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Tapasree R. Sarkar

Purdue University, tapasree@rediffmail.com

Joseph Irudayaraj

Birck Nanotechnology Center, Purdue University, josephi@purdue.edu

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Notes & Tips

Carboxyl-coated magnetic nanoparticles for mRNA isolation and extraction of supercoiled plasmid DNA

Tapasree Roy Sarkar^{a,b}, Joseph Irudayaraj^{a,b,c,*}

^a Purdue Cancer Center, Purdue University, West Lafayette, IN 47907, USA

^b Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907, USA

^c Birck Nanotechnology Center, Purdue University, West Lafayette, IN 47907, USA

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ABSTRACT

Carboxyl-coated magnetic nanoparticles (MNPs) were used to demonstrate dual functionality: isolation of messenger RNA (mRNA) from mammalian cells and extraction of the supercoiled (sc) form of plasmid DNA (pDNA) from agarose gel. These MNPs were attached with 5'-NH₂-tagged oligo-(dT)₂₅ primer and were used to isolate mRNA from breast cancer cells. The isolated mRNA was used for amplification of β-actin to confirm the compatibility. These MNPs were also used to extract the sc form of pDNA from agarose gel. The compatibility of the pDNA was demonstrated by restriction digestion. Both of these methodologies are simple, inexpensive (compared with existing kits), and efficient.

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During the past decade, nanotechnology has developed to such an extent that it has become possible to design and tailor the functional properties of nanoparticles for a variety of biomedical applications. Besides using these particles for drug delivery and magnetic resonance imaging, magnetic nanoparticles (MNPs)¹ have been used for protein purification [1], DNA [2–7], and messenger RNA (mRNA) isolation (First Track MAG mRNA Purification Kit, Invitrogen, Carlsbad, CA, USA; Direct mRNA dembeads Purification Kit, Ademtech, Pessac, France). Naked MNPs have been used as a solid-phase support in the isolation of genomic DNA from mammalian cells and from agarose gel [6,7]. Hawkins and coworkers [2] showed that surface carboxyl-coated MNPs produced higher extraction yields of DNA from cell lysate than did naked MNPs. Recently, arginine chromatography has been used to separate different isoforms of plasmid DNA (pDNA) from solution [8]. In the current study, we demonstrate for the first time the use of carboxyl-coated MNPs for the extraction of specific pDNA from agarose gel and isolation of mRNA using a simple and inexpensive process.

In a typical mammalian cell, the majority of RNA molecules are present in the form of transfer RNA (tRNA) and ribosomal RNA (rRNA), with mRNA accounting for 1 to 5% of the total RNA [9].

Conventional methods for mRNA isolation consists of hybridization of poly(A)-containing RNAs to oligo-(dT) molecules connected to a carrier, washing off the nucleic acids that bind to oligo-(dT) non-specifically, and final elution of poly(A) tail-containing mRNA from the oligo-(dT) carrier. Existing approaches of mRNA isolation using paramagnetic beads are quite expensive. Here we demonstrate the use of carboxyl-coated MNPs for mRNA isolation from mammalian cells using a simple, inexpensive (one-third the current cost), and efficient procedure.

pDNA, one of the key gene delivery vectors, consists of supercoiled (sc) and open circular (oc) isoforms. The sc form of pDNA is believed to deliver genetic information more efficiently, and it is desirable to have the sc content in pDNA at a much higher level [10,11]. Therefore, it is useful to separate the sc form of pDNA by its various biomolecular applications (e.g., cloning). Traditional methods for purification of DNA from agarose gel include organic extraction and electroelution, which either result in low yields of recovered DNA or render the purified DNA incompatible for further manipulation [12–15] in addition to being expensive. Here we report the use of carboxyl-coated MNPs for the extraction of the sc form of pDNA from agarose gel using a simple and inexpensive process.

