Functional integration of DNA purification and concentration into a real time micro-PCR chip†

Junhong Min,[a] Joon-Ho Kim,[b] Youngsun Lee,[b] Kak Namkoong,[b] Hae-Cheon Im,[c] Han-Nah Kim,[c] Hae-Yeong Kim,[c] Nam Huh*[b] and Young-Rok Kim*[c]

Received 21st August 2010, Accepted 4th October 2010
DOI: 10.1039/c0lc00320d

Microfluidic devices for on-chip amplification of DNA from various biological and environmental samples have gained extensive attention over the past decades with many applications including molecular diagnostics of disease, food safety and biological warfare testing. But the integration of sample preparation functions into the chip remains a major hurdle for practical application of the chip-based diagnostic system. We present a PCR-based molecular diagnostic device comprised of a microfabricated chip and a centrifugal force assisted liquid handling tube (CLHT) that is designed to carry out concentration and purification of DNA and subsequent amplification of the target gene in a single chip. The reaction chamber of the chip contains an array of pillar structures to increase the surface area for capturing DNA from a raw sample of macro volume in the presence of kosmotropic agents. The CLHT was designed to provide an effective interface between sample preparation and the microfluidic PCR chip. We have characterized the effect of various fluidic parameters including DNA capture, amplification efficiency and centrifugal pressure generated upon varying sample volume. We also evaluated the performance of this system for quantitative detection of *E. coli* O157:H7. From the samples containing 10^1 to 10^4 cells per mL, the C_T value linearly increased from 25.1 to 34.8 with an R^2 value greater than 0.98. With the effectiveness and simplicity of operation, this system will provide an effective interface between macro and micro systems and bridge chip-based molecular diagnosis with practical applications.

Introduction

The polymerase chain reaction (PCR) is a powerful molecular diagnostic tool for identifying and monitoring the source of infectious diseases or genetic disorders in patients.[1,2] The time and cost required for the analysis of PCR results have greatly been reduced by the development of real-time PCR.[3] Real-time PCR utilizes fluorescent dye molecules in a reaction mixture to monitor the amount of PCR products that are produced during the course of thermal cycles. Therefore, it does not require time-consuming post-PCR manipulations, such as gel electrophoresis, staining and visualization with fluorescence setup. The advent of real-time PCR facilitated the development of a portable instrument for genetic tests that could be used in the field and is capable of rapid detection and identification of infectious organisms.[4,5] But the labor-intensive and time-consuming sample preparation processes have been a limiting factor for the actual application of PCR outside of the research facilities.[6–8] Sample preparation includes extraction and concentration of target analytes and it depends on the nature of the sample. The target analytes should be concentrated to enhance detection efficiency in a small volume. In addition all the impurities that can interfere with the analysis process should be removed. Most clinical samples contain various PCR inhibitors that severely deteriorate the specificity and sensitivity of PCR.[9,10] For this reason, the inhibitors should be removed from the sample during the sample preparation process.

Starting with Boom technology a number of solid-state DNA purification methods had been developed to improve the traditional labor-intensive and hazardous-liquid-based isolation protocols that use ethanol, phenol, chloroform or 2-propanol.[11] Solid-state DNA purification is particularly useful when designing a chip-based analytical system. Boom technology uses chaotropic salts for dehydrating the silica surface to induce the adsorption of DNA on silica through the hydrogen bonding between the hydroxyl functional groups of silica and DNA.[12,13] Charge switch technology (CST) is another commercialized nucleic acid isolation technology that utilizes the chargeable organic layer on a solid surface whose charge can be controlled by pH of solution due to its pK_a values. On the other hand, solid-phase reversible immobilization technologies (SPRI) use a relatively high concentration of salt such as NaCl and polyethylene glycol (PEG) to bind DNA on the surface coated with carboxyl functional groups.[14] These solid-state nucleic acid isolation technologies have been applied to a membrane-type kit and provide simple and fast protocols for the purification of nucleic acids. But there are several technical challenges present for

[a]*College of Bionano Technology, Kyungwon University, Seongnam, 461-701, Korea*
[b]*Bio Lab, Samsung Advanced Institute of Technology, Yongin, 446-712, Korea. E-mail: namhuh@samsung.com; Fax: +82-31-280-8277; Tel: +82-31-280-6939*
[c]*Institute of Life Science and Resources & Department of Food Science and Biotechnology, Kyung Hee University, Yongin, 446-701, Korea. E-mail: youngkim@khu.ac.kr; Fax: 82-31-204-8116; Tel: +82-31-201-3830*
† Electronic supplementary information (ESI) available: Fabrication of PCR chip, binding efficiency of DNA according to pH and concentration of a kosmotropic salt or chaotropic salt. See DOI: 10.1039/c0lc00320d

integrating nucleic acids isolation technology to a chip-based system. First, chaotropic salts or other organic solvents are not preferable because it requires a relatively large volume of solution for washing and thus make the process unsuitable for miniaturized microfluidic application. Second, a specific surface modification should be minimized when integrating nucleic acids isolation modules with other analytical units since it could negatively interfere with the amplification or other enzymatic reactions. In addition, all nucleic acids in the sample should effectively be collected and concentrated to a small volume (a few microliters or below) that is compatible with subsequent analytical reaction in the microfluidic chamber. It is particularly important to enhance the detection sensitivity for samples containing rare analytes.

