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Cite this: *Lab Chip*, 2011, **11**, 3649

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PAPER

Miniaturized bead-beating device to automate full DNA sample preparation processes for Gram-positive bacteria†

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Received 27th July 2011, Accepted 15th August 2011
DOI: 10.1039/c1lc20692c

We have developed a miniaturized bead-beating device to automate nucleic acids extraction from Gram-positive bacteria for molecular diagnostics. The microfluidic device was fabricated by sandwiching a monolithic flexible polydimethylsiloxane (PDMS) membrane between two glass wafers (*i.e.*, glass–PDMS–glass), which acted as an actuator for bead collision *via* its pneumatic vibration without additional lysis equipment. The Gram-positive bacteria, *S. aureus* and methicillin-resistant *S. aureus*, were captured on surface-modified glass beads from 1 mL of initial sample solution and *in situ* lyzed by bead-beating operation. Then, 10 μL or 20 μL of bacterial DNA solution was eluted and amplified successfully by real-time PCR. It was found that liquid volume fraction played a crucial role in determining the cell lysis efficiency in a confined chamber by facilitating membrane deflection and bead motion. The miniaturized bead-beating operation disrupted most of *S. aureus* within 3 min, which turned out to be as efficient as the conventional benchtop vortexing machine or the enzyme-based lysis technique. The effective cell concentration was significantly enhanced with the reduction of initial sample volume by 50 or 100 times. Combination of such analyte enrichment and *in situ* bead-beating lysis provided an excellent PCR detection sensitivity amounting to *ca.* 46 CFU even for the Gram-positive bacteria. The proposed bead-beating microdevice is potentially useful as a nucleic acid extraction method toward a PCR-based sample-to-answer system.

1. Introduction

A nucleic acid-based micro-total analysis system (μTAS) is a promising molecular diagnosis approach since all the analytical procedures (*i.e.*, sample preparation, amplification, and detection) are integrated and automated on a microchip format.[1–3] Real-time polymerase chain reaction (RT-PCR) has been widely accepted as both amplification and detection techniques because it provides higher accuracy and wider dynamic range than conventional PCR by determining the threshold cycle (Ct) directly proportional to the initial copy number. Moreover, it facilitates the integration of the PCR technique into a microfluidic system by removing post-PCR processes such as sample transfer, reagent addition, gel-electrophoresis and fluorescence imaging.[1,4,5] However, the DNA sample preparation process to acquire PCR-quality DNA from the raw samples remains as a critical hurdle toward the integration of full analytical steps into μTAS.[6–8] In order to achieve the successful amplification, nucleic acids should be properly extracted from clinical samples without potential PCR inhibitors because they have a direct effect on PCR performance (*i.e.*, sensitivity and specificity).[9–11] In addition, it is highly desirable to have the capability of enriching target analyte from a large volume of initial sample (*e.g.*, mL) into a microchamber during the sample preparation processes. By doing so, the large sample volume will be properly interfaced with micro-scaled devices, which can increase the detection sensitivity through the target analyte concentration. This would be one of the most important characteristics of μTAS which can compete with the conventional benchtop analytical methods. Therefore, incorporation of the above functions into the nucleic acid sample preparation device would be the key to the practical application of μTAS.

Among nucleic acids sample preparation steps, cell lysis is the fundamental step to release them by disrupting the cell

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† Electronic supplementary information (ESI) available: Figures for illustrating experimental set-up (S1), microfluidic components (S2), isotropically etched membrane valve structure (S3), Ct values as a function of PDMS membrane actuation time (S4), respectively, and a video clip of demonstrating bead-beating in a microdevice (S5). See DOI: 10.1039/c1lc20692c

membrane. In particular, the Gram-positive bacteria possess a thicker peptidoglycan layer in cell wall, and thus it is harder to lyze them than the Gram-negative ones.[12] *Staphylococcus aureus* (*S. aureus*), *streptococcus pneumonia*, and *enterococcus* species, *etc.* are classified as Gram-positive cells. They cause hospital-acquired infectious diseases such as MRSA (methicillin-resistant *Staphylococcus aureus*), VRE (vancomycin-resistant *enterococcus*), sepsis, *etc.* Although the mechanical or enzymatic methods have been largely employed to disrupt Gram-positive bacteria,[13–18] their incorporation into the miniaturized platform remains challenging. For example, for the enzymatic lysis of *S. aureus* using a commercial Qiagen kit protocol,[13] it requires at least two types of enzymes (lysostaphin and lysozyme), temperature control for enzyme reaction, and long processing time (*ca.* 2 h). Further purification steps are needed because the left enzymes and some chemicals (EDTA and detergents, *etc.*) are inhibitory to PCR amplification. These complex processes could not be easily installed in a microfluidic system.

