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Silica-Coated Iron Oxide Nanoparticles for DNA Isolation for Molecular Genetic Studies in Hematology

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Aim: To develop magnetic nanoparticles (MNPs) based on iron oxide for DNA isolation from blood cells for quantitative molecular genetic analyses of the V617F mutation in the Januskinase 2 (*JAK2*) gene.
Materials and Methods: MNPs were synthesized by the coprecipitation method and coated with tetraethyl orthosilicate (TEOS). The size and shape of the complexes were estimated using transmission electron microscopy. Twenty blood samples from patients with myeloproliferative disorders were used for DNA isolation with the MNPs. DNA quality and compatibility for molecular genetic studies of the *JAK2* V617F mutation were investigated by gel electrophoresis and real-time polymerase chain reaction (RT-PCR).
Results: The average amount of DNA isolated from 150 μL of whole blood was 75.2 ng when MNPs were used and 72.5 ng when standard silica sorbent was used. There was no DNA damage observed after interaction with MNPs. RT-PCR demonstrated similar values for the *JAK2* V617F mutant DNA ratios in the samples after DNA isolation with MNPs and by standard sorption on silica.
Conclusions: MNPs with silicate capsules of sufficient thickness were obtained and the undesirable damaging effect of iron oxides on nucleic acids during isolation from cells were eliminated. Designed MNPs allow obtaining intact DNA for molecular genetic studies using the example of the *JAK2* V617F for study.

Keywords: magnetic nanoparticles, DNA isolation, myeloproliferative neoplasms, *JAK2* V617F

Introduction

Nucleic acid isolation from cells is a mandatory and very important stage of molecular genetic research in medicine. The quantity and quality of the isolated nucleic acid largely determines the reliability of the results obtained, and, consequently, the correctness of the diagnosis. Nuclear-containing blood cells are one of the most accessible materials for genetic disorder studies in many diseases. But it is of particular importance in hematology, where breakdowns in the genome are the causes of such pathologies as myeloproliferative neoplasms, acute and chronic leukemias, lymphomas, etc.

The V617F point mutation of the Januskinase 2 (*JAK2*) gene is one of the important genetic disorders in hematology. This is due to the fact that *JAK2* V617F is a single mutation associated with a whole group of myeloproliferative diseases. This mutation occurs in 90–95% of cases of polycythemia vera, 50–70% of cases of essential thrombocythemia, 40–50% of cases of primary myelofibrosis (Tefferi *et al.*,

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2005), and rarely in some other hematologic diseases (Jelinek *et al.*, 2005). Moreover, it is very important to estimate *JAK2* V617F mutant allele burden in blood sample, because it reflects the proportion of oncotransformed cells. It has been shown that *JAK2* mutant cells in an amount of 1% or less occur in people without cancer and are not an indication for therapy (Sidon *et al.*, 2006), whereas higher values of *JAK2* V617F mutant allele burden affect hematologic parameters and the severity of oncologic course (Kim *et al.*, 2017) (Vannucchi *et al.*, 2011). The detection of *JAK2* V617F in leukocytes is included in the clinical guidelines and allows to diagnose chronic myeloproliferative neoplasms with a high degree of probability.

So, the assessment of the *JAK2* V617F allele burden is an important diagnostic and prognostic study in a number of myeloproliferative diseases, and growing quantities of such studies determine the need to develop test systems for automated testing.

Magnetic separation is one of the promising methods for DNA isolation. Unlike standard adsorption methods, magnetic separation does not require centrifugation to collect the sorbent, replacing it with a magnetic field. This opens up more possibilities for automating the process. In addition, the use of various coating materials and methods of magnetic core forming makes it possible to expand the possibilities of magnetic separation and to perform specific sorption of nucleic acids by unique nucleotide sequence. Thus, the immobilization of specific primers to the chimeric BCR/ABL1 gene on the surface of magnetic nanoparticles (MNPs) made it possible to increase the polymerase chain reaction (PCR) sensitivity and specificity in the diagnosis of chronic myeloid leukemia (Jangpatarapongsa *et al.*, 2011). Yang *et al.* (2011) have proposed a new way to detect DNA molecules and single nucleotide polymorphisms using immunomagnetic reduction assay. MNPs are considered promising agents for the isolation and enrichment of nucleic acids, highly sensitive indicators for detecting DNA or searching for mutations. They can be useful for DNA libraries preparing for high-throughput methods such as NGS (Tang *et al.*, 2020).