Iron oxide nanoparticles bearing –COOH groups were prepared [16,17] and used for bioconjugation. Iron(II) chloride and iron(III) chloride were used to prepare MNPs [16], which were later separated and modified with surface –COOH groups by reacting with 2-carboxyethyl phosphonic acid [17]. The carboxyl-coated MNPs (Fig. 1A) were used to demonstrate the dual functionality: isolation of mRNA from MDA-MB-231 breast cancer cells and extraction of hypoxia-responsive element (HRE) pDNA from agarose gel using simple, inexpensive, and efficient processes.

* Corresponding author. Fax: +1 765 496 1115.

E-mail address: josephi@purdue.edu (J. Irudayaraj).

¹ Abbreviations used: MNP, magnetic nanoparticle; mRNA, messenger RNA; pDNA, plasmid DNA; tRNA, transfer RNA; rRNA, ribosomal RNA; sc, supercoiled; oc, open circular; HRE, hypoxia-responsive element; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; FA, formaldehyde agarose; RT-PCR, reverse transcription-polymerase chain reaction; HIF-1, hypoxia-inducing factor 1; MMTV, mouse mammary tumor virus; PEG, polyethylene glycol.

mRNA isolation from MDA-MB-231 cells was performed as follows. The surface –COOH groups of MNPs were activated by incubating freshly prepared 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) solution (0.4 M) with nanoparticles at 25 °C for 10 min. Excess EDC was removed by centrifugation, and nanoparticles were resuspended in RNase-free water. To conjugate 5'-NH₂-capped oligo-(dT)₂₅ primer (IDT, Coralville, IA, USA) with activated nanoparticles, oligo-(dT)₂₅ primer was added to the nanoparticles and the solution was agitated overnight at 4 °C in a sonicator. After removing the free oligo-(dT)₂₅ primers by centrifugation, the oligo-(dT)₂₅-conjugated nanoparticles were washed with RNase-free 1 × phosphate-buffered saline (PBS, pH 7.4) and resuspended in RNase-free water.

Different volumes of oligo-(dT)₂₅-conjugated nanoparticles (50, 100, and 200 μl) were equilibrated with 100 μl of lysis buffer (20 mM Tris-HCl [pH 8.0], 140 mM NaCl, 1.5 mM MgCl₂, 0.5% Nonidet P-40, 1 mM ethylenediaminetetraacetic acid [EDTA], and 1 mM dithiothreitol [DTT]) for 3 to 5 min. MDA-MB-231 cells were grown in RPMI 1640 (with 10% fetal bovine serum) in a T75 flask. The cells were trypsinized using 0.25% trypsin, detached from the flask, and placed into an RNase-free centrifuge tube. The cells were centrifuged at 900 rpm for 5 min, and the cell supernatant was removed. The cell pellet was washed with PBS and suspended in lysis buffer. The sample was homogenized by passing through the needle (21-gauge) four or five times. The resulting solution was incubated at 70 °C for 5 min to denature the secondary structures of RNA. The cell lysate was incubated with different concentrations of MNPs and agitated at room temperature for 10 min. The MNPs were separated using a magnetic rack, and the supernatant was removed from the solution. The nanoparticles were washed twice with wash buffer (20 mM Tris-HCl [pH 8.0], 140 mM NaCl, 1.5 mM MgCl₂, 0.1% Nonidet P-40, 1 mM EDTA, and 1 mM DTT) and resuspended with RNase-free water (50 μl) by flicking the tube. The resulting particles were captured magnetically, and the mRNA was transferred into an RNase-free tube.

The quality of isolated mRNA was assessed by formaldehyde agarose (FA) gel electrophoresis (Fig. 1B). Two sharp bands of rRNA (28S and 18S) were clearly visible, corresponding to the RNA isolated using an RNeasy Mini Kit (QIAGEN Inc., Valencia, CA, USA), but mRNA isolated using nanoparticles showed a smear on the gel, with the intensity of the smear increasing with increasing amounts of nanoparticles. The concentration was determined by reading the absorbance in a spectrophotometer at 260 and 280 nm. Approximately 30 μg of mRNA was purified from MDA-MB-231 cells using 200 μl (200 μg) of nanoparticles with an OD_{260nm}/OD_{280nm} value of 1.85. To check the compatibility of isolated mRNA, reverse transcription–polymerase chain reaction (RT-PCR) was performed for amplification of the commonly used

housekeeping gene β-actin (Fig. 1C). To check the reproducibility of this method, mRNA was isolated more than three times, and the results were found to be consistent. The proposed method does not require a purification column or different buffer solutions and is inexpensive compared with the existing mRNA isolation kits.