In comparison, the mechanical lysis techniques such as bead-beating and ultrasonic methods are simpler and more efficient than the enzyme-based technique, despite that they require specialized laboratory instruments. Indeed, there have been some trials to implement the mechanical lysis into a microchip platform. For example, an ultrasonic horn was installed in an analytical system, which delivered an ultrasonic energy to disrupt bacterial cells.[19,20] Also, the mechanical shearing effect was applied by flowing a liquid sample through porous polymer monolith or sharp microstructures under mixed chaotrope and detergent solution.[21,22] Nonetheless, a real active device based on "bead-beating" is still needed because the impact force occurring on bead collision along with the shear effect is important to lyze Gram-positive bacteria or yeast. This would be one of the reasons why studies on detection sensitivity of Gram-positive bacteria have been rarely reported with a microfluidic platform.[21] Although some attempts have been made on a centrifugal CD-based microfluidic device, their analytical performance such as detection sensitivity remains unknown.[23,24] In the context of the microchip-based approach, a stand-alone flexible bead-beating chamber installed with an external actuator has been demonstrated to induce bead-beating cell lysis.[25]

In this study, we have developed a bead-beating microdevice through the pneumatic vibration of an elastomeric membrane (PDMS). It was fabricated by sandwiching a PDMS membrane between two glass chips (glass–PDMS–glass). In order to automate the full DNA extraction processes efficiently, the proposed microdevice and biological protocol were designed to have the following features: first, a monolithic flexible PDMS membrane, typically used as a flow-regulating tool,[26,27] was further employed as an actuator for bead collision *via* its pneumatic vibration. Namely, both manipulation of solutions (pumping and valving) and bead-beating for mechanical cell lysis were simultaneously implemented on a microchip using a single pneumatic source without any specialized lysis equipments. Also, valve seat and weir for bead trapping were designed to have the same microstructure, thereby simplifying the overall fabrication processes. In the aspect of the biological protocol, surface-modified glass beads were used to capture bacterial cells, which also acted as grinding media to disrupt the captured cells on their surface. Furthermore, the present microdevice transformed the captured cells from 1 mL of initial sample into an analyte volume of 10 or 20 μL DNA solution. It reduced the initial sample volume by 100 or 50-fold, thereby concentrating the target analyte into a microscale volume that is more compatible for subsequent PCR amplification. As a result, our microdevice showed an excellent DNA extraction performance similar to the conventional benchtop vortexing machine or the enzymatic method, allowing for successful PCR detection of Gram-positive bacteria. Here, we present the analytical performance of the bead-beating device together with microfluidic integration for valving and pumping.

2. Materials and methods

2.1. Cell strain and culture

Bacterial strains used in this study were methicillin-susceptible *Staphylococcus aureus* (ATCC No. BAA 1718, *S. aureus*) and methicillin-resistant *Staphylococcus aureus* (ATCC No. 33591, MRSA). They were grown in 50 mL trypticase soy broth (Becton, Dickinson and Company) at 37 °C overnight and suspended to appropriate concentrations with sodium acetate buffer (pH 4, 50 mM) after washing twice with 1× PBS solution (pH 7.4). The optical density of 0.2 OD was found to be $\sim 10^8$ CFU (colony forming unit) mL^{-1}.

2.2. Bead surface modification

Glass beads (Polysciences) with a diameter of 30∼50 μm were cleaned in piranha solution. After thorough washing with DI water, they were filtered and vacuum-dried. The solution for bead surface modification was prepared to contain 5% (v/v) of trimethoxysilylpropyl modified polyethyleneimine (Gelest) in ethanol. Glass beads were immersed in the prepared solution and allowed to react for 2 h with gentle mixing. Afterwards, they were filtered and rinsed with the fresh ethanol. It was repeated three times. Finally, the recovered glass beads were incubated at 110 °C for 50 min.

2.3. Testing instrumentation

PDMS membrane operation was controlled by applying the positive or negative pressure in pneumatic displacement chambers through an array of solenoid valves (S070-5DC, SMC), which were interconnected with electro-pneumatic-regulator (ITV0030-3BL, SMC) and LabVIEW software (National Instruments). The valve operation coupled with fluid transport was visualized at each step in the LabVIEW interface to monitor the nucleic extraction procedure. The testing instrument to automate DNA extraction processes was constructed as shown in Fig. S1†.

