Development of DNA extraction system from pathogenic bacteria using dendrimer modified bacterial magnetic particles for medical diagnostics

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Microfluidic devices using lab-on-a-chip technology have the capability to perform a number of analytical operations on an integrated device. These devices have been applied to genetic analysis, biosensors, cell sorting and medical diagnostics. For development of nucleic acid based medical diagnostic devices, integration of all processes such as cell lysis, DNA extraction and purification, polymerase chain reaction (PCR), detection and analysis of amplified DNA are required. Extraction and purification of nucleic acids from target cells is crucial step for an integrated device since sensitivity of detection depends on recovery ratio and purity of extracted DNA.

Fully automated genetic diagnostic system using bacterial magnetic particles (BMPs) for micro-well plate based assay have been developed, and the efficacy of using magnetic particles in genetic analysis was demonstrated [1-3]. In this study, DNA extraction from *Escherichia coli* and pathogenic bacteria, *Mycobacterium bovis* using amino-dendrimer modified BMPs was investigated for development of a microdevice for genetic diagnostics.

Amino-dendrimer modified BMPs for capturing DNA after cell lysis were synthesized by doubling reaction of amino groups on BMPs using methylacrylate and ethylenediamine after 3-[2-(2-aminoethylamino) ethylamino] propyl-trimethoxysilane (AEEA) coating of BMPs. The number of introduced amino groups on BMPs were $2x10^5$ groups per particle (diameter: 50～100 nm). Prepared dendrimer modified BMPs have high dispersity in water by positive charges of amino groups. Capturing of DNA using dendrimer modified BMPs was investigated using fluorescent intercalater, SYBR Green. The efficiency of capturing DNA was about 1 μg (30 fmol) of λ DNA (48,502 bp) using 10 μg of dendrimer modified BMPs (Fig. 1).

DNA extraction from *E. coli* and *M. bovis* using dendrimer modified BMPs was investigated. Cell lysis was performed in 20 mM Tris-HCl buffer (pH 7.0) containing 5% Triton X-100 and 0.3 mg/ml proteinase K (cell lysis solution) and incubated for 30 min at 50°C. Then, 10 μg of dendrimer modified BMPs was added in cell lysate, and the DNA-magnetic particle conjugates was washed, magnetically separated and finally resuspended in ultra pure water. The recovery ratio of extracted DNA from *M. bovis* estimated using fluorescent intercalater, Pico Green was 86.6 % (Table). Fig. 3 shows PCR amplification of a 184 bp fragment of the gyrase B gene that can be applied for identification of *Mycobacterium* species. It was shown that DNA extraction was successfully conducted using dendrimer modified BMPs.

DNA extraction in polydimethylsiloxane (PDMS) micro-chamber was investigated. Cell lysis was performed in a micro-chamber using cell lysis solution. After recovery of DNA, PCR amplification of *gyrB* fragment was confirmed using DNA captured on dendrimer modified BMPs as the template DNA.

In conclusion, a method for DNA extraction using dendrimer modified BMPs was constructed. This method can be applied to development of microdevices for genetic diagnostics of pathogenic bacteria.

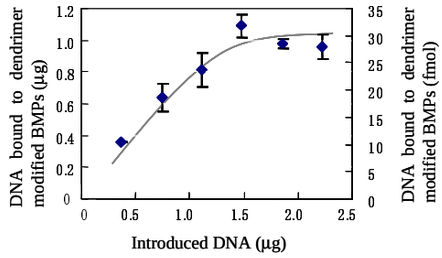

Fig. 1 Amount of DNA bound to 10 μg of dendrimer modified BMPs in 200 μl of 20mM Tris-HCl buffer (pH7.0).
The amount of bound DNA (λDNA:48,502 bp) was determined using SYBR Green.
(Amount of DNA bound to dendrimer modified BMPs) = (Amount of introduced λDNA) – (Amount of DNA in the supernatant; after addition of dendrimer modified BMPs and magnetic separation).

Table The amount of DNA bound to dendrimer modified BMPs after DNA extraction from *M. bovis*.

Amount of DNA in lysate (ng)	Bound DNA using 10 μg of dendrimer modified BMPs (ng)	Recovery ratio (%)
4.38	3.80	86.6

The amount of DNA was determined using Pico Green.
The amount of DNA bound to dendrimer modified BMPs was calculated as follows:
(The amount of DNA bound to dendrimer modified BMPs) =
(The amount of DNA in lysate) – (The amount of DNA in supernatant after addition of dendrimer modified BMPs and magnetic separation).

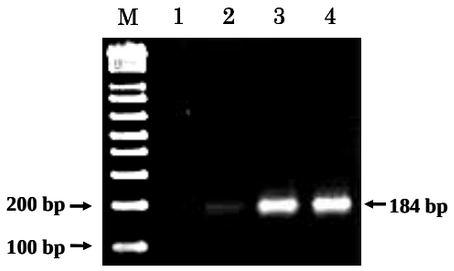

Fig. 2 PCR amplification of extracted DNA using 10 μg of dendrimer modified BMPs from *M. bovis* cells at various dilutions. Cell lysis condition was 5% TritonX-100 containing 0.3 mg/ml proteinase K.

M: 1kb ladder
1: 1/10000 dilution
2: 1/1000 dilution
3: 1/100 dilution
4: 1/10 dilution

References

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