J. Flow Injection Anal., Vol. 30, No. 1(2013) 29–35

Application of Compact Disc-type Microfluidic Platform to Biochemical and Biomedical Analysis

Shuai Guo, Toshihiko Imato

Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, Fukuoka, 819-0395, Japan

Abstract

Compact disc (CD)-type microfluidic platforms, where a solution in a reservoir is flowed by the centrifugal force driven by rotating the platform, have attracted much concern in various fields, especially for the biochemical analysis and diagnostics, due to the special advantages as follows. First, centrifugal force is used for driving samples to flow on a CD-type microfluidic platform, which is suitable for biomolecules since it is simple and no additional component is needed. Second, a dramatic reduction of sample volume can be realized on a CD-type microfluidic platform with the micro-scaled reservoirs and channels, which is very suitable for biochemical analysis due to the limit of samples. The third advantage is due to the high surface-to-volume ratio of microfluidics, the diffusion length is significantly decreased and this leads to a rapid time-to-result, which is also essential for biochemical analysis. Finally, CD-type microfluidic platform is capable for integrating several procedures, such as sample separation, mixing, reaction and detection on a normal CD-sized device, so that the volume of the analytical system is highly decreased. Additionally CD-type microfluidic platform have the advantage of capability for a multi-sample analysis by designing a proper microchannels and by controlling the rotation speed induced centrifugal force for the multi-sample. In this review, we will introduce the application of CD-type microfluidic platforms on biochemical analysis and diagnostics, such as cell lysis and blood sedimentation, DNA hybridization and immunoassay on chip.

Keywords CD-type microfluidic platform, cell lysis, blood sedimentation, DNA hybridization, immunoassay

1. Introduction

Micro total-analytical system (μ-TAS) has been highly developed and applied to various of scientific fields over last decades, due to the unique advantages such as the high integration (sample injection, mixing, reaction and detection can all be integrated to a single chip), the short time-to-result (the diffusion length decreased in micro-scaled chambers and reservoirs) and the reduced volume of sample reagents. A new type of μ-TAS as compact disc-type microfluidic platform was advanced in last decades [1-5], and several commercial products are already available for the researchers [6-9]. Besides the advantages of the normal μ-TAS, a CD-type microfluidic platform is especially suitable for the biochemical and biomedical analysis, since the centrifugal force generated form the rotation works as the driving force to flow the solution. Compared with other driving forces, such as electro-osmotic force which needs a high electric field and limit for biomolecules, or mechanical pump which is hard to miniaturize, the centrifugal force is simple, and no additional component is required.

Compact disc-type microfluidic platforms have the advantages to make them attractive for biochemical diagnostics and analysis, such as cell lysis and blood sedimentation [10-39], DNA hybridization [40-58], immunoassay [59-68] and other applications [69, 70]. Individual designs of the reservoirs and microchannels are used for the sample preparation, separation, reaction and detection. In this review, some unique designs of CD-type microfluidic platform for cell lysis and blood sedimentation [36-39], DNA hybridization [53-58] and immunoassay [5, 66-68] are typically introduced here. Compared with the conventional methods, CD-type microfluidic platform is expected to be a miniaturized, integrated and portable analytical instrument for biochemical and biomedical analysis.

2. Cell lysis and blood sedimentation

Cell lysis and blood sedimentation is the first step for all molecular biology and molecular diagnostic applications. Several technologies have already been used for the cell lysis and roughly can be divided into two main groups, chemical/biological methods and physical methods. For the chemical/biological methods, techniques such as enzymatic lysis [29, 30] (application of enzymes to digest cell walls), viral lysis [31] (introduce of virus to compromise the cell walls), chemical lysis [32] (utilization of detergents to dissolve the cell membranes) and other methods have already been used. However, a major limitation of these methods is that they may leave behind the residual substances which can result in amplification process so that they must be removed. This causes the problem of an increased complexity of the application on the microfluidic devices. On the other hand, for the physical cell lysis methods, such as freeze-thaw lysis [33] (rapid freeze-thaw cycles in buffers to destroy the cell walls), thermal lysis [34] (application of autoclave method), plasma lysis (electrical discharge is used to disrupt the cell membranes), although requiring additional instruments, little or no residual chemicals are left in such systems. Compared with the chemical/biological methods, the physical methods are often faster and more efficient. Within these physical methods, mechanical method is the most effective method for breaking down cells which have thick cell walls. One mechanical method, called “bead-beating” [35] is the most efficient in this respect, and has already been applied on compact disc-type microfluidic platform and integrated with the downstream process.

