CONTINUOUS MICROFLUIDIC IMMUNOSENSING WITH ANTIBODY CONJUGATED PARAMAGNETIC BEADS
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ABSTRACT
A microdevice assay is being developed to continuously measure specific antigen concentrations within a sample stream. This device will allow real-time measurement of systemic protein concentrations within blood plasma, at high sample rates not possible with existing bench-top assays. Magnetic actuation of antibody coated paramagnetic microbeads between sample and wash streams leads to highly controlled residence times for accurate and reproducible measurements. A two-stage antigen sandwich assay is used to determine the antigen concentration based on fluorescence intensity of a secondary antibody. The microdevice can act as an on-chip Enzyme-Linked Immuno-Sorbent Assay (ELISA) with real time tracking of antigen concentration.

KEYWORDS: Immunosensing, ELISA, Magnetic, Microfluidic, Anaphylatoxin

INTRODUCTION
Studies have shown that cardiopulmonary bypass (CPB) procedures induce systemic inflammatory responses characterized by the release of anaphylatoxins. Evidence suggests that systemic inflammation causes many postoperative complications including vital organ dysfunction [1]. Current methods to assess anaphylatoxin concentration rely on bench-top assays requiring relatively large sample volumes. Due to limitations on the amount of blood that can be drawn from the patient (especially neonates) during CPB, the achievable sample rate of these assays is very low, which hinders the study of the inflammatory response. A microfluidic assay will allow real-time measurements of antigen concentration at much higher sample rates. This technology will allow researchers to gain a better understanding of inflammatory responses, leading to better intervention and treatment techniques. The microdevice uses a magnetic actuation scheme to manipulate paramagnetic microbeads, which are used to measure antigen concentration. This has proven more robust and simpler to implement that previous designs relying on hydrodynamics alone [2]. The device has been tested for an analogous reaction, and an assay to measure anaphylatoxins has been designed.

THEORY
The designed microdevice assay is based on an antigen sandwich technique, where the primary antibody is immobilized on a paramagnetic bead and the secondary antibody is conjugated with the fluorescent marker, phycoerythrin (PE). It is designed to measure typical systemic anaphylatoxin concentrations seen before and during CPB. The amount of antigen bound to the bead surface after incubation is a function of both antigen concentration and bead incubation time. Thus, incubation time within the microdevice must be controlled so that the amount of bound antigen is linearly dependent on concentration within a proper dynamic range.

Prior to running the microdevice assay, streptavidin coated paramagnetic beads are coated with a biotinylated monoclonal antibodies to the antigen of interest. All further bead processing occurs within the device in a common straight channel. The beads are introduced in a carrier fluid to the center stream of a 2 cm long microchannel. Running
adjacent to the beads are the antigen stream and the wash streams, which are localized through hydrodynamic focusing. Magnets on opposite sides of the channel direct the beads into the appropriate stream as they travel through the device. The beads are first pulled into the antigen stream, where they roll along the wall allowing antibody-antigen binding to occur for the predetermined incubation time. At a fixed point downstream, the magnet on the opposite side of the device pulls the beads across the channel into the wash stream, which transfers them to a second stage free of unbound antigen. In the second stage, the PE-conjugated secondary antibody stream and second wash stream are introduced alongside the stream of beads. Again, the beads are pulled magnetically into an incubation stream containing the PE-conjugated secondary antibody and then to the wash stream. As the beads flow through the wash outlet they pass over the focused beam of an argon ion laser with an emission of 488nm. A photomultiplier tube detects the intensity of fluorescent emission from the beads, which is proportional to the amount of antigen adsorbed on on the bead surface. Since beads are continuously introduced into the device they are able to detect time-varying changes in antigen concentration, allowing real-time measurements. Figure 1 shows a conceptual rendering of the microdevice.

Figure 1. Conceptual rendering of two-stage immunosensor device

EXPERIMENTAL

The physical operation of the microdevice assay has been tested with an analogous reaction, binding streptavidin coated beads with biotinylated FITC. The incubation and measurement was done on-chip in a single stage with magnetic actuation and PMT detection. Figure 2 shows a conceptual rendering of the device as well as a composite micrograph showing the beads entering the FITC solution, and a photograph of the device.

Figure 2. Rendering of FITC device, composite micrograph of beads in channel being magnetically pulled into the antigen stream, photograph of complete device

The biological aspect of the assay was tested to show that biotin-streptavidin binding could be replaced by antibody-antigen-antibody binding in a two-stage process with an anaphylatoxin (C3a) as the antigen. This was done by bench incubating over varying
times with C3a concentrations in the range expected from systemic samples, generally 1000 to 2500ng/mL [3]. The mean intensity of each sample was used to find a time scale that produced a linear relationship between fluorescence and concentration.

RESULTS AND DISCUSSION

Results from the streptavidin-biotin microdevice show the ability to measure antigen concentration with fully on-chip processing. The incubation time for this assay was 18 seconds in a 1 cm long microchannel at a flow rate of 300 nL/min. Bench-top testing of the two-stage incubation with the anaphylatoxin C3a showed that an incubation time around 45 seconds for each stage would produce good dynamic range for distinguishable fluorescence intensity measurements using standard flow cytometry. Thus, a two-stage device with a 2 cm long channel (1 cm for each incubation step) would require about one third this flow rate, or 100 nL/min, for incubation times that will achieve the proper dynamic range. Figure 3 shows the mean fluorescence versus biotin-FITC concentration for the biotin-streptavidin microdevice, as well as the mean fluorescence versus C3a concentration from bench-top incubation.

CONCLUSIONS

It has been shown that a microfluidic assay based on magnetic actuation of antibody coated beads is capable of direct, on-chip, and real-time measurement of pro-inflammatory cytokines at systemic concentrations. The microdevice can provide previously unattainable, high sample rate data related to inflammatory response and other systemic reactions involving the release of signalling proteins into the bloodstream.

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REFERENCES