Over the past ten years, many efforts have been made to simplify and miniaturize molecular diagnostic processes, including sample preparation, taking advantage of the rapidly advancing micro/nano fabrication technologies, and notable progresses have been made.[6,15–19] Lab-on-a-chip devices that contain functional components, such as microfluidic mixers, valves, pumps, channel networks, and chambers were developed to carry out sample preparation, amplification and detection of target genes.[15,16] They greatly enhanced the portability of molecular diagnostic system for handy operations in the field. But their approaches were still subject to the traditional concepts of microelectromechanical systems (MEMS) that require complicated external control units and expensive fabrication processes.

Here we present a PCR-based molecular diagnostic device comprised of a microfabricated chip and centrifugal force assisted liquid handling tube (CLHT) that is designed to carry out concentration and purification of DNA and subsequent amplification of the target gene in a single chip. Our approach eliminated several of the major problems with the current technologies associated with the use of hazardous chemicals/organic solvents and surface modification which could impose limitations in lab-on-a-chip applications. The reaction chamber of the chip contains an array of pillar structures to increase the surface area for capturing DNA from the raw sample of macro volume in the presence of kosmotropic agents. We employed kosmotropic salt solution to capture DNA to the surface of a silicon oxide pillar chamber. Kosmotropic salts are known to enhance the interaction between water molecules and hydrophilic surface as well as the interaction between water molecules to generate strong water molecule structures on the hydrophilic surface.[20] Nucleic acids bind indirectly to the hydration layer on the silica surface through hydrogen bonding in the presence of a kosmotropic salt whereas chaotropic salts induced direct binding of DNA to silica surface.[21] Kosmotropic salts were easily removed from the chip by a simple washing step and did not affect the downstream enzymatic reaction. The bound DNA was efficiently released when a neutral or slightly alkaline aqueous solution, such as a PCR mixture (pH 9.0), was introduced to the chamber, allowing high availability of template DNAs for the subsequent amplification reaction.

PCR efficiency was inversely proportional to the surface area of the pillar chamber.[22–25] Therefore the size and pitch of the pillar array were optimized to capture the most DNA and yet to allow PCR reaction. At the same time, the PCR reagents were elaborately modified to change the pillar-packed chamber into a PCR-friendly environment. CLHT was designed to provide an effective interface between sample preparation and the microfluidic PCR chip. The inlet of the microfluidic device is in fluid communication with the exit of the fluid introduction reservoir and a fluid from the fluid introduction reservoir is introducible into the microfluidic device in a controlled manner using a centrifugal force generated by rotation of the tube.

We have characterized the effect of various fluidic parameters including DNA capture, amplification efficiency and centrifugal pressure generated upon varying sample volumes. A dynamic range of the device was tested for quantitative detection of *E. coli* O157:H7. All the processes from sample preparation to PCR-based detection were carried out in a single chamber, eliminating potential cross-contamination between samples and simplifying device operations. This system provides a cost-effective solution to a simple and rapid genetic analysis, and is expected to have wide applications in the field of point-of-care genetic analysis.

Materials and methods

Materials

Sodium sulfate, sodium acetate, Tris (Trizma base), ethylenediaminetetraacetic acid (EDTA), Triton X-100, Casein and phosphate-buffered saline (PBS), pH 7.4, were obtained from Sigma-Aldrich (St. Louis, MO). Bovine serum albumin (BSA) 100 mg ml^{-1}, *Taq* buffer and *Taq* DNA polymerase were obtained from SolGent (Daejeon, South Korea). SYBR-Green was obtained from Invitrogen (Carlsbad, CA). Luria–Bertani media was obtained from BD Difco (Franklin Lakes, NJ). *E. coli* BL21 (Invitrogen-Carlsbad, CA) and *E. coli* O157:H7 (ATCC 43894) were grown in Luria–Bertani (LB) media at 37 °C to an optical density at 600 nm of 1.0. Cells were suspended to appropriate concentrations after washing with phosphate-buffered saline (PBS, pH 7.4).

Microdevice design and fabrication

The PCR chip was fabricated using standard photolithography techniques on a 6 inch silicon wafer. Briefly, the wafers were spin coated with a photoresist (AZ-GXR601) and patterned using an EVG 620 mask aligner (EV Group., St. Florian am inn, Austria). The patterned photoresist was developed and the wafer was dry etched using inductively coupled plasma (ICP) in a DRIE (Deep Reactive Ion Etching) etcher (STS Co., Newport, UK) to 100 μm depth. Then, 300 nm of silicon dioxide was grown on the surface of the reaction chamber. The processed silicon wafers were anodically bonded with the glass cover with inlet/outlet holes formed by a sand blast technique. The resulting PCR chips contain microchambers with a volume of 2–4.5 μl. The microchambers were filled with an array of pillars of various diameters and gap sizes (Table 1). A centrifugal force assisted liquid handling tube (CLHT) was fabricated with Teflon and composed of the upper and lower part. The upper part includes a chip receiver and a fluid introduction reservoir. The lower part includes a chip supporter and a collection tube. The exit of fluid introduction reservoir has a tight contact with the inlet of the PCR chip. The chip supporter in the lower part supports the second part of the PCR chip. The PCR chip is disposed between

Table 1 The dimensions of the pillar structure and chamber in tested PCR chips

Chip no.	1	2	3	4	5
Pillar diameter (μm)	25	25	50	100	25
Gap size (μm)	8	17	9	50	30
Pillar height (μm)	100	100	100	100	100
Chamber volume (μl)	2	3	1.3	2.5	3.6
Surface area (mm^2)	452	313	284	130	220
Surface to volume ratio	11.6	5.33	11.1	2.95	3
Pillar image					

the two parts and the solution in the fluid introduction reservoir is introduced into the chip and then discharged into the collection tube by centrifugal force.

