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One-stop Genomic DNA Extraction by Salicylic Acid Coated Magnetic Nanoparticles

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Abstract

Salicylic acid coated magnetic nanoparticles were prepared via a modified, one-step synthesis and used for a one-stop extraction of genomic DNA from mammalian cells. The synthesized magnetic particles were used for magnetic separation of cells from the media by non-specific binding of the particles, as well as extraction of genomic DNA from the lysate. The quantity and quality were confirmed by agarose gel electrophoresis and polymerase chain reaction. The entire process of extraction and isolation can be completed within 30 min. Compared to traditional methods based on centrifugation and filtration, the established method is fast, simple, reliable, and environmentally-friendly.

Keywords

Salicylic acid; Magnetic nanoparticles; genomic DNA; extraction; simple and rapid

In recent years, the use of magnetic nanomaterials for the separation of biomolecules has attracted significant interest because of the wide range of possible applications in biomedicine and biology. Magnetic nanoparticles (MNPs) have been used as solid supports to enrich and purify cells, cell organelles and biomolecules (including, nucleic acids, proteins and xenobiotics) directly from chemical or biological suspensions [1; 2]. The use of MNPs provide a number of advantages over other traditional techniques specifically in biological separations and extraction [1]. Traditional separation techniques used in molecular biology, biochemistry, and chemistry rely on centrifugation and/or filtration. Although these define the standards in a variety of applications and processes, neither centrifugation nor filtration are amenable to automation or rapid and often times require intense mechanical forces for separation. Magnetic separation techniques have the potential to be fast and selective and does not require elaborate protocols, expensive equipment or filtration devices [1].

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Magnetic separation and purification of cells and nucleic acids offer various benefits over conventional methods, especially in its ease of use and the possibility of an environmentally friendly extraction process (does not require a high volume of toxic chemicals) at ambient temperature conditions. Molecular biology approaches, such as PCR amplification and restriction digestion, require high-molecular-weight DNA. Compared with centrifugation and filtration based techniques that exert shearing forces on the DNA, magnetic separation exerts only a magnetic drag, which is nondestructive and nonintrusive on the biomolecules.

Today, DNA based assays are the most common in molecular biology and are fundamental to life science research. Separation assays based on MNPs can provide DNA with improved quality for use in enzymatic digestion, polymerase chain reaction, detection of epigenetic marks, and sequencing [3; 4; 5; 6; 7], because the method is simple and is almost free of toxic chemicals [7] or force-based separation protocols as in centrifugation. However, the steps involved in the preparation of functionalized and monodispersed MNPs and the cost of using commercialized magnetic separation kits hinder routine application of this facile technology for biochemical or clinical screening.

Salicylic acid (SA) is a chemical ligand with a carboxylic and a phenolic functional site, commonly used as the modifier in chelating resins and has shown to possess excellent sorption properties [8]. It has also been used to coat MNPs and for solid phase extraction and in concentrating heavy metal ions from various impure samples [8; 9]. However, the application of salicylic acid coated magnetic nanoparticles (SAMNPs) as a solid phase, for genomic DNA (gDNA) separation has not been reported. Here we report on a modified, easy and reliable technique to synthesize SAMNPs, and demonstrate its utility as a robust one-stop gDNA extraction process from crude cell culture media. The quality and quantity of SAMNPs purified gDNA were confirmed by agarose gel electrophoresis and PCR amplification.

