DNA recovery from a single bacterial cell using charge-reversible magnetic nanoparticles

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ABSTRACT

Highly efficient DNA recovery from a single bacterial cell was performed by means of imidazole-modified magnetic nanoparticles (Imi-MNPs). The modification by imidazole was confirmed by Fourier transform infrared spectroscopy. The Imi-MNPs were highly efficient at DNA extraction owing to the charge-reversible properties of Imi-MNPs, whereby DNA is attached to the particles at low pH and eluted at high pH because of electrostatic interactions. The DNA recovery ratio was determined by real-time PCR, and it revealed that complete recovery was guaranteed at $\geq 10^3$ genome copies of *Bacillus subtilis*. Extraction of DNA from single bacterial cells was followed by PCR amplification of 16S rDNA and capillary electrophoresis. We achieved detection of single bacterial cells with a detection rate of 80%. We believe that our DNA recovery strategy may serve as a powerful tool for efficient DNA extraction and should be useful for quality control of cosmetics, foods, and pharmaceutical products.

Key Words

DNA recovery

Magnetic nanoparticles

Imidazole

Single bacterial cell

1. Introduction

Detection of contamination with microorganisms is crucial for a wide range of industries including production of foods, cosmetics, and pharmaceuticals [1-3]. Identification of pathogenic microorganisms is also of great importance in clinical diagnosis. The traditional colony formation test on selective media is time-consuming, labor-intensive, and poorly reproducible. Genetic analysis by PCR may be an alternative technology that enables simple and rapid detection of bacterial DNA. Theoretically, PCR can amplify target fragments from samples containing an extremely low concentration of template DNA (1 to several genomes per milliliter). On the other hand, template DNA extraction and concentration into a small volume of a PCR mixture (10–100 μ L) are still necessary for reliable amplification [4-6].

Because of easy handling, magnetic nanoparticles (MNPs) have been widely used as a solid phase for DNA recovery [7-12]. MNPs can disperse rapidly in a reaction mixture and can then be easily recovered by an external magnetic field. This property allows for the development of fully automated systems [4, 13-16]. A number of studies have shown successful DNA isolation using silica-coated [7] or amine-modified [11] MNPs, which are responsible for DNA adsorption. These studies were focused only on the purification of no less than several micrograms of DNA (corresponding to approximately 10^9 bacterial genomes). The failure of DNA recovery at the level of $1-10^3$ genomes was ignored, but industrial detection of DNA deals with this range of small amounts of DNA [17]. Therefore, it would be a serious problem if such tiny amounts of DNA could not be captured by MNPs, or if the adsorbed DNA could not be eluted from MNPs after magnetic concentration. Rational design of MNPs for this purpose is still at an early stage, and its development has just begun [4, 6].

In our previous study, we attempted to develop a method of DNA recovery from a single bacterial cell using polyamidoamine dendron-functionalized MNPs (PAMAM-MNPs) [5]. This PAMAM-MNP-based DNA extraction system was demonstrated to be highly effective at recovering a large amount of bacterial DNA on a microgram scale [18-20]. This success may be due to the efficient DNA adsorption by hierarchically assembled amine groups on the MNPs as well as the efficient DNA release through the phosphate replacement after addition of excess deoxyribonucleotides (dNTP) [19]. Nonetheless, the DNA

1 recovery ratio for a small amount of DNA (picogram levels: $\sim 10^3$ bacterial genomes) is significantly
2 lower: <50%. Although this recovery efficiency was still better than that of commercially available
3 magnetic particles, the subsequent PCR with a single bacterial genome was successful twice per 7 trials
4 [5]. That study also revealed that the DNA desorption process is more problematic than DNA adsorption
5 because of the strong interaction between DNA and PAMAM-MNPs, which lowers the recovery
6 efficiency for small amounts of DNA.