Supercoiled pDNA extraction from agarose gel was performed as follows. Hypoxia-inducing factor 1 (HIF-1)-responsive elements or HRE-like motif (5'-ACGTG-3') is responsive to hypoxia. The promoter activity in hypoxia is regulated through the interaction of the transcriptional regulator HIF-1 with an HRE. The HRE elements and luciferase gene-containing plasmid constructs, such as pGV-HP-E and HP2K [18], are widely used for reporter assay. In this study, pHLuc plasmid (American Type Culture Collection [ATCC], Rockville, MD, USA), which consists of pXP2 and mouse mammary tumor virus (MMTV) promoter with a functional HRE, was used. The plasmid was hosted and amplified in *Escherichia coli* DH5α and isolated using a Qiagen Plasmid Mini Kit. The HRE plasmid was run on a 0.8% low-melt agarose gel (Fig. 2A). The plasmid DNA was then visualized with a UV transilluminator. From the gel, the sharp band of sc pDNA was excised using a sterile blade (from lane 2) and transferred into a sterile 1.5-ml centrifuge tube. After adding 4 volumes of SSC buffer (0.75 M NaCl and 0.0075 M sodium citrate, pH 7.0), the centrifuge tube containing agarose gel was incubated at 80 °C for 5 to 7 min to allow the gel to melt. Immediately after incubation, 100 μl of nanoparticles (6 mg/ml) was added into the centrifuge tube along with 200 μl of binding buffer (1.25 M NaCl, 10% polyethylene glycol [PEG] 8000) [2]. The suspension was mixed by inversion, and the tube was incubated at room temperature for 3 to 5 min. The MNPs were separated using the magnetic rack, and the supernatant was discarded. The particles were then washed twice with 5 M NaCl and later with wash buffer (25 mM Tris-acetate [pH 7.8], 100 mM KOAc, 10 mM Mg₂OAc, and 1 mM DTT). Finally, the MNPs were resuspended in 20 μl of water. The water-containing nanoparticles were then magnetically separated, and the supernatant containing the plasmid DNA was transferred into a fresh tube and used for restriction digestion. It was reported that under conditions of high PEG and salt concentration, the DNA attached to the surface of carboxyl-coated MNPs is eluted in water [2]. The current method of extracting pDNA magnetically was compared with the conventional spin column extraction of the sc form of pDNA from agarose gel by cutting the sc pDNA from lane 3 (Fig. 2B).

To check the compatibility of the isolated HRE plasmid DNA by the magnetic method, restriction digestion was done with *Bam*HI. Fig. 2C shows a successful restriction digestion where the sc pDNA was found to be linearized on *Bam*HI digestion. This result indicates that nanoparticles were able to elute more than 75% of sc plasmids from agarose gels, which was higher than the pDNA iso-

