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Magnetic Nanoparticles for Extracting DNA from Blood Cells

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Abstract—A technique for extracting DNA from blood cells using magnetic particles offers the advantage of saving time and prospects of automating the extraction process. A way of obtaining magnetic iron nanoparticles for extracting DNA from blood cells is developed. Magnetic nanoparticles with characteristics suitable for extracting genomic DNA from leukocytes are obtained and investigated.

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INTRODUCTION

The molecular genetic study of blood cells plays an important role in modern medicine, particularly in hematology. Identifying genetic disorders or changes in obtaining genetic information is a crucial diagnostic and prognostic factor in the treatment of blood diseases. Chronic myeloleukemia is diagnosed in 95% of all cases from the presence of a special chimeric BCR-ABL1 RNA molecule in tumor cells, the number of which allows us to judge the degree to which the disease has progressed and the severity of the response to treatment [1]. A great many different genetic modifications are of key importance when studying, e.g., acute leukemia and myeloproliferative diseases [2]. Identifying mutations in the DNA of malignant blood cells also allows us to select an individual regimen of treatment for each case of the disease [3, 4].

Extracting nucleic acids (DNA or RNA) is the first important stage of most molecular genetic studies. The validity of test results and the correctness of conclusions depend on the number and quality of purified molecules. There are now several ways of extracting nucleic acids from blood, both intracellular molecules and nucleoprotein complexes that circulate outside cells [5]. These techniques allow for the characteristics of a material, but each one has its advantages and drawbacks [6]. One of the most promising of these is to use magnetic particles with special coatings for capturing nucleic acid molecules. The advantages of this technique are less time consumed than with precipitation, less toxicity of the reagents than with phenol–chloroform extraction, and fewer requirements for laboratory equipment (no need for centrifugation). The use of magnetic particles ensures high purity of the nucleic acids for studies that employ highly productive and demanding techniques (microchipping and new-generation sequencing). Finally, the use of magnets opens up prospects for automating nucleic acid extraction. Despite these advantages and intense investigations [7], however, reagents for the extraction of nucleic acids on magnetic particles remain underdeveloped and undervalued in Russia, much like lensless blood microscopy [8]. Many kits are purchased abroad, increasing their cost. The main difficulties that must be overcome when using this technique are the ability of magnetic nanoparticles to agglomerate [9], which reduces their functional area for capturing nucleic acid molecules, and the ability of iron compounds to affect subsequent reactions with purified molecules.

The aim of this work was to develop an easy way of synthesizing magnetic iron nanoparticles for extracting DNA from blood cells in molecular genetic investigations.

EXPERIMENTAL

Magnetite particles were obtained from iron chlorides $FeCl_3$ and $FeCl_2 \cdot 6H_2O$, taken in a molar ratio of 2 : 1 using the technique described by Pertakova et al. in [10]. Magnetite particles were deposited upon alkalizing the solution to pH 10 with ammonia liquor (25%) by rapidly introducingf the alkali in a thin

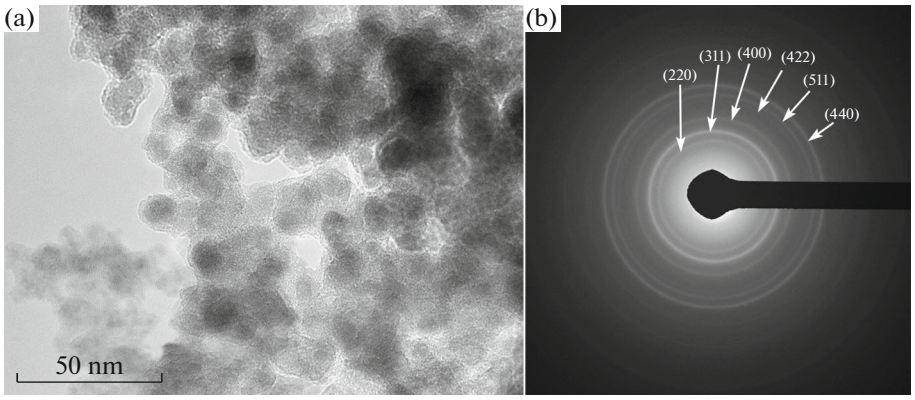

Fig. 1. High-resolution transmission electron microscope images of $Fe_3O_4@SiO_2$ nanoparticles synthesized with mechanical stirring: (a) appearance of the particles and (b) microdiffraction pattern.

stream through the needle of a syringe. After adding the ammonia liquor, the container with the reaction mixture was sealed and incubated with 30 min of continuous stirring.