Quantification of captured DNA on the surface of the pillar packed PCR chip

E. coli BL21 was grown in LB media at 37 °C to an optical density at 600 nm of 1.0. Cells were centrifuged at 5000*g* for 5 min and resuspended in PBS. Bacterial DNA was prepared by phenol–chloroform extraction and ethanol precipitation.[11] DNA concentration was measured with PicoGreen reagent according to the manufacturer's instruction. 100 μl solution containing 800 ng bacterial DNA was mixed with an equal volume of binding buffer containing 1 M sodium sulfate and 1 M sodium acetate (pH 3.0). And then the mixture was introduced into the microchip by centrifugation at 100 g for 1 min. The chip was then washed by introducing 200 μl of washing buffer containing 10 mM sodium sulfate and 10 mM sodium acetate (pH 3.0) by centrifugation at 100 g for 1 min followed by additional centrifugation at 10 000 g for 1 min. The amount of DNA in the flown through washing buffer was measured with PicoGreen reagent. λ-phage DNA (New England Biolabs) was used as a reference for the quantification. Captured DNA on the surface of the pillar packed PCR chip was calculated by subtracting the amount of DNA in the flown through washing buffer from the total DNA in the introduced solution.

Concentration of DNA and amplification of the target gene in a pillar chip

Concentration of DNA in a pillar chip was conducted in the following manner. Lysed *E. coli* cells were mixed with an equal volume of binding buffer (BB) containing 1 M sodium sulfate and 1 M sodium acetate (pH 3.0), and introduced into the microchip by centrifugation at 100*g* for 1 min. The chip was then washed by introducing 200 μl of washing buffer containing 10 mM sodium sulfate and 10 mM sodium acetate (pH 3.0) by centrifugation at 100*g* for 1 min followed by additional centrifugation at 10 000*g* for 1 min to remove all the residual washing buffer in the PCR chip. 3μl of PCR reaction mixture was added to the reservoir and introduced into the chamber by centrifugation at 100*g* for 30 s. The PCR reaction mixture consisted of 0.3 U μl^{-1} *Taq* polymerase (Solgent, Korea), 75 mM Tris-HCl (pH 9.0), 15 mM $(NH_4)_2SO_4$, 5 mM $MgCl_2$, 200 μM dNTP mixture, 0.5X SYBR-Green (Invitrogen), a set of 200 nM PCR primers, 1 mg ml^{-1} BSA, and 5% (w/v) of polyethylene glycol (MW = 8000). Primers to a 204-bp conserved region of the 16S ribosomal RNA of the *E. coli* genome were used for concentration and amplification experiments (*E. coli* 16s-F: 5′-YCC AKA CTC CTA CGG GAG GC-3′; *E. coli* 16s-R: 5′-GTA TTA CCG CRR CTG CTG GCA C-3′). Primers to a 160-bp region of the Shiga toxin gene stx_1 were used for the detection of *E. coli* O157:H7 (stx_2-F: 5′-AGT TCT GCG TTT TGT CAC TGT C-3′; stx_2-R: 5′-CGG AAG CAC ATT GCT GAT T-3′).[26] After the chip was loaded with PCR reagent, the chip was transferred to the GenSpector® TMC-1000 system (SAIT, Korea) for real-time PCR analysis.[4] PCR reactions for the 16S ribosomal RNA gene were cycled under the following conditions: 95 °C denaturation for 60 s, 30 cycles of 94 °C for 5 s, 62 °C for 5 s, 72 °C for 20 s. For Shiga toxin gene in *E. coli* O157:H7, the reactions were cycled under the flowing conditions: 95 °C denaturation for 60 s, 40 cycles of 94 °C for 5 s, 54 °C for 5 s, 72 °C for 25 s. The fluorescence was monitored during the 72 °C extension step of each cycle. DNA amplification was confirmed by electrophoresis in 2.5% agarose gel.

