One-step Pathogen Specific DNA Extraction from Whole Blood On a Centrifugal Microfluidic Device

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ABSTRACT

We have fully integrated complex microfluidic operational steps such as specific pathogen DNA extraction from whole blood on a CD. By combining the TS-LIMBS (Target Separation and Laser-Irradiated Magnetic Bead System) and centrifugal microfluidics using the novel LIFM (The Laser Irradiated Ferrowax Microvalves), the total process of the plasma separation, mixing with magnetic beads conjugated with target specific antibodies, removal of plasma residual, washing, and DNA extraction was finished within 12 minutes with only one manual step of adding 100 μL of whole blood. Real-time PCR results showed that the concentration of DNA prepared on the fully automated CD was as good as the samples prepared in conventional bench top method. Furthermore, a portable device (213.0 x 272.3 x 165.8 mm, 1,865 g) equipped with a small laser diode and a disc mounting stage like CD player was developed.

Keywords: lab-on-a-chip, DNA extraction, whole blood, centrifugal microfluidics

1 INTRODUCTION

Recently we reported a novel cell lysis method, Laser-Irradiated Magnetic Bead System (LIMBS). Addition of magnetic beads to the pathogen containing solution accelerated the heating speed. Therefore, DNA from various types of pathogens including both Gram-negative and Gram positive bacteria and hepatitis B viruses were effectively extracted by simply applying 40 seconds of laser (808 nm, 1.0 W) irradiation [1]. However, direct DNA extraction using LIMBS from raw samples such as whole blood and the concentration of cells or DNA were not possible.

In this paper, we report a fully integrated pathogen specific DNA extraction device utilizing centrifugal microfluidics on a polymer based CD. The design principles are schematically shown in **Fig. 1.** As a model study, DNA extraction from whole blood spiked with HBV was conducted using a CD pre-loaded with reagents.

"Lab-on-a-CD" platform in which the centrifugal pumping is the basic physical principle to transfer liquid in microfluidic structures has been investigated by various groups. The majority of centrifugal microfluidic platforms utilized either hydrophobic or capillary valves. The fabrication and the simultaneous actuation of multiple valves were relatively simple. However, for the robust control of the valving operation, fine tuning of the spin speed as well as the local surface properties or dimension of the microchannels were required. Furthermore, these valves can function only as opening valves; i.e. from normally closed state to open state, not vice versa. As a result, only a limited number of diagnostic tests that do not require complex fluidic design have been developed on a CD platform and launched to the market.

We have demonstrated an innovative Laser Irradiated Ferrowax Microvalves (LIFM) that are based on phase transition of ferrowax, parrafin wax embedded with 10 nm sized ferrooxide nanoparticles [2]. Compared to the conventional phase change based microvalves, the control of multiple microvalves was simple by using single laser diode instead of multiple embedded microheaters. Furthermore, LIFM is not very sensitive to rotation speed

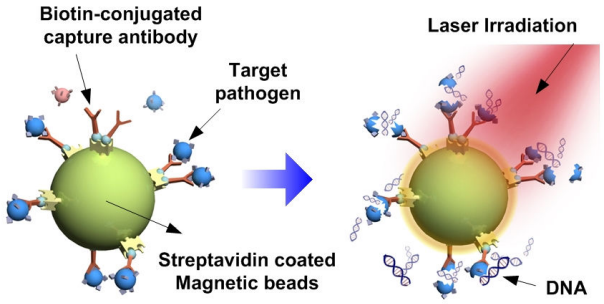

Fig 1. Schematic diagram of the reaction principle. The magnetic beads conjugated with target specific antibody are mixed with sample solution. Target pathogens are selectively captured on the magnetic beads and the waste materials such as plasma residue are washed away. Simple irradiation of laser (808 nm, 1.5 W) for 30 seconds could effectively extract PCR-ready DNA from captured target pathogens.

A

B

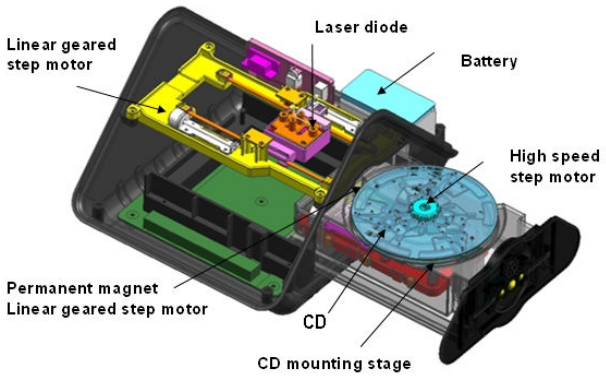

Fig 2 (A) A photo image of the portable device to operate a Lab-On-a-Disc for rapid DNA extraction from whole blood using Laser-Irradiated Magnetic Bead System (LIMBS). Single laser diode was used not only to operate multiple Laser Irradiated Ferrowax Microvalves (LIFM) but also to extract DNA from pathogens. **(B)** Schematic diagram showing the inside of the portable Lab-on-a-Disc system. A laser diode is mounted on a linear geared step motor. A permanent magnet is located on the other linear geared step motor located under the CD mounting stage. High speed step motor is used to run the spin program.

