CENTRIFUGAL MICROFLUIDICS

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ABSTRACT

"Lab-on-a-disc", in which centrifugal force is used to pump fluid, has been a powerful tool for biological and chemical analysis due to its capability to integrate and automate all the process into a disc-shaped device that can be operated by simple, small-sized, and cost-efficient instrument. We report various examples of fully integrated lab-on-a-disc for biomedical applications such as pathogen specific DNA extraction to test infectious diseases, enzyme-linked immuno-sorbent assay (ELISA), simultaneous detection of blood chemistry and immunoassay, and multiplexed immunoassays starting from whole blood.

KEYWORDS: Lab-on-a-disc, POCT, DNA Extraction, Immunoassay, Blood Analysis

INTRODUCTION

There has been great advances in incorporating microfluidics for point-of-care diagnostic applications during the last decade. Despite significant improvement in sensitivity, throughput, cost and analysis time due to the scale-down effects, the full integration from the raw sample input to the final read-out for the biomedical diagnostics has been rare [2]. One of the major bottlenecks to realize "sample-to-answer" type analysis has been in the capability of on-chip handling of real biological specimen, e.g. blood, saliva, and urine etc. The fluidic control of biological samples and multiple kinds of reagents in microfluidic devices can be complicated. Lab-on-a-disc can be a simple alternative because it requires only single motor to control multiple fluidic transports [1]. A range of biological assays such as immunoassays [3], cell lysis for DNA analysis [4] and whole blood analysis [5] have been demonstrated on a centrifugal microfluidic platform.

EXPERIMENTAL

Recently, we have developed a unique phase change based active type of valve, i.e., the laser irradiated ferrowax microvalve (LIFM), that is based on the phase transition of ferrowax, paraffin wax embedded with 10 nm sized iron oxide nanoparticles [1]. Both Normally Opened LIFM (NO-LIFM) and Normally Closed LIFM (NC-LIFM) are possible as shown in Figure 1. It has been demonstrated that optical control of multiple microvalves is fast, robust, simple to operate, and requires minimal chip space and thus is well suited for fully integrated lab-on-a-chip applications.

The detail information of the disc fabrication is reported elsewhere [1, 3-5]. In brief, the microfluidic channels and

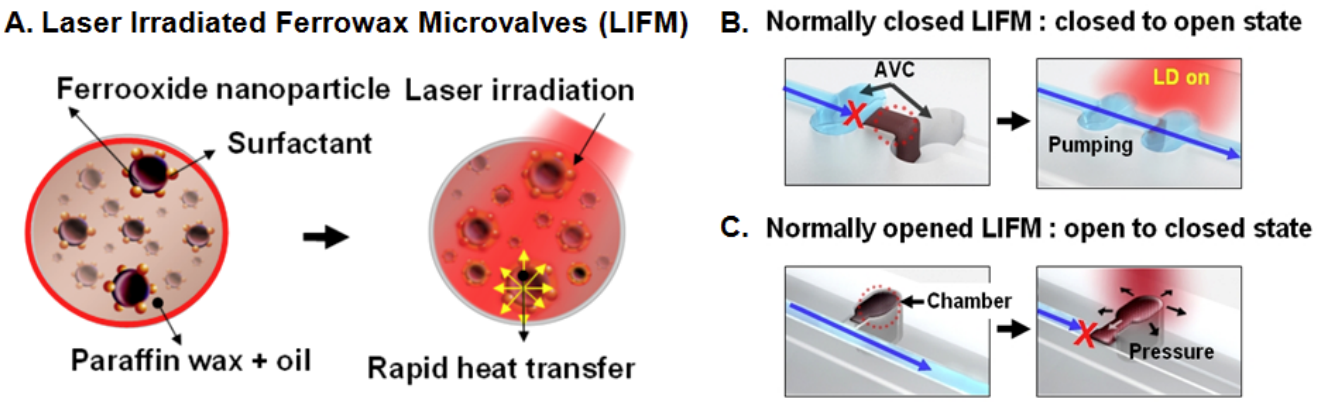

Figure 1: Schematic diagram of LIFM operation. (A) Heated nanoparticles rapidly melt the paraffin wax matrix upon laser irradiation. (B) Molten ferrowax plug placed in the channel by capillary action is solidified and the channel is normally closed. The channel can be open on-demand by laser irradiation. (C) The molten ferrowax is introduced to a reservoir adjacent to the main channel and the normally open channel cab be closed by laser irradiation.[1]

