



Evaluation of Genomic DNA Extraction Using Monolayer and Bilayer Magnetic Nanoparticles

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Abstract

Background: There are different methods for genomic DNA extraction. Magnetic nanoparticles (MNPs) exhibit many important features making them suitable for DNA extraction.

Objectives: The aim of this study was to compare the effect of monolayer and bilayer MNPs on genomic DNA extraction.

Materials and Methods: The genomic DNA was extracted from the *Staphylococcus aureus* strain ATCC 25923 and clinical isolates using monolayer MNPs SiO₂ and Fe₃O₄ and bilayer MNPs SiO₂/Fe₃O₄. Then, the quality and quantity of the obtained genomic DNA were compared with both NPs.

Results: The obtained results showed the concentration and purity of the extracted genomic DNA using bilayer magnetic NPs was significantly higher in comparison to the extracted genomic DNA by monolayer MNPs.

Conclusion: In general, surface-coated MNPs are much more efficient than naked MNPs for genomic DNA extraction.

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Background

Magnetic nanoparticles (MNPs) with magnetic properties are found to have many applications in nanomedicine including therapeutic and diagnostic uses.¹

In terms of diagnosis, these NPs can be used both for in vitro and in vivo applications such as in immobilization and detection of biomolecules, cell separation, purification, and gene transfer.²⁻⁵ In addition, MNPs exhibit many important features such as a high specific surface area, chemical stability, low intraparticle, a diffusion rate, high loading capacity, and superparamagnetism.¹ Further, they have been widely used as a solid phase for DNA recovery.⁶ After the surface modification of MNPs due to positively charged ligands, negatively charged nucleic acids are absorbed on the surface of MNPs by electrostatic interactions.¹ In recent years, magnetic iron oxide NPs have received much attention for their interesting properties and applications in organic synthesis, biotechnology, and medicine. For instance, silica MNPs are used as drug carriers due to their stability and low toxicity.^{7,8} Furthermore, these MNPs are applied for DNA

extraction because of their ease of operation, simplicity, and affordability.⁹ DNA extraction is a major step in molecular studies on pathogenic bacteria.¹⁰ *Staphylococcus aureus* is one of the most important pathogens that cause extremely important infections.¹¹ In recent years, the important role of *S. aureus* in causing nosocomial and community infections has led to increased molecular research on this bacterium.¹² Accordingly, this study compared the extracted genomic DNA of *S. aureus* by SiO₂ and Fe₃O₄ as monolayer MNPs, as well as SiO₂/Fe₃O₄ as bilayer MNPs.

Materials and Methods

Monolayer MNPs SiO₂ and Fe₃O₄ and bilayer MNPs SiO₂/Fe₃O₄ were prepared from Kashan University of Medical Sciences. The standard strain of *S. aureus* ATCC 25923 was also obtained from the *Pasteur Institute of Iran*. Moreover, 5 clinical samples of *S. aureus* were obtained from the microbiology laboratory of Kashan University of Medical Sciences. Then, standard biochemical tests including catalase, coagulase, mannitol fermentation,

and DNase assay were used to confirm the diagnosis of *S. aureus* isolates.

Extraction of Genomic DNA by MNPs

To extract genomic DNA, the *S. aureus* strain ATCC 25923 and the clinical isolates of this bacterium were cultured on the Luria-Bertani agar medium (Merck, Germany). After 24 hours, pure *S. aureus* colonies were transferred to the Tryptic Soy broth medium (Merck, Germany), placed in a shaking incubator for 24 hours, and then centrifuged at 10 000 rpm for 5 minutes. After adding 500 μ L suspension buffer and 200 μ L of the lysis buffer, and 1 hour of incubation at 37°C, proteinase K was added at a concentration of 10 mg/mL and incubated at 55°C for 1 hour. Additionally, 200 μ L binding buffer (i.e., NaCl 1M, polyethylene glycol 60%, distilled water, and 0.8 mg MNPs) was added to the supernatant after centrifuging at 10,000 rpm for 10 minutes. Next, the magnetic rack was used to separate the particles for 30 minutes and washed with 200 μ L ethanol 70%. The pellet was dried at room temperature, then 50 μ L of the TE buffer was added and incubated at 55°C for 10 minutes. Finally, MNPs were separated with the magnetic rack, and the supernatant containing the DNA was transferred to a new microtube.