2.4. Device fabrication

Microfluidic features such as valve seats and channels were fabricated on a glass wafer using conventional photolithography and wet etching techniques.[26,27] Briefly, 6-inch borosilicate glass wafers (700 μm thickness) were piranha-cleaned and 500 nm of amorphous polysilicon was deposited using a low pressure chemical vapor deposition. Then, the wafers were coated with photoresist, patterned, and developed. The exposed polysilicon layer was dry-etched, the photoresist was stripped, and the

resulting exposed glass was isotropically etched to have a final depth of *ca.* 100 μm and width of *ca.* 200 μm with HF (hydrofluoric acid, 49%) solution. After removing the remained polysilicon, the glass wafers were patterned with dry film resist on the etched side, and then the bead chamber and holes (fluidic and pneumatic access) were generated by the sand-blasting technique. The bead chamber and its corresponding pneumatic displacement chamber were an oval type. The bead chamber had the volume of *ca.* 15 μL and the size of *ca.* 6 mm × 4 mm × 0.75 mm (longest × shortest × depth), respectively. The two pneumatic displacement chambers for bead-beating had the total volume of *ca.* 3 μL and the size of *ca.* 6 mm × 4 mm × 0.2 mm (longest × shortest × depth), respectively, and the spacing between two wells was *ca.* 0.4 mm. The manufactured glass wafers were diced into chips (27.4 mm × 12 mm). The resulting fluidic and pneumatic manifold chips were plasma-cleaned (Harrick) and bonded permanently by sandwiching plasma-activated PDMS membrane sheet (254 μm thickness, Rogers). About 15∼16 mg of surface-modified glass beads were weighed within the bead chamber and PCR-compatible adhesive tape (Applied biosystems) was sealed to close the open chamber. The polycarbonate plate (1 cm × 1 cm × 0.7 cm) was additionally glued over the adhered adhesive tape to prevent its deflection during DNA extraction processes.

2.5. DNA extraction

1 mL of sodium acetate buffer (50 mM, pH 4, Sigma-Aldrich) containing *S. aureus* or MRSA (typically 10^6 CFU mL^{-1}), 0.5 mL of TE buffer (10 mM, pH 8, Ambion) for washing, and 10 μL or 20 μL of NaOH solution (0.02 N, Sigma-Aldrich) for lysis were previously dispensed into liquid reservoirs. The liquid solutions were transported in a pressure-driven manner and their operating fluid pressures were determined in preliminary experiments. The initial sample solution passed through the bead-packed chamber with a flow rate of *ca.* 200 μL min^{-1} at 30 kPa while pressurizing PDMS membrane of bead chamber upward at 150 kPa. The flown-through solution was recovered to evaluate the cell capture capability. After initial loading, the bead-packed chamber was washed at a flow rate of *ca.* 500 μL min^{-1} (80 kPa), and N_2-dried for 30 s with 100 kPa. For lyzing the captured cells, 6 μL of NaOH was injected and valves at both sides of the bead-packed chamber were closed. The pressures of two pneumatic displacement chambers alternate asynchronously with one chamber positive (80 kPa) and the other negative (−80 kPa) for the first half of a cycle and they were reversed for the second half of a cycle. The PDMS membrane vibrated at a frequency of 10 Hz for 5 min. The extracted DNA was eluted by injecting another 4 μL or 14 μL of NaOH solution with a fluid pressure of 100 kPa, and 10 μL or 20 μL of DNA solution was obtained as a result. The overall process time took less than 20 min. No further DNA purification steps were performed. Detailed description of microdevice components was provided in Fig. S2†.

2.6. Positive and negative lysis controls (benchtop DNA extraction)

Two types of benchtop lysis techniques (*i.e.*, enzymatic and bead-beating methods) were carried out as a positive lysis control (PLC). 1 mL of *S. aureus* samples having two different cell concentrations (10^4 CFU mL^{-1} and 10^6 CFU mL^{-1}) were centrifuged in microcentrifuge tubes at 13200 rpm for 20 min and the supernatant was carefully discarded. The remained cell pellets were further treated with the above techniques, respectively. In the case of an enzymatic method, the cell pellet was incubated with lysostaphin solution (200 μg mL^{-1}, Sigma) at 37 °C for 30 min before application to the Qiagen DNA extraction kit (Cat 51304, QIAamp DNA Mini Kit), and 20 μL of purified DNA solution was acquired as described in its protocol. For a benchtop bead-beating lysis, after adding both 30 mg of bare glass bead and 20 μL of lysis solution (0.02 N NaOH solution or DI water) to the cell pellet, vortexing was vigorously performed at the full speed for 5 min (GENIE 2, Fisher Scientific). The extracted DNA solution was recovered following brief centrifugation. As a negative lysis control (NLC), the cell pellet was vortexed with DI water only (in the absence of glass beads). The resulting lysis control samples (PLC and NLC) were amplified in comparison with the obtained DNA solution from the bead-beating microdevice. For the accurate comparison, the total number of *S. aureus* injected and the final DNA elution volume were always controlled to be the same with those samples.

