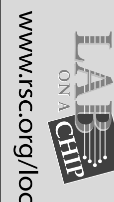

Microfluidic chip for high efficiency DNA extraction

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A high efficiency DNA extraction microchip was designed to extract DNA from lysed cells using immobilized beads and the solution flowing back and forth. This chip was able to increase the extraction efficiency by 2-fold when there was no serum. When serum existed in the solution, the extraction efficiency of immobilized beads was 88-fold higher than that of free beads. The extraction efficiency of the microchip was tested under different conditions and numbers of *E. coli* cells. When the number of *E. coli* cells was between 10^6 and 10^8 in 25 μl of whole blood, the extraction efficiency using immobilized beads was only slightly higher than that using free beads (10^0 to 10^1 fold). When the number of *E. coli* cells was in the range 10^4 to 10^6 in 25 μl of whole blood, the extraction efficiency of immobilized beads was greater than that of the free beads (10^1 to 10^2 fold). When the number of *E. coli* cells was lower, in the range 10^3 to 10^4 in 25 μl of whole blood, the extraction efficiency of immobilized beads was much higher than that of the free beads (10^2 to 10^3 fold). This study indicated that DNA could be efficiently extracted even when the number of bacterial cells was smaller (10^5 to 10^3). This microfluidic extraction chip could find potential applications in rare sample genomic study.

1 Introduction

The use of nucleic acid technologies has considerably enhanced preparation and diagnostic procedures in the life sciences. DNA sequencing and analysis have been developed in a chip format by DNA hybridization.[1–3] The amplification of DNA has become faster because of the improvement in micro PCR systems.[4–8] Sample preparation is an important process for DNA sequence analysis and genetic recombination. Automation of sample preparation processes is required in experimental and clinical fields. It involves cell lysis, DNA binding and release. The quality of extraction is a basic criterion for successful application in this technology. DNA-extracting beads and buffers have been developed to be commercial products. They provide benefits over conventional method due to quick processing times, reduced chemical requirements, easy separation from the beads and ease of automation. Clinical disease diagnosis generally involves the detection of a very small amount of sample (*e.g.* bacteria) with an excess of undesired constituents (*e.g.* protein, red blood cells).

There are many investigations concerning the extraction of nucleic acid. Chemical methods for the extraction of genomic DNA depend mostly on the utilization of lysozyme and other lipolytic and/or proteolytic enzymes.[9–13] However, several bacteria are resistant to the action of lysozyme.[14] 70% ethanol treatment for increasing the susceptibility of the cells to subsequent lysis had been suggested.[15] This led to a modified procedure for the extraction of high-quality genomic DNA that is rapid, simple and biologically non-hazardous.[16] Polyvinyl-alcohol-based magnetic beads were used for the separation of genomic DNA and specific nucleic acid purification.[17] A DNA extraction method using bacterial magnetic particles based on the ionic interactions between DNA and cationic elements has been developed.[18] A hyper-branched poly-amidoamine dendrimer has been synthesized to improve the extraction of DNA from fluid suspensions, and the binding and release efficiencies increased with the number of generations.[19] DNA extraction of the samples requires high yields. Polysaccharides, glycoproteins and lipopolysaccharides, however, can interrupt the DNA extraction process by absorbing the DNA.[20,21] Polysaccharides co-precipitate with the DNA during the extraction processes and will lead to problems, such as poor yields and/or subsequent interference with restriction endonuclease digestion.[22,23] A simple method for the extraction of high quality DNA from marine bacteria that produce extracellular materials has been proposed.[24] A DNA extraction method consisting of micro-particle-beating with SDS (sodium dodecyl sulfate) for cell lysis, followed by polyethylene glycol precipitation for primary DNA purification and chromatography for final DNA purification has been developed.[25] However, 63% of the original DNA was lost during purification. Therefore, DNA extraction is still challenging.

On a larger scale, it is easy to completely extract the DNA from samples by using various chemicals, precipitation and centrifugation. The conventional methods for DNA extraction are either column-based techniques or include centrifugation and precipitation steps having the disadvantage of being time consuming, difficult to automate or difficult to scale down to small sample volumes. However, at the microscales, high concentration solutions (*e.g.* whole blood) will impede the fluid flow and the process becomes time-consuming. In particular, the centrifugation and precipitation processes are difficult to perform in chips. Even though the DNA-extracting beads have been developed, the residual solution cannot be totally exhausted because the beads are free to move. This also means that the beads can be easily exhausted at the same time. These observations indicate that the extraction efficiencies of free beads cannot be very high, especially when various constituents existed. Therefore, a new method for improving the DNA extraction involving only an immobilization process and flowing solution, and requiring no extra reagents, is developed in this study. Using the immobilized beads and making the solution flow, the above major limitations can be avoided. The device,

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which is based on the concept of microfluidics, is simple in design, cost-effective and can easily increase extraction rates. It results in fast and efficient sample preparation and enables the automation of the process.

