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FIELD-DEPLOYABLE MICROFLUIDIC IMMUNOASSAY DEVICE FOR PROTEIN DETECTION

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ABSTRACT

We present a field-deployable microfluidic immunoassay device in response to the need for sensitive, quantitative, and high-throughput protein detection at point-of-need. The portable microfluidic system facilitates eight magnetic bead-based sandwich immunoassays from raw samples in 45 minutes. An innovative bead actuation strategy was incorporated into the system to automate multiple sample process steps with minimal user intervention. The device is capable of quantitative and sensitive protein analysis with a 10 pg/ml detection limit from interleukin 6-spiked human serum samples. We envision the reported device offering ultrasensitive point-of-care immunoassay tests for timely and accurate clinical diagnosis.

KEYWORDS

Microfluidics, field-deployable, immunoassay, protein detection, automated sample preparation

INTRODUCTION

Identifying specific protein biomarkers is a critical clinical procedure for timely and cost-effective diagnosis. Therefore, the development of mobile and low-cost technology for sensitive protein detection is of interest in the clinical setting [1]. Qualitative lateral flow strips are commonly used for rapid, portable immunoassay tests. However, their sensitivity highly depends on the types of the sample matrix. The qualitative readout of lateral flow strips is inadequate for some conditions that require quantitative analysis for a correct decision. On the contrary, the enzyme-linked immunosorbent assay (ELISA) is a gold standard technique for sensitive and quantitative detection of proteins, allowing detection limit in some cases down to femtomolar concentration [2]. However, complicated sample handling steps, long turnaround time, and bulky benchtop scanners limit its use at the point-of-need [3]. Alternatively, the microfluidic approach is well suited for on-site clinical diagnosis because of its inherent attributes of portability, low sample/reagent volumes, and automated processing [4]. To this end, extensive research efforts have been made to translate laboratory-quality immunoassays into a microfluidic format over the past decade [5-7].

Microfluidic immunoassays frequently involve using solid-phase particles, so-called bead-based ELISA, because a high surface-to-volume ratio promotes antigen-antibody binding. Use of beads significantly reduces the assay time while improving the sensitivity, making bead-based assays ideal for sequential immunoassay procedures (i.e., streamlined incubation, purification, and reaction). However, the primary consideration to implementing bead-based immunoassays in microfluidics relies on the strategic actuation of beads and liquid droplets in a controllable manner.

The most intuitive approach is a flow-based microfluidic immunoassay platform [5]. The sequence was performed by sequentially merging reagent inflows to bead-containing droplets via T-junction, then extracting beads using a magnet. Despite its automated capability, tubing and infusion pumps are not preferred for portability. As an alternative, a centrifugal microfluidic platform can drive the beads for the streamlined immunoassay process without peripheral devices [6]. However, centrifugal force can only direct the beads in one direction. Therefore, a complicated sample sequence that requires multi-directional bead operation (e.g., agitation) is limited. Electrowetting-based digital microfluidics (DMF) enables flexible discrete droplet actuation (move, split, and merge) on the hydrophobic surface [7], this method holds great promise for adapting magnetic bead-based immunoassay. However, active magnetic bead actuation for resuspension and agitation is challenging to implement in a droplet, especially with a stationary magnetic trap. In addition, the adsorption of proteins and detergents causes biofouling or cross-contamination, affecting surface wettability and creating challenges for reproducibility with complex sample matrices. As opposed to controlling droplets against stationary beads, active magnetic actuation on beads can alleviate such problems. For example, Chiou et al. developed a system that electromagnetically actuates magnetic beads in stationary droplets [8]. However, the system is only compatible with certain types of beads (e.g., large size, high susceptibility) because small electromagnets cannot provide sufficient magnetic fields to induce strong magnetic forces on small beads. The necessity to use large beads is at odds with benefit of high surface-to-volume ratio to maximize sensitivity. Although numerous works have been reported, integrating reliable, robust, and automated front-end immunoassay is still a common hurdle for most existing point-of-care microfluidic devices.