Analysis of Extracted DNA

The NanoDrop spectrophotometer 2000 (Thermo Fisher Scientific, the USA) was used to evaluate the concentration and purity of the extracted genomic DNA, and a 260/280 ratio of \sim 1.7 was considered as pure.

In addition, the extracted DNA by SiO₂/Fe₃O₄ was analyzed using the polymerase chain reaction (PCR) assay and electrophoresis on 1% agarose gel. Next, the PCR was performed in the final volume of 50 μ L using primers (Table 1).¹³ The thermal conditions were the initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 15 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes.

Results

The concentration and purity of the extracted genomic DNA of the *S. aureus* strain ATCC 25923 using monolayer MNPs SiO₂ and Fe₃O₄ and bilayer MNPs SiO₂/Fe₃O₄ were measured by the NanoDrop spectrophotometer and the results showed that all were acceptable in purity. However, the obtained DNA with bilayer MNPs had the highest concentration (Table 2). Based on the results, the extracted genomic DNA of the clinical samples of

S. aureus had acceptable purity although the obtained DNA by SiO₂ showed less purity in some isolates. In addition, DNA concentrations were lower compared to the standard strain (Table 3). Figure 1 shows the results of the PCR amplification of the *spa* gene using specific primers and the extracted DNA by SiO₂, Fe₃O₄, and SiO₂/Fe₃O₄ among the *S. aureus* strain ATCC 25923 and the clinical isolates of *S. aureus*.

Discussion

The extraction of genomic DNA with high quality is a critical step for molecular studies.¹⁰ In this regard, different methods can be performed, including phenol-chloroform and DNA extraction kits. However, more appropriate methods are needed due to the use of toxic substances, especially phenol and the high cost of commercial kits. In the current study, monolayer (SiO₂ and Fe₃O₄) and bilayer (SiO₂/Fe₃O₄) MNPs were used to extract the genomic DNA of *S. aureus* isolates.

In their study, Chao et al isolated genomic DNA from four sources (i.e., the bacterium, human blood, yeast, and virus) using MNPs and DNA extraction kits and then

Table 2. DNA Concentration and Absorbance Ratios of 260/280 nm for the *Staphylococcus aureus* Strain ATCC 25923 Using NanoDrop

Magnetic Nanoparticles	DNA Concentration (ng/mL)	260	280	260/280
SiO ₂	77.2	1.4	0.8	1.8
Fe ₃ O ₄	83.6	2.0	1.0	2.0
SiO ₂ /Fe ₃ O ₄	131.6	2.6	1.5	1.7

Note. SiO₂: Silicon dioxide; Fe₃O₄: Iron oxide.

Table 3. DNA Concentration and Absorbance Ratios of 260/280 nm for the Clinical Isolates of *Staphylococcus aureus* Using NanoDrop

MNPs*	Clinical Isolates	DNA Concentration (ng/mL)	260	280	260/280
SiO ₂	1	50.6	1.3	0.7	1.9
	2	77.2	1.4	0.8	1.8
	3	62.1	1.3	0.7	1.9
	4	48.9	1.2	0.7	1.7
	5	58.7	1.5	0.8	1.9
Fe ₃ O ₄	1	73.4	1.4	0.7	1.8
	2	82.5	1.6	1.0	1.6
	3	72.1	1.4	0.7	1.8
	4	61.4	1.2	0.7	1.6
	5	73.7	1.4	0.9	1.6
SiO ₂ /Fe ₃ O ₄	1	91.4	1.9	1.1	1.7
	2	99.3	2.2	1.2	1.8
	3	102.8	2.4	1.3	1.8
	4	88.9	1.7	1.0	1.7
	5	96.7	1.8	1.0	1.8

Note. * MNPs: Magnetic nanoparticles; SiO₂: Silicon dioxide; Fe₃O₄: Iron oxide.

Table 1. The Primers Used for PCR Assays

Gene	Primer Sequence (3-5)	PCR product (bp)	Reference
<i>spa</i> . R	AAAGACGACCCAAGCCTTCC	160	13
<i>spa</i> . F	ACCATTGCGTTGTTCTTCG	160	

Note. PCR: Polymerase chain reaction.