Kido *et al*. [36] developed a novel centrifugal microfluidic system for the cell lysis and sample homogenization by applying a bead-beating technique. Bead-beating is accomplished by combining a liquid or solid sample with milling beads in a closed chamber and exposing the entire

mixture of grinding media, buffer and tissue to intense mixing by rapid and abrupt motion of the container. Lysis occurs predominantly as a result of two types of collisions between the milling beads and the cells, puncture by direct collision and friction-based shearing. The instrumentation in this research is shown in Fig. 1. The system implements magnetic field actuated microfluidics in a plastic disk. Individual chambers within the CD assembly harbor flat ferromagnetic metal disks and user-defined bead-beating matrices. Tissue homogenization and cell lysis are achieved via the relative motion of these metal disks and bead matrices in an oscillating magnetic field. The mechanical impaction and shear forces achieved are capable of disrupting even the most difficult to lyse cells. Residual solids are eliminated by centrifugation and clarified supernatants are captured vis siphoning into individual collection chambers fabricated into the CD assembly.

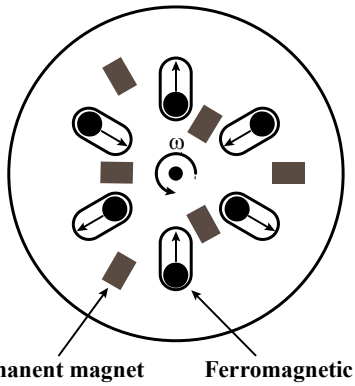

Fig. 1 Simplification illustration of the movement of the ferromagnetic blades in the lysis chambers relative to the magnets

In order to evaluate the performance of the magneto-hydrodynamic disk-based homogenizer, two kinds of samples, *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) were processed. Genomic DNA (gDNA) was processed in the FastPrep-24 device, which is considered to be the most advanced orbital bead-beating tissue homogenization system. The results were compared with that isolated using the FastDNA purification kit by DNA gel electrophoresis and imaging. Different rotation speeds and operation times were tested and according to the DNA yield results and gel electrophoresis images, it suggested that under all conditions the magneto-hydrodynamic disk-based homogenizer was successfully employed. The authors demonstrated that this system can be utilized for semi-automated molecular biology sample preparation on a micro and nano scale sample volume to provide genomic materials.

Steigert *et al*. [37] introduced a design of compact disc-type microfluidic platform for the sedimentation of whole blood and the plasma extraction, by using a hydrophilic siphon-based structure with a centrifugal overflow. Sedimentation and extraction are integrated with the metering of alcohol concentration in human whole blood by a total internal reflection (TIR) structure combined with the colorimetric method. The design of platform is shown in Fig. 2.

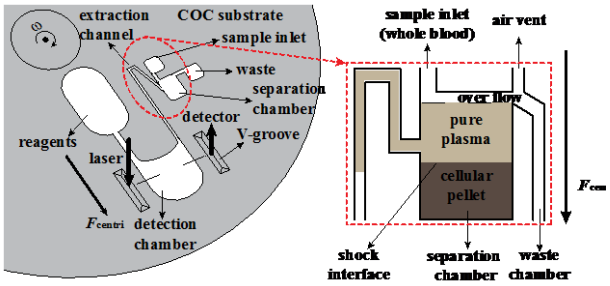

Fig. 2 Diagram of the microfluidic platform with integrated optical structures to perform an enzymatic absorption assay for detecting the alcohol concentration in human whole blood