or surface properties. Both Normally Closed (NC) – LIFM and Normally Opened (NO) – LIFM were demonstrated and various fluidic functions such as valving, metering, mixing, and distribution were demonstrated using centrifugal microfluidic pumping.

In this report, single laser diode was used for dual purposes; for the multiple LIFM control as well as for the cell lysis. Because the laser beam is effectivley absorbed on magnetic beads or ferrooxide nanoparticles and the heat is generated very rapidly, both response times to extract DNA from pathogens and to operate microvalves were dramatically reduced. Furthermore, a hand-held type sample preparation device was developed as shown in **Fig. 2.**

2 MATERIALS AND METHODS

2.1 Instrumentation

As shown in **Fig. 2**, a hand-held type sample preparation device was fabricated using high power laser diode (BS808T2000C-MOUNT, Best-Sources Industry (HK) Co., Ltd, China) and high speed step motor (19TM-J802, Minebea Co., Ltd). The energy source of the device is 6 lithium ion battery pack (3.6 V 2200 mAh, LG 18650, LG CHEM, Korea). A microprocessor (PIC16F74, Microchip Technology. Inc.USA) and a main control board (Analog Research System, Korea) are used to regulate laser power, high speed step motor, and linear geared step motor for the positioning of the laser diode and permanent magnet. Spin program can be downloaded from PC and stored in EEPROM of the device via RS-232 communication.

As shown in **Fig. 3**, the CD is made of polycarbonate (PC) plates bonded with a double sided adhesive tape (Flexmount DFM 200 Clear V-95 150 POLY H-9 V-95 4, FLEXcon Inc., MA, USA). The inlet holes and microfluidic layouts were produced by conventional CNC machine (computer-controlled machine, Sirius 550, Hwacheon Inc. Seoul Korea). Each CD has three identical DNA extraction units.

2.2 Target specific pathogen concentration and DNA extraction using laser irradiation

The DNA extraction process is composed of plasma separation, mixing with magnetic beads conjugated with target specific antibodies, washing the magnetic beads, and lysis using laser irradiation. HBV solution (3×10^6 copies mL^{-1}) is spiked in human whole blood with the volume ratio of 1:2. The final HBV concentration of the sample was $10^4 \sim 10^6$ copies mL^{-1}. The detailed procedure of the antibody conjugation and real-time PCR is described elsewhere [3].

3 RESULTS AND DISCUSSION

3.1 CD design & spin program

As shown in **Fig. 3C**, a microfluidic layout was designed to fully integrate the TS-LIMBS method on a CD. The CCD image as shown in **Fig. 3D** was obtained during the rotation using the CCD camera and strobe light.

The total process of plasma separation, mixing with magnetic beads conjugated with target specific antibodies, removal of plasma residual, washing, and DNA extraction were automatically controlled by custom-designed software and takes less than 12 minutes. The detailed spinning program with captured images is discussed elsewhere [3].