Herein, we present a field-deployable microfluidic immunoassay device for sensitive protein detection with autonomous sample preparation capability. The system consists of a mobile analyzer and a compact microfluidic chip. The platform integrates electromechanical and optical subsystems, and the microfluidic chip contains eight spatially isolated testing units spaced for 8-channel pipette compatibility. An embedded programmable planar electromagnetic microactuator array actuates the permanent magnet. Thus, magnetic beads can be reliably manipulated against the stationary droplets by strong magnetic interaction, enabling an autonomous sample process. The system facilitates the whole immunoassay process (i.e., incubation, purification, and chemiluminescent reaction) from raw samples in 45 minutes total processing time for 8 samples. The device achieved a detection limit of 10 pg/ml with Interleukin-6 (IL-6) in human serum. The sensitive, quantitative, and high-throughput immunoassay device will enable timely and accurate diagnosis at the clinical site.

MATERIALS AND METHODS

Instrumentation

Fig. 1A shows a system overview of the microfluidic immunoassay system. The platform was designed in SolidWorks, 3D printed and assembled in the lab. The fully integrated system has a footprint of 11×12×8 cm and contains optical and electromechanical subsystems. ***Optical subsystem:*** A low-noise CMOS imager (ULS24, Anitoa Systems) was integrated into the system to measure the chemiluminescent signal. The image sensor surface is directly in contact with the microfluidic channel to maximize the collection of blue emission light (peak wavelength: 425 nm). In this optical configuration, the sensing area (3.6×3.6 mm) sufficiently covers the detection zone of the individual testing unit without an optical lens or filter. We attached mylar reflective

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film on top of the permanent magnet located at the bottom of the detection zone during the chemiluminescent measurement to further increase the light collection. ***Electromechanical subsystems:*** Integrated electromechanical subsystems include linear, rotary, and electromagnetic actuators. For a linear actuator, a rack-and-pinion mechanism converts the rotary motion of the stepper motor into a linear motion using in-house fabricated rack/pinion gears. A unipolar stepper motor enables the precision positioning of the microfluidic chip. On the right edge of the rack, a high-torque servomotor was installed as a rotary joint to rotate the microfluidic chip (Fig. 2 - step 2). A custom-printed planar electromagnetic microactuator array is used to actuate a neodymium magnet (grade: N52) that further manipulates the magnetic beads in a microfluidic channel in a programmed manner. Each rectangular-shaped planar coil has a copper trace with a width of 170 µm, a thickness of 36 µm, and a pitch of 170 µm. Adjacent planar coils are partially overlapped in different printed circuit board (PCB) layers with a 4.5 mm gap. It is noteworthy that magnetic force follows an inverse square relationship with distance [9]. Therefore, a neodymium magnet close to the coil center can be actuated by a small electromagnetic field with low power consumption (<2.5W). To transport a neodymium magnet from one location to another, the localized electromagnetic field is generated from the coil one at a time by sequentially switching the transistor. A customized PCB with an embedded microcontroller unit (MCU) operates all integrated electronic components. The entire system is powered by either a USB connection or a DC power adapter (5V, 500 mA)

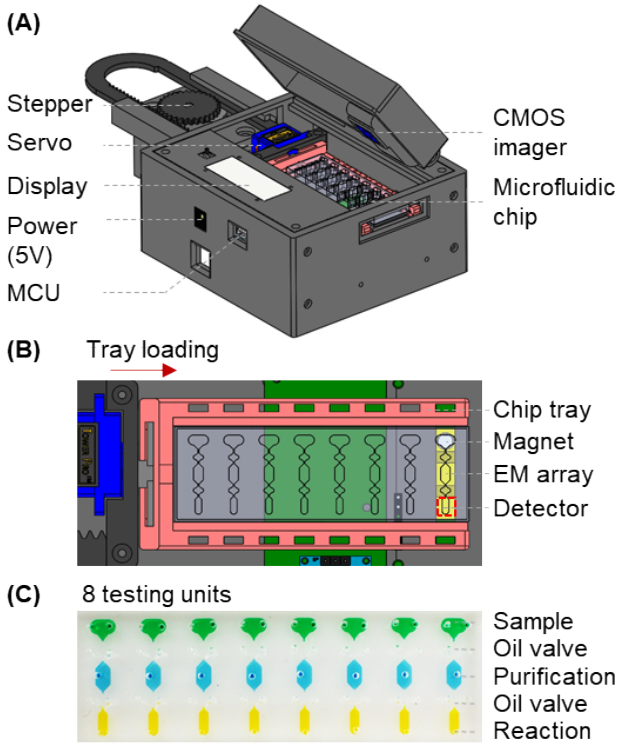


Figure 1: System overview. (A) Platform design. (B) Top-down view. Rack/pinion linear actuator moves microfluidic chip in the x-direction to align with planar electromagnetic microactuator array. The detector vertically aligns with the reaction chamber (red square) (C) Photo of a microfluidic chip showing eight independent testing units. Before the test, all reagents were preloaded in a ready-to-use fashion (food dyes were loaded for illustration purposes).