chambers are usually fabricated on the bottom disc by CNC-micromachining (Osan, Korea). The top disc has injection holes and the ferrowax loading chambers. The ferrowax are printed on the loading chambers by the custom-designed wax dispensing machine (Hanra Precision Eng. Co. LTD., Incheon, Korea). The disc is fabricated using either poly (carbonate), poly (methyl methacrylate), or cyclic olefin copolymer. For the rapid prototype fabrication in the laboratory, the top and bottom plates were bonded by using double-sided adhesive tape prepared by cutting plotter (Graphtec CE3000-60 MK2, Graphtec, USA). The fluid control on the rotating disc could be visualized by using CCD camera and strobe light which frequency was synchronized with the spinning speed.

RESULTS AND DISCUSSION

Pathogen specific DNA extraction from whole blood on a disc

As shown in Figure 2, PCR-ready pathogen specific DNA from whole blood could be prepared within 12 minutes with single manual operation [4]. Three blood samples could be analyzed simultaneously (Figure 2A). The plasma sample is prepared by centrifugation of whole blood at 3600 rpm for 3 minutes (Figure 2B). The automatically metered amount of plasma, 30 μL, is transferred to the mixing chamber and mixed with the magnetic beads pre-modified with specific antibody to capture the target virus. After washing the beads with the washing buffer, the total volume was reduced by several steps of valve actuation and fluid transfer by centrifugation. Then, PCR-ready DNA is prepared by simple laser irradiation on the magnetic beads (Figure 2C). The centrifugal microfluidic layout for the fully integrated analysis is given in Figure 2D and the final PCR results were compared with the conventional lysis methods as shown in Figure 2E. We could amplify DNA from HBV spiked in whole blood sample with the concentration of 10 copies/μL, which is as good as the commercially available methods even though the sample volume (100 μL vs. 1 mL), buffer volume (200 μL vs. 3 mL), and analysis time (12 min vs 2 hours) were significantly reduced.