The whole blood was deposited in the separation chamber under the effect of centrifugal force, and the plasma was transferred into the detection chamber by using a hydrophilic siphon. A total internal reflection (TIR) was achieved by using two V-grooves beside the detection chamber. With a certain rotation frequency protocol, the sedimentation, extraction and metering were realized via the microfluidic device. An integrated colorimetric absorption assay was conducted by a fully automated frequency protocol and the reaction was based on a standard two-step enzymatic reaction using the well-established alcohol oxidase (AOX) method. To carry out the absorption assay, the extracted plasma in the detection chamber was efficiently mixed with enzymatic reagents by using "shake-mode" mixing, and then the absorbance which quantified the alcohol concentration was monitored in real-time during constant (low) spinning with the introduced optical total internal reflection (TIR) concept.

By benchmarking with calibrated commercial samples, the results held a lower limit of 0.05‰ with a standard deviation of 5.3% and a linearity of R^2=0.996. Additionally, the time-to result was reduced from 8 min with standard kits to 150 s, which is very essential in the medical diagnostics. This performance is comparable to the common breath analyzers while avoiding the ambiguous correlation between the alcohol concentration in the breath and the whole bold.

Recently Kim *et al*. [38] has done a study of the geometry effects on the blood separation rate on a rotating CD-type microfluidic platform. The authors designed a straight channel and various tilted channels with different angles from the radial direction to compare the separation rates of the plasma. According to the authors, the particle sedimentation distance is shorter in a slanted channel than in a straight channel, and they designed microchannels with different title angles (0º~75º) and widths (1~4 mm) and compared the separation rates of blood under each condition. The results indicated that the separation rate of the title angle of 75º was the highest. Also the samples with lower cell fraction and higher spin speed could enhance the blood separation rate while the channel depth or the rotating direction not. The experimental data was fitted to the Ponder-Nakamura-Kuroda (PNK) theory to explain the Boycott effect on the separation rate of blood, and good agreement was achieved between the experimental results and the theoretical study.

Similarly Kearney *et al*. [39] have done a study of Spira Mirabilis enhanced density gradient centrifugation on the CD-type microfluidic platform, with a similar designed structure. In this study, the authors compared the time required for the blood separation with straight chambers which have

been inclined at 0º, 15º, 30º, 45º and 60º (measured at the point closest to the center of rotation) and the chambers which closely approximate a Spira Mirabilis at the same pitches. According to the results, the Spira Mirabilis significantly increases the rate of blood sedimentation in the chambers by up to 25% (for the 45º chamber) compared to conventional linear chambers.

3. DNA hybridization

With the development of microfluidic devices, microfluidic technologies have been applied more and more on DNA microarrays. The introduction of microfluidic holds many important advantages compared with the conventional DNA arrays. First, since microfluidic technologies deal with the transfer and control of a small amount of fluids in the microchannels, the sample volume used in DNA arrays can be significantly reduced, from milliliter scale in the conventional methods to a volume as little as μL or pL. The second advantage of using microfluidic devices is that the surface hybridization of target nucleic acids can be accelerated greatly. The diffusion coefficients for nucleic acids in aqueous solutions are on the order of 10^{-7} cm^2s^{-1} [51]. For a 24 h of analysis time, the typical distance moved by the target nucleic acids is about 1 mm, with a hybridization efficiency of 0.2% [52]. By using microfluidic devices the diffusion distance could be reduced due to the high surface-to-volume ratio in the microchannel and the hybridization process can be accelerated. The microfluidic devices also offer an advantage of multi-sample capabilities on one single chip. By properly designing the microchips, a high-throughput multi-sample analysis can be achieved.

Jia *et al*. [53] developed a dynamic DNA hybridization microfluidic system on a compact disc-type platform. The CD was used as the fluidic platform for the sample and reagent manipulation using centrifugal force. Self-assembled DNA oligonucleotide monolayers were used on the gold pads patterned on the glass slides, and a set of PDMS flow cells were aligned with and sealed against the glass slides to form the DNA hybridization units. The design of the platform is shown in Fig. 3.