Microfluidic chip

A microfluidic chip was constructed by casting polydimethylsiloxane (PDMS) into a 3D-printed reusable master mold. A negative mold was printed with a commercial resin-based stereolithography 3D printer (Form 3, Formlabs). After printing, the mold was sonicated with isopropyl alcohol, then post-cured in a UV box at 60 °C for 30 minutes. The mold surface was silane-modified following oxygen plasma treatment to avoid PDMS sticking to the mold surface and prevent uncured resin residues from interfering with PDMS curing. To fabricate the PDMS device, curing and elastomer agents were thoroughly mixed in 1:10 (m/m), degassed in a vacuum desiccator, then cured at 65 °C for 2 hours. Reagent-loading holes were punched on a PDMS stamp and irreversibly bonded to a glass slide following oxygen plasma treatment.

The compact reagent microfluidic chip (25×70 mm) has eight spatially isolated testing units spaced for standard 8-channel pipette compatibility (Fig. 1C). Each testing unit has a sample, purification, and reaction chambers. These three chambers contain 15 µl of sample diluent with 25 µg of functionalized magnetic beads, 15 µl of washing buffer (PBS with 0.5% TWEEN-20), and 8 µl of chemiluminescent substrate, respectively. In addition, two oil valves hold 10 µl of mineral oil with 1% ABIL to separate the chambers. All reagents required for magnetic bead-based sandwich immunoassay were pre-loaded into the chip before the test.

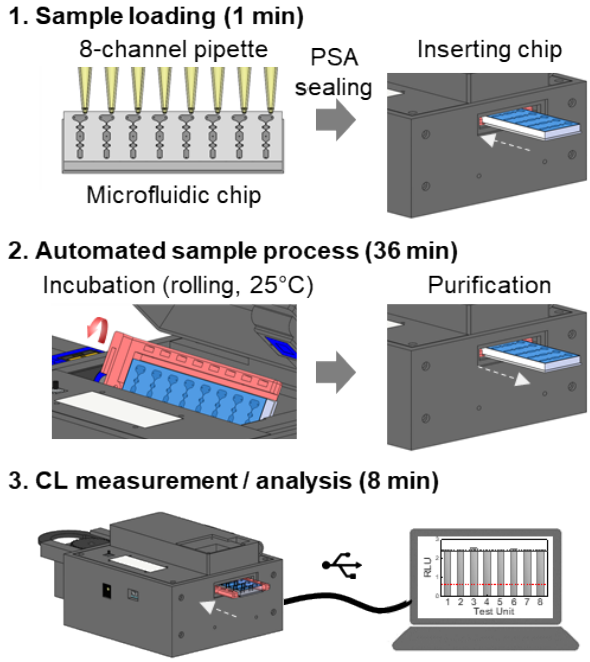


Figure 2. Overall operation workflow. Within 45 minutes, the automated immunoassay platform can process eight tests from a sample matrix (e.g., human serum) with minimal user intervention.

Workflow

The workflow of the device comprises sample loading, automated sample process, and chemiluminescent (CL) measurement/analysis (Fig. 2). Briefly, 15 µl of human serum is loaded into each sample chamber using an 8-channel pipette, then sealed with clear pressure-sensitive adhesive (PSA). Once a prepared microfluidic chip is inserted, the platform facilitates the automated sample process (i.e., incubation and purification). During the incubation, the microfluidic chip tumbles to prevent the

bead settlement and promote the formation of capture antibody-coupled magnetic beads, antigen, and HRP-linked antibody complex for CL detection. After the sample preparation, the CL signal from each testing unit is measured one at a time; then analyzed using a custom-developed program.

