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NEW MATERIALS FOR DNA ISOLATION

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НОВЫЕ МАТЕРИАЛЫ ДЛЯ ВЫДЕЛЕНИЯ ДНК

Except silicon dioxide, commonly used for DNA isolation, such metal oxides (V) as Ta₂O₅, Nb₂O₅ and V₂O₅ were proposed for this purpose. A method for covering magnetic iron-based nanoparticles with metal oxides (V) was suggested. The synthesized nanoparticles were used for DNA extraction and the amount of isolated DNA was compared with the commercially available magnetic particles coated with silicon dioxide. Magnetic nanoparticles covered with Ta₂O₅ and Nb₂O₅ showed a greater adsorption capacity as compared with the same nanoparticles covered with silica and commercial samples. The synthesized nanoparticles can be suitable for DNA extraction in clinical or research laboratories. The functional thin films for fast and effective DNA extraction are of great interest for neurobiology investigations.

SOLGEL; THIN FILMS; DNA, MAGNETICAL NANOPARTICLES; XPS.

За исключением диоксида кремния, обычно используемого для выделения ДНК, для этой цели были предложены такие оксиды металлов (V), как Ta₂O₅, Nb₂O₅ и V₂O₅. В данной статье рассмотрен метод покрытия этими оксидами магнитных наночастиц на основе железа, исследованы химические и структурные свойства. Синтезированные наночастицы были использованы для выделения ДНК, проведено сравнение с имеющимися в продаже магнитными частицами, покрытыми диоксидом кремния. Магнитные наночастицы, покрытые Ta₂O₅ и Nb₂O₅ показывают наибольшую адсорбционную способность по сравнению с наночастицами, покрытыми диоксидом кремния и с коммерчески доступными. Синтезированные наночастицы могут быть использованы для экстракции ДНК в клинических или научно-исследовательских лабораториях. Функциональные тонкие пленки для быстрой и эффективной экстракции ДНК представляют большой интерес для исследований в нейробиологии.

ТОНКИЕ ПЛЕНКИ; ЗОЛЬ-ГЕЛЬ; ДНК; МАГНИТНЫЕ НАНОЧАСТИЦЫ; РФЭС.

Introduction

Over the last decade the studies on the magnetic nanoparticles (MNP) have become popular in wide range of biomedical applications, such as biosensors, contrast agents for magnetic resonance imaging, drug delivery, etc [1–5]. One of the most common applications of magnetic nanoparticles is DNA isolation. In that application the surface of magnetic nanoparticles is covered with materials which are active for DNA isolation. So, at the first step MNP surface actively absorbs DNA molecules and at the next step MNP are efficiently separated from chemical or biological suspensions with the magnetic field [6]. Magnetic

separation is recognized as simple and effective method for nucleic acids purification.

On the other hand materials with high surface area, such as nanoparticles, are preferred for the nucleic acids binding. Magnetic particles with shape of nanospheres, are more preferred in the process of selection, as they have a greater ability to bind-conductive [7]. Many different DNA isolation kits with magnetic particles are presents in the market, such as: AGOWA® mag, Dynabeads® DNA and other [8]. In general, surface cover materials of these magnetic nanoparticles is silicon dioxide SiO₂ or a compound based on it [9–13].

The principle of DNA isolation on silicon dioxide matrix is based on the high-affinity of negatively charged DNA strands to the positively charged sodium ions, which are in turn bound to negatively charged particles of silicon dioxide. Sodium ions act as a cation bridges, which attract negatively charged oxygen of the phosphates in the nucleic acid chain. Sodium ions treat the bonds between hydrogen in water and negatively charged oxygen ions on silica surface under high salt ($pH \leq 7$). Thus, DNA is firmly connected to the matrix. Purified DNA molecules can be eluted by low salt solution using Elution buffer or distilled water [14–16](fig. 1).