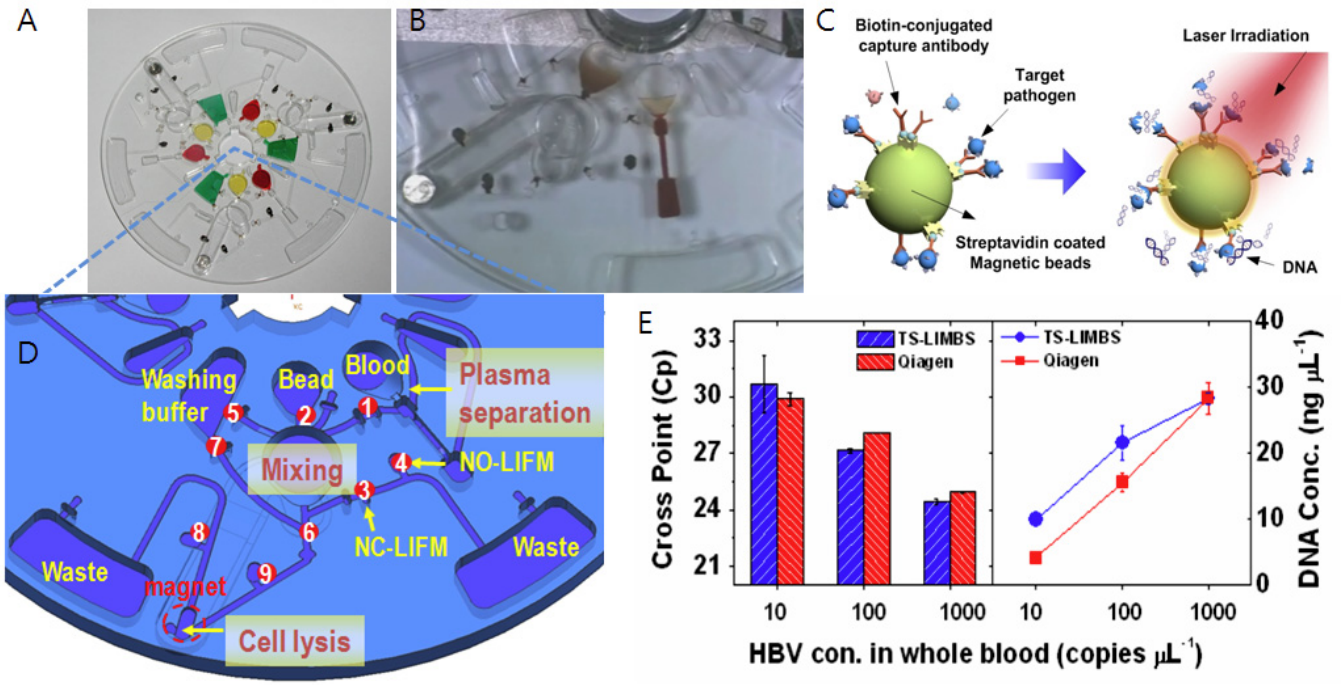

Figure 2: Pathogen specific DNA extraction from whole blood (A) Photo image of the fabricated disc loaded with color dye solution for clear demonstration. (B) Visualization of the centrifugal microfluidic control on the disc. Red blood cell is sediment during the centrifugation. (C) Schematic diagram of the DNA extraction from specific target cells by laser irradiation. (D) Centrifugal microfluidic layout to show the total process (E) Real-time PCR results of DNA prepared by the laser lysis on lab-on-a-disc compared with the sample prepared by conventional chemical lysis method.[4]

Enzyme-linked immuno-sorbent assay (ELISA) on a disc

A fully automated, microbead-based suspension type enzyme-linked immuno-sorbent assay (ELISA) (Figure 3A) system has been demonstrated to test infectious diseases from whole blood using the centrifugal microfluidic device [3]. The concentrations of the antigen and the antibody of Hepatitis B virus (HBV), HBsAg and Anti-HBs respectively, were measured using the lab-on-a-disc. All the necessary reagents are preloaded on the disc and the whole process of plasma separation, incubation with target specific antigen or antibody coated microbeads, multiple steps of washing, enzyme reaction with substrates, and the absorbance detection could be finished within 30 minutes (Figure 3B). The calibration curve for HbsAg and Anti-HBs is shown in Figure 3C and Figure 3D, respectively. Compared to the conventional ELISA, the operation time was dramatically reduced from over 2 hours to less than 30 minutes while the limit of detection was kept similar; *e.g.* the limit of detection of Anti-HBs tests were 8.6 mIU mL^{-1} and 10 mIU mL^{-1} for the disc-based and the conventional ELISA respectively.