2.7. Real-time PCR amplification

In order to evaluate the cell lysis performance and the resulting extracted DNA quality, real-time PCR (polymerase chain reaction) assays were performed on the GenSpector® TMC-1000 instrument (Samsung Electronics).[5] Primer sets specific to SA442 region (forward: 5′-GTT GCA TCG GAA ACA TTG TGT-3′ and reverse: 5′-ATG ACC AGC TTC GGT ACT ACT AAA GAT-3′, GeneBank accession number AF033191) of *S. aureus* genome and mecA fragment (forward: 5′-ACG AGT AGA TGC TCA ATA-3′ and reverse: 5′-GGA ATA ATG ACG CTA TGA T-3′, GeneBank accession number EF190335.1) of MRSA genome were designed by Primer3 software (Whitehead Institute/MIT Center for Genome Research), respectively. The PCR reaction mixture (*ca.* 2 μL) was prepared to possess the following final concentrations: 0.4 μM Taqman probe (FAM–5′-TGT ATG TAA AAG CCG TCT TG-3′–MGB–NFQ for *S. aureus* and FAM–5′-CCA ATC TAA CTT CCA CAT ACC ATC T-3′–BHQ1 for MRSA, respectively), 1× Z-Tag buffer (Takara Bio), 1 μM each primer (Applied Biosystems or Sigma), 0.05 U Z-Taq polymerase (Takara Bio), 0.2 mM dNTP (Takara Bio), 0.5 μL PCR grade water (Ambion), and 1 μL extracted DNA solution. After loading into PCR chip, the thermocycling was performed with a denaturation step at 95 °C for 1 s and an extension step at 60 °C for 4 s. The size of PCR amplicons was designed to be 72 and 98 base pairs for *S. aureus* and MRSA, respectively. They were further confirmed by gel-electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies).

3. Results and discussion

3.1. Device layout and characterization

The bead-beating microdevice presented here is based on monolithic integration of polymer membrane valve and two glass layers.[25,26] It consists of fluidic (top glass), PDMS, and pneumatic (bottom glass) layers, which is schematically illustrated in Fig. 1.

About 15 μL of cell lysis chamber was constructed to accommodate surface-modified glass beads in the fluidic layer so that they were in direct contact with liquid solutions. The installed glass beads were designed to act both as cell capture and grinding media. In order to establish the bead-beating operation within a microfluidic platform, pneumatic vibration of PDMS membrane was carried out to actuate glass beads, thereby inducing vigorous collision and shear stress. Two pneumatic displacement chambers were fabricated to apply a positive and a negative pressure alternatively. In addition, the PDMS membrane was permanently bonded to the two glass wafers because the previous reversible bonding has shown limitations on valve operation under a fluid pressure of 75 kPa.[26,27] Because the plasma-activated irreversible PDMS bonding causes typical PDMS sticking onto the valve seat, the valve seat was isotropically etched to be separated from the PDMS mating surface by *ca.* 20 μm (see Fig. 1a, 2 and S3†). It turned out that the suggested alteration on the membrane valve structure made it operable in a wider range of fluid pressures (~300 kPa) without any solution leakage and membrane sticking.[28] Moreover, the same isotropically etched microstructures were also used as weirs to isolate the glass beads (*ca.* 30~50 μm) within the microchamber (Fig. 1a and 2c). Such dual-functional device components could make possible facile construction of operating components as well as simplification of fabrication processes.

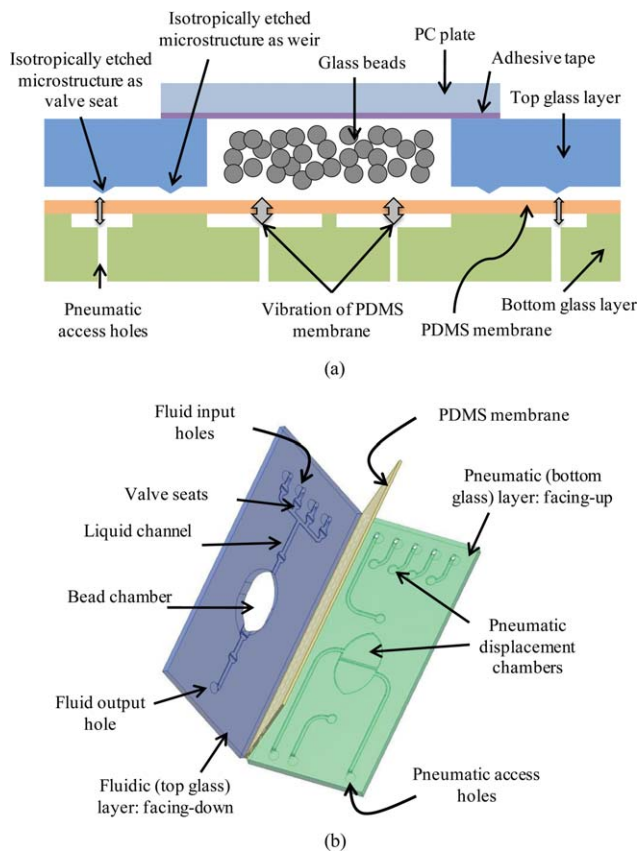

Fig. 1 (a) Cross-sectional view of the miniaturized bead-beating device *via* vibration of PDMS membrane. (b) Exploded view of the three-layer monolithic glass–PDMS–glass microdevice and its fluidic and pneumatic components.