7 To overcome this problem, we fabricated novel MNPs modified with imidazole moieties (Imi-
8 MNPs). The surface charge of the Imi-MNPs can be zero at neutral pH; therefore, it would be easy to
9 convert the surface charge from positive to negative by changing pH. Positively charged Imi-MNPs can
10 electrostatically adsorb DNA, whereas negatively charged ones are expected to release DNA due to the
11 electrostatic repulsion. The pH-responsive charge inversion of solid-phase surfaces has been already
12 utilized for isolation of biomolecules including DNA [9, 21-24] and proteins [25, 26], but to the best of
13 our knowledge, there are no such studies dealing with small amounts of DNA, at the level of a single
14 bacterial genome. In this study, we analyzed the phenomena of DNA absorption and desorption on the
15 Imi-MNPs by changing pH of the medium. The DNA recovery ratio was evaluated when a small amount
16 of DNA ($\geq 10^3$ bacterial genomes) was extracted, and the performance of Imi-MNPs was compared with
17 that of PAMAM-MNPs. Furthermore, DNA recovery from a sample containing a single bacterial genome
18 and subsequent successful PCR detection were demonstrated on *Bacillus subtilis* as a model bacterium.

20 2. Experimental Section

21 2.1. Preparation of Imi-MNPs

22 Magnetic nanoparticles without surface modifications (MNPs, 10 mg, Ferrotec Corporation, Tokyo,
23 Japan) were treated with $\text{H}_2\text{O}:\text{H}_2\text{O}_2:\text{NH}_4\text{OH}$ (5:1:1, volume ratio) at room temperature for 10 min to
24 activate the surface hydroxyl groups. The resulting MNPs were collected using a columnar neodymium-
25 boron (Nd-B) magnet, and incubated with 20 mL of a 2% solution of (3-aminopropyl)triethoxysilane

(APTES) in ethanol at room temperature for 20 min. After a wash with dimethylformamide (DMF) by sonication, APTES-modified MNPs (APTES-MNPs) were incubated at 120°C in 20 mL of DMF for 30 min with sonication every 5 min. After that, the particles were washed with methanol 3 times. In a new tube, 4-imidazolecarboxylic acids (20 mM) were reacted with *N*-hydroxysuccinimide (NHS, 20 mM) and *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide (EDC, 20 mM) in 6 mL of 10 mM 2-(*N*-morpholino)ethanesulfonate (MES) buffer (pH 5) at room temperature for 15 min. Then, the reaction mixture was diluted with 14 mL of phosphate-buffered saline (PBS, pH 7.4) and mixed with the APTES-MNPs (9 mg). The particle suspension was incubated for 3 h at room temperature with sonication every 15 min. After 3 washes with methanol, the resulting MNPs (Imi-MNPs) were stored in methanol at 4°C. The hydrodynamic size and zeta potential of Imi-MNPs were analyzed on a laser particle analyzer (ELS-8000, Otsuka Electronics, Osaka, Japan) at various pH levels (10 mM MES buffer, pH 5–7; 10 mM Tris-HCl buffer, pH 8; or 10 mM carbonate buffer, pH 9 or 10). Transmission electron microscopy (TEM) was performed by means of a JEOL JEM1400 microscope at an acceleration voltage of 100 kV. For comparison, PAMAM-MNPs were prepared as described elsewhere [5].

2.2. Fourier transform infrared (FT-IR) spectroscopy

A fine powder of potassium bromide (KBr) was prepared using a pestle and mortar made of agate and dried at 150°C for 12 h. MNPs and Imi-MNPs were freeze-dried in a lyophilizer (Labconco Co., FreeZone 6 Plus, Kansas city, MO, USA) overnight. KBr (200 mg) and the dried particles were mixed by means of the pestle and mortar made of agate and molded into pellets under pressure of 54.9 MPa for 10 min with a hand pump (Shimadzu Co., Kyoto, Japan). The resulting pellets were then subjected to FT-IR spectroscopy on a FT-IR 420 instrument (Shimadzu Co., Kyoto, Japan).

