CONTINUOUS MAGNETIC BEAD BASED MICRO-IMMUNOSENSING WITH EXTENDED INCUBATION VIA SPIRAL CHANNEL DESIGN

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ABSTRACT

This work describes the development of a continuous flow magnetic bead based microfluidic biosensor for antigen concentration tracking. These devices will ultimately be applied to clinical applications such as for monitoring systemic inflammation during cardiac surgeries. A novel magnetic actuation scheme is used to enable autonomous on-chip serial reaction bead processing and detection. Previously reported devices used a single layer device with a straight incubation channel. A novel implementation is presented using a similar magnetic actuation scheme within multi-layer devices with spiral incubation channels. These devices offer reaction incubation times up to 40 times longer than previous designs with continuous bead motion for lower antigen concentration detection limits.

KEYWORDS: Immunoassay, Magnetic Actuation, Spiral Channel, Microbeads

INTRODUCTION

An autonomous, magnetically actuated, bead-based microimmunofluorocytometry assay for continuous protein concentration monitoring was previously reported [1]. This technology is being developed with the goal of monitoring inflammatory markers found in the blood during cardiac surgeries. A two-stage magnetically actuated immunosensor with straight incubation channels has been able to measure the concentration of the inflammatory marker complement C3a in an untagged sample stream. This technology has been expanded using a novel microchannel design, which provides much longer bead incubation times for detecting analytes found at lower concentrations. A spiral incubation channel is used to provide incubation times up to 30 minutes at a flow rate of 100 nl/min, 40 times longer than previous designs, while the incubation time can be simply adjusted by altering the flow rate. Since the device operates with continuous bead injection and processing, it is also capable of providing temporal measurements at a much higher sample rate than traditional immunoassays. The spiral channel is the most efficient use of chip space for providing a long incubation channel yet does not have the bead sticking/aggregation problems which can occur with channel geometries involving sharp turns.

THEORY

Both the straight channel and spiral incubation devices use magnetic actuation to autonomously manipulate paramagnetic streptavidin coated microbeads between adjacent reactant streams. Each can be implemented as either a single incubation stage, or dual incubation stage device. The single-stage devices have been used for benchmarking the technology with a representative binding of a fluorescently tagged biotin molecule (biotin-FITC) to the streptavidin coated microbeads. The two-stage devices are designed for direct measurement of protein concentration from a native sample stream. This is accomplished with an antigen-sandwich immunofluorocytometry assay. In the first stage of this assay the antibody coated beads are incubated with the sample fluid and the antigen binds to the bead surface, while in the second stage the antigen coated beads are incubated with a fluorescently tagged secondary antibody. In devices with a straight incubation channel [1], the beads are pulled to the side of

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the device where they roll along the wall at a velocity much slower than the mean velocity of the channel while incubation occurs. Subsequent to the incubation, another magnet pulls the beads across the channel into a wash stream. These devices are optimal for incubation times of a few seconds up to 1 minute.

In the spiral incubation devices (Figure 1), the same magnetic actuation is used, but a long spiral channel is added to increase the incubation time and sensitivity of the assay. These devices are optimal for incubation times of a few minutes to half an hour. All of the devices allow tuning of the incubation time through flow rate adjustments. After the beads are pulled by the magnet from the bead carrier stream to the sample stream, they enter the spiral incubation region from the center by transferring to a lower layer of the device. Subsequent to the spiral, the beads return to a straight channel where the same magnet pulls them into the next fluid stream. For the single-stage device this is a wash stream, whereas for the two-stage device it is the fluorescently labeled secondary antibody stream. Beads in two-stage device then transfer to an incubation spiral in the third layer before being pulled by the magnet into a wash stream. Once in the final wash stream, fluorescence detection occurs. Concentration measurements are made by measuring the fluorescence intensity of each bead and applying the mean intensity to a calibration curve. For temporal tracking, a moving average filter is used to determine the mean bead intensity.