But, despite the advantages, the use of MNPs has some limitations, and the development of new magnetic composites requires a number of important aspects to be taken into account.

Thus, various studies have shown the dose-dependent effect of iron oxide nanoparticles on the level of reactive oxygen species in the cell, which contributes to the occurrence of oxidative stress with damage to structures and molecules, including DNA (Alarifi *et al.*, 2014; Gaharwar *et al.*, 2017; Ansari *et al.*, 2019). This creates the need to design a nanoparticle with the magnetic core being packed in a dense shell that prevents the damaging effect of iron oxide on the adsorbed molecules.

The aim of the study was to develop MNPs based on iron oxide for the DNA separation from blood cells for further quantitative molecular genetic analysis of the V617F mutation in the *JAK2* gene.

Materials and Methods

Iron oxide MNPs coated with amorphous silicon oxide were synthesized according to the protocol described earlier (Komina *et al.*, 2020) with a modification of the ratio of magnetic particles and tetraethyl orthosilicate (TEOS) at the coating stage. For this, 30 mg of magnetic particles (dry mass) were mixed with 500 μL of TEOS. The presence of magnetic inclusions in the silicate capsule was confirmed by transmission electron microscopy on Hitachi HT7700 instrument (accelerating voltage 100 kV) of the Krasnoyarsk Regional Center of Research Equipment of Federal Research Center «Krasnoyarsk Science Center» SB RAS.

The biological material for the study was provided by the hematology department of the Regional Clinical Hospital (Krasnoyarsk) and consisted of whole blood from patients with myeloproliferative neoplasms ($n = 20$).

To isolate DNA from leukocytes, 400 μL of red blood cell lysis solution (Formula Gena LLC, Russia) was preliminarily added to 150 μL of whole blood and incubated at room temperature for 10 min. Then the samples were centrifuged for 5 min at 2000 rpm, and leukocyte sediment was used for DNA isolation. The DNA-sorb B Kit (AmpliSens, Russia) was used as reagent for isolation. In this case, the silicate sorbent from the kit was replaced to MNPs at the amount of 2 mg per sample. After sorption, the magnetic particles with DNA were washed once with a solution for washing 1 and twice with a solution for washing 2 supplied in the kit. Furthermore, the magnetic sorbent was dried at room temperature (not heated) until the visible drops of ethanol disappeared, and the elution solution (TE buffer) preheated to 65°C was added. Elution incubation of the samples with magnetic particles was carried out at room temperature. The particles from the suspension were collected using a neodymium magnet. The reagent kit, ''DNA sorb B,'' itself was used as an isolation control in accordance with the manufacturer's instructions, using 6.5 mg of silicate sorbent per sample.

Relative amount and integrity evaluation of the isolated DNA was performed by electrophoresis in 1% agarose gel with ethidium bromide staining (0.8 mg/L). A quantitative study of mutant *JAK2* V617F DNA copies was carried out by real-time PCR (RT-PCR) on a CFX96 PCR System (BioRad). The reagent kit ''Myeloscreen'' (LLC ''Formula Gena,'' Russia) was used to estimate the number of DNA copies with the V617F mutation.

Analysis of the correspondence of PCR data obtained using DNA isolated on MNPs and on silica was performed using the Pearson's correlation criterion.

Results

The developed MNPs were aggregates of magnetic cores covered with silicon oxide shell. The coating thickness reached 20 nm (Fig. 1). So, the size of such particles was 100 nm and more.

DNA isolated using MNPs demonstrated single DNA band on the electrophoretogram as evidence of DNA integrity (Fig. 2A). The average amount of DNA isolated using 2 mg of nanoparticles was 75.2 ng, whereas 6.25 mg of silicate sorbent released an average of 72.5 ng of molecules. Thus, 1 mg of a standard silicate sorbent released on average 12.87 ± 1.40 ng of DNA, and 1 mg of the developed nanoparticles—37.60 ± 11.06 ng of DNA, which is 2.9 times more.