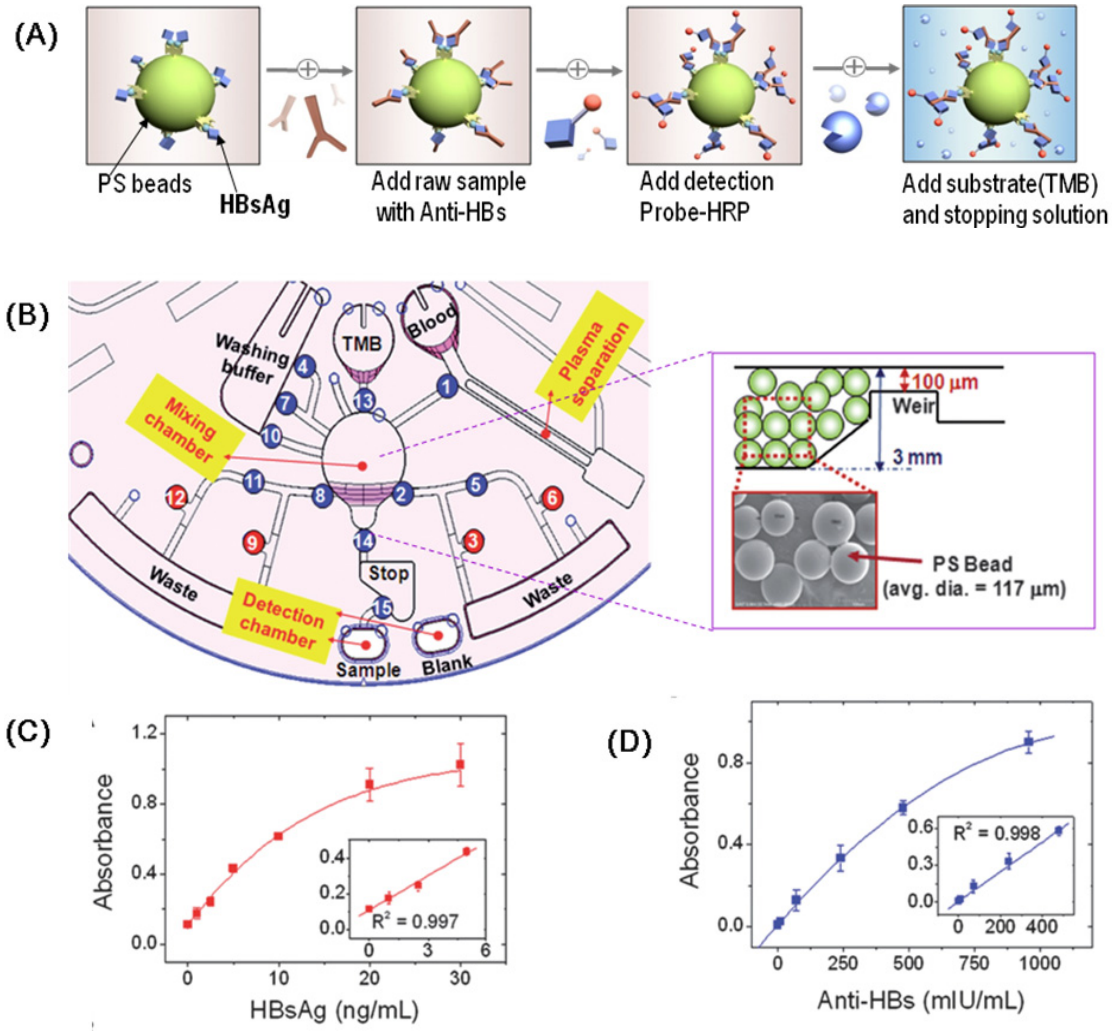

Figure 3. Schematic diagram showing (A) Principle of the bead-based immunoassay, (B) A disc layout showing the fully integrated bead-based immunoassay, Absorbance measured by the blood analyzer. Calibration curves of HBsAg (C) and Anti-HBs (D) measured by using the fully automated lab-on-a-disc. Each data point is the average of the 6 measurements obtained by 6 separate discs. The lines are a polynomial curve fitting. The inset shows the linear dynamic range [3].

Simultaneous analysis of blood chemistry and immunoassay on a disc

In order to have better diagnostics, the analysis of different kinds of target molecules such as enzymes, hormones, antibodies, and antigens are often necessary. For example, analysis of enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) in addition to the measurement of Anti-HBs and HBsAg are often required for the test for liver-related diseases. However, blood chemistry analysis for AST, ALT, GGT, and ALP and immunoassay for Anti-HBs and HBsAg are normally performed by using two separate instruments,