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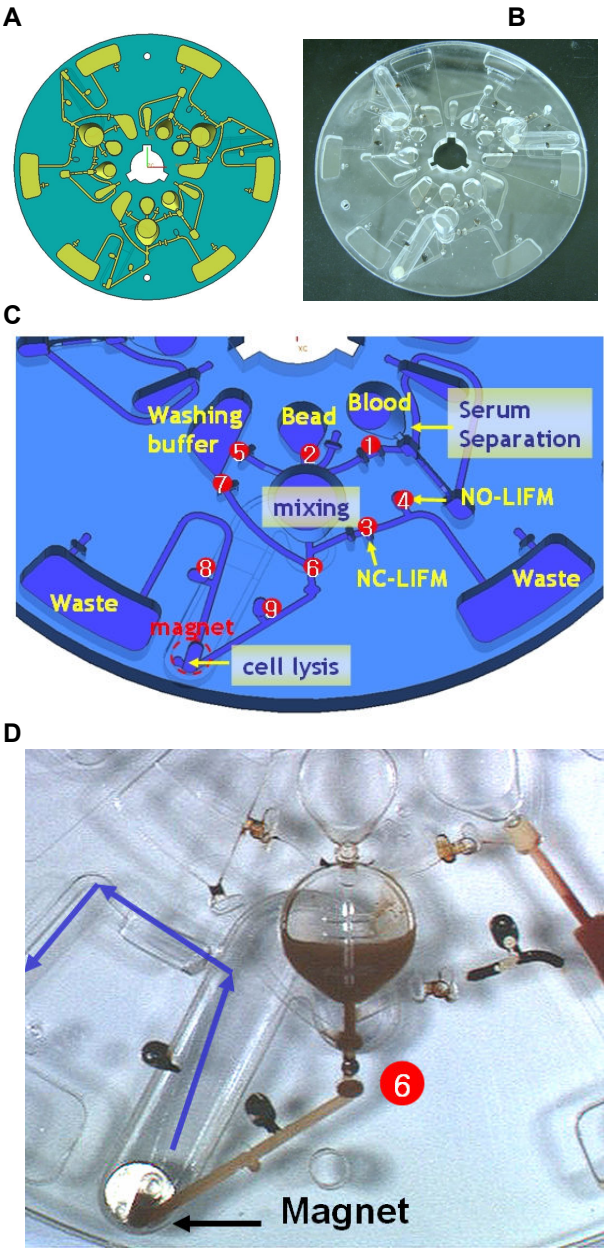

Fig. 3 Schematic diagram of the CD fabrication and microfluidic layout. The DNA extraction CD is composed of 3 parts; top and bottom parts are made of polycarbonate (PC) plates and bonded by a double sided adhesive tape. Schematic diagram **(A)** and photo image **(B)** of the fully assembled CD. The black spots in the photo image (B) are LIFM **(C)** CD design showing the detailed microfluidic layout and functions. The number indicates the order of the LIFM operation. **(D)** CCD images captured during the magnetic beads capture step before the cell lysis.

3.2 Control of magnetic beads on a rotating CD using a magnet moving rail

Though various biological assays have been tried on a CD platform, it is the first trial to integrate magnetic beads based assay on a CD platform. In order to control the position of the magnetic beads on a rotating CD, the following magnet moving rail system has been developed.

In order to control the location of the magnetic beads on a rotating CD, two permanent magnet (Nd-Fe-B Magnet, JungWoo, Korea) were used. One magnet was inserted on a magnet moving railroad attached on the bottom of the CD. The other magnet was fixed on the CD mounting stage and the position could be controlled by using a linear geared step motor. Both magnets were aligned to be attractive.

Depending on the direction and spin speed and the position of the magnet at the bottom, the location of the magnet inserted on the CD could be determined. For example, when the CD is rotated with a spin speed of 3 Hz to the clockwise direction, the magnet moves to the counter clokwise direction and stays at the position A as schematically shown in **Fig. 5A**. When the spin speed increased to 8 Hz, the magnet moves to the position B. On the other hand, if the CD spins to the counter clockwise direction with the spin speed of 8 Hz, the magnet moves to the position C and if the spin speed is larger than 10 Hz, the centrifugal force wins over the magnetic force and thus the magnet moves to the position D.

At small spin speed, the magnet inserted on a rotating CD is moved to the position where the bottom magnet is located; the magnetic force is larger than the centrifugal force. However, if the spin speed is fast and the direction of the spin is clockwise, the magnet moves to the direction of counter clockwise due to the inertia force, vise versa. If the spin speeds are too fast, i.e. the centrifugal force is larger than the magnetic force, the magnet moves to the radial direction to the end of the magnet moving railroad. Using this mechanism of force balance between the magnetic force and centrifugal force, the location of the magnet on a rotating CD could be controlled. In order to further investigate the force balance between the magnetic force and the centrifugal force, we have calculated the magnetic force using a commercial numerical simulation package CFD-ACE+ (ESI Group). As shown in **Fig. 5B**, depending on the rotation speed and the vertical distance between magnets, the equilibrium horizontal distance between magnets could be determined.

3.3 Virus concentration and DNA extraction

The DNA preparation efficiency of our TS-LIMBS was compared with a commercial kit (Qiagen, QIAamp MinElute virus vacuum kit, 57714). The MinElute virus vaccum kit requires 500 µL of serum or plasma sample and takes over one hour with many manual steps of adding various buffers. Our TS-LIMBS method uses only 30 µL of serum or plasma sample and takes 12 minutes with only one step.