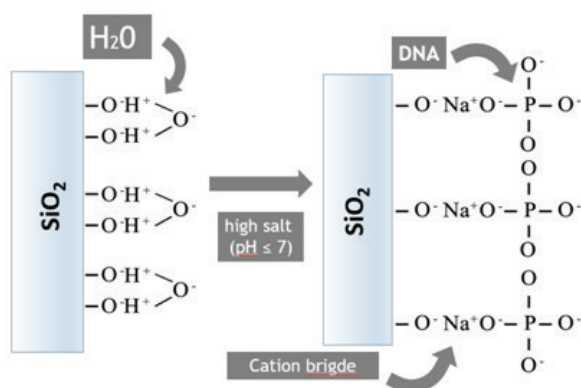


Fig. 1. SiO_2 -DNA interaction under high salt ($pH \leq 7$)

In the previous work [17] it was assumed that using of metal oxides (V) (tantalum, niobium and vanadium) would provide more effective DNA extraction than silicon dioxide. Since their crystal lattices close to stoichiometric ratio 2:5, while the silicon dioxide stoichiometric composition is close to 1:2. More effective DNA extraction can be explained by the ability of metal oxide (V) surface to form 25% more chemical bonds than SiO_2 according to oxides stoichiometry. In recent study we proposed a technological framework for fabrication of the magnetic iron-based nanoparticles covered by SiO_2 and Ta, Nb, V oxides in order to verify this assumption. Synthesised magnetic nanoparticles with bioactive surface were used for DNA extraction and quantitative characteristics of isolated DNA [18] were compared with commercial Syntol® magnetic particles.

Materials and Methods

The non-agglomerative spherical magnetic nanoparticles of Fe_3O_4 were produced by aerosol CVD synthesis method. The experimental setup for the

CVC synthesis of nanoparticles has been described elsewhere [19, 20]. Briefly, the liquid precursor, $\text{Fe}(\text{CO})_5$, was heated in a bubbler, evaporate and transported by inert gas (argon or helium) flow into a heated tubular furnace. The tubular furnace provides a heat source for the controlled decomposition of the precursor. The product of the precursor decomposition was collected in a vacuum chamber on the surface of a rotating chiller cooled by liquid nitrogen. An iron particle passivation process was achieved by dosing oxygen before opening the chamber to air. The precursor decomposition temperature was set at 400 °C. After the synthesis initial Fe-based nanoparticles have core-shell structure: core is pure iron and shell is 1-2 nm thick magnetite. SEM micrograph of as-produced nanoparticles are shown in the fig. 2.

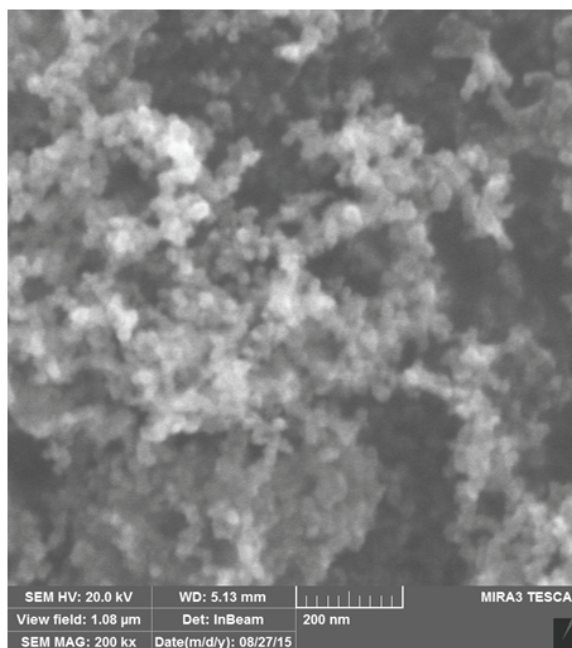


Fig. 2. SEM electron micrograph of Fe_3O_4 MNPs

Magnetic nanoparticles we covered with oxides using sol-gel synthesis method. MNPs were covered with metal oxides in the following compositions: $\text{Fe}_3\text{O}_4/\text{SiO}_2$; $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{Nb}_2\text{O}_5$; $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{Ta}_2\text{O}_5$; $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{V}_2\text{O}_5$. All reagents and materials are commercially available in Sigma Aldrich: tantalum (V) chloride, niobium (V) chloride, vanadium (V) oxide, isoamyl alcohol, thionyl chloride and ethanol.