3.2. Evaluation of PLC samples (benchtop DNA extraction)

The threshold cycle (Ct) values of PLC samples were obtained as shown in Table 1. Generally, the benchtop bead-beating method has been applied in combination with lysis solution containing detergent or chemical to enhance lysis efficiency. Here, NaOH solution (0.02 N) was chosen because it did not inhibit PCR amplification at all without further purification steps (data not shown). The effect of bead-beating was more distinct with NaOH solution than DI water, suggesting that NaOH contributed to the DNA extraction yield by disrupting the cell membrane chemically. For enzymatic lysis of *S. aureus*, lysostaphin was utilized because it is capable of specifically cleaving the crosslinking pentaglycine bridges in the cell walls of *Staphylococci*.[13,29] As shown in Table 1, the benchtop bead-beating method with NaOH solution performed better than or similar to the enzyme-based DNA extraction in terms of Ct values. Therefore, the performance of the miniaturized bead-beating device was evaluated by comparing it to the benchtop vortexing machine. As the measurement of optical density is a rough cell quantification technique, the Ct value of PLC was varied daily with a standard deviation of *ca.* 1.5 even at the identical optical density. For comparison, the NLC vortexed with DI water only showed Ct of *ca.* 31.5 and *ca.* 37.0 for 10^6 CFU mL^{-1} and 10^4 CFU mL^{-1} samples, respectively.

3.3. Cell capture

The basic operation of the present microdevice is as follows: (1) cell capture on glass beads, (2) washing and drying, (3) cell lysis using *in situ* bead-beating, and (4) elution of extracted DNA solution. The bacterial cells can be captured on the solid substrate in specific or non-specific manners. Along with the cell-specific immunoaffinity technique,[30,31] nonspecific cell capture methods using surface thermodynamics[32–34] or electrostatic interaction[35–37] have demonstrated their ability to capture pathogenic bacteria. In this work, the long-range Coulombic electrostatic interaction[35–37] was exploited by modifying glass beads electropositive. Specifically, the surface of glass beads was derivatized so as to possess the positive amine groups in reaction with organosilane compound containing polyethyleneimine. After packing the surface-modified glass beads inside the microchamber (Fig. 2), 1 mL of initial sample solution with *S. aureus* (10^6 CFU mL^{-1}) passed through them. At first, the role of electrostatic interaction was assessed by comparing the modified glass beads with the bare ones. It was found that the Ct value of eluted DNA from the modified glass beads was lower than that of unmodified ones by 2. These results indicated that the cell capture efficiency was greatly increased with the surface modification by a factor of 4 (*i.e.*, 1 Ct difference indicates 2-fold difference in initial template copy numbers). To obtain a more quantitative comparison on cell capture capability, the passed-through sample solution was collected at the outlet of the microdevice. Then, *S. aureus* in the solution, not captured on the bead surface, was subject to benchtop vortexing with bare glass beads as with the case of PLC samples. The Ct value of passed-through *S. aureus* was compared to that of the eluted DNA solution obtained from the operation of bead-beating microdevice. Finally, their Ct difference was used as a measure to

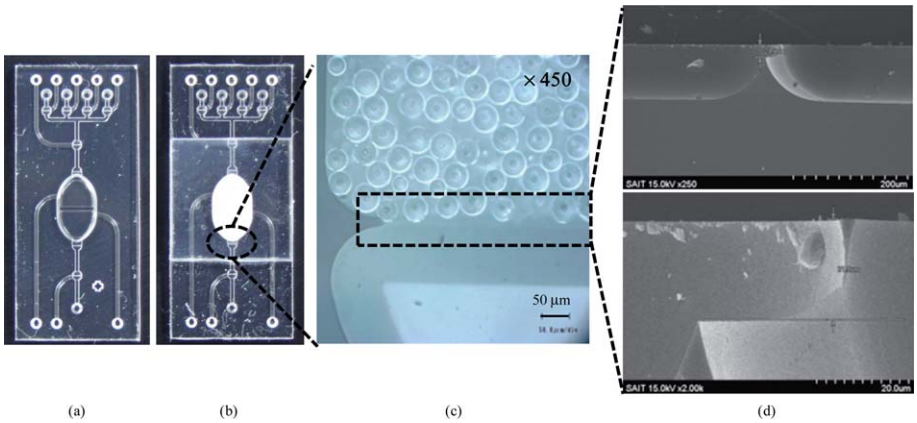

Fig. 2 Digital pictures and SEM images of the fabricated bead-beating microdevice: (a) assembled microchip through PDMS-interface bonding (27.4 mm × 12 mm), (b) sealed microchip packed with glass beads (*ca.* 30–50 μm), (c) one of the isotropically etched microstructures working as a weir for isolating glass beads, and (d) etching depth of *ca.* 20 μm from the bonding surface.