Bead-based immunoassay for protein detection

The integrated magnetic bead-based sandwich immunoassay has three components: capture bead, detector antibody, and target protein samples. ***Capture bead preparation:*** 30 µg of target-specific polyclonal antibody was covalently coupled to 1 mg of epoxy-activated superparamagnetic bead surface using a commercially available antibody-coupling kit (14311D, Invitrogen). After overnight incubation at room temperature, antibody-coupled beads were thoroughly washed three times with purification buffer to remove the unbound antibody excess and then blocked with SuperBlock agent (37518, Thermo Scientific) to prevent non-specific binding. The final stock concentration of the capture bead was set to 10 mg/ml. ***HRP-labeled detector preparation:*** monoclonal antibody was labeled with HRP conjugation kit (ab102890, Abcam). The final concentration of the HRP-labeled detector was adjusted to 1 µg/ml with phosphate buffer saline (PBS) with 0.05% tween-20. ***Target protein sample preparation:*** protein was spiked into PBS and human serum to validate the assay and sample matrix effect.

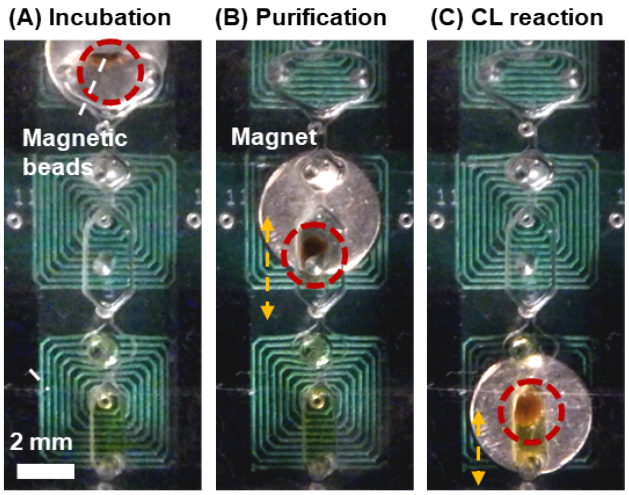


Figure 3. Automated sample preparation. The planar electromagnetic array actuates the permanent magnet to drag magnetic bead into desired working chamber through channel.

Automated sample preparation

For automated microfluidic sample preparation, magnetic beads were actuated against the stationary reagent droplets in the fluidic channel/chamber by controlled neodymium magnet motion on electromagnet micro-actuators. The integrated sample preparation consists of incubation, purification, and CL reaction steps (Fig. 3). During the incubation step, functionalized bead specifically captures the complex of the target protein and HRP-conjugated detection antibody into the sample chamber (Fig. 3A). After the incubation, the neodymium magnet drags the target-captured magnetic beads to the purification chamber. For purification (Fig. 3B), magnetic beads were agitated back and forth to remove the reagent carryovers (i.e., unbound analyte and detector antibodies) from the sample chambers. Thoroughly washed beads were then transferred to the reaction chamber for CL measurement (Fig. 3C).

A funnel-shaped microfluidic channel between chambers and valve assists in forming the magnetic bead cluster and maximizes the magnetic force acting on the beads for efficient bead capture. Note that the mass of the magnetic bead cluster is proportional to the magnetic force [10]. The addition of 1% ABIL EM-90 surfactant in mineral oil lowers the interfacial tension at the aqueous-to-oil interface; thus, magnetic beads can easily transport from chamber to chamber without significant loss.

RESULTS AND DISCUSSION

Uniformity of eight testing units

Since the microfluidic immunoassay device facilitates eight tests, unit-to-unit consistency is a prerequisite for quantitative comparison. We used an IL-6 detection assay as a model to assess uniformity between channels. An identical concentration of purified IL-6 aliquot was loaded into each testing unit, and then processed using the automated protocol. The quantitative results among eight testing units showed excellent uniformity with slight variation (Fig. 4, black-dotted lines), implying good optical alignment (i.e., CMOS sensor, final position of beads in reaction chamber). The unit-to-unit consistency indicates robust reagent mixing and washing during the sample preparation, and consistent transfer of magnetic beads throughout the process. While manual sandwich immunoassays on microplates (e.g., ELISA) is prone to human errors due to iterative liquid exchanging steps, the integrated autonomous sample process mitigates such errors and allows eight tests on the mobile immunoassay platform.