The resulting precipitate was separated using a neodymium magnet. Particles were stabilized with 5 mM sodium citrate (pH 6–7) and washed with deionized water to obtain a pure wash liquor.

Silicon oxide in the form of tetraethoxysilane (TEOS) was chosen as a coating accessible to magnetic nanoparticles. To form the coating, magnetite particles were suspended in the ethanol : water (9 : 1) mixture in concentrations of 20 and 10 mg/mL and stirred for 10 min. TEOS was then introduced (70 μL per 200 mg of nanoparticles).

Two modes of processing were used in forming the coating: ultrasonic breakdown in the cavitation mode (1 h) and mechanical stirring. All variants of magnetic nanoparticles coated with silicon oxide were washed with distilled water, separated on a neodymium magnet, and stored in ultrapure water (18 mΩ/cm^3) at a concentration of 400 mg/mL.

The resulting samples were examined in the mid-IR region of 380–4000 cm^{-1} using the Hitachi HT7700 transmission electron microscope and the Bruker VERTEX 80V vacuum Fourier-transform spectrometer at the Krasnoyarsk Scientific Center's regional shared resource center.

An AmpliSens DNK-sobr-V reagent set (Moscow, Russia) was used to extract DNA with the obtained magnetic nanoparticles. Extraction was performed according to the manufacturer's protocol, but with magnetic nanoparticles instead of the silicate sorbent supplied in the set. DNA was extracted from leukocytes obtained with 130 μL of human blood. The amount and quality of the genomic DNA was estimated via electrophoresis in 1.5% agarose gel.

RESULTS AND DISCUSSION

Results from the high-resolution transmission electron microscopy of $Fe_3O_4@SiO_2$ nanoparticles obtained with mechanical stirring are presented in Fig. 1. Conglomerates of iron nanoparticles with common silicate shells formed upon coating them with silicon oxide under mechanical stirring. The average particle size with mechanical stirring was ~10 nm. Due to aggregation, however, the overall size of particles with coating grew to hundreds of nanometers. According to the microdiffraction pattern, the nanoparticle material is magnetite with a spinel structure.

Figure 2 compares the FTIR spectrum of $Fe_3O_4@SiO_2$ nanoparticles obtained via mechanical stirring to the spectrum for Fe_3O_4. In the spectrum of the $Fe_3O_4@SiO$ nanoparticle samples, we observed very strong vibrations with a maximum at 1091 cm^{-1} that belonged to the asymmetric stretching vibrations of silaxane groups

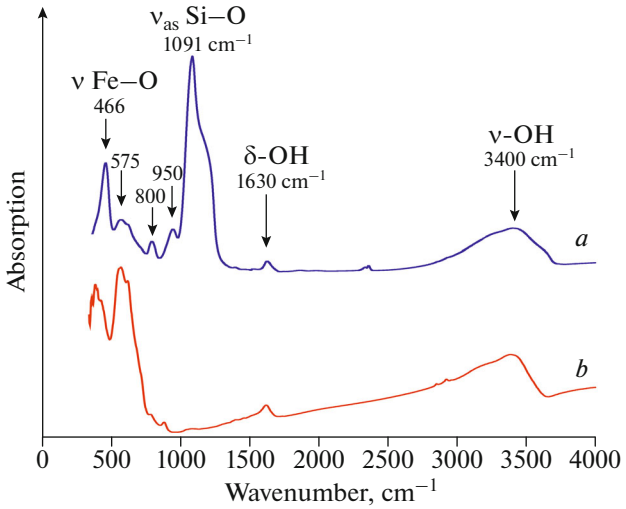

Fig. 2. Room-temperature FTIR spectra for (*a*) $Fe_3O_4@SiO_2$ and (*b*) Fe_3O_4 nanoparticles.