2 Materials and methods

2.1 Principles of operation

It is known that DNA can bind to some kinds of beads whose surfaces have organic groups. When the ion concentration of the solution is changed, the bound DNA can be released into the solution. If the beads are dispersed in the solution, the probability of collision between the beads and the DNA will be low due to the existence of other large molecules (*e.g.* protein) in the solution along with the DNA. Even if one makes the solution in the channel move back and forth, the relative locations are still unchanged, so the probability of collision between the beads and the DNA is still limited. If the beads are immobilized on the surface of the channel, and the solution flows back and forth, the probability of collision will be dramatically increased leading to a higher extraction efficiency. Polysaccharides, glycoproteins and lipopolysaccharides can interrupt the DNA extraction process by absorbing the DNA, which will cause some problems, such as poor yields.[20–23] However, the above-mentioned explanation only suggests a possible reason for an increase in extraction efficiencies; the real reasons need to be further explored.

2.2 Design and fabrication of microfluidic extraction device

The microfluidic extraction chip was designed (Fig. 1a) and fabricated onto a poly-methylmethacrylate (PMMA) substrate with flow channels, which are 2 mm wide and 1 mm deep. Machined and bead-immobilized PMMA substrate and blank PMMA were bonded together to form the device. Beads immobilized on the surface of an Eppendorf pipette were used as a macroscale model system for comparative experiments. The beads used for DNA extraction (Bead #1) were obtained from Magic Bead Inc. (USA). A plasma generator (VVS-55, Victor Taichung, Taiwan) was used to perform the surface treatment. Plasma source gas consisting of a mixture of ammonia (75%) and oxygen (25%) was used to activate the surface of PMMA substrate. The treatment time was about 3 minutes.

One gram of the bead powder was added and dispersed into 20 ml of distilled deionized water. 50 μl of this solution was injected into the channel. After one hour of mild shaking, most of the beads in solution were immobilized onto the channels. The channel area covered by the immobilized beads was 50 mm^2 (2 mm wide × 25 mm long). The surface of the immobilized beads appeared light white, but nearly transparent. The area occupied by the total fluid flowing back and forth (exposed area) was 100 mm^2 (2 mm wide × 50 mm long). The ratio of the surface area of immobilized beads to that of the free beads was about 0.7. The photo image of the microchip is shown in Fig. 1b. A bead coated Eppendorf was also prepared by the same method. 50 μl of the solution was added into the Eppendorf. The preparation was completed by gentle shaking. The photomicrographs of the channel without and with immobilized beads are shown in Fig. 1c and 1d, respectively.

2.3 Materials

Escherichia coli was cultured in 5 ml of LB medium (NaCl: 10 g l^{-1}, Tryptone: 10 g l^{-1}, yeast extract: 5 g l^{-1}) in 15 ml tubes at 37 °C and 225 rpm. After 16 h, the optical density (OD) of the culture was measured in a spectrophotometer (U-2100, Hitachi, Japan). The number of *E. coli* cells or the amount of DNA was calculated from an OD *versus* cell number or OD *versus* weight of DNA correlations. The culture was then diluted with distilled water to obtain varying numbers (10^1–10^8) of *E. coli* cells per microlitre.

Blood from a female member of our group was used for DNA extraction experiment using the microchip. Whole blood was directly used without any pretreatment. DNA extraction efficiency of the device was also tested using serum obtained by the centrifugation of blood.

2.4 Experimental setup and analysis

A laboratory-made bi-directional microfluidic driving system[26] was connected to the devices and used to induce oscillations to the sample flow inside the channel. The sample flowed forward and backward with the immobilized beads at a frequency of 0.5 Hz inside the channel. The average velocity and total lasting time of flow in both directions for the devices was 1 cm s^{-1} and 10 min,

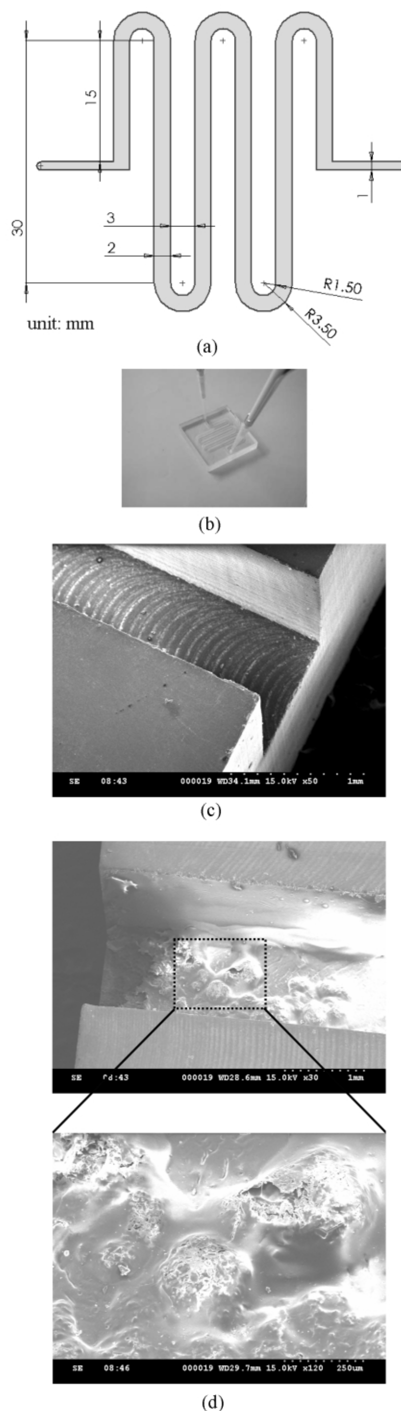

Fig. 1 Schematic drawing and photo image of the microfluidic device. (a) Layout of the flow channel in the device, unit: mm, (b) photograph of the microfluidic chip, (c) photomicrograph of the channel without immobilized beads, (d) photomicrograph of the channel with immobilized beads.