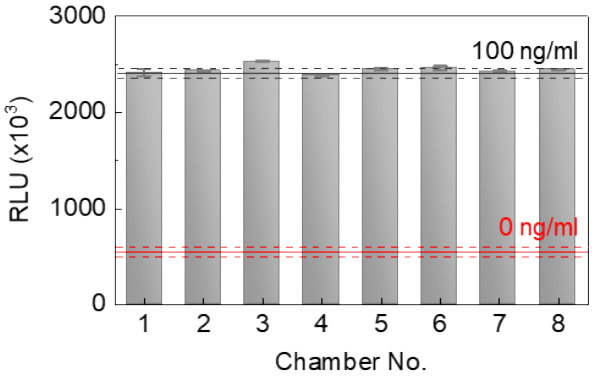


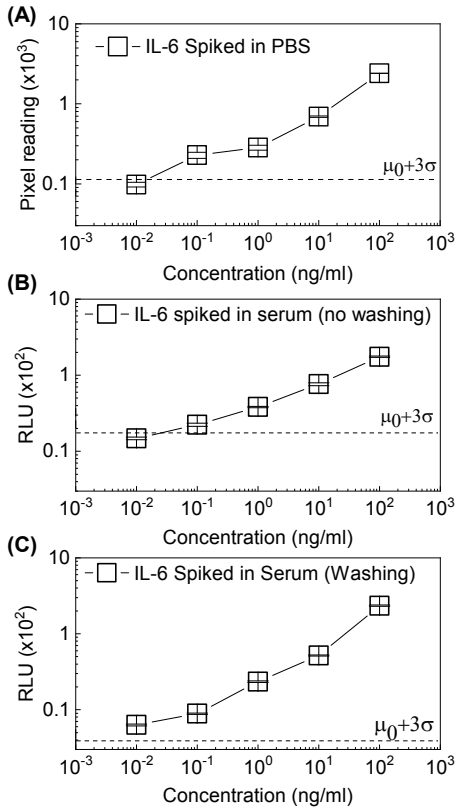
Figure 4. Chamber-to-chamber consistency among eight testing units (100 ng/ml: 2410 ± 50, 0 ng/ml: 548 ± 20. Clear CL signal differences were observed between 0 and 100 ng/ml IL-6 samples.

Sensitivity test

We next validated and optimized the analytical sensitivity of the device using an IL-6 detection assay. 10-fold serial dilution of IL-6 (10^{-3} – 10^{3} ng/ml) was spiked in human serum to mimic complex protein samples. The device automatically carried out the sandwich immunoassay on the microfluidic chip. The detection limit was determined as the lowest analyte concentration with a 3×standard deviation higher than the signal background. As shown in Fig 5, the linear standard curve showed the device's quantitative ability. The detection limit of 10 pg/ml was achieved with the presence of sample matrix. This level of sensitivity is comparable to a benchtop plate reader [2].

We further investigated how the integrated sample purification process enhances the device performance against the sample matrix effect. Without the on-chip sample purification, an order of magnitude higher detection limit (i.e., ~480 pg/ml) and decreased signal-to-background ratios were observed for pure PBS and complex serum spiked samples, indicating the clear presence

of the sample matrix effect. More specifically, the small amount of unbound HRP-labeled antibodies was carried over to the reaction chamber with beads due to viscous human serum. The incorporated washing step mitigates such sample matrix hindrance and enables sensitive protein detection.



	in PBS (no wash)	in serum (no wash)	in serum (wash)
S:B	4.2	2.8	5.2
LOD (pg/ml)	480	480	10

Figure 5. Sensitivity test results with/without built-in washing process. Signal-to-background (S:B). Error bars were obtained from triplicates.

CONCLUSION

In summary, we developed a microfluidic immunoassay device for sensitive and high-throughput detection of proteins in the field. The system consists of a mobile analyzer and a reagent-compact microfluidic chip. The embedded electromagnetic microactuator actuates the permanent magnet in a programmed manner to facilitate the sequential incubation and purification during the immunoassay process. Within 45 minutes, the system automatically prepares eight samples for CL measurement from human serum. The excellent unit-to-unit consistency indicated eight testing units' robust sample process with minimal bead loss and optical uniformity. Besides, the linear standard curve showed the quantitative ability of the platform. The device achieved a detection limit of 10 pg/ml with IL-6 spiked in human serum. Finally, we confirmed that integrated sample purification reduced the non-specific signal originating from the sample matrix effect, thus, improving the detection sensitivity. Although we only tested the IL-6 detection assay for device validation, it can be adaptable to identify various proteins by conjugating capture antibodies specific for the target of interest on magnetic beads. The reported device will enable highly sensitive immunoassays for accurate and timely diagnosis at point-of-need.

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