Water dispersible SAMNPs were prepared via a one-step wet chemical strategy, modified from a previous method by Unal et al. [9]. Briefly, a solution of a mixture of NaOH and SA was added to a sterilized three necked-bottle to increase the pH to ~11 under vigorous stirring with Ar gas, followed by the addition of an aqueous solution comprising of a mixture of Fe(III) and Fe(II) salts with a molar ratio of 2 Fe(III): 1Fe(II): 4 SA until the formation of a black suspension. After refluxing at 90°C for 4 h, a dark brown suspension was noted. Using sodium hydroxide instead of ammonium hydroxide was found to be effective in controlling liquid flooding. Moreover, the prepared SAMNPs by this method were much smaller than the previously reported ones. The smaller size renders the SAMNPs with higher surface area-to-volume ratio to maximize the interaction with the environment. The final product (Fig. 1A) was separated from the aqueous solution by magnetic separation and further washed with pure water until a neutral pH was reached. The SAMNPs were then dispersed in pure water at a concentration of 10 mg/ml. Figure 1A shows a representative TEM image of ~10 nm SAMNPs dispersed in water.

Dynamic light scattering analysis of the same samples revealed that the synthesized SAMNPs were highly monodispersed and had a hydrodynamic diameter of ~122.7 nm [10]. Zeta potential analysis confirmed that the zeta potential was 38.1 mV in water, data not

shown. Samples with zeta potentials between -30 mV and $+30$ mV typically tend to aggregate, because higher level of zeta potential results in greater electro-static repulsion between the particles, minimizing aggregation [11]. According to Unal et al, the chemisorptions of SA onto Fe_3O_4 nanoparticles via the carboxylic acid oxygen, which are symmetrically bonded to the Fe_3O_4 nanoparticle surface, allows these particles to be easily dispersible in water due to the exposed OH group.

The ability to produce nanocomposite materials that are water soluble and monodispersible in water is a highly desirable feature because of its utility in a wide range of biomedical applications and treatments, whereas other forms of MNPs currently are not. The high surface area-to-volume ratio of the evenly distributed SAMNPs and its monodispersability makes it highly attractive for experiments related to cells and DNA using these particles, compared to their counterparts.

Genomic DNA (gDNA) purification from crude HeLa cell culture media was performed as follows. HeLa cells were grown in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 10 $\mu\text{g}/\text{ml}$ streptomycin, in a 5% CO_2 incubator at 37°C . When the cells were $\sim 80\%$ confluent, 1 ml trypsin was added to suspend the adhered cells at 37°C for 3 min, and the trypsin digestion ceased with additional culture media. Cell concentration was determined with a hemocytometer as $\sim 5 \times 10^5$ cells/ml. Without specific methion, the subsequent experiment used the same concentration of cells. The cells were then transferred to a 1.5 ml tube containing 10 μl of SAMNPs. The mixture was incubated at room temperature for 5 min to form cell-nanoparticle complexes, which were then immobilized to a magnetic separation stand followed by aspiration of the supernatant and then discarded. Immobilized cells were lysed by the addition of 50 μl of lysis buffer (3 M NaI; 5 M urea; 40 g/l Triton X-100; 10 nM EDTA, 25 nM Tris-HCl, pH 6.5) prior to incubation at room temperature for 5 min. 50 μl of isopropanol, which facilitates binding of the released DNA to the SAMNPs, was added to the suspension (and incubated for 3 min at room temperature). After magnetic separation of the DNA-nanoparticle complexes, the supernatant was discarded, and the immobilized DNA was rinsed once with 100 μl of cold 70% ethanol solution. After removal and evaporation of ethanol, the DNA was eluted in 20 μl TE buffer (10 mM EDTA, 25 mM Tris-HCl, pH 8.0) at room temperature for 10 min. RNase digestion was performed if necessary. The SAMNPs were then immobilized with the supernatant transferred to a DNase/RNase free EP tube for further analysis. The results obtained were compared with traditional phenol-chloroform extraction [7; 12]. Each experiment was repeated in triplicate.