respectively. The extractions were carried out under identical conditions except that the control for the device with free beads contained the same weight of beads as that in the device with immobilized beads. The bead-coated Eppendorf with the fluid was spun in a vortex (VSM-3Mixer, Shelton, USA) for 10 min to improve the collision probability between beads and DNA.

E. coli cells were treated with a buffer (B1+B2, Magic Bead, USA) to lyse the cells and to release the DNA. Before the DNA was extracted by the beads, the channels were washed and cleaned by pumping distilled deionized water (*i.e.* pre-experimental wash). After extracting DNA and the fluid being exhausted, 10 mM Tris-HCl buffer (pH = 7.6) was pumped through the channel of the device in order to remove any unbound substances (*i.e.* rinsing before elution). Both these steps formed the negative controls. Then the beads were eluted by distilled deionized water to release the DNA into the solution.

The DNA extracted using the microchips was amplified by PCR (PCR system 9700, Gene Amp, USA). In order to identify whether the DNA fragments were amplified at random and not a particular sequence, the effect of different primers on the extraction was investigated. Three different primer pairs were used. Pair 1: the forward and reverse primers used were 5′-CAGGATTAGATACCCTGGTAG-3′ and 5′-GACGTCATCCCCACCTTCCTCC-3′, respectively, and the length of the PCR product was 413 bp. Pair 2: the forward and reverse primers used were 5′-AGTGGCGGACGGGTGAGTAA-3′ and 5′-GACGTCATCCCCACCTTCCTCC-3′, respectively, and the length of the PCR product was 1095 bp. Pair 3: the forward and reverse primers used were 5′-CAGGATTAGATACCCTGGTAG-3′ and 5′-TTCCCCTACGGTTACCTTGTT-3′, respectively, and the length of the PCR product was 716 bp. The PCR conditions using primer pairs 1, 2 and 3 were the same, *i.e.*, one cycle of 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 40 s at 58 °C and 40 s at 72 °C, and one cycle of 10 min at 72 °C. All three PCR products corresponded to the amplified 16S rRNA in the DNA fragments of *E. coli* genomic DNA. DNA ladders (SM0633, Taiwan Flow, Taiwan; DM100-2, Bioman, Taiwan) were used as markers in the gel electrophoresis. The overall experimental process is shown in Fig. 2. The PCR products were analyzed qualitatively in a Mupid-2 electrophoresis equipment (Advance, Japan) and quantitatively in an Agilent 2100 bioanalyzer (Agilent Technologies, USA).

3 Results and discussion

3.1 DNA extraction in the solution containing serum

The gel electrophoresis fluorescence images (Fig. 3a) of the DNA extracted from 10^5 *E. coli* cells in 75 µl of solution after PCR amplification were used to compare the extraction efficiencies using both immobilized and free beads. Since the molecular weight of genomic DNA in *E. coli* cells is about 3.1×10^9 daltons (g mol^{-1}), there was about 0.5 ng genomic DNA in 10^5 *E. coli* cells. The experimental conditions are summarized in Table 1. Each set of conditions of the experiments was repeated at least 5 times, and the average error was less than 15%. In Fig. 3a, Lanes 1 to 12 indicated the results of DNA extraction. Lanes 1 to 3 showed the results of using no beads in the devices with the solutions flowing back and forth. The differences between them are described as follows. In Lane 1, serum was not added while 25 µl and 50 µl of serum were added in Lanes 2 and 3, respectively. Since there were no DNA-extracting beads, there were no fluorescence bands in Lanes 1 to 3. The results of using the devices with the free beads are shown in Lanes 4 to 8. While 25 µl and 50 µl of serum were added in Lanes 5 and 6, respectively, there was no serum addition in Lane 4. Since there was no serum in Lane 4, the free beads could efficiently