estimate the cell capture efficiency and capacity. When 1 mL of the various concentrations of *S. aureus* (10^4, 10^5, 10^6, 10^7, and 10^8 CFU mL^{-1}) was applied, Ct difference was maintained to be over 5 in the range of 10^3 CFU mL^{-1} to 10^7 CFU mL^{-1} and it dropped to about 2.5 for the 10^8 CFU mL^{-1}. Therefore, the capture efficiency would be over 90% as evidenced by the ten-fold difference in the initial template copy numbers accompanying the Ct change of *ca.* 3.3 (*i.e.*, verified while evaluating the limit of detection in the final section). In addition, the cell capture efficiency was further confirmed by the colony count method.[32,34] It appears that the fabricated bead-packed microdevice displays the capacity over ~10^7 CFU of *S. aureus*. After cell capture, the bead-packed microchamber was washed and N_2-dried such that the complete exchange to lysis solution was achieved. These results demonstrated the potential of *in situ* bead-beating lysis of the captured cells instead of releasing them. Other cell capture techniques such as immunoaffinity-based ones can also be incorporated into the present device by adopting proper solid surface modification chemistry.

3.4. Miniaturized bead-beating lysis

The bead-beating cell lysis takes place when the captured cells on bead surface are disrupted by inter-bead collision or the shear effect. Some controllable parameters are conceivable that can affect bead motion such as membrane vibration frequency (5~10 Hz), membrane actuation pressure (20~80 kPa), and depth of pneumatic displacement chamber (100~200 μm). When the bead chamber is fully filled with the lysis solution, it turned out that the above parameters did not improve the cell lysis efficiency notably on the basis of the PCR Ct values (data not shown). From these results, it could be thought that the incompressible liquid solution would inhibit the membrane deflection and bead motion in a confined chamber, which fails to induce the bead-beating lysis. To investigate the effect of liquid amount participating in the bead-beating lysis, the liquid volume fraction (f_L) was defined as the ratio of lysis solution volume to void volume of lysis chamber. The void volume of lysis chamber was calculated to be *ca.* 12 μL by subtracting net volume of glass bead (*ca.* 6 μL) from that of lysis chamber (*ca.* 18 μL, including pneumatic displacement volume of the chamber). The final eluted DNA volume was controlled to be the same (20 μL) by adjusting the additional NaOH solution when eluting the extracted DNA from the bead chamber. As shown in Fig. 3, f_L was found to be the key factor to determine PCR Ct values. The Ct value became close to that of PLC when f_L approached *ca.* 0.3.

To gain an understanding on this behavior, an attempt was made to separate the bead-beating and the chemical effect from the obtained lysis results. The use of DI water instead of NaOH appears the most direct way to exclude the effect of chemical lysis caused by NaOH. However, it was hindered by the fact that the extracted DNA (*i.e.*, containing phosphate groups) adsorbed significantly on the positively charged bead surface at neutral pH (*i.e.*, Ct was *ca.* 28). These results implied that NaOH neutralized the bead surface by increasing the solution pH above the pK_a of the amine groups and induced the cell membrane breakage to some extent. Thus, NaOH-driven chemical lysis was indirectly estimated from the cell pellet as with the case of PLC. Lysis with NaOH without beads yielded *ca.* 3 Ct decrease (*i.e.*, the maximum chemical lysis effect) from NLC Ct, which turned out to be almost equivalent to ΔCt decrease (*ca.* 3.7, NLC Ct–Ct) when $f_L = 1$ (Fig. 3). These results indicated that the bead-beating lysis was nearly negligible when the lysis chamber was full of NaOH solution. Also, Ct at $f_L = 0$ would represent the bead-beating lysis only without the chemical effect. The effect of chemical lysis would increase with increasing f_L until NaOH solution soaks the entire surface of the glass bead. Assuming that the packing ratio of glass beads is 50% when the PDMS membrane presses them upward, 6 μL of lysis solution is required

Table 1 Ct values of PLC samples[a]

Number of applied *S. aureus* cell/CFU	Benchtop bead-beating method		Enzymatic method (lysostaphin)
	NaOH (0.02 N)	DI water	
~10^4	30.5 ± 0.35	34.5 ± 0.26	31.6 ± 0.56
~10^6	23.7 ± 0.29	26.7 ± 0.15	25.7 ± 1.43

[a] Three repetitions were performed for each cell number.