TEM was used to characterize the cells captured by SAMNPs, with images obtained with a CM-100 instrument operating at 80 kV, as shown in Fig. 2. Compared to our previous study which used carboxyl coated MNPs to extract urine DNA [7], the yield of cell collection by SAMNPs was higher since many more cells were present in the cell culture media compared to that in urine. The ratio of immobilized cells compared to that in the media was determined by a hemocytometer as 68%. Fig. 2 clearly shows that SAMNPs were attached to the surface of HeLa cells; some were even trapped in the cell membrane, allowing for the labeling of the particle ingested cells in the media to be easily recovered with an external magnet (SuperMag Multitube SeparatorTM). It should be noted that the SAMNPs were randomly

deposited on the surface of mammalian cells, in a manner similar to that observed with the carboxyl coated nanoparticles, indicating the non-specific nature of their interaction. The high yield of cells harvested by SAMNPs is expected to ensure a high yield of gDNA extracted from the samples.

During the initial lysis process, the SAMNPs attached to the cell surface need not be removed because the SAMNP-cell complexes can be directly lysed. For DNA extraction, the same MNPs were used. An equal volume of isopropanol was added to form DNA/nanoparticle complexes. Since the enrichment of mammalian cells and the adsorption of gDNA can both be realized with the same nanoparticles, many residual impurities and enzyme inhibitors present in the cell culture media or cell lysate can be easily removed. Because of the non-specific interaction of DNA and SAMNPs, there is a possibility of proteins being bound to SAMNPs contributing to a possible loss of purity of the DNA. However, agarose gel electrophoresis confirmed that negligible proteins were observed in the final eluted DNA samples in TE buffer, as shown in Fig. 3A. gDNA concentration from 10, 20, 50 and 100 μ l of culture media of mammalian cells was determined by a spectrophotometer as 66.7, 90.2, 149.0 and 160.6 μ g/ μ l, respectively. The OD₂₆₀/OD₂₈₀ ratio ranged from 1.79 to 1.83, which also is indicative of the high purity of the extracted gDNA. The gDNA extracted by the traditional phenol extraction process displayed similar electrophoretic pattern of DNA fragments, but with lower yields, which is in agreement with a previous study [13].

The quality of extracted gDNA was determined by PCR amplification of the four fragments (Table 1) with different sizes derived from genes possibly located at different chromatin using a Bio-Rad MyCycler Thermal Cycle (Bio-Rad Laboratories, Hercules, CA). Amplification reactions were carried out in a final volume of 25 μ l containing 12.5 μ l 2 x PCR master mix, 1 μ l of extracted DNA template, 1 μ l of each of the PCR primers and pure water. Amplification profile for all of the four genes was the same: 95°C for 5 min, followed by 34 cycles 60s at 94°C, 30s at 53°C, 60s at 72°C, and a final extension performed at 72°C for 5 min. The final amplicons (5 μ l) were loaded onto a 2.5% agarose gel in TBE buffer for electrophoresis, with bands visualized under ultraviolet light by RGB staining.

PCR was performed on K-ras, GAPDH, CYP3A4 and GDF5 genes with amplicon sizes ranging from 214–1106 bps, as shown in the agarose gel image (Fig. 3B). The PCR reactions show excellent agreement, verifying the high quality of DNA extracted by the present method. All of the negative controls show no binding in the gel image. All of the PCR templates contained 1 μ l of gDNA extracted from 50 μ l of HeLa cell suspensions and eluted in 20 μ l of TE buffer. To further investigate the sensitivity of the gDNA preparation method, a 556-bp DNA sequence of GAPDH gene was amplified using different sample volumes (10, 20, 50 and 100 μ l) tested by PCR, as shown in Fig. 3C (I). As expected, the intensities of PCR bands became stronger with increased sample volume (from 10 to 50 μ l). The high extraction performance can be attributed to the high binding affinity of SAMNPs to mammalian cells. Our results strongly suggest that the method developed is suitable for small-scale and PCR-ready mammalian gDNA extraction. Another set of PCR reaction was also performed using 1 μ l of 5, 25, 125, and 625 times diluted gDNA extracted from 100 μ l

suspension. As expected, the intensities of PCR bands became weaker and weaker as the dilutions increased (from 5 to 625).