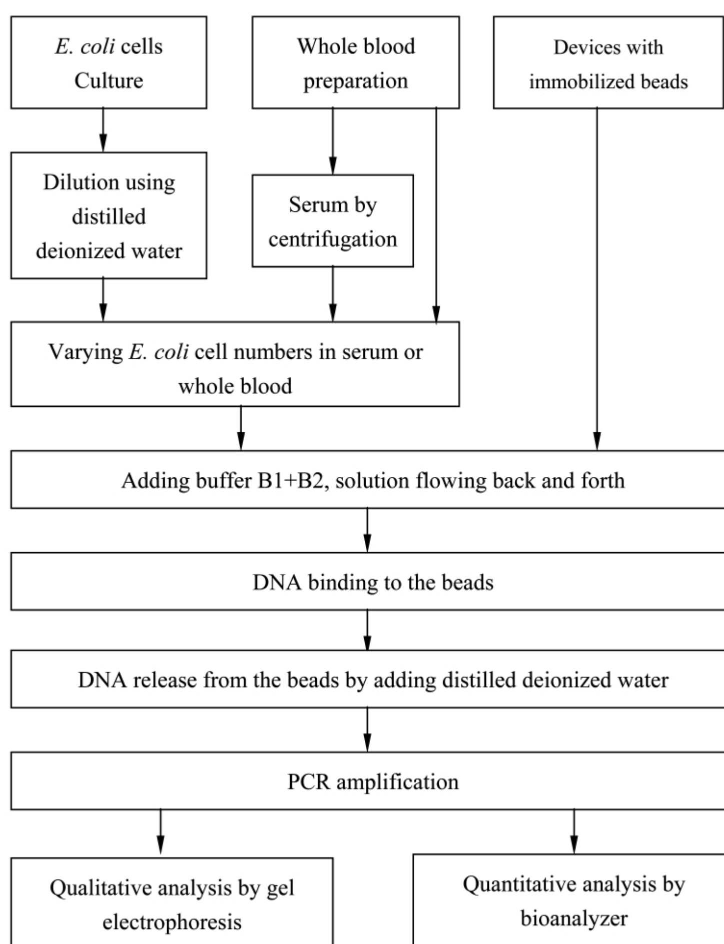

Fig. 2 Outline of DNA extraction process.

extract the DNA, as indicated by the fluorescence band. However, when serum existed in the solution (Lanes 5 and 6), the free beads could hardly extract any DNA because the larger molecules (*e.g.* protein) hindered the collision between the DNA and the beads. Lane 7 indicated the result of extracting the DNA from half (*i.e.* 5×10^4 cells) of the *E. coli* cells as in the Lanes 1 to 6 without serum. The fluorescence intensity was lower than that of Lane 4. The result of DNA extraction under static conditions without serum is indicated in Lane 8. The probability of collision between the DNA and the beads was decreased since there was no flow. Consequently, the fluorescence intensity of Lane 8 was lower than that of Lane 4. Lane 9 was the result of directly adding 0.5 ng (corresponding to 10^5 cells) *E. coli* genomic DNA into the non-bead treated Eppendorf. The fluorescence intensity of Lane 9 was the highest in Fig. 4. Except that the beads were immobilized, the experimental conditions for Lanes 10 to 12 were similar to that of Lanes 4 to 6, respectively. Lanes 10 to 12 indicated the results of devices with immobilized beads and the fluid flowing back and forth. Compared with the extraction using free beads (Lane 4), the fluorescence intensity of Lane 10 was higher than that of Lane 4, because the collision probability was higher. The fluorescence intensities of Lanes 11 and 12 were much higher than those of Lanes 5 and 6, with similar amounts of serum existing in these lanes. It is apparent that the extraction efficiencies using immobilized beads were much higher than that using free beads.

A quantitative analysis of the PCR product weight corresponding to each lane in Fig. 3a is shown in Fig. 3b. Lane 9 was the result of direct addition of *E. coli* genomic DNA into the Eppendorf. PCR product weight in Lane 9 was assigned to be 1.00 since it is the highest and corresponded to the genomic DNA weight of the same *E. coli* cell number, thus being suitable to compare the efficiency of extraction in other lanes. Relative PCR product weights in the various lanes were defined as the percentage of the PCR product weight in Lane 9. With no serum present, the extraction efficiency of the immobilized beads (Lane 10) was 2.1-fold higher than that of using free beads (Lane 4). Further, when serum was added, the average extraction efficiency of immobilized beads was 88-fold higher than that of free beads, as shown by the PCR product weight of Lanes 5 to 6 and Lanes 11 to 12 in Fig. 3b. These results indicated that the extraction efficiency of the immobilized beads was higher than that of the free beads, especially when there was serum in the solution.