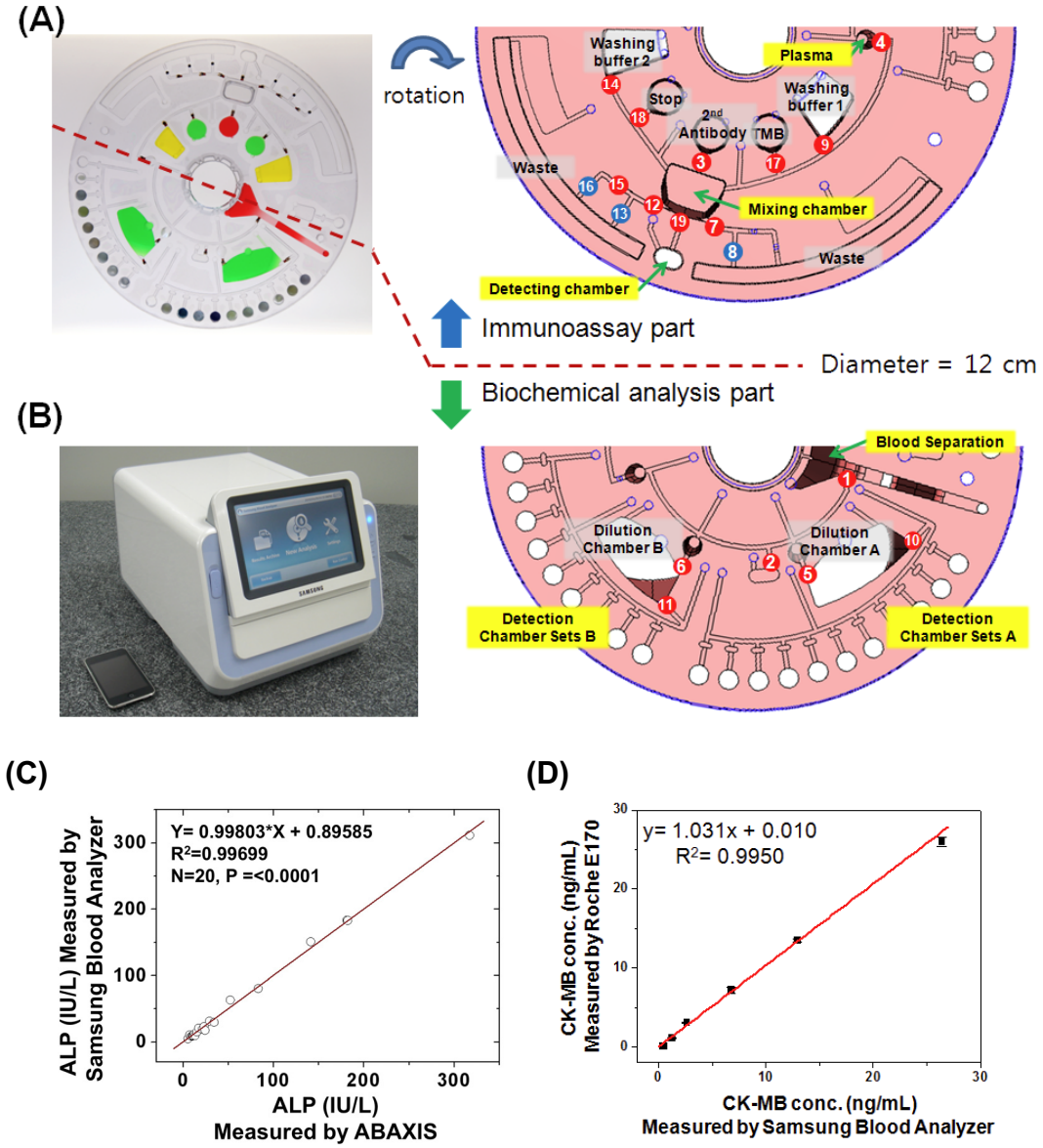

Figure 4. (A) Photograph of a disc. Detection wells on the clinical chemistry side are preloaded with lyophilized reagents. Other chambers for liquid type reagent are loaded with food dye solution for demonstration. In right hand side, the disc design shows the detailed microfluidic layout. The number indicates the order of the LIFM operation. The top half of the disc for the immunoassay part is rotated for easier demonstration. The blue circles with numbers are (NO)-LIFM. The other half of the disc for the clinical chemistry analysis part is shown in the bottom. (B) A photo image of the Samsung Blood Analyzer (25(W) x 35(D) x 25(H) cm) (C) ALP concentration measured by the proposed lab-on-a-disc and a commercialized blood analyzer, ABAXIS. (D) The concentration of CK-MB measured by the proposed lab-on-a-disc and a conventional analyzer, Roche E170. [5]