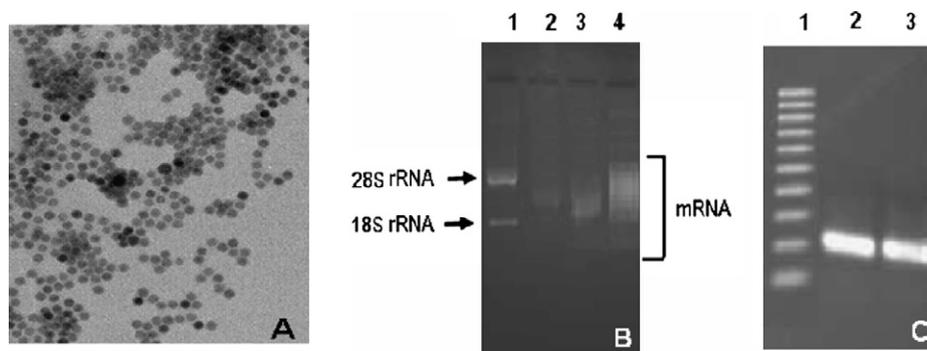


Fig. 1. (A) Transmission electron microscope image of carboxyl-coated MNPs. (B) mRNA isolation from MDA-MB-231 cells using MNPs. Lane 1: RNA isolated using Qiagen RNeasy Mini Kit; lane 2: mRNA isolated with 50 μg MNPs; lane 3: mRNA isolated with 100 μg MNPs; lane 4: mRNA isolated with 200 μg MNPs. (C) Agarose gel of PCR products showing amplification of β-actin. Lane 1: DNA marker; lanes 2 and 3: β-actin from mRNA isolated using 200 and 100 μg MNPs, respectively.

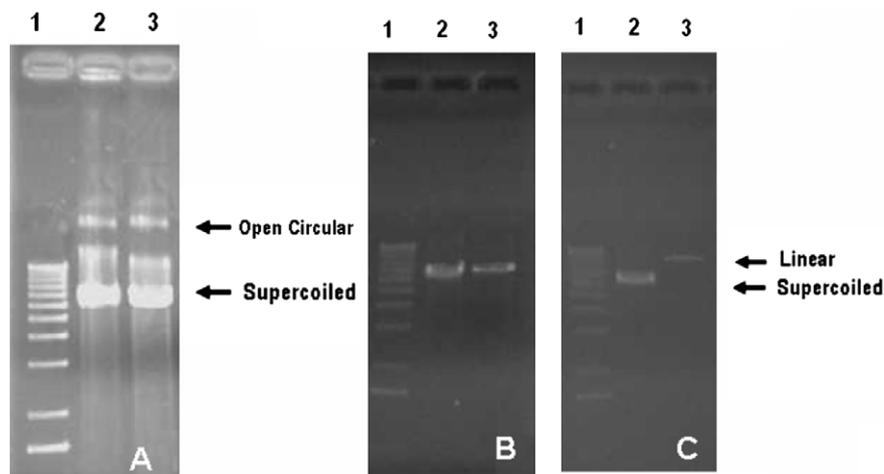


Fig. 2. (A) Agarose gel electrophoresis of HRE plasmid extracted from *E. coli* cell lysate. Lane 1: DNA molecular weight marker; lanes 2 and 3: HRE plasmid (6.2 kb) isolated from *E. coli* cells and run on agarose gel. The arrows indicate the presence of different forms of pDNA. (B) Agarose gel electrophoresis of the extracted sc form of pDNA eluted from agarose gel. Lane 1: DNA molecular weight marker; lane 2: pDNA eluted with carboxyl-coated MNPs; lane 3: pDNA eluted with conventional spin column. (C) Agarose gel electrophoresis of extracted plasmid eluted from agarose gel and restriction digestion of the sc form of HRE plasmid. Lane 1: DNA molecular weight marker; lane 2: sc form of HRE plasmid eluted with MNPs; lane 3: sc form of HRE plasmid digested with *Bam*HI.

lated from agarose gel using the conventional spin column (Fig. 2B), and the isolated pDNA can be used for downstream applications. This method of plasmid elution is simple, inexpensive, and quick, and it does not require the use of any centrifugation or expensive organic solvents. The DNA isolated by this process can easily be used for further experiments.

In summary, the carboxyl-coated magnetic nanoparticles have been used for two different biomolecular applications: mRNA isolation from MDA-MB-231 breast cancer cells and sc HRE plasmid DNA extraction from agarose gel. The methods described here are simple and inexpensive, and they do not require the use of organic solvents or expensive spin columns. The quality of isolated mRNA and pDNA was validated using PCR and restriction digestion, respectively.

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