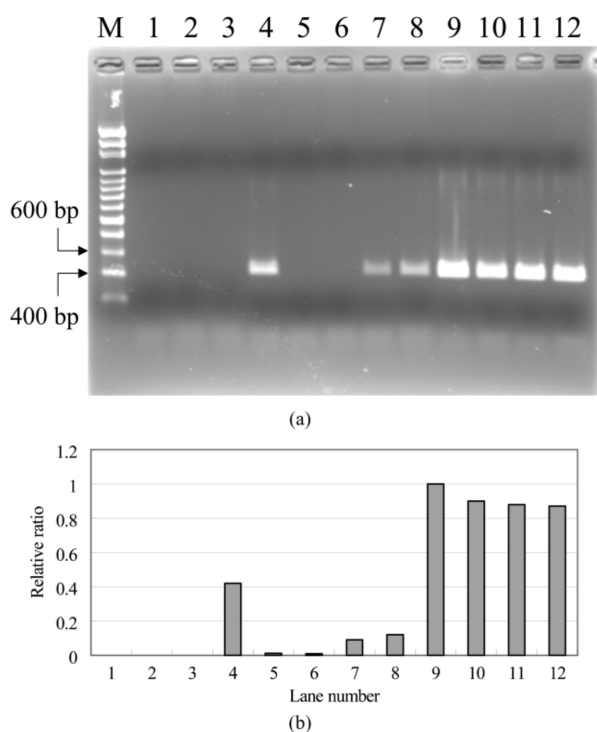

Fig. 3 DNA extraction results using 10^5 *E. coli* cells in 75 µl solution. (a) Agarose gel electrophoresis of extraction experiments using 10^5 *E. coli* cells in 75 µl solution (except Lane 9) after PCR amplification: Lane 1: no beads, no serum; Lane 2: no beads, 25 µl serum; Lane 3: no beads, 50 µl serum; Lane 4: free beads, no serum; Lane 5: free beads, 25 µl serum; Lane 6: free beads, 50 µl serum; Lane 7: free beads, half the number of *E. coli* cells; Lane 8: free beads, static solution and no serum; Lane 9: without beads, vortexed solution and 0.5 ng *E. coli* genomic DNA; Lane 10: immobilized beads, no serum; Lane 11: immobilized beads, 25 µl serum; Lane 12: immobilized beads, 50 µl serum. (b) Comparison of relative quantitative PCR product weight (PCR product weight of different Lane/PCR product weight of Lane 9) at the same conditions as in (a).

3.2 DNA extraction in whole blood

The influence of whole blood on the extraction efficiency of free beads and immobilized beads was tested. 10^8 *E. coli* cells were added into 50 µl of whole blood and extracted using the devices. The experimental conditions are summarized in Table 2. The results of the studies are shown as an electrophoresis fluorescence image in Fig. 4a. The DNA fragment after PCR is 1095 bp. Extraction using free beads showed a bright fluorescence in Lane 1. Lanes 2 to 5 were the results of DNA extraction using bead-coated Eppendorfs. The results of the pre-experimental wash and rinsing before elution (Lanes 2 to 3) corresponded to the negative control conditions. The fluorescence images of first and second elution (Lanes 4 and 5) were bright; indicating that the extraction efficiency using immobilized beads in the bead-coated Eppendorf was qualitatively similar to that of the free beads in the channel of the device (Lane 1). Lanes 6 to 9 were the results of using immobilized beads in the device. The results of Lanes 6 and 7 were similar to those of Lanes 2 and 3, the negative controls. From the fluorescence image, there was no distinguishable difference between the immobilized beads in the micro-channel of the device (Lanes 8 and 9) and immobilized beads in the coated Eppendorf (Lanes 4 and 5). After quantitative analysis in the bioanalyzer, the results are shown in Fig. 4b. The PCR product of Lane 8 was assumed to be 1.00. Comparing the data of Lanes 1 and 8, the extraction efficiency under immobilized bead conditions was only 1.5-fold higher than that under free bead conditions. These results indicated that when the number of *E. coli* cells was 10^8, there was no apparent difference in the extraction efficiency between immobilized beads and free beads. Lanes 10, 11 and 13 corresponded to the negative control, and Lane 14 corresponded to the positive control. Next, the lysis efficiency on boiling the *E. coli* cells was tested since the cell wall and membrane would be

Table 1 Summary of the experimental conditions in Fig. 3

Lane number	1	2	3	4	5	6	7	8	9	10	11	12
Bead condition[a]	No	No	No	Fr	Fr	Fr	Fr	Fr	No	Im	Im	Im
Container[b]	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Epd	Dvc	Dvc	Dvc
Fluid condition[c]	Fl	Fl	Fl	Fl	Fl	Fl	Fl	St	Vrt	Fl	Fl	Fl
E. coli cell number	10^5	10^5	10^5	10^5	10^5	10^5	5×10^4	10^5	0	10^5	10^5	10^5
Weight of *E. coli* genomic DNA/ng	0	0	0	0	0	0	0	0	0.5[d]	0	0	0
Serum volume/µl	0	25	50	0	25	50	0	0	0	0	25	50
Total volume/µl	75	75	75	75	75	75	75	75	75	75	75	75

[a] Fr: Free, Im: Immobilized. [b] Dvc: Device, Epd: Eppendorf. [c] Fl: Flows back and forth, St: Static, Vrt: Vortex. [d] 0.5 ng genomic DNA corresponds to 10^5 cells.

destroyed by heat. The broad band in Lane 12 resulted from the boiling of *E. coli* cells and indicated a lower lysis efficiency as compared to that with the buffer B1+B2.