which results longer analysis time and higher cost. In this study, a portable diagnostic device that can perform not only immunoassays but also multiple kinds of biochemical analyses simultaneously on a disc starting from whole blood is developed as shown in Figure 4 [5]. Whole blood can be directly applied to the disposable "lab-on-a-disc" containing different kinds of reagents for the blood chemistry analysis as well as the immunoassay. The concentrations of different kinds of analytes could be reported within 20 minutes by simply inserting a disc into a portable device. Using the innovative laser irradiated ferrowax microvalves together with the centrifugal microfluidics, the total process of plasma separation, metering, mixing, incubation, washing, and detection was fully automated. The analyzer is equipped with an optical detection module to measure absorbance at 10 different wavelengths to accommodate the various kinds of reaction protocols. Compared to the conventional blood analysis done in clinical laboratories, it is advantageous for point-of-care applications because it requires a smaller amount of blood (350 μL vs 3 mL), takes less time (30 min vs several days), does not require specially trained operators or expensive instruments to run biochemical analysis and immunoassay separately.

Multiplex immunoassay on a disc

Diagnostics based upon the analysis of multiple biomarkers could significantly improve the detection accuracy in many biomedical applications. In this study, a lab-on-a-disc that is capable of simultaneous detection of three kinds of biomarkers,