A noteworthy feature of our work is that the MNP approach developed can greatly simplify the process of direct DNA template preparation for PCR because of enrichment of the cell population by MNP targeting and separation. DNA template from 100 and 200 μ l of media was obtained and the PCR amplification was successful for GDF5 gene with an amplicon size of ~1106 bp (Fig. 3C). The negative control did not have any DNA template. An important point to note is that the nanoparticles attached on the cell membrane did not produce any adverse effect on the PCR amplification [14]. Further, the entire process can be completed within 7 min, a highly desirable feature for quick and simple PCR-based screening.

In conclusion, SAMNPs proved to be a superior solid phase carrier for gDNA purification. Compared to other functional MNPs used as solid phase carriers for DNA extraction, the preparation of SAMNPs was easy and did not require complex surface coating steps. Our method embraces a green chemistry approach because we attempt to reduce or eliminate the use or generation of hazardous/toxic chemicals. The synthesized SAMNPs nonspecifically binds to mammalian cells which are separated and further binds to the released gDNA to complete the one-step extraction process. The entire procedure could be complete in less than 30 min without any lag in the process, and the extraction could be accomplished in one tube. Thus the developed method can be termed as “one-tube, one-stop genomic DNA extraction” that does not require elaborate sample preparation or handling or use hazardous reagents such as phenol or chloroform. The setup cost associated with the current method is much less compared to that associated with centrifugation-dependent methods and are well suited for routine biochemical or clinical screening.

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Fig. 1.
(A) Transmission electron microscopy (TEM) image of SAMNPs. The scale bar represents 20 nm; (B) Dynamic light scattering curves of the dispersion of SAMNPs in pure water.

Fig. 2. Transmission electron microscopy (TEM) image of mammalian cells captured with SAMNPs. The scale bar represents 5 μm . Note that a few aggregates of SAMNP (indicated by black arrows) are visible at the surface of cells. The mammalian nucleus was indicated by white arrow.

Fig. 3.

(A) Agarose gel electrophoresis of mammalian genomic DNA (gDNA). Lane 1, 2, 3 and 4: gDNA extracted by SAMNPs from 10, 20, 50 and 100 µl of culture media of mammalian cells ($\sim 5 \times 10^5$ cells/ml), respectively. Lane 5: gDNA extracted by traditional phenol-chloroform method from 1 ml of culture media from mammalian cells. (B) Agarose gel electrophoresis of PCR products obtained after amplification of gDNA extracted from 50 µl of cell suspension. (C) Agarose gel electrophoresis of PCR products. (I) GAPDH gene with a size of ~ 556 bps as amplified by PCR with 1 µl of DNA templates extracted from 10, 20, 50 100 µl of culture media with mammalian cells. (II) K-ras gene with a size of ~ 214 bps was amplified by PCR with 1 µl of 5, 25, 125, and 625 times diluted DNA from 100 µl of culture media with mammalian cells. (III) GDF5 gene with size 1106 bp was amplified by directly using purified cells by magnetic separation. The cells were from 50, 100, and 200 µl of culture media, respectively. All of the negative controls show a lack of binding to the gel image.

Table 1

Gene location, PCR primers and predicted sizes of amplified products.

Gene name	Primers (5' → 3')	Location	Product size
K-ras	Forward: GTACACATGAAGCCATCGTATA Reverse: CCACTTGTACTAGTATGCCTTAAG	chr12(p12.1)	214 bp
GAPDH	Forward: ACCACAGTCCATGCCATCAC Reverse: TCCACCACCCTGTTGCTGTA	Chr12(p13.31)	556 bp
CYP3A4	Forward: AACAGGGGTGGAAACACAAT Reverse: CTTTCCTGCCCTGCACAG	chr7(q21.1)	592 bp
GDF5	Forward: GGTGAGGTTGCAGGGAAT Reverse: CAGGGGAATTGTGGATAA	Chr20(q11.2)	1106 bp