Results and discussion

Liquid handling of microfluidic chip by CLHT

The CLHT is composed of a liquid introduction part and a collection tube (Fig. 1). The liquid introduction part has a chip receiver and a fluid introduction reservoir. The collection tube has a chip supporter. The microfluidic chip is easily mounted to and removed from the CLHT by hand. Once the chip is inserted into the chip receiver, the exit of fluid introduction reservoir has a tight contact with the inlet of the pillar packed PCR chip. The chip supporter in the collection tube supports the pillar packed PCR chip in the right position. The PCR chip is fixed between the two parts and the solution in the fluid introduction reservoir is introduced into the chip and then discharged into the collection tube through the chip outlet by centrifugation using a conventional microcentrifuge. When a centrifugal force is generated in the direction marked by an arrow in Fig. 1B, the pressure of the liquid at the exit of the fluid introduction reservoir is increased. The liquid in the introduction reservoir is flowed through the microfluidic PCR chamber and then into the collection tube by the pressure. The flow rate can be controlled by the rotation speed of the microcentrifuge. Fig. 2A illustrates a relationship between a centrifugal force and a centrifugal pressure according to the volume of a fluid in the fluid introduction device. It shows

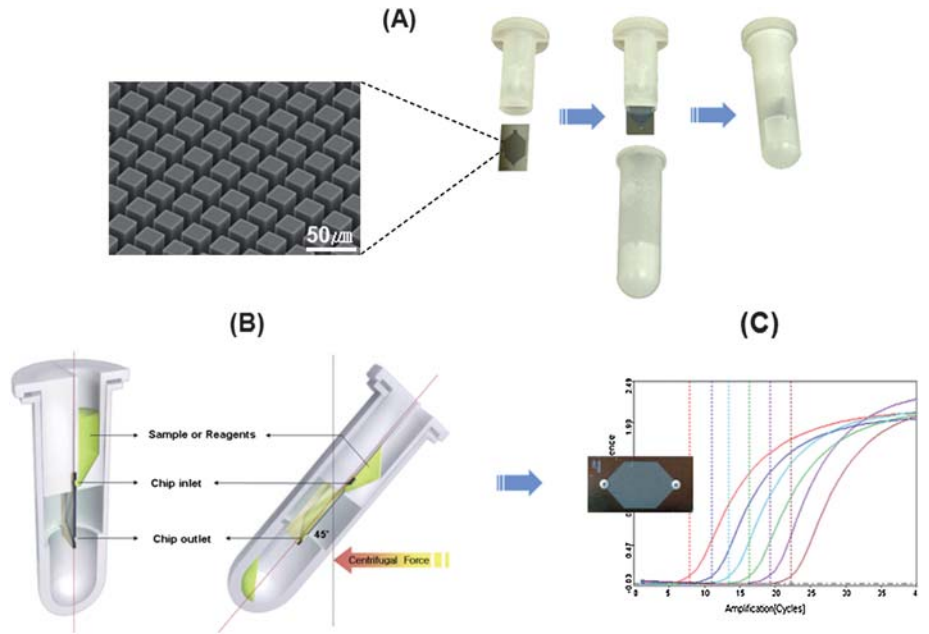

Fig. 1 Schematic drawing of an integrated real-time PCR chip and liquid handling device for the concentration and purification of DNA, and subsequent amplification of target DNA in the same chip. (A) Assembly of real-time PCR chip into the CLHT. Inset shows an SEM image of the PCR chamber packed with pillar structures with height, width and gap of 100, 25, 17 μm, respectively. (B) Scheme for the liquid handling device. Sample and reagents are sequentially introduced into the chip for concentration and purification of bacterial DNA and amplification of target genes. (C) The chip is now ready for real-time PCR analysis.

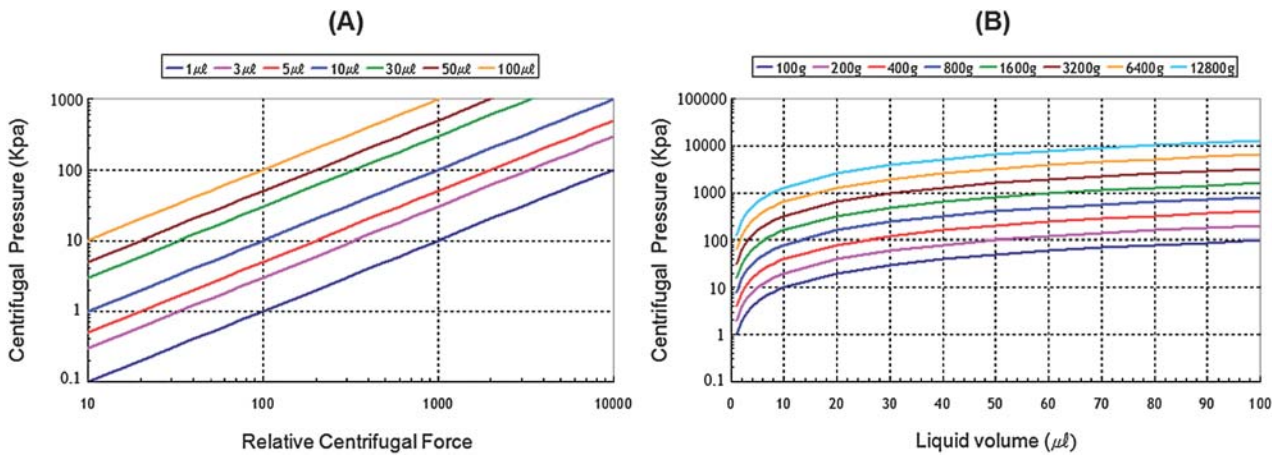

Fig. 2 A graph illustrating the relationship between centrifugal force and centrifugal pressure according to the volume of a fluid applied to the inlet of fluid introduction apparatus (A). The graph (B) shows the relationship between fluid volume and centrifugal pressure according to a relative centrifugal force applied to the fluid introduction tube.