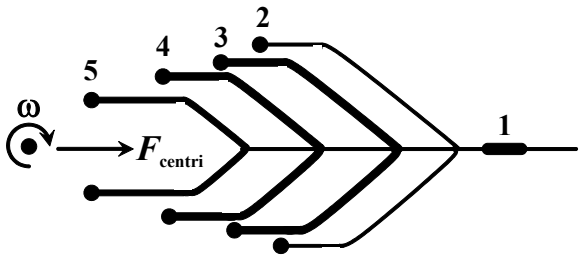

Fig. 3 Schematic diagram of the flow cell for the DNA hybridization

As shown in Fig. 3, the cells with No. 1 to 5 are the hybridization column, the sample chamber, the washing buffer chamber, the blocking buffer chamber and the enzyme chamber respectively. The depth of the microchannel is 25 μm and the depth of the chamber is 250 μm. Self-assembled monolayers of oligonucleotide-alkane thiols on the gold pads were obtained by using methodology mentioned in Herne's work [54]. Two different 25-mer probes were used and placed in the chambers, and by using the centrifugal force, the sample solutions were sent to the hybridization column and an enzyme-labeled fluorescence (ELF 97) technology was used for the detection. Passive assays were also carried out for comparison with the flow-through hybridization detection results. The results show that at each detected concentration, the fluorescence intensities from the flow-through assay were higher than that from the passive method. As the sample concentration decreases, a fluorescence intensity increasing up to threefold was observed in the flow-through hybridization unit compared to the passive hybridization assays. At the lowest sample concentration (100 pM), the fluorescence intensity from the passive assay is at the same level of the background while the signal from the flow-through assay was significantly above the noise level. This increase was due to the high surface-to-volume ratio in the hybridization column to accelerate the hybridization process.

Li *et al*. [55] designed a special compact disc-type microfluidic platform to realize a rapid discrimination of single-nucleotide mismatches based on the reciprocating flow in the microchannel. A perfect-match probe and three mismatched probes containing a discriminate SNM at various positions were designed to investigate the ability of the device to discriminate SNM. The design of the compact disc-type microfluidic platform is shown in Fig. 4.

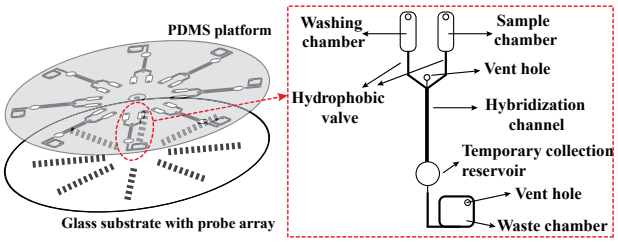

Fig. 4 Schematic of the overall device (left); Schematic of a single SNM assay unit (right).

As Fig. 4 shows, the system involved two platforms, the glass substrate with probe array and the PDMS platform with chambers and microchannels. The sample solution and the washing solution were placed in each chamber, and the sample solution was sent into the microchannel for hybridization with the probe by the centrifugal force, followed by a flow of washing solution.

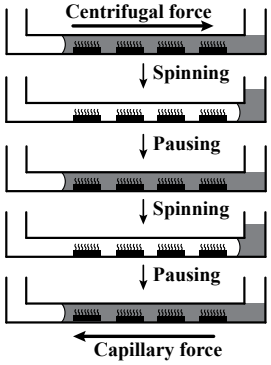

Fig. 5 Schematic diagram of the reciprocating process for improving the DNA hybridization

A reciprocating process was realized by the alternately spinning and pausing of the CD device in the hydrophilic

channel, as shown in Fig. 5. While spinning, the centrifugal force concentrated the solution downstream, and while pausing, the capillary force expanded the solution again cross the microchannel. The results show that after pausing the rotation, it only took 12 s for the solution to recover the whole microchannel. By using this reciprocating flow process, the hybridization was significantly accelerated. The needed time for discriminating SNM by using this system under the optimized washing conditions is only 400 s. Only sub-microliter volume of samples and reagents are required per assay unit. This platform holds promise to be developed into a fully automated, portable, and high-throughput platform for the rapid gene diagnostics.