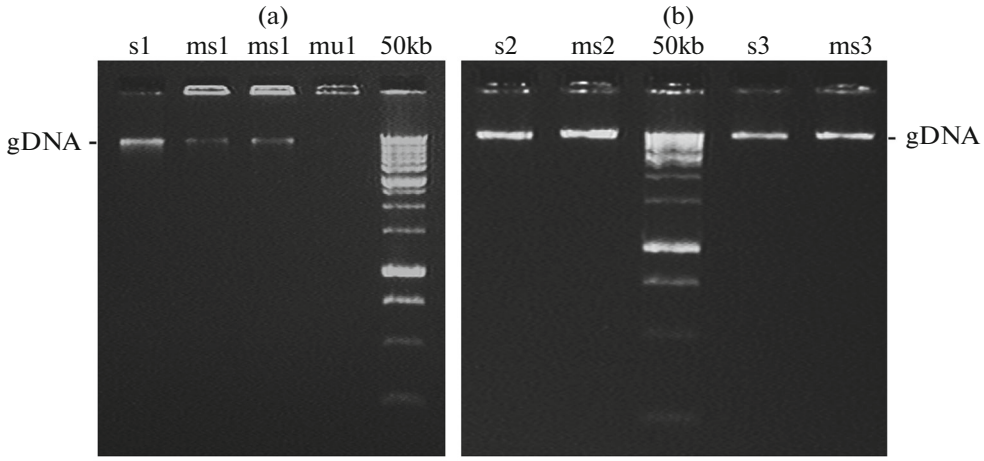

Fig. 3. Agarose gel electrophoresis of purified genomic DNA extracted using a set of DNA-sorb B reagents and different sorbents: a commercial silicate sorbent (C1-3); magnetic nanoparticles coated with silicon oxide during mechanical stirring upon adding 18 mg (ms1) and 15 mg (ms2, ms3) of nanoparticles to a sample; and magnetic nanoparticles coated with silicon oxide using ultrasound (mu1). gDNA is genomic DNA and 50kb is the DNA marker.

(ν_{as} Si–O–Si) [11]. In the spectra of the investigated samples, the ν–OH stretching vibrations formed a high-intensity band in the range of 3200–3600 cm^{-1}. The position and character of this band depended on the participation of the hydroxyl group in the hydrogen bond; the broad weakly structured band in our case testifies to the wide diversity of the states of water molecules in the samples. The band of deformational vibrations of water δ–OH was located in the range of 1600–1650 cm^{-1}. The weak vibrations at 950 and 800 cm^{-1} in the spectrum of Fe_3O_4@SiO_2 nanoparticles were associated with the bending and stretching vibrations of silanol groups Si–OH, respectively (Fig. 2a). It is difficult to correlate the bands at frequencies below 600 cm^{-1} with specific vibrations, but they were somehow associated with the vibrations of Fe–O bonds. In addition, it is well known that the vibrations of Fe–O and Si–O bonds differ negligibly in their oxide compounds. The lack of an absorption band at ~3700 cm^{-1} indicates successful modification over free silanol groups. Analysis of the infrared spectra suggests that the siloxane groups participated in bonding of Fe_3O_4 magnetic particles and the silica layer, while the silanol groups were most active on the surface and chemically active in the forming of covalent bonds.

CONCLUSIONS

When DNA was extracted by particles with a coating formed via mechanical stirring, the amount of obtained DNA was comparable to the one extracted using a commercial kit with a silicate sorbent (Fig. 3a). At the initial test stages, however, a change in the properties of purified molecules was noted when assessing the quality of the DNA obtained via electrophoresis. The change was reflected in the incomplete embedding of DNA into the agarose gel (Fig. 3). This could have been due to the dose-dependent effect of magnetic iron nanoparticles on DNA molecules, which can interact with a molecule's bases and change its structure [12]; or to the presence of impurities in the solution. A way of overcoming the damaging effect magnetite has on the molecules of a living organism was described in [13, 14], where we proposed chemically modifying nanoparticles during synthesis. In this work, however, an attempt was made to avoid additional components when working with individual molecules. The effect of magnetic particles was notably weakened during electrophoresis when the number of magnetic particles introduced into the sample during extraction was reduced from 18 to 15 mg, and the uniform embedding of the purified DNA into the agarose gel was observed (Fig. 3). The amount of DNA extracted by nanoparticles coated using ultrasound was appreciably smaller (Fig. 3) and, in some experiments, equal to the trace level, despite the expected drop in aggregation and an increase in the ratio of the silicate coating's surface area to the particle volume. The origin of this effect requires more thorough investigations, since a more detailed study of these particles was not made here due to the lack of the desired result from DNA extraction. However, the effect acoustic cavitation has on the structure of ferrihydrite particles [15] suggests similar processes in this case as well.

Magnetic iron oxide nanoparticles for the effective extraction of DNA under laboratory conditions can be obtained via simple mechanical stirring without the ultrasonic disaggregation of particles. At the same time, the reduced effect iron nanoparticles have on DNA can be controlled by changing the number of particles.

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