2.3. DNA recovery analysis with purified DNA

Prior to the trials of direct recovery of DNA from single bacterial cells, performance of Imi-MNPs was evaluated by means of already purified DNA. Genomic DNA was purified from *B. subtilis*

ATCC6051 by treatment of the bacterial cells with 1 mg/mL lysozyme (50°C for 5 min) and 0.4 mg/mL proteinase K (65°C for 5 min). The resulting samples were subjected to cetyltrimethylammonium bromide (CTAB) treatment and phenol chloroform extraction to remove polysaccharides and proteins, followed by ethanol precipitation for DNA isolation. The DNA concentration was measured with a DNA intercalator, PicoGreen (Life Technologies, Tokyo, Japan), and the fluorescence intensity was quantified on a fluorescence microplate reader (FLUO Star Galaxy, BMG Labtechnologies Inc., Offernburg, Germany; excitation at 502 nm, emission at 523 nm) as described previously [19]. Calibration curves for the DNA quantification were built with λ DNA as a standard in appropriate buffers. We calculated the copy number of *B. subtilis* genomes assuming that a single genome is composed of 4,215,610 bp (i.e., 2.17×10^{14} genomes/g, NCBI reference sequence: NC_020507.1).

To explore the adsorption behavior of DNA, Imi-MNPs (10 μ g) were mixed with λ DNA (1 μ g) in 10 mM MES buffer (50 μ L, pH 5 or 7) or 10 mM carbonate buffer (50 μ L, pH 10) and incubated for 10 min at room temperature. The DNA-MNP complexes were magnetically separated. The supernatant (~50 μ L) was collected, and then 1 μ L of each supernatant was subjected to quantitative PCR (qPCR) as described below. The amount of the adsorbed DNA was estimated by subtracting the DNA quantity remaining in the supernatant from that initially added.

To analyze the DNA desorption, λ DNA (1 μ g) or bacterial genomic DNA (10^3 – 10^6 genomes of *B. subtilis*; ATCC6051) was adsorbed on the Imi-MNPs at pH 5 as described above. The DNA-MNP complexes were resuspended in the buffers with various pH levels (pH 7–10) at 80°C or with pH 8 at various temperatures (27–80°C) and incubated for 20 min. After magnetic separation, the DNA that was released from Imi-MNPs was quantified by qPCR. The DNA recovery was calculated using Eq. 1:

$$\text{DNA recovery (\%)} = [\text{DNA amount desorbed from Imi-MNPs}] \div [\text{DNA amount introduced}] \times 100 \quad (\text{Eq. 1})$$

2.4 Quantitative PCR

During this procedure, 1 μ L of a DNA template was mixed with 10 μ L of the Fast SYBR Green Master Mix (2 $\times$, Life Technologies, Tokyo, Japan), 0.4 μ L of each primer, and 7.6 μ L of nuclease-free

water. The primer pairs for quantification of λ DNA (forward primer: 5'-TTT ATG CGT TTT CAT GTT GC-3', reverse primer: 5'-AGG CAT TAT GCT CGC AGA-3') and *B. subtilis* genomic DNA (forward primer: 5'- CTT AGT TGC CAG CAT TCA GTT G-3', reverse primer: 5'- ATA AGG GGC ATG ATG ATT TGA C-3') target the gene of terminase large subunit A and 16S rRNA gene, respectively. PCR was conducted under the following cycling conditions: the initial denaturation step (95°C for 20 s), followed by 40 cycles of denaturation (95°C for 1 s) and annealing (59°C for 20 s) on a ViiA™ 7 Real-Time PCR System (Life Technologies, Tokyo, Japan).