Besides radial hybridization microchannels on the microfluidic devices, the spiral microchannels were also taken into applications on DNA microarray by some researchers. Peng *et al*. [56] generated a compact disc-type microfluidic platform to realize a multi-sample-multi-probe array by combining the radial probe lines and the spiral microchannels. The device also includes two same sized platforms. On the substrate platform, the radial pre-immobilized DNA probe lines were introduced before spinning. On the top PDMS platform, numerous spiral microchannels were properly designed and fabricated. The intersection of the radial probe lines and each single spiral microchannel formed a microarray of DNA hybridization which covered the whole platform surface. The design is shown in Fig. 6.

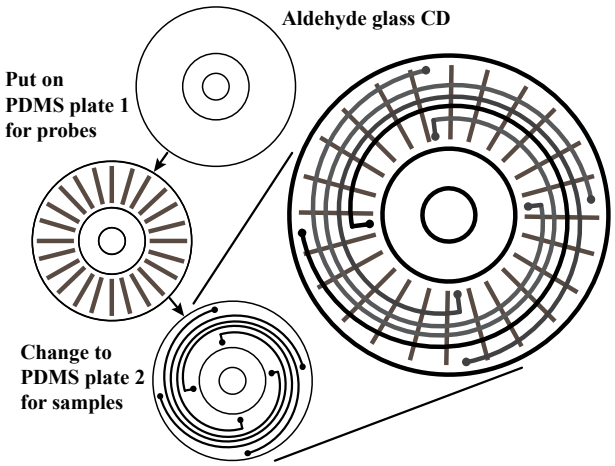

Fig. 6 Schematic diagram of the DNA microarray by using spiral microchannels on the platform

As Fig. 6 shows, with the PDMS plate 1, 24 radial DNA probes were immobilized, and with the PDMS plate 2, 4 samples were hybridized to the 24 probe lines. This structure could form a 24×4 array. The authors actually applied a 96×96 array in the experiments by interacting 96 radial probe lines and 96 spiral channels. Two kinds of plant pathogens *Didymella bryoniae* (A) and *Botrytis cinerea* (B) were used as the probes and were modified at the 5′-end via an amine group with a C6 linker. Samples A′ and B′ were the complementary sequences of A and B, respectively, and they were labeled with fluorescein at the 5′-end. A confocal laser fluorescent scanner was used to detect the hybridization at the intersections between the DNA samples and the radial probe lines. The results indicated that this structure has the potential for developing to be a high-throughput device for the biomedical diagnostics.

Wang *et al*. [57] improved such kind of compact disc-type microfluidic platform using spiral channels by optimizing the experimental conditions, such as the mark molecules, the channel depth and the hybridization temperature. In this work, the probes and samples were the same with the research mentioned above [56], and Cy5 was used for mark instead of fluorescein. Sensitivity was compared by using the two different mark molecules. Also microchannels with depth of 75 μm and 24 μm were applied for the hybridization. For discrimination of single nucleotide mismatch, the hybridization temperature was also estimated for the discrimination ratio.

According to the authors, the hybridization signals could be enhanced by using Cy5-labeld oligonucleotides due to higher hybridization efficiency and less photobleaching, with the same oligonucleotide concentration of 0.1 μM. The single-to-noise ratio increased in the microchannels with a lower depth, probably due to the shorter diffusion length, which resulted in higher hybridization efficiency. For the single nucleotide mismatch detection, at the highest experimental temperature of 62 ℃, the highest discrimination ratio between the perfect match (PM) and mismatch (MM) was obtained. The reason was that the mismatched duplex had a lower melting temperature, and at the temperature of 62 ℃, the mismatch signals were the lowest and were comparable to the background, indicating the best discrimination ratio. The optimized experimental conditions, including a shallower spiral channel plate (24 μm depth), using of Cy5 labels and a higher temperature for SNM detection, are essential for the further application of spiral-radial compact disc-type microfluidic platform on DNA microarray.

