity with analysis time around 10 minutes but the procedure based on sequential capture and release of MB remains laborious, requires specific materials and is still not adapted for high throughput and multiplexing.

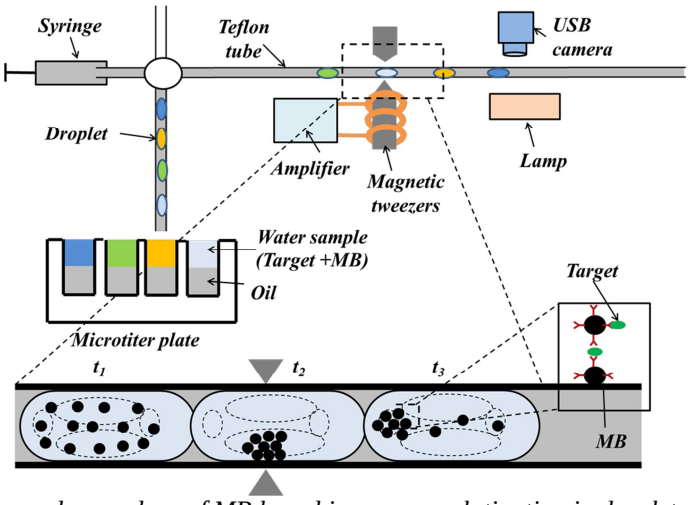

Figure 1. Experimental set up and procedure of MB based immuno-agglutination in droplets. Droplets were generated in a Teflon tubing by sequentially aspirating defined volumes of oil and sample (containing the MB and the analyte) from a microtiter plate. (t1) The droplets containing free MB were further transported towards magnetic tweezers. (t2) The confinement induced by the magnetic field enhances aggregates formation. (t3) Beyond magnetic tweezers, the MB were redispered thanks to internal recirculation flows (dotted lines) and droplets were visualized using USB camera.

In this paper we developed a fully automatized and low cost platform dedicated to one step magnetic enhanced immuno-agglutination in droplets. The first step consists in capturing the analyte using antibody coated superparamagnetic particles . After incubation, confined droplets containing the MB and the analyte were generated and injected in the tubing towards magnetic tweezers. When passing in between the tweezers, MB were magnetically

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confined thus improving collision probability and aggregation rate. After this magnetic confinement step, droplets internal recirculation flows ensure MB redispersion. The detection method consists in measuring the MB spatial distribution in the droplet using a USB camera. Indeed MB redispersion is limited by the creation of specific interaction between MB that depends on analyte concentration in the sample. Several parameters influencing the agglutination have been evidenced such as the percentage of surfactant in the carrying oil, magnetic flux density, flow rate and MB concentration. The easy and fully automatized droplets generation ensures serial and high throughput analyses using low cost materials while keeping good sensitivity.

EXPERIMENTAL

The principle of the immuno-agglutination assay is depicted on Figure 1. 90 µL of Streptavidin coated 1 µm magnetic beads (MB, Chemicell) at different concentrations (from 1 to 3 mg/mL) were mixed with 10 µL of biotinylated phosphatase alkaline (b-PA, Thermo Fisher Scientific) used as a model analyte (concentration ranging from 50 to 10.000 pM). After 5 minutes incubation, the droplets were generated and transported in a Teflon tubing (Sigma) using flow rates ranging from 0.4 to 1 µL/min. [5] When passing in between the magnetic tweezers, the MB were magnetically confined by applying a current from 0.2 A (20 mT) to 1 A (85 mT).[6] Once passing the tweezers, the internal recirculation flows in the droplet induce shear forces that favor MB redispersion. In presence of the analyte, the formation of specific binding will keep MB in aggregated state. However, when reducing analyte concentration, the number of specific interactions will decrease as well as the number of MB engaged in the cluster thus favoring MB redispersion in the droplet. The detection step consists in measuring the projection of the surface occupied by the MB in absence (S_{blank}) or presence (S_{assay}) of the analyte in the sample. USB camera placed at 5 cm after magnetic tweezers was used to take droplet pictures. The MB were observed in transmission and induced a contrast represented by dark pixels. After imposing a grey level threshold, the surface occupied by the MB was determined through image J software. The signal was determined as: $1-(S_{assay}/S_{blank})$. The entire procedure was performed at 21°C.