2.5. Direct recovery of DNA from samples containing a single bacterial cell

B. subtilis ATCC6051 was cultured in lysogeny broth (LB) at 37°C overnight. After bacterial cell counting on a hemocytometer, bacterial cell suspensions were prepared by serial dilution to 1–10³ cells per 50 μ L. For bacterial cell lysis, 4 μ L of lysis buffer (7.5 mg/mL lysozyme and 25% [v/v] Triton X-100 in 10 mM Tris-HCl, pH 5) was mixed with 50 μ L of each cell suspension, and the reaction mixture was incubated for 5 min at 50°C. Subsequently, 7 μ L of 20 mg/mL proteinase K was added, with subsequent incubation at 65°C for 5 min, followed by 98°C for 10 min. The resulting lysate was mixed with Imi-MNPs (10 μ g) and incubated for 10 min at room temperature. Imi-MNPs were then magnetically collected and washed with 10 mM MES buffer (pH 7) 3 times. Imi-MNPs were then resuspended in the DNA desorption solution (10 μ L of 10 mM carbonate buffer, pH 10) and incubated at 80°C for 20 min.

In order to confirm the success of direct DNA extraction from the bacterial cells, the whole supernatant was transferred to a new PCR tube containing the PCR mixture (1 $\times$ PrimeStar® Max DNA polymerase, 0.3 μ M forward primer [5'-CCC TTA TGA CCT GGG CTA C-3'] and reverse primer [5'-TGT GGG ATT GGC TTA ACC TC-3'] targeting the 97-bp fragment of 16S rDNA, final volume 50 μ L) after magnetic separation of Imi-MNPs. PCR was conducted under the following cycling conditions: the initial denaturation step (98°C for 2.5 min), followed by 35 cycles of denaturation (98°C for 10 s), annealing (59°C for 5 s), and extension (72°C for 5 s), and then final extension at 72°C for 5 min. The

PCR products were analyzed by capillary electrophoresis (2100 Bioanalyzer, Agilent Technologies, CA, USA).

3. Results and Discussion

3.1. Characterization of surface properties of the Imi-MNPs

Imidazole modification of the MNPs was confirmed by FT-IR spectroscopy. Before the modification, FT-IR signals at 627 and 584 cm^{-1} were observed (Fig. 1A): they correspond to the Fe–O bond of bulk magnetite [27]. In contrast to the unmodified MNPs, enhancement of the FT-IR signals in the region 1150–950 cm^{-1} was observed after the modification (Fig. 1B). These signals could correspond to the SiO–H and Si–O–Si stretching [27], and this was likely to be caused by the silane-coupling reaction. Furthermore, the modification by imidazole yielded 2 new peaks: at 1654 and 1647 cm^{-1} (Fig. 1B, inset), which could be assigned to amide carbonyl stretching [28] derived from the cross-linking of APTES-MNPs to 4-imidazolecarboxylic acids. The peaks near 3000 cm^{-1} also appeared after modification by imidazole, and 4-imidazolecarboxylic acid also has peaks in a similar region (Fig. S1). These data support successful immobilization of imidazole moieties on the MNPs. TEM analysis revealed that Imi-MNPs were polydisperse in size (14.7 ± 3.1 nm, mean $\pm$ SD, $n = 20$; Fig. S2). Imi-MNPs aggregation was examined by means of TEM and dynamic light scattering analysis (Fig. S3).

Electrostatic interactions are believed to be a major driving force behind DNA adsorption onto amine-modified MNPs [5, 18-20]. On the other hand, too stable electrostatic bonds can hamper efficient DNA desorption from the MNP surface, thus causing low recovery of DNA [5, 11, 29]. Imi-MNPs were designed to resolve this dilemma by reversing the surface charge in response to pH in the medium. Protonated imidazole on an MNP can interact with DNA via electrostatic forces at low pH, and deprotonation of imidazole can attenuate the electrostatic interaction, thereby facilitating the elution of DNA. To test whether Imi-MNPs can reverse the surface charge as expected, zeta potentials of Imi-MNPs