The structure of the materials was analyzed by scanning electron microscopy (SEM, Leo DSM 982 Gemini and JEOL JSM_7500F microscopes). The

phase composition of the materials was studied by X-ray photoelectron spectroscopy (XPS) on a Thermo Scientific K-alpha spectrometer (USA) with monochrome radiation source (Al K α). Ion etching of samples was carried out until the element content became constant as a function of the etching depth.

The biological material obtaining and DNA isolation process was carried out in the Immanuel Kant Baltic Federal University.

Real-time polymerase chain reaction (RT-PCR) was carried out using PCR CFX96 Real-Time PCR Detection System («Bio-Rad», USA). PCR tube contained 20 μ l reaction mixture: DNA-polymerase buffer Taq («Evrogen», Russia) – 1x, 2,5 mM MgCl₂ («Syntol», Russia, 0,25 mM of each dNTPs («Syntol», Russia, Taq-polymerase («Syntol», Russia) – 1x, the neurotrophin receptor gene primer - TrkB - 0,5 μ M. The amplification reaction was started with the following conditions: 95 °C for 3 min (once), 95 °C for 10 s, 63 °C for 40 s, (50 cycles).

Results and discussion

Preparation of metal oxides (V) thin film on the surface of MNPs precursors

At the first step the nanoparticles were covered by silicon dioxide thin films by sol-gel synthesis method by using the well-known process described in [21]. We mixed 15 mg of MNPs, 500 μ l ethanol, 500 μ l deionized and deoxygenated water, 50 μ l ammonium solution (25% wt) and 3 μ l tetraethoxysilane. Mixture was shaken on vortex and sonicated for 0,5 h. The tube with MNPs was placed in the magnetic rack/ After the separation particles, liquid was removed by pipetting.

Silicon dioxide thin film on the surface of MNPs was used as a substrate for formation of metal oxide (V) thin-films. For the synthesis of thin layers of metal oxides on the silica surface of MNPs corresponding etoxides (Nb(C₂H₅O)₅; Ta(C₂H₅O)₅) were used as precursors for Ta and Nb oxides coating and vanadium oxychloride (VOCl₃) was used for vanadium ones. Precursors were synthesized in laboratory using reaction between metal chlorides and absolute ethanol [22–24], excess of HCl was removed by reaction with gaseous ammonium.

For the synthesis 15 mg MNPs (Fe/SiO₂) - coated by SiO₂ (described above), was added 0.7 ml deionized water, 0,05 ml 25% ammonia solution for HCl

neutralizing (which released during VOCl₃ hydrolysis process) and 0.01 ml ethylenglycol (is a complexing agent). In the resulting solution dropwise 0.03 ml 10% solution corresponding precursor – (Nb(C₂H₅O)₅; Ta(C₂H₅O)₅ – wherein the metal oxides are sol. The mixture was stirred for 10 min and placed on an ultrasonic bath under room temperature for 10 min - 3 times. The tube with MNPs was placed in the magnetic rack, after the separation particles, liquid was removed by pipetting. Then MNPs were washed three times with 1 ml deionized water under magnetic separation process.

As the results four type of coating had been synthesized on the surface of MNPs: 1) Fe/SiO₂; 2) Fe/SiO₂/Ta₂O₅; 3) Fe/SiO₂/Nb₂O₅; 4) Fe/SiO₂/V₂O₅. These particles were used in further experiments.

Particles characterization

Chemical composition of MNP's surface with different coatings was studied by X-ray photoelectron spectroscopy (XPS) The X-ray beam size was 400 microns. Overview spectra were obtained with electron transmission energy 200 eV with step 1,0 eV, the number of scans was 15. High-resolution spectra (Si, Nb, Ta, V, O) were obtained with electron transmission energy 50 eV with step 1,0 eV, the number of scans 7. Cleaning of the samples after air contamination was carried out by Ar⁺ ion etching under at 200 eV for 30 sec. XPS spectra before and after ion etching are shown in fig. 3. The ion beam with 20 mm diameter had a uniform radial distribution of ion current.