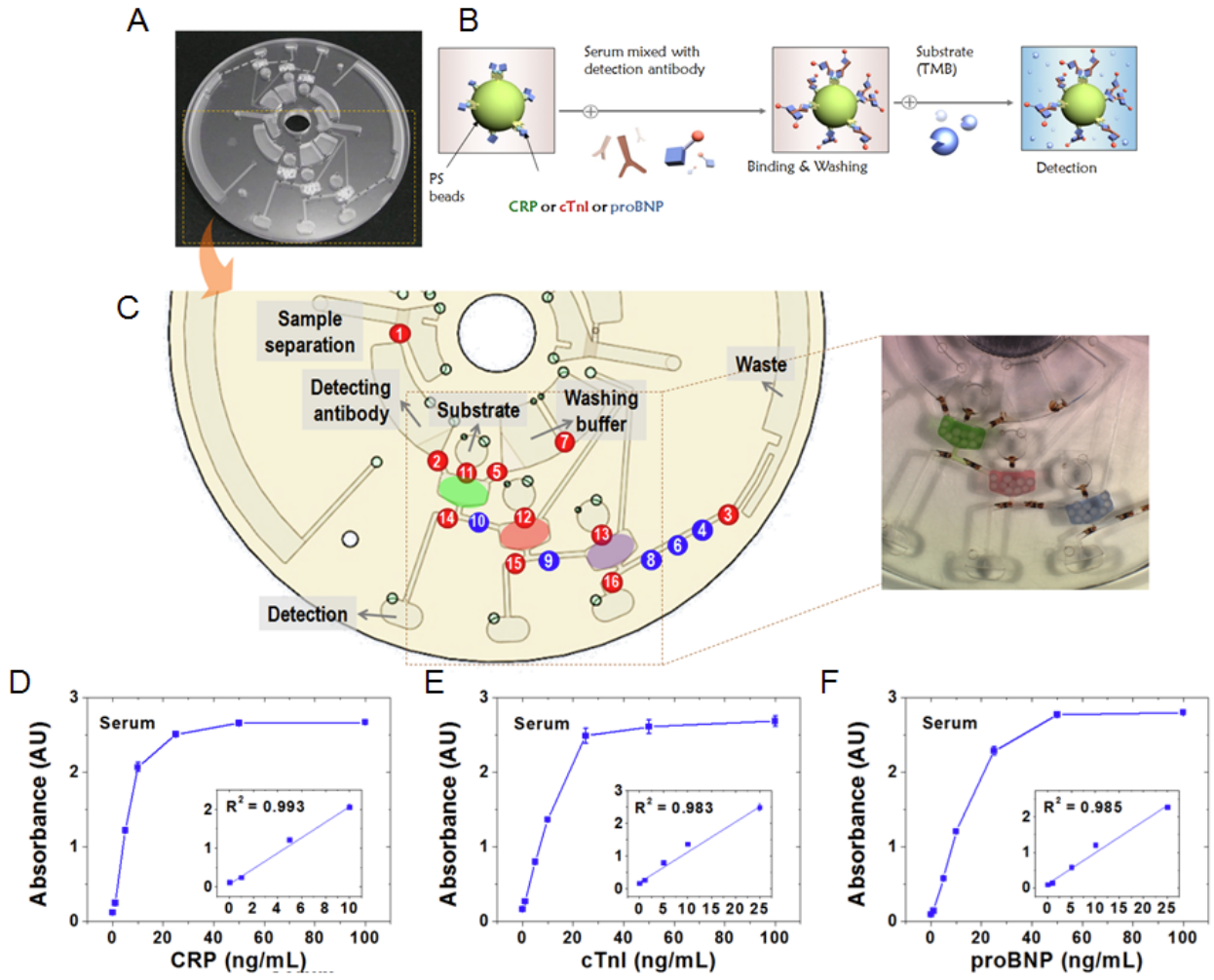

Figure 5. (A) Photograph of a disc for multiplex immunoassay. (B) Schematic diagram to show the concept of the bead-based immunoassay. (C) The disc design shows the detailed microfluidic layout. The number indicates the order of the LIFM operation. The blue and the red circles with numbers are (NO)-LIFM and (NC)-LIFM, respectively. Experimental results of the immunoassay to measure the concentration of CRP (D), cTnI (E), and proBNP (F) spiked in serum.

Troponin I, C-reactive protein (CRP), and proBNP from a sample is introduced. As shown in Figure 5A, each reaction chamber is preloaded with the polystyrene (PS) beads modified with capture antibodies. The bead-based immunoassay (Figure 5B) is used not only because it can provide more efficient mixing conditions but also it is versatile to accommodate other kinds of bioassays by simply replacing the beads. The detailed layout for the centrifugal microfluidic control is shown in Figure 5C. During the binding reaction with target solution and washing steps, the three chambers are interconnected. However, the chambers are isolated for the independent reaction steps such as the enzyme reaction by closing the NO-LIFM located between chambers. There was no significant cross-reactivity among the different sets of antibody and antigen. The calibration curves of the simultaneous detection of CRP, cTnI, and proBNP is shown in Figure. 3D-F. The limit of the detection and the dynamic range were as good as the conventional ELISA for all three targets even though the total reaction time and the reagents volume were significantly reduced; e.g. a few hours vs. 20 minutes of total reaction time, over a few mL vs. 300 μL of washing buffer.

CONCLUSION

We have developed various kinds of fully automated lab-on-a-disc for biomedical applications that can directly use the raw samples such as whole blood, urine, and whole saliva. The required reagents are preloaded on a disposable disc and there is no user intervention during the test, which may provide additional benefits such as increased specificity, reduced analysis time and total cost. So far, we have demonstrated pathogen specific DNA extraction, single and multiplexed immunoassay, and simultaneous analysis of blood chemistry and immunoassay. The binding efficiency was significantly enhanced due to the increased surface area as well as the efficient mass transfer due to the 3D mixing of the beads on a rotating disc. Assay performance values such as LOD or the dynamic range were optimized to be as good as the conventional methods even though the total assay time and the sample volume were significantly reduced. In addition, the sensitivity could be further improved by incorporating fluorescence and/or electrochemical detection. Furthermore, new kinds of applications such as the nucleic acids analysis starting from real biological samples and the environmental monitoring from raw samples such as seawater or soil are under development. The fluidic designs are based on the fundamental understanding of the centrifugal microfluidics which is a function of the dimension, shape, location, and surface chemistry of the channels, spin program including rotation speed, direction and acceleration, and their combination with other kinds of external thermal, and electromagnetic forces. In conclusion, the centrifugal microfluidics can provide simple and innovative alternative methods for micro total analysis systems.

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