were measured at various pH levels (from 5 to 10; Fig. 2). Imi-MNPs are positively charged between pH 5 and 7. This charge is inverted from positive to negative (or vice versa) between pH 7 and 8 and becomes more negative from pH 8 to 10. Variation of zeta potentials depending on pH is determined by the protonation/deprotonation balance of functional moieties on MNPs (e.g., the hydroxyl group from magnetite, the primary amine from APTES, and imidazole groups). Magnetite is an amphoteric solid; Fe-OH can be protonated (Fe-OH_2^+) or deprotonated (Fe-O^-), and the point of zero charge is pH 7.3 [30]. In contrast, a primary amine and imidazole can only be protonated, and the acid dissociation constant (pK_a) is approximately 7.0 and 9.3, respectively [31, 32]. Therefore, the positive charge of Imi-MNPs at $\text{pH} < 7$ could be attributable to protonation of all 3 moieties. In contrast, the negative charge at $\text{pH} > 8$ could be attributable to deprotonation of hydroxyl groups on the magnetite surface.

3.2. DNA adsorption and desorption characteristics of Imi-MNPs

DNA adsorption from liquid samples onto the surface of a solid phase is the initial step of our DNA isolation procedure. Because surface properties of Imi-MNPs drastically vary depending on the pH of the medium, the nature of DNA adsorption should be analyzed under different conditions. Here, we quantified the DNA adsorbed onto Imi-MNPs at pH 5, 7, or 10, where Imi-MNPs have a highly positive charge (28.1 ± 0.7 mV), slightly positive (3.6 ± 3.4 mV), or a strongly negative charge (-28.0 ± 0.6 mV), respectively. When an excess amount (1 μg) of λDNA was added to the Imi-MNPs suspension at room temperature, the amounts of λDNA adsorbed on 1 mg of Imi-MNPs were 25.3 ± 2.8 μg at pH 5, 19.7 ± 1.0 μg at pH 7, and 5.0 ± 1.9 μg at pH 10. The maximal adsorption value at pH 5 (Fig. S4) is of the same order of magnitude as the DNA adsorption capacity of PAMAM-MNPs (56.6 μg of λDNA per milligram of PAMAM-MNPs) [18]. The hydrodynamic size of Imi-MNPs increased when DNA was added (Fig. S3). Aggregation of Imi-MNPs may be induced by DNA adsorption. Unexpectedly, the DNA adsorption capacity at pH 7 remained at 78% of that at pH 5 although Imi-MNPs almost lost the surface

charge at pH 7. Furthermore, Imi-MNPs at pH 10 still interacted with negatively charged DNA at room temperature. Although there is no doubt that electrostatic interactions play a major role in the DNA adsorption, the above results suggest that electrostatic interactions are not the only force causing the DNA binding. Other interactions including π - π stacking between imidazole and DNA nucleobases [33] as well as the dehydration effect [34] may promote the bridging of Imi-MNPs and DNA at high pH levels. In further experiments, we used pH 5 for DNA adsorption.

DNA desorption from Imi-MNPs is the next step to be analyzed, and this step tends to be the bottleneck of a DNA recovery process [5]. Imi-MNPs binding the maximal amount of λ DNA were incubated at pH 7–10 for 20 min. In this experiment, we used 80°C as an incubation temperature according to our previous study [5]. As a result, complete desorption of DNA from Imi-MNPs was observed only at pH 10, indicating that the negatively charged surface of Imi-MNPs enabled efficient DNA release as expected (Fig. 3[A]). At pH 9, DNA desorption drastically decreased although Imi-MNPs still remained negatively charged (-17.1 ± 1.4 mV). Additionally, a heating effect on DNA desorption was examined. The λ DNA-MNP complexes were incubated at pH 8 at various temperatures (27–80°C) for 20 min. We found that incubation at 80°C was necessary for complete desorption (Fig. 3[B]). These results are consistent with the assumption mentioned above: aside from electrostatic interactions, other binding forces may contribute to the DNA adsorption. The electrostatic repulsive force at pH 9 may not be sufficient, and at pH 10, heating at 80°C is required to overcome those interactions.