the pressures generated by a predetermined volume of a fluid stored in the fluid introduction reservoir under relative centrifugal forces. The equation below defines the relationship between the centrifugal force and the pressure that is applied to the inlet of the microfluidic chip in CLHT.

$$p_{\text{centrifugal}} = \left(\frac{\rho V}{\pi r_{\text{i}}^2}\right)\left(\frac{r_{g,\text{inlet}}}{r_{g,\max}}\right)(\text{R.C.F})$$

The variable ρ denotes fluid density, V denotes fluid volume, r_{i} denotes an inner radius of the exit of the introduction member, $r_{g,\text{inlet}}$ denotes a rotation radius of the inlet around a center of rotation, $r_{g,\max}$ denotes a maximum rotation radius of a part of the tube further from the center of rotation, and R.C.F. denotes the relative centrifugal force. For example, when the minimum pressure necessary to introduce a fluid into the inlet of the microfluidic chip is 3 kPa and there is a fluid of 10 μl in the fluid introduction reservoir, a centrifugal force of approximately 40 gravities (g) is required to introduce the fluid, and if there is a fluid of 1 μl in the fluid introduction reservoir, a centrifugal force of approximately 300g is required to introduce the fluid. Fig. 2B illustrates the relationship between fluid volume and

centrifugal pressure according to a relative centrifugal force applied to the fluid introduction tube. It shows the variations of the pressure as the volume of fluid in the fluid introduction reservoir decreases under a constant centrifugal force. If the centrifugal force increases according to the volume of residual fluid in the fluid introduction reservoir during the fluid introduction into the microfluidic chip, the pressure can be kept constant.

First, the chip is mounted in the chip receiver of the fluid introduction part. A sample containing cell lysates or DNA is introduced from the fluid introduction reservoir to the microfluidic chamber through the inlet using a weak (100*g*) centrifugal force. Here, the DNA is bound to the inner surface of the microchamber and the rest of the sample is discharged through the outlet. Next, a buffer solution for washing is introduced into the microfluidic chamber in the same manner as the sample was introduced as described above. Impurities on the surface of the microfluidic chamber were eliminated in this washing process. The chip was then subject to centrifugation at 10 000*g* for 1 min to remove all the reagents that might have remained in the chip. The flow through solutions generated during each process may be decanted from the collection tube. Next, a PCR mixture (3 μl) was introduced into the microfluidic chamber by centrifugation at 100*g* for 30 s. The PCR mixture was readily introduced into the hydrophilic microchamber by capillary force but remained within the chip since the low centrifugal force (100*g*) was not enough to drive the solution out of the microchamber when the fluid introduction reservoir was empty. The microfluidic chip filled with the PCR mixture was transported to a real-time PCR equipment to amplify the target DNA.

PCR in pillar packed chips with various dimensions

5 different chips with various pillar dimensions were fabricated to maximize the binding capacity of DNA while having a lower C_T (threshold cycle) value (Table 1). In comparison to the PCR chip with an open chamber, the surface to volume ratio of chip #1 was 11.6 whereas that of chip #4 was 2.95. The chamber volume ranged from 1.27 μl (chip #3) to 3.57 μl (chip #5). As expected, the DNA binding capacity was proportional to the surface to volume ratio of the tested chip. Chip #1 and Chip #3 had a higher DNA binding capacity compared with the other chips. On the other hand, amplification efficiency was inversely proportional to the surface to volume ratio of the chip when the same amount of template DNA (0.7 ng μl^{-1}) was used for PCR reaction. amplification efficiency was determined by measuring C_T and fluorescence intensity.

The C_T values of PCR in chip #1 and chip #3 which have a high surface to volume ratio could be reduced by increasing the concentration of PEG in the reaction mixture but the fluorescence intensity decreased significantly when PEG concentration increased over 5% (v/v). Fig. 2 shows the percentage (%), by volume, of the initially loaded DNA that is bound in each chip. As the surface to volume ratio of the chip increases, the volume of DNA bound also increases. According to the C_T value, chip #2 provided the best amplification efficiency (Fig. 3). Considering that the DNA binding capacities of chip #1, #2 and #3 were not significantly different, chip #2 with pillar diameter of 25 μm and gap size of 17 μm was found to have the best performance in DNA concentration and PCR among the tested chips.

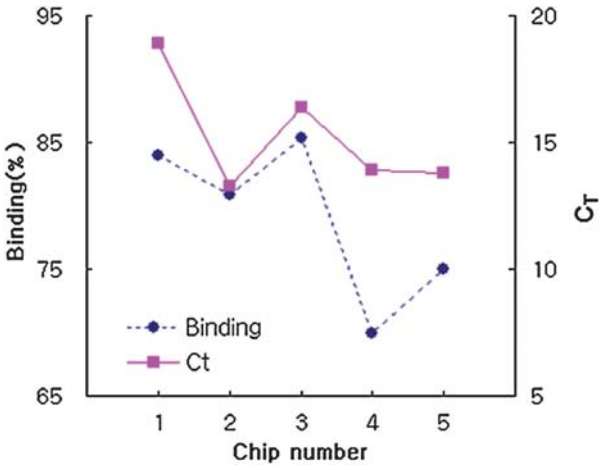

Fig. 3 Comparison of DNA binding and C_T value of the tested PCR chips with various pillar dimensions. 0.7 ng of *E. coli* BL21 genomic DNA was used as a template for all PCR reactions. Chip 2 packed with pillars of 25 μm diameter and 17 μm gap provided the best conditions for PCR along with high binding capacity.