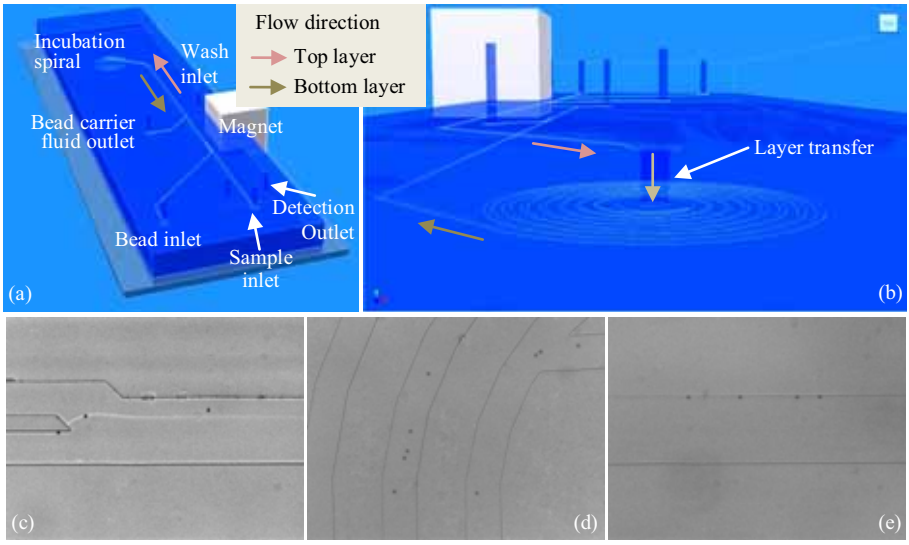

Figure 1. a)Conceptual rendering of single-stage device with spiral incubation b)Conceptual rendering of spiral incubation channel and layer transfer c)Micrograph of beads being pulled into sample stream and against wall by magnet d)Micrograph of beads entering and progressing through the incubation spiral e)Micrograph of beads rolling along wall in wash fluid after the second separation

EXPERIMENTAL

Both the single and dual stage devices have been fabricated by soft lithography. Each layer is separately fabricated and the inlet and outlet ports are punched with an 18 gauge needle. The second layer is then bonded to the top layer, and its inlet and outlet ports are punched again so that they continue through the top layer to the top surface of the device. For a two-stage, three layer device, the third layer is then

bonded to the second, and the inlets and outlets are punched through the two upper layers. Finally, a 75 mm x 25 mm glass slide is bonded to the lowest layer.

RESULTS AND DISCUSSION

The single-stage device was tested to show the incubation adjustability range of the devices. The device was infused with a 10 ng/ml Biotin-FITC solution, streptavidin coated microbeads suspended in a carrier fluid, and a wash fluid, all at equal flow rates. The device flow rate was varied and fluorescence intensity measurements were made on the incubated beads. The data (Figure 2) show a linear increase in fluorescence with increased incubation time. Each measurement is based on the average fluorescence intensity of approximately 300 beads. This device, with a 29 cm long, 100 μm channel width incubation spiral, is capable of incubation times from 4 minutes to 14 minutes. A second implementation of the device has a 59 cm long, 200 μm channel width spiral, and is capable of incubations up to 30 minutes at 100 μl/min. A three-layer version of the device has also been fabricated and shown to appropriately actuate beads for a two-stage immunoassay. The two-stage spiral device will be used to measure concentrations of inflammatory markers (complements and cytokines) found in low concentrations in the blood. Quantum dots are also being tested as a fluorescent tag. It is expected that using different colored quantum dots conjugated to a variety of secondary antibodies will enable a multiplexed micro-immunoassay can to measure a range of inflammatory markers simultaneously.

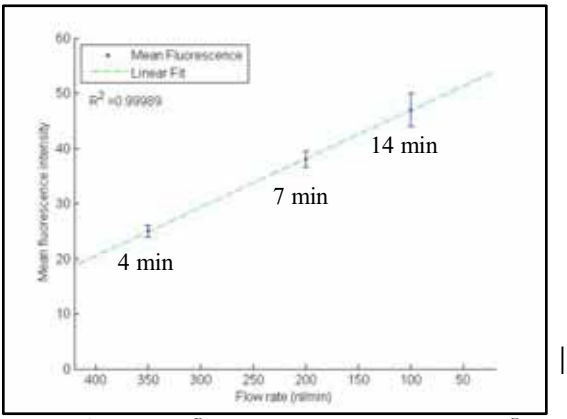

Figure 2. Mean fluorescence intensity versus flow rate from spiral incubation device for a 10 ng/ml (15μM) sample of biotin-FITC. Approximate incubation times are shown for each flow rate.

CONCLUSIONS

The magnetically actuated microimmunosensor technology presented enables integrated autonomous fluorocytometry with single and dual incubation stages for high sample rate measurements of analyte concentration. Devices using this technology have been implemented with incubation times from under a minute to 30 minutes, allowing the detection of antigens found in a wide range of concentrations. Ultimately, these devices will be applied to clinical monitoring applications.

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REFERENCES

[1] L.A. Sasso and J.D. Zahn. Continuous Microfluidic Immunosensing with Antibody Conjugated Paramagnetic Beads. Proceedings of MicroTAS 2008, San Diego, California, USA, October 12-16. pp. 77-79.