As shown **Fig. 6**, the real time PCR results prepared by the proposed TS-LIMBS method were at least as good as

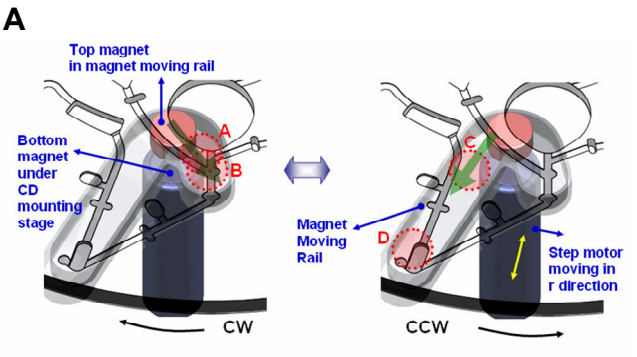

Fig. 5. (A) The principle of the control of the location of the magnet inserted on a CD using a magnet moving railroad cap. Depending on the direction and spin speed and the position of the magnet at the bottom, the location of the magnet inserted on the CD is determined. **(B)** The magnetic force is dependent of the vertical and horizontal distance between magnets. The radial component of the magnetic forces and the centrifugal forces were calculated as a function of the horizontal distances between the two Nd-Fe-B magnets.

the commercially available kit. The limit of the detection was 10 copiesμL^{-1} for both of the preparation methods. It is noteworthy that the cut-off range for the HBV DNA test in current clinical diagnostics is 100 copiesμL^{-1}.

4 CONCLUSION

By combining the TS-LIMBS method with the novel LIFM and centrifugal microfluidics, we could, for the first time, fully integrate the complex microfluidic operational steps such as specific virus DNA extraction from whole blood on a CD. Furthermore, a portable device equipped with a small laser diode and CD mounting stage like CD player is developed.

The TS-LIMBS method is advantageous because it is

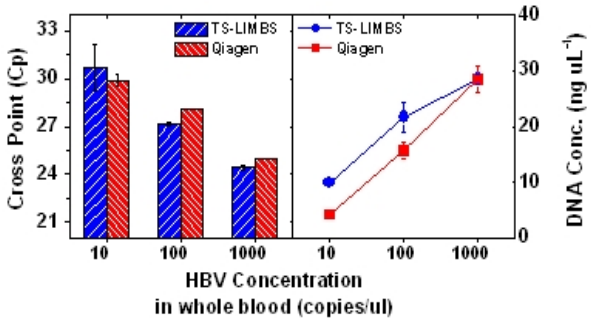

Fig. 6 The DNA preparation efficiency of our TS-LIMBS (Target Separation and Laser-Irradiated Magnetic Bead System) was compared with the results obtained by using a commercial virus DNA preparation kit (Qiagen, QIAamp MinElute virus vacuum kit, 57714). The blue and red symbols represent the results obtained by using TS-LIMBS and Qiagen kit, respectively. Both real-time PCR results and DNA concentration measured by TMC-1000 and Agilent Bioanalyzer 2100 respectively showed that the newly proposed TS-LIMBS method is at least as good as the commercially available virus DNA preparation kit.

capable of target specific cell separation and concentration from raw samples, and DNA extraction and protein removal could be done in a short time without the requirement of the large volume of lysis buffer.

In the present report, we have only demonstrated pathogen DNA extraction from whole blood on a CD. However, many other kinds of novel biological assays, e.g. RNA preparation for cancer marker tests, molecular diagnostics of infectious diseases, genomic DNA preparation, various kinds of immunoassays, and blood chemistry analysis etc., are also possible using the same centrifugal microfluidics with the novel ferrowax valve control.

REFERENCES

[1] J.-G. Lee, K. H. Cheong, N. Huh, S. Kim, J.-W. Choi, and C. Ko, "Microchip-based one step DNA extraction and the real-time PCR in one chamber for rapid pathogen identification", Lab Chip, 6, 886-895, 2006.

[2] J.-M. Park, Y.-K. Cho, J.-G. Lee, B.-S. Lee, and C. Ko, "Multifunctional Microvalves Control by Optical illumination on Nanoheaters and its Application in Centrifugal Microfluidic Devices", Lab Chip, DOI: 10.1039/b616112j, 2007.

[3] Y.-K. Cho, J.-G. Lee, J.-M. Park, B.-S. Lee, Y.-S. Lee and C. Ko, "One-step Pathogen Specific DNA Extraction from Whole Blood On a Centrifugal Microfluidic Device", Lab Chip, DOI: 10.1039/b616115d, 2007.