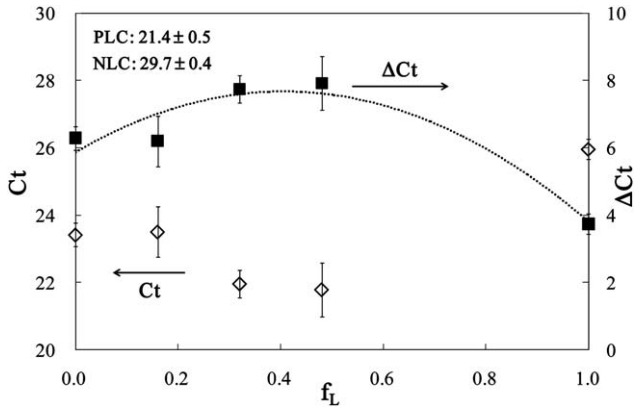

Fig. 3 Effect of liquid volume fraction (f_L) on bead-beating lysis in the fabricated microdevice. (a) Obtained Ct values (◇) and ΔCt (NLC Ct–Ct, ■) as a function of f_L. Three repetitions were performed for each f_L. 1 mL of *S. aureus* (10^6 CFU mL^{-1}) was applied.

to soak the whole glass bead surface, which corresponds to f_L of *ca.* 0.5 (*i.e.*, 6 divided by 12). Also, this would be the maximum f_L value where the membrane deflection was not hindered by the liquid solution because there was void space to accommodate it. However, a Ct value approaching that of PLC at *ca.* $f_L = 0.3$ indicates that NaOH-driven chemical lysis would start to be saturated at this point. As f_L was increased from *ca.* 0.5 to 1, the increasing incompressible liquid solution would start to disturb the vibration of the membrane. Therefore, the maximum cell lysis efficiency would be achieved with f_L in the range of 0.3 and 0.5 by maximizing both bead-beating cell lysis caused by membrane deflection and NaOH-driven chemical lysis. On the basis of these results, all subsequent experiments were performed by adjusting f_L at *ca.* 0.5 (see Video S5†).

3.5. DNA extraction using miniaturized bead-beating lysis

We further characterized the bead-beating microdevice by investigating the effect of PDMS actuation time and limit of detection (LOD). At first, the real-time PCR results were displayed as a function of PDMS actuation time (see Fig. S4†). It was observed that most of the captured cells on beads surface were lyzed within 3 min (*i.e.*, its Ct value became close to PLC samples) and further actuation did not influence Ct significantly. Considering both cell capture and lysis performance, it could be inferred that a considerable degree of the achieved 50-fold volume reduction was translated into the DNA enrichment effect. In order to measure the LOD, 1 mL of serially diluted *S. aureus* samples (10^5, 10^4, 10^3, 10^2 CFU mL^{-1} and 0 CFU mL^{-1}) were applied and 20 μL of DNA solution was obtained. Until now, the attempt to detect Gram-positive bacteria on a miniaturized platform has been rarely made, and even its reported detection sensitivity was lower than that of Gram-negative bacteria by one or two orders-of-magnitude.[21] As shown in Fig. 4a, the typical S-curved shape of PCR amplification was obtained for the tested samples, and furthermore 10^2 CFU samples were successfully detected. 0 CFU mL^{-1} and no template control (PCR-grade water) samples were not amplified. These results suggest that the *in situ* bead-beating lysis in combination with flow-through cell capture could increase the effective analyte concentration, allowing for higher detection sensitivity for the Gram-positive bacteria. Such target analyte enrichment is one of the potential benefits of the beat-beating microdevice, which was fully exploited in the present approach. Noting that the cycle interval separating each 10-fold difference in the initial template concentration was calculated to be about 3.36 (Fig. 4b), a PCR efficiency, defined as the (number of copies at cycle n + 1)/(number of copies at cycle n), was as high as 1.9.[38]

Lastly, we validated the present microdevice by detecting the low number of MRSA in a high background of *S. aureus*, which is closer to that in complex clinical samples. Various amounts of MRSA were mixed with the constant amount of *S. aureus* (10^5 CFU mL^{-1}) to have the final MRSA concentrations of 4.6×10^4, 4.6×10^3, 4.6×10^2, 4.6×10^1 and 0 CFU mL^{-1}. For this test, 1 mL of initial sample was introduced and DNA was extracted with a total volume of 10 μL, and therefore a 100-fold volume reduction was achieved. The total bacterial DNA (*S. aureus* and MRSA) was recovered and mecA region of MRSA was amplified. As shown in Fig. 5, the detection of an extremely low number of MRSA, *ca.* 46 CFU, was successfully demonstrated. The sample possessing 0 CFU of MRSA was not amplified. Also, these results implied that the non-specific cell capture would be applicable to a PCR-based diagnostic system because the specificity could be derived from PCR. The