Next, we compared the DNA isolation performance of Imi-MNPs and PAMAM-MNPs using bacterial genomic DNA. When we used 10^3 to 10^6 copies of genomic DNA purified from *B. subtilis* (approximately 5 pg to 5 ng of bacterial DNA dissolved in 50 μ L of MES buffer: 100 pg to 100 ng per milliliter), PAMAM-MNPs showed decrease in DNA recovery with the decreasing amounts of added DNA, as we reported previously [5]. In contrast, Imi-MNPs did not show a significant decrease in DNA recovery with the decreasing amounts of added DNA, down to 10^3 genome copies (Fig. 4). This

experiment clearly showed an advantage of Imi-MNPs over PAMAM-MNPs in terms of recovery of trace amounts of DNA and prompted us to attempt DNA extraction from a single bacterial cell using Imi-MNP.

N-methylimidazolium-modified magnetic nanoparticles (MIm-MNPs) have also been used for DNA extraction [12]. For MIm-MNPs, the maximum capacity of DNA adsorption is 25 µg/mg of MNPs, comparable with that of Imi-MNPs [12]. In contrast, the DNA recovery ratio is 79.7%, which is lower than that of Imi-MNPs [12]. DNA desorption from Imi-MNPs is performed by reversing the surface charge of the particles, while desorption from MIm-MNPs is performed by phosphate ion replacement. This comparison points to advantages of the charge-reversible strategy for efficient DNA recovery.

3.3. Evaluation of DNA extraction from a single bacterial cell using Imi-MNPs

Because of the efficient DNA recovery, we applied the Imi-MNPs to DNA extraction from a single cell of *B. subtilis*, and the efficiency was evaluated by PCR-based detection where the 16S rDNA gene was used as an amplification target [5]. *B. subtilis* was selected as a model microorganism because *Bacillus* species can be frequently detected in industrial settings [17, 35, 36]. The bacterial samples were prepared by serial dilution (1, 5, 10, 10², and 10³ cells per 50 µL), with subsequent cell lysis. The resulting whole-cell lysate was reacted with Imi-MNPs at pH 5, and then magnetic separation was performed, followed by elution at pH 10. DNA extraction from the cells was confirmed by amplification of 16S rDNA (10 copies are present in the *B. subtilis* genome). The amplified DNA fragment was analyzed by capillary electrophoresis. The signals increased with the increasing cell number, as expected (Fig. S5[A]). The signals in the electrophoretic experiments were calculated from the values of band intensity (Fig. S5[B]). We determined that the samples with/without the cells can be distinguishable when the signals from the samples containing the cells are greater than the average signal + 3-fold standard deviations from a sample without the cells (background signals). We repeated the experiments on different days and collected the data from 10 single-cell samples. As a result, 8 of 10 experiments

1 successfully distinguished the single-cell samples from the blank samples (Table 1). This detection rate
2 was better than that in our previous study involving PAMAM-MNPs [5].

3 The reason why DNA extraction failed in 2 of the 10 experiments may be the following: the dilution-
4 based preparation of samples containing a single bacterial cell is imprecise, and thus it is impossible to
5 eliminate the samples without a cell [37]. To test this hypothesis, we analyzed our dilution method by
6 qPCR. *B. subtilis* genomic DNA was diluted to the concentration of 0.4 genomes/ μL as in the above
7 experiments. Subsequently, 1 μL of the DNA samples was transferred to 50 tubes; according to a simple
8 calculation, 20 of 50 tubes were estimated to include 1 genome. Then qPCR was carried out to confirm
9 how many tubes contained DNA. It should be noted that qPCR can detect a single bacterial genome when
10 sample volume is small enough [5]. As a result, 15 samples were found to contain DNA. When the same
11 experiment was performed without the DNA template, no significant qPCR signals were detected.
12 Because 20 samples are expected to contain DNA, this finding indicates that 25% of the samples tend to
13 lack DNA after dilution by our method. These data are consistent with the DNA extraction ratio of Imi-
14 MNPs and support the hypothesis that the dilution step is the main cause of the failure of the detection.
15 Given this complexity of the sample preparation, our DNA extraction method with the detection rate of
16 80% (8/10) could serve as a reliable protocol for detection of a single bacterial cell. The improvement of
17 the detection rate is attributable to the better DNA desorption from Imi-MNPs as compared to PAMAM-
18 MNPs. To the best of our knowledge, this result is the highest detection rate of stand-alone cells [4, 6].