The slope of the linear part of the fluorescence curve during RT-PCR reflects the amplification efficiency when

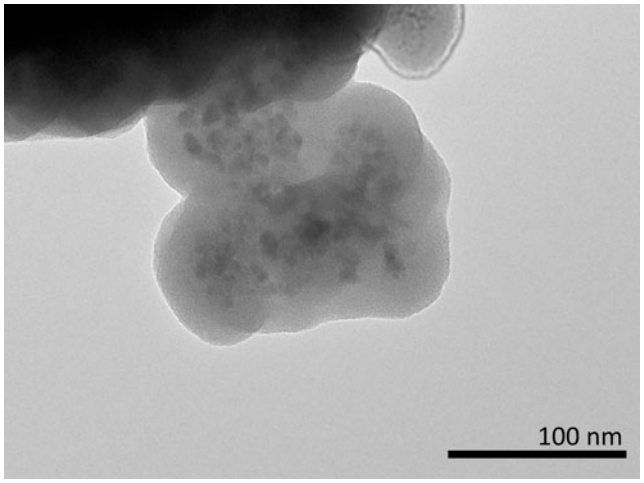

FIG. 1. TEM image of iron oxide magnetic nanoparticles coated with TEOS. TEOS, tetraethyl orthosilicate.

calculated by the formula $E = 10^{tg\alpha}$, where E is the amplification efficiency, and $tg\alpha$ is the slope of the linear part of the curve presented on a logarithmic scale (Lapa *et al.*, 2019). The reaction with the DNA isolated using the MNPs demonstrated an angle of inclination equal to that in the control reaction (Fig. 2B).

Evaluation of the conformity of the analysis results of DNA isolated on MNPs with the data obtained by the standard method of DNA extraction showed a high degree of consistency (Pearson's correlation coefficient $R = 0.99$) (Fig. 3). Regardless of the DNA extraction method, the result of genetic analysis showed similar values for *JAK2* V617F mutant DNA proportions, allowing for an identical clinical interpretation.

Discussion

Quantitative methods place increased demands on the quality of the material. The presence of reaction inhibitors can impede PCR or hinder the normal detection of the result. Damage or fragmentation of the studied molecules during sample preparation can affect the ratio of the products, distorting the test result. The development of MNPs for molecular and cellular biology has particular limitations, since any object that is alien to a biological object (cells, molecules) can elicit an undesirable response from it. Despite the relative biosafety, iron oxide nanoparticles affect DNA molecules both in living cells and in a tube, causing its oxidative damage, fragmentation, contributing to the occurrence of mutations (Rim *et al.*, 2013; Dissanayake *et al.*, 2015). One of the ways to overcome this influence is to encapsulate the magnetic core inside the shell for minimizing its chemical contact with the external environment. Bai *et al.* (2016) described a method for magnetic core encapsulation with a silicate shell using a two-component coating with TEOS and 3-aminopropyltriethoxysilane (APTES) and demonstrated the absence of inhibition by adding such particles to a PCR mixture. However, the presence of amino groups on the surface of APTES-coated particles facilitates the attachment of protein or other active molecules, but much less effective for DNA sorption. In this work, the coating was produced only by TEOS, but in a ratio with iron oxide particles, which helps to isolate the magnetic core, while retaining the sorption properties of silicon oxide.

The identification of individual genetic alterations in blood cells makes it possible to perform differential diagnostics of oncohematological diseases; the need to monitor their course and response to therapy makes it important to carry out an increasing number of quantitative molecular genetic studies in this area. In this case, it is especially important for the sample preparation method to not cause a damaging effect on DNA, ensuring the reliability of the results obtained. Silicon oxide is considered to be one of the least toxic materials that does not cause significant harm to living objects with the right combination of particle size and concentration. In this work, it is shown that the use of the developed MNPs for DNA isolation from blood cells provides good comparability with the results of using standard commercial methods. The absence of DNA damage allows suggesting that DNA isolated