SEM electron micrographs of MNPs coated by silica and niobium oxide are shown in fig. 4.

SEM electron micrographs confirmed the fact that coated MNPs do not form agglomerates in fluids. The average MNP size is 25 nm, MNPs shape is close to spherical.

Testing MNP's for DNA isolation

The sorption efficiency of metal oxides (V) was analysed by conducting real-time polymerase chain reaction. For this purpose we isolated DNA using standard protocol based on magnetic particles DNA isolation method, described in [10]. In order to determine the optimal conditions for DNA binding by metal oxides (V) we have changed some parameters of standard protocol.

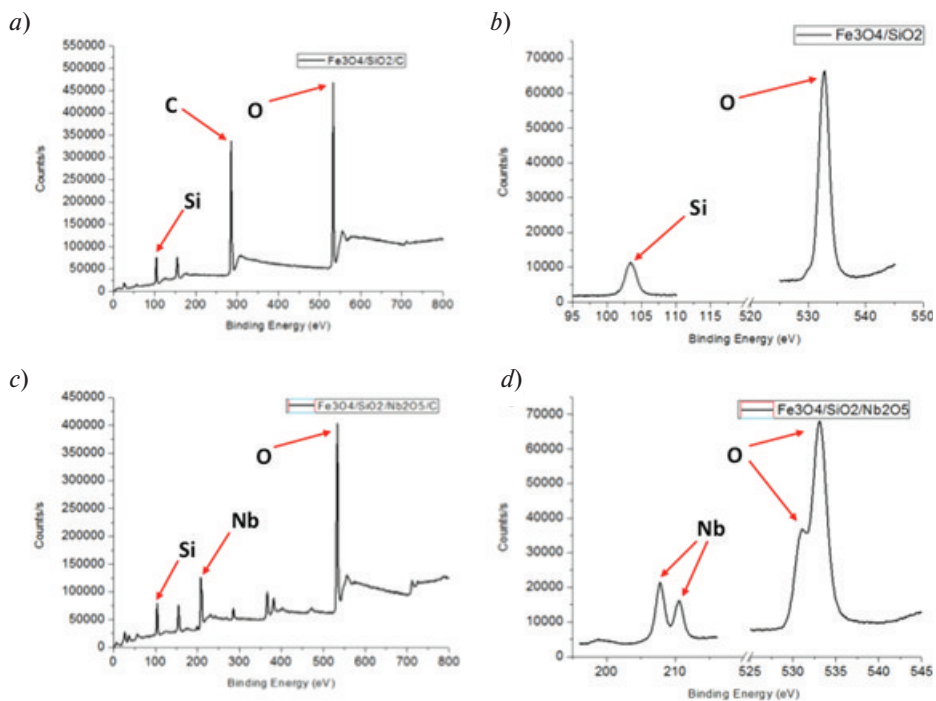


Fig. 3. XPS spectra of $\text{Fe}_3\text{O}_4/\text{SiO}_2$ and $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{Nb}_2\text{O}_5$ before (a, c) and after (b, d) etching

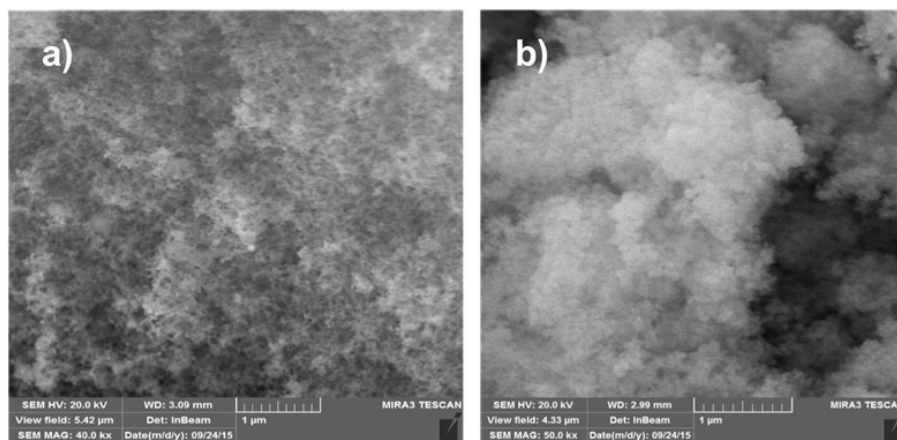


Fig. 4. SEM electron micrographs of: a) MNPs coated SiO_2 , b) MNPs coated $\text{SiO}_2/\text{Nb}_2\text{O}_5$.