20 4. Conclusion

21 We demonstrated DNA recovery from a single bacterial cell using imidazole-modified MNPs.
22 Reversal of the surface charge when environmental pH changed was also confirmed. Via the reversal of
23 the surface charge, DNA adsorption/desorption onto/from nanoparticles can be controlled efficiently.
24 DNA recovery by Imi-MNPs at the level of 10^3 bacterial genomes was more efficient than that by
25 previously developed amine dendrimer-modified MNPs. Furthermore, the combination of DNA recovery

1 by Imi-MNPs, 16S rDNA amplification by PCR, and capillary electrophoresis allowed us to detect single
2 bacterial cells at a high detection rate. These results indicate that the DNA recovery using Imi-MNPs is a
3 promising method for highly sensitive detection of bacterial contamination. We believe that this approach
4 can be useful in a wide range of industries for identification of contaminating microorganisms.

5

Figure legends

Fig. 1. Fourier transform infrared (FT-IR) spectra of unmodified magnetic nanoparticles (MNPs) (A) and imidazole-modified magnetic nanoparticles (Imi-MNPs) (B). Insets: magnified spectra of the box; the peaks that are indicated with arrows in (B) are newly generated signals: at 1654 and 1647 cm^{-1} .

Fig. 2. Zeta potentials of imidazole-modified magnetic nanoparticles (Imi-MNPs) at different pH levels. Imi-MNPs (10–15 $\mu\text{g/mL}$) were resuspended in 10 mM 2-(N-morpholino)ethanesulfonate (MES) buffer (pH 5–7), 10 mM Tris-HCl buffer (pH 8), or 10 mM carbonate buffer (pH 9 or 10). Error bars indicate standard deviation ($n = 3$).

Fig. 3. DNA desorption from imidazole-modified magnetic nanoparticles (Imi-MNPs) as a function of pH or temperature. Imi-MNPs binding the maximal amount of λ DNA were subjected to a variety of desorption conditions. DNA recovery is the ratio of desorbed DNA to introduced DNA. (A) DNA recovery at different pH levels after desorption at 80°C. The experiments were performed in 10 mM 2-(N-morpholino)ethanesulfonate (MES) buffer (pH 7), 10 mM Tris-HCl buffer (pH 8), or 10 mM carbonate buffer (pH 9 or 10). Error bars indicate standard deviation ($n = 3$). (B) DNA recovery at different temperatures during the desorption process at pH 10. Error bars indicate standard deviation ($n = 3$).

Fig. 4. Comparison of DNA recovery by imidazole-modified magnetic nanoparticles (Imi-MNPs; gray bars) and polyamidoamine dendron-functionalized MNPs (PAMAM-MNPs; white bars). Various numbers of *Bacillus subtilis* genome copies were mixed with the nanoparticles for adsorption. At 10^6 genomes, unrecovered DNA was undetectable with both types of nanoparticles; thus, DNA recovery in this condition was 100%, and recovery under other conditions is indicated as a ratio. For DNA desorption, Imi-MNPs were incubated at pH 10 and 80°C, whereas PAMAM-MNPs were incubated with 50 mM deoxyribonucleotides in a 2% solution of dimethyl sulfoxide. Error bars indicate standard deviation ($n = 3$).

1

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Table 1. Repeated trials of the PCR-based detection of *Bacillus subtilis* using imidazole-modified

3

magnetic nanoparticles (Imi-MNPs) for DNA extraction

Number of cells	1	5	10	10 ²	10 ³
Detection rate (positive/trial)	8/10	8/10	5/5	4/4	4/4

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Figure 1

Figure 2

Figure 3

Figure 4

Graphical Abstract