Further, the effect of *E. coli* cell number on the efficiency of extraction was investigated. The experimental conditions are summarized in Table 3. In Fig. 5a, Lanes 1 and 2 indicate the result of using fresh free beads with 10^5 and 10^7 *E. coli* cells, respectively, in 50 μl of whole blood. It could be seen that the free beads were able to efficiently extract DNA when the number of *E. coli* cells was 10^7, but could hardly extract any DNA when the number of *E. coli* cells was 10^5 in 50 μl of whole blood. Lanes 3 to 5 indicate the results with the bead-coated Eppendorf. The result of rinsing before elution is shown in Lane 3 and it corresponds to the pre-elution test. Lanes 4 and 5 were the results of first and second elution, respectively. It revealed that the bead-coated Eppendorf could efficiently extract DNA when the number of *E. coli* cell was 10^5, and the efficiency in the second elution was larger than that in the first one (quantitative data shown in Fig. 5b). Lanes 6 to 9 were the results of using immobilized beads with the fluid flowing back and forth in the channel of the device. Lanes 6 and 7 were the results of pre-experimental washing and rinsing before elution, respectively. It corresponds to the requirements of the pre-elution test. Lanes 8 and 9 were the results of the first and second elution. It showed that the immobilized beads in the device could extract DNA, and the efficiency of the first elution was higher than that of the second elution. Quantitative analysis and results are shown in Fig. 5b. The PCR product of Lane 8 was assigned to be 1.00. The extraction efficiency of immobilized beads in the device was higher than that of the bead-coated Eppendorf, as shown in Lanes 8 and 9 and Lanes 4 and 5 in Fig. 5b. Quantitative data indicated that the average extraction efficiency of the former was about 1.8-fold higher than that of the latter. Since the number of *E. coli* cells in Lane 2 (10^7) was much larger than that in Lane 8 (10^5), the weight of PCR product in Lane 2 was also higher. The results of Lanes 10 and 11 correspond to the negative control, and the positive control, respectively.

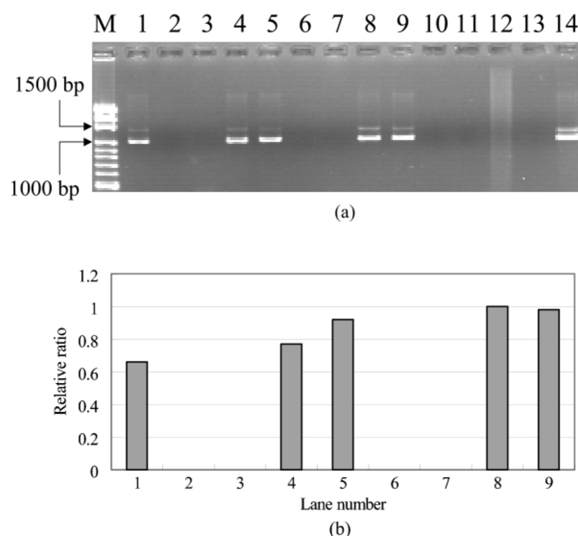

Fig. 4 (a) Agarose gel electrophoresis of the extraction experiments using 10^8 *E. coli* cells (Lanes 1–9, 12), without *E. coli* cell (Lanes 10–11, 13) and 50 ng *E. coli* genomic DNA (Lane 14) in 50 μl of whole blood after PCR amplification: Lane 1: free beads; Lane 2: pre-experimental wash, bead-coated Eppendorf; Lane 3: rinsing before elution, bead-coated Eppendorf; Lane 4: first elution, bead-coated Eppendorf; Lane 5: second elution, bead-coated Eppendorf; Lane 6: pre-experimental wash, immobilized beads; Lane 7: rinsing before elution, immobilized beads; Lane 8: first elution, immobilized beads; Lane 9: second elution, immobilized beads; Lane 10: rinsing before elution, bead-coated Eppendorf, no *E. coli* cell; Lane 11: first elution, bead-coated Eppendorf, no *E. coli* cell; Lane 12: boiling *E. coli* cells for 5 min; Lane 13: distilled deionized water, no *E. coli* cell; Lane 14: 50 ng *E. coli* genomic DNA. (b) Comparison of relative PCR product weight (PCR product weight of different Lane/PCR product weight of Lane 8) under the same conditions as in (a).

3.3 Correlation between extraction efficiency and *E. coli* cell number in the whole blood

The extraction efficiencies of the devices were tested for the lower limit of *E. coli* cell number in the whole blood. Different numbers of *E. coli* cells were added into 25 μl of whole blood. The experimental conditions are summarized in Table 4. As shown in Fig. 6a, Lane 1 indicated extraction using free beads without *E. coli*, and it corresponded to the negative control. Lanes 2 and 4 to 7 indicated extraction using free beads in the device as 10^1, 10^2, 10^3, 10^4 and 10^5 *E. coli* cells were added. It showed that the free beads could efficiently extract DNA when the number of *E. coli* cells was higher than 10^5, but could hardly extract any DNA when the number was smaller than 10^4 in 25 μl of whole blood. Lanes 3 and 8 to 13 indicated the immobilized bead conditions in the device when the numbers of *E. coli* cells were 10^1, 10^2, 10^3, 10^4, 10^5, $2 \times$

Table 2 Summary of the experimental conditions in Fig. 4

Lane number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Bead conditions[a]	Fr	Ct	Ct	Ct	Ct	Im	Im	Im	Im	Ct	Ct	No	No	No
Container[b]	Dvc	Epd	Epd	Epd	Epd	Dvc	Dvc	Dvc	Dvc	Epd	Epd	Epd	Epd	Epd
Fluid conditions[c]	Fl	Vrt	Vrt	Vrt	Vrt	Fl	Fl	Fl	Fl	Vrt	Vrt	Vrt	Vrt	Vrt
Experimental conditions[d]	FE	PW	RE	FE	SE	PW	RE	FE	SE	RE	FE	BL	PW	AD
E. coli cell number	10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	0	0	10^8	0	0
Weight of *E. coli* genomic DNA/ng	0	0	0	0	0	0	0	0	0	0	0	0	0	50[e]
Whole blood volume/μl	50	50	50	50	50	50	50	50	50	50	50	50	50	0