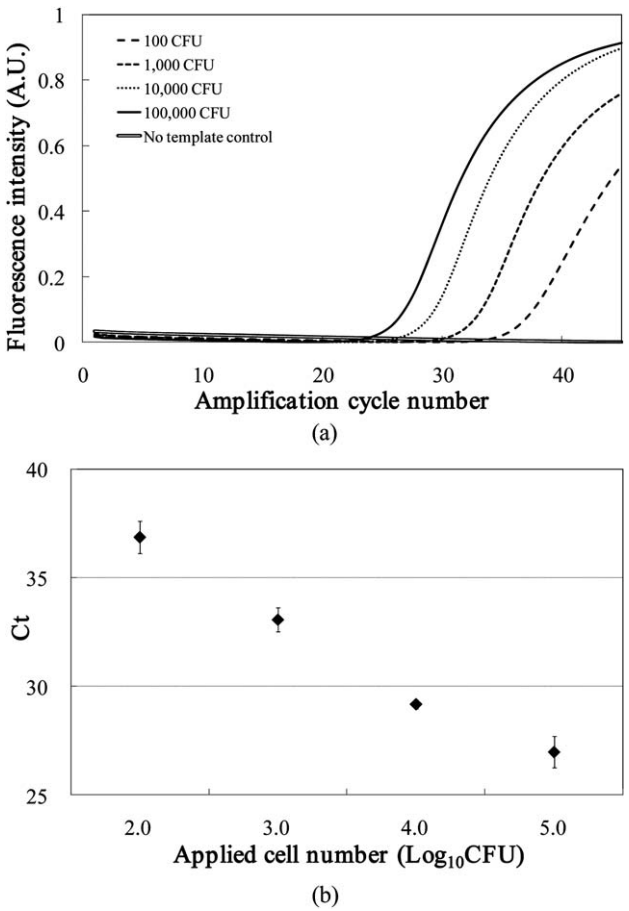

Fig. 4 Real-time PCR detection of *S. aureus* using the bead-beating microdevice: (a) representative real-time PCR amplification curves and (b) obtained Ct values as a function of the applied *S. aureus* number. Three repetitions were performed for each cell number.

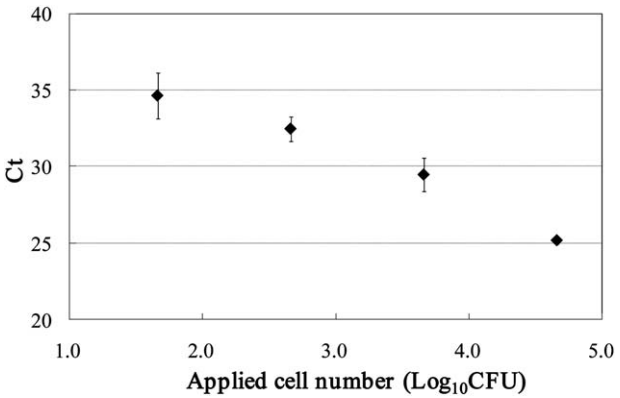

Fig. 5 Real-time PCR detection of MRSA using the bead-beating microdevice in a high background of *S. aureus*. Three repetitions were performed for each cell number.

installation of the bead-beating cell lysis technique into flow-through microdevice capable of processing a large sample volume allowed for the excellent detection performance for Gram-positive bacteria. Application to the detection of MRSA in a nasal swab is in progress.

4. Conclusions

We have demonstrated a miniaturized bead-beating device to lyze Gram-positive bacteria by means of the vibration of a monolithic PDMS membrane. The PDMS membrane was designed to be dual-functional as a valving component as well as a bead actuator, so that the single pneumatic source coupled with solenoid valves could implement the fluidic manipulation of liquid samples and bead-beating lysis without any additional specialized lysis equipment. The flow-through cell capture on glass beads from a large sample volume and subsequent *in situ* bead-beating lysis of captured cells on its surface gave rise to the significant DNA enrichment, which was translated into the high detection sensitivity (*ca.* 46 CFU) even for the Gram-positive bacteria. Also, the liquid volume fraction played a crucial role in producing the maximum DNA yield because it dominated the membrane deflection, bead motion, and ultimately lysis capability. The bead-beating microfluidic device presented here is potentially useful for the development of μTAS aiming at the detection of various pathogens including Gram-positive bacteria.

Acknowledgements

This work was supported by the World Class University program (R31-2008-000-10083-0), the Korea Research Foundation Grant (KRF-J03003) and the Basic Science Research Program (2010-0027955) through the National Research Foundation (NRF) funded by the Korea government (MEST).

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