Recently Amasia *et al*. [58] developed a centrifugal microfluidic platform for rapid PCR amplification of a *Bacillus anthracis gene* by using integrated thermoelectric heating and ice-valving structure. The system used in this study automates the fluidic pumping, valving, and rapid thermocycling required for polymerase chain reaction. According to the authors, this research is the first utilization of ice-valving in an integrated centrifugal microfluidic system as a method for sealing the thermocycling chamber to reduce fluid loss due to evaporation. Fluidic thermal profiles were monitored and optimized to minimize non-specific amplification, and according to the results, the total amplification time was reduced from 110 min down to 53 min, without compromising the specificity and efficiency of the amplification process.

4. Immunoassay

The use and development of immunoassay technology have drawn a great deal of interest because of the high selectivity and sensitivity. Microfluidic devices are also tried to be carried out of immunoassay due to the special advantages such as the low reagent consumption, the short diffusion length, the rapid time-to-result and the high integration, compared with the conventional immunoassay methods, which usually involve a tedious and labor-intensive protocol that often results in large errors and inconsistent results. Using microfluidic devices could enhance the reaction efficiency, simplify the procedures, reduce the assay time and the sample or reagent consumptions, and provide highly portable systems.

Lai *et al*. [5] carried out an integrated microfluidic device on a compact disk (CD) to perform enzyme-linked immunosorbent assay (ELISA) for rat IgG from hybridoma cell structure. The samples were integrated in the reservoirs on a 12 cm diameter

sized CD-type platform and each reservoir was linked by the microchannels. The design is shown in Fig. 7.

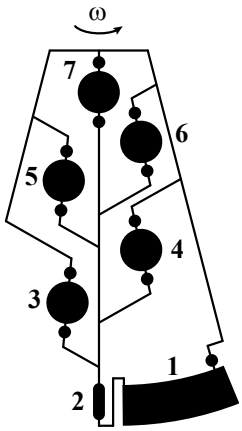

Fig. 7 Schematic diagram of a five-step flow sequencing CD

As Fig. 7 shows, the reservoirs of No. 1 to 7 were used for loading waste, detection, antigen, washing, second antibody, washing, and substrate, respectively. Capillary valve was used for forming a flow sequence by an increased rotation speed with the different reservoir locations, the channel sizes and the solution heights. A sandwich ELISA method was used for the detection of rat IgG and HPPA was used as the substrate of horseradish peroxidase (HRP) in the experiment. ELISA process with conventional 96-well microtiter plate was also carried out and the results were compared. According to the authors, the microchip-based ELISA had the same detection range as the conventional method on the 96-well microtiter plate, while due to the high surface-to-volume ratio of the microfluids, a much shorter assay time as 250 s was enough compared with that of 180 min on the 96-well microtiter plate.

Riegger *et al*. [67] developed another kind of immunoassay on a centrifugal microfluidic platform. Fluorescence immunoassays carried out by multiplexed beads were used and detected by a color camera. 3 kinds of quantum dots were used as the carrier of the different antibodies, and a monolayer of beads was formed on the platform, shown in Fig. 8.

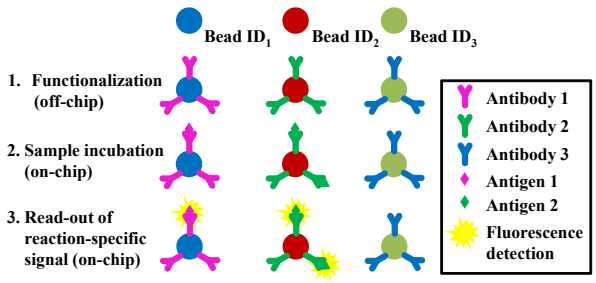

Fig. 8 Principle of a threefold multiplexed fluorescence immunoassay based on optically encoded beads (ID_1, ID_2, ID_3)

According to the authors, beads were first functionalized according to their color tag (the bead ID) with distinct capture proteins, mixed, and then loaded into the disc-based detection chamber. Next, the serum containing the target antigens was transported into the detection chamber and the specific antigen–antibody complex was formed. After washing, the detection antibody was coupled to these antigen–antibody complexes and the reaction-specific fluorescence signals were detected. The design of the platform is shown in Fig. 9.