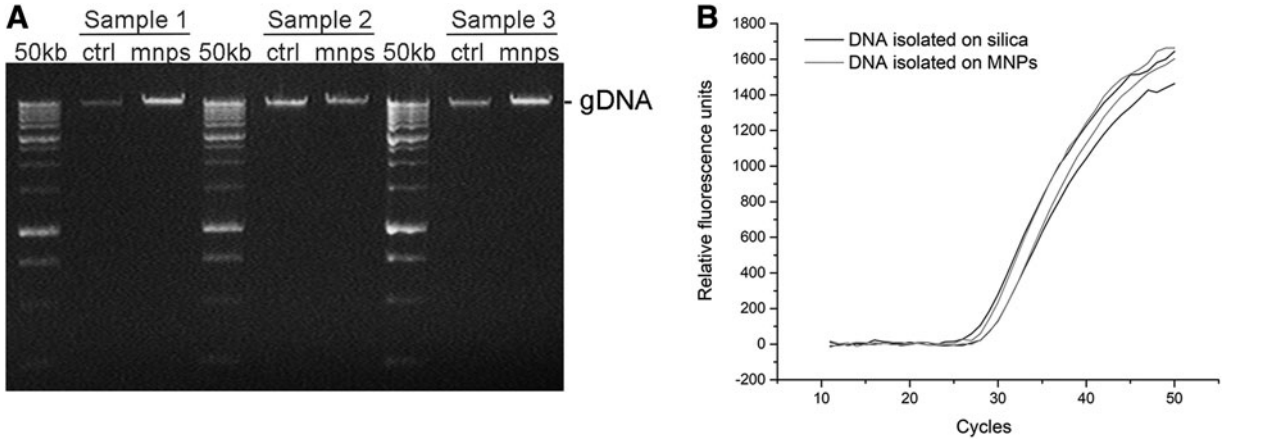

FIG. 2. The results of integrity and reactivity studying of DNA isolated using magnetic nanoparticles in comparison with silica: **(A)** electrophoresis in 1% agarose gel: single DNA bands on each lane indicate the absence of fragmentation; **(B)** graph of fluorescent signal accumulation during real-time PCR: the equal slope of the linear parts of the curves allows us to assert the equal reaction efficiency.

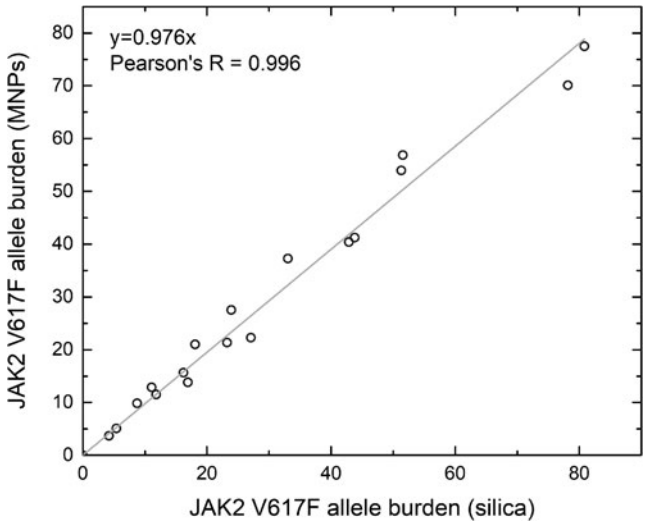

FIG. 3. Scatter plot of paired data on mutant *JAK2* V617F DNA percentage obtained after DNA isolation using magnetic nanoparticles and standard silica. *JAK2*, Januskinase 2.

using the MNPs may also be suitable for detecting other mutations, such as ones in the *ABL1* gene associated with drug resistance in chronic myeloid leukemia. But this requires further confirmation.

Conclusions

Thus, the use of a silicate capsule of sufficient thickness can completely eliminate the undesirable effect of iron oxides on nucleic acids in the process of isolation from the cell and ensure the obtaining of intact DNA for molecular genetic studies using the example of *JAK2* V617F study.

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Author Disclosure Statement

No competing financial interests exist.

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