RESULTS

The feasibility of the agglutination assay in droplets was demonstrated using 1µm streptavidin coated MB and biotinylated phosphatase alkaline as model analyte. We first evidenced that aggregation rate and kinetics from passive agglutination, *i.e.* without magnetic flux density (***B***), were very poor. In absence of magnetic field (figure 2 A,D), the presence of the analyte (2 nM) has no significant influence on MB behavior as compared to the blank (0 pM). Diffusion and convection processes due to droplet internal recirculation flows were not sufficient to induce MB collision and bridging. As in passive agglutination, agglutination is limited by MB collision frequency and contact time. Electrostatic and steric repulsions between MB could also explain the slow process of passive agglutination.

In order to improve agglutination rate and kinetics, magnetic tweezers have been developed to magnetize and confine MB in droplets (Figure 1). We observed experimentally that the application of ***B*** improved MB collision probability and contact time. In the presence of high analyte concentrations (figure 2 F) large aggregates of MB were observed while progressive dissociation and redispersion was observed in blank experiments. These results demonstrate the specificity of the interaction. We investigated the influence of the magnetic field on the agglutination test. As shown on figure 2G, above 20 mT, the value of ***B*** has no more influence on the signal.

In order to perform a high throughput assay, the influence of the flow rate on the signal was also studied for a given analyte concentration. Four different flow rates from 0.4 to 1µL/min were investigated. Figure 2H shows that the signal decreased when increasing the flow rate. A careful attention on MB redispersion reveals two different regimes. From 0.4 to 0.6 µL/min, the increase of droplet velocity reduces the magnetic confinement time and therefore the MB capture efficiency. It resulted in a decrease of agglutination efficiency. However, from 0.6 to 1 µL/min we observed a different behavior : here the droplet velocity is so high that MB stay packed at the rear of the droplet with or without analyte in the sample. Therefore a flow rate of 0.4 µL/min was chosen as it offers the higher signal and a scanning rate of 7 droplets per minute.

The influence of MB concentration on the signal is highlighted on figure 2I. It was shown that the assay sensitivity was improved when decreasing the MB concentration. Indeed, when the MB concentration decreases the number of captured target per MB increases thus increasing the percentage of MB engaged in the aggregates. A linearity was obtained in a concentration range of 100-900, 200-1100 and 500-1700 pM when using 1,2 and 3 mg/mL of MB respectively. The assay limit of detection (LOD), which was determined as three standard deviation above the background, was estimated at 100 pM with a high reproducibility (RSD of 4.8% with 5 repetitions) when using a MB concentration of 1 mg/mL. This LOD meets the sensitivity required for most of immunodiagnostics. The assay specificity was evaluated using BSA as interfering molecule; the results indicate the absence of non-specific agglutination. In addition to high sensitivity, the possibility to generate the droplets in large number gives access to reliable and high throughput analyses. Including target capture, droplet generation, magnetic confinement and droplet visualization, this approach offers an analysis rate of 300 assays per hour.