[a] Fr: Free, Ct: Coated, Im: Immobilized. [b] Dvc: Device, Epd: Eppendorf. [c] Fl: Flows back and forth, Vrt: Vortex. [d] PW: Pre-experimental Wash, RE: Rinsing before Elution, BL: Boiling, FE: First Elution, SE: Second Elution, AD: Added Directly. [e] 50 ng genomic DNA corresponds to 10^7 cells.

Table 3 Summary of the experimental conditions in Fig. 5

Lane number	1	2	3	4	5	6	7	8	9	10	11
Bead conditions[a]	Fr	Fr	Ct	Ct	Ct	Im	Im	Im	Im	No	No
Container[b]	Dvc	Dvc	Epd	Epd	Epd	Dvc	Dvc	Dvc	Dvc	Epd	Epd
Fluid conditions[c]	Fl	Fl	Vrt	Vrt	Vrt	Fl	Fl	Fl	Fl	Vrt	Vrt
Experimental conditions[d]	FE	FE	RE	FE	SE	PW	RE	FE	SE	PW	AD
E. coli cell number	10^5	10^7	10^5	10^5	10^5	10^5	10^5	10^5	10^5	0	0
Weight of *E. coli* genomic DNA/ng	0	0	0	0	0	0	0	0	0	0	50[e]
Whole blood volume/μl	50	50	50	50	50	50	50	50	50	50	0

[a] Fr: Free, Ct: Coated, Im: Immobilized. [b] Dvc: Device, Epd: Eppendorf. [c] Fl: Flows back and forth, Vrt: Vortex. [d] PW: Pre-experimental Wash, RE: Rinsing before Elution, FE: First Elution, SE: Second Elution, AD: Added Directly. [e] 50 ng genomic DNA corresponds to 10^7 cells.

10^4 and 2×10^3. It showed that the immobilized beads could efficiently extract DNA when the number of *E. coli* cells was larger than 10^3, but could hardly extract any DNA when the number was lower than 10^2 in 25 μl of whole blood. Lane 14 indicated immobilized bead conditions without *E. coli*, and it corresponded to the negative control. Lanes 15 and 16 were the result of adding 0.05 ng and 5 ng *E. coli* genomic DNA, respectively, and it corresponded to the positive control. After analysis in the bioanalyzer, the results were as shown in Fig. 6b. The PCR product of Lane 11 was assumed to be 1.00. Quantitative analysis indicated that when the number of *E. coli* cells in the devices with immobilized and free beads was in the range 10^4 to 10^6, the extraction efficiency of former was higher (15 to 100-fold), and in the range 10^3 to 10^4, the extraction efficiency of former was much higher (100 to 400-fold). These results showed that extraction using immobilized beads was more effective than that using free beads when the solution flowed back and forth, especially when the number of *E. coli* cells was 10^3 to 10^6 in 25 μl of whole blood.

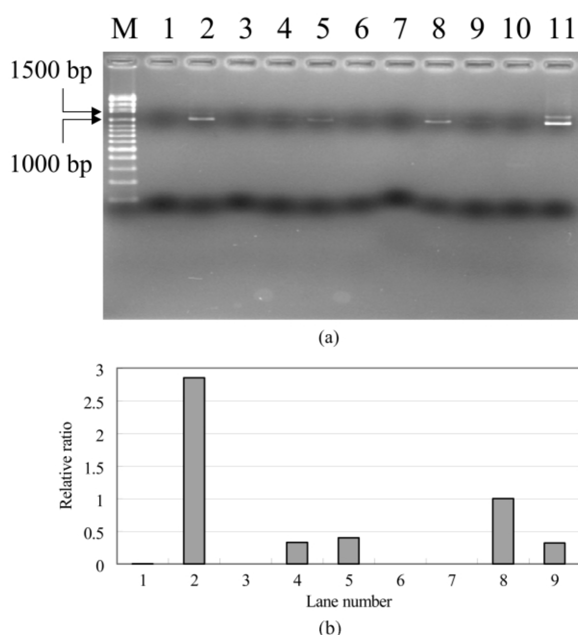

Fig. 5 (a) Agarose gel electrophoresis of the extraction experiments with 10^5 *E. coli* cells (Lanes 1, 3–9), 10^7 *E. coli* cells (Lane 2), without *E. coli* cell (Lane 10) and 50 ng *E. coli* genomic DNA (Lane 11) in 50 μl of whole blood after PCR amplification: Lane 1: free beads; Lane 2: free beads; Lane 3: rinsing before elution, coated Eppendorf; Lane 4: first elution, coated Eppendorf; Lane 5: second elution, coated Eppendorf; Lane 6: pre-experimental wash, immobilized beads; Lane 7: rinsing before elution, immobilized beads; Lane 8: first elution, immobilized beads; Lane 9: second elution, immobilized beads; Lane 10: distilled deionized water, no *E. coli* cell; Lane 11: 50 ng *E. coli* genomic DNA. (b) Comparison of relative PCR product weight (PCR product weight of different Lane/PCR product weight of Lane 8) at the same conditions as in (a).