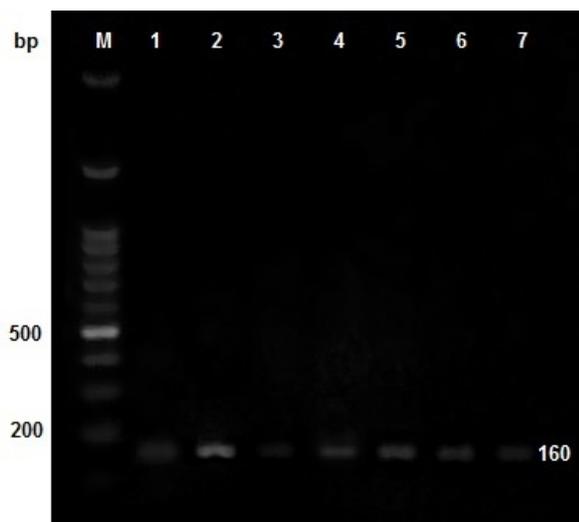


Figure 1. PCR Analysis of Genomic DNA Extracted by Bilayer MNPs and Monolayer MNPs. M: 3 kb molecular size marker; 1, 3: Genomic DNA extracted from clinical isolates of *Staphylococcus aureus* by monolayer MNPs; 2: Genomic DNA extracted from *S. aureus* strain ATCC 25923 by bilayer MNPs; 4, 6: Genomic DNA extracted from clinical isolates of *S. aureus* by bilayer MNPs; 5: Genomic DNA extracted from *S. aureus* strain ATCC 25923 by monolayer MNPs.

compared them, and finally, MNPs were introduced as a convenient method for extracting DNA from all the above-mentioned sources.¹⁴

Likewise, Intorasoot et al applied gelatin-coated MNPs to extract the genomic DNA of *S. aureus* and compared the time of extraction, along with the amount and quality of the obtained DNA with the extracted DNA by the phenol-chloroform and kit method.¹⁵ Their results confirmed the use of gelatin-coated MNPs for the extraction of DNA as an appropriate method in comparison to the phenol-chloroform and kit method.¹⁵ In addition, Chen et al employed phenol-chloroform and silica-coated MNPs to access the genomic DNA of *S. aureus* in milk¹⁶ and found that these NPs can extract DNA in a shorter time compared to the phenol-chloroform method.¹⁶ Similarly, in this study, the genomic DNA of *S. aureus* was isolated in less time by applying NPs. In another research, monolayer and bilayer MNPs including Fe_3O_4 and $\text{SiO}_2/\text{Fe}_3\text{O}_4$ were used to extract pBI121 plasmid DNA. The obtained results revealed that the quantity and purity of the extracted DNA using coated MNPs were higher compared to naked MNPs,¹⁷ which is consistent with the results of our study, demonstrating that the extracted DNA by bilayer MNPs $\text{SiO}_2/\text{Fe}_3\text{O}_4$ had higher concentrations and purity in comparison to monolayer MNPs. This could be related to an increase in the surface-to-volume ratio in bilayer NPs.

Moreover, Langyanchen et al employed bilayer $\text{SiO}_2/\text{Fe}_3\text{O}_4$ MNPs to extract the genomic DNA of *S. aureus* and concluded that the bacterial genomic DNA was obtained in a short period of time,¹⁸ which corroborates with the findings of the current study, representing that the genomic DNA of *S. aureus* was obtained in a short time.

It has been proven that DNA binds to the solid surface of NPs by electrostatic interaction, and as the surface of NPs changes, their function in absorbing the DNA molecule demonstrates a change as well.¹⁹ Similar to the above-mentioned finding, in our study, the concentration of the extracted genomic DNA was negligible when using naked MNPs. However, the adsorption rate of the DNA molecule increased significantly as the NP surface represented a change.

Conclusion

Based on the results of this study and their comparison with those of other existing methods for genomic DNA extraction, surface-coated bilayer MNPs are much more efficient than naked monolayer MNPs in this regard.

Authors' Contributions

Study concept and design: FF and MZ; Data acquisition: HR, FF, and MZ; Data analysis and interpretation: FF, MZ, and IS.

Ethical Approval

All stages of the plan were carried out in accordance with the Ethics Standards of Islamic Azad University, Science and Research Branch, Tehran, Iran.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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