Concentration and purification of DNA using a CLHT

All the processes from sample preparation to PCR detection were carried out in a single chip. Fig. 4 illustrates the initial cell density and threshold cycle (C_T) when a CLHT was used for DNA concentration, purification and real time PCR. As shown in Fig. 4, 90 μl of the raw sample containing various input cell concentrations was mixed with equal volume of binding buffer and flown through the microfluidic chip mounted on CLHT. The DNAs present in the cell lysates were driven to the inner surface of the microfluidic chip and most of other components were flowed through the chip. The remaining components that might inhibit the following PCR reaction could be further removed by washing. Flowing 180 μl of sample through the 3 μl PCR chamber would result in 60-fold concentration of DNA in comparison to running a standard 3 μl PCR reaction comprising of 1.5 μl raw sample and 1.5 μl PCR reaction mixture. In addition to a concentration effect, this system also provides a purification function. When 1.5 μl of raw sample containing 10^4 cells per μl was directly used for PCR, the C_T value was 25.5 (Fig. 4B). But concentrating 90 μl of the same sample using this system resulted in a C_T value of 16.5. The C_T value of 16.5 was equivalent to that was obtained from standard PCR reaction with 1.5 μl of sample containing 3×10^6 cells per μl. According to the enhancement of C_T value, the concentration and purification effect was equivalent to 300-fold in comparison to the original sample. The purification effect was believed to be responsible for the additional 5-fold increase in addition to a 60-fold volumetric concentration. The purification effect of the washing step may be more significant with other clinical samples such as whole blood.

In this study, we employed a kosmotropic salt solution to capture DNA to the surface of a silicon oxide pillar chamber through hydrogen bonding. In the case of a chaotropic salt, a solid support surface is dehydrated and DNAs are directly

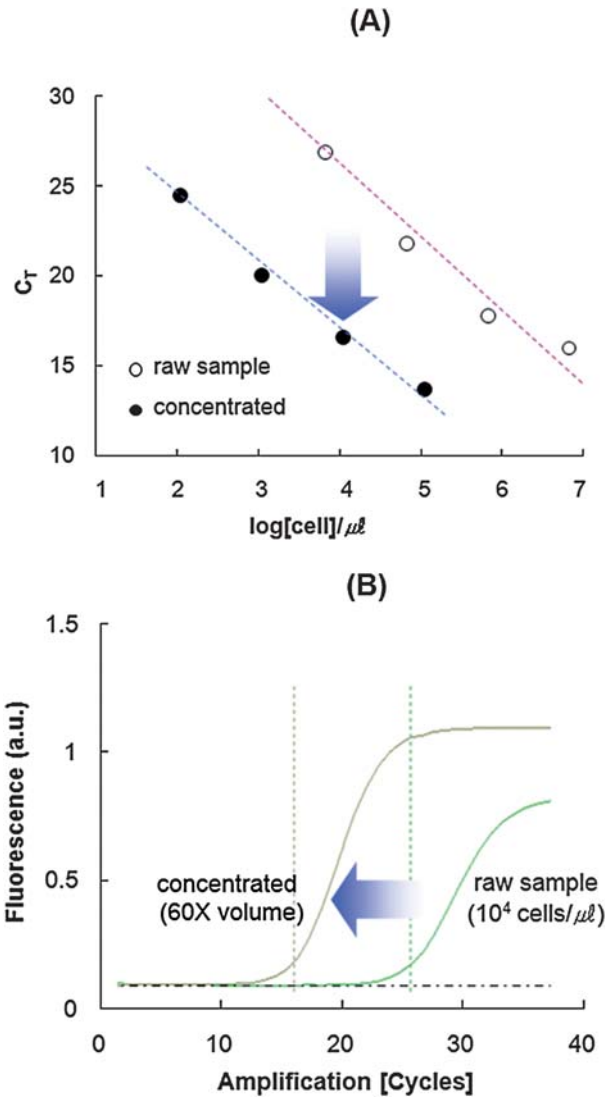

Fig. 4 The enhancement of PCR detection of *E. coli* by DNA concentration and purification using a CLHT. (A) A sample containing a known number of *E. coli* was tested before (○) and after (●) the concentration using a CLHT assisted real time PCR chip. 90μl of raw samples containing various input cell concentrations were tested for concentration and purification of DNA, which would bring a 60-fold concentration of initial sample volume in comparison to a standard reaction that uses 1.5 μl raw sample mixed with an equal volume of PCR premix solution. (B) Amplification plot showing enhancement of C_T value from 25.5 to 16.5 by the centrifugal tube assisted real time PCR chip. The C_T value of 16.5 is equivalent to 3×10^6 cells per μl, which is 300-fold higher than the original cell concentration.

bound to the solid support by hydrogen bonding, therefore the pH of the solid support is important. However, in the case of a kosmotropic salt, the solid support surface is hydrated so that a stable water layer is formed. Nucleic acids are considered to be hydrated on the hydrated solid support surface, and it stems from a salting-out effect by hydrophilic interaction. Therefore, nucleic acid binding with a solid support does not require acidic conditions that are generally used for nucleic acid binding, and the solid support surface need not be limited to silica. As shown in Fig. S1†, the binding efficiency of DNA shows little difference at pH 4 and pH 10 in the presence of a high concentration of kosmotropic salt. On the other hand, the binding efficiency of DNA notably decreases as pH increases for a chaotropic salt of the same concentration. It is believed that the kosmotropic salt hydrates the substrate surface so that it enhances a water network, and the strong network of water layer on the silica surface plays a critical role when DNA is bound to the surface by hydrogen bonding. Therefore, when binding DNA to the surface using a kosmotropic salt, DNA can be efficiently bound regardless of pH by a proper amount of the kosmotropic salt.