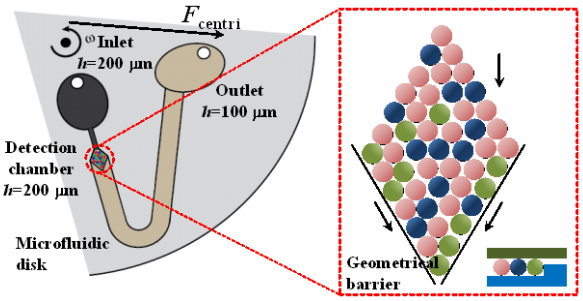

Fig. 9 The chip which follows the format of a compact disk (CD) features only passive microfluidic elements (left). In a preparative step of the sealed disk, the beads are aggregated in form of a periodic monolayer at a geometrical barrier (right).

As Fig. 9 shows, the inlet chamber and the outlet drain were 100 µm in depth, while the detection chamber is 200 µm in depth. The colored beads were trapped in the detection chamber passively to form a monolayer. The spectral information of the tagged beads was acquired with a CCD camera while being illuminated to identify the optical tag. Also the solid phase was excited by the LED-light with the peak wavelength and the fluorescence intensity was measured to determine the assay results for each optical tag. As a consequence, only zones of captured antigens emit a fluorescence signal with emission wavelength.

A standard human serum containing a calibrated concentration of antibodies (hepatitis A or tetanus) with 20 µL of sample was measured with this platform while a constant concentration of detection antibodies and FluoSpheres® were applied. The results clearly allowed distinguishing the state of long-term protection by tetanus or hepatitis A. A CV of 11% with an LOD of 215 mIU/mL for the hepatitis A and a CV of 11.1% with an LOD of 158 mIU/mL for the tetanus were obtained. The major benefits of this technology are the full process integration including an automated read out as well as its modular setup composed of conventional optical components, a standard centrifuge drive, and a disposable polymer disk. These features are keys to meet the technical and economic demands of diagnostic point-of-care applications.

Recently Nwankire *et al*. [68] developed an integrated and miniaturized CD-type microfluidic platform for monitoring the bioprocess (industrial human IgG (hIgG) product) with a fluorescence-linked immunosorbent assay (FLISA). The reagents used in the FLISA process, including the hIgG samples, the washing buffer, the BSA solution and the antibody-dye conjugate, were flowed sequentially by using a serial siphoning structure with different number of crests on a CD-type microfluidic platform. A supercritical angle fluorescent (SAF) scanner was used to measure the fluorescence signals specifically emitted from the antibody-dye conjugate combined with the hIgG, which was initially immobilized on the surface of the SAF chip. Also a conventional SAF monitoring on a 96-well microtiter plate was done to compare with the built microfluidic FLISA process on a CD-type microfluidic platform. The results indicated that the novel system holds a significant reduction of the time-to-result, only about 30 min compared with that of 5 h in the

conventional FLISA process.

5. Conclusion

In this review, we introduced some unique applications of CD-type microfluidic platform to the biochemical and biomedical analysis. Besides these given examples, the CD-type microfluidic platforms are also applied to other scientific fields, and various components can be combined with the device to realize numerous specific functions for improving the biochemical and biomedical analysis. Although the development and application of CD-type microfluidic platform is still in the early stage, and some standards have not been fully build up for such kind of a device, the CD-type microfluidic platform has the unlimited potential to be developed into a portable, simple to use, and cost-effective analytical method.

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(Received June 20, 2013)
(Accepted July 4, 2013)