4 Conclusions

A detailed study of a newly designed microfluidic chip for enhancing DNA extraction was conducted. The extraction efficiency was improved by immobilizing the beads on the channel surface of the device and the solution flowing back and forth. The extraction efficiencies of the immobilized beads and the free beads were tested and compared qualitatively using gel electrophoresis images and quantitatively by PCR product weight. Compared with the free beads, a 2-fold increase in extraction efficiency was obtained using the proposed method when there was no serum. When serum was present in the fluid, the extraction efficiency of immobilized beads was 88-fold higher than that of free beads. Experimental results showed that the immobilized beads play an important role in enhancing DNA extraction.

When the number of *E. coli* cells was 10^6 to 10^8 in 25 μl of whole blood, there was no distinguishable difference in the extraction efficiencies of immobilized and free beads (10^0 to 10^1 fold). When the number of *E. coli* cells was 10^4 to 10^6 in 25 μl of whole blood, the extraction efficiency of immobilized beads was several-fold larger than that of free beads (10^1 to 10^2 fold). When the number of

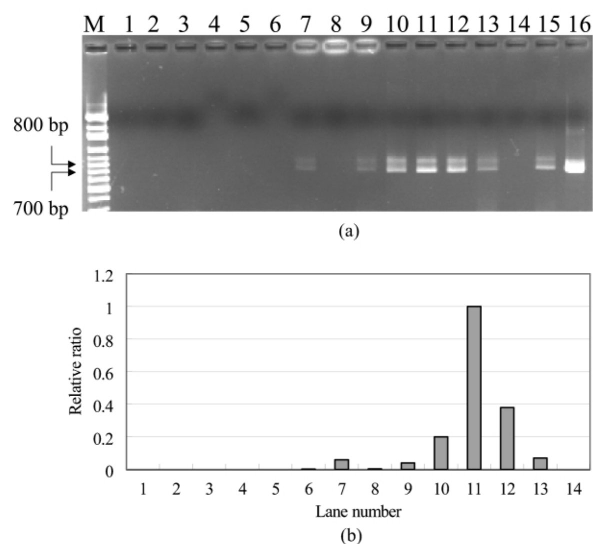

Fig. 6 (a) Agarose gel electrophoresis of the extraction experiments with different *E. coli* cell numbers in 25 μl of whole blood after PCR amplification: Lane 1: free beads, no *E. coli* cell; Lane 2: free beads, 10^1 *E. coli* cells; Lane 3: immobilized beads, 10^1 *E. coli* cells; Lane 4: free beads, 10^2 *E. coli* cells; Lane 5: free beads, 10^3 *E. coli* cells; Lane 6: free beads, 10^4 *E. coli* cells; Lane 7: free beads, 10^5 *E. coli* cells; Lane 8: immobilized beads, 10^2 *E. coli* cells; Lane 9: immobilized beads, 10^3 *E. coli* cells; Lane 10: immobilized beads, 10^4 *E. coli* cells; Lane 11: immobilized beads, 10^5 *E. coli* cells; Lane 12: immobilized beads, 2×10^4 *E. coli* cells; Lane 13: immobilized beads, 2×10^3 *E. coli* cells; Lane 14: immobilized beads, no *E. coli* cell; Lane 15: 0.05 ng *E. coli* genomic DNA; Lane 16: 5 ng *E. coli* genomic DNA. (b) Comparison of relative PCR product weight (PCR product weight of different Lane/PCR product weight of Lane 11) at the same conditions as in (a).

Table 4 Summary of the experimental conditions in Fig. 6

Lane number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bead conditions[a]	Fr	Fr	Im	Fr	Fr	Fr	Fr	Im	Im	Im	Im	Im	Im	Im	No	No
Container[b]	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Dvc	Epd	Epd
Fluid conditions[c]	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Fl	Vrt	Vrt
E. coli cell number	0	10^1	10^1	10^2	10^3	10^4	10^5	10^2	10^3	10^4	10^5	2×10^4	2×10^3	0	0	0
Weight of *E. coli* genomic DNA/ng	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5×10^{-2}[d]	5[d]
Whole blood volume/ μl	25	25	25	25	25	25	25	25	25	25	25	25	25	25	0	0

[a] Fr: Free, Im: Immobilized. [b] Dvc: Device, Epd: Eppendorf. [c] Fl: Flows back and forth, Vrt: Vortex. [d] 5×10^{-2} ng genomic DNA corresponds to 10^4 cells, 5 ng genomic DNA corresponds to 10^6 cells

E. coli cells was 10^3 to 10^4 in 25 μl of whole blood, the extraction efficiency of immobilized beads was much larger than that of free beads (10^2 to 10^3 fold). Based on the results of this investigation, this microfluidic extraction chip could find potential applications in rare sample genomic study.

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