Kosmotropic salts were easily removed with a washing step and did not affect the downstream enzymatic reaction. The bound DNA was efficiently released when the PCR mixture (pH 9.0) was introduced to the chamber, allowing high availability of template DNAs for the subsequent amplification reaction. The same type of chip coated with aluminium oxide showed a similar DNA concentration effect. But the amplification efficiency of aluminium oxide coated chip was much lower compared with that of the silicon oxide chip based on C_T value (data not shown). We speculate that DNAs are more easily released from the surface of the silicon oxide chip than that of the aluminium oxide chip upon introduction of the PCR reaction mixture and it provides better conditions for PCR.

Quantitative detection of *E. coli* O157:H7

Here we evaluated the performance of this system for quantitative detection of *E. coli* O157:H7, one of the most virulence pathogenic bacteria in food. *E. coli* O157:H7 has been known as an important human pathogens associated with a spectrum of diseases ranging from diarrhea to hemorrhagic colitis and hemolytic-uremic syndrome.[27] Shiga toxin is a major virulence factor of the strain and is responsible for hemolytic-uremic syndrome.[28] Here we employed a primer set that was previously designed for the detection of Shiga toxin gene 2 (*stx*$_2$) of *E. coli* O157:H7.[26] Cultures of *E. coli* O157:H7 were serially diluted to obtain between 10^4 to 10^1 cells per ml and lysed simply by heating at 95 °C for 5 min. The samples were then applied to the fluid introduction reservoir of CLHT. 500 μl of cell lysate mixed with an equal volume of binding buffer were injected into the microfluidic PCR chip in the same manner as described above for concentration of DNA. Most of the cell debris such as proteins, lipids and other cellular components were removed during the following washing step. After loading the microfluidic PCR chamber with 3 μl of PCR master mix including the specific primer set, the chip was transferred to a GenSpector® TMC-1000 system for quantitative assay. The dynamic range of the system was evaluated with 4 different concentrations from 10^4 to 10^1 cells per ml (Fig. 5). From the raw samples containing 10^4 to 10^1 cells per ml, the C_T value linearly increased from 25.1 to 34.8 with an R^2 value greater than 0.98. The sample containing 0 cells did not generate a C_T value and the result was evident in the agarose gel electrophoresis image (Fig. 5B). The size of the PCR products from the *E. coli* O157:H7 positive sample was equivalent to the target region of *stx*$_2$ gene which is 160 bp. From the results, the CLHT is shown to provide a simple and effective means of handling large volumes of samples and reagents for a microfluidic chip in a controlled manner. Here we successfully demonstrated concentration and purification of DNA and

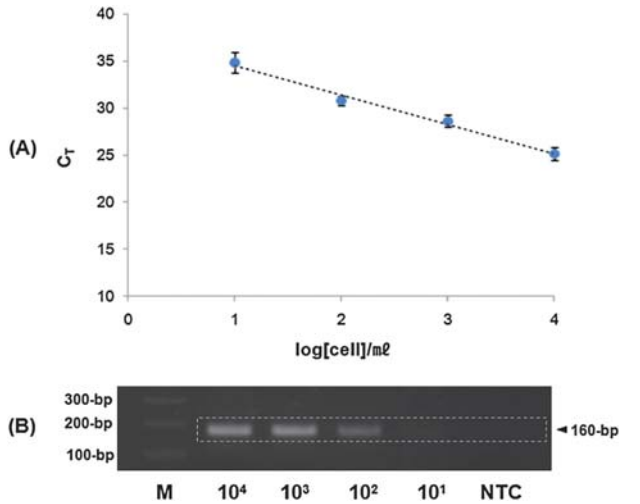

Fig. 5 Result of real time PCR for *stx*$_2$ gene of *E. coli* O157:H7 using a CLHT. (A) The average C_T value from three separate experiments was plotted as a function of log (cell number per ml). (B) The PCR products from the chips were analyzed by 2.5% agarose gel electrophoresis to confirm the right amplification of the target gene (160 bp).

subsequent amplification of target genes in a single chip for quantitative detection of pathogenic bacteria, *E. coli* O157:H7.