DNA isolation was carried out with standard protocol, described previously, with metal oxides (V) and commercially available sorbents (“Syntol”, Russia). The resulting amplification curves are shown in fig. 5.

Basing on the values of average DNA threshold cycles (fig. 5) we can conclude that MNPs covered by niobium oxide (V) thin films have the highest sorption properties.

The worst result was shown by commercial MNPs produced by «Syntol» (Russia). The average difference in threshold cycles between the MNPs covered by metal oxides (V) and MNPs covered by silicon dioxide («Syntol») was about 3 cycles.

This phenomenon can be explained by the difference in amount of formed chemical bonds available for DNA binding and it turn to decreased the quantity of extracted DNA.

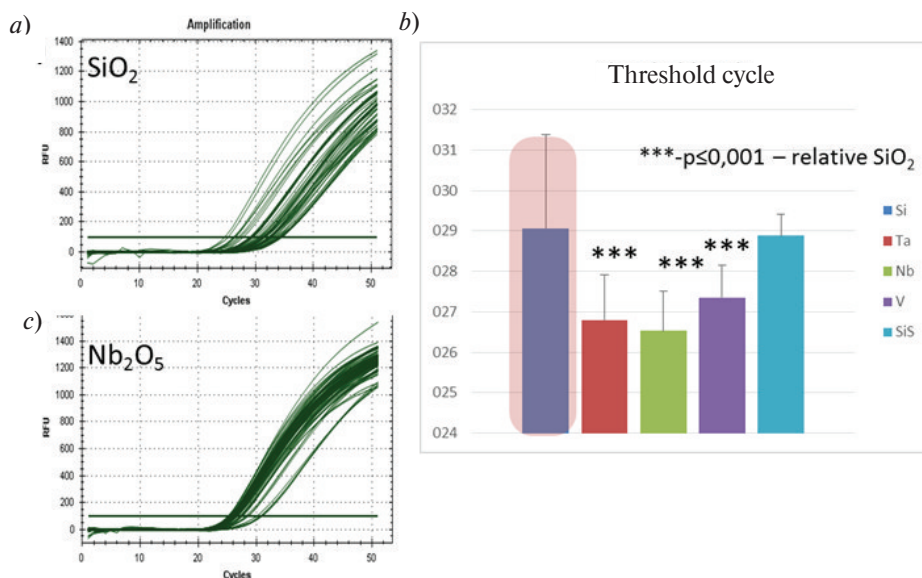


Fig. 5. The amplification curves obtained during the real-time PCR for isolated DNA using the standard protocol with SiO₂ and Nb₂O₅ as sorption materials (a) and (c) respectively and average DNA threshold cycles with silicon dioxide; tantalum oxide (V); niobium oxide (V); vanadium oxide (V); silicon dioxide («Syntol») as sorption materials

Conclusions

The metal oxides (V) have ability to form additional chemical bonds, which increase sorption capacity of MNPs covered by the metal oxides (V) thin film for DNA binding. However, the total usable area of surface is a dominant factor for achieving high sensitivity and efficiency of DNA extraction using MNPs. This fact was confirmed by the example of MNPs obtained by aerosol synthesis with silica compound as a sorbent, obtained by sol-gel method and commercial magnetic particles “Syntol”. Furthermore, obtained results suggest that the developed

thin film materials based on metal oxides (V) are suitable for the DNA extraction in clinical or research laboratories. Described above investigation results are relevant for future developments of micro- and nanodiagnostic systems, which might be realized “on-chip”, since the thin film materials are shown prospective for DNA isolation.

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