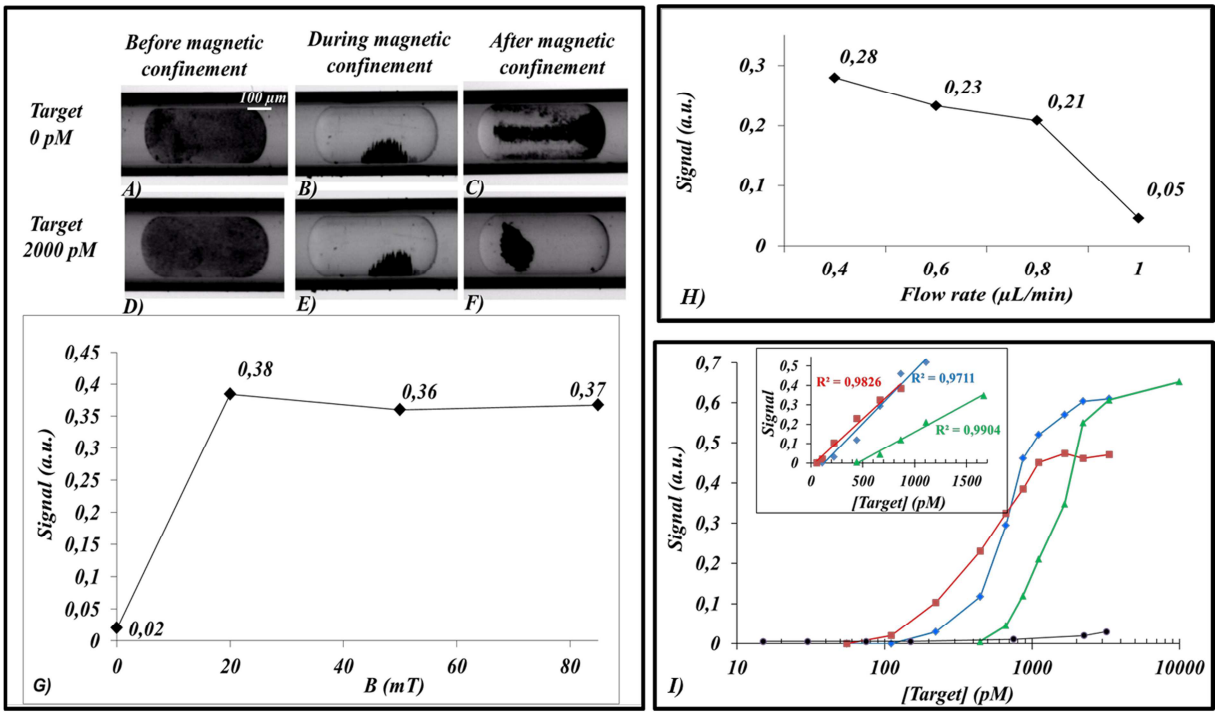

*Figure 2. Sequence of MB aggregation without (A,B,C) or with (D,E,F) analyte. Influence of magnetic field **B** on the signal (G). Influence of the flow rate on the signal (H). Calibration curve for three different MB concentrations: 1(square), 2 (diamond), 3(triangle) mg/mL, circle represent non- specific analyte (BSA) with 2 mg/mL of MB (I).*

CONCLUSION

In this work we have shown the implementation of a promising low cost and high throughput agglutination assay combining the advantages of MB, magnetic tweezers and droplets. High gradient magnetic fields improve agglutination rate and kinetics while water in oil droplets provide a significant of the reaction volumes (100 nL), multiplexed analyses and automated high throughput analyses (300 analyses /hour).

REFERENCES

[1] D. Godfrin, H. Sénéchal, J.P. Sutra, J.M. Busnel, F.X. Desvaux, G. Peltre, *A modified enzyme-linked immunosorbent assay adapted for immunodetection of low amounts of water-insoluble proteins*, J. Immunol. Methods, vol. 326, pp. 83–92, (2007).

[2] K. Sato, M. Tokeshi, H. Kimura, T. Kitamori, *Determination of Carcinoembryonic Antigen in Human Sera by Integrated Bead-Bed Immunoasay in a Microchip for Cancer Diagnosis*, Anal. Chem., vol. 73, pp. 1213–1218, (2001).

[3] J. Baudry, C. Rouzeau, C. Goubault, C. Robic, L. Cohen-Tannoudji, A. Koenig, E. Bertrand, J. Bibette, *Acceleration of the recognition rate between grafted ligands and receptors with magnetic forces*, Proc. Natl. Acad. Sci., vol.103, pp16076–16080, (2006).

[4] R. Afshar,Y. Moser, T. Lehnert, M. A. M. Gijs, *Three-Dimensional Magnetic Focusing of Superparamagnetic Beads for On-Chip Agglutination Assays*,Anal. Chem., vol. 83, pp. 1022–1029, (2011).

[5] M. Chabert, K.D. Dorfman, P. de Cremoux, J. Roeraade, J-L. Viovy, *Automated microdroplet platform for sample manipulation and polymerase chain reaction*, Anal. Chem., vol. 78, pp.7722-7728, (2006).

[6] A. Ali-Cherif, S. Begolo, S. Descroix, J-L. Viovy, L. Malaquin, *A programmable magnetic tweezers and droplet microfluidic platform for high throughput nanoliter multi-steps assays*, Angew. Chem., (2012) - Accepted.

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