Conclusions

We have presented a chip-based diagnostic system that is designed to carry out DNA concentration, purification and amplification of the target gene in a single chip. A centrifugal force assisted liquid handling tube was found to offer a great means of controlling liquid samples and reagents for the microfluidic PCR chip by a conventional microcentrifuge. Microfluidic devices for on-chip amplification of DNA from various biological samples have gained extensive attention over the past decades. But integration of sample preparation functions into the chip remains a major hurdle for practical application of the chip-based diagnostic system. The CLHT was designed to provide an effective interface between sample preparation and chip-based molecular diagnosis. The inlet of the microfluidic device is in fluid communication with the exit of the fluid introduction reservoir and the fluid from the fluid introduction reservoir is introducible into the microfluidic device in a controlled manner using a centrifugal force generated by rotation of the tube. We demonstrated concentration and purification of DNA in micro real-time PCR chip from raw samples of macro volume for subsequent PCR assay. The results were comparable with those obtained with a sophisticated syringe pump. A plastic-based CLHT is suitable for mass production and will find wide applications in other chip-based molecular diagnostic systems.

Acknowledgements

This work was supported by a grant (PJ007074) from BioGreen 21 program, Rural Development Administration, Republic of Korea.

Notes and references

1 K. Mullis, R. Faloona, S. Scharf, R. Saiki, G. Horn and H. Erlich, *Cold Spring Harbor Symp. Quant. Biol.*, 1986, **51**, 263–273.
2 H. Erlich, D. Gelfand and J. J. Sninsky, *Science*, 1991, **252**, 1643–1651.
3 C. A. Heid, J. Stevens, K. J. Livak and P. M. Williams, *Genome Res.*, 1996, **6**, 986–994.
4 Y. K. Cho, J. Kim, Y. Lee, Y. A. Kim, K. Namkoong, H. Lim, K. W. Oh, S. Kim, J. Han, C. Park, Y. E. Park, C. S. Ki, J. R. Choi, H. K. Myeong and C. Ko, *Biosens. Bioelectron.*, 2006, **21**, 2161–2169.
5 R. W. Chen, H. Piiparinen, M. Seppanen, P. Koskela, S. Sarna and M. Lappalainen, *J. Med. Virol.*, 2001, **65**, 250–256.
6 Y. Huang, E. L. Mather, J. L. Bell and M. Madou, *Anal. Bioanal. Chem.*, 2002, **372**, 49–65.
7 L. Chen, A. Manz and P. J. R. Day, *Lab Chip*, 2007, **7**, 1413–1423.
8 C. A. Batt, *Science*, 2007, **316**, 1579–1580.
9 K. Y. Hwang, H. K. Lim, S. Y. Jung, K. Namkoong, J. H. Kim, N. Huh, C. Ko and J. C. Park, *Anal. Chem.*, 2008, **80**, 7786–7791.
10 N. C. Cady, S. Stelick and C. A. Batt, *Biosens. Bioelectron.*, 2003, **19**, 59–66.
11 J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989.
12 R. Boom, C. J. Sol, M. M. Salimans, C. L. Jansen, P. M. Wertheim-van Dillen and J. van der Noordaa, *J. Clin. Microbiol*, 1990, **28**, 495–503.
13 K. A. Melzak, C. S. Sherwood, R. F. B. Turner and C. A. Haynes, *J. Colloid Interface Sci.*, 1996, **181**, 635–644.
14 M. M. DeAngelis, D. G. Wang and T. L. Hawkins, *Nucleic Acids Res.*, 1995, **23**, 4742–4743.
15 R. H. Liu, J. Yang, R. Lenigk, J. Bonanno and P. Grodzinski, *Anal. Chem.*, 2004, **76**, 1824–1831.
16 N. C. Cady, S. Stelick, M. V. Kunnavakkam and C. A. Batt, *Sens. Actuators, B*, 2005, **107**, 332–341.
17 J. G. Lee, K. H. Cheong, H. Nam, S. Kim, J. W. Choi and C. Ko, *Lab Chip*, 2006, **6**, 886–895.
18 C. Lui, N. C. Cady and C. A. Batt, *Sensors*, 2009, **9**, 3713–3744.
19 Y.-K. Cho, J.-G. Lee, J.-M. Park, B.-S. Lee, Y. Lee and C. Ko, *Lab Chip*, 2007, **7**, 565–573.
20 E. A. Galinski, M. Stein, B. Amendt and M. Kinder, *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.*, 1997, **117**, 357–365.
21 *US Pat.*, US20070238109, 2007.
22 M. A. Shoffner, J. Cheng, G. E. Hvichia, L. J. Kricka and P. Wilding, *Nucleic Acids Res.*, 1996, **24**, 375–379.
23 B. C. Giordano, E. R. Copeland and J. P. Landers, *Electrophoresis*, 2001, **22**, 334–340.
24 A. R. Prakash, M. Amrein and K. V. I. S. Kaler, *Microfluid. Nanofluid.*, 2008, **4**, 295–305.
25 K.-Y. Hwang, S.-Y. Jeong, Y.-R. Kim, K. Namkoong, H.-K. Lim, W.-S. Chung, J.-H. Kim and N. Hun, *Sens. Actuators B: Chem.*, 2010, in press, DOI: 10.1016/j.snb.2009.1011.1005.
26 S. D. Belanger, M. Boissinot, C. Menard, F. J. Picard and M. G. Bergeron, *J. Clin. Microbiol.*, 2002, **40**, 1436–1440.
27 R. E. Besser, P. M. Griffin and L. Slutsker, *Annu. Rev. Med.*, 1999, **50**, 355–367.
28 P. M. Griffin and R. V. Tauxe, *Epidemiol. Rev.*, 1991, **13**, 60–97.